# Reproducible Analysis: The Two-Wing admixed structure of environmental microbial communities

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```
library(ggplot2)       # for generating plots
library(latex2exp)     # for plot text latex
library(cowplot)       # for merging plots
library(gridExtra)     # for griding plots
library(grid)          # for griding plots
library(stringr)       # for uppercase first letter
library(ggh4x)         # for grid plot with free axis
```

## Preface

This document provides reproducible research records for our manuscript about the Two-Wing admixed structure of environmental microbial communities. We provide step-by-step instructions for main results in both the original article and the supplemental materials.

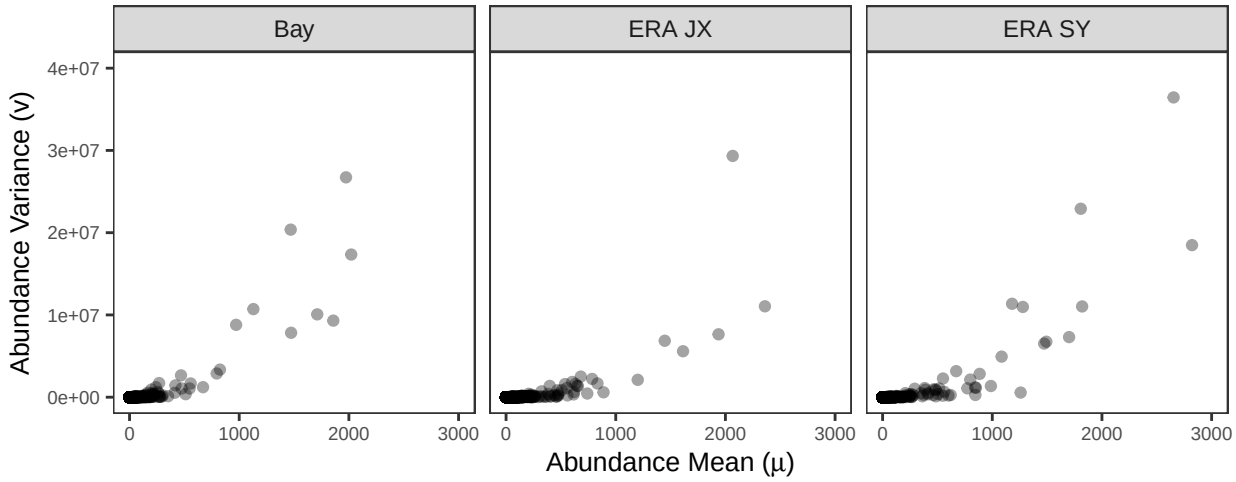
## Main Figures

### Figure 1. Observed abundance distribution

#### The over-dispersion pattern of microbial communities

```
load('../output/HZ/hz_mean_var.RData')
dat_plt$Region <- factor(dat_plt$Region, levels=c('Bay','ERA JX','ERA SY'))
ggplot(dat_plt, aes(x=miseqmean, y=miseqvar)) +
    geom_point(alpha=0.36, size=1.5) +
    xlab(TeX('Abundance Mean ($\\mu$)')) +
    ylab(TeX('Abundance Variance ($v$)')) +
    xlim(0,3000) +
    ylim(0,4e7) +
    theme_bw() +
    theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank(),
          axis.title.x = element_text(size = 10),
          axis.title.y = element_text(size = 10),
          axis.text.x = element_text(size = 7),
          axis.text.y = element_text(size = 7)) +
```

```
    theme(aspect.ratio=1) +
    facet_grid(~Region)
```




```
load('../output/AO_ASV/ao_mean_var.RData')
dat_plt$Industry <- factor(dat_plt$Industry, levels=c('Dye','Pharmaceutical','Pesticide'))
dat_plt$Process <- factor(dat_plt$Process, levels=c('Influent','Anoxic','Oxic','Effluent'))
ggplot(dat_plt, aes(x=miseqmean, y=miseqvar)) +
    geom_point(alpha=0.36, size=1.5) +
    xlab(TeX('Abundance Mean ($\\mu$)')) +
    ylab(TeX('Abundance Variance ($v$)')) +
    xlim(0,1000) +
    ylim(0,4e6) +
    theme_bw() +
    theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank(),
          axis.title.x = element_text(size = 10),
          axis.title.y = element_text(size = 10),
          axis.text.x = element_text(size = 7),
          axis.text.y = element_text(size = 7)) +
    theme(aspect.ratio=1) +
    facet_grid(Industry~Process)
```

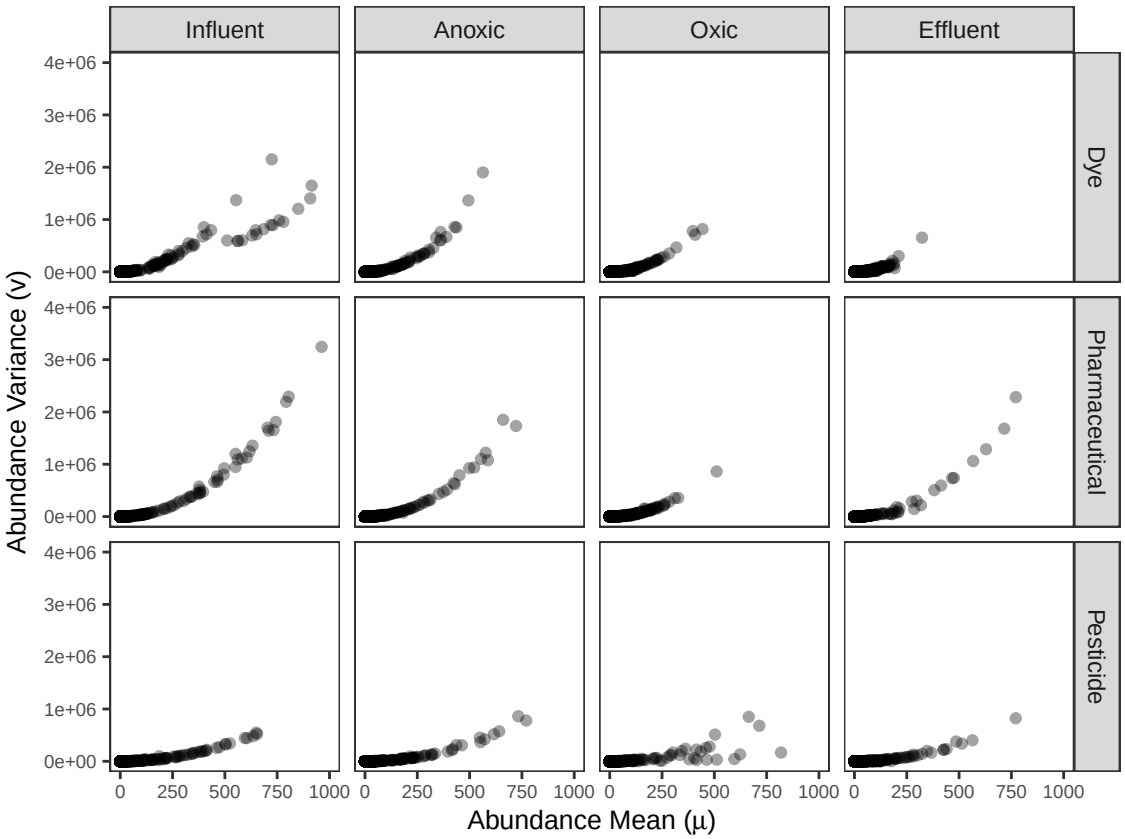
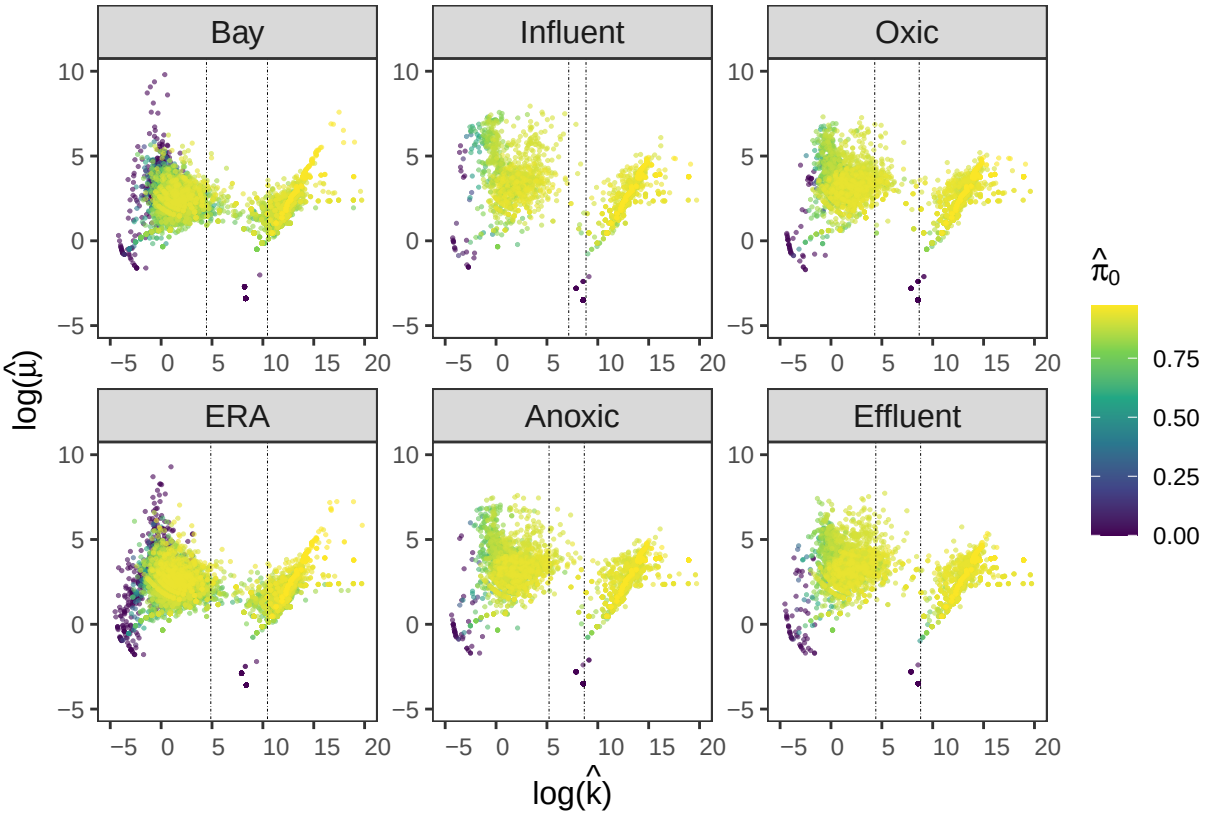

Influent
Anoxic
Oxic
Effluent
Dye
Pharmaceutical
Pesticide
Abundance Variance (v)
Abundance Mean (μ)
0e+00
1e+06
2e+06
3e+06
4e+06
0
250
500
750
1000

**Figure 2. The *Two-Wing* admixed structure**

**Before boundary refining (Figure 2A)**




**After boundary refining (Figure 2B)**

```
# load parameters mle
vec_process <- c('influent','anoxic','oxic','effluent')
param_plt <- data.frame()
for(item in vec_process){
  load(paste0('../output/AO_ASV/param_trio_asv_',item,'.RData'))
  load(paste0('../output/AO_ASV/mic_od_',item,'.RData'))
  param_trio$ID <- rownames(param_trio)
  # extract gamma-poisson distributed microbes
  param_gpm <- param_trio[param_trio$k!=Inf,]
  # annotate procedure
  param_gpm$Procedure <- str_to_title(item)
  # annotate taxa category
  load(paste0('../output/AO_ASV/mic_id_mu_k_',item,'.RData'))
  param_gpm$Wing <- ifelse(param_gpm$ID %in% id_mu, 'mu', 'Other')
  param_gpm$Wing <- ifelse(param_gpm$ID %in% id_k,  'k', param_gpm$Wing)
  param_plt <- rbind(param_plt, param_gpm)

}

vec_process <- c('bay','era')
```

```r
for(item in vec_process){
  load(paste0('../output/HZ/param_trio_',item,'.RData'))
  load(paste0('../output/HZ/mic_od_',item,'.RData'))
  param_trio$ID <- rownames(param_trio)
  # extract gamma-poisson distributed microbes
  param_gpm <- param_trio[param_trio$k!=Inf,]
  # annotate procedure
  param_gpm$Procedure <- str_to_title(item)
  # annotate taxa category
  load(paste0('../output/HZ/mic_id_mu_k_',item,'.RData'))
  param_gpm$Wing <- ifelse(param_gpm$ID %in% id_mu, 'mu', 'Other')
  param_gpm$Wing <- ifelse(param_gpm$ID %in% id_k, 'k', param_gpm$Wing)

  param_plt <- rbind(param_plt, param_gpm)

}

rownames(param_plt) <- c(1:nrow(param_plt))
param_plt$Procedure <- ifelse(param_plt$Procedure=='Era','ERA',param_plt$Procedure)

param_plt$Procedure <- factor(param_plt$Procedure,
                              levels=c('Bay','Influent','Oxic',
                                       'ERA','Anoxic','Effluent'))
param_plt$Wing <- factor(param_plt$Wing,
                             levels=c('mu','k', 'Other'))

ggplot() +
  geom_point(data=param_plt,
             aes(x=log(k), y=log(mu), color=Wing), alpha=0.36,size=0.6) +
  ylab(TeX('\\log{(\\hat{\\mu})}')) +
  xlab(TeX('\\log{(\\hat{k})}')) +
  ggtitle(TeX('')) +
  xlim(-5,20) +
  ylim(-5,10) +
  theme_bw() +
  theme(aspect.ratio=1) +
  facet_wrap(Procedure~., scale='free') +
  scale_colour_manual(values = c('#984ea3','#4daf4a'),
                      labels = unname(TeX(c('$\\mu$','$k$')))) +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size = 9, vjust = 0.5, hjust = 0),
        axis.text.y = element_text(size=9),
        strip.text = element_text(size = 12),
        legend.title = element_text(color = "black", size = 10),
        legend.text = element_text(color = "black", size = 9)) +
  guides(color = guide_legend(override.aes = list(size = 1.8)))
```

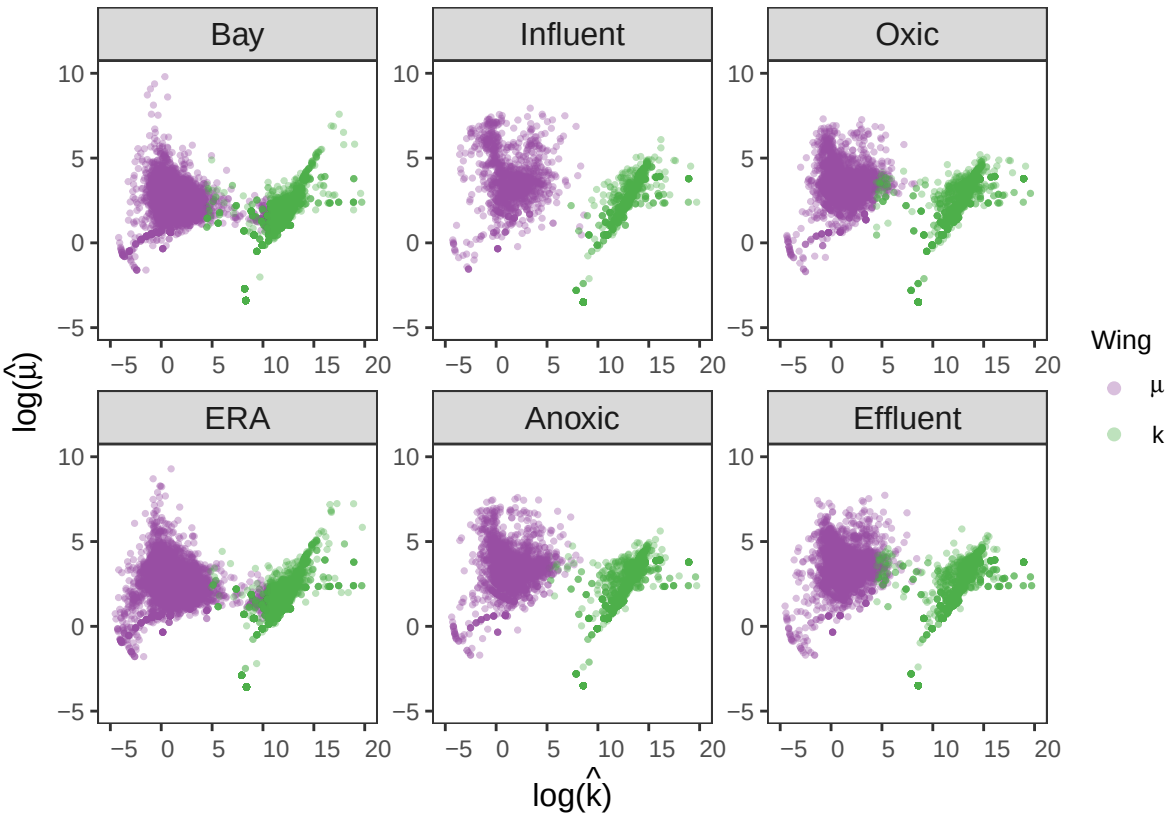


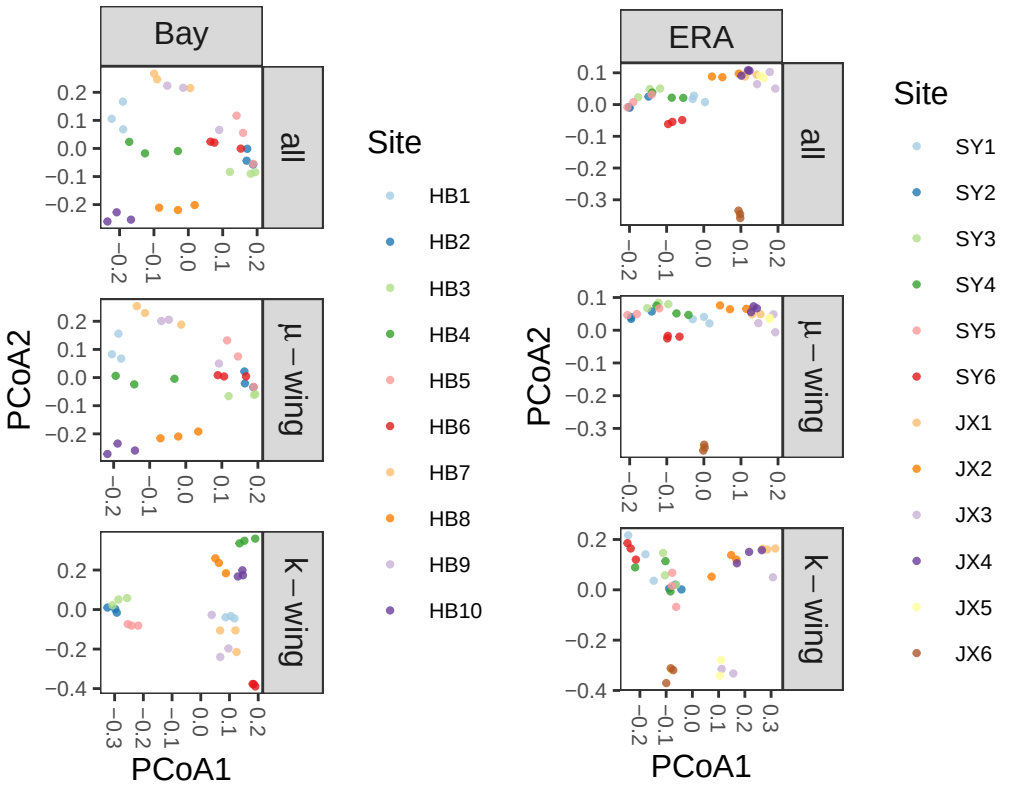

**Figure 3. Dimensionality reduction analysis of $\mu$-wing and $k$-wing sub-community**

**PCoA**

```
# for bay
bay_plt <- data.frame()
lab_site <- c(rep(c('HB1','HB2','HB3','HB4','HB5','HB6','HB7','HB8','HB9','HB10'), each=3))
load('../output/HZ/pcoa_bay.RData')

bay_plt <- rbind(bay_plt, data.frame(PCoA1=pcoa_mu$vectors[,1],
                                     PCoA2=pcoa_mu$vectors[,2],
                                     Site=lab_site,
                                     Wing='mu-wing',
                                     Region='Bay'))
bay_plt <- rbind(bay_plt, data.frame(PCoA1=pcoa_k$vectors[,1],
                                     PCoA2=pcoa_k$vectors[,2],
                                     Site=lab_site,
                                     Wing='k-wing',
                                     Region='Bay'))
bay_plt <- rbind(bay_plt, data.frame(PCoA1=pcoa_all$vectors[,1],
                                     PCoA2=pcoa_all$vectors[,2],
                                     Site=lab_site,
                                     Wing='all',
                                     Region='Bay'))
bay_plt$Site <- factor(bay_plt$Site,
                       levels=c('HB1','HB2','HB3','HB4','HB5','HB6','HB7','HB8','HB9','HB10'))
```

```
bay_plt$Wing <- factor(bay_plt$Wing,
                       levels=c('all','mu-wing','k-wing'))

g_bay <- ggplot(bay_plt, aes(x=PCoA1, y=PCoA2, color=Site)) +
  geom_point(size=0.8, alpha=0.8) +
  theme_bw() +
  xlab(TeX('PCoA1')) +
  ylab(TeX('PCoA2')) +
  ggtitle('') +
  theme(plot.title=element_text(hjust=0, size=rel(0.0)),
        aspect.ratio=1,
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size = 8, angle=-90, vjust=0.5, hjust=0),
        axis.text.y = element_text(size = 8),
        strip.text = element_text(size = 12),
        legend.title = element_text(color = "black", size = 12),
        legend.text = element_text(color = "black", size = 8)) +
  scale_colour_manual(values=c('#a6cee3','#1f78b4','#b2df8a','#33a02c',
                               '#fb9a99','#e31a1c','#fdbf6f','#ff7f00',
                               '#cab2d6','#6a3d9a')) +
  facet_grid2(Wing~Region, scales='free',
              independent = "all",labeller='label_parsed')

# for era
era_plt <- data.frame()
lab_site <- c(rep(c('SY1','SY2','SY3','SY4','SY5','SY6',
                    'JX1','JX2','JX3','JX4','JX5','JX6'), each=3))
load('../output/HZ/pcoa_era.RData')

era_plt <- rbind(era_plt, data.frame(PCoA1=pcoa_mu$vectors[,1],
                                     PCoA2=pcoa_mu$vectors[,2],
                                     Site=lab_site,
                                     Wing='mu-wing',
                                     Region='ERA'))
era_plt <- rbind(era_plt, data.frame(PCoA1=pcoa_k$vectors[,1],
                                     PCoA2=pcoa_k$vectors[,2],
                                     Site=lab_site,
                                     Wing='k-wing',
                                     Region='ERA'))
era_plt <- rbind(era_plt, data.frame(PCoA1=pcoa_all$vectors[,1],
                                     PCoA2=pcoa_all$vectors[,2],
                                     Site=lab_site,
                                     Wing='all',
                                     Region='ERA'))
era_plt$Site <- factor(era_plt$Site,
                       levels=c('SY1','SY2','SY3','SY4','SY5','SY6','JX1','JX2','JX3','JX4','JX5','JX6'
era_plt$Wing <- factor(era_plt$Wing,
                       levels=c('all','mu-wing','k-wing'))

g_era <- ggplot(era_plt, aes(x=PCoA1, y=PCoA2, color=Site)) +
```

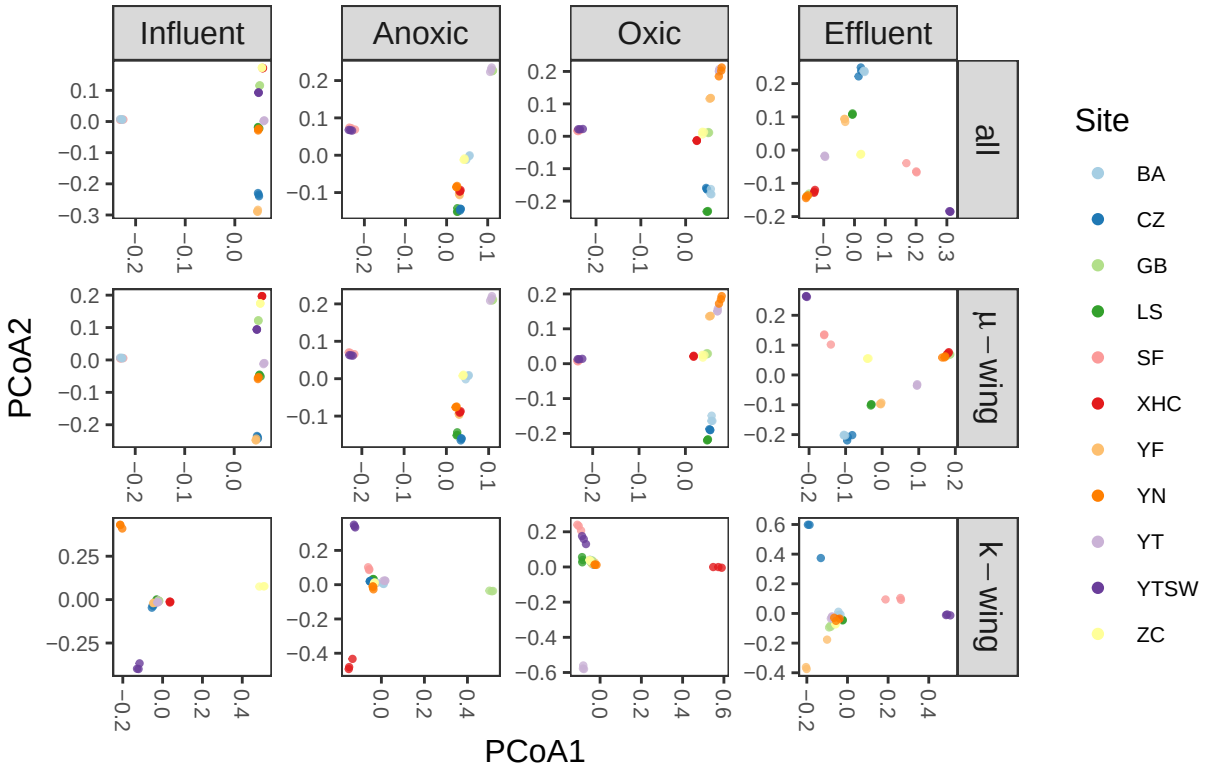
```
  geom_point(size=0.8, alpha=0.8) +
  theme_bw() +
  xlab(TeX('PCoA1')) +
  ylab(TeX('PCoA2')) +
  ggtitle('') +
  theme(plot.title=element_text(hjust=0, size=rel(0.0)),
        aspect.ratio=1,
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size = 8, angle=-90, vjust=0.5, hjust=0),
        axis.text.y = element_text(size = 8),
        strip.text = element_text(size = 12),
        legend.title = element_text(color = "black", size = 12),
        legend.text = element_text(color = "black", size = 8)) +
  scale_colour_manual(values=c('#a6cee3','#1f78b4','#b2df8a','#33a02c',
                               '#fb9a99','#e31a1c','#fdbf6f','#ff7f00',
                               '#cab2d6','#6a3d9a','#ffff99','#b15928')) +
  facet_grid2(Wing~Region, scales='free',
              independent = "all",labeller='label_parsed')



plot_grid(NULL, g_bay, g_era, NULL,
          rel_widths = c(1,5,5,1), nrow=1, align='v')
```




```
dat_plt <- data.frame()
vec_process <- c('influent','anoxic','oxic','effluent')
```

```
lab_site <- c(rep(c('LS','SF','CZ','BA','YF','GB','YTSW','XHC','ZC','YT','YN'), each=3))
for(item in vec_process){
  load(paste0('../output/AO_ASV/pcoa_',item,'.RData'))
  dat_plt <- rbind(dat_plt, data.frame(PCoA1=pcoa_mu$vectors[,1],
                                       PCoA2=pcoa_mu$vectors[,2],
                                       Site=lab_site,
                                       Wing='mu-wing',
                                       Procedure=str_to_title(item)))
  dat_plt <- rbind(dat_plt, data.frame(PCoA1=pcoa_k$vectors[,1],
                                       PCoA2=pcoa_k$vectors[,2],
                                       Site=lab_site,
                                       Wing='k-wing',
                                       Procedure=str_to_title(item)))
  dat_plt <- rbind(dat_plt, data.frame(PCoA1=pcoa_all$vectors[,1],
                                       PCoA2=pcoa_all$vectors[,2],
                                       Site=lab_site,
                                       Wing='all',
                                       Procedure=str_to_title(item)))

}

dat_plt$Wing <- factor(dat_plt$Wing, levels=c('all','mu-wing','k-wing'))
dat_plt$Procedure <- factor(dat_plt$Procedure, levels=str_to_title(vec_process))


g_pre <- ggplot(dat_plt, aes(x=PCoA1, y=PCoA2, color=Site)) +
  geom_point() +
  theme_bw() +
  xlab(TeX('PCoA1')) +
  ylab(TeX('PCoA2')) +
  ggtitle('') +
  theme(plot.title=element_text(hjust=0, size=rel(0.0)),
        aspect.ratio=1,
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size = 8, angle=-90, vjust=0.5, hjust=0),
        axis.text.y = element_text(size = 8),
        strip.text = element_text(size = 12),
        legend.title = element_text(color = "black", size = 12),
        legend.text = element_text(color = "black", size = 8)) +
  scale_colour_manual(values=c('#a6cee3','#1f78b4','#b2df8a','#33a02c',
                               '#fb9a99','#e31a1c','#fdbf6f','#ff7f00',
                               '#cab2d6','#6a3d9a','#ffff99')) +
  facet_grid2(Wing~Procedure, scales='free',
              independent = "all",labeller='label_parsed')

g_leg <- get_legend(g_pre)


g_main <- ggplot(dat_plt, aes(x=PCoA1, y=PCoA2, color=Site)) +
  geom_point(size=0.8, alpha=0.8) +
```

```
  theme_bw() +
  xlab(TeX('PCoA1')) +
  ylab(TeX('PCoA2')) +
  ggtitle('') +
  theme(plot.title=element_text(hjust=0, size=rel(0.0)),
        aspect.ratio=1,
        legend.position="none",
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size = 8, angle=-90, vjust=0.5, hjust=0),
        axis.text.y = element_text(size = 8),
        strip.text = element_text(size = 12)) +
  scale_colour_manual(values=c('#a6cee3','#1f78b4','#b2df8a','#33a02c',
                               '#fb9a99','#e31a1c','#fdbf6f','#ff7f00',
                               '#cab2d6','#6a3d9a','#ffff99')) +
  facet_grid2(Wing~Procedure, scales='free',
              independent = "all",labeller='label_parsed')

plot_grid(g_main, g_leg,
          rel_widths = c(5, 1), nrow=1)
```




### PCA

Follow similar algorithm as above. Specifically, run `../figures/vignettes/pca_hz.Rmd` and `../figures/vignettes/pca_ao.Rmd`

### CCA

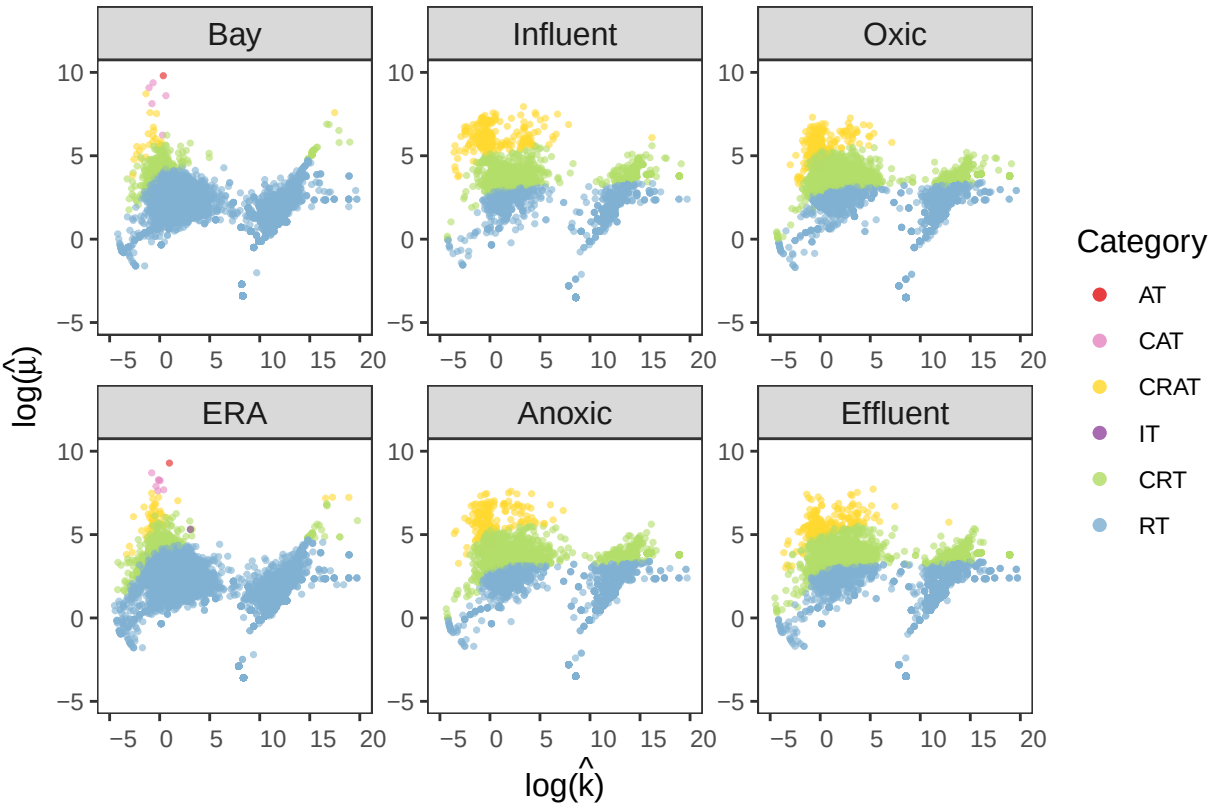
Follow similar algorithm as above. Specifically, run `../figures/vignettes/cca_ao_ori.Rmd`

## Figure 4. The *Two-Wing* structure generalizes relative-abundance-based categorizing method

### Using *Method 0* to identify microbial categories

```
# load parameters mle
vec_process <- c('influent','anoxic','oxic','effluent')
param_plt <- data.frame()
for(item in vec_process){
  load(paste0('../output/AO_ASV/param_trio_asv_',item,'.RData'))
  param_trio$ID <- rownames(param_trio)
  # extract gamma-poisson distributed microbes
  param_gpm <- param_trio[param_trio$k!=Inf,]
  # annotate procedure
  param_gpm$Procedure <- str_to_title(item)
  # annotate taxa category
  load(paste0('../output/AO_ASV/taxa_category_',item,'.RData'))
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_rt, 'RT', 'Other')
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_mt, 'IT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_at, 'AT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_crt, 'CRT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_cat, 'CAT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_crat, 'CRAT', param_gpm$Category)

  param_plt <- rbind(param_plt, param_gpm)

}

vec_process <- c('bay','era')
for(item in vec_process){
  load(paste0('../output/HZ/param_trio_',item,'.RData'))
  param_trio$ID <- rownames(param_trio)
  # extract gamma-poisson distributed microbes
  param_gpm <- param_trio[param_trio$k!=Inf,]
  # annotate procedure
  param_gpm$Procedure <- str_to_title(item)
  # annotate taxa category
  load(paste0('../output/HZ/taxa_category_',item,'.RData'))
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_rt, 'RT', 'Other')
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_mt, 'IT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_at, 'AT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_crt, 'CRT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_cat, 'CAT', param_gpm$Category)
  param_gpm$Category <- ifelse(param_gpm$ID %in% id_crat, 'CRAT', param_gpm$Category)

  param_plt <- rbind(param_plt, param_gpm)

}
```

```
rownames(param_plt) <- c(1:nrow(param_plt))
param_plt$Procedure <- ifelse(param_plt$Procedure=='Era','ERA',param_plt$Procedure)

param_plt$Procedure <- factor(param_plt$Procedure,
                              levels=c('Bay','Influent','Oxic',
                                       'ERA','Anoxic','Effluent'))
param_plt$Category <- factor(param_plt$Category,
                             levels=c('AT','CAT','CRAT',
                                      'IT','CRT','RT'))

ggplot() +
  geom_point(data=param_plt,
             aes(x=log(k), y=log(mu), color=Category), alpha=0.6,size=0.6) +
  geom_point(data=param_plt[param_plt$Category=='IT',],
             aes(x=log(k), y=log(mu), color=Category), alpha=0.6,size=0.6) +
  ylab(TeX('\\log{(\\hat{\\mu})}')) +
  xlab(TeX('\\log{(\\hat{k})}')) +
  ggtitle(TeX('')) +
  xlim(-5,20) +
  ylim(-5,10) +
  theme_bw() +
  theme(aspect.ratio=1) +
  facet_wrap(Procedure~., scale='free') +
  scale_colour_manual(values = c('#e41a1c','#e78ac3','#ffd92f',
                                 '#984ea3','#b3de69','#80b1d3')) +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size = 9, vjust = 0.5, hjust = 0),
        axis.text.y = element_text(size=9),
        strip.text = element_text(size = 12),
        legend.title = element_text(color = "black", size = 12),
        legend.text = element_text(color = "black", size = 9)) +
  guides(color = guide_legend(override.aes = list(size = 1.8)))
```




**Using *Method a-c* to identify microbial categories**

The algorithm is similar as above. Specifically, run `../figures/vignettes/main/mu_k_category_abcd.Rmd`

```
sessionInfo()
```

```
## R version 4.2.0 (2022-04-22)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Catalina 10.15.3
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
## [1] ggh4x_0.2.1     stringr_1.4.0   gridExtra_2.3   cowplot_1.1.1
## [5] latex2exp_0.9.4 ggplot2_3.3.6
##
## loaded via a namespace (and not attached):
##  [1] highr_0.9         pillar_1.8.0      compiler_4.2.0    tools_4.2.0
##  [5] digest_0.6.29     viridisLite_0.4.0 evaluate_0.15     lifecycle_1.0.1
```

```
##  [9] tibble_3.1.8      gtable_0.3.0      pkgconfig_2.0.3   rlang_1.0.4
## [13] cli_3.3.0         rstudioapi_0.13   yaml_2.3.5        xfun_0.31
## [17] fastmap_1.1.0     withr_2.5.0       dplyr_1.0.9       knitr_1.39
## [21] generics_0.1.3    vctrs_0.4.1       tidyselect_1.1.2  glue_1.6.2
## [25] R6_2.5.1          fansi_1.0.3       rmarkdown_2.14    farver_2.1.1
## [29] purrr_0.3.4       magrittr_2.0.3    scales_1.2.0      htmltools_0.5.3
## [33] colorspace_2.0-3  labeling_0.4.2    utf8_1.2.2        stringi_1.7.8
## [37] munsell_0.5.0
```