Distinct Compositional Profiles of Gut Microbiota over Hepatic Steatosis Severities in Non-Diabetic Obese Women

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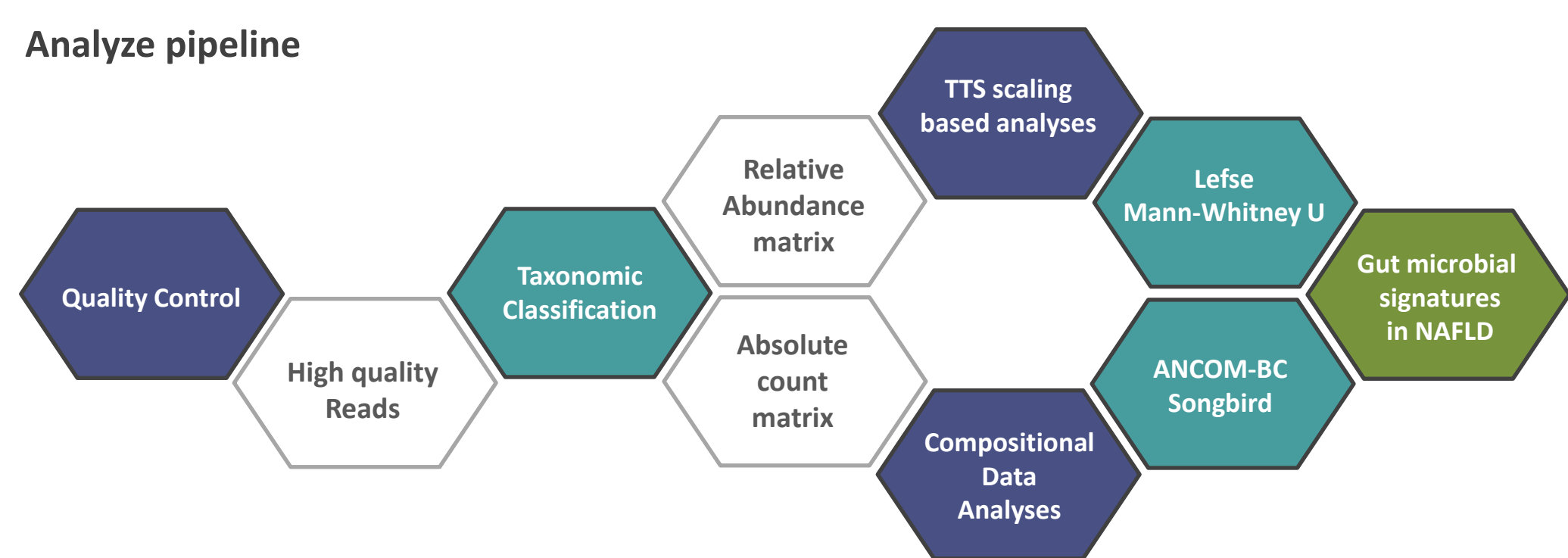
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Summary

Dysfunction of the gut microbiota has been implicated in the development of hepatic steatosis, which is generally a symptom at the early stage of metabolic fatty liver disease (MAFLD), known as non-alcoholic fatty liver disease (NAFLD). Elucidating gut microbial signatures associated with MAFLD has been urged to treat dysbiosis and to alleviate steatosis severities. Here, we analyzed 57 whole metagenomics sequencing (WGS) samples of non-diabetic obese women with biopsy-proven hepatic steatosis in Spain and Italy. We separated those samples into 'absent and slight' and 'moderate or severe' group, less than 33% and more than 33% steatosis, respectively. We found that microbial diversities were significantly reduced in the group with more steatosis. In addition, the beta diversity between two groups was significantly discriminant at the species taxonomy rank. We selected 113, 137 and 641 significantly differentially abundant taxa across all taxonomic ranks between the two groups, through Wilcoxon rank-based method, Lefse, and ANCOM-BC, respectively. Finally, 6 microbial taxa that were discovered across all the methods were then filtered, which consist of order – Christensenellales, Monoglobales_A, Opitutales, Victivallales, Family – QALW01, and Genus – UMGS1338. We statistically confirmed that the abundance of those 6 taxa were significantly enriched in the group showing more severe steatosis. Our study implicates there are significant associations between hepatic steatosis and gut microbial compositions, which might be utilized to further clinical applications. Nevertheless, out study might be suffered from the confounders such as country, BMI, and age, which makes the test results unreliable. Thus, the future study need to be conduction in careful consideration of any confounders that researchers can handle to elicit more reliable and applicable conclusions.
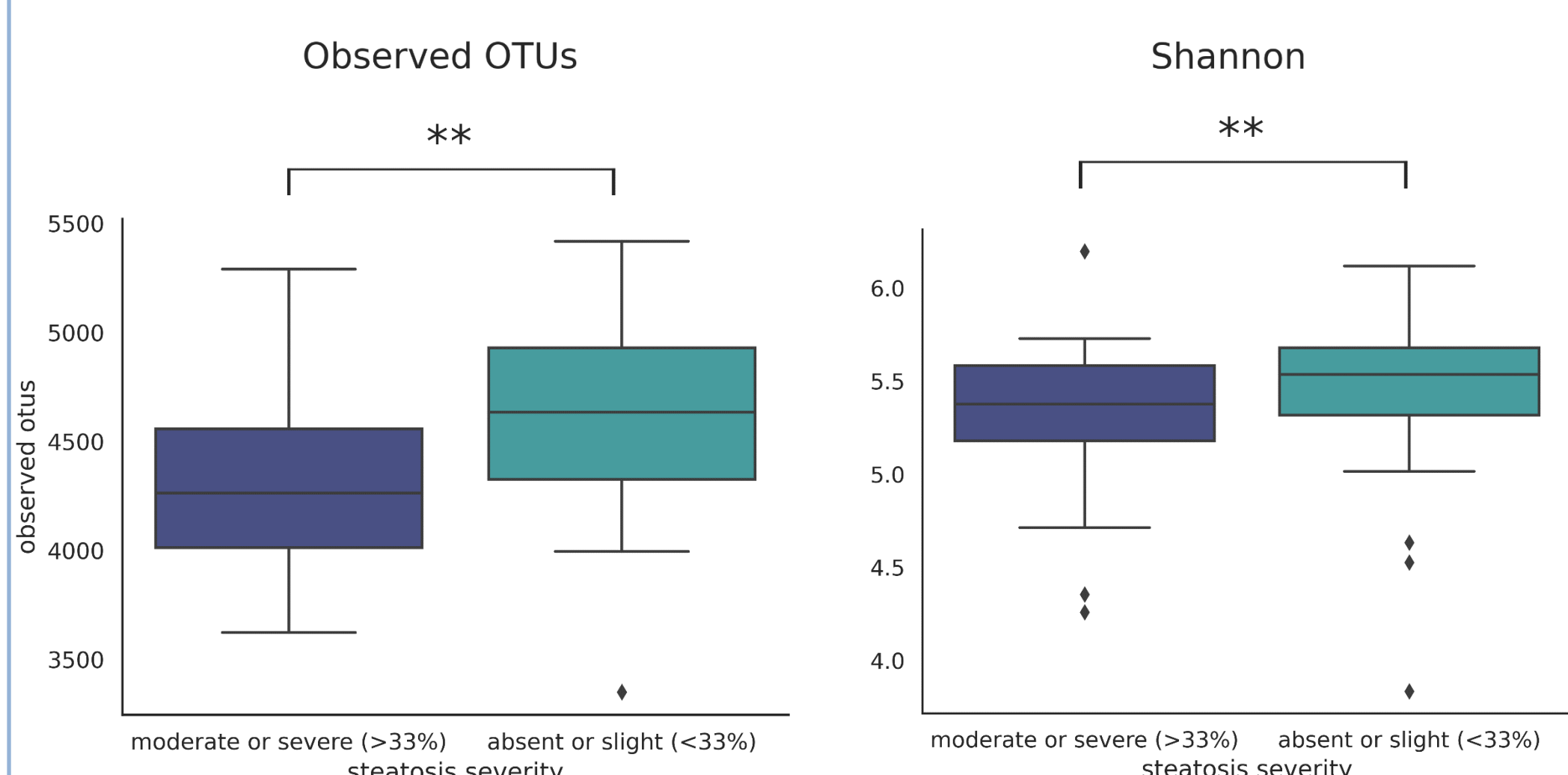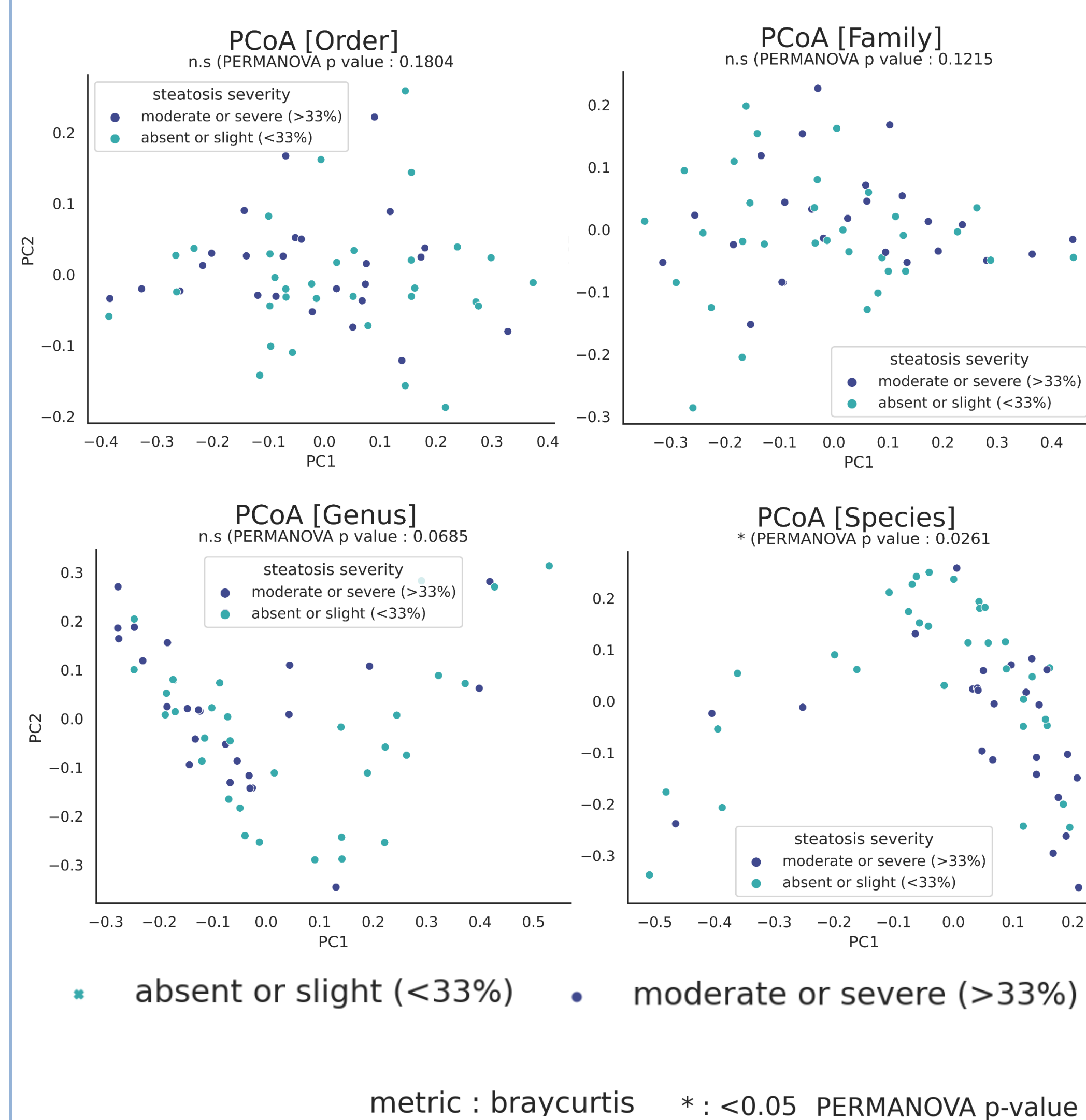
Methods

[Pipeline] Analyze pipeline: Quality Control → High quality Reads → Taxonomic Classification → Relative Abundance matrix / Absolute count matrix → TTS scaling based analyses → Compositional Data Analyses → Lefse Mann-Whitney U / ANCOM-BC Songbird → Gut microbial signatures in NAFLD

Results

Diversity

Alpha diversity

Observed OTUs — Shannon — ** < 0.01 Mann-Whitney U test

Beta diversity

PCoA [Order] n.s PERMANOVA p value : 0.1804
PCoA [Family] n.s PERMANOVA p value : 0.1215
PCoA [Genus] n.s PERMANOVA p value : 0.0685
PCoA [Species] * PERMANOVA p value : 0.0261

absent or slight (<33%)　moderate or severe (>33%)
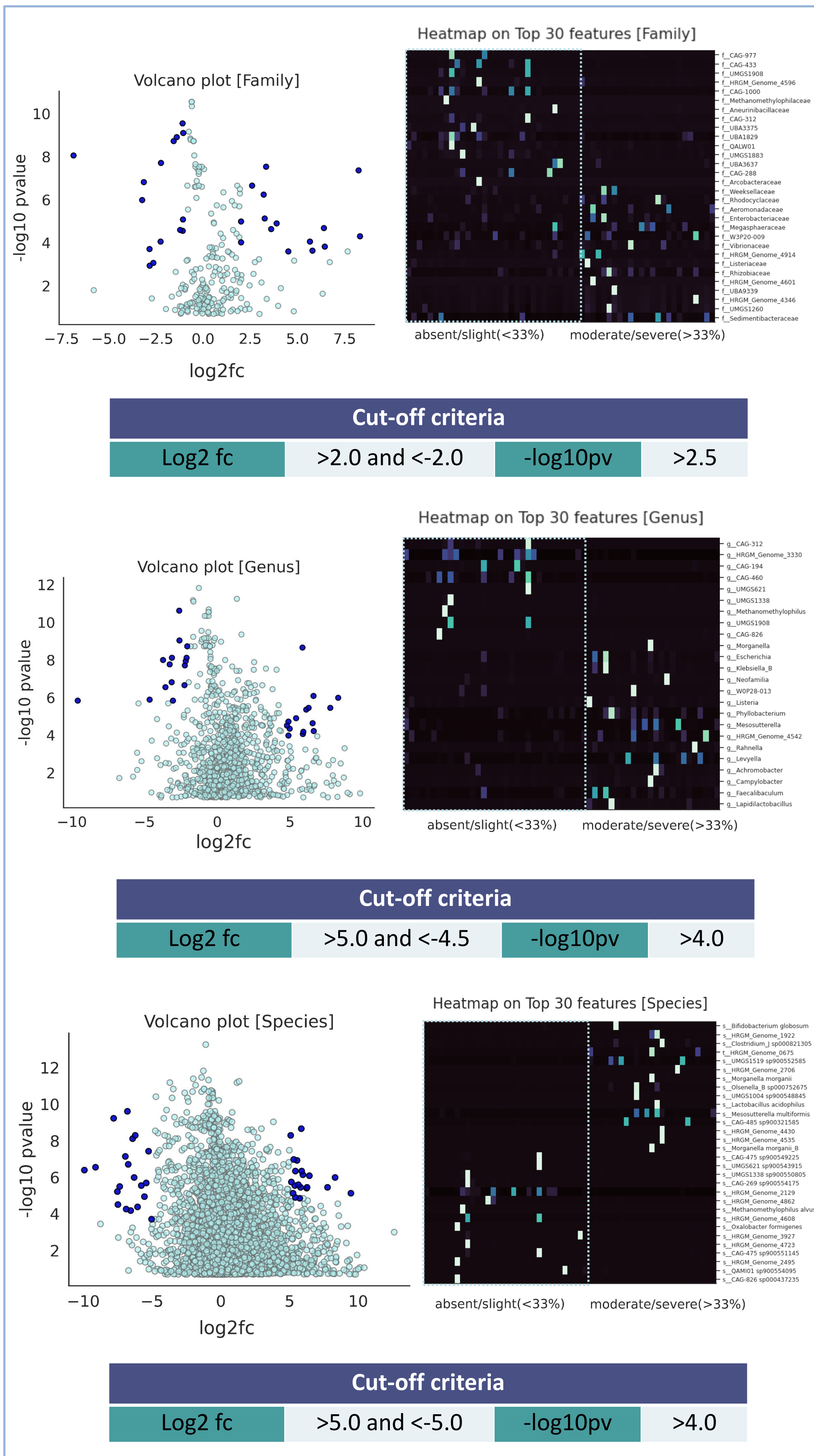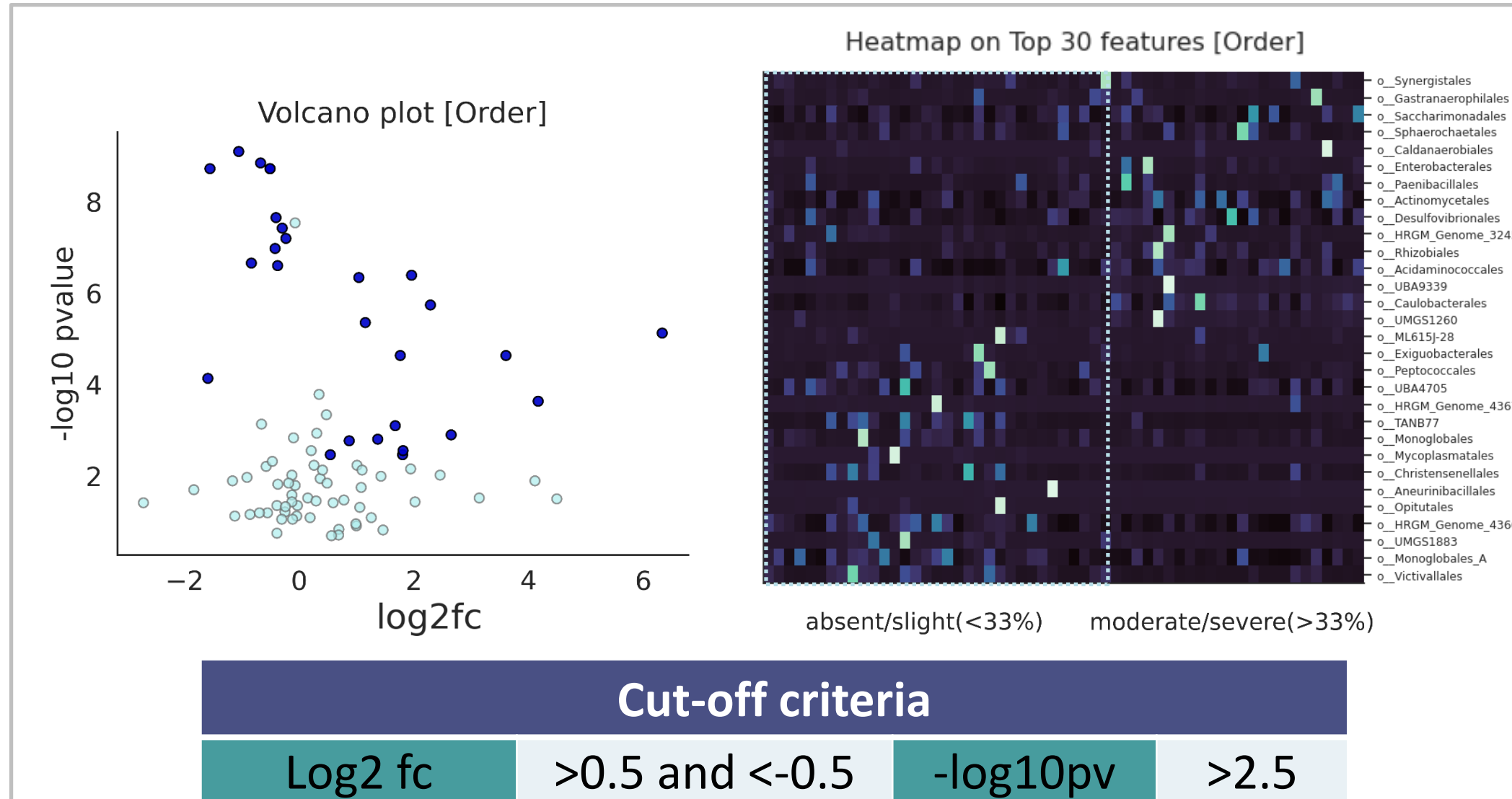
metric : braycurtis　* : <0.05 PERMANOVA p-value

Differential abundance analyses

Volcano plots and Heat map on Top 30 highest p-value

Volcano plot [Order] — Heatmap on Top 30 features [Order]

Cut-off criteria			
Log2 fc	>0.5 and <-0.5	-log10pv	>2.5

Volcano plot [Family] — Heatmap on Top 30 features [Family]

Cut-off criteria			
Log2 fc	>2.0 and <-2.0	-log10pv	>2.5

Volcano plot [Genus] — Heatmap on Top 30 features [Genus]

Cut-off criteria			
Log2 fc	>5.0 and <-4.5	-log10pv	>4.0

Volcano plot [Species] — Heatmap on Top 30 features [Species]

Cut-off criteria			
Log2 fc	>5.0 and <-5.0	-log10pv	>4.0

Lefse (Linear discriminant analysis Effect Size)

LDA score plot — moderate/severe(>33%) / absent/slight(<33%)

Lefse cladogram
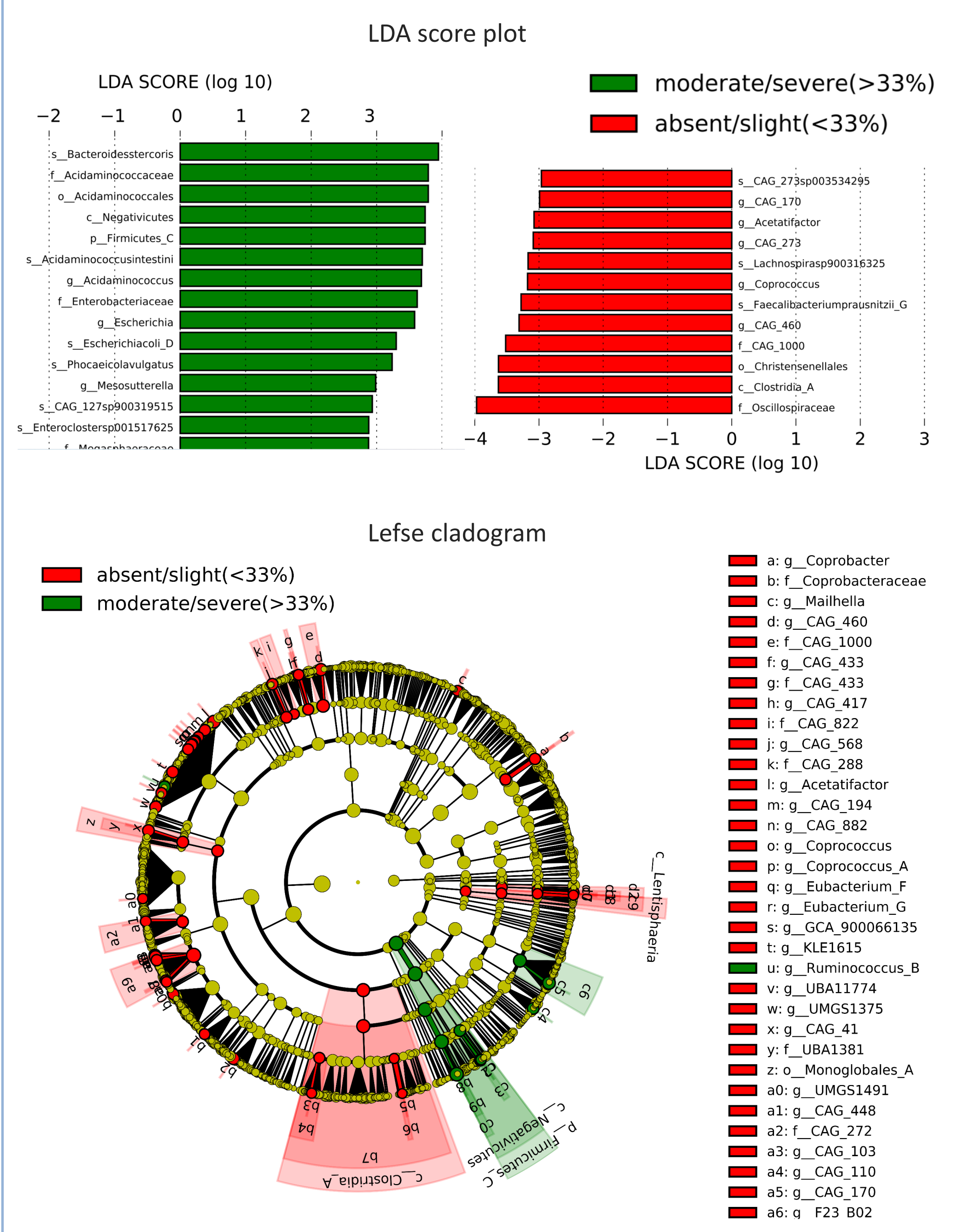
Analysis of compositions of microbiomes with bias correction [ANCOM-BC]

ANCOM-BC Log2 fold change at each rank — Differentially expressed False/True

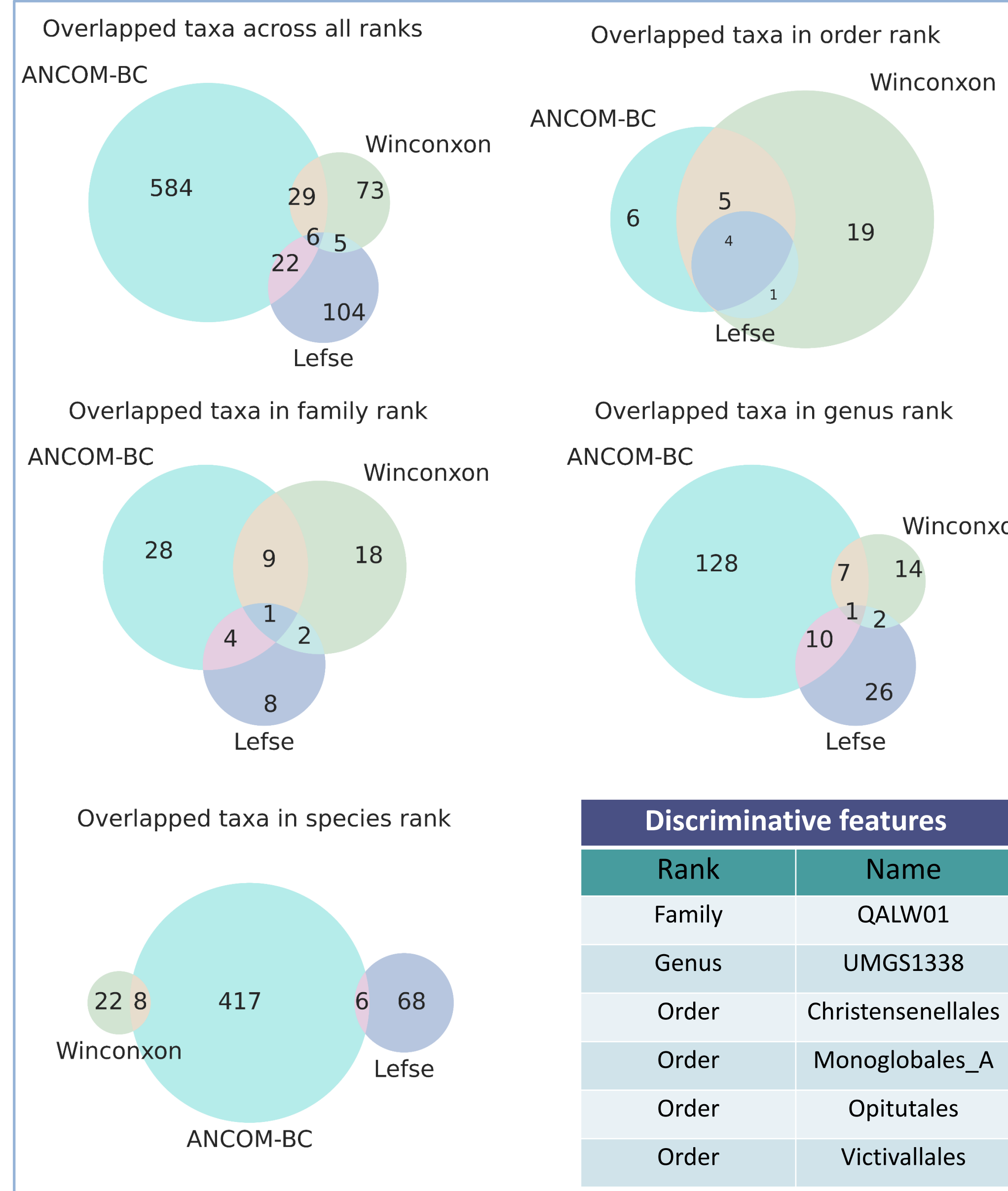ANCOM-BC Number of the differentially expressed — Differentially expressed False/True
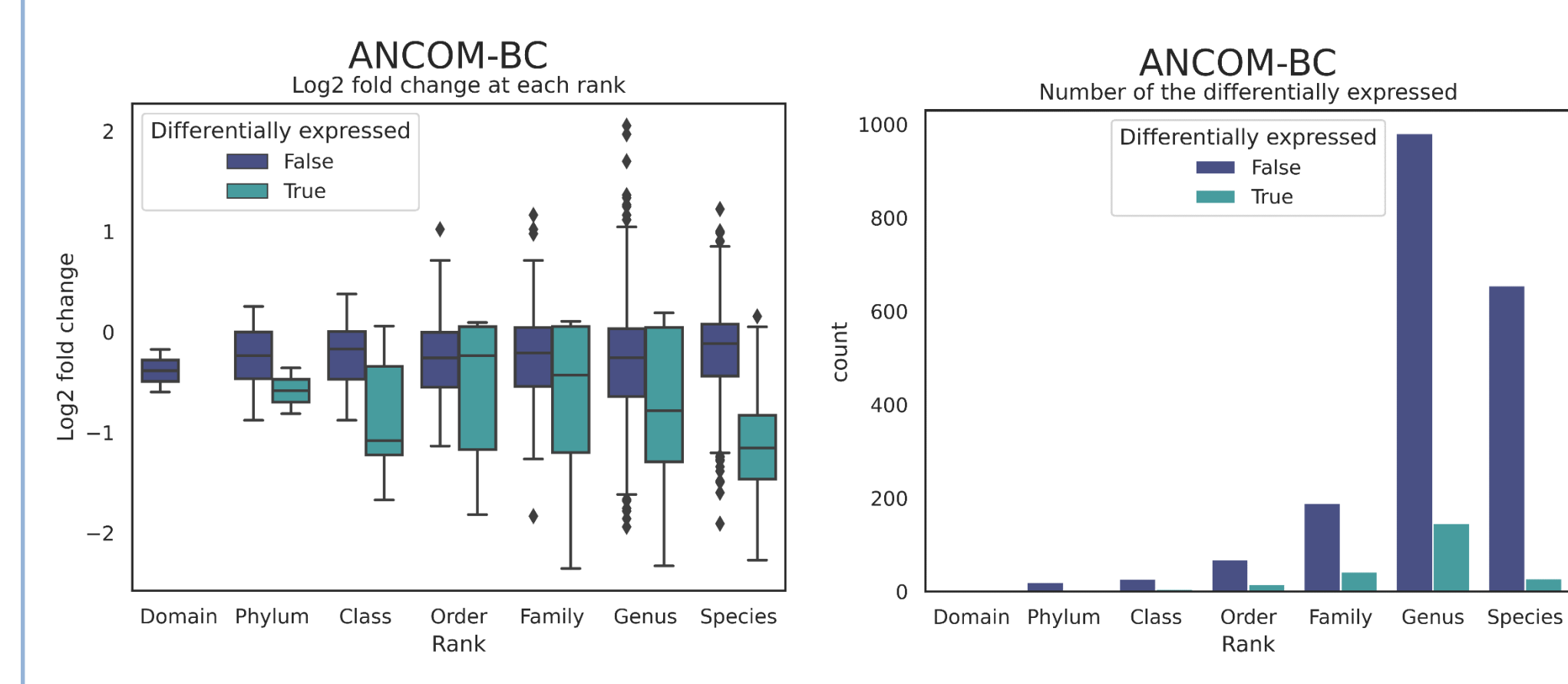
Overlapped significantly discriminative features

Overlapped taxa across all ranks — ANCOM-BC 584, Winconxon 73, 29, 5, 22, 104, Lefse

Overlapped taxa in order rank — ANCOM-BC 6, Winconxon 19, 5, 4, 1, Lefse

Overlapped taxa in family rank — ANCOM-BC 28, Winconxon 18, 9, 1, 4, 2, 8, Lefse

Overlapped taxa in genus rank — ANCOM-BC 128, Winconxon 14, 7, 1, 2, 10, 26, Lefse

Overlapped taxa in species rank — Winconxon 22, 8, ANCOM-BC 417, Lefse 6, 68

Discriminative features	
Rank	Name
Family	QALW01
Genus	UMGS1338
Order	Christensenellales
Order	Monoglobales_A
Order	Opitutales
Order	Victivallales

Conclusion

6 most discriminative features — steatosis severity: moderate/severe (>33%) / absent/slight (<33%)

f_QALW01 *** g_UMGS1338 *** o_Christensenellales ** o_Monoglobales_A *** o_Opitutales ** o_Victivallales ***

** < 0.01 Mann-Whitney U test
***: <0.001 Mann-Whitney U test

References

1. Hoyles, L., Fernandez-Real, J. M., Federici, M., Serino, M., Abbott, J., Charpentier, J., ... & Dumas, M. E. (2018). Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. Nature medicine, 24(7), 1070-1080.

2. Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. Nature communications, 11(1), 1-11.

3. Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. Genome biology, 12(6), 1-18.