mock_microbiome_analysis

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Setup

```
library("tidyverse")
library("phyloseq")
library("ggpubr")
library("DESeq2")
library("ggsci")
library("colorspace")
library("flextable")
library("pracma")
library("purrr")
```

Summarize MetaScope Results

```
ground_truth_taxonomy <- read.csv("data_processed/kozich_2013/ground_truth_taxonomy.csv",
                      head = TRUE)
species_ground_truth <- ground_truth_taxonomy$species
accessions_path <- "reflib/accessionTaxa.sql"

## Code used to generate ground truth taxids
#ground_truth_taxids <- ground_truth_taxonomy |>
#  tidyr::pivot_longer(everything(), names_to = "Taxonomy Level", values_to = "taxon") |>
#  dplyr::distinct(taxon, .keep_all = TRUE) |>
#  mutate(taxid = taxonomizr::getId(taxon, sqlFile = accessions_path)) |>
#  rowwise() |>
#  mutate(taxid = as.numeric(strsplit(taxid, split = ",")[[1]][1]))
#write.csv(ground_truth_taxids,
#          file = "data_processed/kozich_2013/ground_truth_taxids.csv",
#          row.names = FALSE)
ground_truth_taxids <- read.csv("data_processed/kozich_2013/ground_truth_taxids.csv",)

# Function used to read MetaScope outputs and generate cleaned accuracy metrics
create_summary_df <- function(csv_paths, pipeline_name) {
  summary_df <- data.frame(
    Species = c("No Call", "Incorrect Call",species_ground_truth))
  n = 1
  for (i in csv_paths) {
    # Get Sample Names
```

```
    sample_name <- sub("\\..*$", "", basename(i))
    # Clean MetaBlast Table
    metascope_df <- read.csv(i, head = TRUE)
    head(metascope_df)
    metascope_df <- metascope_df |>
      dplyr::select(Genome, read_count) |>
      dplyr::mutate_if(is.numeric, ~ . / sum(.))
    colnames(metascope_df) <- c("Species", sample_name)
    summary_df <- dplyr::left_join(summary_df, metascope_df, by = "Species")
    summary_df[is.na(summary_df)] <- 0

    no_call.metascope <- metascope_df |>
      dplyr::filter(is.na(Species)) |>
      dplyr::select(sample_name) |>
      sum()
    correct_call.metascope <- metascope_df |>
      dplyr::filter(Species %in% species_ground_truth) |>
      dplyr::select(sample_name) |>
      sum()
    incorrect_call.metascope <- 1 - correct_call.metascope - no_call.metascope

    summary_df[1,n+1] <- no_call.metascope
    summary_df[2,n+1] <- incorrect_call.metascope
    n = n + 1
  }


  summary_df_long <- tidyr::pivot_longer(
    summary_df,
    cols = c(2:ncol(summary_df)),
    values_to = "prop"
  )
  summary_df_long$Species <- factor(summary_df_long$Species,
                                    levels = c("No Call", "Incorrect Call", species_ground_truth))
  summary_df_long$name <- factor(summary_df_long$name)
  summary_df_long$pipeline <- factor(rep(pipeline_name, nrow(summary_df_long)))
  return(summary_df_long)
}

## Generate Summary dataframes for all metascope outputs
ms_df <- create_summary_df(list.files(path = "data_processed/kozich_2013/results",
                               pattern = ".metascope_id.csv",
                               full.names = TRUE,
                               recursive = TRUE),
                  pipeline = "MetaScope")
```

```
## Warning: Using an external vector in selections was deprecated in tidyselect 1.1.0.
## i Please use ‘all_of()‘ or ‘any_of()‘ instead.
##   # Was:
##   data %>% select(sample_name)
##
##   # Now:
##   data %>% select(all_of(sample_name))
```

```
##
## See <https://tidyselect.r-lib.org/reference/faq-external-vector.html>.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
ms_p_df <-create_summary_df(list.files(path = "data_processed/kozich_2013/results_priors",
                            pattern = ".metascope_id.csv",
                            full.names = TRUE,
                            recursive = TRUE),
                  pipeline = "MetaScope Priors")
ms_p_b_df <-create_summary_df(list.files(path = "data_processed/kozich_2013/results_metablast_priors_1.
                            pattern = ".metascope_id.csv",
                            full.names = TRUE,
                            recursive = TRUE),
                  pipeline = "MetaBlast")
summary_df <- rbind(ms_df, ms_p_df, ms_p_b_df)

## Add Ground Truth to summary dataframe
ground_truth_df <- data.frame(
  Species = c("No Call", "Incorrect Call",species_ground_truth))
ground_truth_df <- cbind(ground_truth_df,
                         as.data.frame(do.call(cbind, replicate(33, c(0,0,rep(1/21, 21)), simplify = FA
colnames(ground_truth_df) <-  c("Species", as.character(unique(summary_df$name)))
ground_truth_df <- tidyr::pivot_longer(
  ground_truth_df,
  cols = c(2:34),
  values_to = "prop"
)
ground_truth_df$pipeline <- "Ground Truth"
summary_df <- rbind(summary_df, ground_truth_df)
```

Summarize DADA2 Results

```
##Left joining with aggregate because duplicate species names
dada2_files <- list.files(path = "data_processed/kozich_2013/dada2_results",
                          full.names = TRUE)
taxonomy_cols <- c("Species", "Genus", "Family", "Order", "Class", "Phylum", "Kingdom")

## Join DADA2 Files together
dada2_df <- purrr::map_dfr(dada2_files, function(x){
  sample_name <- sub("\\..*$", "", basename(x)) |> strsplit(split = "dada2_")
  sample_name <- sample_name[[1]][2]
  res <- read.csv(x) |>
    dplyr::mutate(Species = ifelse(is.na(Species), NA, paste0(Genus, " ", Species))) |>
    dplyr::mutate_if(is.numeric, ~ . / sum(.)) |>
    dplyr::mutate(pipeline = "DADA2-NB", name = sample_name) |>
    dplyr::rowwise() |>
    dplyr::mutate(
      taxon = {
        tax_vals <- c_across(all_of(taxonomy_cols))
```

```
        non_na_vals <- tax_vals[!is.na(tax_vals)]
        if (length(non_na_vals) > 0) non_na_vals[1] else NA_character_
      }
    ) |>
    dplyr::ungroup()
})

## Clean up old taxonomy names
dada2_df$taxon[dada2_df$taxon == "Bacteroides vulgatus"] <- 'Phocaeicola vulgatus'
dada2_df$taxon[dada2_df$taxon == 'Actinomyces odontolyticus'] <- "Schaalia odontolytica"
dada2_df$taxon[dada2_df$taxon == "Propionibacterium acnes"] <- 'Cutibacterium acnes'
dada2_df$taxon[dada2_df$taxon == "Rhodobacter sphaeroides"] <- 'Cereibacter sphaeroides'
dada2_df$taxon[dada2_df$taxon == "Clostridium sensu stricto 1 beijerinckii"] <- 'Clostridium beijerinck
dada2_df$taxon[dada2_df$taxon == "Escherichia-Shigella"] <- 'Escherichia' # Assuming DADA2 meant Escher
dada2_df$taxon[dada2_df$taxon == "Prevotella_9"] <- 'Segatella'
dada2_df$taxon[dada2_df$taxon == "Prevotella_9 copri"] <- 'Segatella copri'
dada2_df$taxon[dada2_df$taxon == "Rhodobacteraceae"] <- 'Paracoccaceae'
dada2_df$taxon[dada2_df$taxon == "Bacteroides massiliensis"] <- 'Phocaeicola massiliensis'
dada2_df$taxon[dada2_df$taxon == "Ruminococcaceae"] <- 'Oscillospiraceae'
dada2_df$taxon[dada2_df$taxon == "Clostridium sensu stricto 1"] <- 'Clostridium'
dada2_df$taxon[dada2_df$taxon == "Planococcaceae"] <- 'Caryophanaceae'


## Add NCBI taxonomy ids
dada2_df <- dada2_df |>
  mutate(taxid_raw = taxonomizr::getId(taxon, sqlFile = accessions_path)) |>
  rowwise() |>
  mutate(taxid = as.numeric(strsplit(taxid_raw, split = ",")[[1]][1])) |>
  ungroup() |>
  mutate(taxid = if_else(is.na(taxid), 9999999, taxid)) |> # REPLACING ALL UNKNOWN TAXONS WITH TAXID 99
  dplyr::select(!taxid_raw)
```

```
## Warning: There was 1 warning in `mutate()`.
## i In argument: `taxid_raw = taxonomizr::getId(taxon, sqlFile =
##   accessions_path)`.
## Caused by warning in `taxonomizr::getId()`:
## ! Multiple taxa ids found for Bacillus, Serratia. Collapsing with commas
```

```
## Adding categories
dada2_df_cats <- dada2_df |>
  dplyr::mutate(category = case_when(
    (taxid %in% ground_truth_taxids$taxid) & !is.na(Species) ~ taxon,
    (taxid %in% ground_truth_taxids$taxid) & is.na(Species) ~ "No Call",
    !(taxid %in% ground_truth_taxids$taxid) ~ "Incorrect Call")) |>
  dplyr::group_by(category, name) |>
  dplyr::summarise(prop = sum(reads_count))
```

```
## `summarise()` has grouped output by 'category'. You can override using the
## `.groups` argument.
```

```
dada2_df_cats$pipeline = "DADA2-NB"
colnames(dada2_df_cats) <- c("Species", "name", "prop", "pipeline")
```

```
#write.csv(dada2_df_cats, file = "data_processed/kozich_2013/dada2_df.csv",
#          row.names = FALSE)

summary_df <- rbind(summary_df, dada2_df_cats)
```

Summarize QIIME2 Results

```
summary_df_qiime2 <- data.frame(
  Species = c("No Call", "Incorrect Call",species_ground_truth))

qiime2_otu_table <- read.table("data_processed/kozich_2013/qiime2_results/feature_table.tsv",
           header = TRUE, sep = "\t") |>
  dplyr::mutate_if(is.numeric, ~ . / sum(.)) |>
  pivot_longer(cols = !OTU.ID)

taxonomy_cols <- c("Species", "Genus", "Family", "Order", "Class", "Phylum", "Superkingdom")
qiime2_tax_table <- read.table("data_processed/kozich_2013/qiime2_results/taxonomy.tsv",
                               header = TRUE, sep = "\t") |>
  separate(Taxon, into = c("Superkingdom", "Phylum", "Class",
                           "Order", "Family", "Genus", "Species"),
           sep = ";", fill = "right", extra = "drop") |>
  mutate(across(Superkingdom:Species, ~ str_remove(., "^\\s*[a-z]__"))) |>
  mutate(Species = sub("_", " ", Species)) |>
  rowwise() |>
  mutate(
    taxon = {
      tax_vals <- c_across(all_of(taxonomy_cols))
      non_na_vals <- tax_vals[!is.na(tax_vals)]
      if (length(non_na_vals) > 0) non_na_vals[1] else NA_character_0}) |>
  ungroup()

# Manually Address NAs
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Clostridium_sensu_stricto_1"] <- "Clostridium"
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Escherichia-Shigella"] <- "Escherichia" # Escherichia
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Bacteroides vulgatus"] <- "Phocaeicola vulgatus"
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Bacteroides massiliensis"] <- "Phocaeicola massiliens
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Alistipes obesi"] <- "Alistipes communis"
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Candidatus_Udaeobacter"] <- "Candidatus Udaeobacter"
qiime2_tax_table$taxon[qiime2_tax_table$taxon == "Ruminococcaceae"] <- "Oscillospiraceae"

# Add taxids to QIIME2 taxonomy table
qiime2_tax_table <- qiime2_tax_table |>
  mutate(taxid_raw = taxonomizr::getId(taxon, sqlFile = accessions_path)) |>
  rowwise() |>
  mutate(taxid = as.numeric(strsplit(taxid_raw, split = ",")[[1]][1])) |>
  ungroup() |>
  mutate(taxid = if_else(is.na(taxid), 9999999, taxid)) |> # REPLACING ALL UNKNOWN TAXONS WITH TAXID 99
  dplyr::select(!taxid_raw)
```

```
## Warning: There was 1 warning in `mutate()`.
```

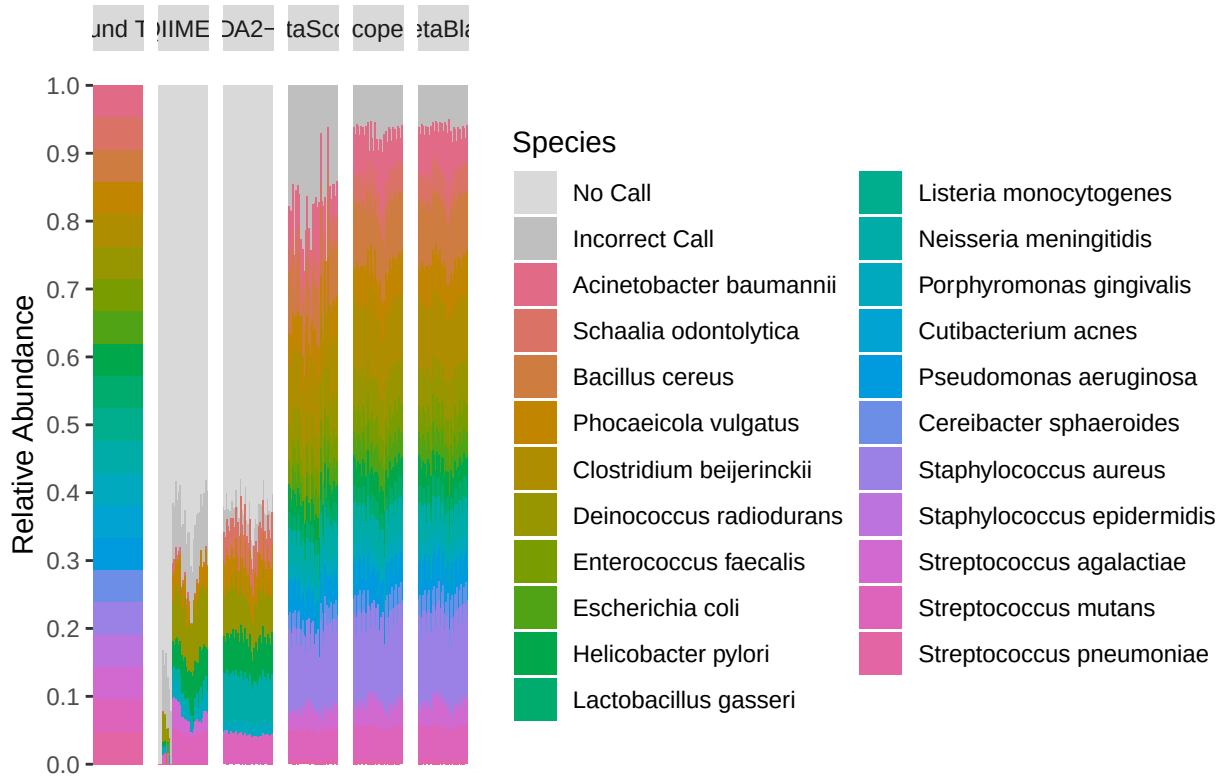
```
## i In argument: `taxid_raw = taxonomizr::getId(taxon, sqlFile =
##   accessions_path)`.
## Caused by warning in `taxonomizr::getId()`:
## ! Multiple taxa ids found for Bacillus. Collapsing with commas
```

```
qiime2_df <- full_join(qiime2_otu_table, qiime2_tax_table, by=join_by(OTU.ID == Feature.ID))


qiime2_df_cats <- qiime2_df |>
  dplyr::mutate(category = case_when(
    (taxid %in% ground_truth_taxids$taxid) & !is.na(Species) ~ taxon,
    (taxid %in% ground_truth_taxids$taxid) & is.na(Species) ~ "No Call",
    !(taxid %in% ground_truth_taxids$taxid) ~ "Incorrect Call")) |>
  dplyr::group_by(category, name) |>
  dplyr::summarise(prop = sum(value))
```

```
## `summarise()` has grouped output by 'category'. You can override using the
## `.groups` argument.
```

```
qiime2_df_cats$pipeline <- "QIIME2"
colnames(qiime2_df_cats) <- c("Species", "name", "prop", "pipeline")

summary_df_qiime2 <-
  expand.grid(Species = c("No Call", "Incorrect Call", species_ground_truth),
              name = unique(qiime2_df_cats$name),
              stringsAsFactors = TRUE) |>
  merge(qiime2_df_cats, by = c("Species", "name"), all.x = TRUE) |>
  replace_na(list(prop = 0, pipeline = "QIIME2"))

#write.csv(summary_df_qiime2, file = "data_processed/kozich_2013/qiime2_df.csv",
#          row.names = FALSE)
summary_df <- rbind(summary_df, summary_df_qiime2)
```

Plotting Relative Abundance of Mock Microbiome



Accuracy Tables

```
## Warning: fonts used in ‘flextable‘ are ignored because the ‘pdflatex‘ engine is
## used and not ‘xelatex‘ or ‘lualatex‘. You can avoid this warning by using the
## ‘set_flextable_defaults(fonts_ignore=TRUE)‘ command or use a compatible engine
## by defining ‘latex_engine: xelatex‘ in the YAML header of the R Markdown
## document.
```

Profiler	Correct Call	No Call	Incorrect Call
QIIME2	0.211 ± 0.116	0.708 ± 0.135	0.081 ± 0.028
DADA2-NB	0.347 ± 0.023	0.623 ± 0.022	0.03 ± 0.005
MetaScope	0.821 ± 0.047	0 ± 0	0.179 ± 0.047
MetaScope Priors	0.929 ± 0.013	0 ± 0	0.071 ± 0.013
MetaBlast	0.936 ± 0.009	0 ± 0	0.064 ± 0.009

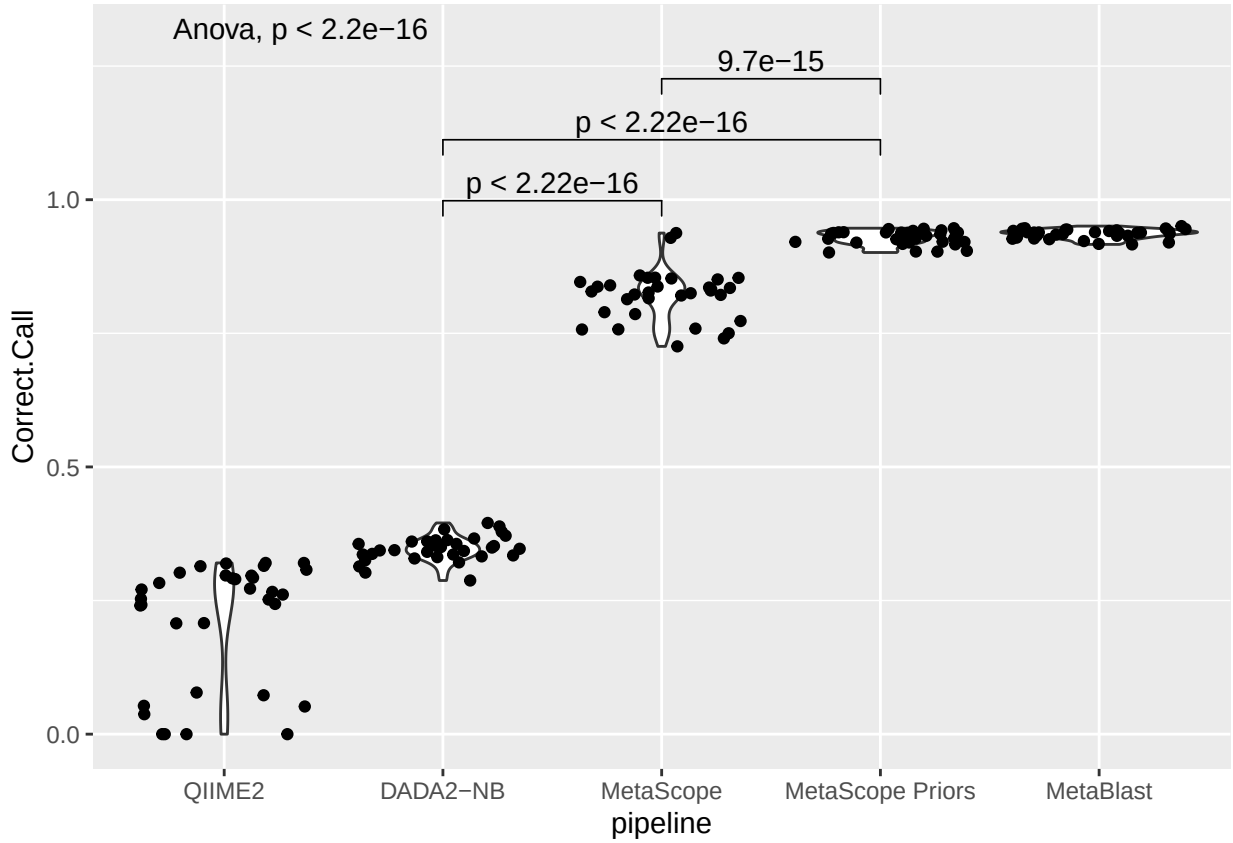
```
# This metascope is k - 25
p_bar_with_stats<- summary_df |> dplyr::filter(Species %in% c("No Call", "Incorrect Call")) |>
  tidyr::pivot_wider(names_from = Species, values_from = prop, names_repair = "universal") |>
```

```
  dplyr::mutate(Correct.Call = 1 - (No.Call + Incorrect.Call)) |>
  dplyr::filter(pipeline != "Ground Truth") |>
  dplyr::group_by(pipeline) |>
  dplyr::group_by(name) |>
  ggplot(aes(x = pipeline, y = Correct.Call)) +
  geom_violin() +
  geom_jitter() +
  ylim(0,1.3) +
  stat_compare_means(label.y = 1.3, method = "anova", paired = TRUE) +
  stat_compare_means(comparisons = list(c("DADA2-NB", "MetaScope"),
                                        c("DADA2-NB", "MetaScope Priors"),
                                        c("MetaScope", "MetaScope Priors"),
                                        c("MetaScope Priors", "MetaBlast")),
                     method = "t.test",
                     paired = TRUE)
```

```
## New names:
## * `No Call` -> `No.Call`
## * `Incorrect Call` -> `Incorrect.Call`
```

```
p_bar_with_stats
```

```
## Warning: Removed 3 rows containing missing values or values outside the scale range
## (`geom_signif()`).
```



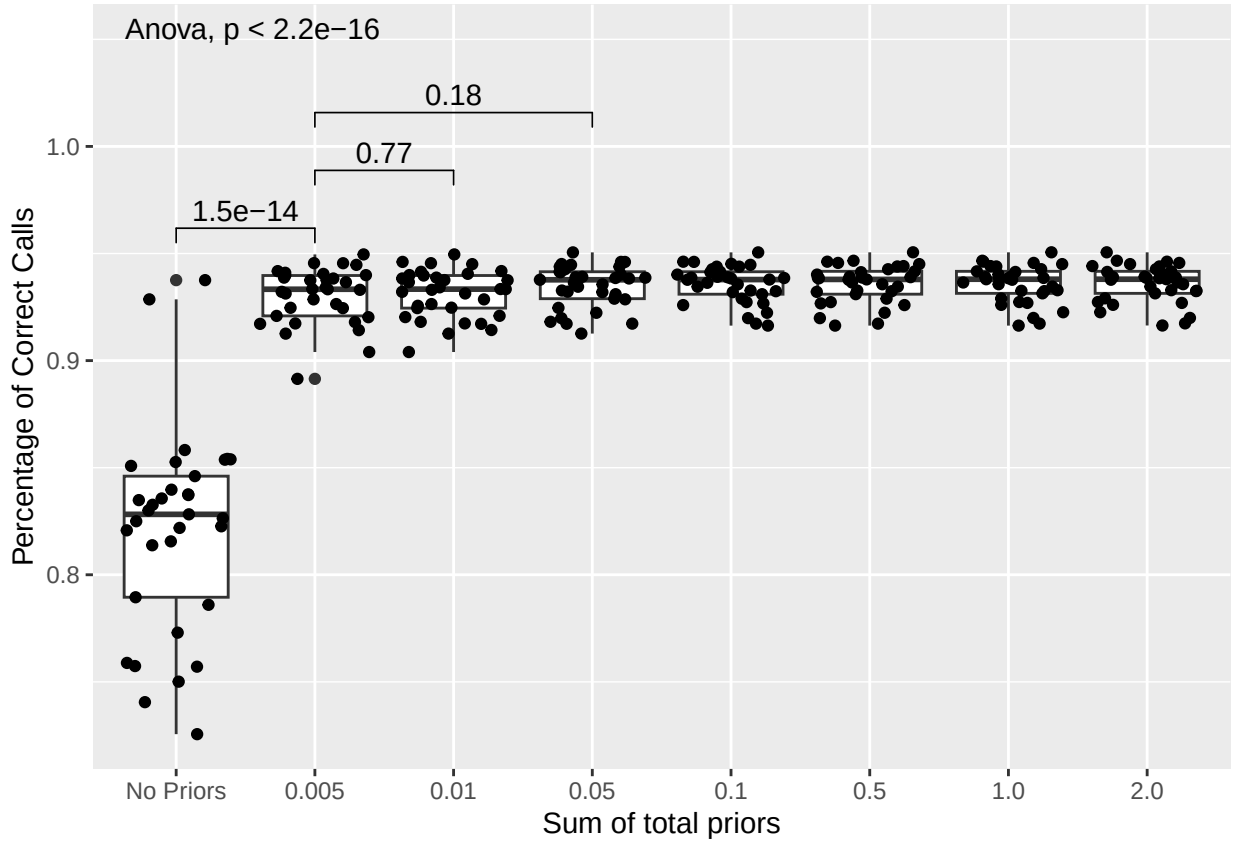
Sensitivity Analysis

```
priors_0<- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "No Priors"
)

priors_0.005 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_0.005",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "0.005"
)

priors_0.01 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_0.01",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "0.01"
)

priors_0.05 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_0.05",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "0.05"
)

priors_0.1 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_0.1",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "0.1"
)

priors_0.5 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_0.5",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
```

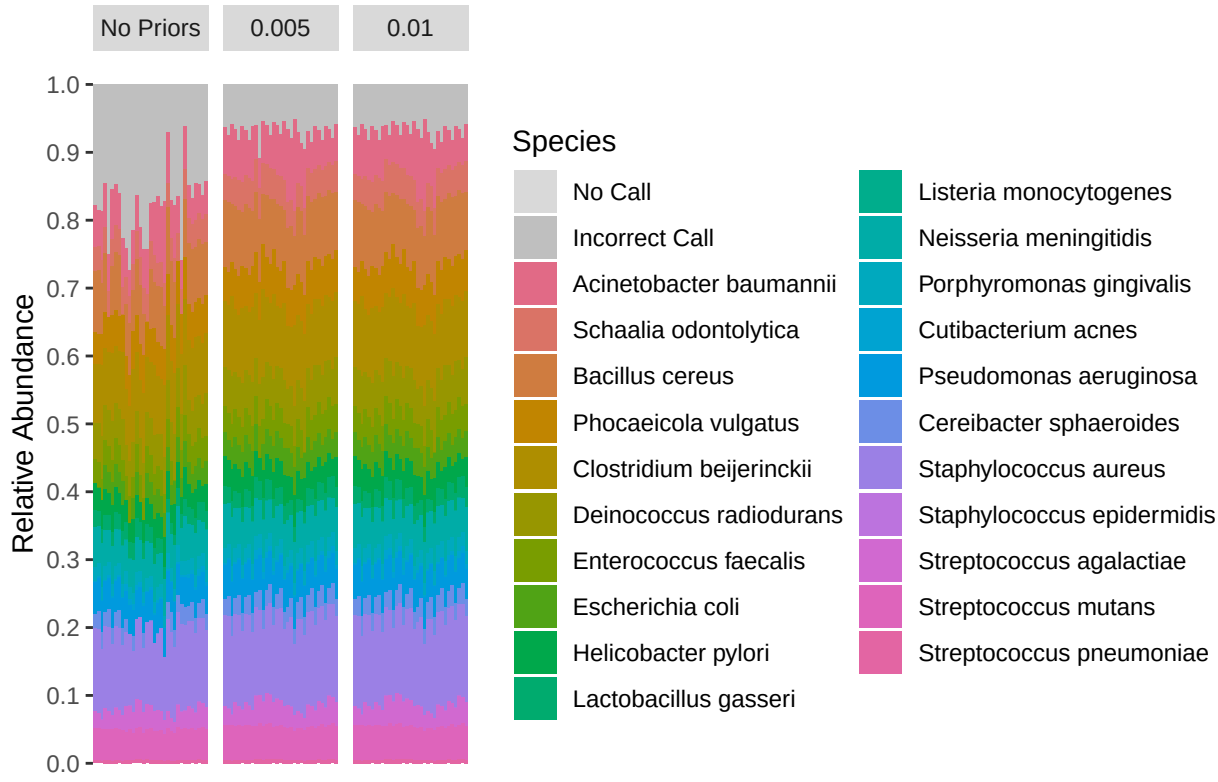
```
  pipeline_name = "0.5"
)

priors_1.0 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_1.0",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "1.0"
)

priors_2.0 <- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_priors_2.0",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "2.0"
)


summary_df_sensitivity <- rbind(priors_0, priors_0.005, priors_0.01, priors_0.05, priors_0.1, priors_0.5

summary_df_sensitivity |> dplyr::filter(Species %in% c("Correct Call", "Incorrect Call")) |>
  tidyr::pivot_wider(names_from = Species, values_from = prop, names_repair = "universal") |>
  dplyr::mutate(Correct.Call = 1 - as.numeric(Incorrect.Call)) |>
  dplyr::filter(pipeline != "Ground Truth") |>
  dplyr::group_by(pipeline) |>
  dplyr::group_by(name) |>
  ggplot(aes(x = pipeline, y = Correct.Call)) +
  geom_boxplot() +
  geom_jitter() +
  #theme(axis.text.x = element_text(angle = 90)) +
  xlab("Sum of total priors") +
  ylab("Percentage of Correct Calls") +
  stat_compare_means(label.y = 1.05, method = "anova", paired = TRUE) +
  stat_compare_means(comparisons = list(c("No Priors", "0.005"),
                                        c("0.005", "0.01"), c("0.005", "0.05")),
                     method = "wilcox.test",
                     paired = FALSE)
```

```
## New names:
## * `Incorrect Call` -> `Incorrect.Call`
```

```
## Warning in wilcox.test.default(c(0.936613634323669, 0.92448846455617,
## 0.941782795512704, : cannot compute exact p-value with ties
```




```
summary_df_final_3 <- rbind(priors_0, priors_0.005, priors_0.01)
p3 <- ggplot(data = summary_df_final_3  , aes(fill = Species, y = prop, x = name)) +
  geom_bar(position ="stack", stat = "identity")+
  scale_fill_manual(values = wheel_colors, name = "Species") +
  ylab("Relative Abundance") +
  xlab("") +
  scale_y_continuous(breaks = seq(0,1, by = 0.1)) +
  facet_grid(~pipeline) +
  theme(axis.text.x=element_blank(),
        axis.ticks.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank())
plot(p3)
```



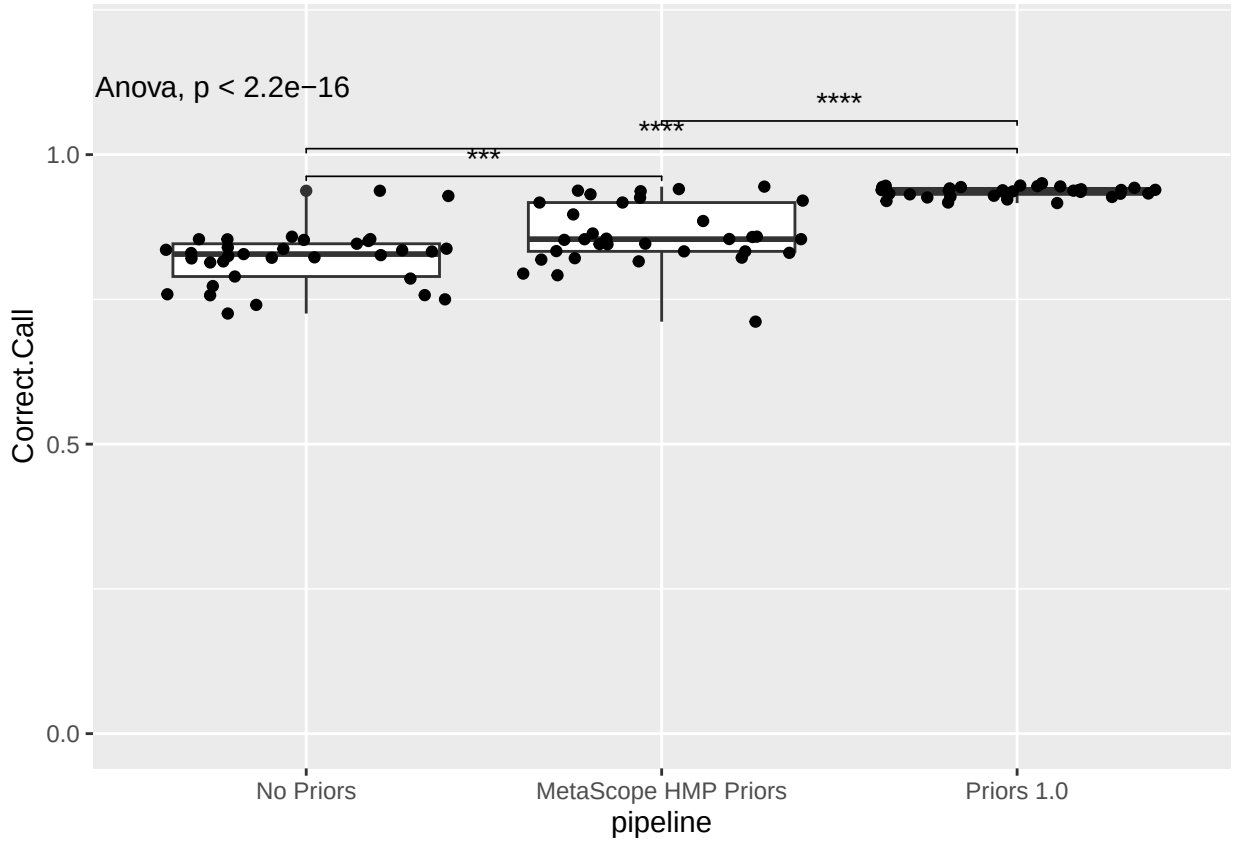
#HMP priors

```
hmp_priors<- create_summary_df(
  list.files(
    path = "data_processed/kozich_2013/results_hmp_priors",
    pattern = ".metascope_id.csv",
    full.names = TRUE,
    recursive = TRUE),
  pipeline_name = "MetaScope HMP Priors"
)


hmp_test <- rbind(priors_0, hmp_priors, priors_1.0) |>
  dplyr::filter(Species %in% c("No Call", "Incorrect Call")) |>
  tidyr::pivot_wider(names_from = Species, values_from = prop, names_repair = "universal") |>
  dplyr::mutate(Correct.Call = 1 - (No.Call + Incorrect.Call))
```

```
## New names:
## * `No Call` -> `No.Call`
## * `Incorrect Call` -> `Incorrect.Call`
```

```
ggplot(hmp_test, aes(x=pipeline, y=Correct.Call)) +
  geom_boxplot() +
  stat_compare_means(label.y = 1.1, label.x = 0.55, method = "anova", paired = TRUE) +
  stat_compare_means(comparisons = list(c("No Priors", "MetaScope HMP Priors"),
```

```
                                        c("No Priors", "1.0"), c("MetaScope HMP Priors", "1.0")),
                    method = "wilcox.test",
                    paired = TRUE,
                    step.increase = .2,
                    symnum.args = list(cutpoints = c(0, 0.0001, 0.001, 0.01, 0.05, 1),
                                       symbols = c("****", "***", "**", "*", "ns"))) +
  geom_jitter() +
  scale_x_discrete(labels=c("1.0" = "Priors 1.0")) +
  scale_y_continuous(breaks=c(0,0.5,1),
                     limits = c(0,1.2))
```



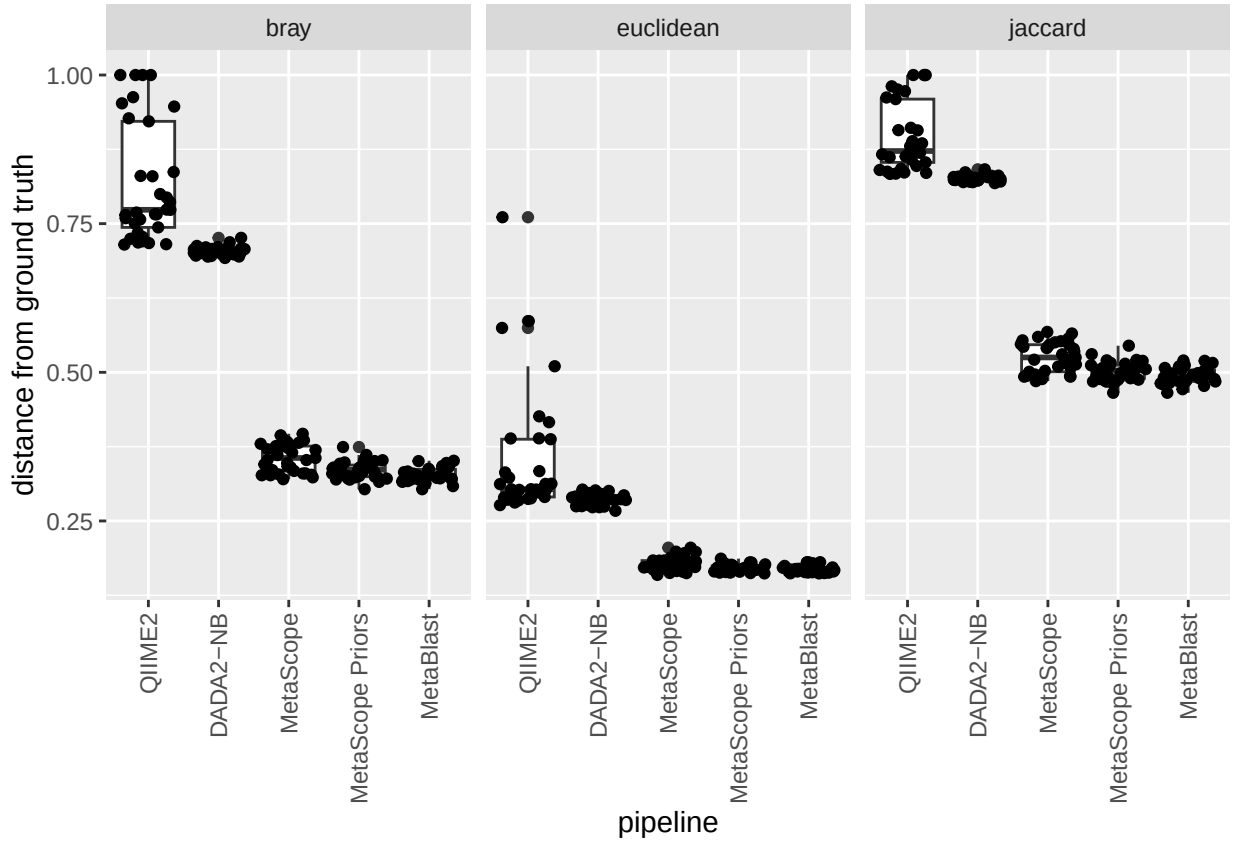
Using Vegan to determine distance matrices

```
ground_truth_taxonomy_taxids <-
  taxonomizr::getId(ground_truth_taxonomy$species,
                            accessions_path)

# Helper function to generate distance metrics from metascope files
dist_ms_to_ground_truth <- function(file_paths, pipeline, distance_metric) {
  dfs_list <- file_paths |>
    set_names(function(x) sub("\\..*$", "", basename(x))) |>
    map(function(x) {
```

```
      df <- read.csv(x)
      sample_name <- sub("\\..*$", "", basename(x))
      df <- df |>
        select(TaxonomyID,Genome,readsEM) |>
        dplyr::rename(!!paste0(sample_name,".",pipeline) := readsEM)
      return(df)
    })
  dfs_merged <- purrr::reduce(dfs_list, full_join, by = c("TaxonomyID", "Genome")) |>
    mutate(across(where(is.numeric), ~ replace_na(.x, 0))) |>
    dplyr::select(-Genome) |>
  tibble::column_to_rownames("TaxonomyID") |>
  dplyr::mutate_if(is.numeric, ~ . / sum(.))

  # Generate Matrix used for vegan::vegdist and full join ground_truth taxonomy
  ms_dist_mat <- full_join(dfs_merged |>
            tibble::rownames_to_column("TaxonomyID") |>
            mutate(TaxonomyID = as.numeric(TaxonomyID)),
          data.frame(TaxonomyID = as.numeric(ground_truth_taxonomy_taxids),
                     Ground_Truth = rep(1/length(ground_truth_taxonomy_taxids),
                                        length(ground_truth_taxonomy_taxids))),
          by = "TaxonomyID") |>
  mutate(across(everything(), ~ replace_na(.x, 0))) |>
  column_to_rownames("TaxonomyID") |>
  filter(rowSums(across(where(is.numeric))) > 0) |>
  t()

  dist_mat <- vegan::vegdist(ms_dist_mat, method = distance_metric) |> as.matrix() |>
    as.data.frame() |>
    filter(row_number() <= n()-1) |>
    pull(Ground_Truth)

  return(dist_mat)

}

## Function to generate distances for all outputs
dist_from_ground_truth <- function(distance_metric) {
  ms_dist <- dist_ms_to_ground_truth(list.files(path = "data_processed/kozich_2013/results",
                                pattern = ".metascope_id.csv",
                                full.names = TRUE,
                                recursive = TRUE),
                     pipeline = "MetaScope",
                     distance_metric = distance_metric)
  ms_p_dist <- dist_ms_to_ground_truth(list.files(path = "data_processed/kozich_2013/results_priors",
                                pattern = ".metascope_id.csv",
                                full.names = TRUE,
                                recursive = TRUE),
                     pipeline = "MetaScope Priors",
                     distance_metric = distance_metric)
  ms_p_b_dist <- dist_ms_to_ground_truth(list.files(path = "data_processed/kozich_2013/results_metablas
                                pattern = ".metascope_id.csv",
                                full.names = TRUE,
                                recursive = TRUE),
```

```
                  pipeline = "MetaBlast",
                  distance_metric = distance_metric)


dada2_dist <- dada2_df |> select(name, taxon, taxid, reads_count) |>
  group_by(taxon, taxid, name) |>
  summarise(reads_count = sum(reads_count), .groups = "drop") |>
  pivot_wider(names_from = name, values_from = reads_count, values_fill = 0) |>
  full_join(data.frame(taxid = as.numeric(ground_truth_taxonomy_taxids),
                       Ground_Truth = rep(1/length(ground_truth_taxonomy_taxids),
                                          length(ground_truth_taxonomy_taxids))),
            by = "taxid") |>
  mutate(taxon = if_else(is.na(taxon), taxonomizr::getTaxonomy(taxid, accessions_path, desiredTaxa = c
                         taxon)) |>
  mutate(across(everything(), ~ replace_na(.x, 0))) |>
  tibble::column_to_rownames("taxon") |> # Using taxon for rowname instead of taxid because I labeled
  select(-taxid) |>
  t() |>
  vegan::vegdist(method = distance_metric) |>
  as.matrix() |>
  as.data.frame() |>
  filter(row_number() <= n()-1) |> # Remove the last row which contains ground_truth vs ground_truth
  pull(Ground_Truth)

qiime2_dist <- qiime2_df |>
  select(name, taxon, taxid, value) |>
  group_by(taxon, taxid, name) |>
  summarise(reads_count = sum(value), .groups = "drop") |>
  pivot_wider(names_from = name, values_from = reads_count, values_fill = 0) |>
  full_join(data.frame(taxid = as.numeric(ground_truth_taxonomy_taxids),
                       Ground_Truth = rep(1/length(ground_truth_taxonomy_taxids),
                                          length(ground_truth_taxonomy_taxids))),
            by = "taxid") |>
  mutate(taxon = if_else(is.na(taxon), taxonomizr::getTaxonomy(taxid, accessions_path, desiredTaxa = c
                         taxon)) |>
  mutate(across(everything(), ~ replace_na(.x, 0))) |>
  tibble::column_to_rownames("taxon") |> # Using taxon for rowname instead of taxid because I labeled
  select(-taxid) |>
  t() |>
  vegan::vegdist(method = distance_metric) |>
  as.matrix() |>
  as.data.frame() |>
  filter(row_number() <= n()-1) |> # Remove the last row which contains ground_truth vs ground_truth
  pull(Ground_Truth)

merged_res <- tibble(
  "QIIME2" = qiime2_dist,
  "DADA2-NB" = dada2_dist,
  "MetaScope" = ms_dist,
  "MetaScope Priors" = ms_p_dist,
  "MetaBlast" = ms_p_b_dist,
  "distance_method" = distance_metric) |>
pivot_longer(cols = c("QIIME2","DADA2-NB","MetaScope","MetaScope Priors", "MetaBlast"),
```

```
                names_to = "pipeline", values_to = "distance_from_ground_truth")
  merged_res$pipeline <-factor(merged_res$pipeline,
                               levels = c("QIIME2", "DADA2-NB", "MetaScope",
                                          "MetaScope Priors", "MetaBlast"))
  return(merged_res)
}

distance_res <- map_dfr(c("bray", "jaccard", "euclidean"), dist_from_ground_truth)


ggplot(distance_res, aes(x = pipeline, y = distance_from_ground_truth)) +
  geom_boxplot() +
  geom_jitter() +
  facet_grid(~distance_method) +
  xlab("pipeline") +
  ylab("distance from ground truth") +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))
```




```
distance_res |>
  group_by(distance_method, pipeline) |>
  summarise(mean = mean(distance_from_ground_truth),
            sd = sd(distance_from_ground_truth))
```

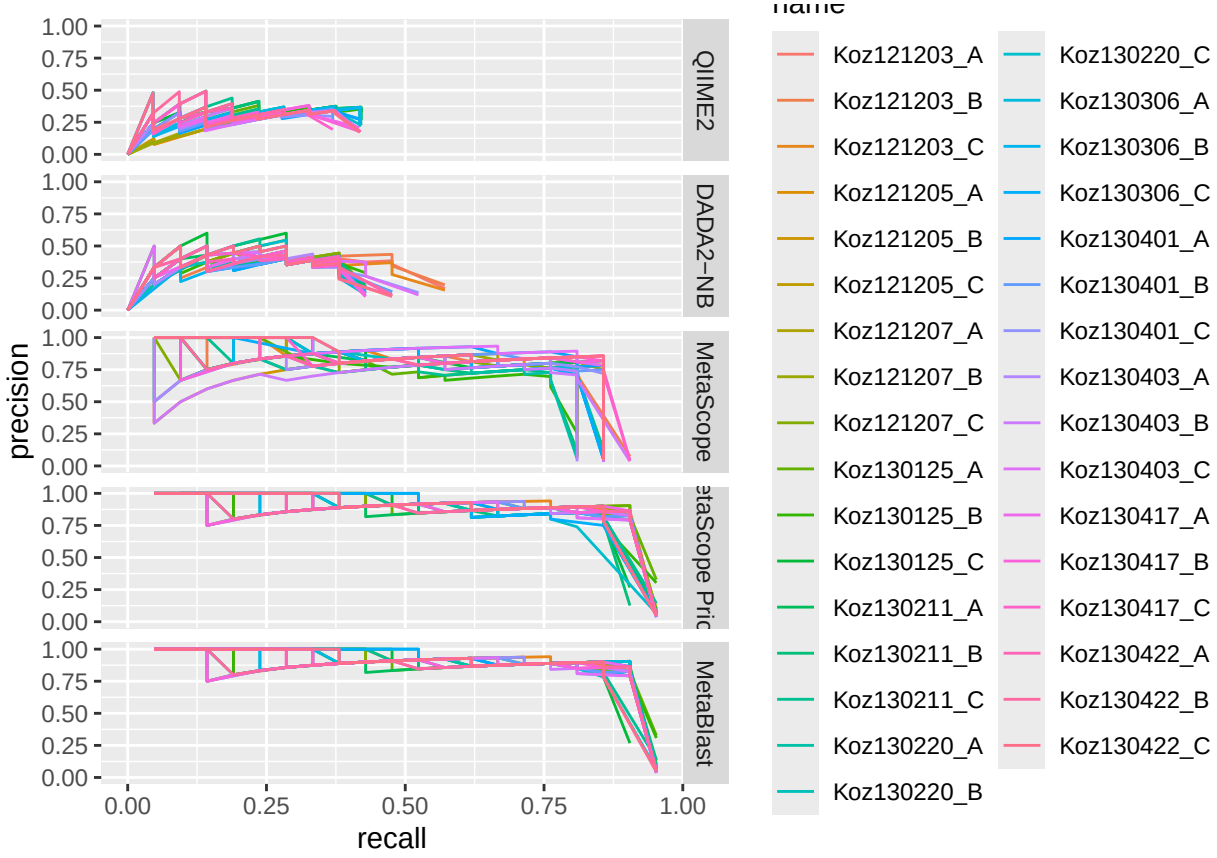
```
## `summarise()` has grouped output by 'distance_method'. You can override using
## the `.groups` argument.
```

```
## # A tibble: 15 x 4
## # Groups:   distance_method [3]
##    distance_method pipeline          mean      sd
##    <chr>           <fct>            <dbl>   <dbl>
##  1 bray            QIIME2           0.818 0.100
##  2 bray            DADA2-NB         0.703 0.00716
##  3 bray            MetaScope        0.357 0.0235
##  4 bray            MetaScope Priors 0.335 0.0145
##  5 bray            MetaBlast        0.328 0.0117
##  6 euclidean       QIIME2           0.353 0.109
##  7 euclidean       DADA2-NB         0.287 0.00898
##  8 euclidean       MetaScope        0.178 0.0109
##  9 euclidean       MetaScope Priors 0.171 0.00598
## 10 euclidean       MetaBlast        0.169 0.00549
## 11 jaccard         QIIME2           0.897 0.0583
## 12 jaccard         DADA2-NB         0.826 0.00491
## 13 jaccard         MetaScope        0.525 0.0256
## 14 jaccard         MetaScope Priors 0.502 0.0162
## 15 jaccard         MetaBlast        0.494 0.0132
```

```r
# Helper function to read MetaScope results
read_ms_results <- function(file_paths, pipeline) {
  dfs_list <- file_paths |>
    set_names(function(x) sub("\\..*$", "", basename(x))) |>
    map(function(x) {
      df <- read.csv(x)
      sample_name <- sub("\\..*$", "", basename(x))
      df <- df |>
        select(TaxonomyID,Genome,readsEM) |>
        dplyr::rename(!!sample_name := readsEM)
      return(df)
    })
  dfs_merged <- purrr::reduce(dfs_list, full_join, by = c("TaxonomyID", "Genome")) |>
    mutate(across(where(is.numeric), ~ replace_na(.x, 0))) |>
    dplyr::select(-Genome) |>
  tibble::column_to_rownames("TaxonomyID") |>
  dplyr::mutate_if(is.numeric, ~ . / sum(.))
  return(dfs_merged)
}

# Helper Function to calculate precision, recall, and F1 and species level
#  relative abundance threshold
calc_prf <- function(abundance_threshold) {
  dada2_prf <- dada2_df |>
    group_by(name, taxid) |>
    summarise(value = sum(reads_count), .groups = "drop") |>  # Merge same sample and taxids together
    filter(value > abundance_threshold) |>
    mutate(abund_weight = 1 - abs(value - 1/21),
           is_tp = taxid %in% ground_truth_taxonomy_taxids) |> # weight by distance from ground truth a
    group_by(name) |>
    summarise(
      TP = sum(is_tp),
      precision = TP / n(),
      recall = TP / length(ground_truth_taxonomy_taxids),
```

```
    F1 = 2 * precision * recall / (precision + recall)) |>
  mutate(pipeline = "DADA2-NB")

qiime2_prf <- qiime2_df |>
  group_by(name, taxid) |>
  summarise(value = sum(value), .groups = "drop") |>
  filter(value > abundance_threshold) |>
  mutate(abund_weight = 1 - abs(value - 1/21),  # weight by distance from ground truth abundance
         is_tp = taxid %in% ground_truth_taxonomy_taxids) |>
  group_by(name) |>
  summarise(
    TP = sum(is_tp * abund_weight),
    precision = TP / n(),
    recall = TP / length(ground_truth_taxonomy_taxids),
    F1 = 2 * precision * recall / (precision + recall)) |>
  mutate(pipeline = "QIIME2")



ms_prf <- read_ms_results(list.files(path = "data_processed/kozich_2013/results",
                                     pattern = ".metascope_id.csv",
                                     full.names = TRUE,
                                     recursive = TRUE),
                          pipeline = "MetaScope") |>
  tibble::rownames_to_column("taxid") |>
  pivot_longer(-taxid, names_to = "name", values_to = "value") |>
  group_by(name) |>
  filter(value > abundance_threshold) |>
  mutate(abund_weight = 1 - abs(value - 1/21),
         is_tp = taxid %in% ground_truth_taxonomy_taxids) |> # weight by distance from ground truth a
  summarise(
    TP = sum(is_tp),
    precision = TP / n(),
    recall = TP / length(ground_truth_taxonomy_taxids),
    F1 = 2 * precision * recall / (precision + recall)) |>
  mutate(pipeline = "MetaScope")

ms_p_prf <- read_ms_results(list.files(path = "data_processed/kozich_2013/results_priors",
                                     pattern = ".metascope_id.csv",
                                     full.names = TRUE,
                                     recursive = TRUE),
                          pipeline = "MetaScope Priors") |>
  tibble::rownames_to_column("taxid") |>
  pivot_longer(-taxid, names_to = "name", values_to = "value") |>
  group_by(name) |>
  filter(value > abundance_threshold) |>
  mutate(abund_weight = 1 - abs(value - 1/21),
         is_tp = taxid %in% ground_truth_taxonomy_taxids) |> # weight by distance from ground truth a
  summarise(
    TP = sum(is_tp),
    precision = TP / n(),
    recall = TP / length(ground_truth_taxonomy_taxids),
    F1 = 2 * precision * recall / (precision + recall)) |>
```

```
    mutate(pipeline = "MetaScope Priors")

  ms_p_b_prf <- read_ms_results(list.files(path = "data_processed/kozich_2013/results_metablast_priors_
                                pattern = ".metascope_id.csv",
                                full.names = TRUE,
                                recursive = TRUE),
                      pipeline = "MetaBlast") |>
    tibble::rownames_to_column("taxid") |>
    pivot_longer(-taxid, names_to = "name", values_to = "value") |>
    group_by(name) |>
    filter(value > abundance_threshold) |>
    mutate(abund_weight = 1 - abs(value - 1/21),
           is_tp = taxid %in% ground_truth_taxonomy_taxids) |> # weight by distance from ground truth a
    summarise(
      TP = sum(is_tp),
      precision = TP / n(),
      recall = TP / length(ground_truth_taxonomy_taxids),
      F1 = 2 * precision * recall / (precision + recall)) |>
    mutate(pipeline = "MetaBlast")

  merged_prf <- rbind(dada2_prf, qiime2_prf, ms_prf, ms_p_prf, ms_p_b_prf) |>
    #pivot_longer(cols = c(precision, recall, F1), names_to = "metric_scores", values_to = "value") |>
    select(-TP) |>
    replace_na(list(value = 0))
  merged_prf$pipeline <- factor(merged_prf$pipeline,
                                levels = c("QIIME2", "DADA2-NB", "MetaScope",
                                           "MetaScope Priors", "MetaBlast"))
  merged_prf$abundance_threshold <- abundance_threshold
  return(merged_prf)
}


threshold_vals <- c(0,1e-6,5e-6,1e-5,5e-5,1e-4,5e-4,1e-3,5e-3,1e-1,5e-1, 1)
threshold_vals <- seq(0,0.1, 0.001)
pr_curve_df <- map_dfr(threshold_vals,calc_prf)

# Generate Precision Recall curve
ggplot(pr_curve_df, aes(x = recall, y = precision, color = name)) +
  geom_path() +
  facet_grid(rows = vars(pipeline))
```




```
# Calculate AUC for precision recall curve
pr_curve_df |>
  group_by(name, pipeline) |>
  arrange(recall) |>
  summarize(cum_area = trapz(recall, precision)) |>
  ungroup() |>
  group_by(pipeline) |>
  summarize(aauc = mean(cum_area))
```

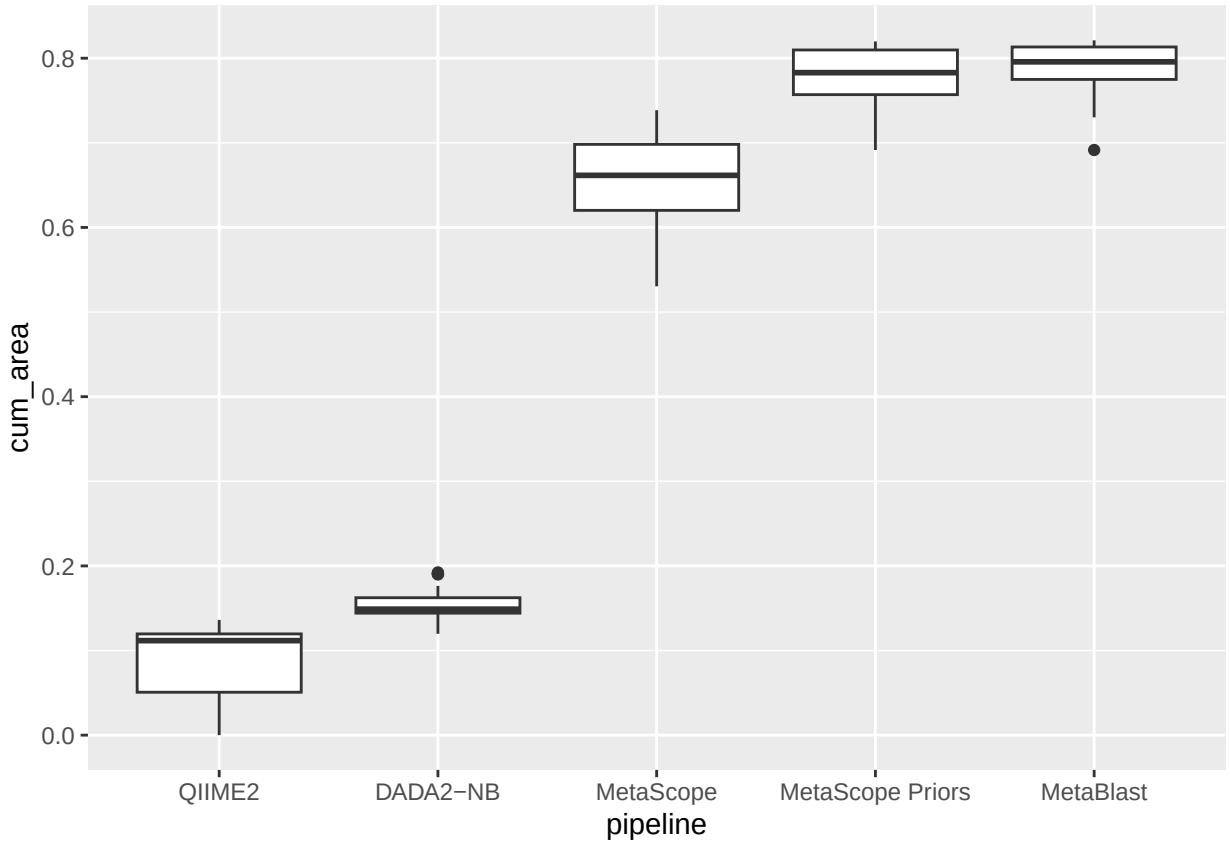
```
## `summarise()` has grouped output by 'name'. You can override using the
## `.groups` argument.
```

```
## # A tibble: 5 x 2
##   pipeline           aauc
##   <fct>             <dbl>
## 1 QIIME2           0.0894
## 2 DADA2-NB         0.155
## 3 MetaScope        0.656
## 4 MetaScope Priors 0.778
## 5 MetaBlast        0.787
```

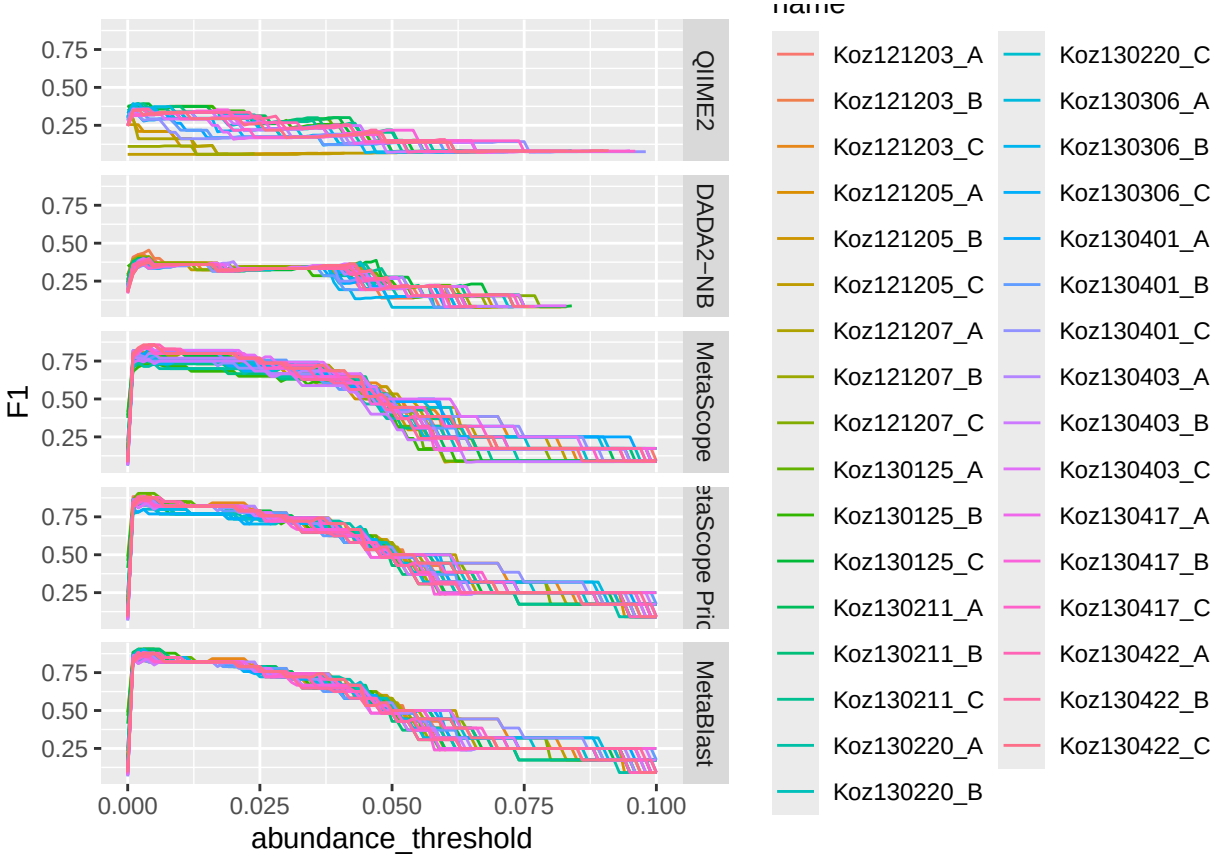
```
pr_curve_df |>
  group_by(name, pipeline) |>
  arrange(recall) |>
  summarize(cum_area = trapz(recall, precision)) |>
```

```
  ggplot(aes(x = pipeline, y = cum_area)) +
  geom_boxplot()
```

```
## `summarise()` has grouped output by 'name'. You can override using the
## `.groups` argument.
```




```
# F1 Curve
ggplot(pr_curve_df, aes(x = abundance_threshold, y = F1, color = name)) +
  geom_path() +
  facet_grid(rows = vars(pipeline)) +
  xlim(0,0.1)
```




```
pr_curve_df |>
  filter(precision == 0, recall == 0) |>
  group_by(pipeline) |>
  summarise(min_abund = min(abundance_threshold),
            max_abund = max(abundance_threshold))
```

```
## # A tibble: 2 x 3
##   pipeline min_abund max_abund
##   <fct>        <dbl>     <dbl>
## 1 QIIME2       0           0.1
## 2 DADA2-NB     0.055       0.1
```