Longitudinal VOCs profiling by direct-injection mass spectrometry reveals the interaction between the gut microbiota and dietary fibers *in-vitro*: Supplementary Figures

IMMEDIATE

1. SUPPLEMENTARY FIGURES

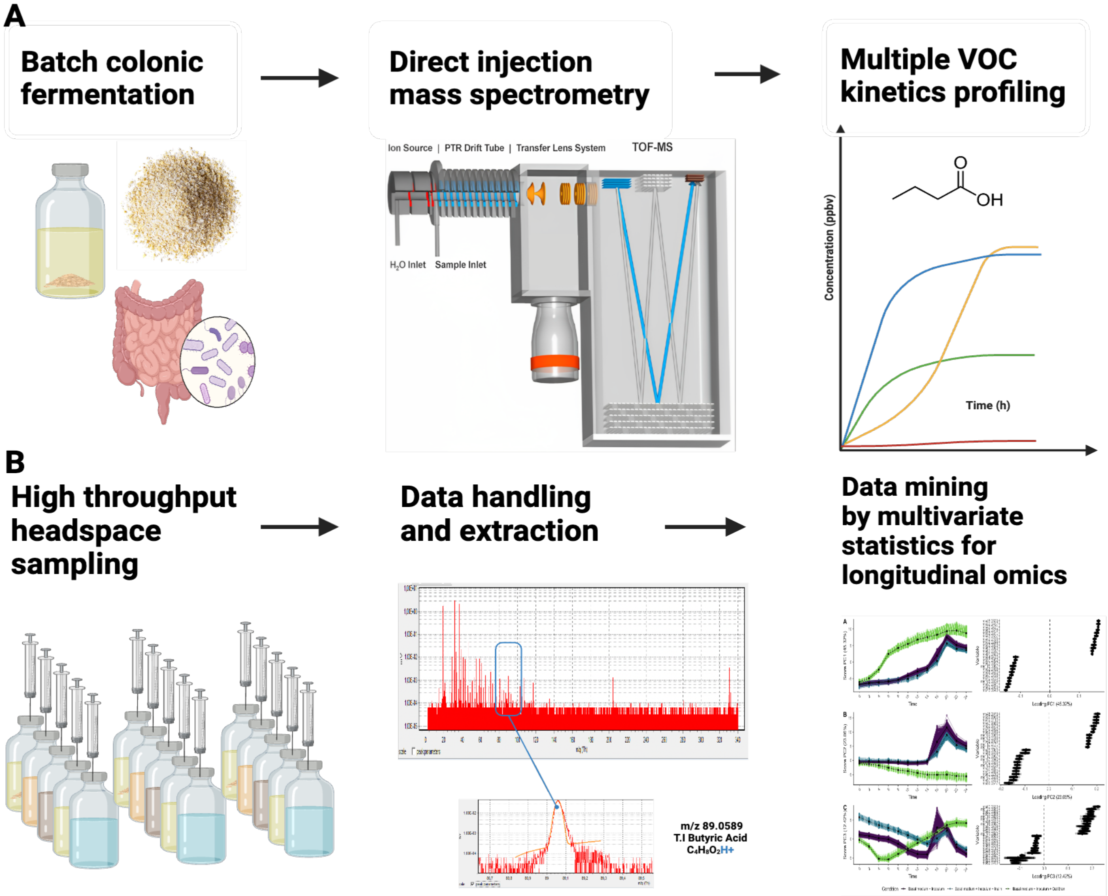

Fig. S1. Workflow of the study illustrating the experimental setup and analytical approaches used to investigate volatile organic compounds (VOCs) during colonic fermentation. **(A)** Batch colonic fermentation with substrates M, OB, I, and C, followed by VOC analysis through direct injection mass spectrometry to obtain kinetic data of VOC concentrations over time. **(B)** VOC collection using headspace sampling, followed by data acquisition and multivariate statistical analysis for longitudinal volatilomics. The figure highlights the identification of a specific VOCs and the application of multivariate statistics for data interpretation.

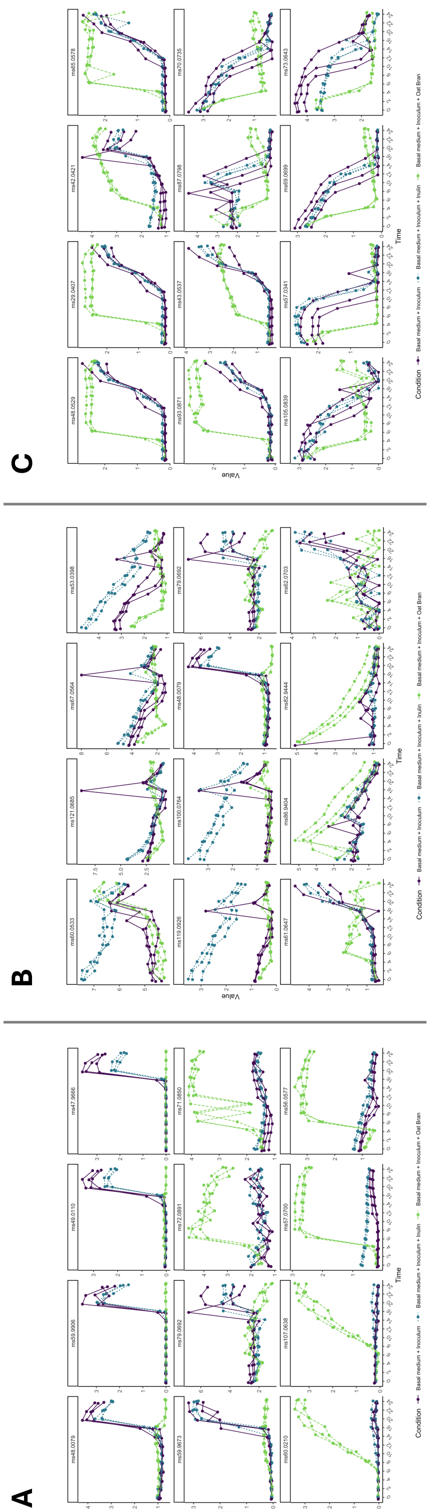

Fig. S2. Abundance-time curves for selected metabolites determined by RM-ASCA during anaerobic in-vitro batch fermentation over 24 hours. Panel A, B and C represent the PC1, PC2 and PC3 from the RM-ASCA Model. Metabolites are displayed for the three experimental conditions: BM, I, OB. The represented curves are the result of three independent biological replica (n=3). Curves highlight key metabolites showing significant time-dependent changes in abundance for each condition.

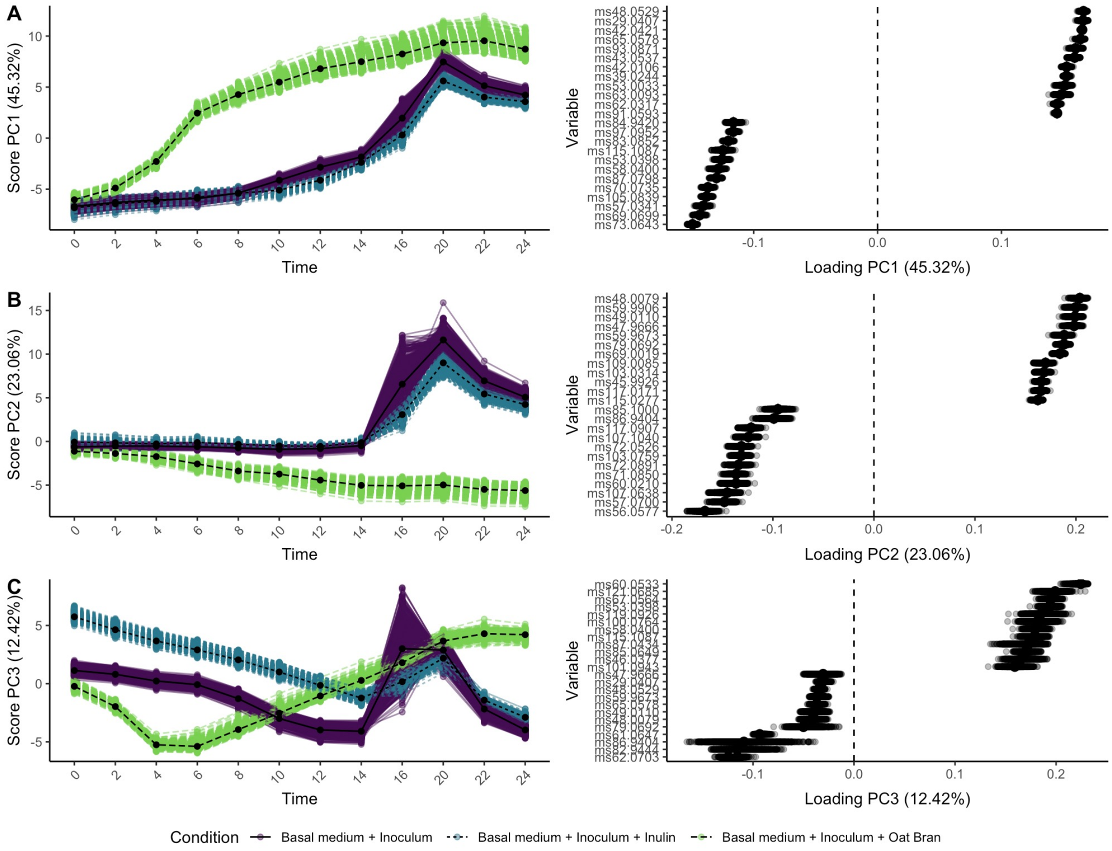

Fig. S3. RM-ASCA model validation using bootstrapping (n=1000). The left panels (A, B, C) show the time-dependent score curves for PC1 (45.32%), PC2 (23.06%), and PC3 (12.42%) for different experimental conditions: : BM, I, OB. The right panels display the corresponding loadings for PC1, PC2, and PC3 across selected metabolites. The bootstrapping approach confirms the stability and reproducibility of the RM-ASCA model, supporting the temporal separation of conditions based on metabolite profiles.

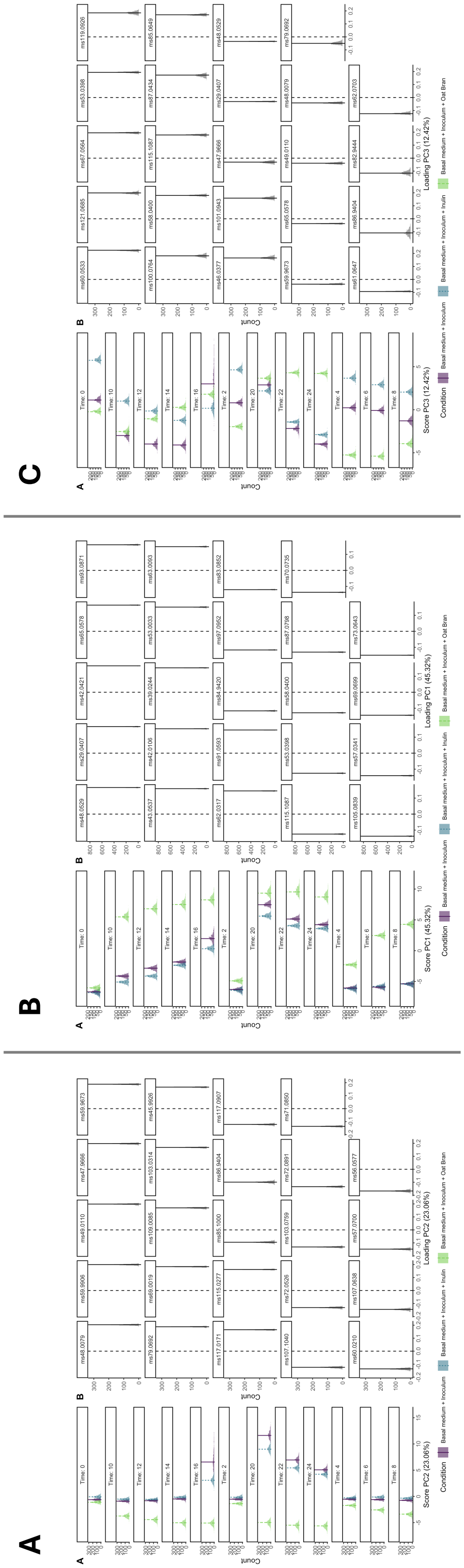

Fig. S4. Distribution of scores for PC1, PC2, and PC3 presented as histograms from the RM-ASCA model (Panel A, B and C). The represented curves are the result of three independent biological replica (n=3). The distribution of scores was obtained using bootstrapping validation (n=1000). This approach was used to assess the robustness of the RM-ASCA model and the temporal separation of the conditions across principal components.