**Data extraction**

#Load shinydigitise

devtools::install\_github("EIvimeyCook/ShinyDigitise", force = TRUE)

install.packages("promises")

df<-shinyDigitise("C:/Users/muhta/OneDrive/Desktop/Meta analysis/paper/figure")

shinyDigitise::shinyDigitise()

# === Load Required Packages ===

install.packages("metafor") # Only if not already installed

library(metafor)

library(readxl)

**Alpha diversity**

# === Load Data ===

data <- read\_excel("AlphaDiversity\_MetaAnalysis\_Input.xlsx")

# === Convert Columns to Numeric ===

data$m1i <- as.numeric(data$m1i) # Treatment mean

data$sd1i <- as.numeric(data$sd1i) # Treatment SD

data$n1i <- as.numeric(data$n1i) # Treatment N

data$m2i <- as.numeric(data$m2i) # Control mean

data$sd2i <- as.numeric(data$sd2i) # Control SD

data$n2i <- as.numeric(data$n2i) # Control N

# === Calculate Effect Sizes (Hedges' g) ===

res <- escalc(measure = "SMDH",

m1i = m1i, sd1i = sd1i, n1i = n1i,

m2i = m2i, sd2i = sd2i, n2i = n2i,

data = data)

# === Run Random-Effects Meta-Analysis ===

model <- rma(yi, vi, data = res, random = ~ 1 | Study\_ID, method = "REML")

# === Summary Output ===

summary(model)

# === Forest Plot ===

forest(model,

slab = paste(data$Study\_ID),

xlab = "Effect Size (Hedges' g)",

main = "Meta-Analysis of Shannon Diversity")

# === Funnel Plot ===

funnel(model,

xlab = "Effect Size (Hedges' g)",

main = "Funnel Plot of Effect Sizes")

# === Heterogeneity Stats ===

cat("\n=== Heterogeneity Statistics ===\n")

cat("Between-study variance (tau²): ", summary(model)$tau2, "\n")

cat("I²: ", summary(model)$I2, "%\n")

cat("Q statistic: ", summary(model)$QE, "\n")

cat("Q-test p-value: ", summary(model)$QEp, "\n")

# === Meta-regression on Mean Sample Size ===

res$mean\_n <- (data$n1i + data$n2i) / 2

meta\_reg <- rma(yi, vi, mods = ~ mean\_n, data = res, method = "REML")

summary(meta\_reg)

# === Meta-regression on Study ID (categorical) ===

meta\_reg\_cat <- rma(yi, vi, mods = ~ Study\_ID, data = res, method = "REML")

summary(meta\_reg\_cat)

# Initialize a data frame to store results

#leave one

# === Load Required Packages ===

library(metafor)

library(readxl)

library(dplyr)

library(ggplot2)

# === Load Dataset ===

data <- read\_excel("MetaAnalysis\_Shannon\_ByPaper.xlsx")

# === Ensure Numeric Columns Are Properly Set ===

data <- data %>%

mutate(across(c(m1i, sd1i, n1i, m2i, sd2i, n2i), as.numeric))

# === Calculate Effect Sizes (Hedges' g) ===

res <- escalc(measure = "SMDH",

m1i = m1i, sd1i = sd1i, n1i = n1i,

m2i = m2i, sd2i = sd2i, n2i = n2i,

data = data)

# === Run Random-Effects Meta-Analysis Using Paper as Group ===

model <- rma(yi, vi, data = res, random = ~1 | Paper, method = "REML")

# === Leave-One-Paper-Out Sensitivity Analysis ===

unique\_papers <- unique(res$Paper)

results <- data.frame()

for (paper in unique\_papers) {

res\_subset <- res %>% filter(Paper != paper)

model\_lopo <- rma(yi, vi, data = res\_subset, method = "REML")

results <- rbind(results, data.frame(

Paper\_Excluded = paper,

Estimate = model\_lopo$b,

CI\_lb = model\_lopo$ci.lb,

CI\_ub = model\_lopo$ci.ub

))

}

# === Plot the LOPO Results ===

ggplot(results, aes(x = Paper\_Excluded, y = Estimate)) +

geom\_point(size = 3) +

geom\_errorbar(aes(ymin = CI\_lb, ymax = CI\_ub), width = 0.2) +

theme\_minimal() +

labs(title = "Leave-One-Paper-Out Sensitivity Analysis",

x = "Study (Paper) Excluded",

y = "Effect Size (Hedges' g)") +

theme(axis.text.x = element\_text(angle = 45, hjust = 1))

**Relative abundance meta-analysis (Log response ratio)**

install.packages(c("readxl", "metafor", "dplyr", "ggplot2"))

# Load packages

getwd()

# Load necessary packages

library(readxl)

library(dplyr)

library(metafor)

# Step 1: Read the Excel file

file\_path <- "C:/Users/muhta/OneDrive/Desktop/Meta analysis/paper/data/relativeabundance.xlsx"

df <- read\_excel(file\_path)

# Step 2: Calculate proportions and F:B ratio

df <- df %>%

mutate(

p\_firm\_control = Control.Firmicutes / (Control.Firmicutes + Control.Bacteroidetes),

p\_firm\_treat = Treatment.Firmicutes / (Treatment.Firmicutes + treatement.Bacteroidetes),

p\_bact\_control = Control.Bacteroidetes / (Control.Firmicutes + Control.Bacteroidetes),

p\_bact\_treat = treatement.Bacteroidetes / (Treatment.Firmicutes + treatement.Bacteroidetes),

fb\_control = Control.Firmicutes / Control.Bacteroidetes,

fb\_treat = Treatment.Firmicutes / treatement.Bacteroidetes

)

# Step 3: Log response ratios (effect sizes)

df <- df %>%

mutate(

yi\_firm = log(p\_firm\_treat / p\_firm\_control),

yi\_bact = log(p\_bact\_treat / p\_bact\_control),

yi\_fb = log(fb\_treat / fb\_control)

)

# Step 4: Delta-method SEs

df <- df %>%

mutate(

se\_firm = sqrt(

((1 - p\_firm\_treat) / (Treatment.Sample \* p\_firm\_treat))^2 +

((1 - p\_firm\_control) / (Control.Sample \* p\_firm\_control))^2

),

se\_bact = sqrt(

((1 - p\_bact\_treat) / (Treatment.Sample \* p\_bact\_treat))^2 +

((1 - p\_bact\_control) / (Control.Sample \* p\_bact\_control))^2

),

se\_fb = sqrt(

((1 / treatement.Bacteroidetes)^2 + (1 / Treatment.Firmicutes)^2) / Treatment.Sample +

((1 / Control.Bacteroidetes)^2 + (1 / Control.Firmicutes)^2) / Control.Sample

)

)

# Step 5: Meta-analysis

res\_firm <- rma(yi = yi\_firm, sei = se\_firm, data = df, method = "REML")

res\_bact <- rma(yi = yi\_bact, sei = se\_bact, data = df, method = "REML")

res\_fb <- rma(yi = yi\_fb, sei = se\_fb, data = df, method = "REML")

# Step 6: Summary

summary(res\_firm)

summary(res\_bact)

summary(res\_fb)

# Step 7: Forest plots

forest(res\_firm, slab = paste(df$Study\_ID, df$Treatment), xlab = "Log Ratio (Firmicutes)", main = "Firmicutes Effect Size")

forest(res\_bact, slab = paste(df$Study\_ID, df$Treatment), xlab = "Log Ratio (Bacteroidetes)", main = "Bacteroidetes Effect Size")

forest(res\_fb, slab = paste(df$Study\_ID, df$Treatment), xlab = "Log Ratio (F:B Ratio)", main = "Firmicutes:Bacteroidetes Ratio")

#Plot

# Load library

library(ggplot2)

# ========== Firmicutes ==========

loo\_firm <- leave1out(res\_firm)

loo\_firm\_df <- as.data.frame(loo\_firm)

loo\_firm\_df$Study <- paste(df$Study\_ID, df$Treatment)

ggplot(loo\_firm\_df, aes(x = reorder(Study, estimate), y = estimate)) +

geom\_point() +

geom\_errorbar(aes(ymin = ci.lb, ymax = ci.ub), width = 0.2) +

geom\_hline(yintercept = res\_firm$b[1], linetype = "dashed", color = "red") +

coord\_flip() +

labs(

title = "Leave-One-Out Analysis (Firmicutes)",

x = "Excluded Comparison",

y = "Effect Size (Log Ratio)"

) +

theme\_minimal()

# ========== Bacteroidetes ==========

loo\_bact <- leave1out(res\_bact)

loo\_bact\_df <- as.data.frame(loo\_bact)

loo\_bact\_df$Study <- paste(df$Study\_ID, df$Treatment)

ggplot(loo\_bact\_df, aes(x = reorder(Study, estimate), y = estimate)) +

geom\_point() +

geom\_errorbar(aes(ymin = ci.lb, ymax = ci.ub), width = 0.2) +

geom\_hline(yintercept = res\_bact$b[1], linetype = "dashed", color = "red") +

coord\_flip() +

labs(

title = "Leave-One-Out Analysis (Bacteroidetes)",

x = "Excluded Comparison",

y = "Effect Size (Log Ratio)"

) +

theme\_minimal()

# ========== F:B Ratio ==========

loo\_fb <- leave1out(res\_fb)

loo\_fb\_df <- as.data.frame(loo\_fb)

loo\_fb\_df$Study <- paste(df$Study\_ID, df$Treatment)

ggplot(loo\_fb\_df, aes(x = reorder(Study, estimate), y = estimate)) +

geom\_point() +

geom\_errorbar(aes(ymin = ci.lb, ymax = ci.ub), width = 0.2) +

geom\_hline(yintercept = res\_fb$b[1], linetype = "dashed", color = "red") +

coord\_flip() +

labs(

title = "Leave-One-Out Analysis (F:B Ratio)",

x = "Excluded Comparison",

y = "Effect Size (Log Ratio)"

) +

theme\_minimal()