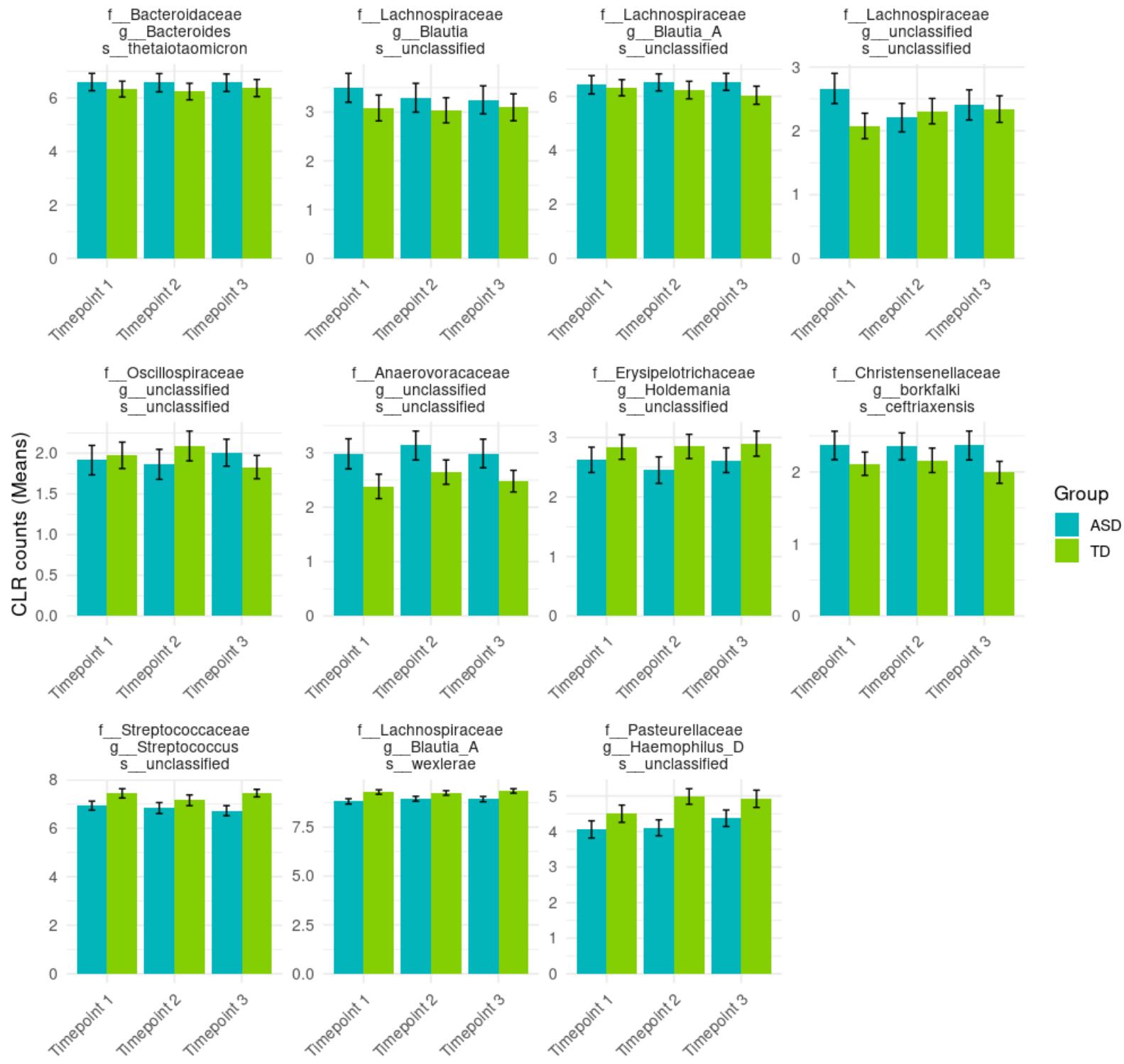


Supplementary File 16: Bray Curtis distance between samples from the same family. DESeq2 minimizes the distance between samples expected to be close due to lifestyle, dietary, and physical proximity.

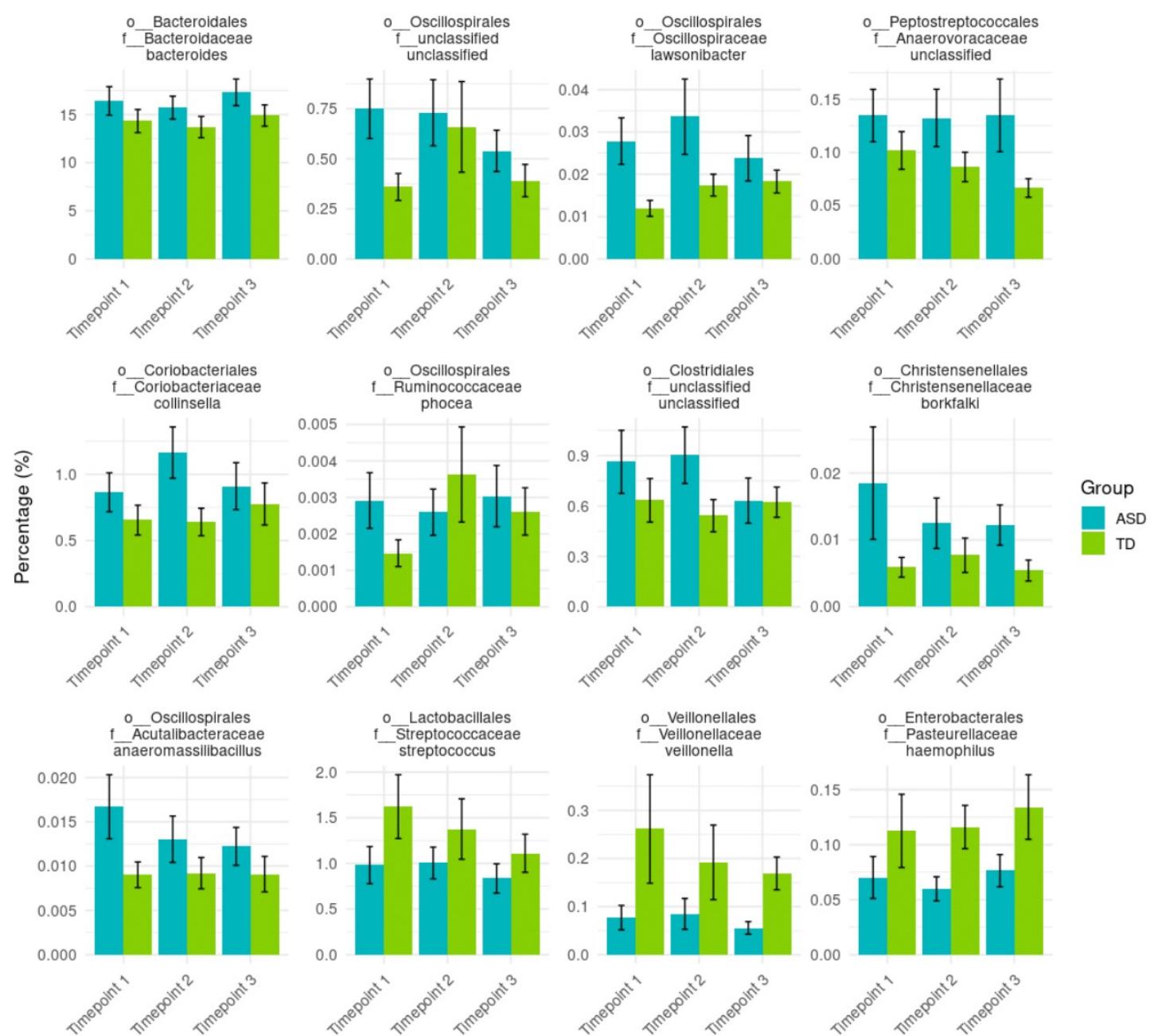


Supplementary File 3: Centered log transform ASVs significantly associated with the ASD cohort in two independent contrast methods. ASVs taxonomic annotation of the 16S amplicon (at the family, genus, and species) and the corresponding centered log transform abundance for the 11 taxa identified in at least two independent contrast methods (ANCOM and/or MetagenomeSeq and/or DESeq2) over the three time points.

A

Order	Family	Genus	Enrichment	Method
<i>Bacteroides</i>	Bacteroidaceae	<i>Bacteroides</i>	ASD	DESEQ2
<i>Oscillospirales</i>	Unclassified	Unclassified	ASD	DESEQ2
<i>Oscillospirales</i>	Oscillospiraceae	<i>Lawsonibacter</i>	ASD	DESEQ2, Mtgseq
<i>Peptostreptococcales</i>	Anaerovoracaceae	Unclassified	ASD	DESEQ2, Mtgseq
<i>Coriobacteriales</i>	Coriobacteriaceae	<i>Collinsella</i>	ASD	Mtgseq
<i>Oscillospirales</i>	Ruminococcaceae	<i>Phocea</i>	ASD	Mtgseq
<i>Clostridiales</i>	Unclassified	Unclassified	ASD	DESEQ2
<i>Christensenellales</i>	Christensenellaceae	<i>Borkfalki</i>	ASD	Mtgseq
<i>Oscillospirales</i>	Acutalibacteraceae	<i>Anaeromassilibacillus</i>	ASD	Mtgseq
<i>Lactobacillales</i>	Streptococcaceae	<i>Streptococcus</i>	TD	Mtgseq
<i>Veillonellales</i>	Veillonellaceae	<i>Veillonella</i>	TD	DESEQ2, Mtgseq, ANCOM
<i>Enterobacteriales</i>	Pasteurellaceae	<i>Haemophilus</i>	TD	Mtgseq, ANCOM

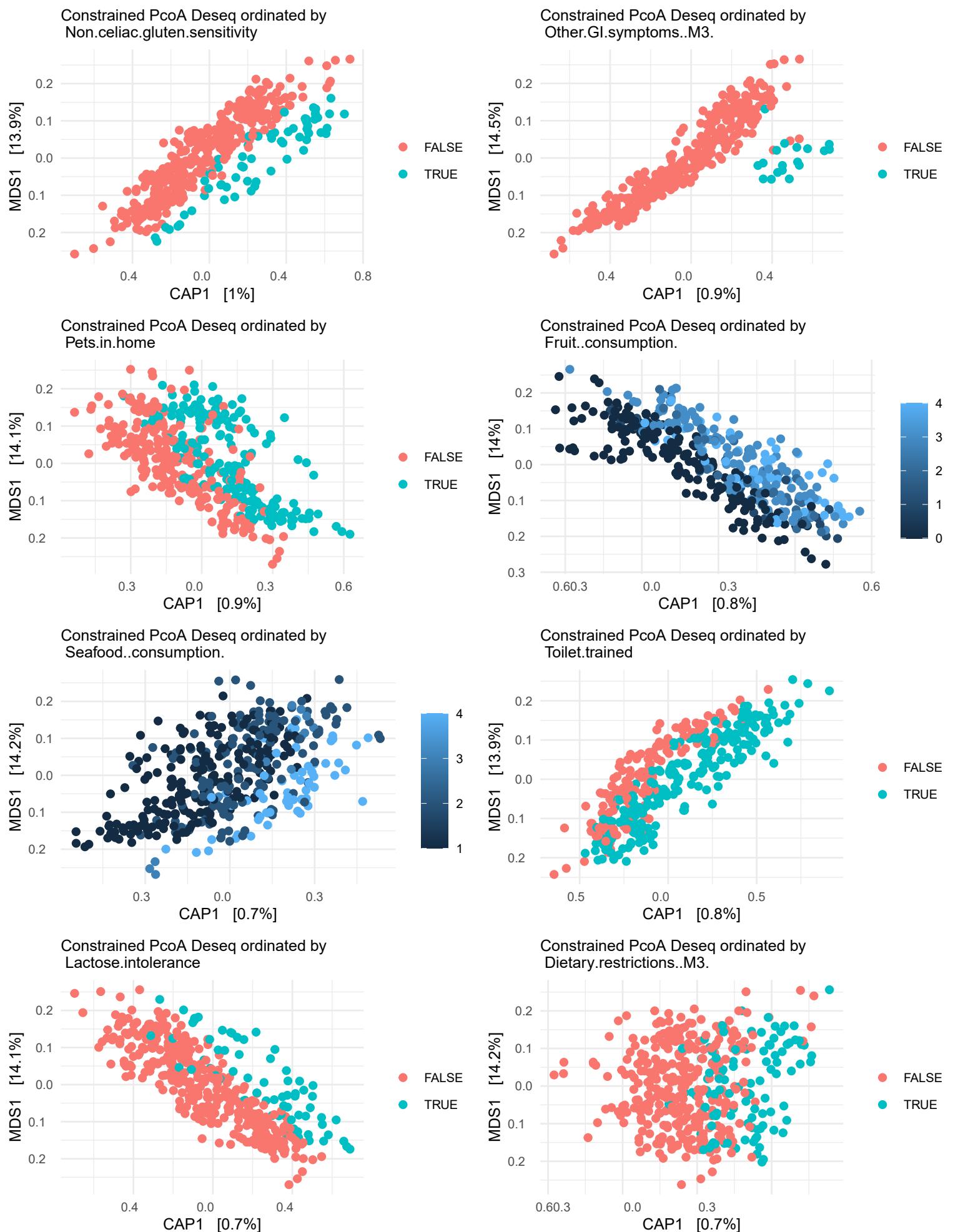
B



Supplementary Figure 4: Differentially abundant genera in ASD over 3 time points. ASV counts were aggregated by genus level annotation, ASVs that could not be assigned an annotation using the StrainSelect database were removed. Supp. 4A shows the taxonomic annotation of the genus, the phenotype the genus was enriched in, and the analysis method that identified it. Supp. 4B shows the relative percent of that genus in either group ASD or TD. MtgSeq refers to MetagenomeSeq.

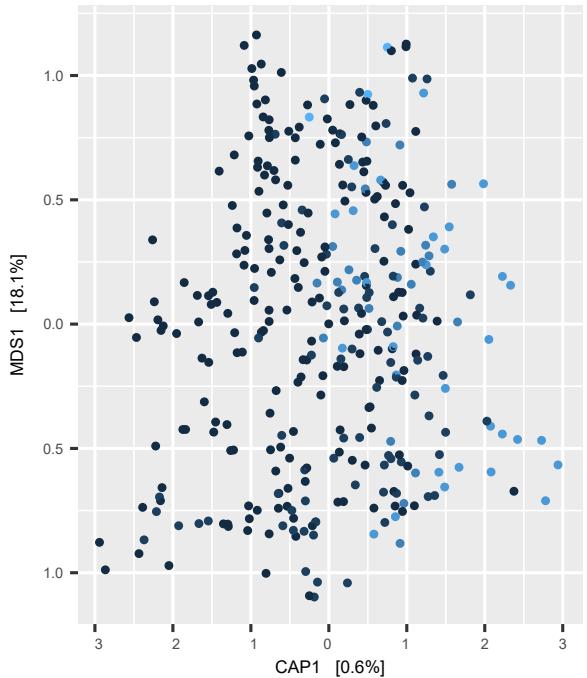
Variable	Factor class	Total samples	R ²	q-value
Vegetable consumption frequency (longitudinal)	numeric	405	0.027	0.002
Fruit consumption frequency*	numeric	432	0.012	0.002
Outdoor pet	categorical	366	0.011	0.002
Non celiac gluten sensitivity	categorical	432	0.011	0.002
Other GI symptoms	categorical	366	0.01	0.002
Sugary food consumption frequency	numeric	432	0.01	0.002
Pets in home	categorical	432	0.009	0.002
Dairy consumption frequency (longitudinal)*	numeric	405	0.009	0.002
Toilet trained	categorical	405	0.008	0.002
Lactose intolerance	categorical	432	0.008	0.002
Dietary restrictions*	categorical	432	0.008	0.002
Seafood consumption frequency	numeric	429	0.007	0.002
Dietary supplement*	categorical	366	0.007	0.003
Multivitamin	categorical	432	0.007	0.002
Fermented vegetable consumption frequency	numeric	432	0.007	0.002
GI symptoms within 3 months*	categorical	432	0.006	0.004
GI issues this week*	categorical	432	0.005	0.003
GI issues two weeks ago	categorical	432	0.004	0.013

Supplementary File 5: Lifestyle and dietary features significantly associated with overall microbiome composition (as determined by a PERMANOVA test) but NOT significantly different between the ASD and TD cohort.

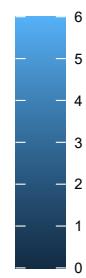
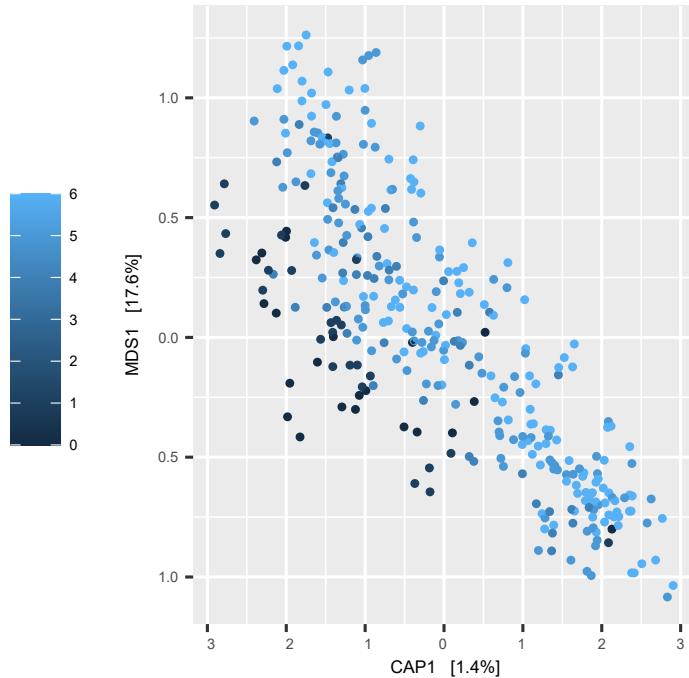


Supplementary File 6: PCoAs using bray curtis distance constrained by phenotype (ASD or TD) along with the variable in each plot title. Colors correspond to the variable in each plot title. Only those variables that were determined to be significantly associated with microbiome composition (PERMANOVA) are depicted.

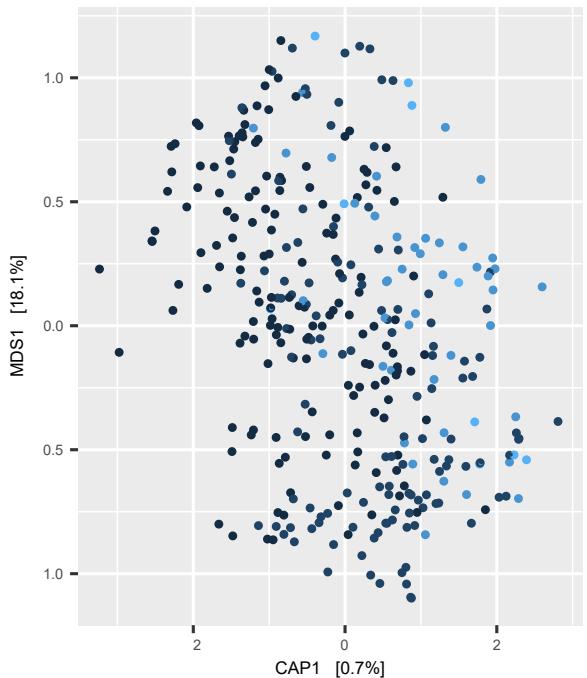
Fermented.vegetable..consumption.frequency.



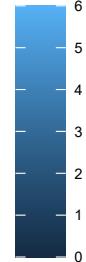
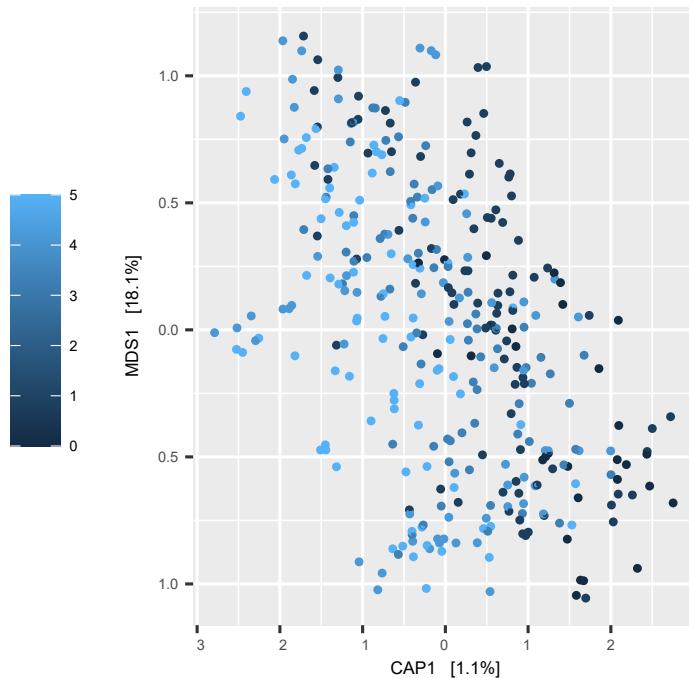
Fruit..consumption.frequency.



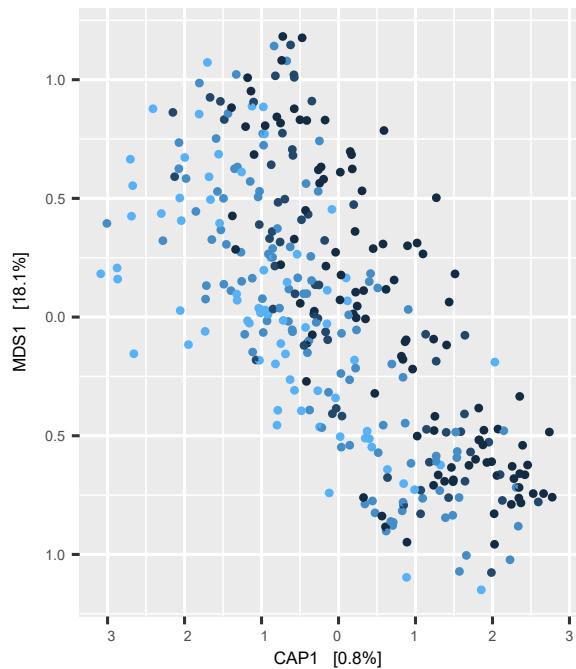
Seafood..consumption.frequency.



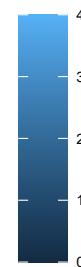
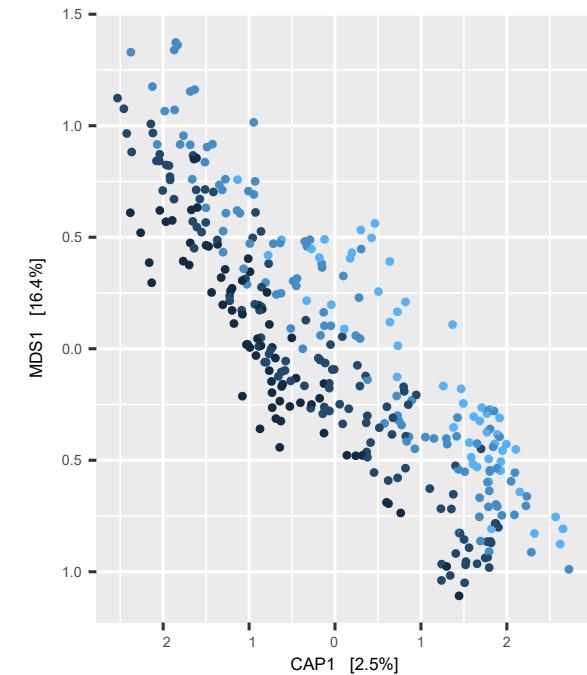
Sugary.food..consumption.frequency.



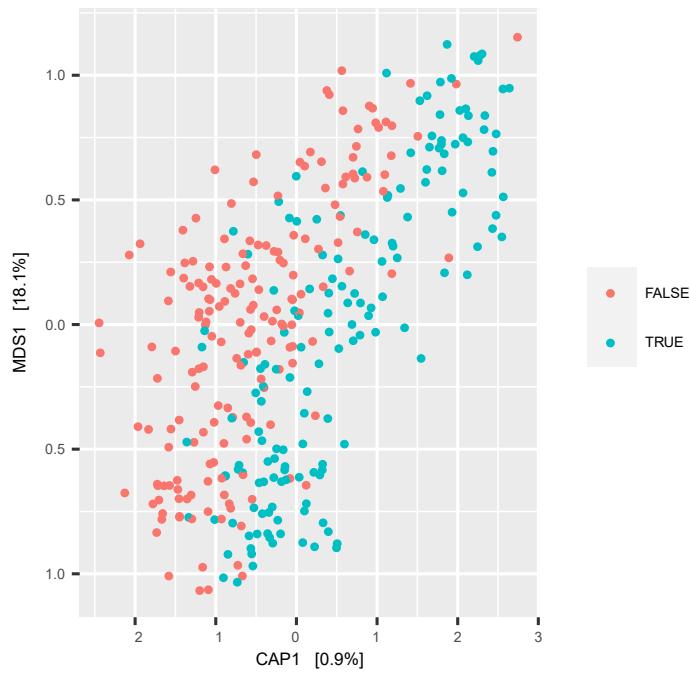
Dairy..consumption.frequency...longitudinal.



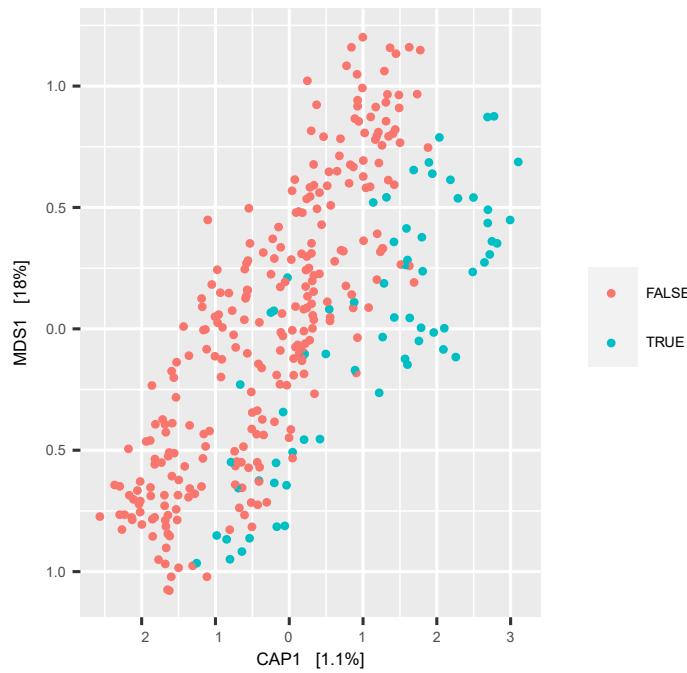
Vegetable..consumption.frequency...longitudinal.



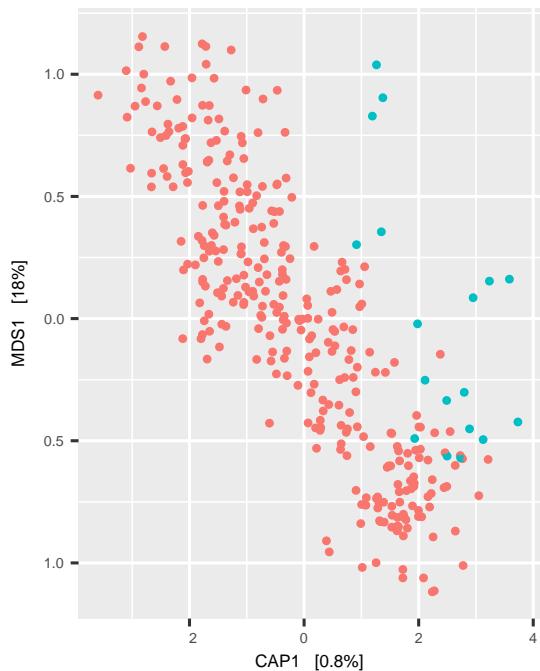
Pets.in.home



Outdoors.pet



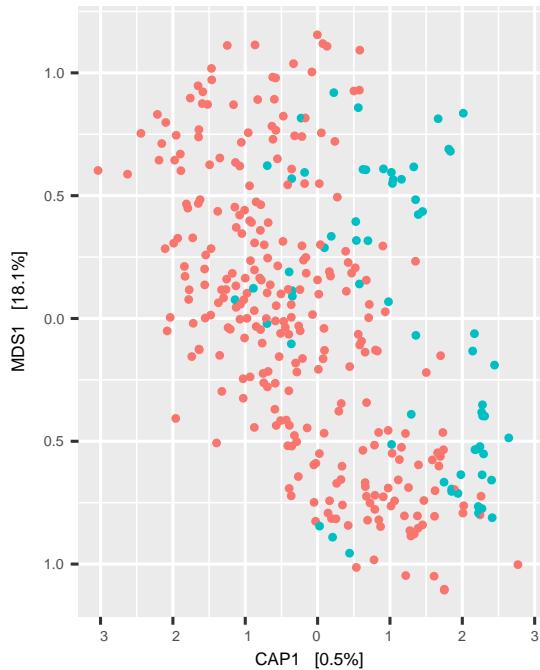
Other.GI.symptoms..M3.



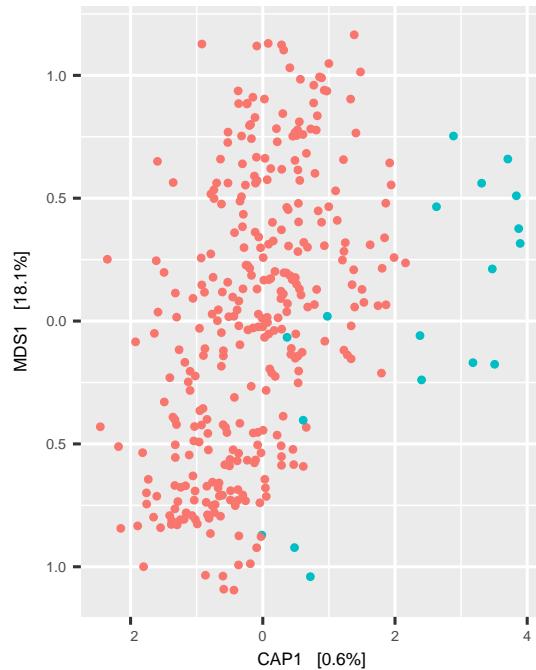
GI.symptoms.within.3.months..M3.

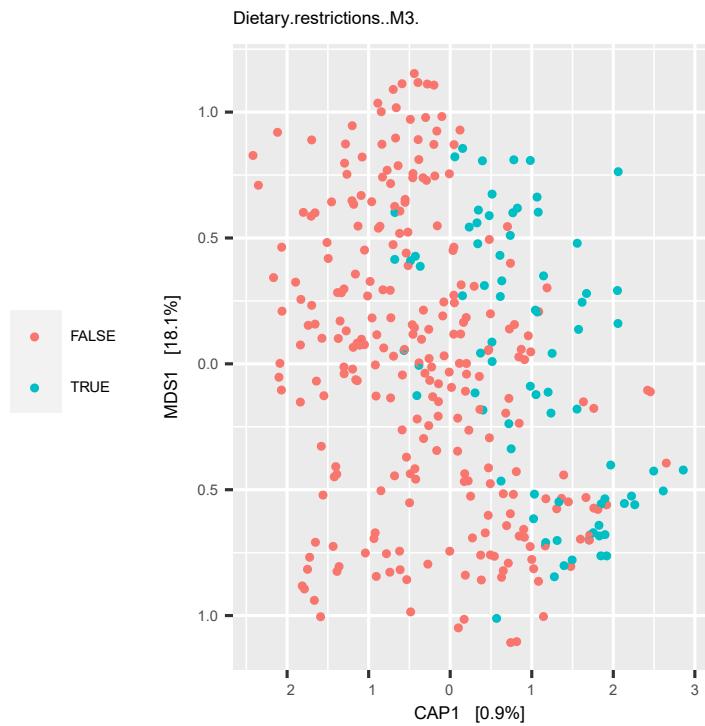
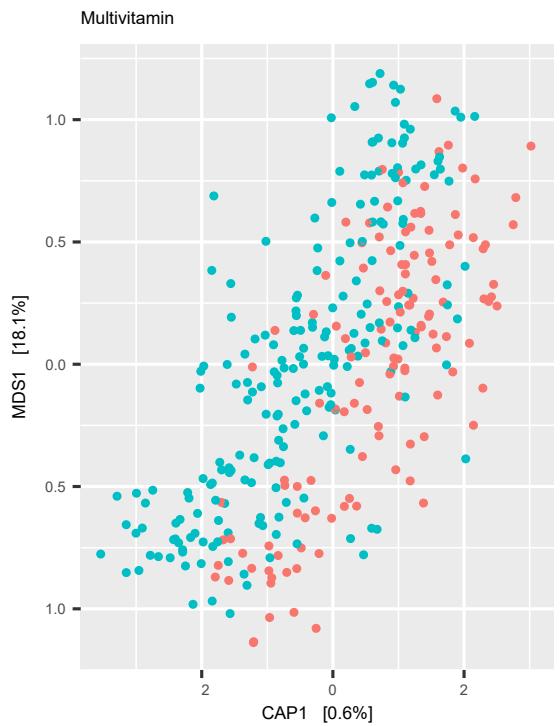
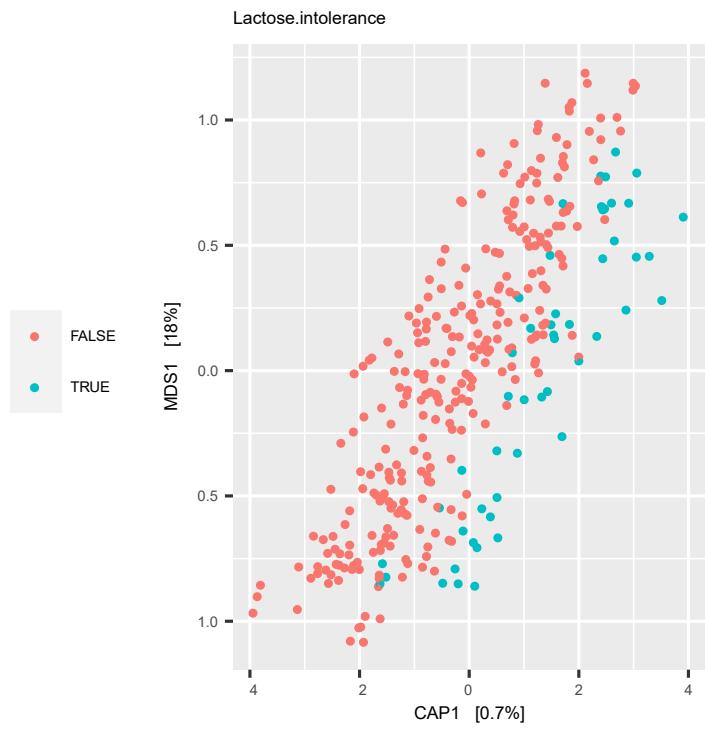
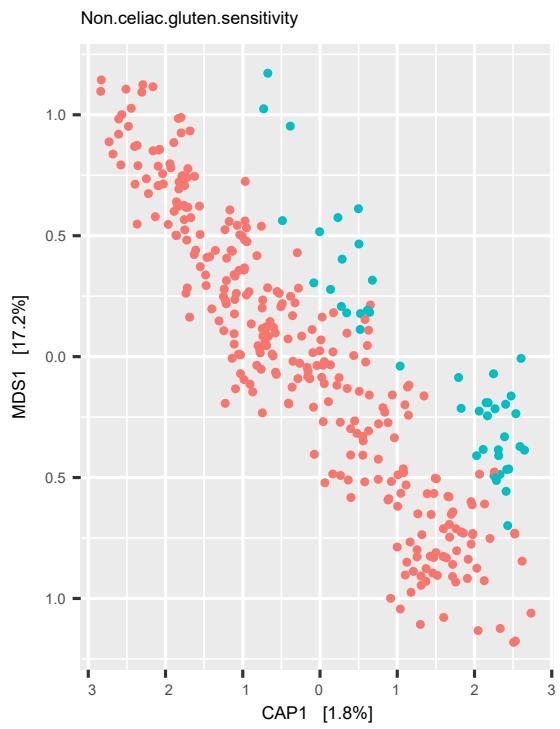


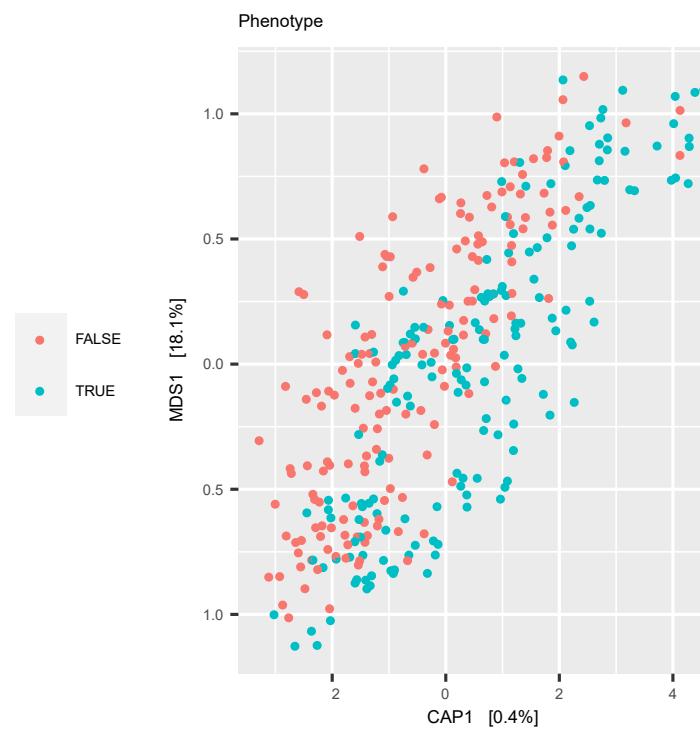
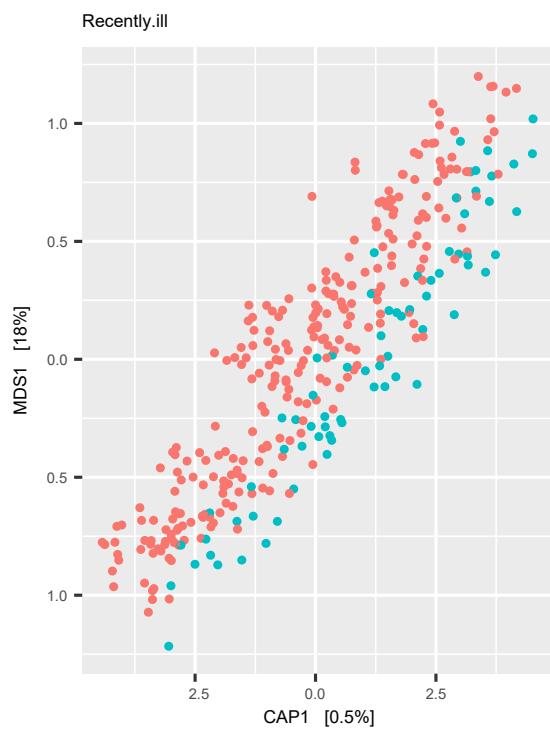
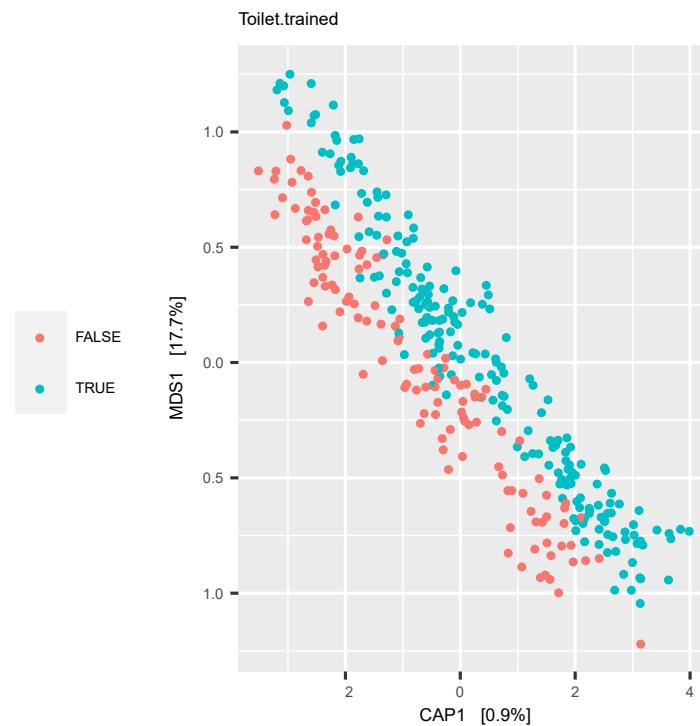
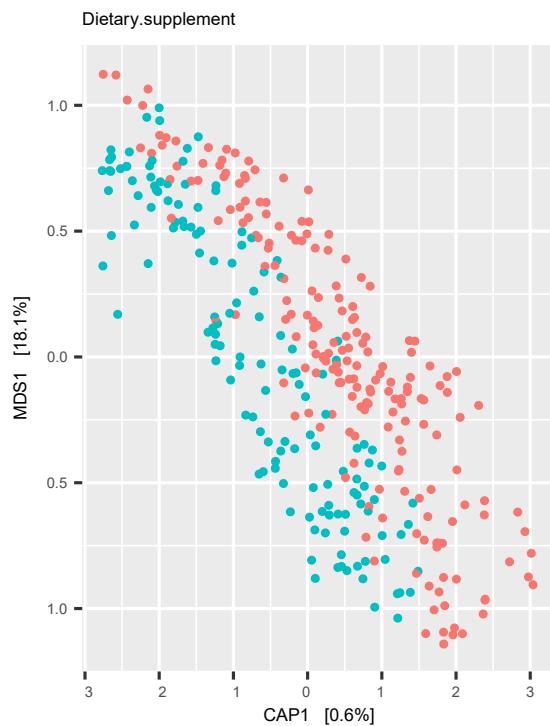
GI.issues.this.week..M3.



GI.issues.two.weeks.ago..M3.

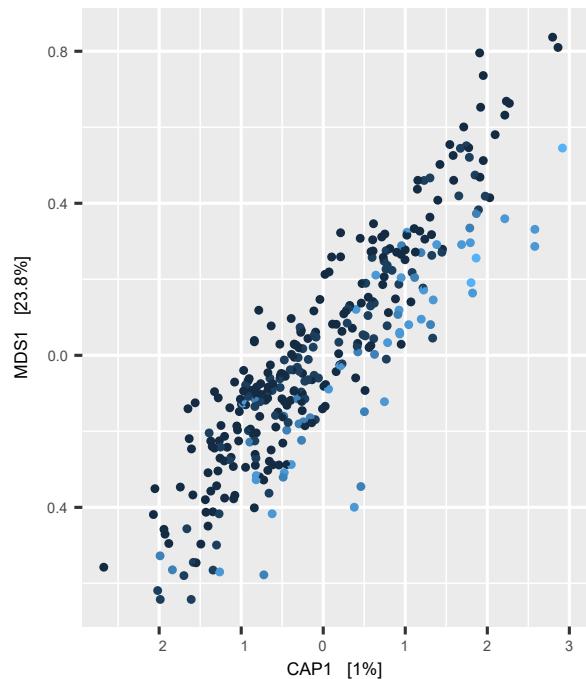




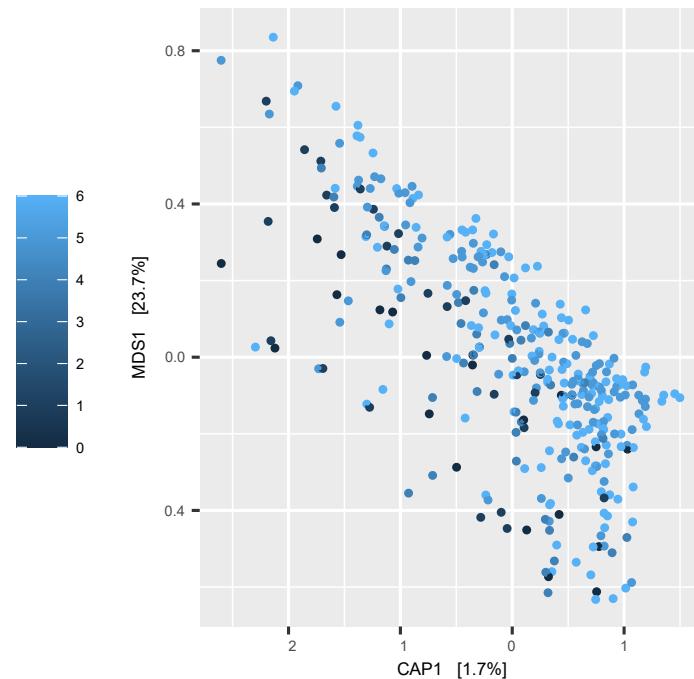


Supplementary File 7: PCoA using weighted UniFrac distances constrained by the variable in each plot title. Colors correspond to the variable in each plot title.

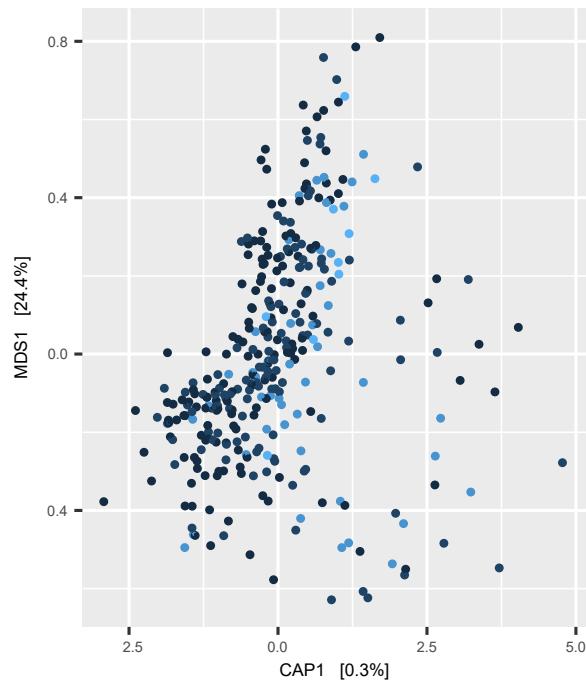
Fermented.vegetable..consumption.frequency.



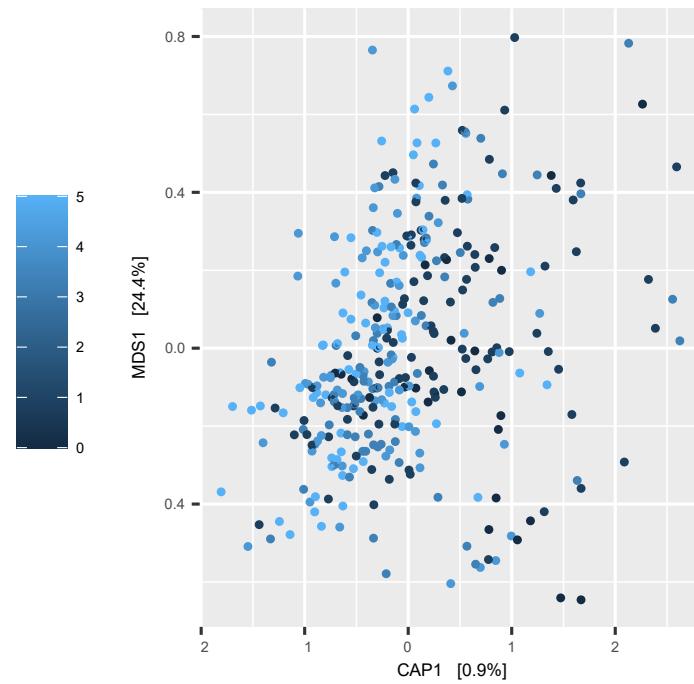
Fruit..consumption.frequency.



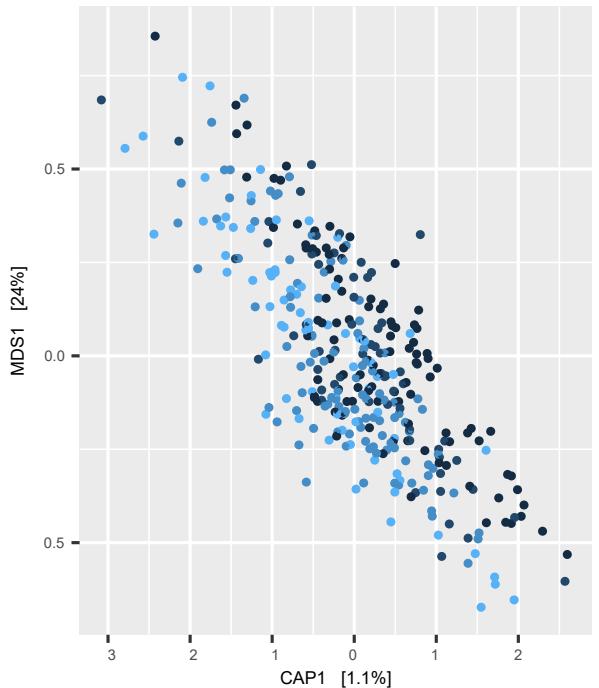
Seafood..consumption.frequency.



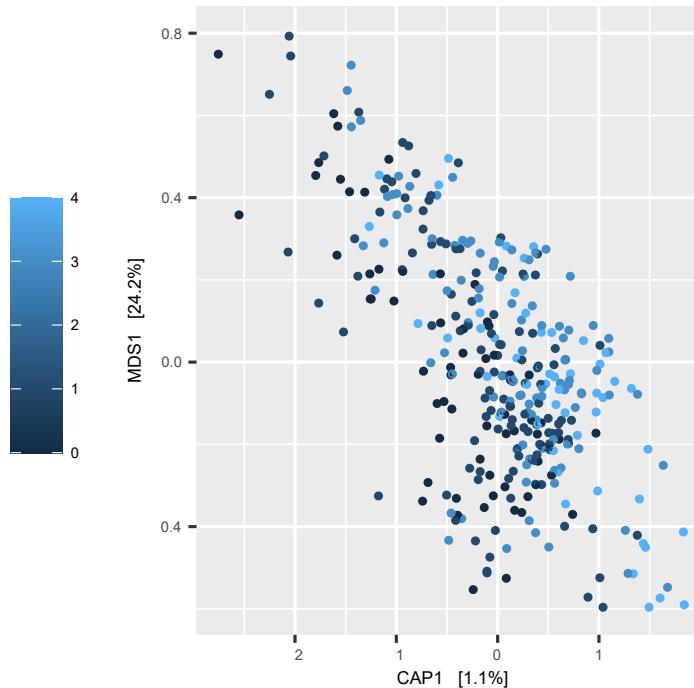
Sugary.food..consumption.frequency.



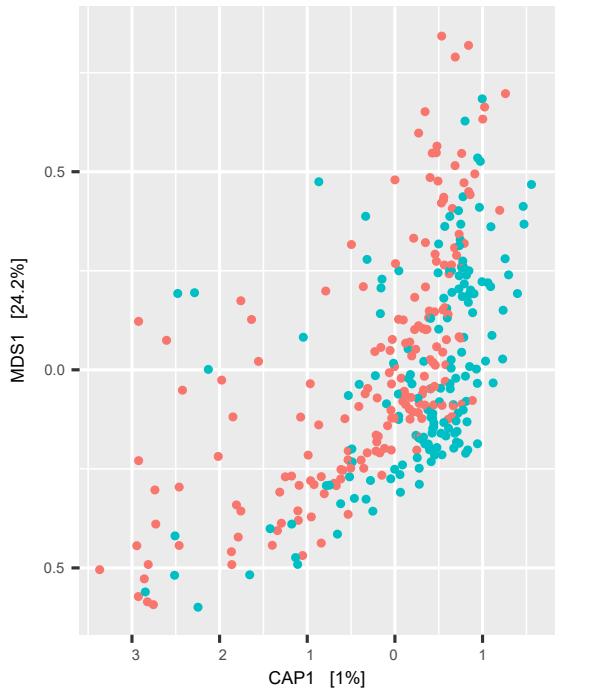
Dairy..consumption.frequency...longitudinal.



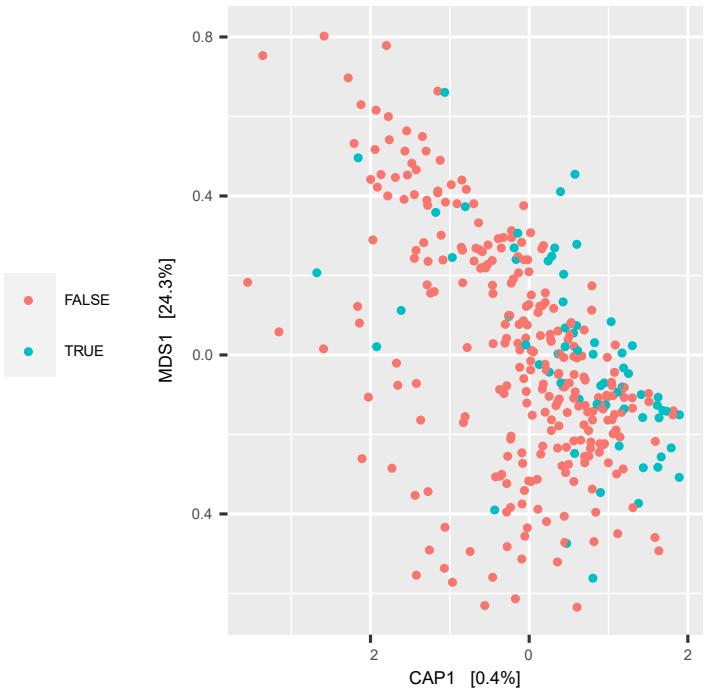
Vegetable..consumption.frequency...longitudinal.

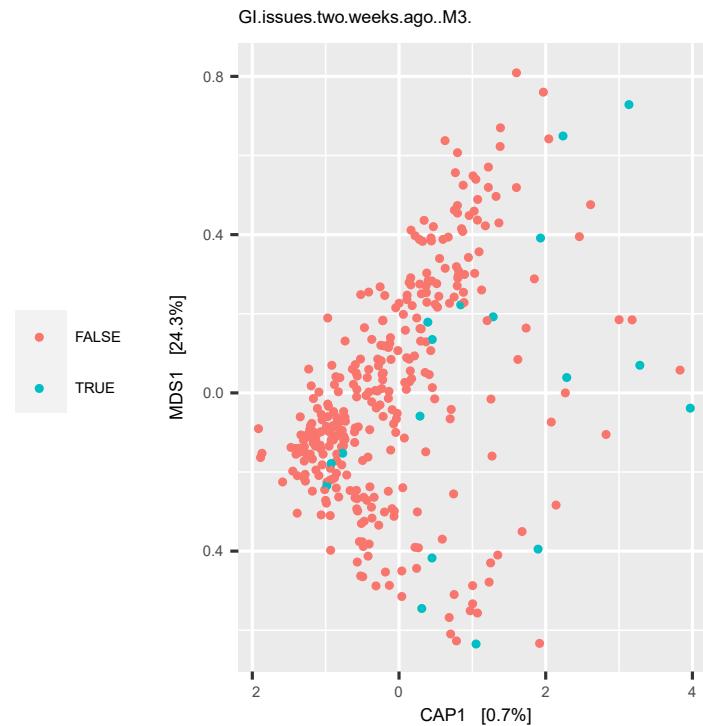
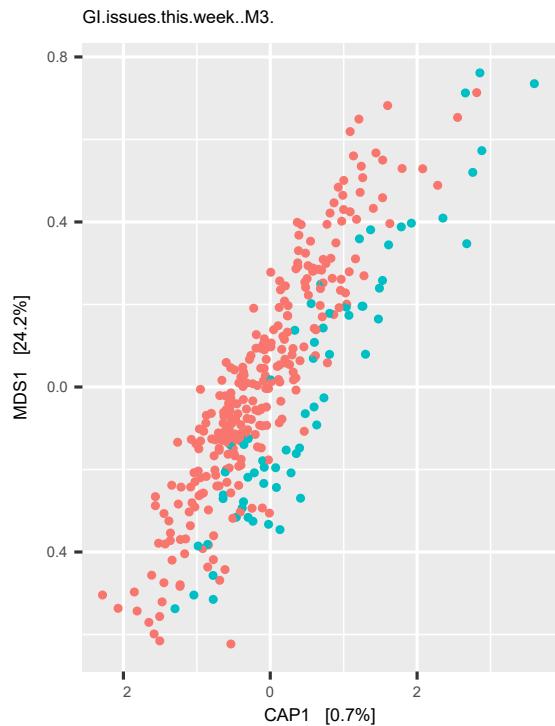
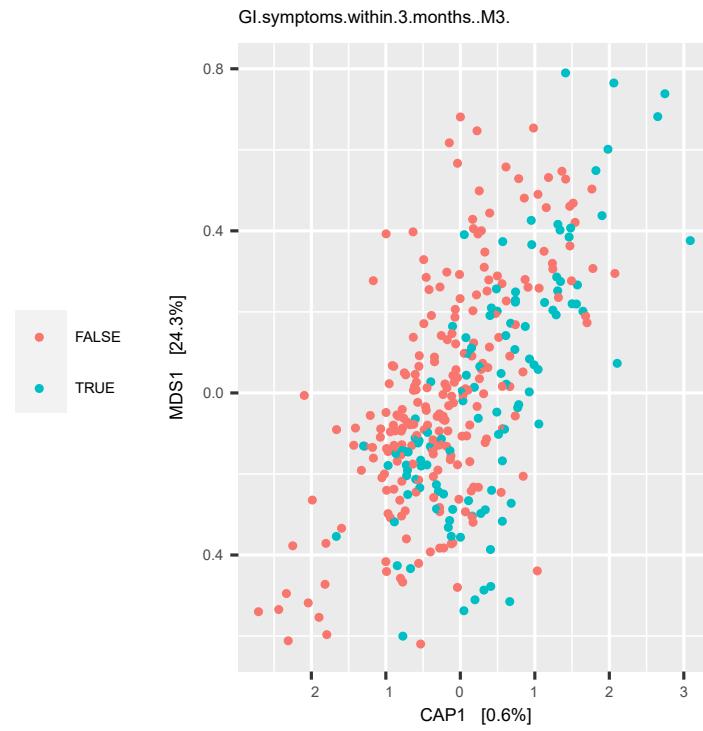
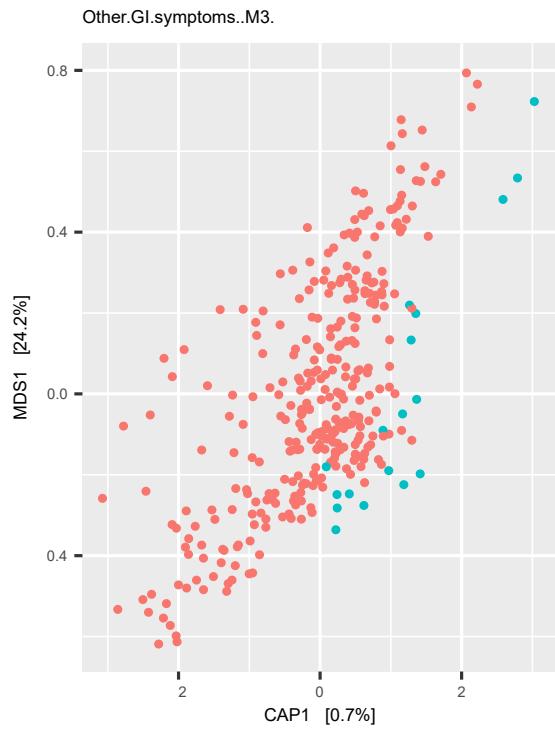


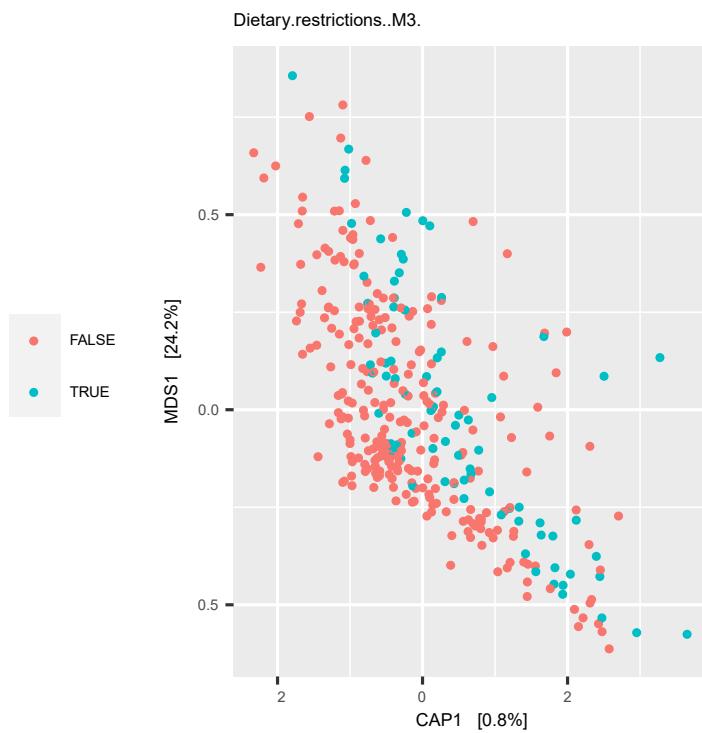
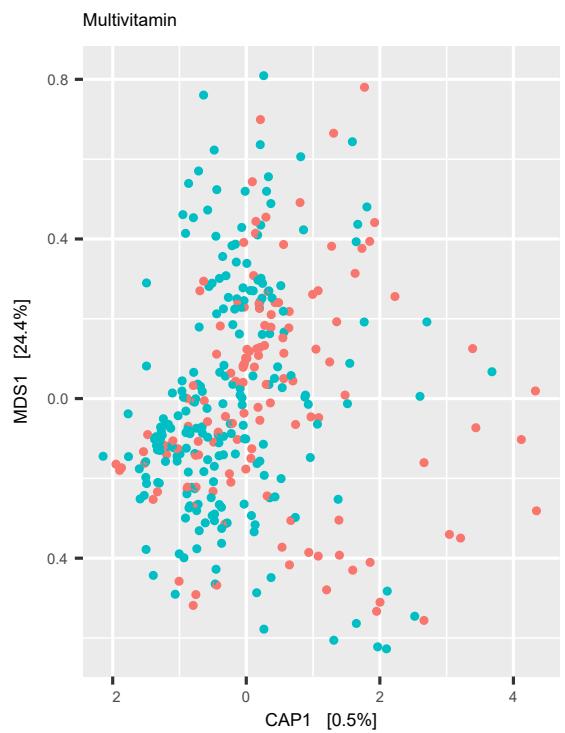
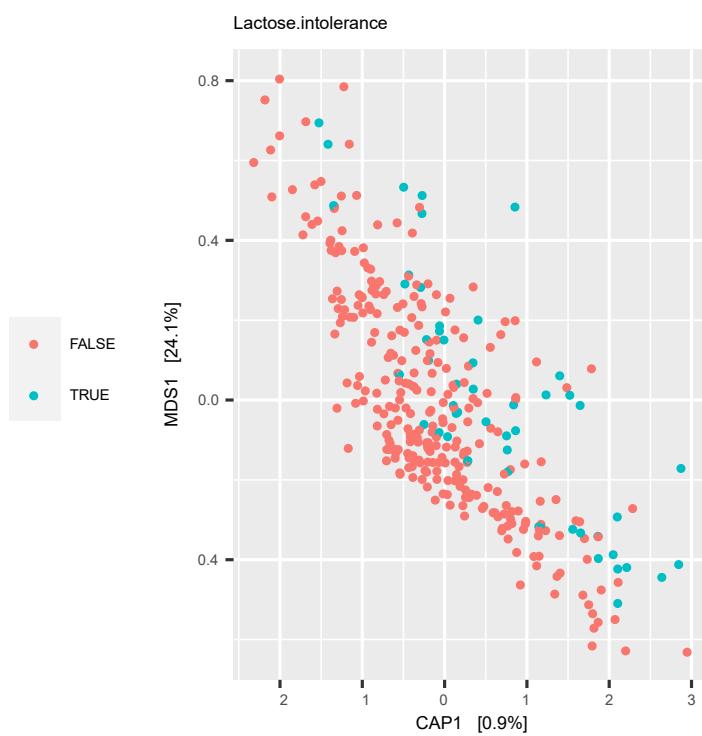
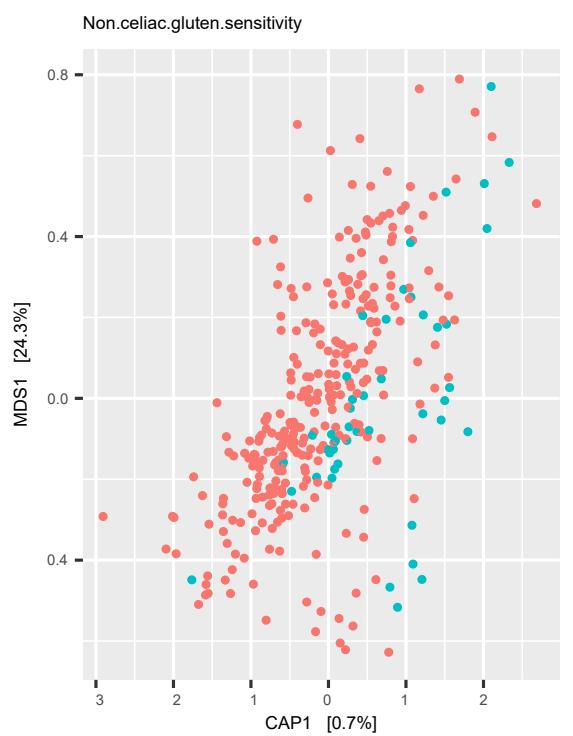
Pets.in.home

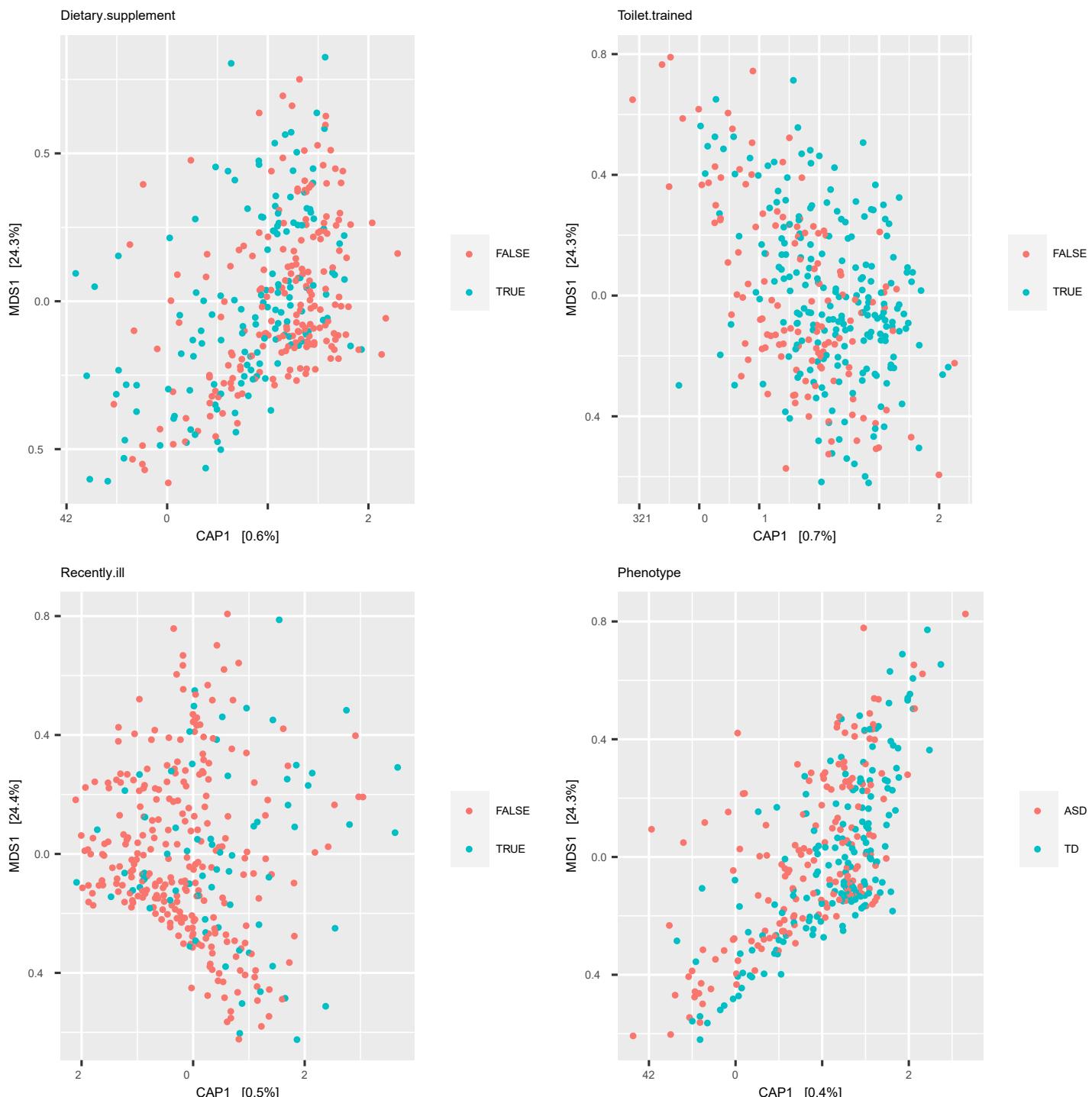


Outdoors.pet

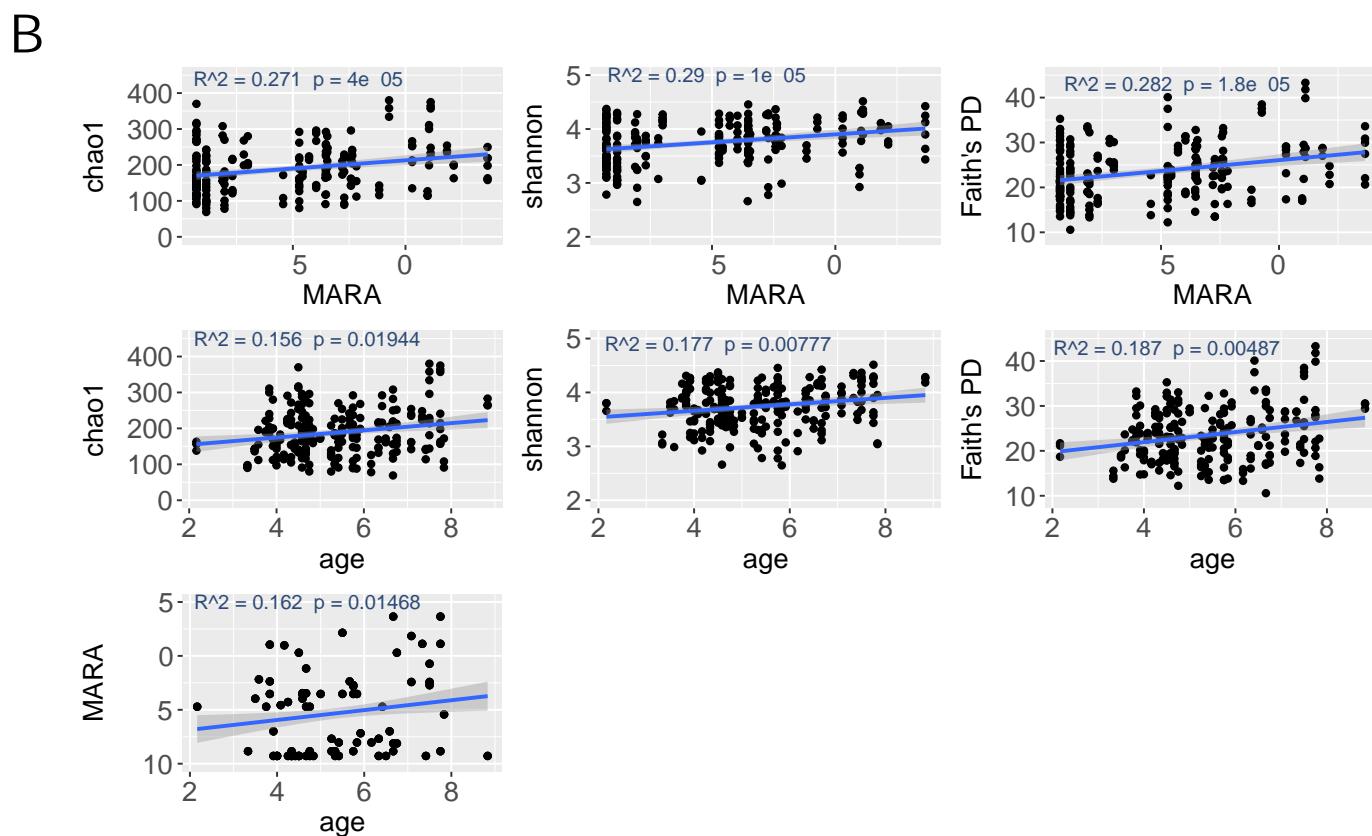
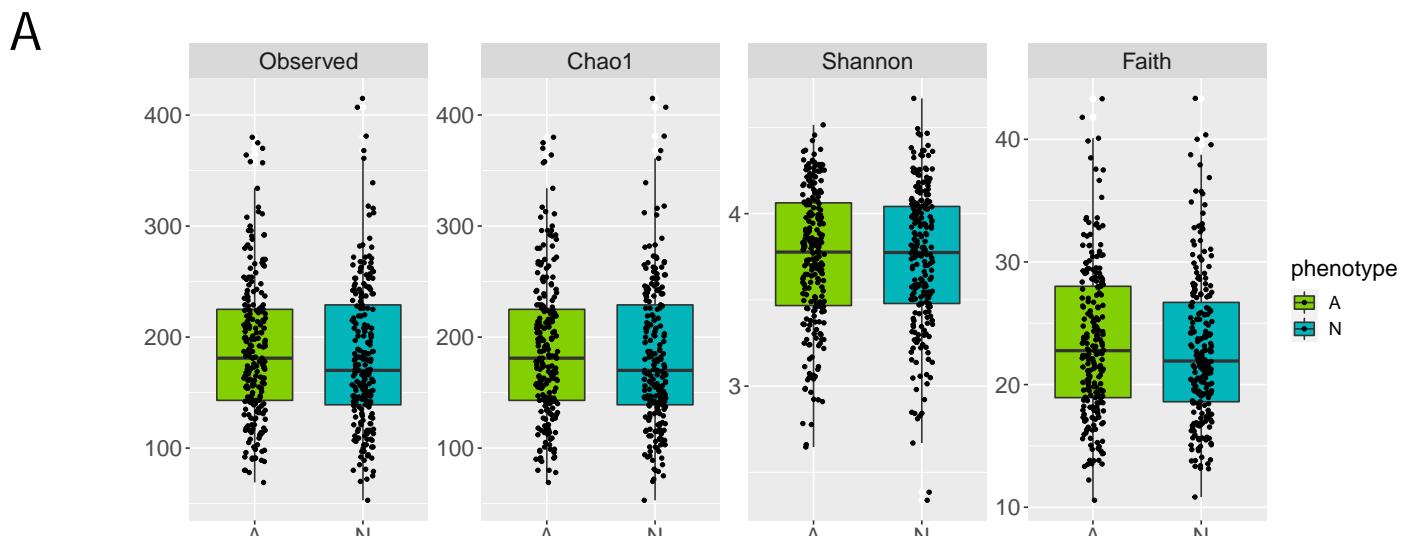






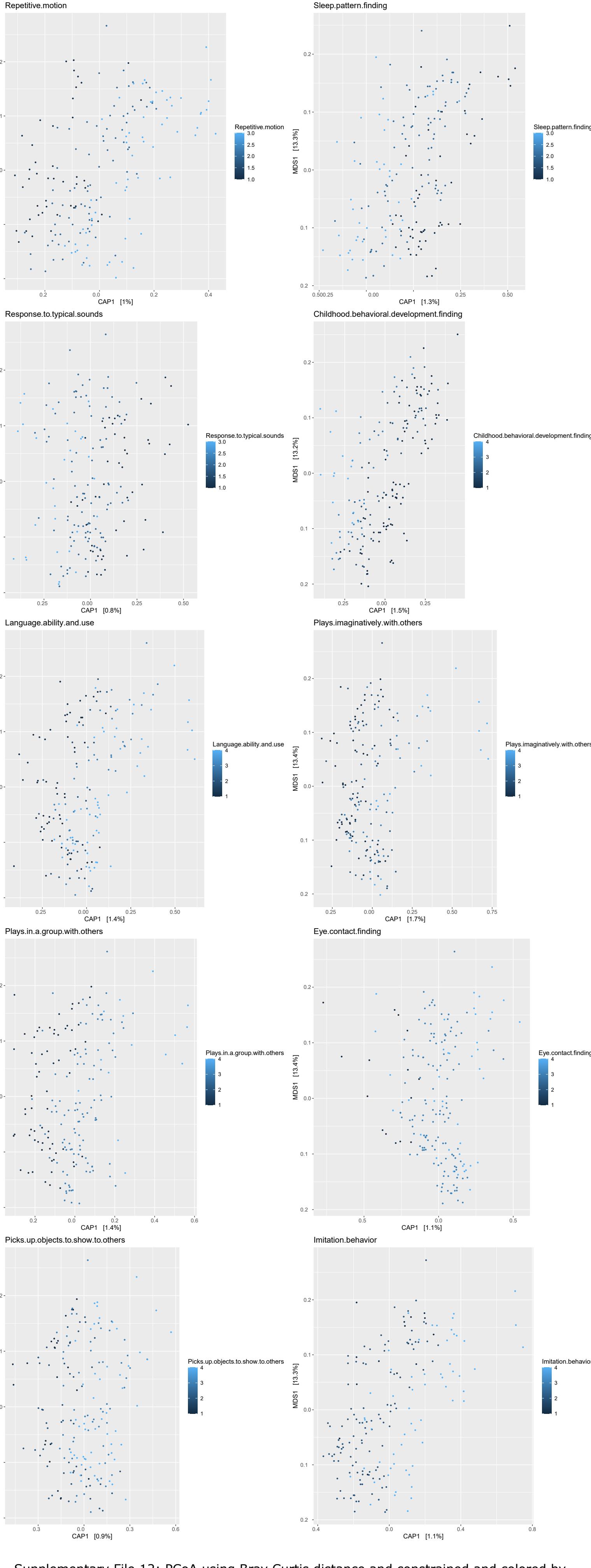


Supplementary File 8: PCoA using unweighted UniFrac distances constrained by the variable in each plot title. Colors correspond to the variable in each plot title

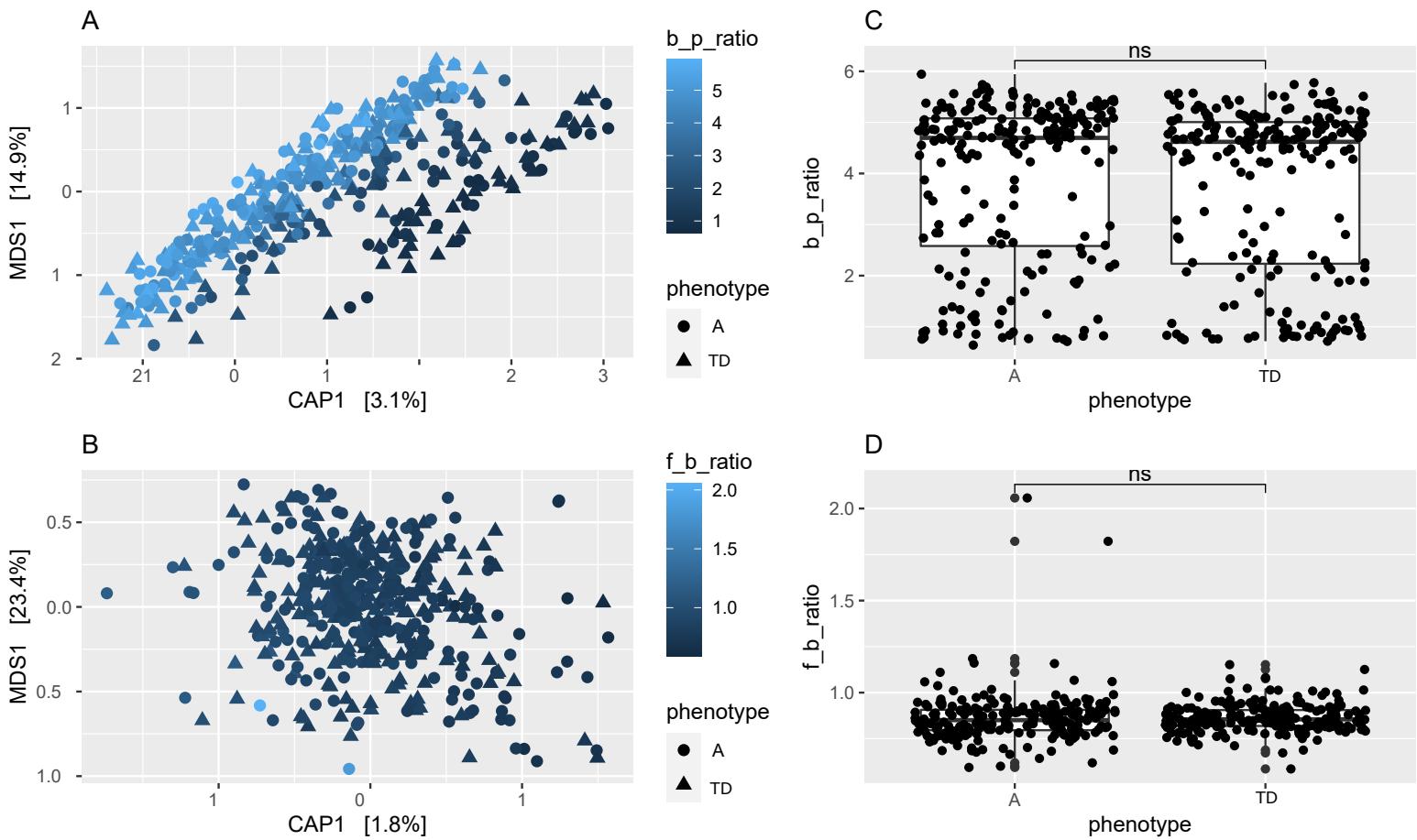


Supplementary File 11:

- A) Diversity metrics Chao1, Shannon, and Faith's Phylogenetic Diversity indices, as compared between ASD and TD. None of the metrics showed statistical significance between groups as determined by a t-test and Mann-Whitney rank sum test.
- B) Spearman correlations show statistical significance between diversity metrics, MARA score (ASD severity), and age (measured in years). Negative MARA score represents more severe ASD behavioral symptoms.

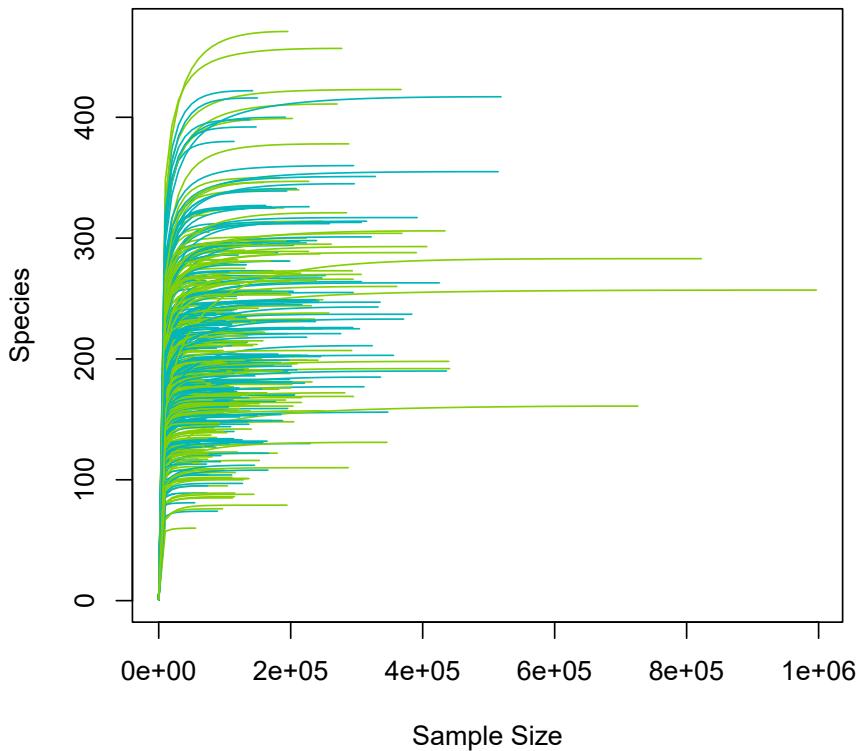


Supplementary File 12: PCoA using Bray Curtis distance and constrained and colored by behavioral traits in the title of each plot. Samples from ASD participants only are used.



Supplementary File 14:

- PCoA plot constrained by the ratio of Bacteroides to Prevotella
- PCoA plot constrained by the ratio of Firmicutes to Bacteroidetes
- Bacteroides to Prevotella ratio is not distinctly different between ASD and TD (rank sum test)
- Firmicutes to Bacteroidetes ratio is not different between ASD and TD (rank sum test)



Supplementary File 15: Rarefaction curve pre-filtering showing the read depth of each sample (sample size) and the corresponding number of detected species.