Supplementary Figures: Predicting the impact of dietary fibers on distinct microbial communities: a multi-omics study on lean and obese pooled faecal samples

Dell’Olio et.al (2024)

1. SUPPLEMENTARY FIGURES

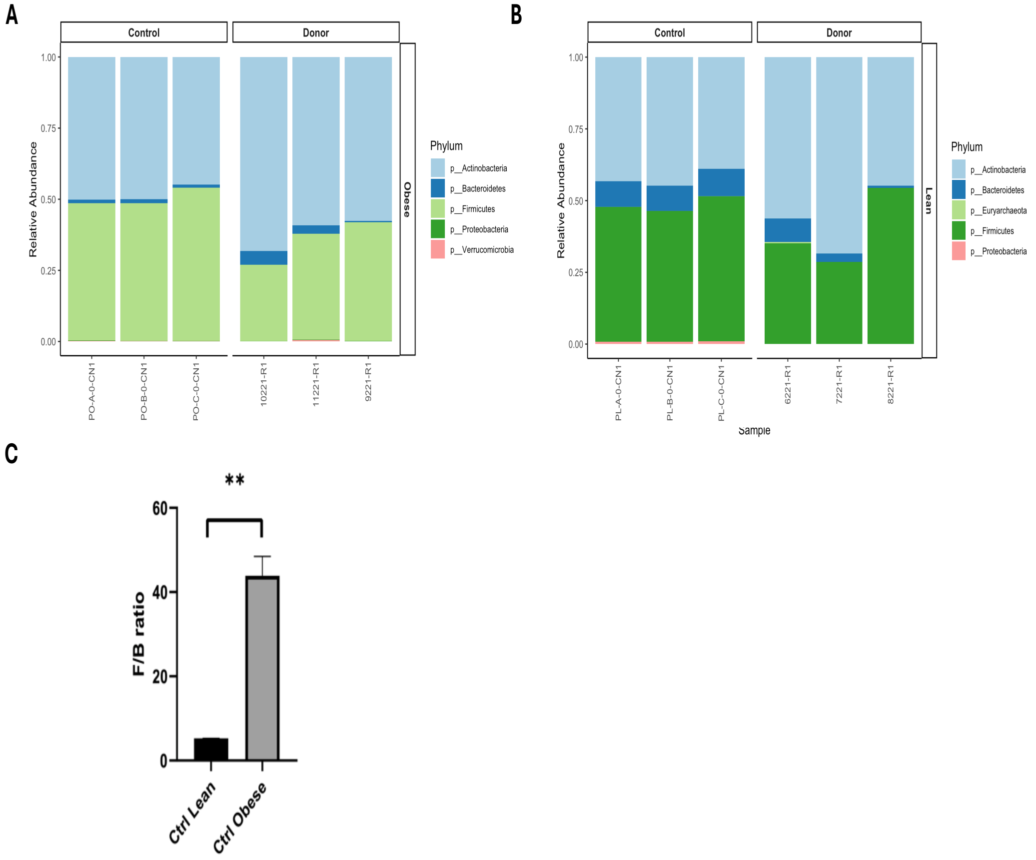


Fig. S1. a) Obese donors and pooled faecal samples (control) at phylum level b) Lean donors and pooled faecal samples (control) at phylum level. c) Obese pooled faecal sample shows an higher *Firmicutes*:*Bacteroidetes* ratio. To determine statistical significance among the treatments, multiple t-tests with Tukey correction for multiple comparisons were performed. Significance levels are denoted by asterisks above the horizontal lines connecting relevant bars, with the following conventions: a single asterisk (*) for $p \leq 0.05$, two asterisks (**) for $p \leq 0.01$, three asterisks (***) for $p \leq 0.001$, and four asterisks (****) for $p \leq 0.0001$, indicating increasingly significant differences between the treatment effects. Data are representative of three independent experiments (n=3).

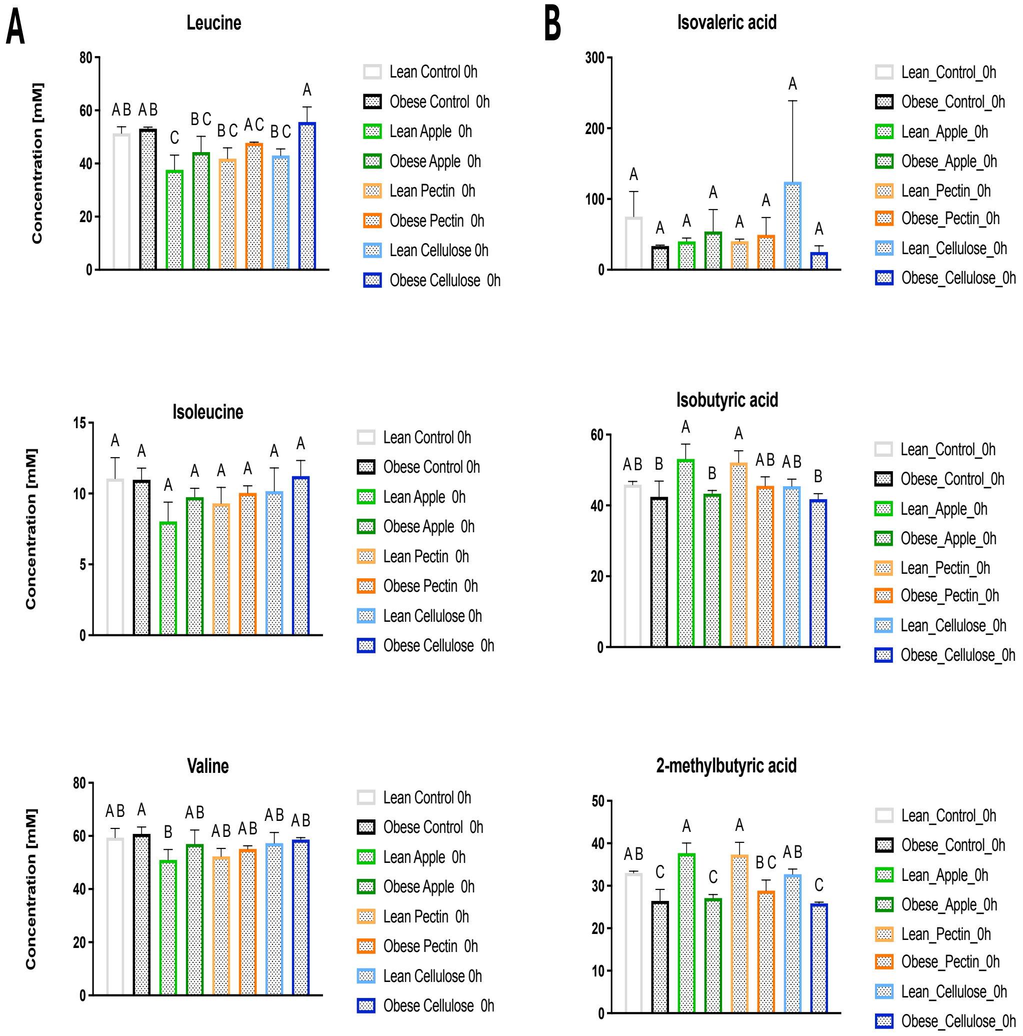
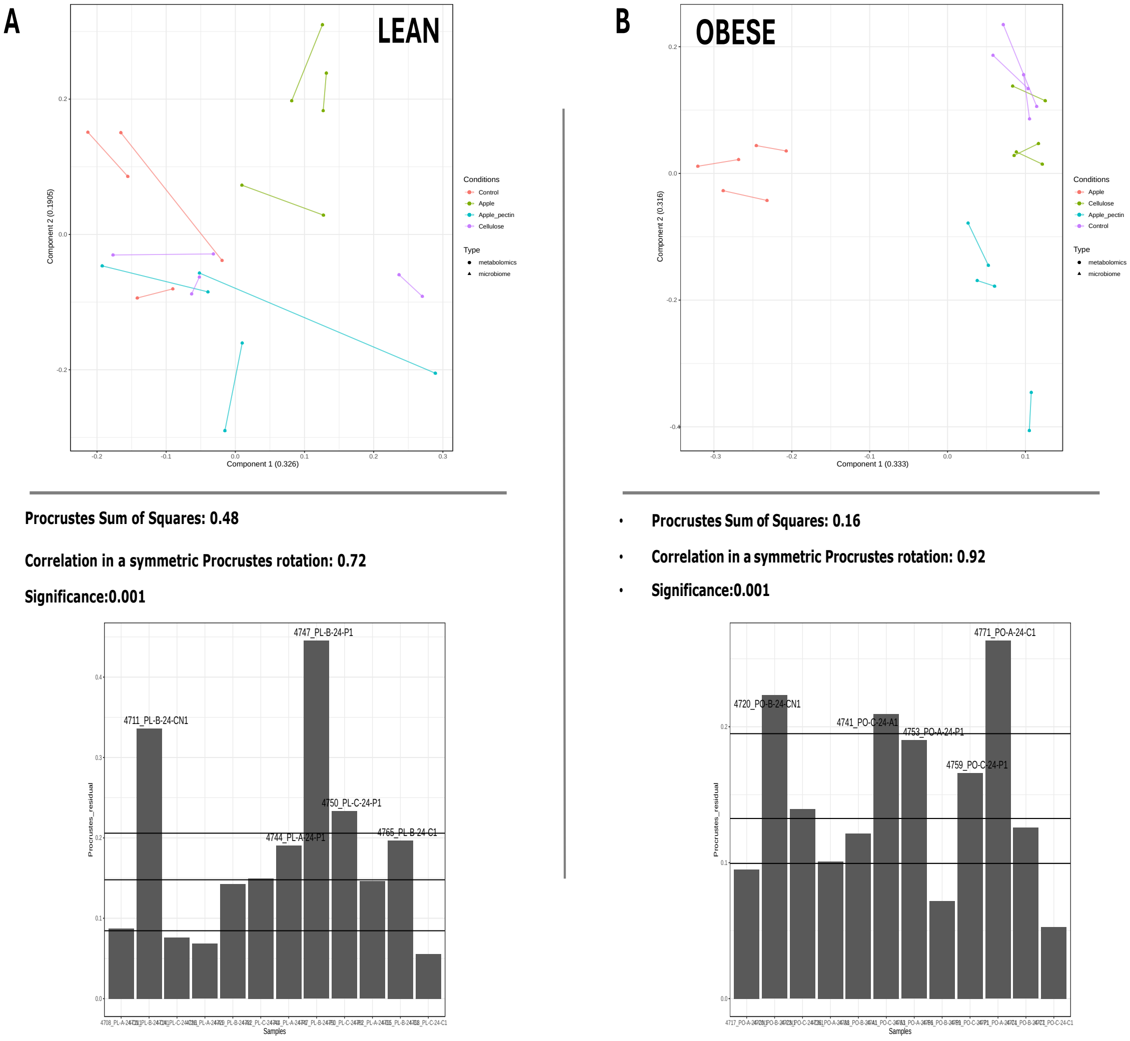


Fig. S2. **(A)** Concentrations of BCAAs in lean and obese microbial communities before being exposed to the feeding conditions (t = 0 h): this figure provides a graphical representation comparing the concentrations of BCAAs - leucine, isoleucine, and valine - in both lean and obese microbial communities before a specific feeding condition. Each bar in the graph represents the average concentration observed for a particular group and condition, with error bars depicting the standard deviation to illustrate the variability within each group. To identify statistical significance among the microbial communities and conditions, the analysis involved multiple t-tests with Tukey correction for multiple comparisons. The results are visually indicated by different letters above each bar. Bars that share the same letter do not differ significantly from each other at a p-value ≤ 0.05, suggesting that the treatments had comparable effects on the concentrations of BCAAs in these groups. Conversely, bars adorned with different letters signify that the treatment groups exhibit statistically significant differences in BCAA concentrations, emphasizing the distinct impact of the treatments. **(B)** Concentrations of BCFAs in lean and obese microbial communities before being exposed to the studied feeding conditions (t = 0 h): this figure provides a graphical representation comparing the concentrations of BCFAs - isovaleric acid, 2-methylbutyric acid, and isobutyric acid - in both lean and obese groups before and after the administration of specific dietary treatments. Similar to the BCAA graph, each bar represents the average concentration for a particular group and condition, with error bars showing the standard deviation. Statistical significance was assessed using multiple t-tests with Tukey correction, and different letters above each bar indicate significant differences (p-value ≤ 0.05). Data are representative of three independent experiments (n=3).



Procrustes Sum of Squares: 0.48

Correlation in a symmetric Procrustes rotation: 0.72

Significance:0.001

- Procrustes Sum of Squares: 0.16
- Correlation in a symmetric Procrustes rotation: 0.92
- Significance:0.001

Fig. S3. Procrustes similarity test for lean and obese metabolomics and metagenomics datasets. The analysis is depicted in two panels: **Panel A**: Procrustes analysis for lean subjects' metabolomics and metagenomics datasets, with a Procrustes Sum of Squares of 0.48, a correlation in a symmetric Procrustes rotation of 0.72, and a significance of 0.001. **Panel B**: Procrustes analysis for obese subjects' metabolomics and metagenomics datasets, with a Procrustes Sum of Squares of 0.16, a correlation in a symmetric Procrustes rotation of 0.92, and a significance of 0.001. Data are representative of three independent experiments (n=3)
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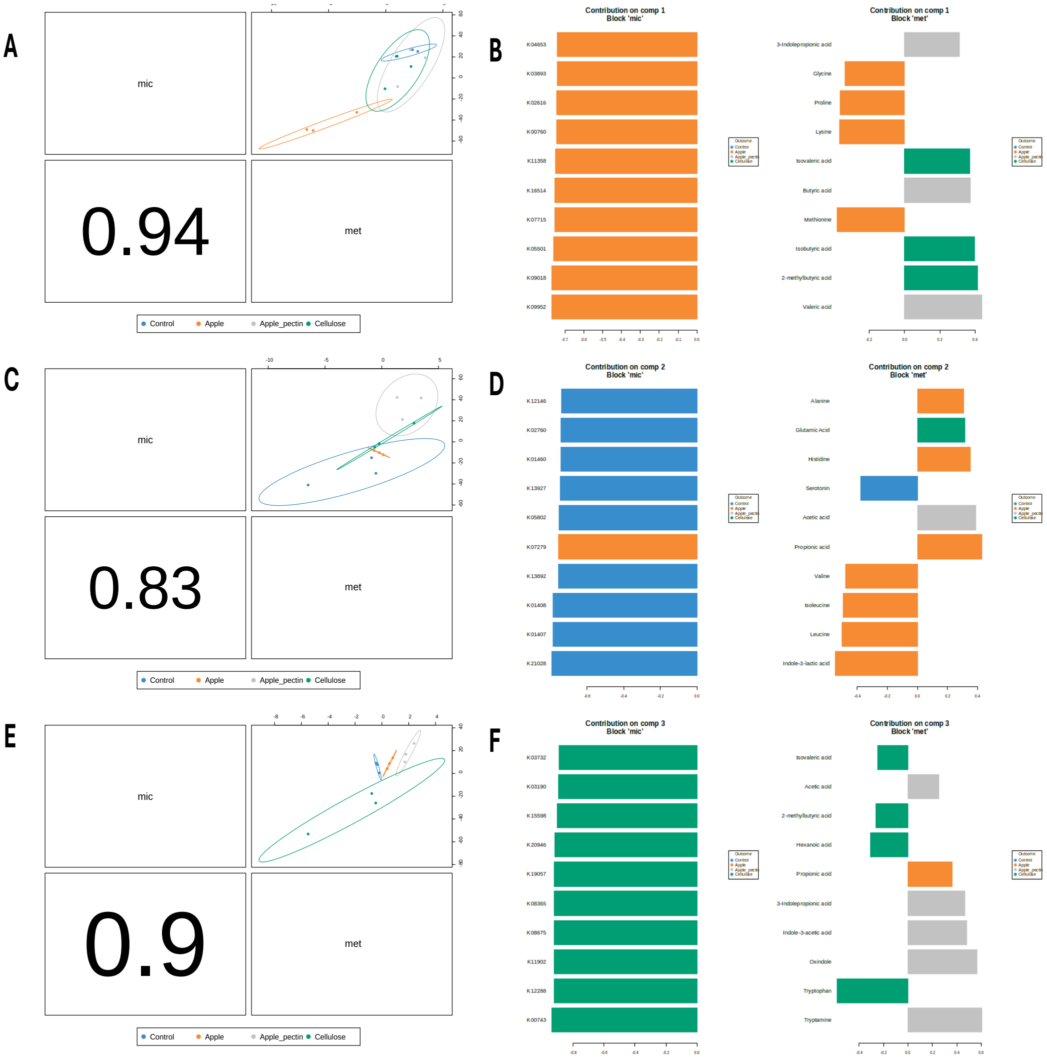
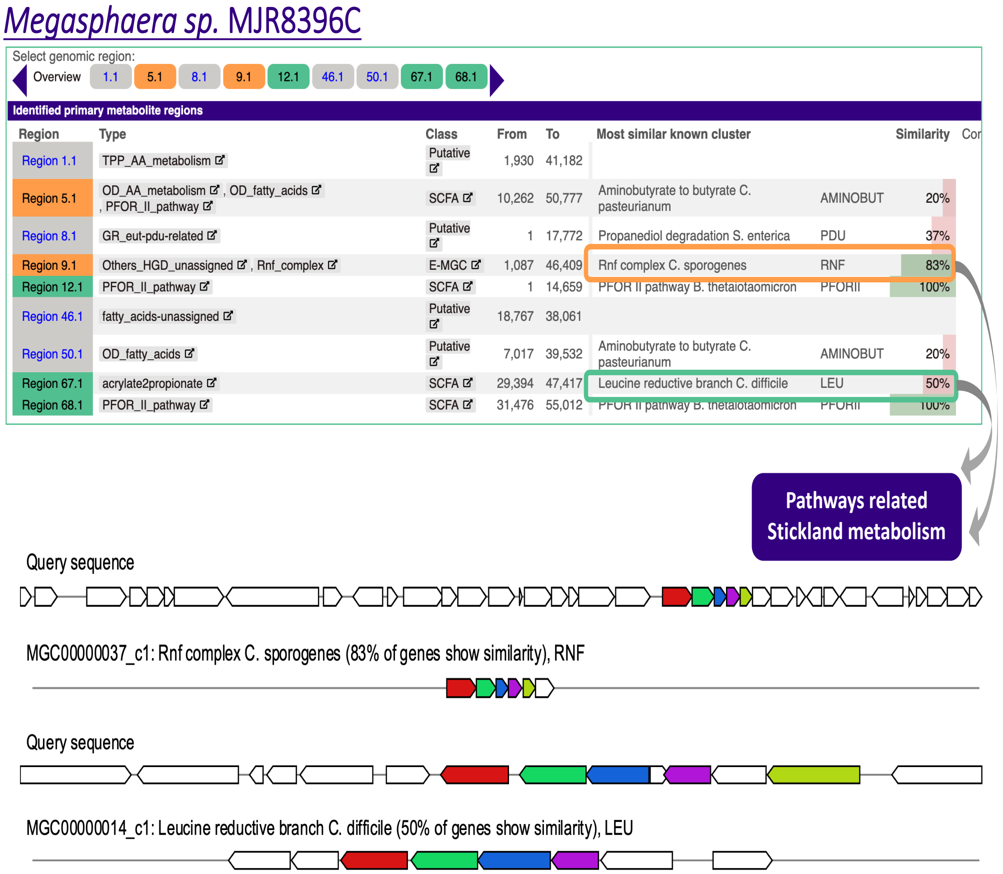


Fig. S4. This figure shows that DIABLO perform dimensionality reduction to select a minimal set of features selected by DIABLO across data types (microbiome, metabolome) that could discriminate between dietary treatments in lean. Panel A shows a distinct set of these features separated by apple (DIABLO component 1) from apple pectin (DIABLO component 2) and cellulose (DIABLO component 3). Panel B shows how the analysis selects, therefore, features jointly across data types, resulting in the identification of features with strong associations across datasets. Data are representative of three independent experiments (n=3).



Region	Type	Class	From	To	Most similar known cluster		Similarity	Cor
Region 1.1	TPP_AA_metabolism	Putative	1,930	41,182				
Region 5.1	OD_AA_metabolism , OD_fatty_acids , PFOR_II_pathway	SCFA	10,262	50,777	Aminobutyrate to butyrate C. pasteurianum	AMINOBUT	20%	
Region 8.1	GR_eut-pdu-related	Putative	1	17,772	Propanediol degradation S. enterica	PDU	37%	
Region 9.1	Others_HGD_unassigned , Rnf_complex	E-MGC	1,087	46,409	Rnf complex C. sporogenes	RNF	83%	
Region 12.1	PFOR_II_pathway	SCFA	1	14,659	PFOR II pathway B. thetaiotaomicron	PFORII	100%	
Region 46.1	fatty_acids-unassigned	Putative	18,767	38,061				
Region 50.1	OD_fatty_acids	Putative	7,017	39,532	Aminobutyrate to butyrate C. pasteurianum	AMINOBUT	20%	
Region 67.1	acrylate2propionate	SCFA	29,394	47,417	Leucine reductive branch C. difficile	LEU	50%	
Region 68.1	PFOR_II_pathway	SCFA	31,476	55,012	PFOR II pathway B. thetaiotaomicron	PFORII	100%	

Fig. S5. A graphical representation of GutSMASH result from the analyzed *Megasphaera sp.* MJR8396C species. The analysis reveals the presence of an homolog of RNF complex from *C.sporogenes* and Leucine reductive branch from *C.difficile* pointing towards a clostridium like metabolism.