Full account of statistical analysis performed in R

The effect of gut microbiome composition on human immune responses - interference of helminth infections

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All datasets to reproduce the following statistical analyses are available in the following Github address: https://github.com/IvonneMartin/Helminths_GutMicrobes_Cytokine. These datasets are the count of microbiota phyla of 150 Ende samples together with its metadata and cytokine responses(16S_cytokine.txt), the count of microbiota phyla from Jakarta samples (DatJkt.txt) and from USA samples (DatUS.txt).

1 Microbiome composition and diversity at three different demographical areas

First load the datasets from subjects in Ende with complete measurement of microbiome and cytokine responses, microbiome data from Jakarta samples and HMP samples. We also loaded all packages needed for the analyses.

```
library(lme4);
```

```
## Warning:  package 'lme4' was built under R version 3.4.4
## Loading required package:  Matrix
```

```
library(lmerTest);
```

```
##
## Attaching package:  'lmerTest'
## The following object is masked from 'package:lme4':
##
##    lmer
## The following object is masked from 'package:stats':
##
##    step
```

```
library(MASS);
library(vegan);
```

```
## Loading required package:  permute
## Loading required package:  lattice
## This is vegan 2.4-5
```

```
library(HMP);
```

```
## Warning:  package 'HMP' was built under R version 3.4.4
## Loading required package:  dirmult
##
## Attaching package:  'HMP'
## The following object is masked from 'package:dirmult':
##
##    weirMoM
```

```
library(dirmult);
library(plotrix);
```

```
## Warning:  package 'plotrix' was built under R version 3.4.4
```

```
library(ggplot2);
```

```
## Warning:  package 'ggplot2' was built under R version 3.4.4
```

```
library(reshape2);
library(scales);
```

```
##
## Attaching package:  'scales'
## The following object is masked from 'package:plotrix':
##
##    rescale
```

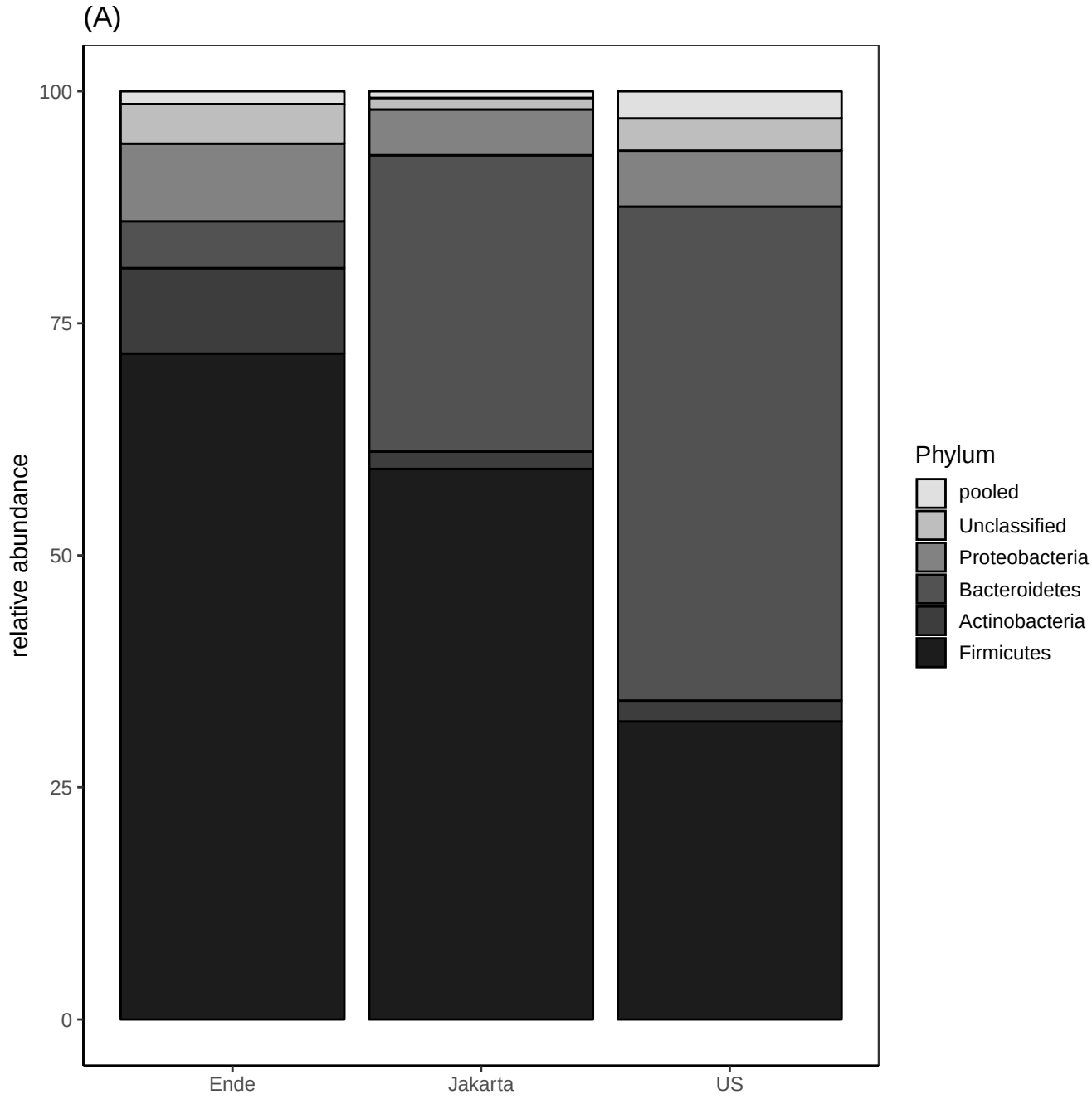
```
dat.mic <- read.table("16S_cytokine.txt",header=TRUE)
Dat.Jkt <- read.table("DatJkt.txt",header = TRUE)
us.dat <- read.table("DatUS.txt",header=TRUE)
```

The following code is to produce the microbiome composition of three different demographical areas. Note that all samples in Jakarta and USA are adult (older than 18 years old). Hence, we selected helminth-uninfected subjects in Ende who are older than 18 years old at pre-treatment.

```
## prepare the analyses for the Ende data

dat08 <- dat.mic[which(dat.mic[,2] == "2008"), ]
dat10 <- dat.mic[which(dat.mic[,2] == "2010"), ]

## Removing subjects with unobserved cytokine at pre-treatment.

RS08 <- rowSums(is.na(dat08[,35:40] == 1))
idnulc <- which(RS08 == 6)
d08 <- dat08[-idnulc,]
d10 <- dat10[-idnulc,]


d081 <- d08[which(d08$Age > 18 & d08$inf == 0), ]

## 1. plotting the comparison between three different demographical areas.

c.poolE <- c(15,18:21,23:26,28:32,34)
d081$pooled <- rowSums(d081[,c.poolE])
Phy.E <- c(22,16,17,27,33,41)


# select Jakarta data

pool.phy = c(4,7:10,12:15,17:21,23)
Dat.Jkt$pooled = rowSums(Dat.Jkt[,pool.phy])
phy.J = c(11,5,6,16,22,24)
Jkt.d = Dat.Jkt[,phy.J]
colnames(Jkt.d) = c("Firmicutes","Actinobacteria","Bacteroidetes","Proteobacteria",
                    "Unclassified_Bacteria","pooled")
```

```
## select the US data

pooled.phy = c(2,5:11,13:16,18:23)
us.dat$pooled = rowSums(us.dat[,pooled.phy])
phy.us <- c(12,3,4,17,24,25)



He.EndeBase = dirmult(d081[,Phy.E])

## Iteration 1:  Log-likelihood value:  -33998.9717219476
## Iteration 2:  Log-likelihood value:  -33988.0815512191
## Iteration 3:  Log-likelihood value:  -33984.1585171095
## Iteration 4:  Log-likelihood value:  -33983.3058665516
## Iteration 5:  Log-likelihood value:  -33983.2420532435
## Iteration 6:  Log-likelihood value:  -33983.2415738662
## Iteration 7:  Log-likelihood value:  -33983.241573836

Jkt = dirmult(Dat.Jkt[,phy.J])

## Iteration 1:  Log-likelihood value:  -29201.3494185736
## Iteration 2:  Log-likelihood value:  -29191.8097188891
## Iteration 3:  Log-likelihood value:  -29188.0215559517
## Iteration 4:  Log-likelihood value:  -29187.2231919037
## Iteration 5:  Log-likelihood value:  -29187.1765451067
## Iteration 6:  Log-likelihood value:  -29187.176336681
## Iteration 7:  Log-likelihood value:  -29187.1763366764

US = dirmult(us.dat[,phy.us])

## Iteration 1:  Log-likelihood value:  -23032.8452512908
## Iteration 2:  Log-likelihood value:  -23014.5584041081
## Iteration 3:  Log-likelihood value:  -23001.8761145087
## Iteration 4:  Log-likelihood value:  -22994.7310084698
## Iteration 5:  Log-likelihood value:  -22992.0216037761
## Iteration 6:  Log-likelihood value:  -22991.5671642258
## Iteration 7:  Log-likelihood value:  -22991.5519149936
## Iteration 8:  Log-likelihood value:  -22991.5518933628

df <- as.data.frame(cbind(He.EndeBase$pi,Jkt$pi,US$pi))
CN <- c("Ende", "Jakarta", "US")
colnames(df) <- CN
phylum_colors <- c("grey11","grey24","grey32","grey51","grey","grey88")


dfm <- melt(cbind(df, ind = rownames(df)), id.vars = c('ind'))
colnames(dfm) <- c("Phylum","variable","value")
levels(dfm$Phylum)

## [1] "Actinobacteria"        "Bacteroidetes"         "Firmicutes"
## [4] "pooled"                "Proteobacteria"        "Unclassified_Bacteria"

dfm$Phylum <- factor(dfm$Phylum, levels = c("Firmicutes","Actinobacteria","Bacteroidetes",
                                            "Proteobacteria","Unclassified_Bacteria","pooled"))


ggplot(dfm,aes(x = variable, y = value,fill = Phylum)) +
  theme_bw() +
```

```
theme(panel.grid.major = element_blank(),
      panel.grid.minor = element_blank(),
      axis.line = element_line(size = 0.5, linetype = "solid",
                               colour = "black"))+
geom_bar(position = position_fill(reverse=TRUE),colour="black",stat = "identity") +
scale_y_continuous(labels = c(0,25,50,75,100)) +
scale_fill_manual(values = phylum_colors,
                  labels = expression(Firmicutes,Actinobacteria,Bacteroidetes,
                                      Proteobacteria,Unclassified,pooled)) +
theme(legend.text.align = 0)+
guides(fill = guide_legend(reverse = TRUE, keywidth = 1, keyheight = 1)) +
ylab("relative abundance") +
theme(axis.text.x = element_text(angle = 0, hjust = 0.5))+
xlim(CN)+
theme(axis.title.x=element_blank())+
ggtitle('(A)') + theme(plot.title=element_text(hjust=0))
```



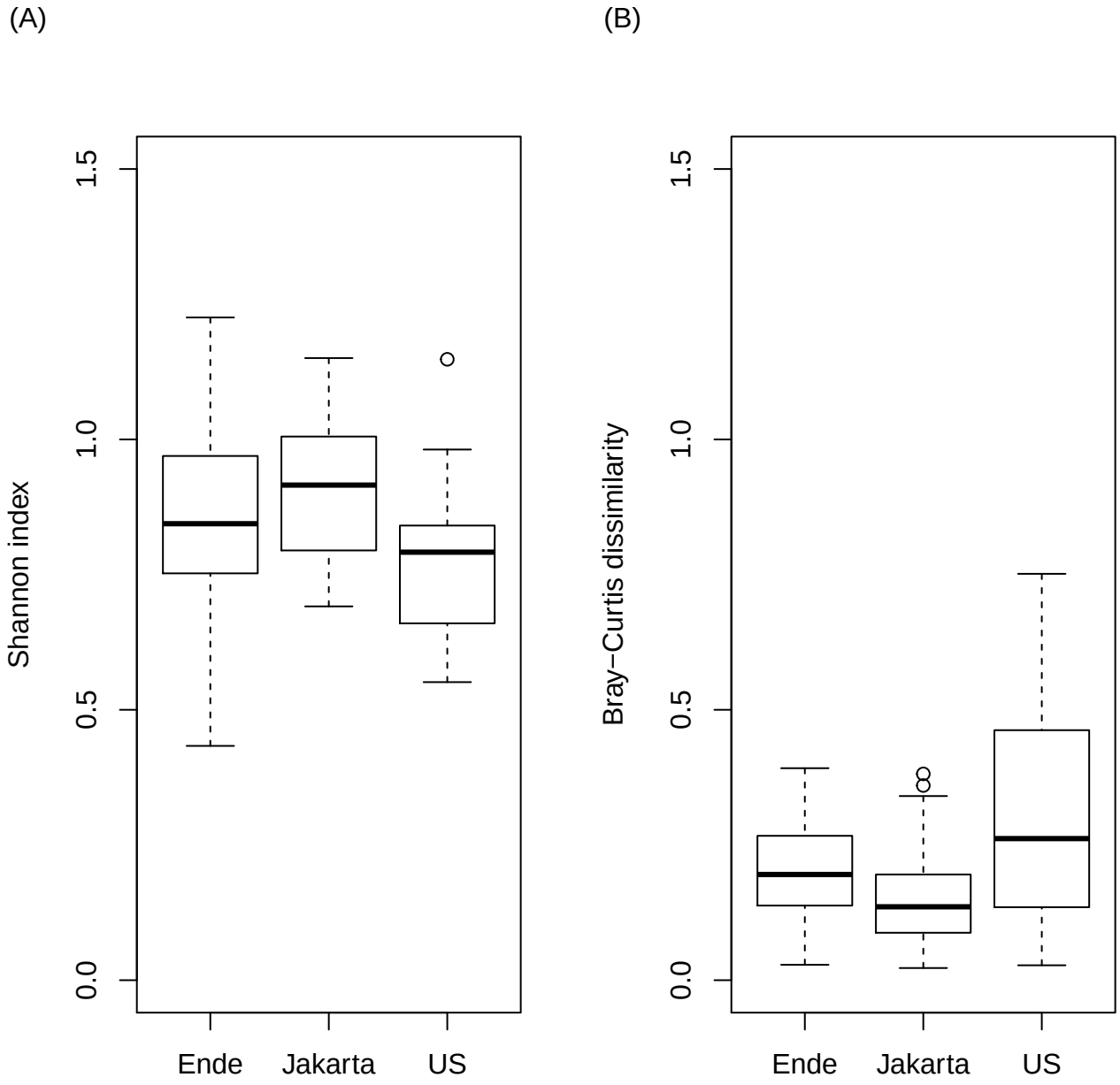
Testing the null hypothesis that the microbiome composition between these three areas are the same.

```
colnames(Dat.Jkt)[phy.J] <- c("Firmicutes","Actinobacteria","Bacteroidetes","Proteobacteria",
                              "Unclassified_Bacteria","pooled")
colnames(us.dat)[phy.us] <- c("Firmicutes","Actinobacteria","Bacteroidetes","Proteobacteria",
                              "Unclassified_Bacteria","pooled")

Xdc.sevsample(list(d081[,Phy.E],Dat.Jkt[,phy.J],us.dat[,phy.us]),est="mle")
```

```
## $`Xdc statistics`
## [1] 200.9424
##
## $`p value`
## [1] 0
```

The comparison at the diversity level.

```
d081$shannon <- diversity(as.matrix(d081[,15:34]),index = "shannon")
Dat.Jkt$shannon <- diversity(as.matrix(Dat.Jkt[,4:23]),index="shannon")
us.dat$shannon <- diversity(as.matrix(us.dat[,2:23]),index="shannon")


par(mfrow=c(1,2))
boxplot(d081$shannon,Dat.Jkt$shannon,us.dat$shannon,ylab="Shannon index",xaxt = "n",ylim = c(0,1.5))
corner.label(label='(A)',x=-1,figcorner=T,cex=1)

## $x
## [1] -0.7955752
##
## $y
## [1] 1.817442

ticks = c(1:3)
axis(1,at = ticks,label = c("Ende","Jakarta","US"))

MatEnde <- vegdist(as.matrix(d081[,15:34]),"bray")
MatJkt <- vegdist(as.matrix(Dat.Jkt[,4:23]),"bray")
MatUS <- vegdist(as.matrix(us.dat[,2:23]),"bray")

boxplot(MatEnde,MatJkt,MatUS,ylab = "Bray-Curtis dissimilarity",xaxt = "n",ylim = c(0,1.5))
corner.label(label='(B)',x=-1,figcorner=T,cex=1)

## $x
## [1] -0.7955752
##
## $y
## [1] 1.817442

ticks = c(1:3)
axis(1,at = ticks,label = c("Ende","Jakarta","US"))
```



2 Analysing the relationship between bacterial proportion, helminth infection and cytokine responses

All analyses in the following use dataset from Ende with 66 subjects. For this purpose, we combine the dataset at 2008 and 2010.

```
dat <- NULL
  for (i in 1:66){
    dat <- rbind(dat,d08[i,],d10[i,])
  }
```

```
# adding the dummy variable of timepoint: 0 for baseline and 1 for post-treatment
 dat$t.point <- rep(0:1,66)

# adding the diversity index (Shannon index)
 dat$shannon <- diversity(as.matrix(dat[,15:34]),index = "shannon")
```

Our interest here is to model the proportion of Actinobacteria, Bacteroidetes and Firmicutes. The format of these variable in this dataset are still the count of sequence reads. We need to transform it into proportion by dividing each bacterial categories of interest with total sequence read.

```
matProp <- as.matrix(dat[,c(16,17,22)])/2000
dat1 <- as.data.frame(cbind(dat,matProp))
colnames(dat1)[43:45] <- c("p.Actino","p.Bactero","p.Firmi")
```

2.1 The analysis of the main effect of bacterial proportion and diversity

The following code are for the Actinobacteria proportion. The analysis for the other bacterial proportion were done by replacing the variable "bacp" with other bacterial proportion or diversity index.

```
for (i in 1:6){

  bacp <- dat1$p.Actino
  MainM <- lmer(dat1[,34+i] ~ t.point + bacp + (1|Ind.ID),REML = FALSE, data = dat1)
  MS <- summary(MainM)

  cat(sprintf("%s; %.2f (%.2f, %.2f); %.3f\n", colnames(dat1)[34+i],MS$coefficients[3,1],
              MS$coefficients[3,1]-1.96*MS$coefficients[3,2],MS$coefficients[3,1]+
                1.96*MS$coefficients[3,2],MS$coefficients[3,5]))
}
```

```
## lnil10lps; 0.20 (-0.58, 0.98); 0.614
## lntnflps; 0.55 (-0.35, 1.44); 0.236
## lnil5asc; -1.02 (-2.78, 0.74); 0.258
## lnifnasc; -1.03 (-2.45, 0.39); 0.160
## lnil5pha; -0.04 (-1.55, 1.46); 0.956
## lnifnpha; -0.57 (-2.12, 0.98); 0.473
```

2.2 The analysis of bacterial proportion and diversity effect on cytokine responses stratified by helminth infection

The following code are for Bacteroidetes proportion. The analysis for the other bacterial proprotion were done by replacing the variable "bacp" with other bacterial proportion or diversity index.

```
for (i in 1:6){

  bacp <- dat1$p.Bactero
  Model <- lmer(dat1[,34+i] ~ t.point + inf*bacp +
                  (1|Ind.ID), data = dat1,REML = FALSE)
  MS <- summary(Model)

  model <- Model
  VC <- vcov(model)
  SEI <- sqrt(VC[4,4]+VC[5,5] + 2*(VC[4,5]))
```

```
  Ifc <- sum(summary(model)$coefficients[c(4,5),1])
  Hea <- summary(model)$coefficients[4,1]
  SEH <- sqrt(VC[4,4])

  cat(sprintf("%s; %.2f (%.2f, %.2f); %.3f; helminth(+)\n%s; %.2f (%.2f, %.2f); %.3f; helminth(-)\n",
              colnames(dat1)[34+i],Ifc,Ifc-1.96*SEI,Ifc + 1.96*SEI,MS$coefficients[5,5],
              colnames(dat1)[34+i],Hea,Hea-1.96*SEH,Hea+1.96*SEH,MS$coefficients[4,5]))

}
```

```
## lnil10lps; -0.03 (-0.59, 0.53); 0.002; helminth(+)
## lnil10lps; -1.96 (-3.05, -0.87); 0.001; helminth(-)
## lntnflps; -0.18 (-0.86, 0.50); 0.485; helminth(+)
## lntnflps; 0.35 (-0.97, 1.67); 0.605; helminth(-)
## lnil5asc; -0.04 (-1.41, 1.32); 0.768; helminth(+)
## lnil5asc; 0.38 (-2.08, 2.84); 0.762; helminth(-)
## lnifnasc; -0.03 (-1.12, 1.05); 0.472; helminth(+)
## lnifnasc; 0.78 (-1.15, 2.70); 0.432; helminth(-)
## lnil5pha; 0.33 (-0.81, 1.46); 0.903; helminth(+)
## lnil5pha; 0.18 (-1.83, 2.20); 0.858; helminth(-)
## lnifnpha; -0.71 (-1.85, 0.43); 0.109; helminth(+)
## lnifnpha; 1.20 (-0.85, 3.25); 0.254; helminth(-)
```

Figure 2 was created using the following code.

```
f.name <- c("IL-10 to LPS",expression(paste("TNF- ",alpha," to LPS")),
            "IL-5 to AscAg",expression(paste("IFN-",gamma," to AscAg")),
            "IL-5 to PHA",expression(paste("IFN-",gamma," to PHA")))
par(mfrow=c(2,2))
i = 1
  bacp <- dat1$p.Bactero
  Model <- lmer(dat1[,34+i] ~ t.point + inf*bacp +
                  (1|Ind.ID), data = dat1,REML = FALSE)
  MS <- summary(Model)

  model <- Model
  VC <- vcov(model)
  SEI <- sqrt(VC[4,4]+VC[5,5] + 2*(VC[4,5]))
  Ifc <- sum(summary(model)$coefficients[c(4,5),1])
  Hea <- summary(model)$coefficients[4,1]
  SEH <- sqrt(VC[4,4])

  plotCI(c(1:2),c(Hea,Ifc),ui = c(Hea+1.96*SEH,Ifc+1.96*SEI),
         li = c(Hea-1.96*SEH,Ifc-1.96*SEI),lwd=2,xaxt = "n",xlab = "",
         ylab= "Estimated effect of Bacteroidetes",
         ylim=c(-4,4),xlim = c(0,3),main= f.name[i])
  corner.label(label='(C)',x=-1,figcorner=T,cex=1)
```

```
## $x
## [1] -1.012482
##
## $y
## [1] 7.30073
```

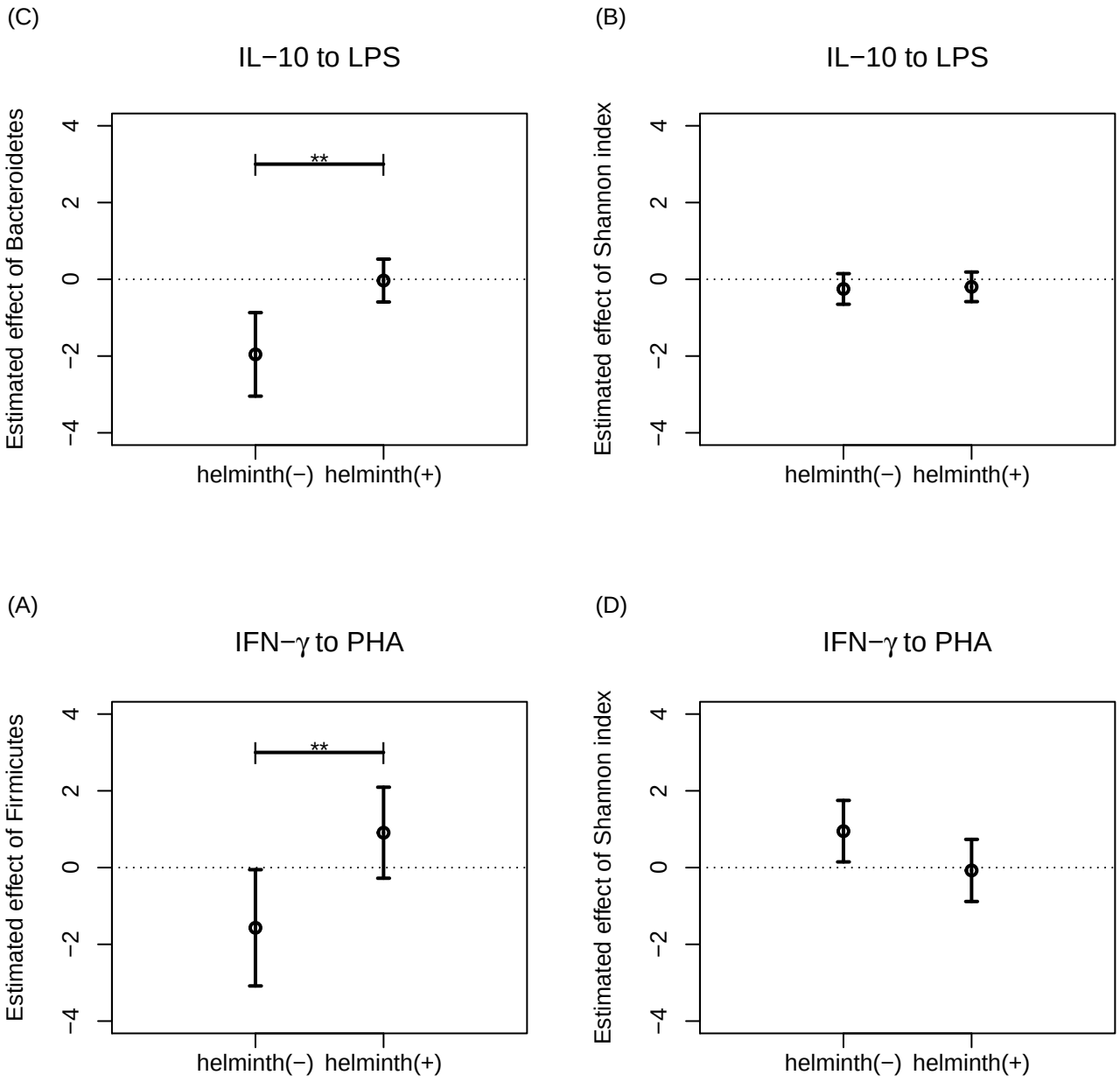
```
  abline(h = 0,lty = 3)
```

```
    lines(c(1,2),c(3,3),lwd=2)
    points(c(1,2),c(3,3),pch=c("|","|"),lwd=2)
    text(1.5,3.2,"**",cex = 1.05)
  ticks = c(1:2)
  axis(1,at=ticks, labels = FALSE,tick=TRUE)
  text(1:2, par("usr")[1]-5, srt = 0, adj = 0.5, labels = c("helminth(-)","helminth(+)"), xpd = TRUE)

## diversity
  bacp <- dat1$shannon
  Model <- lmer(dat1[,34+i] ~ t.point + inf*bacp +
                  (1|Ind.ID), data = dat1,REML = FALSE)
  MS <- summary(Model)

  model <- Model
  VC <- vcov(model)
  SEI <- sqrt(VC[4,4]+VC[5,5] + 2*(VC[4,5]))
  Ifc <- sum(summary(model)$coefficients[c(4,5),1])
  Hea <- summary(model)$coefficients[4,1]
  SEH <- sqrt(VC[4,4])

  plotCI(c(1:2),c(Hea,Ifc),ui = c(Hea+1.96*SEH,Ifc+1.96*SEI),
         li = c(Hea-1.96*SEH,Ifc-1.96*SEI),lwd=2,xaxt = "n",xlab = "",
         ylab= "Estimated effect of Shannon index",
         ylim=c(-4,4),xlim = c(0,3),main= f.name[i])
  corner.label(label='(B)',x=-1,figcorner=T,cex=1)
```

```
## $x
## [1] -1.012482
##
## $y
## [1] 7.30073
```

```
  abline(h = 0,lty = 3)

  ticks = c(1:2)
  axis(1,at=ticks, labels = FALSE,tick=TRUE)
  text(1:2, par("usr")[1]-5, srt = 0, adj = 0.5, labels = c("helminth(-)","helminth(+)"), xpd = TRUE)

i = 6

bacp <- dat1$p.Firmi
Model <- lmer(dat1[,34+i] ~ t.point + inf*bacp +
                (1|Ind.ID), data = dat1,REML = FALSE)
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEI <- sqrt(VC[4,4]+VC[5,5] + 2*(VC[4,5]))
Ifc <- sum(summary(model)$coefficients[c(4,5),1])
Hea <- summary(model)$coefficients[4,1]
SEH <- sqrt(VC[4,4])

plotCI(c(1:2),c(Hea,Ifc),ui = c(Hea+1.96*SEH,Ifc+1.96*SEI),
       li = c(Hea-1.96*SEH,Ifc-1.96*SEI),lwd=2,xaxt = "n",xlab = "",
       ylab= "Estimated effect of Firmicutes",
       ylim=c(-4,4),xlim = c(0,3),main= f.name[i])
```

```
corner.label(label='(A)',x=-1,figcorner=T,cex=1)

## $x
## [1] -1.012482
##
## $y
## [1] 7.30073

abline(h = 0,lty = 3)

lines(c(1,2),c(3,3),lwd=2)
points(c(1,2),c(3,3),pch=c("|","|"),lwd=2)
text(1.5,3.2,"**",cex = 1.05)
ticks = c(1:2)
axis(1,at=ticks, labels = FALSE,tick=TRUE)
text(1:2, par("usr")[1]-5, srt = 0, adj = 0.5, labels = c("helminth(-)","helminth(+)"), xpd = TRUE)

## diversity
bacp <- dat1$shannon
Model <- lmer(dat1[,34+i] ~ t.point + inf*bacp +
                (1|Ind.ID), data = dat1,REML = FALSE)
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEI <- sqrt(VC[4,4]+VC[5,5] + 2*(VC[4,5]))
Ifc <- sum(summary(model)$coefficients[c(4,5),1])
Hea <- summary(model)$coefficients[4,1]
SEH <- sqrt(VC[4,4])

plotCI(c(1:2),c(Hea,Ifc),ui = c(Hea+1.96*SEH,Ifc+1.96*SEI),
       li = c(Hea-1.96*SEH,Ifc-1.96*SEI),lwd=2,xaxt = "n",xlab = "",
       ylab= "Estimated effect of Shannon index",
       ylim=c(-4,4),xlim = c(0,3),main= f.name[i])
corner.label(label='(D)',x=-1,figcorner=T,cex=1)

## $x
## [1] -1.012482
##
## $y
## [1] 7.30073

abline(h = 0,lty = 3)

ticks = c(1:2)
axis(1,at=ticks, labels = FALSE,tick=TRUE)
text(1:2, par("usr")[1]-5, srt = 0, adj = 0.5, labels = c("helminth(-)","helminth(+)"), xpd = TRUE)
```



2.3 The analysis of bacterial proportion and diversity on cytokine response at pre-treatment and at post-treatment in placebo and albendazole group

As before, the following code are for the diversity index. The analysis for bacterial proprotion were done by replacing the variable "bacp" with bacterial proportion.

```
# define treat_t1 as binary variable which is 1 if subjects receive albendazole at time 1
dat1$treat_t1 <- (dat1$Treatment == 1)*(dat1$t.point == 1)
for (i in 1:6){
bacp <- dat1$shannon
Model <- lmer(dat1[,34+i] ~ t.point + bacp*t.point + treat_t1*bacp +
                (1|Ind.ID), data = dat1,REML = FALSE)
```

```
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEPre <- sqrt(VC[3,3])
SEP <- sqrt(VC[3,3] + VC[5,5] + 2*VC[3,5])
SEA <- sqrt(VC[3,3]+ VC[5,5] + VC[6,6] + 2*(VC[3,5]+VC[3,6] +VC[5,6]))
Pla <- sum(MS$coefficients[c(3,5),1])
Alb <- sum(MS$coefficients[c(3,5,6),1])
bac0 <- MS$coefficients[3,1]

cat(sprintf("%s; %.2f (%.2f, %.2f); %.3f; pre\n
            %s; %.2f (%.2f, %.2f); %.3f; post-pla\n
            %s; %.2f (%.2f, %.2f); %.3f; post-alb\n",
            colnames(dat1)[34+i],bac0,bac0-1.96*SEPre,bac0 + 1.96*SEPre,MS$coefficients[3,5],
            colnames(dat1)[34+i],Pla,Pla-1.96*SEP,Pla + 1.96*SEP,MS$coefficients[5,5],
            colnames(dat1)[34+i],Alb,Alb-1.96*SEA,Alb+1.96*SEA,MS$coefficients[6,5]))
}
```

```
## lnil10lps; -0.32 (-0.72, 0.08); 0.124; pre
##
##             lnil10lps; -0.14 (-0.57, 0.30); 0.535; post-pla
##
##             lnil10lps; -0.20 (-0.91, 0.50); 0.878; post-alb
## lntnflps; -0.41 (-0.87, 0.05); 0.082; pre
##
##             lntnflps; 0.27 (-0.22, 0.76); 0.040; post-pla
##
##             lntnflps; 0.75 (-0.06, 1.55); 0.325; post-alb
## lnil5asc; -0.89 (-1.83, 0.05); 0.067; pre
##
##             lnil5asc; 0.17 (-0.81, 1.16); 0.116; post-pla
##
##             lnil5asc; -1.48 (-3.24, 0.28); 0.110; post-alb
## lnifnasc; 0.49 (-0.29, 1.27); 0.219; pre
##
##             lnifnasc; 0.06 (-0.75, 0.87); 0.428; post-pla
##
##             lnifnasc; -0.94 (-2.40, 0.51); 0.236; post-alb
## lnil5pha; 0.59 (-0.22, 1.40); 0.155; pre
##
##             lnil5pha; 1.03 (0.18, 1.87); 0.448; post-pla
##
##             lnil5pha; -0.79 (-2.30, 0.72); 0.041; post-alb
## lnifnpha; 0.63 (-0.23, 1.48); 0.153; pre
##
##             lnifnpha; 0.64 (-0.23, 1.52); 0.975; post-pla
##
##             lnifnpha; -0.95 (-2.53, 0.64); 0.086; post-alb
```

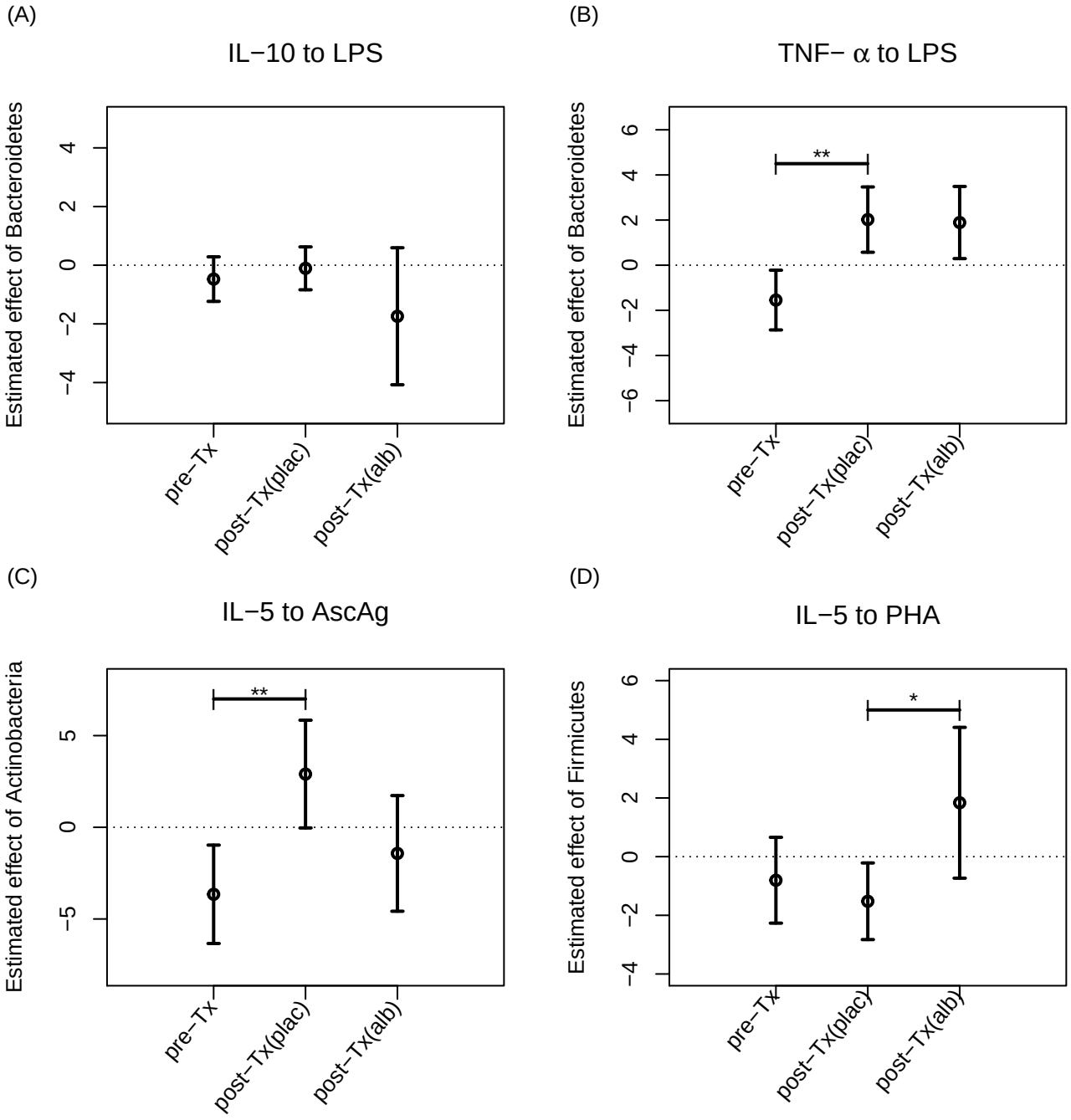
Figure 3 was created using the following code.

```
par(mfrow=c(2,2))
i = 1
```

```
bacp <- dat1$p.Bactero
Model <- lmer(dat1[,34+i] ~ t.point + bacp*t.point + treat_t1*bacp +
                (1|Ind.ID), data = dat1,REML = FALSE)
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEPre <- sqrt(VC[3,3])
SEP <- sqrt(VC[3,3] + VC[5,5] + 2*VC[3,5])
SEA <- sqrt(VC[3,3]+ VC[5,5] + VC[6,6] + 2*(VC[3,5]+VC[3,6] +VC[5,6]))
Pla <- sum(MS$coefficients[c(3,5),1])
Alb <- sum(MS$coefficients[c(3,5,6),1])
bac0 <- MS$coefficients[3,1]

plotCI(c(1:3),c(bac0,Pla,Alb),ui = c(bac0+1.96*SEPre,Pla+1.96*SEP,Alb+1.96*SEA),
       li = c(bac0-1.96*SEPre,Pla-1.96*SEP,Alb - 1.96*SEA),lwd=2,xaxt = "n",xlab = "",
       ylab= "Estimated effect of Bacteroidetes",
       ylim=c(-5,5),xlim = c(0,4),main= f.name[i])
abline(h = 0,lty = 3)
ticks = c(1:3)
axis(1,at=ticks, labels = FALSE,tick=TRUE)
text(1:3, par("usr")[1]-6, srt = 50, adj = 1,
     labels = c("pre-Tx","post-Tx(plac)","post-Tx(alb)"), xpd = TRUE)
corner.label(label='(A)',x=-1,figcorner=T,cex=1)

## $x
## [1] -1.349976
##
## $y
## [1] 9.125912

i = 2
bacp <- dat1$p.Actino
Model <- lmer(dat1[,34+i] ~ t.point + bacp*t.point + treat_t1*bacp +
                (1|Ind.ID), data = dat1,REML = FALSE)
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEPre <- sqrt(VC[3,3])
SEP <- sqrt(VC[3,3] + VC[5,5] + 2*VC[3,5])
SEA <- sqrt(VC[3,3]+ VC[5,5] + VC[6,6] + 2*(VC[3,5]+VC[3,6] +VC[5,6]))
Pla <- sum(MS$coefficients[c(3,5),1])
Alb <- sum(MS$coefficients[c(3,5,6),1])
bac0 <- MS$coefficients[3,1]
plotCI(c(1:3),c(bac0,Pla,Alb),ui = c(bac0+1.96*SEPre,Pla+1.96*SEP,Alb+1.96*SEA),
       li = c(bac0-1.96*SEPre,Pla-1.96*SEP,Alb - 1.96*SEA),lwd=2,xaxt = "n",xlab = "",
       ylab= "Estimated effect of Bacteroidetes",
       ylim=c(-6.5,6.5),xlim = c(0,4),main= f.name[i])
lines(c(1,2),c(4.5,4.5),lwd=2)
points(c(1,2),c(4.5,4.5),pch=c("|","|"),lwd=2)
text(1.5,5,"**",cex = 1.05)
corner.label(label='(B)',x=-1,figcorner=T,cex=1)

## $x
## [1] -1.349976
```

```
##
## $y
## [1] 11.86369

abline(h = 0,lty = 3)
ticks = c(1:3)
axis(1,at=ticks, labels = FALSE,tick=TRUE)
text(1:3, par("usr")[1]-7.5, srt = 50, adj = 1,
     labels = c("pre-Tx","post-Tx(plac)","post-Tx(alb)"), xpd = TRUE)

i = 3
bacp <- dat1$p.Actino
Model <- lmer(dat1[,34+i] ~ t.point + bacp*t.point + treat_t1*bacp + (1|Ind.ID), data = dat1,REML = FALSE)
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEPre <- sqrt(VC[3,3])
SEP <- sqrt(VC[3,3] + VC[5,5] + 2*VC[3,5])
SEA <- sqrt(VC[3,3]+ VC[5,5] + VC[6,6] + 2*(VC[3,5]+VC[3,6] +VC[5,6]))
Pla <- sum(MS$coefficients[c(3,5),1])
Alb <- sum(MS$coefficients[c(3,5,6),1])
bac0 <- MS$coefficients[3,1]

plotCI(c(1:3),c(bac0,Pla,Alb),ui = c(bac0+1.96*SEPre,Pla+1.96*SEP,Alb+1.96*SEA),
       li = c(bac0-1.96*SEPre,Pla-1.96*SEP,Alb - 1.96*SEA),lwd=2,xaxt = "n",xlab = "",
       ylab= "Estimated effect of Actinobacteria",
       ylim=c(-8,8),xlim = c(0,4),main= f.name[i])
lines(c(1,2),c(7,7),lwd=2)
points(c(1,2),c(7,7),pch=c("|","|"),lwd=2)
text(1.5,7.5,"**",cex = 1.05)
corner.label(label='(C)',x=-1,figcorner=T,cex=1)

## $x
## [1] -1.349976
##
## $y
## [1] 14.60146

abline(h = 0,lty = 3)
ticks = c(1:3)
axis(1,at=ticks, labels = FALSE,tick=TRUE)
text(1:3, par("usr")[1]-9.5, srt = 50, adj = 1,
     labels = c("pre-Tx","post-Tx(plac)","post-Tx(alb)"), xpd = TRUE)

i = 5
bacp <- dat1$p.Firmi
Model <- lmer(dat1[,34+i] ~ t.point + bacp*t.point + treat_t1*bacp +
                (1|Ind.ID), data = dat1,REML = FALSE)
MS <- summary(Model)

model <- Model
VC <- vcov(model)
SEPre <- sqrt(VC[3,3])
SEP <- sqrt(VC[3,3] + VC[5,5] + 2*VC[3,5])
SEA <- sqrt(VC[3,3]+ VC[5,5] + VC[6,6] + 2*(VC[3,5]+VC[3,6] +VC[5,6]))
```

```r
Pla <- sum(MS$coefficients[c(3,5),1])
Alb <- sum(MS$coefficients[c(3,5,6),1])
bac0 <- MS$coefficients[3,1]

plotCI(c(1:3),c(bac0,Pla,Alb),ui = c(bac0+1.96*SEPre,Pla+1.96*SEP,Alb+1.96*SEA),
       li = c(bac0-1.96*SEPre,Pla-1.96*SEP,Alb - 1.96*SEA),lwd=2,xaxt = "n",xlab = "",
       ylab= "Estimated effect of Firmicutes",
       ylim=c(-4,6),xlim = c(0,4),main= f.name[i])
lines(c(2,3),c(5,5),lwd=2)
points(c(2,3),c(5,5),pch=c("|","|"),lwd=2)
text(2.5,5.5,"*",cex = 1.05)
corner.label(label='(D)',x=-1,figcorner=T,cex=1)

## $x
## [1] -1.349976
##
## $y
## [1] 10.12591

abline(h = 0,lty = 3)
ticks = c(1:3)
axis(1,at=ticks, labels = FALSE,tick=TRUE)
text(1:3, par("usr")[1]-4.5, srt = 50, adj = 1,
     labels = c("pre-Tx","post-Tx(plac)","post-Tx(alb)"), xpd = TRUE)
```


(A)
IL−10 to LPS
Estimated effect of Bacteroidetes
−4
−2
0
2
4
pre−Tx
post−Tx(plac)
post−Tx(alb)
(B)
TNF− α to LPS
Estimated effect of Bacteroidetes
−6
−4
−2
0
2
4
6
**
pre−Tx
post−Tx(plac)
post−Tx(alb)
(C)
IL−5 to AscAg
Estimated effect of Actinobacteria
−5
0
5
**
pre−Tx
post−Tx(plac)
post−Tx(alb)
(D)
IL−5 to PHA
Estimated effect of Firmicutes
−4
−2
0
2
4
6
*
pre−Tx
post−Tx(plac)
post−Tx(alb)