

SHIME_SCFA_Processing

Contents

1	Load packages	1
2	Loading Data	1
3	Plotting	2
3.1	Lineplots	2
3.2	Lineplots Rhamnolipids & P80	6
4	Testing significance of trends	11

1 Load packages

```
### This script is used to plot the SCFA-data of the SHIME-experiments in which  
### the impact of 3 dietary emulsifiers was tested on the gut microbiota  
### from 2 individuals.
```

```
# install packages & load libraries #
```

```
library(dplyr)  
library(ggplot2)  
library(scales)  
library(tidyr)  
library(pwr)  
library(readxl)  
library(vegan)  
library(car)  
library(mvnormtest)  
library(DescTools)  
library(ggpubr)
```

```
library(xlsx)#handling Excel files  
library(openxlsx)#handling Excel files without Java deps  
library(readxl) #faster handling of excel files
```

```
set.seed(2)
```

2 Loading Data

```
#data.VFA <- read.csv2('SCFA_All_Donors.csv', header= TRUE)  
data.SCFA <- readxl::read_xlsx("20201006_SHIME_SCFA.xlsx",sheet = "Together_mmol")  
colnames(data.SCFA)[5:15] <- c("Acetic_acid","Propionic_acid","Isobutyric_acid",
```

```

        "Butyric_acid","Isovaleric_acid","Valeric_acid",
        "Isocaproic_acid","Caproic_acid","ISTD","Heptanoic_acid",
        "Octanoic_acid")
data.SCFA$Donor <- data.SCFA$SHIME -1
data.SCFA <- unite(data.SCFA, Treatment, c(Emulsifier, EM_Conc), remove=FALSE, sep = ' - ')
data.SCFA <- unite(data.SCFA, TreatmentTime, c(Treatment, Timepoint), remove=FALSE,
        sep = ' - ')
data.SCFA <- unite(data.SCFA, TreatmentDonor, c(Treatment, Donor), remove=FALSE,
        sep = ' - D')

data.SCFA$Emulsifier<- as.factor(data.SCFA$Emulsifier)
data.SCFA$Treatment<- as.factor(data.SCFA$Treatment)
data.SCFA$TreatmentTime<- as.factor(data.SCFA$TreatmentTime)
data.SCFA$EM_Conc<- as.factor(data.SCFA$EM_Conc)
data.SCFA$Donor<- as.factor(data.SCFA$Donor)

```

3 Plotting

3.1 Lineplots

```

### lineplots
data.SCFA.S2 <- dplyr::filter(data.SCFA,SHIME==2)
### total plot
plot_total_S2 <- ggplot(data.SCFA.S2, aes(x = Timepoint, y = Sum, color = Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
        ymax = Inf) +
  geom_line() + geom_point() + facet_grid(Donor~.)+theme_bw()+ylab("Total SCFA (mmol)")+
  xlab("Timepoint (day)")+
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
        "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0, size = 8),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        legend.position = "right",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_total_S2

```

```

data.SCFA.S3 <- dplyr::filter(data.SCFA,SHIME==3)

plot_total_S3 <- ggplot(data.SCFA.S3, aes(x = Timepoint, y = Sum, color = Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
        ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Total SCFA (mmol)")+xlab("Timepoint (day)")+

```

```

scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                              "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_text(size =12,family='sans',angle=0),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_total_S3

plot_total_SCFA <- ggarrange(plot_total_S2+rremove("x.text"), plot_total_S3,
                             heights = c(2,2.2), labels = c("A", "B"),
                             ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_TotalSCFA1.tiff", width=8*dpi,
     height=6*dpi,res=dpi,compression="lzw")
plot_total_SCFA
dev.off()

### Acetate plot
plot_A_S2 <- ggplot(data.SCFA.S2, aes(x = Timepoint, y = Acetic_acid, color = Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
         ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Acetate (mmol)")+xlab("Timepoint (day)")+
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                              "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_text(size =12,family='sans',angle=0),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_A_S2

plot_A_S3 <- ggplot(data.SCFA.S3, aes(x = Timepoint, y = Acetic_acid, color =Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
         ymax = Inf)+ geom_line() + geom_point() + facet_grid(Donor~.)+ theme_bw()+
  ylab("Acetate (mmol)")+xlab("Timepoint (day)")+

```

```

scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                              "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0, size = 8),
      axis.text.y = element_text(size = 10, family='sans', angle=0),
      axis.title.x = element_text(size = 12, family='sans', angle=0),
      axis.title.y = element_text(size=12, family='sans', angle=90),
      legend.text = element_text(size=10, family='sans', angle=0),
      legend.title = element_text(size=10, family='sans', angle=0),
      strip.text = element_text(size=10, angle=0, family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3, "cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"), axis.line=element_line(colour="black"))
plot_A_S3

```

```

plot_A_SCFA <- ggarrange(plot_A_S2+rremove("x.text"), plot_A_S3, heights = c(2,2.2),
  labels = c("A", "B"),
  ncol = 1, nrow = 2, align = "v")

```

```

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Acetate1.tiff", width=8*dpi,
  height=6*dpi, res=dpi, compression="lzw")
plot_A_SCFA
dev.off()

```

Propionate plot

```

plot_P_S2 <- ggplot(data.SCFA.S2, aes(x = Timepoint, y = Propionic_acid, color=Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
    ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Propionate (mmol)") + xlab("Timepoint (day)") +
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                              "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0, size = 8),
      axis.text.y = element_text(size = 10, family='sans', angle=0),
      axis.title.x = element_text(size = 12, family='sans', angle=0),
      axis.title.y = element_text(size=12, family='sans', angle=90),
      legend.text = element_text(size=10, family='sans', angle=0),
      legend.title = element_text(size=10, family='sans', angle=0),
      strip.text = element_text(size=10, angle=0, family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3, "cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"), axis.line=element_line(colour="black"))
plot_P_S2

```

```

plot_P_S3 <- ggplot(data.SCFA.S3, aes(x = Timepoint, y = Propionic_acid, color=Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
    ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Propionate (mmol)") + xlab("Timepoint (day)") +
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",

```

```

      "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_text(size =12,family='sans',angle=0),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_P_S3

```

```

plot_P_SCFA <- ggarrange(plot_P_S2+rremove("x.text"), plot_P_S3,heights = c(2,2.2),
  labels = c("A", "B"),
  ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Propionate1.tiff",width=8*dpi,
  height=6*dpi,res=dpi,compression="lzw")
plot_P_SCFA
dev.off()

```

Butyrate plot

```

plot_B_S2 <- ggplot(data.SCFA.S2, aes(x = Timepoint, y = Butyric_acid, color=Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
    ymax = Inf) +
  geom_line() + geom_point() +
  facet_grid(Donor~.)+theme_bw()+ylab("Butyrate (mmol)")+xlab("Timepoint (day)")+
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
    "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_text(size =12,family='sans',angle=0),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_B_S2

```

```

plot_B_S3 <- ggplot(data.SCFA.S3, aes(x = Timepoint, y = Butyric_acid, color=Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,

```

```

      ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
theme_bw()+ylab("Butyrate (mmol)")+xlab("Timepoint (day)")+
scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
      "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_text(size =12,family='sans',angle=0),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_B_S3

plot_B_SCFA <- ggarrange(plot_B_S2+rremove("x.text"), plot_B_S3,heights = c(2,2.2),
      labels = c("A", "B"),
      ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Butyrate1.tiff",width=8*dpi,
      height=6*dpi,res=dpi,compression="lzw")
plot_B_SCFA
dev.off()

```

3.2 Lineplots Rhamnolipids & P80

```

data.SCFA.S2 <- dplyr::filter(data.SCFA,SHIME==2)
data.SCFA.S3 <- dplyr::filter(data.SCFA,SHIME==3)

data.SCFA.S2.RL.P80 <- dplyr::filter(data.SCFA.S2,Emulsifier %in% c("Control","RL","P80"))
data.SCFA.S3.RL.P80 <- dplyr::filter(data.SCFA.S3,Emulsifier %in% c("Control","RL","P80"))
data.SCFA.RL.P80 <-rbind(data.SCFA.S2.RL.P80,data.SCFA.S3.RL.P80)

### total plot
plot_total_S2_RL.P80 <- ggplot(data.SCFA.S2.RL.P80, aes(x = Timepoint, y = Sum,
      color = Treatment))+
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
      ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+theme_bw()+
  ylab("Total SCFA (mmol)")+xlab("Timepoint (day)")+
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
      "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_text(size =12,family='sans',angle=0),
      axis.title.y = element_text(size=12,family='sans',angle=90),

```

```

legend.text = element_text(size=10,family='sans',angle=0),
legend.title = element_text(size=10,family='sans',angle=0),
strip.text = element_text(size=10,angle=0,family='sans'),
legend.position = "right",
legend.key.size = unit(0.3,"cm"),
panel.grid.major=element_blank(),
panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_total_S2_RL.P80

```

```

plot_total_S3_RL.P80 <- ggplot(data.SCFA.S3.RL.P80, aes(x = Timepoint, y = Sum,
                                                         color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
           ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+theme_bw()+
  ylab("Total SCFA (mmol)")+xlab("Timepoint (day)")+
  scale_color_manual(values = c("Black","#d28eed", "#844da8", "#74dff7", "#125087",
                                "#f5e2a2", "#e3d029", "#F781BF","#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        legend.position = "right",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_total_S3_RL.P80

```

```

plot_total_SCFA_RL.P80 <- ggarrange(plot_total_S2_RL.P80 +rremove("x.text"),
                                   plot_total_S3_RL.P80 ,heights = c(2,2.2),
                                   labels = c("A", "B"),ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_TotalSCFA_RL_P80.tiff",width=8*dpi,
     height=6*dpi,res=dpi,compression="lzw")
plot_total_SCFA_RL.P80
dev.off()

```

Acetate plot

```

plot_A_S2_RL.P80 <- ggplot(data.SCFA.S2.RL.P80, aes(x = Timepoint, y = Acetic_acid,
                                                         color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
           ymax = Inf) + geom_point() + geom_line() + facet_grid(Donor~.)+
  theme_bw()+ylab("Acetate (mmol/L)")+xlab("Timepoint (Day)")+
  scale_color_manual(values = c("Black","#d28eed", "#844da8", "#74dff7", "#125087",
                                "#f5e2a2", "#e3d029", "#F781BF","#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  ylim(10,115)+

```

```

theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_blank(),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_A_S2_RL.P80

```

```

plot_A_S3_RL.P80 <- ggplot(data.SCFA.S3.RL.P80, aes(x = Timepoint, y = Acetic_acid,
                                                    color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
          ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Acetate (mmol/L)")+xlab("Timepoint (Day)")+
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                                "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  ylim(10,115)+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        legend.position = "right",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_A_S3_RL.P80

```

```

plot_A_SCFA_RL.P80 <- ggarrange(plot_A_S2_RL.P80+rremove("x.text"),
                                plot_A_S3_RL.P80,heights = c(2,2.2),
                                labels = c("A", "B"), ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Acetate_RL_P80.tiff",width=8*dpi,
     height=6*dpi,res=dpi,compression="lzw")
plot_A_SCFA_RL.P80
dev.off()

```

Propionate plot

```

plot_P_S2_RL.P80 <- ggplot(data.SCFA.S2.RL.P80, aes(x = Timepoint, y = Propionic_acid,
                                                    color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
          ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Propionate (mmol/L)")+xlab("Timepoint (Day)")+

```

```

scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                              "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
ylim(0,60)+
theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
      axis.text.y = element_text(size = 10,family='sans',angle=0),
      axis.title.x = element_blank(),
      axis.title.y = element_text(size=12,family='sans',angle=90),
      legend.text = element_text(size=10,family='sans',angle=0),
      legend.title = element_text(size=10,family='sans',angle=0),
      strip.text = element_text(size=10,angle=0,family='sans'),
      legend.position = "right",
      legend.key.size = unit(0.3,"cm"),
      panel.grid.major=element_blank(),
      panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_P_S2_RL.P80

plot_P_S3_RL.P80 <- ggplot(data.SCFA.S3.RL.P80, aes(x = Timepoint, y = Propionic_acid,
                                                    color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
          ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Propionate (mmol/L)")+xlab("Timepoint (Day)")+
  scale_color_manual(values = c("Black", "#d28eed", "#844da8", "#74dff7", "#125087",
                              "#f5e2a2", "#e3d029", "#F781BF", "#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  ylim(0,60)+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size = 12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        legend.position = "right",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_P_S3_RL.P80

plot_P_SCFA_RL.P80 <- ggarrange(plot_P_S2_RL.P80 + rremove("x.text"),
                               plot_P_S3_RL.P80,heights = c(2,2.2),
                               ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Propionate_RL_P80.tiff",
     width=8*dpi, height=6*dpi,res=dpi,compression="lzw")
plot_P_SCFA_RL.P80
dev.off()

### Butyrate plot

plot_B_S2_RL.P80 <- ggplot(data.SCFA.S2.RL.P80, aes(x = Timepoint, y = Butyric_acid,

```

```

                                color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 11.5, xmax = 18, ymin = -Inf,
          ymax = Inf) + geom_line() + geom_point() +
  facet_grid(Donor~.)+theme_bw()+ylab("Butyrate (mmol/L)")+
  # xlab("Timepoint (day)")+
  scale_color_manual(values = c("Black","#d28eed", "#844da8" ,"#74dff7", "#125087",
                                "#f5e2a2", "#e3d029", "#F781BF","#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  ylim(0,35)+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_blank(),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        legend.position = "right",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_B_S2_RL.P80

```

```

plot_B_S3_RL.P80 <- ggplot(data.SCFA.S3.RL.P80, aes(x = Timepoint, y = Butyric_acid,
                                color = Treatment)) +
  annotate("rect", fill = "grey", alpha = 0.5, xmin = 9.5, xmax = 16, ymin = -Inf,
          ymax = Inf) + geom_line() + geom_point() + facet_grid(Donor~.)+
  theme_bw()+ylab("Butyrate (mmol/L)")+xlab("Timepoint (Day)")+
  scale_color_manual(values = c("Black","#d28eed", "#844da8" ,"#74dff7", "#125087",
                                "#f5e2a2", "#e3d029", "#F781BF","#f2b385", "#d97914"))+
  scale_x_continuous(breaks=c(0,2,4,6,8,10,12,14,16,18),limits=c(0,18))+
  ylim(0,35)+
  theme(axis.text.x=element_text(angle=90, hjust=1, vjust = 0,size = 8),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        legend.position = "right",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())+
  theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
plot_B_S3_RL.P80

```

```

plot_B_SCFA_RL.P80 <- ggarrange(plot_B_S2_RL.P80 + rremove("x.text"),
                                plot_B_S3_RL.P80,heights = c(2,2.2),
                                ncol = 1, nrow = 2, align = "v")

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Butyrate_RL_P80.tiff",
     width=8*dpi,height=6*dpi,res=dpi,compression="lzw")
plot_B_SCFA_RL.P80

```

```

dev.off()

plot_P_S2_RL.P80 <- plot_P_S2_RL.P80 + theme(legend.position = "none")
plot_P_S3_RL.P80 <- plot_P_S3_RL.P80 + theme(legend.position = "none")

plot_PB_SCFA_RL.P80 <- ggarrange(plot_P_S2_RL.P80 + rremove("x.text"),
                                plot_B_S2_RL.P80 + rremove("x.text"),
                                plot_P_S3_RL.P80, plot_B_S3_RL.P80,
                                heights = c(2,2,2.5,2.5), widths = c(4.5,5.5,4.5,5.5),
                                labels = c("A","B","C","D"), ncol = 2, nrow = 2)

plot_PB_SCFA_RL.P80

plot_PB_SCFA_RL.P80 <- ggarrange(plot_P_SCFA_RL.P80 ,plot_B_SCFA_RL.P80,
                                labels = c("A","B"), ncol = 1, nrow = 2)

plot_PB_SCFA_RL.P80

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/SCFA/Figures/SHIME_Propionate_Butyrate_RL_P80.tiff",
     width=10*dpi,height=6*dpi,res=dpi,compression="lzw")
plot_PB_SCFA_RL.P80
dev.off()

```

4 Testing significance of trends

```

data.SCFA.diff <- readxl::read_xlsx("20210718_SHIME_SCFA_DifferenceWControl.xlsx",
                                   sheet = "Sheet1")

data.SCFA.diff.P80.RL <- dplyr::filter(data.SCFA.diff,EM %in% c("P80","RL"))
data.SCFA.diff.P80.0.05 <- dplyr::filter(data.SCFA.diff.P80.RL,Treatment %in%
                                         c("P80 - 0,05"))
data.SCFA.diff.P80.0.5 <- dplyr::filter(data.SCFA.diff.P80.RL,Treatment %in%
                                         c("P80 - 0,5"))
data.SCFA.diff.RL.0.05 <- dplyr::filter(data.SCFA.diff.P80.RL,Treatment %in%
                                         c("RL - 0,05"))
data.SCFA.diff.RL.0.5 <- dplyr::filter(data.SCFA.diff.P80.RL,Treatment %in%
                                         c("RL - 0,5"))

### P80 - 0,05%

PAc_D1 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Acetate_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,11
PAc_D2 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Acetate_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,029

PProp_D1 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Propiate_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,34
PProp_D2 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Propiate_S2 ~Test,

```

```

                                method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,057

PBut_D1 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Butyrate_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,69
PBut_D2 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Butyrate_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,057

PTot_D1 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Total_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,49
PTot_D2 <- compare_means(data = data.SCFA.diff.P80.0.05, formula = Total_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,34

### P80 - 0,5%

PAc_D1 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Acetate_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,2
PAc_D2 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Acetate_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,69

PProp_D1 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Propiate_S1 ~Test,
                           method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,057
PProp_D2 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Propiate_S2 ~Test,
                           method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,029

PBut_D1 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Butyrate_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,11
PBut_D2 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Butyrate_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,057

PTot_D1 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Total_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,49
PTot_D2 <- compare_means(data = data.SCFA.diff.P80.0.5, formula = Total_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,49

```

```

### RL - 0,05%

PAc_D1 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Acetate_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,89
PAc_D2 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Acetate_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,2

PProp_D1 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Propiate_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,69
PProp_D2 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Propiate_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,029

PBut_D1 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Butyrate_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,029
PBut_D2 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Butyrate_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,029

PTot_D1 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Total_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,2
PTot_D2 <- compare_means(data = data.SCFA.diff.RL.0.05, formula = Total_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,34

### RL - 0,5%

PAc_D1 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Acetate_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,69
PAc_D2 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Acetate_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,69

PProp_D1 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Propiate_S1 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,057
PProp_D2 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Propiate_S2 ~Test,
                        method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,34

```

```

PBut_D1 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Butyrate_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,34
PBut_D2 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Butyrate_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,029

PTot_D1 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Total_S1 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,69
PTot_D2 <- compare_means(data = data.SCFA.diff.RL.0.5, formula = Total_S2 ~Test,
                          method = "wilcox.test", p.adjust.method = "holm")
#Padj = 0,2

```