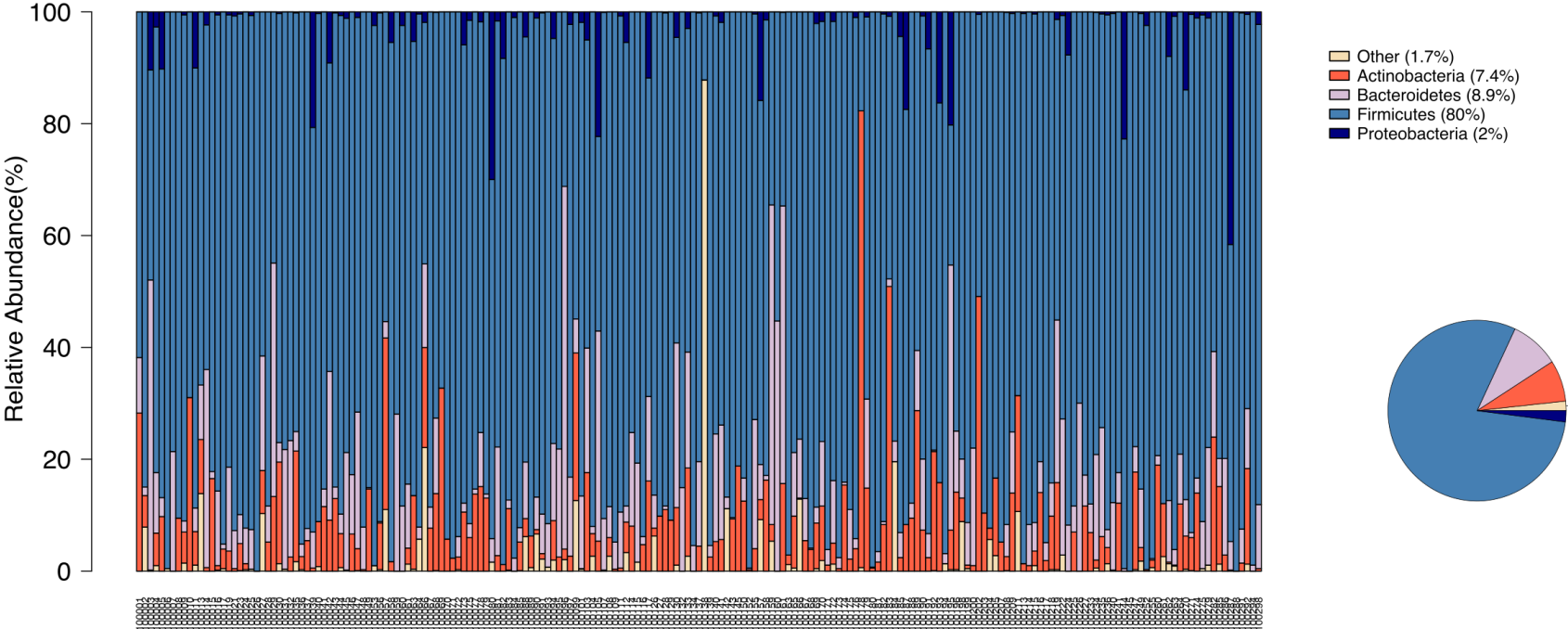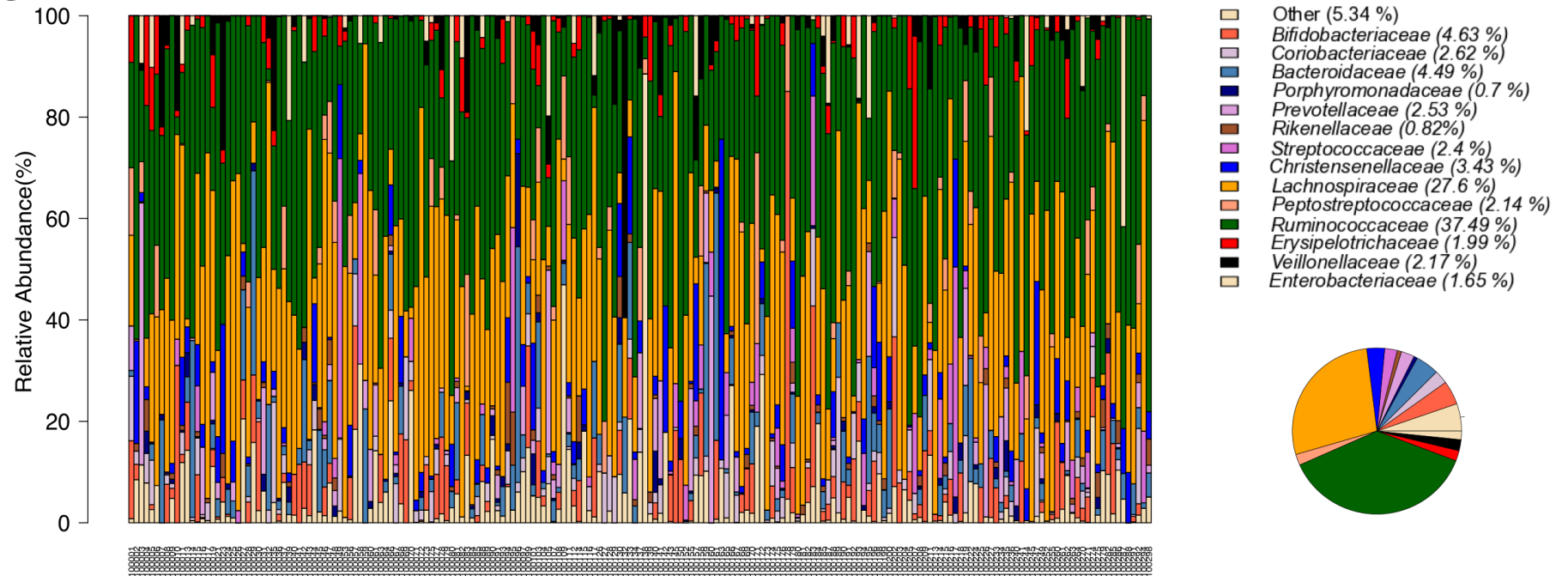# Supplementary material

## a

**b**

Relative Abundance(%)

Other (5.34 %)
*Bifidobacteriaceae* (4.63 %)
*Coriobacteriaceae* (2.62 %)
*Bacteroidaceae* (4.49 %)
*Porphyromonadaceae* (0.7 %)
*Prevotellaceae* (2.53 %)
*Rikenellaceae* (0.82%)
*Streptococcaceae* (2.4 %)
*Christensenellaceae* (3.43 %)
*Lachnospiraceae* (27.6 %)
*Peptostreptococcaceae* (2.14 %)
*Ruminococcaceae* (37.49 %)
*Erysipelotrichaceae* (1.99 %)
*Veillonellaceae* (2.17 %)
*Enterobacteriaceae* (1.65 %)

**c**

Legend (genus level):
- Bifidobacterium (4.63 %)
- Collinsella (1.46 %)
- Bacteroides (4.49 %)
- Parabacteroides (0.23 %)
- Alistipes (0.68 %)
- Streptococcus (2.32 %)
- Christensenellaceae R-7 group (3.41 %)
- Blautia (4.63 %)
- Dorea (1 %)
- Fusicatenibacter (3.14 %)
- Eubacterium rectale group (4.28 %)
- Butyricicoccus (0.49 %)
- Faecalibacterium (7.83 %)
- Ruminiclostridium 5 (0.38 %)
- Ruminococcaceae UCG-002 (2.43 %)
- Ruminococcaceae UCG-005 (0.74 %)
- Ruminococcaceae UCG-013 (0.45 %)
- Ruminococcaceae UCG-014 (1.71 %)
- Ruminococcus 1 (2.76 %)
- Ruminococcus 2 (3.54 %)
- Subdoligranulum (12.5 %)
- Eubacterium coprostanoligenes group (1.43 %)
- Erysipelotrichaceae UCG-003 (0.91 %)
- Dialister (1.31 %)
- Other 33.26 %
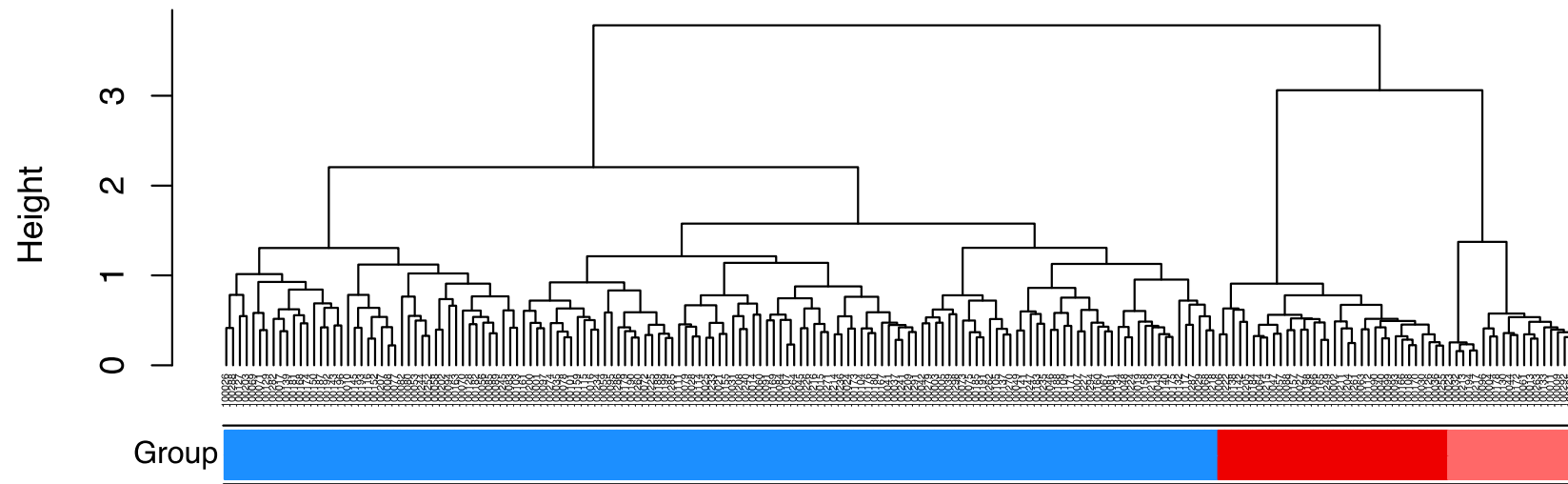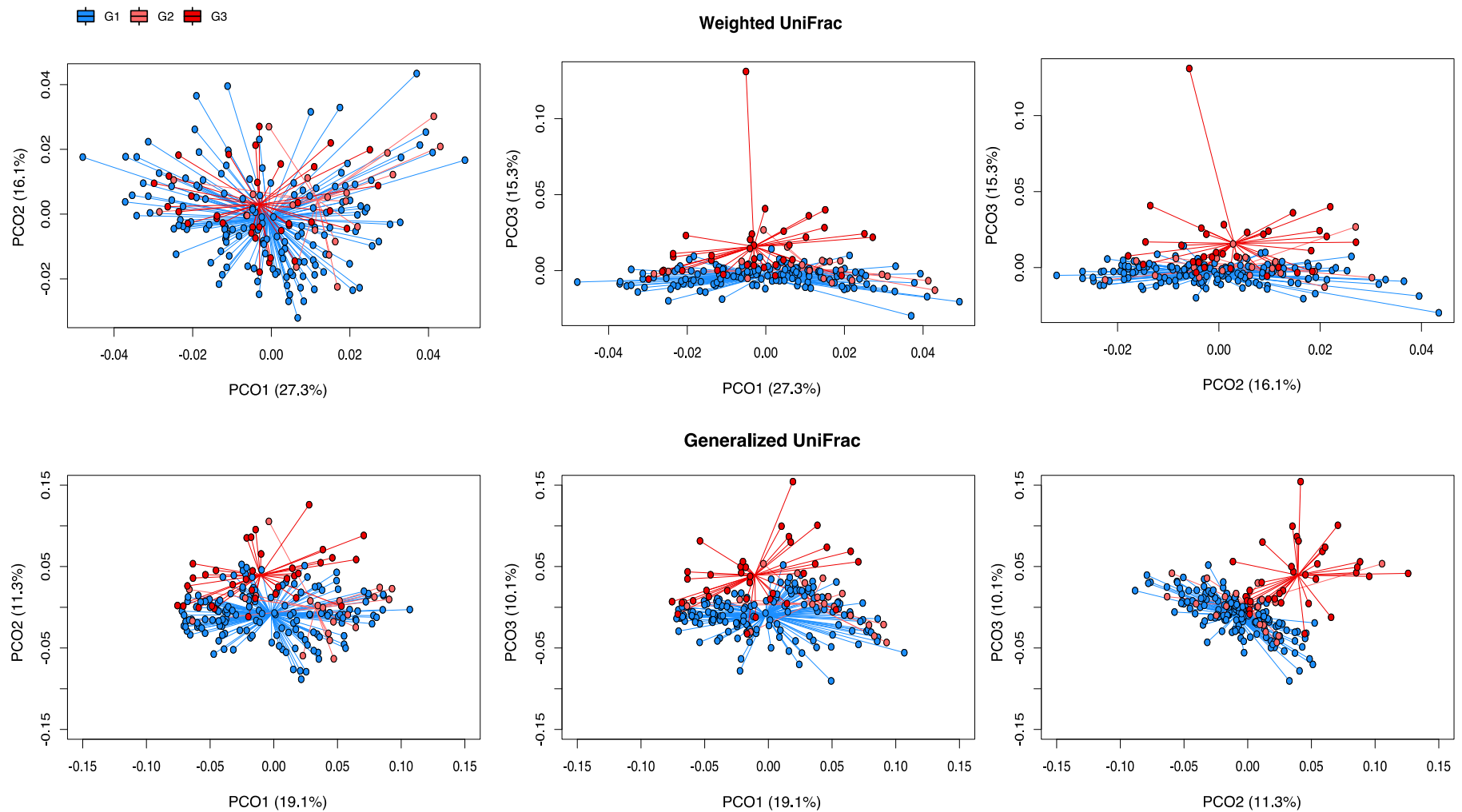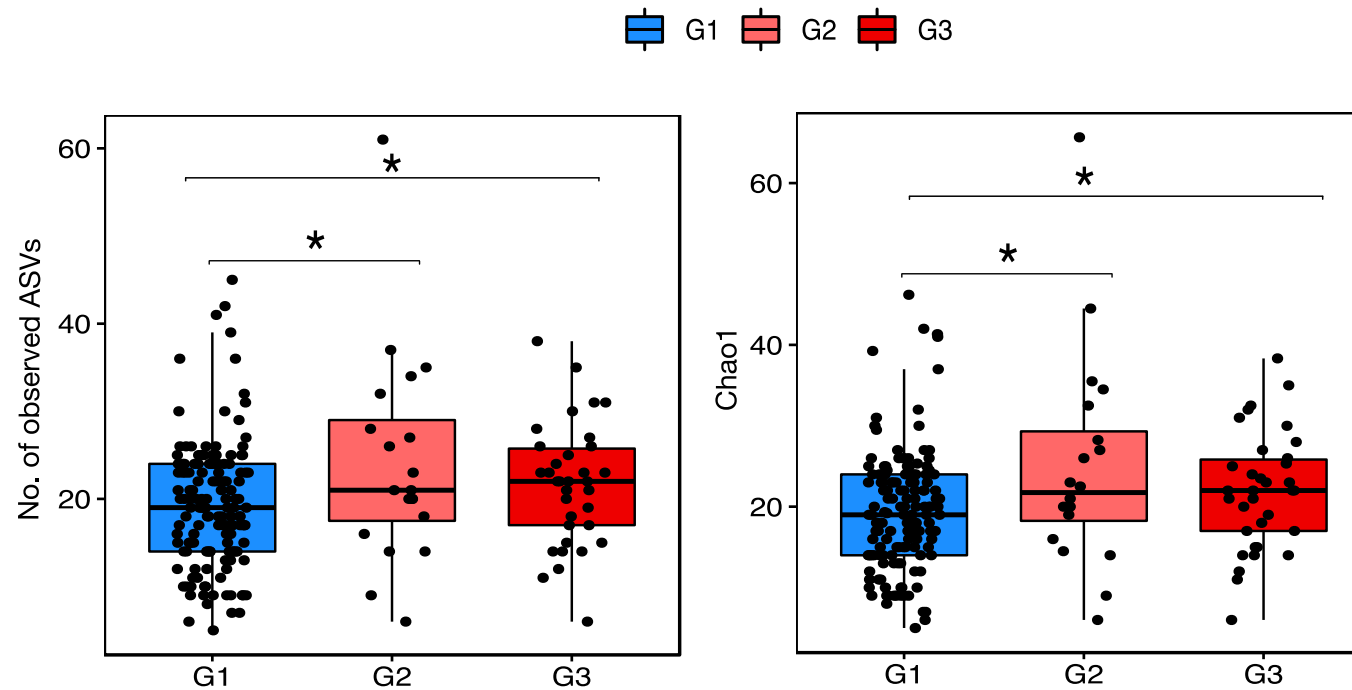
**Figure S1 Relative abundance of ASVs summarized at phylum (a), family (b) and genus (c) level in the faecal samples of 201 Italian elderly.** Bar graphs of the individual profiles of relative abundance, a pie chart with the average values and the colour legend are shown in each panel.
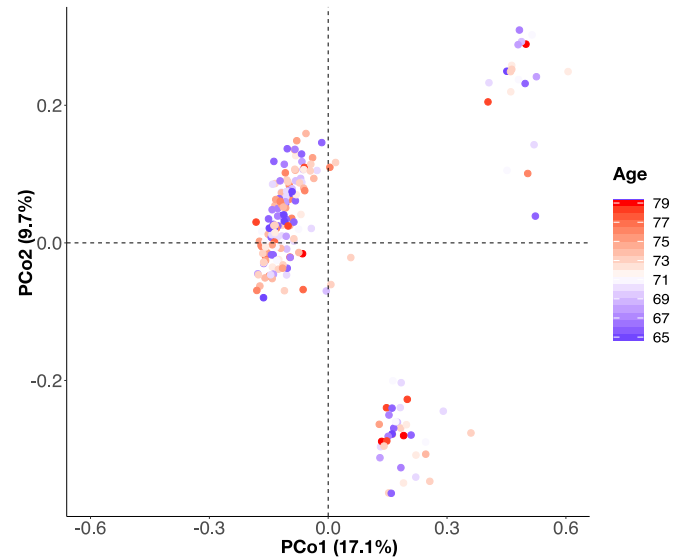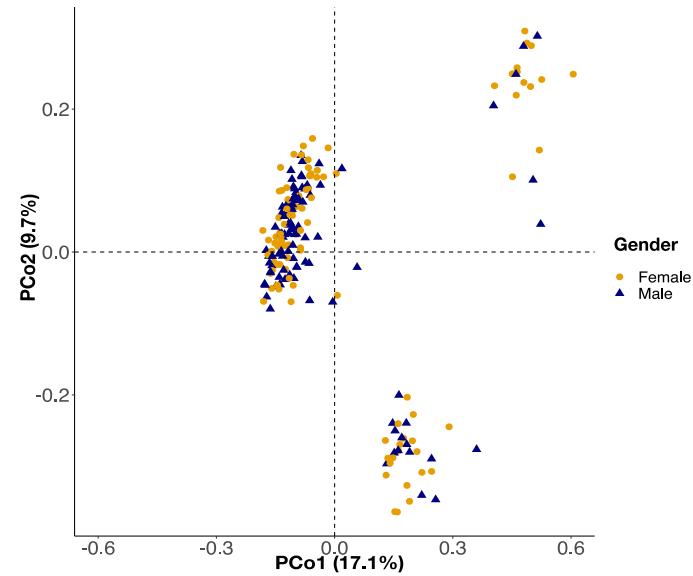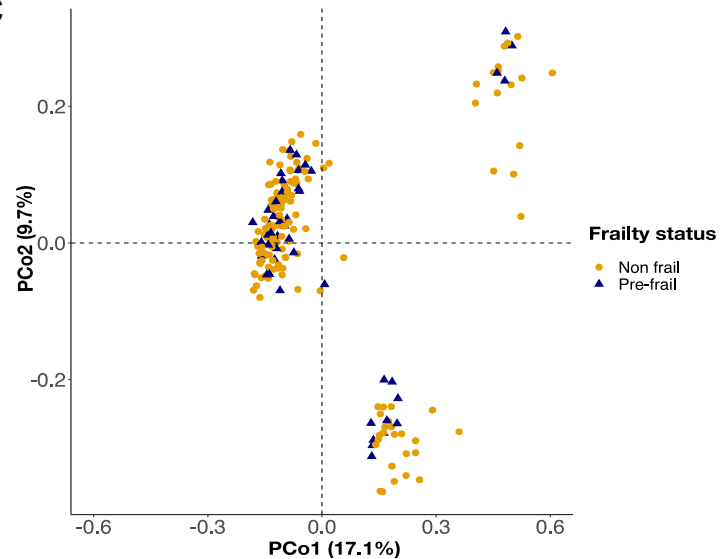
**Figure S2 Hierarchical clustering dendrogram showing the grouping of faecal microbiota profiles of 201 Italian elderly.**
The dendrogram, based on Ward-linkage clustering and computed on unweighted UniFrac distances, shows the same groupings as found in Figure 1a, *i.e.*, G1 (light blue), G2 (coral) and G3 (red) (Fisher's exact test, $p$ value $< 0.0001$). The cluster stability was assessed by using average Jaccard similarities from 1000 bootstrappings (G1, 0.96; G2, 0.95; G3, 0.92).
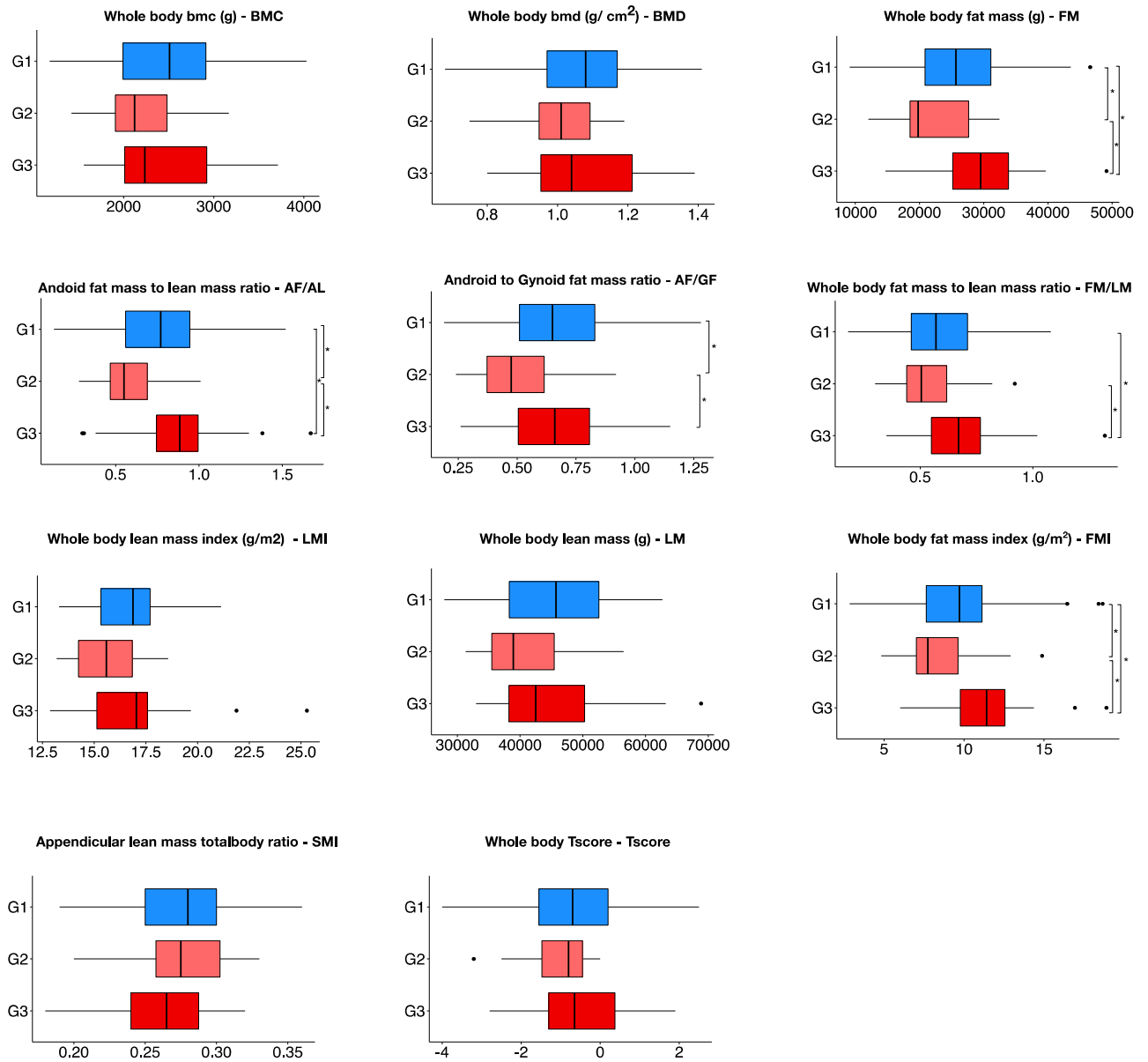
**Figure S3 Principal Coordinate Analysis showing the beta diversity of the faecal microbiota of 201 elderly Italians, with weighted UniFrac and generalized UniFrac distances.** The three microbiome groups (G1 to G3) as identified by PCoA of unweighted UniFrac distances (see Figure 1a) are shown. *P* value = 1e-04 for all analyses, permutation multivariate analysis of variance.

**Figure S4 Alpha diversity of the three microbiome groups.** Box-and-whisker plots showing the distribution of the number of observed ASVs and the Chao1 index in G1 to G3. G2 and G3 samples showed the highest biodiversity (Kruskal-Wallis test, *p* value = 0.05, followed by post-hoc Wilcoxon test, *p* value = 0.045 for both G1 vs G2 and G1 vs G3 comparisons).
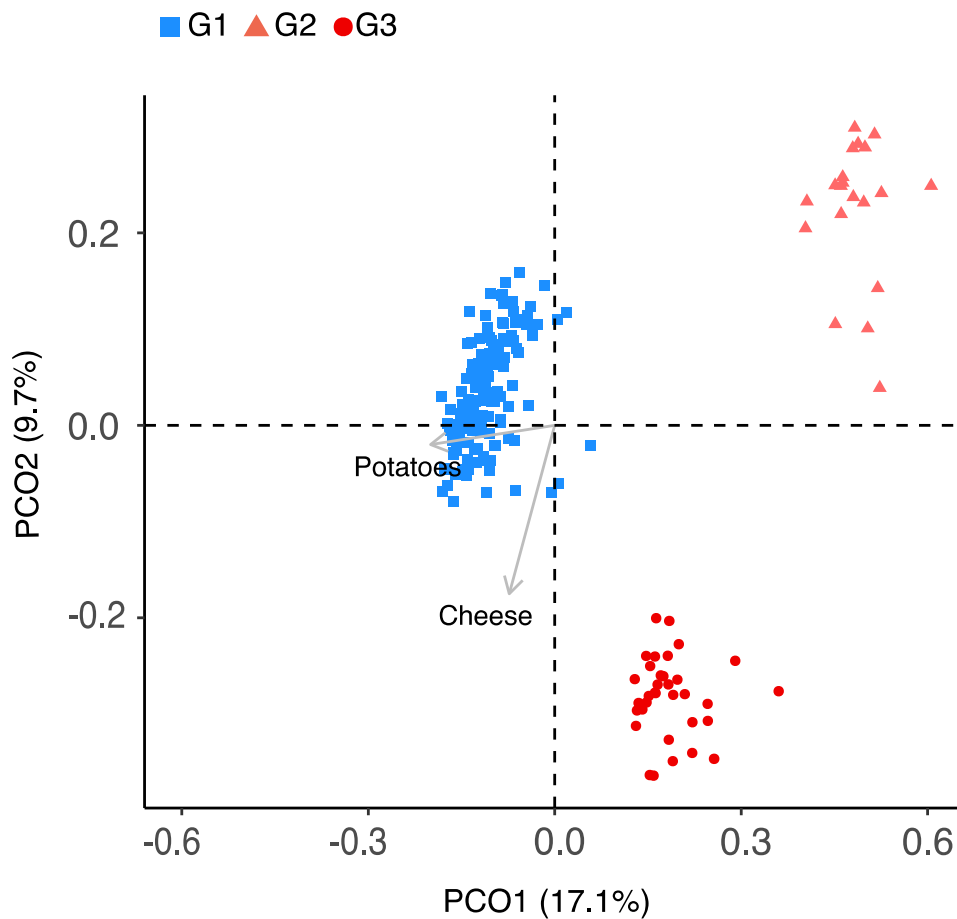
**a**



**b**



**c**



**Figure S5 Age, gender and frailty status distribution in the three microbiome groups (G1 to G3).** Unweighted UniFrac-based Principal Coordinates Analysis showing the age (**a**), gender (**b**), and frailty status (**c**) distribution in the three groups. Samples are coloured and identified with different shapes as described in each legend.
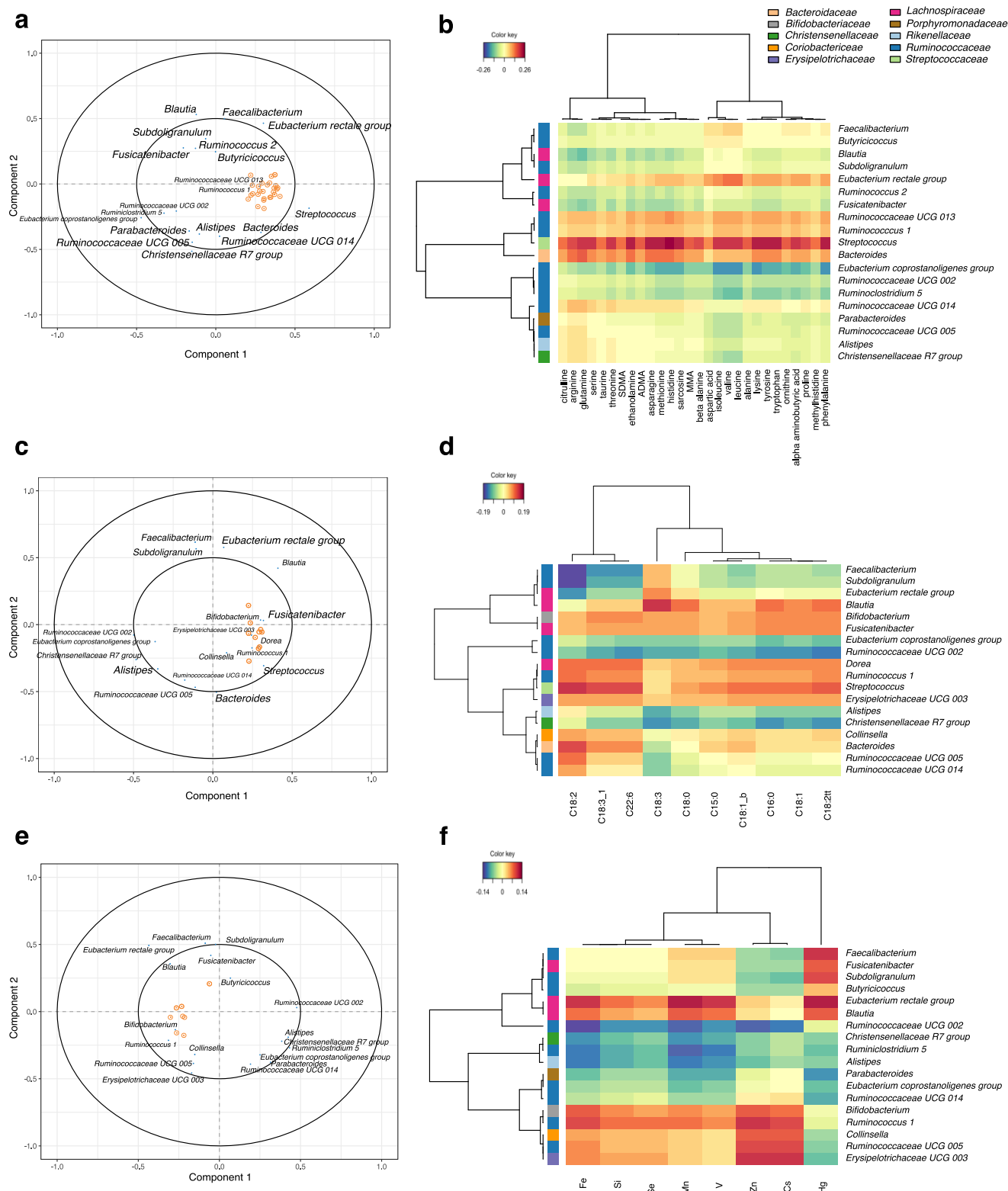
**Figure S6 Distribution of DXA variables in the three microbiome groups (G1 to G3).** Box-and-whisker plots showing the distribution of each variable in the three groups. *, *p* value < 0.05, Wilcoxon test.

**Figure S7 Food groups characterizing the microbiota profiles.** Principal Coordinates Analysis based on unweighted UniFrac distances of the stool microbiota. The consumption of potatoes and cheese (g/day) showed a significant correlation with sample separation (*p* values = 0.021 and 0.025, respectively; permutational correlation test).

**Figure S8 Associations between the elderly gut microbiome and the serum metabolome.** Sparse partial least square (sPLS) regression of microbial abundances at the genus level and metabolites (a-b, amino acids; c-d, fatty acids; e-f, minerals). Left, Correlation circle plot for the first two sPLS components. The two circumferences show correlation coefficient radii at 0.5 and 1.0. The farther from the centre a bacterial genus or metabolite is, the greater the association with the component. Variables projected in the same direction of the plot are positively correlated, while variables in diametrically opposite position are negatively correlated. Variables located perpendicular to each other are not correlated. The variance explained by the genera is 10% on the first component and 5% on the second component. The variance explained by the metabolites is as follows: amino acids, 49% on component 1

and 30% on component 2; fatty acids, 34% on component 1 and 9% on component 2; minerals, 11% on component 1 and 7% on component 2. Right, Hierarchical clustering obtained with complete linkage method and Pearson correlation as distance, was performed on the sPLS regression model retaining the variables shown in the correlation circle plot. For each genus, family level assignment is also shown (see colour legend).

**Table S1. Average daily intake of food groups.** All values were normalized to body weight (kg). Data are shown for the entire cohort as well as for the three microbiome groups (G1 to G3), as identified by PCoA of unweighted UniFrac distances (see Figure 1a). Values are expressed as mean (SD). *P* values were determined by Kruskal-Wallis test, followed by post-hoc Wilcoxon test.

| | All (no.=201) | G1 (no.=147) | G2 (no.=20) | G3 (no.=34) | *p* value |
|---|---|---|---|---|---|
| White grains (g/day) | 1.79 (0.87) | 1.76 (0.85) | 2.31 (0.95) | 1.61 (0.84) | ns |
| Whole grains (g/day) | 0.42 (0.61) | 0.44 (0.64) | 0.38 (0.65) | 0.38 (0.51) | ns |
| Fruit (g/day) | 4.00 (2.30) | 3.88 (1.89) | 5.61 (4.21) | 3.55 (2.03) | ns |
| Vegetables (g/day) | 3.14 (1.66) | 3.16 (1.64) | 3.79 (2.10) | 2.66 (1.32) | 0.09 |
| Fruit plus vegetables (g/day)* | 7.15 (3.28) | 7.07 (2.91) | 9.41 (5.63) | 6.20 (2.34) | ns |
| Legumes (g/day) | 0.18 (0.25) | 0.18 (0.27) | 0.14 (0.18) | 0.19 (0.18) | ns |
| Dairy products (g/day) | 2.34 (2.36) | 2.30 (2.60) | 2.54 (1.55) | 2.38 (1.58) | ns |
| Cheese (g/day) | 0.40 (0.28) | 0.40 (0.29) | 0.35 (0.28) | 0.43 (0.25) | ns |
| Red and processed meat (g/day) | 0.72 (0.42) | 0.72 (0.42) | 0.77 (0.46) | 0.68 (0.37) | ns |
| White meat (g/day) | 0.30 (0.32) | 0.31 (0.31) | 0.28 (0.39) | 0.29 (0.33) | ns |
| Nuts and seeds (g/day) | 0.09 (0.19) | 0.09 (0.20) | 0.05 (0.11) | 0.10 (0.15) | ns |
| Potatoes (g/day)[#] | 0.23 (0.28) | 0.27 (0.30) | 0.10 (0.16) | 0.16 (0.25) | 0.002 |
| Eggs and egg products (g/day) | 0.11 (0.12) | 0.11 (0.12) | 0.10 (0.12) | 0.10 (0.11) | ns |
| Butter and animal fats (g/day) | 0.01 (0.03) | 0.01 (0.03) | 0.01 (0.03) | 0.01 (0.02) | ns |
| Olive oil and other vegetables oils (g/day) | 0.24 (0.12) | 0.23 (0.11) | 0.27 (0.15) | 0.23 (0.10) | ns |
| Sugar-sweetened beverages (g/day) | 0.27 (0.66) | 0.31 (0.70) | 0.10 (0.24) | 0.22 (0.64) | ns |
| Sugar, honey and artificial sweeteners (g/day) | 0.09 (0.12) | 0.10 (0.13) | 0.06 (0.06) | 0.10 (0.09) | ns |
| Sweet, chocolate and snacks (g/day) | 0.69 (0.52) | 0.67 (0.49) | 0.65 (0.63) | 0.80 (0.60) | ns |

*Wilcoxon test: G2 vs G3, *p* value = 0.058.
[#]Wilcoxon test: G1 vs G2, *p* value < 0.05.