

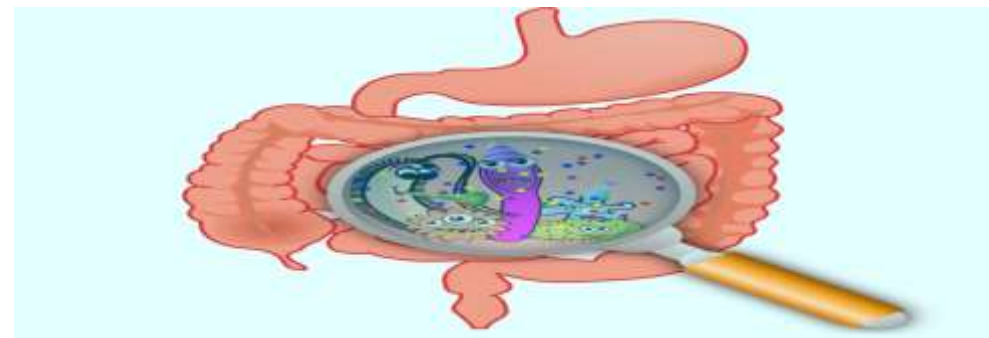


XXVI  
IFSO WORLD  
CONGRESS  
OF BARIATRIC  
& METABOLIC SURGERY



NAPLES, ITALY  
AUGUST 30-SEPTEMBER 1, 2023

Congress President: Prof. Luigi Angrisani



# Gut microbiota's shift 1 year after laparoscopic Roux-en-Y vs One Anastomosis Gastric Bypass

Cristian Boru\*

Flavio De Maio\*, Nunzio Velotti, Danila Capoccia, Giulia Santarelli, Ornella Verrastro, Delia Mercedes Bianco, Brunella Capaldo, Maurizio Sanguinetti, Mario Musella, Marco Raffaelli, Frida Leonetti, Giovanni Delogu\*\* & Gianfranco Silecchia\*\*



SAPIENZA  
UNIVERSITÀ DI ROMA



## No potential conflict of interest to report

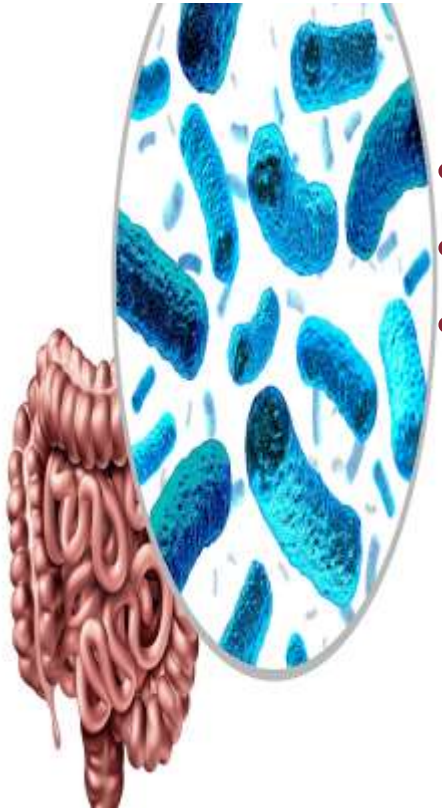


The study was partially funded, unconditionally by

- research grant from the University La Sapienza of Rome Ateneo's Funds 2017
- research grant from the European Association of Endoscopic Surgery (EAES) Research Program 2018
- Italian Society of Diabetology (SID) 2021 Research Reward "Premio SID Diabete e Ricerca"



## BACKGROUND



- GM may contribute to obesity by altering the proportions in bacterial phyla
- GM may mediate some of the metabolic effects of BMS
- changes in its composition and diversity have been observed after RYGB

There are no prospective investigations of GM's SHIFT after OAGB vs RYGB in homogenous cohort of patients with severe obesity.

## AIMS

- to compare GM's composition in subjects with severe obesity eligible for BMS and with / without metabolic syndrome.
- to compare OAGB vs RYGB microbiota profile shift at 1 year and its impact on the metabolic and nutritional status.



## Centers

General Surgery Division, Sant'Andrea Hospital, Department of Medical Surgical Sciences and Translational Medicine General Surgery Unit & Bariatric Center of Excellence IFSO-EC, University "La Sapienza" of Rome

**Prof. G. Silecchia, Dr. CE Boru, Dr. D. Capoccia, Prof. F. Leonetti**

Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

**Flavio De Maio, Prof. M. Sanguinetti**

Department of Advanced Biomedical Sciences, University of Naples "Federico II", Naples, Italy, **Dr N. Velotti, Prof. M. Musella**

Dipartimento di Scienze Biotechologiche di Base, Cliniche Intensivologiche e Perioperatorie – Sezione di Microbiologia, Università Cattolica del Sacro Cuore, Rome, Italy

**G. Santarelli, DM Bianco, Prof. G. Delogu**

Division of Endocrine and Metabolic Surgery, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

**Prof. M. Raffaelli, O. Verrastro**

Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy **Prof. B. Capaldo**

# MATERIAL & METHODS 1

- prospective, randomized study, multicenter: 3 high volume Italian bariatric centers
- Registered on [clinicaltrials.gov](https://clinicaltrials.gov) 2018 (Unique Protocol ID: NCT03412149)
- approved by the local committees
- conducted in accordance with the principles of Good Clinical Practice and STROBE checklist



SAPIENZA  
UNIVERSITÀ DI ROMA



UNIVERSITÀ DEGLI STUDI DI NAPOLI  
FEDERICO II

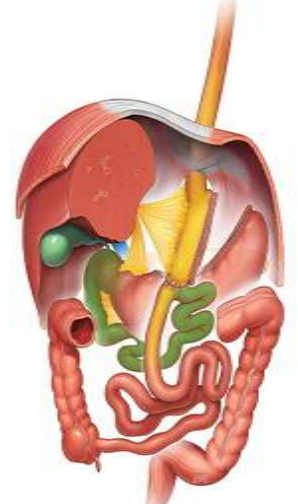


UNIVERSITÀ  
CATTOLICA  
del Sacro Cuore



NAPOLI  
2023

# MATERIAL & METHODS 2



84 patients with severe obesity randomized in 2 groups  
*between 2018 - 2020*

*Fecal (for GM) and blood samples were collected  
preop (T0) and at 12 mo (T1)*



## ***Inclusion criteria***

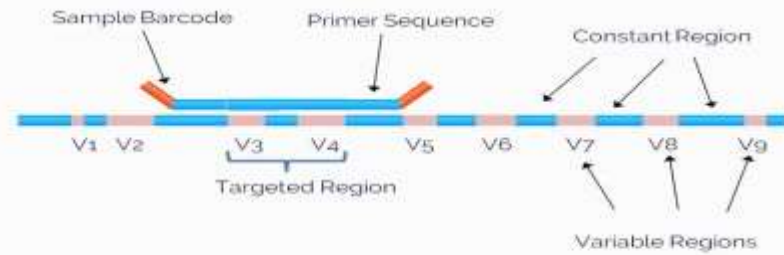
*Aged 18–65 years old  
BMI 40–55 kg/m<sup>2</sup>  
non-smokers  
Informed consensus agreed*



## ***Exclusion criteria***

*corticosteroids, vitamin E, fish oil treatment;  
antibiotics or probiotics treatment 2 months prior to surgery  
chronic gastrointestinal diseases or syndromes;  
previous bariatric and/or HPB surgery; and gallstones  
**severe esophagitis (LA classification > B)**  
**severe GERD and large hiatal hernia***

# MATERIAL & METHODS 3



## Sample processing and 16S rRNA gene sequencing, Bioinformatics and statistics analyses

1. Fresh stool samples were collected before and 1 y after surgical intervention and stored at  $-80^{\circ}\text{C}$  until processing.
2. DNA isolation was performed within a biological safety and sterile cabinet  
The extracted DNA was stored at  $-20^{\circ}\text{C}$  until further analysis.
3. Before library preparation, the isolated DNA was quantitatively evaluated
4. V3–V4 hypervariable regions from the **16S rRNA gene** were amplified by using the primers and conditions  
Extracted DNA (2.0ng) was used as the template in a 50- $\mu\text{L}$  PCR volume
5. Purified amplicons were used to generate DNA libraries, which were submitted to sequencing on the Illumina MiSeq instrument (Illumina). The raw sequencing reads were processed.

# MATERIAL & METHODS 3

6. Bacterial community analysis was performed in R studio version 4.0.2 using the phyloseq software package.

7. This resulted in 8,160,105 reads (median value = 78,519 reads) accounting for a total of 907 bacterial taxa.

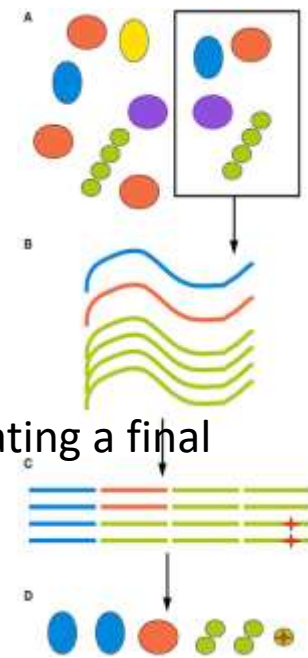
An additional taxonomic filter was applied to remove low-prevalence taxa (Patescibacteria and Tenericutes), generating a final dataset that accounted 8,159,323 reads (median value = 78,519 reads) accounting for 904 taxa.

8. Alpha diversity was measured using the observed species index, Shannon index, Pielou's Evenness and Faith's diversity.

9. Beta diversity was measured by Bray-Curtis and Weighted Unifrac distance and visualized by principal coordinate analysis.

10. Statistical differences were assessed by Kruskal-Wallis test for alpha-diversity or the PERMANOVA test for beta diversity.

Relative abundances were calculated at phylum and genus levels statistical significance was assessed by the Kruskal-Wallis test.



# RESULTS TO

- No differences regarding anthropometric, nutritional parameters, except for vitamin D.
- **The alpha and beta diversity** examinations showed no statistical differences in GM profile.
- **There were no significant differences in the top ten genera.**
- Data on **BACTEROIDES** (inversely related to triglycerides and LDL and directly related to HDL levels) and on **FIRMICUTES** (opposite trend) relative abundances suggest **no differences** among the three conditions: MS, pre-MS and obesity
- No correlation between the relative abundance of the main phyla and plasmatic glucose levels was observed.
- **Conclusions:** In a selected cohort of patients with obesity, MS did not affect the preoperative GM's profile.

**Severe obesity, per se, seems to be an independent condition affecting GM.**

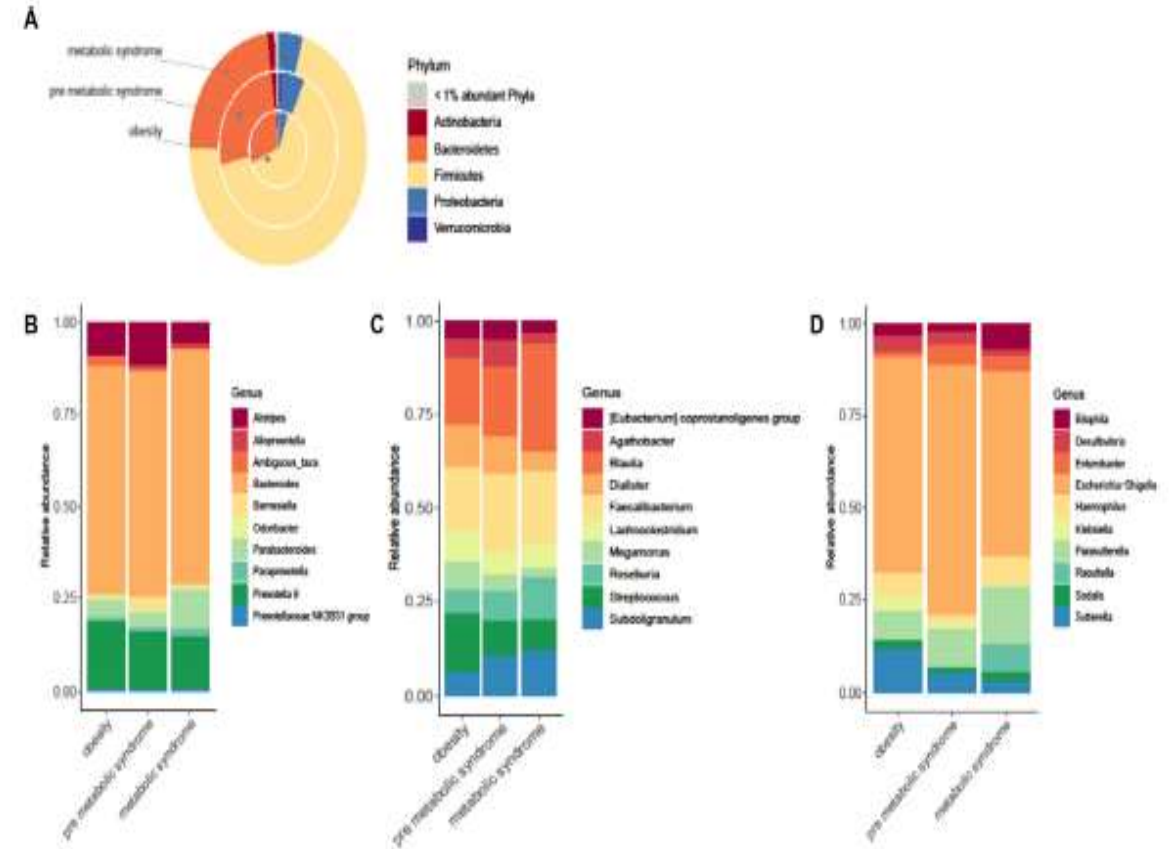


Fig. 2 - Microbiota composition differences between patients with obesity, pre-metabolic and patients with metabolic syndrome. (A) Pie chart showing the relative abundances of the main phyla identified in the three groups. Stacked bar plot showing the average relative abundance of the 10 most represented genera within the major phyla that compose the gut bacterial community of the three groups, Bacteroidetes (B), Firmicutes (C) and Proteobacteria (D).

# RESULTS T1: 54 pts randomized in 2 groups

## SURGICAL

Mortality, conversion rate and hospital readmission for reoperation were nil in both groups.

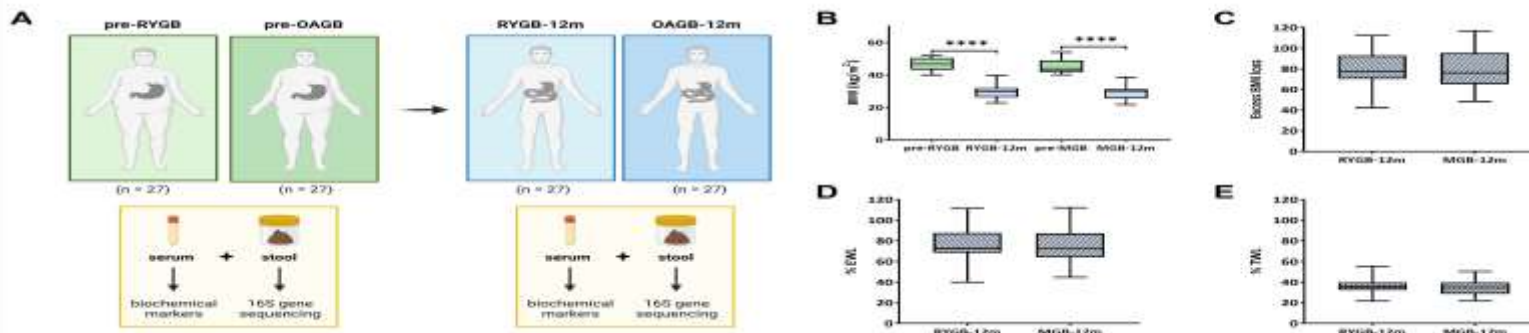
all patients were free of PPI treatment, followed a standard vitamins supplementation, and did not receive antibiotics.

a significant decrease in weight, BMI, neck circumference, waist and hip was observed ( $p < 0.001$ )

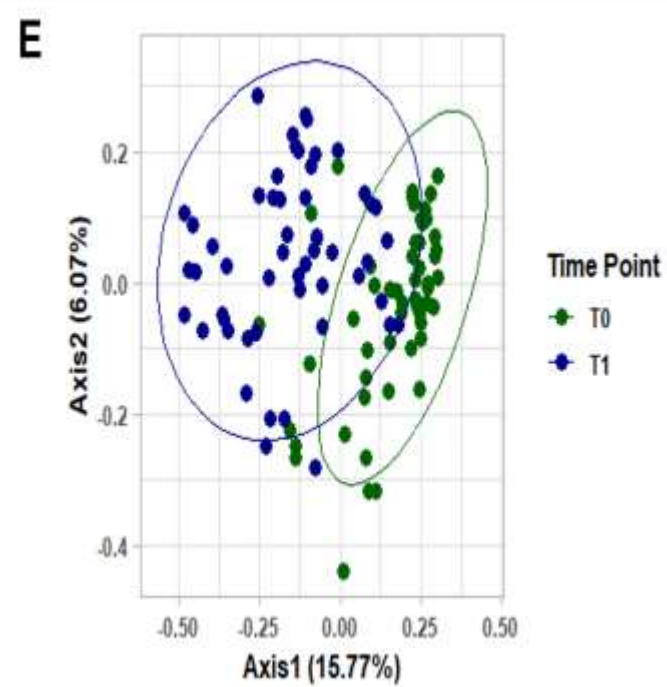
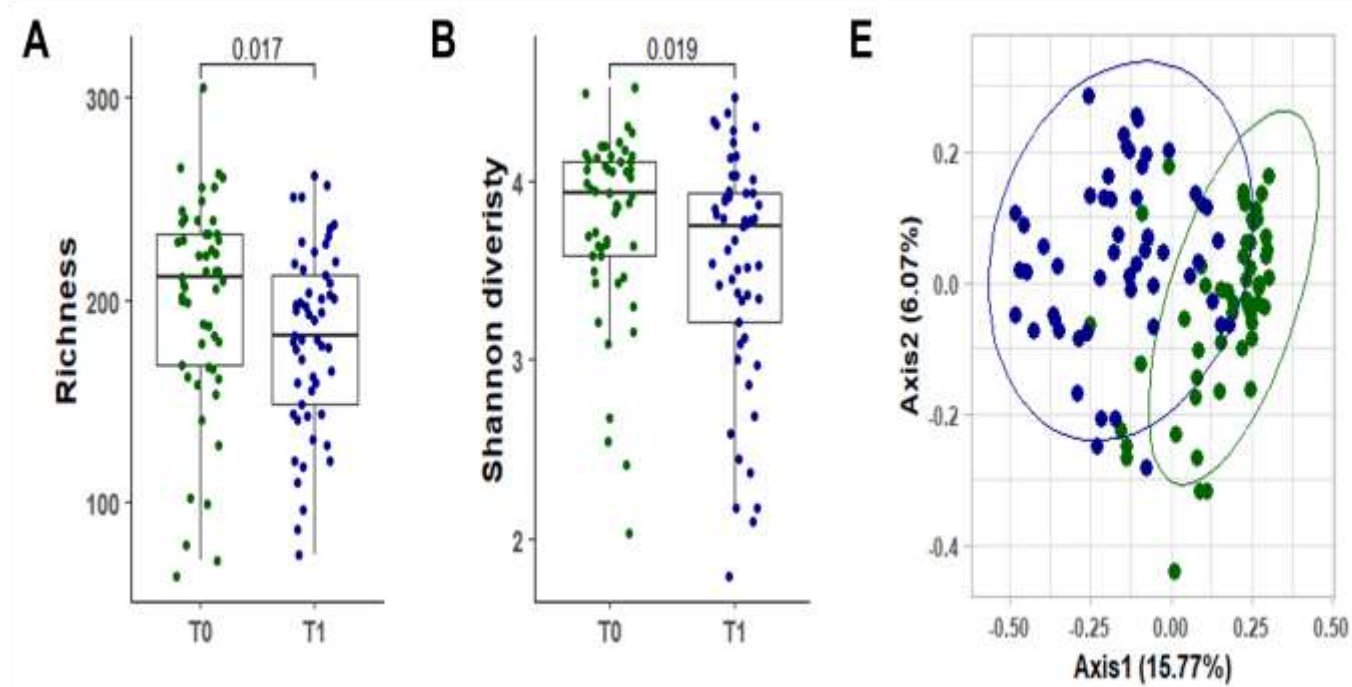
## METABOLIC

hemoglobin, HbA1c, glucose, triglycerides, cholesterol, LDL, ferritin and cortisol were significantly reduced, while vitamin D was significantly increased.

An overall significant variation was detected in anthropometric and serum nutritional parameters at T1.



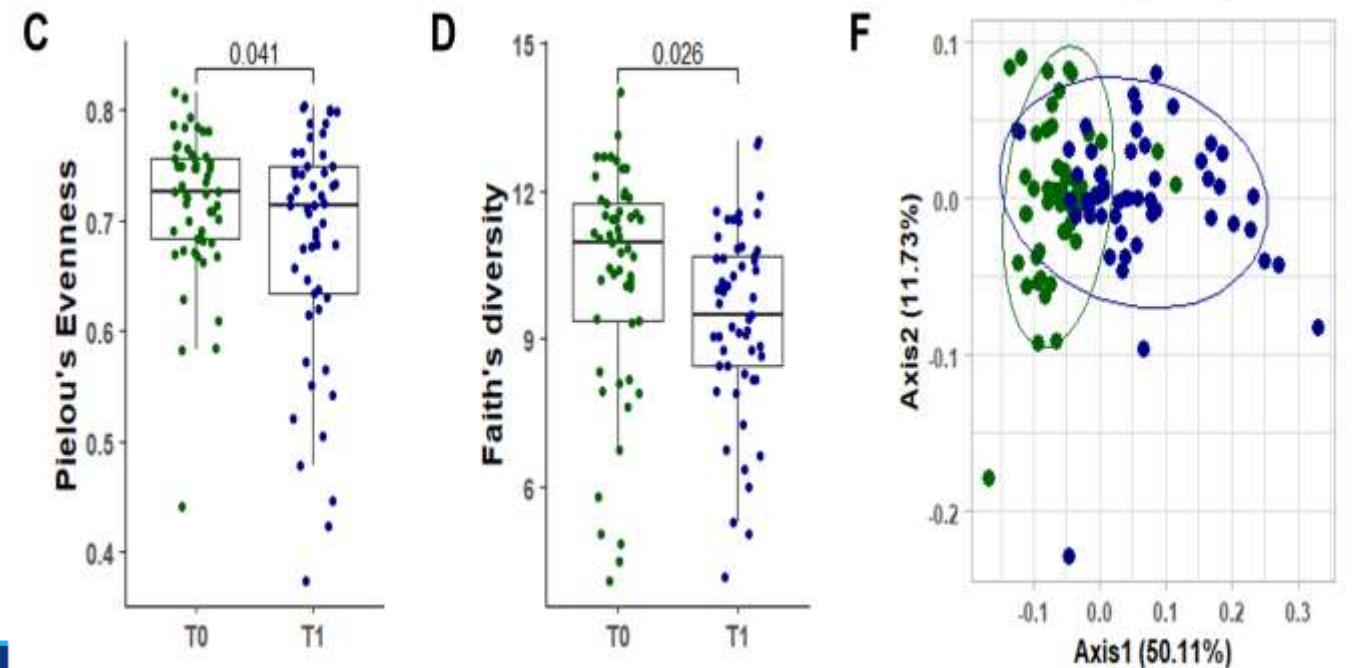
**No differences at T1 were observed between RYGB and OAGB**



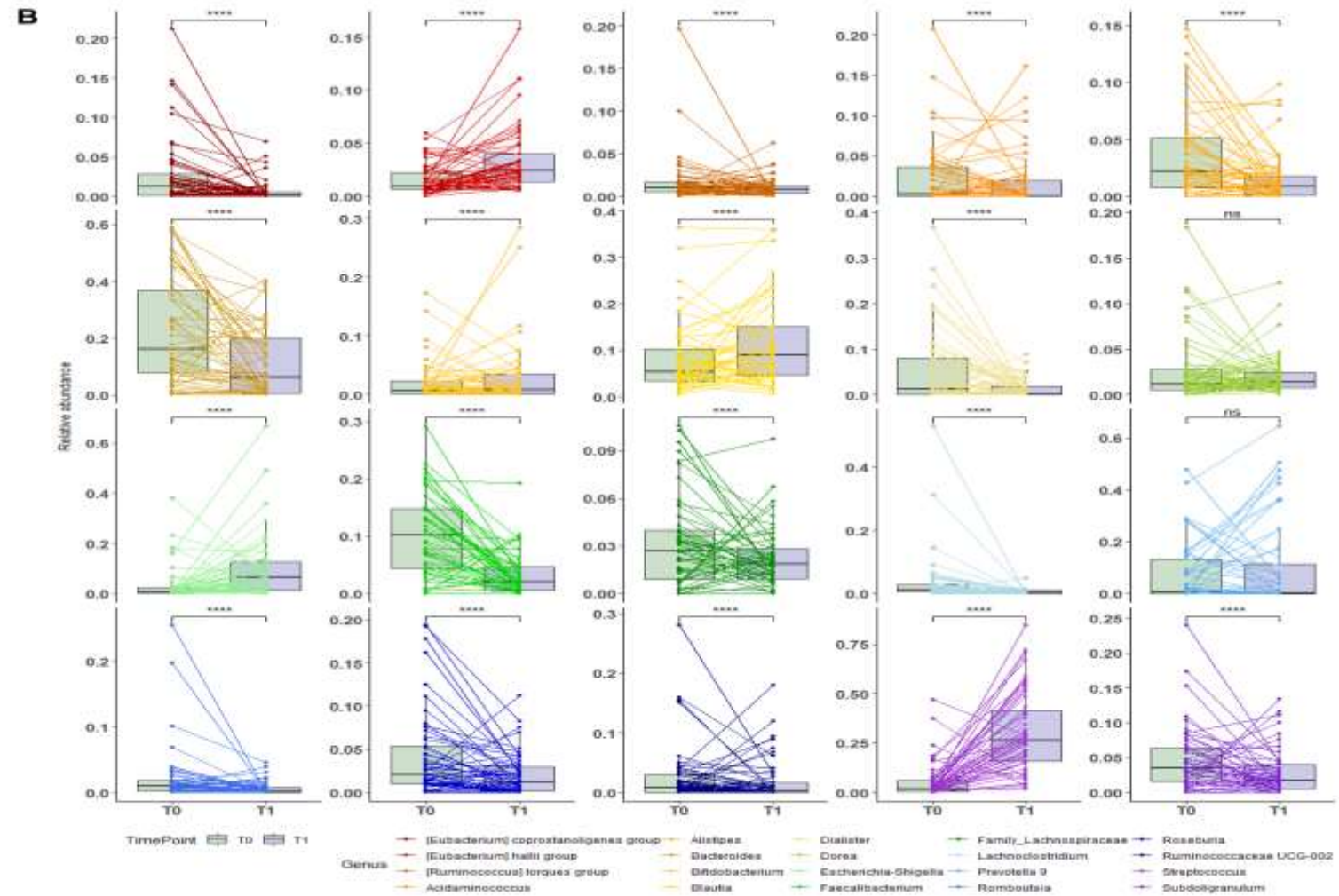
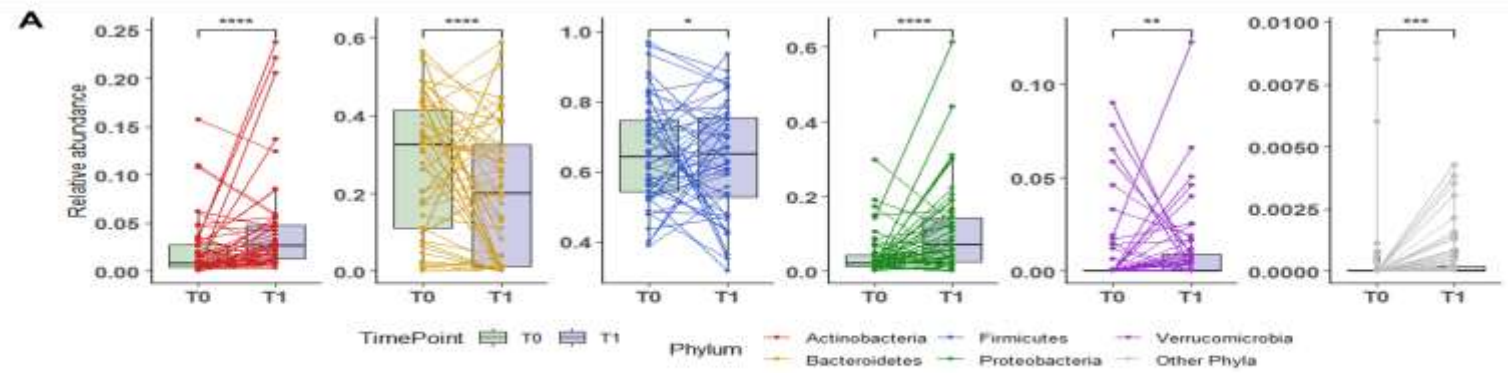
GM composition at 1 year showed  
**a significant decrease in overall microbial diversity**

**An increase of the Firmicutes/Bacteroidetes (F/B) ratio**

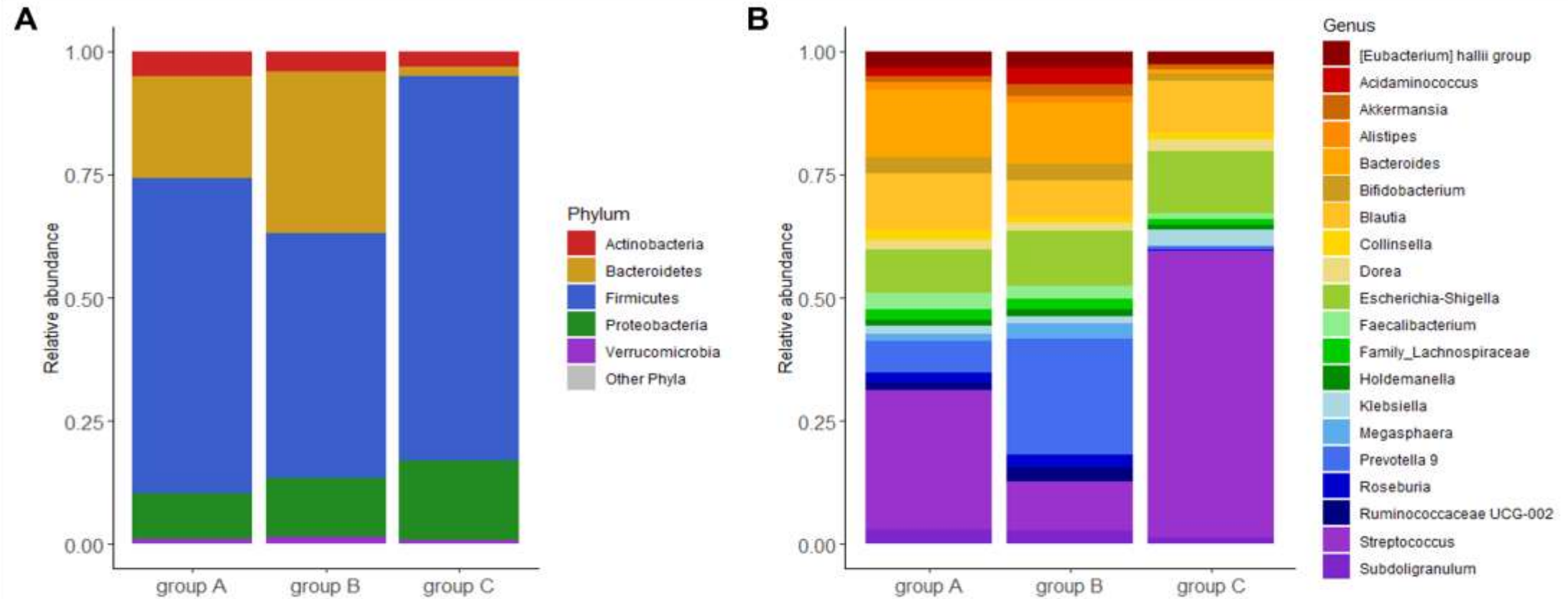
- BMS significantly impacts on gut microbiota alpha diversity (lower values post intervention) and beta diversity



**BMS determines significant changes in the bacterial community phylum (A) and genus level (B).**



# Gut microbial community identifies three groups following bariatric surgery



# Conclusions



*\*Take home message*

BMS promotes a dramatic change in GM composition one year postop.

The bypass technique **Y vs  $\Omega$**  **DID NOT** impact differently on GM,

with similar effect on weight loss and comorbidities

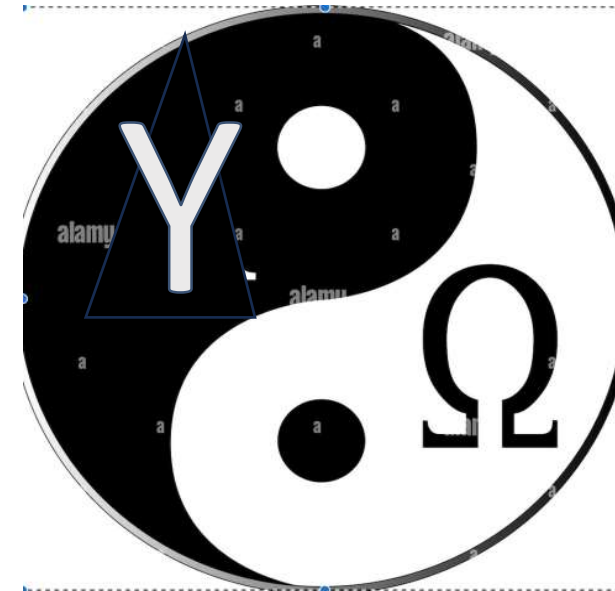
resolution/improvement.



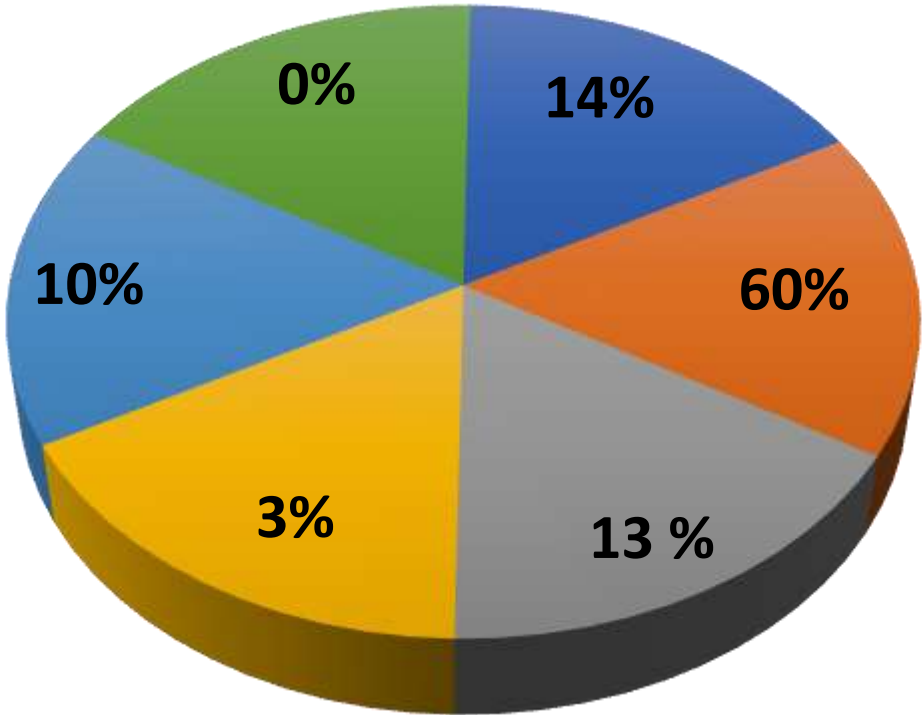
However, we identified three clusters of patients at 1 year across the two

study groups showing different microbial signatures that will be evaluated

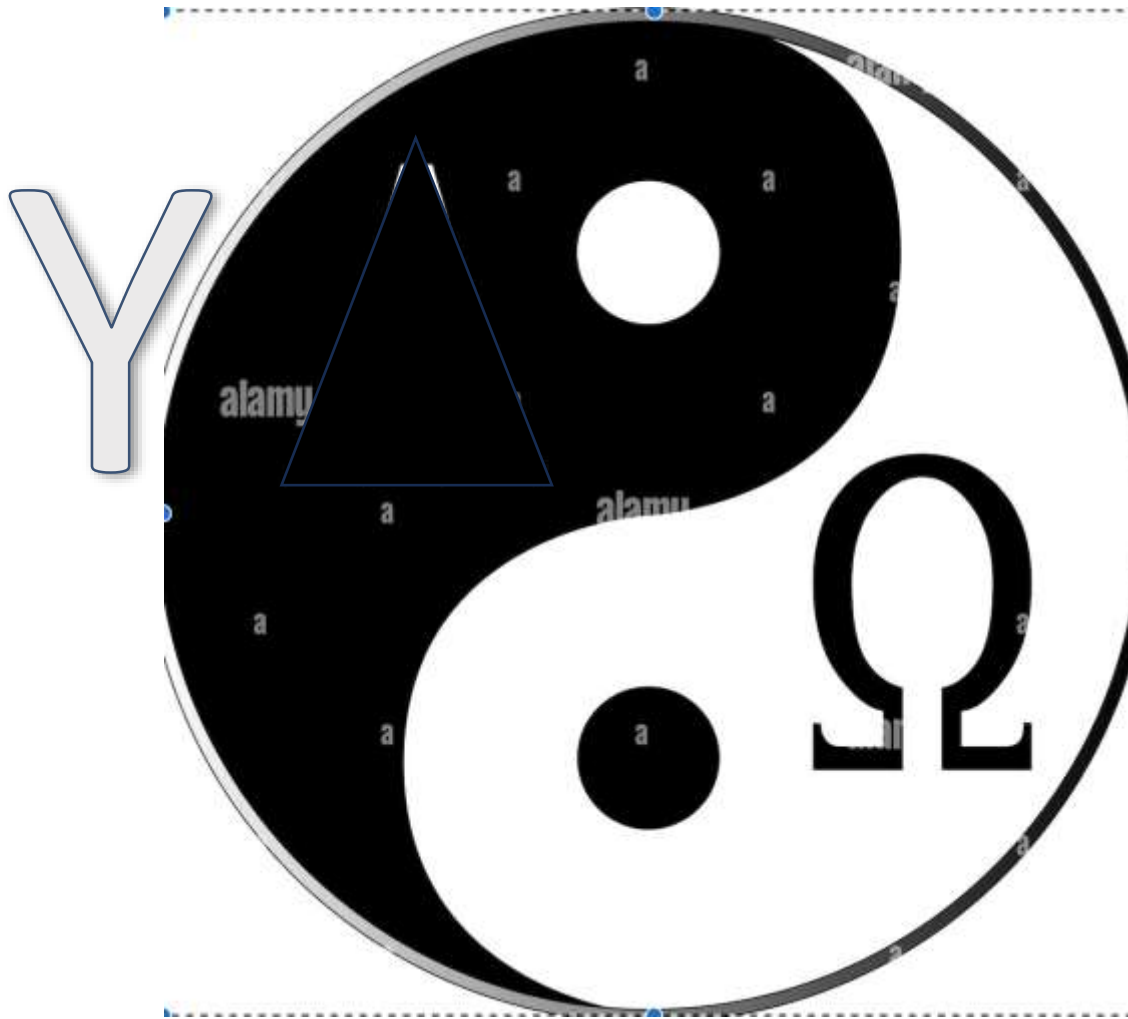
in the long-term to disclose eventual impact on clinical outcomes.



CASE MIX DISCLOSURE



- RYGB
- SG
- OAGB
- DS/SADI-S
- REVISIONAL
- ENDOSCOPIC



NAPOLI  
2023

**Different clusters, showing characteristic alpha and beta diversity and microbial composition, are identified at 12 months post-surgery.**

Dirichlet-Multinomial Mixture Model (DMM) was used to search for groups of samples that were like each other. The number of clusters was identified, sampled sorted following this identification in three groups. Group A (28 samples), group B 14 samples and group C 10 samples. Alpha diversity was assessed as richness (a), Shannon diversity index (b), Pielou's Evenness (c) and Faith's diversity (d) and represented as dot plot chart. Box plot chart reports median and 25<sup>th</sup> and 75<sup>th</sup> percentiles.

Principal coordinates analysis (PCoA) was used to represent *Weighted Unifrac* and *Bray-Curtis* beta diversity (e and f, respectively). Statistical differences between the three were assessed by (PERMANOVA) test. Relative abundances of the main phyla (g) and top 20 genera (h) were analysed and represented as bar chart. Statistical differences were evaluated using Kruskal-Wallis test followed by Bonferroni's correction.

