Supplemental Figure

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Reviewer comment #1

Figure S2 monte carlo test sounds strange for what the authors want to test (page 4 top). If the idea is to test whether the day before was the most similar, why not just test it directly and compare with distance to other days, rather than this permutation test? Am I missing something? And why is Bray-Curtis distance used here?

Reviewer comment #2

The authors state that "each subject's microbiome samples were most similar to their own sample from the previous day compared to all other timepoints from that subject". Was this done also with comparing to all other unrelated samples, or only to samples within the same subject? The proper analysis should be to compare as background to the whole cohort.

To address

The analysis should be redone using the Aitchison's distance.Get each day's distance to the next day within in a person and compare to all of the distances that are 2 or more days apart.

Then test whether the mean difference is <0 with a t-test.
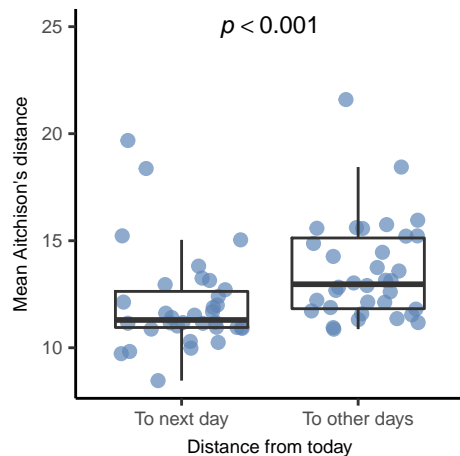
We have seen that diet on the weekend is more variable than on the weekdays.

So, we asked, is the microbiome more variable on Monday-Tuesday (presumably in response to changes in diet on Fri-Sat-Sun)

```
## Warning: Removed 2 rows containing non-finite values (stat_boxplot).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```
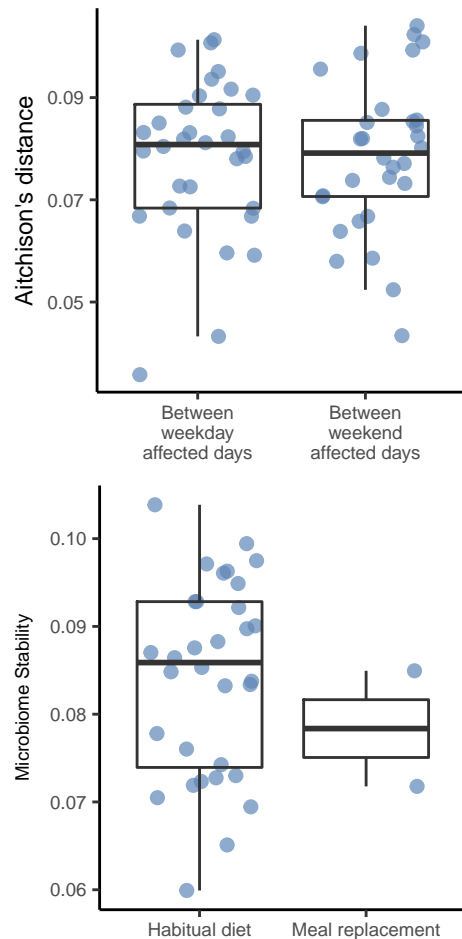


```
names(day1diffall) <- unique(map$UserName)
mydistance <- stack(day1diffall)
names(weekdaysall) <- unique(map$UserName)
mydays <- stack(weekdaysall)


plot <- cbind(mydistance, mydays)
colnames(plot) <- c("Distance", "UserName", "Day", "UserName2")

plot$Diet <- ifelse(plot$UserName %in% c("MCTs11", "MCTs12"), "Shake Drinkers", "Normal Diet")

plot <- subset(plot, !plot$UserName %in% c("MCTs06", "MCTs29"))
plot$Day <- as.factor(plot$Day)
```

```
plot_mean<- aggregate(plot$Distance, by = list(plot$Day, plot$Diet), FUN = mean)
colnames(plot_mean) <- c("Day", "Mean Diet", "Distance")


ggplot(plot, aes(x = Day, y = Distance)) +
  geom_line(aes(group = UserName), color = "grey", aes = 0.75) +
  geom_line(data = subset(plot, plot$UserName == "MCTs11"), aes(group = UserName), color = "#5f86b7", si
  geom_line(data = subset(plot, plot$UserName == "MCTs12"), aes(group = UserName), color = "#5f86b7", si
  #geom_smooth(data = plot_mean, aes(group = `Mean Diet`, color = `Mean Diet`)) +
  ylab("Microbiome distance\nfrom previous day") +
  xlab("Study Day") +
  theme_classic() +
  theme(axis.title = element_text(size = 8))
```

```
## Warning: Ignoring unknown parameters: aes
```