

Supplemental Table 2: 1 year weight-loss and delta BMI after bariatric surgery according to the presence/ absence of psychopathologies (n=132)

	Weight-loss (%)			p-value	Delta BMI (kg/m ²) %			p-value
ACEs	Yes 31.53±0.06	No 28.55±0.03		ns	Yes 14.18±0.04	No 12.60±0.02		ns
								ns
Compulsive eating	Yes 29.82±0,10	No 29.45±0.03		ns	Yes 13.60±0.07	No 12.98±0.02		ns
								ns
Depression	Yes 28.92±0,19	No 28.9±0,04	Symptoms 30.9± 0.07	ns	Yes 12.26±0.12	No 12,75±0.03	Symptoms 14.9±0,05	ns

For ACE and compulsive eating Mann-Whitney test or t-student tests were used depending on variable distribution. For depressive symptoms Kruskal-Wallis test or ANOVA were used depending on variable distribution. ns: p-value>0,05. Results are expressed as mean (± SD) for quantitative variables.

Supplementary Materials and Methods

Metagenomic Analysis

Whole-genome metagenomic sequencing was performed using Oxford Nanopore Technology (ONT) on a MinION sequencer as previously described [1], with flow cells loaded with 12 samples per run. Sequencing was conducted on unamplified DNA over a 48-hour run, and a rarefaction threshold of 10,000 reads per sample was applied for metagenomic analysis. In total, 16 sequencing runs were conducted using the MinION device, generating up to 9.87×10^7 million reads, with an average of 6.17×10^6 reads per run and a mean read length of 2,249 bp. Raw reads were basecalled, quality-filtered, and demultiplexed using Albacore (v2.1.10) and Guppy (v2.1.3).

Taxonomic Binning and Classification

Taxonomic classification of Nanopore reads was performed using a two-step approach following the pipeline generated by Alili et al. (<https://git.ummisco.fr/ebelda/nanopore.v2.0>, last accessed March 2025)

1. **Human Read Removal & Initial Classification:** Reads were first processed with Centrifuge [2] to remove human contaminants and obtain an initial taxonomic classification based on NCBI reference taxonomic identifiers. The precompiled “P_compressed_b + v + h” database available in the Centrifuge distribution was used as a reference.
2. **Species-Level Genome Bin (SGB) Assignment:** Non-human reads were mapped to the MetaPhlAn 4 gene catalog, which consists of 5.1 million marker genes organized into 21,978 species-level genome bins (SGBs)[3]. Mapping was performed using Minimap2 with the map-ont option, optimized for ONT reads [4].

Each MetaPhlAn marker gene was linked to a specific SGB. Gene quantification was performed after normalizing for library size and gene length, following MetaPhlAn guidelines. The robust average approach was applied to estimate SGB abundance.

Principal Coordinates Analysis (PCoA) and Distance-Based Redundancy Analysis (dbRDA)

To assess microbiome variability, a distance-based redundancy analysis (dbRDA) was performed on Principal Coordinates Analysis (PCoA) ordination. The impact of 38 clinical variables was initially evaluated using the `capscale` function from the `Vegan` (v2.6.4; <https://CRAN.R-project.org/package=vegan>) R package. Variables meeting a significance threshold ($\text{capscale } p < 0.05$) were further analyzed using the `ordiR2step` function in `Vegan` to identify those explaining a non-redundant fraction of microbiome variability.

Alpha and Beta-Diversity Calculations

Alpha and beta-diversity metrics were computed using the `Vegan` (v2.6.4; <https://CRAN.R-project.org/package=vegan>) R package:

- Alpha-diversity indexes were calculated using the `diversity` and `specnumber` functions.
- Beta-diversity was assessed using the `vegdist` and `cmdscale` functions.

Normalization and Transformation of SGB Relative Abundances

For regression analyses, SGB relative abundances were transformed using a Centered Log Ratio (CLR) transformation to account for compositional data properties. This was performed with the `clr` function from the `compositions` R package (<https://CRAN.R-project.org/package=compositions>). Before applying the CLR transformation, zero

abundances were imputed using the `cmultRepl` function from the `zCompositions` R package (<https://CRAN.R-project.org/package=zCompositions>), following the zero-count approach.

KML analysis

K-means for longitudinal data clustering on weight at 1, 2, 3, 4 and 5 years after bariatric surgery (BS) was used to identify different weight-loss trajectories after BS. KML method is a partitional clustering approach, meaning that it assigns each trajectory to a single cluster without assuming any underlying distribution within clusters. KML clustering was performed with the KML R package. Euclidian distance was used where 2 and 10 clusters were tested using 20 replications. The optimal number of clusters was determined by using Calinski-Harabasz (CH) index as the primary selection. Two additional information criteria, Ray-Turi and Davies-Bouldin, were evaluated to further assess clustering quality. Highest CH, Ray-Turi and Davies-Bouldin criteria indicated better clustering performance.

Post-Hoc Comparisons and Multiple Testing Correction

To assess the different abundances on different clusters and the SGB associated with weight loss linear regression models were generated with the transformed SGB abundances after adjusting for gender, age, and the number of antidiabetic treatments. The magnitude and direction of the associations was determined with the beta coefficients and the statistical significance was evaluated with the corresponding p values ($p < 0.05$). Multiple testing correction was also applied with the Benjamini-Hochberg correction.

Method reference

1. Alili R, Belda E, Le P, Wirth T, Zucker J-D, Prifti E, et al. Exploring Semi-Quantitative Metagenomic Studies Using Oxford Nanopore Sequencing: A Computational and Experimental Protocol. *Genes* 2021, Vol 12, Page 1496 [Internet]. 2021 [cited 2021 Sep 29]; 12:1496. Available from: <https://www.mdpi.com/2073-4425/12/10/1496/htm>
2. Kim D, Song L, Breitwieser FP, Salzberg SL. Centrifuge: Rapid and sensitive classification of metagenomic sequences. *Genome Res* [Internet]. 2016 [cited 2021 Feb 4]; 26:1721–9. Available from: <http://www.genome.org/cgi/doi/10.1101/gr.210641.116>.
3. Blanco-Míguez A, Beghini F, Cumbo F, McIver LJ, Thompson KN, Zolfo M, et al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology* 2023 41:11 [Internet]. 2023 [cited 2024 Apr 4]; 41:1633–44. Available from: <https://www.nature.com/articles/s41587-023-01688-w>
4. Li H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* [Internet]. 2018 [cited 2025 Mar 5]; 34:3094–100. Available from: <https://dx.doi.org/10.1093/bioinformatics/bty191>