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Coláiste na hOllscoile Corcaigh

Diet determines gut microbiota in the elderly which correlates with health

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Alterations in intestinal microbiota composition are associated with chronic conditions including obesity and inflammatory diseases. The microbiota of older persons displays greater inter-individual variation than that of younger adults. Here we show that the faecal microbiota composition from 178 elderly subjects formed distinct groups correlating with residence location, in the community, day-hospital, rehabilitation, or in long-term residential care. However, clustering of subjects by diet separated them by the same residence location and microbiota groupings. The separation of microbiota composition significantly correlated with measures of frailty, co-morbidity, nutritional status, markers of inflammation and with metabolites in faecal water. The individual microbiota of people in long-stay care was significantly less diverse than that of community dwellers. Loss of community-associated microbiota correlated with increased frailty. Collectively the data support a relationship between diet, microbiota and health status, and indicate a role for diet-driven microbiota alterations in varying rates of health decline upon aging.

The gut microbiota is required for development and for homeostasis in adult life. Compositional changes have been linked with inflammatory and metabolic disorders[1], including inflammatory bowel disease[2,3], irritable bowel syndrome[4,5] and obesity[6] in adults. The composition of the human intestinal microbiota is individual-specific at the level of Operational Taxonomic Units (OTUs) and stable over time in healthy adults[7]. The composition of the intestinal microbiota in older persons (>65 years) is extremely variable between individuals[8], and differs from the core microbiota and diversity levels of younger adults[8,9]. A feature of the ageing process is immuno-senescence or "inflammaging", evidenced by persistent NF-κB-mediated inflammation and loss of naïve $CD4^+$ T-cells[10]. The microbiota is pivotal for homeostasis in the intestine[11], and chronic activation of the innate and adaptive immune system is linked to inflammaging[12]. Correlations have previously been made between specific components of the microbiota and pro-inflammatory cytokine levels, but these did not separate young adults from older persons[9]. Alterations in the microbiota composition have also been associated with frailty[13], albeit in a small cohort from a single residence location.

Deteriorations in dentition, salivary function, digestion, and intestinal transit time[14] may affect the intestinal microbiota upon aging. A controllable environmental factor is diet, which has been shown to influence microbiota composition in animal models, in small-scale human studies[15-20], and over the longer term[21]. However, links between diet, microbiota composition and health in large human cohorts are unclear. To test the hypothesis that variations in the intestinal microbiota of older subjects impact on inflammaging and frailty across the community, we determined the faecal microbiota composition in 178 older persons. We also collected dietary intake information, and measured a range of physiological, psychological and immunological parameters. Dietary groupings were associated with separations in the microbiota and health datasets whereby the healthiest people live in a community setting, eat differently and have a distinct microbiota from those in long-term residential care. Measures of increased inflammation and increased frailty support a diet-microbiota link to these indicators of accelerated aging, and suggest how dietary adjustments could promote healthier aging by modulating the gut microbiota.

Microbiota and residence location

We previously identified considerable inter-individual variability in the faecal microbiota composition of 161 older persons (≥65 years), including 43 receiving antibiotics [8]. To investigate links between diet, environment, health and microbiota, we analyzed 178 subjects, non-antibiotic treated, for whom we also had dietary information, and stratified by community residence setting: (a) community-dwelling, n=83; (b) attending an out-patient day hospital n=20; (c) in short-term (< 6-weeks) rehabilitation hospital care, n=15; (d) in long-term residential care (long-stay), n=60. The mean subject age was 78 (±8 s.d.) years, with a range of 64 to 102 years, and all were of Caucasian (Irish) ethnicity. We included 13 young adults with a mean age of 36 (±6 s.d.) years. We generated 5.4 million sequence reads from 16S rRNA gene V4 amplicons, with an average of 28,099 (±10,891s.d.) reads per subject.

UniFrac β-diversity analysis indicates the extent of similarity between microbial communities[22]. UniFrac PCoA analysis of 47,563 OTUs (grouped at 97% sequence identity) indicated a clear separation between community-dwelling and long-stay subjects using both weighted and un-weighted analysis (Fig. 1A, 1B). Microbiota from the 13 younger controls clustered with community-dwelling subjects. Eighteen other non-UniFrac β-diversity metrics supported microbiota separation by residence location (Supplementary Fig. 1).

When we examined OTU abundance, we identified a cluster comprising the majority of the long-stay subjects separated from the majority of the community-dwelling and young healthy subjects (Fig. 1C). Family-level microbiota assignments showed that long-stay microbiota had a higher proportion of phylum *Bacteroidetes*, compared to a higher proportion of phylum *Firmicutes* and unclassified reads in community-dwelling subjects (Fig. 1C). At genus level, *Coprococcus* and *Roseburia* (of the *Lachnospiraceae* family) were more abundant in the faecal microbiota of community-dwelling subjects (Supplementary Table 1 shows complete list of genera differentially abundant by community location). Genera associated with long-stay subjects included *Parabacteroides*, *Eubacterium*, *Anaerotruncus*, *Lactonifactor* and *Coprobacillus* (Supplementary Table 2). The genera associated with community belonged to fewer families; *Lachnospiraceae* were the most dominant. Thus, the microbiota composition of an individual segregated depending on where they lived within a single ethnogeographic region, in a homogeneous cohort where confounding effects of climate, culture, nationality and extreme environment were not a factor.

Concordance of diet and microbiota

Dietary data (for 168 of the 178 subjects, plus five PEG-fed subjects [percutaneous endoscopic gastrostomy]) was collected through a semi-quantitative, 147-item, food frequency questionnaire (FFQ), weighted by 10 consumption frequencies. This data was visualised with Correspondence Analysis (CoA; Fig. 2A). The first CoA axis described over 11% of the dataset variance and most differences in food consumption between community-dwelling and long stay subjects. The most discriminating food types were vegetables, fruit and meat, whose consumption changed in a gradual manner along the first eigenvector. Procrustes analysis of the FFQ and the microbiota β-diversity was used to co-visualise the data (Fig. 2B). Separations based on either diet or microbiota co-segregated along the first axis of both datasets (unweighted and weighted UniFrac Fig 2B; Monte-Carlo P-value < 0.0001). Application of complete linkage clustering and Euclidean distances to the first eigenvector (Fig. 2C) revealed four Dietary Groups (DGs). DG1 ("low fat/high fibre") and DG2 ("moderate fat/high fibre") included 98% of the community and day hospital subjects, and DG3 ("moderate fat/low fibre") and DG4 ("high fat/low fibre") included 83% of the long-stay subjects. For a complete description of DGs, see Supplementary Notes and Supplementary Table 3.

The Healthy Food Diversity index (HFD[23]) positively correlated with three microbiota diversity indices (Supplementary Fig. 2A), and all four indices showed significant differences between community and long-stay subjects (Supplementary Fig. 2B), suggesting that a healthy, diverse diet promotes a more diverse gut microbiota. Analysing by DGs rather than residence location confirmed that both microbiota and diet were most diverse in DG1, and least diverse in DG3 and DG4 (Suppl. Fig 3). Procrustes analysis similarly showed that the DGs were associated with separations in microbiota composition (Suppl. Fig 3). Furthermore, the microbiota was associated with the duration in long-stay, with residents for more than a year having a microbiota that was furthest separated from community-dwelling subjects (Suppl. Fig 4). For the majority of these longer-term residents, the diet was different from that in more recently admitted subjects (Suppl. Fig 4). Examination of duration of care (Suppl. Fig 4C), showed that diet changed more quickly than the microbiota did, both in the direction away from the community types. After one month in long stay, all subjects had a long-stay diet, but it took a year for the microbiota to be clearly the long-stay type. Collectively the data suggested that the composition of the microbiota was determined by the composition and diversity of the diet.

Community setting and faecal metabolome

Faecal metabolites correlate with microbiota composition and inflammatory scores in Crohn's Disease[24]. We therefore performed metabolomic analysis (NMR spectroscopy) of faecal water from 29 subjects, representative (by UniFrac) of three community settings. (Day-hospital subjects grouped closely to community-dwellers by microbiota and dietary analysis, and were not included). A representative NMR profile is presented in Supplementary Fig 5. Initial PCA analysis showed a trend for separation according to community setting (data not shown). Pair-wise statistical models were therefore constructed according to the cluster groups. Valid and robust models were obtained for comparison of NMR spectra from community and long-stay subjects, and community and rehabilitation subjects (Fig. 3). The major metabolites separating community from long-stay subjects were glucose, glycine and lipids (higher levels in long-stay than community), and glutarate and butyrate (higher levels in community subjects). Co-inertia analysis of the genus-level microbiota and metabolome data revealed a significant relationship (p-value <0.01) between the two datasets (Supplementary Figure 6 and Supplementary Notes). Notwithstanding three long-stay subjects, a diagonal separated community from long-stay in both microbiota and metabolome datasets. Other metabolites of interest were acetate, propionate and valerate, which were more abundant in community dwellers (Supplementary Fig. 6).

To further investigate microbial short-chain fatty acid (SCFA) production, the frequency of microbial genes for SCFA production was investigated by shotgun metagenomic sequencing. We sequenced 125.9 Gb of bacterial DNA from 27 of the 29 subjects, and assembled contigs with a total length of 2.20 Gb, containing 2.51 million predicted genes (Suppl. Table 4). Consistent with reduced microbiota diversity, (Supp. Fig. 3), there were significantly fewer total genes predicted, and higher N50 values, in the assembled metagenomic data of long-stay subjects compared to rehabilitation or community subjects (Supp. Figure 7). The metagenomes were then searched for key microbial genes in butyrate, acetate and propionate production, revealing significantly higher gene counts and coverage for butyrate- and acetate- producing enzymes (BCoAt and ACS, respectively) in community and rehabilitation compared to long-stay subjects (Suppl. Fig 8 and Suppl. Table 5). There was also significantly higher coverage of the propionate related genes (PCoAt) in community compared to long-stay subjects but the higher gene count was not significant (Suppl. Table 5). These observations are consistent with the association of butyrate, acetate and propionate and the direction of the main split between long-stay and

community subjects in the metabolome; candidate genera associated include *Ruminococcus* and *Butyricicoccus* for butyrate production (Supplementary Fig 6), but require validation in larger cohorts. Microbiota function deduced from the metagenome thus corresponded to the measured metabolome for at least one key metabolite that can affect health[25].

Microbiota-health correlations

Markers of inflammation (serum TNFα, IL-6 and IL-8 and C-reactive protein (CRP)) were significantly higher in long-stay and rehabilitation subjects than in community dwellers (Supplementary Fig. 9). Long-stay subjects also scored poorly for diverse health parameters (Supplementary Tables 6 and 7) including the Charlson co-morbidity index (CCI, a robust predictor of survival encompassing 19 medical conditions[26]), the Geriatric Depression Test (GDT), the Barthel Ìndex (BI[27]), Functional Independence Measure (FIM[28]), Mini-mental State Exam (MMSE[29]), and Mini Nutritional Assessment (MNA[30]).

Correlations between health parameters and microbiota composition were examined using quantile (median) regression tests, adjusted for gender, age and community setting with an additive model (Supplementary Methods). Median regression gives less weight to extreme values than the linear regression based on ordinary least squares and consequently, is less influenced by outliers. The model was adjusted for medications that might influence the tested parameters (Supplementary Table 8). The effect of medication was generally small (Supplementary Table 8). Since ethnicity was exclusively Irish Caucasian it did not require model adjustment. The microbiota composition did not differ for males and females after adjusting for age and location.

Significant associations between several health/frailty measurements and the major separations from microbiota UniFrac analysis (Fig. 1) are shown in Table 1. For example, a positive change in microbiota along the full range of the PC1 axis in the un-weighted UniFrac PcoA for long-stay-only subjects was associated with inflammation (CRP increase of 13.9 mg/l), and other inflammatory markers significantly correlated with microbiota (IL-6 and IL-8, whole cohort). As expected, there was minimal variability amongst community-dwelling subjects, but within the long-stay subjects the most significant associations related to functional independence (FIM), Barthel index, and nutrition (MNA), followed by blood pressure, and calf circumference. The latter may be attributable to the influence of diet and/or the microbiota on muscle mass, sarcopaenia[31], and

thereby on frailty. This was supported by investigation of linkage between frailty and faecal metabolites (Probabilistic principal components and covariates analysis; PPCCA[32]). Thus, FIM and Barthel indices were significant covariates with the faecal water metabolome (Supplementary Figure 10) and levels of acetate, butyrate and propionate increased with higher values of both indices (i.e. less frail subjects). Amongst community-dwelling subjects, there was also a strong association between microbial composition and nutrition (MNA) and a weaker link with blood-pressure, for which a relationship with the microbiota has previously been established[33]. There was no correlation between the *Bacteroidetes* : *Firmicutes* ratio and BMI, though there was a correlation with overall microbiota in long-stay subjects. Measures for the Geriatric Depression Test (GDT) showed significant microbiota association with PCoA axis 2 (Table 1). We detected no significant confounding of microbiota-health correlations due to medications, antibiotic treatment (prior to the one-month exclusion window), and diet-health correlations separate from dietary impact on microbiota (Supplementary Notes).

Taken together, the major trends in the microbiota that separated healthy community subjects from less healthy long-stay subjects were associated with markers for increased frailty and poorer health, having adjusted for gender, age and location. Because location largely determines diet (Fig. 2), adjusting for location reduces the effect of diet, and since there was also clear evidence for microbiota-health associations within the long-stay setting, we infer that the causal relationship is in a diet-microbiota-health direction.

Microbiota structure and healthy ageing

Gut microbiota can be assigned to one of three enterotypes[34], driven by *Bacteroides*, *Prevotella* and *Ruminococcus* species. A recent study detected only the *Bacteroides* and *Prevotella* enterotypes, which were associated with diets rich in protein and carbohydrate, respectively[21]. Using those methods, we predicted an optimal number of two clusters using five of six methodologies, albeit with weaker support than previous studies (Suppl. Fig 11). In line with a previous study[21], the two clusters associated with *Bacteroides* and *Prevotella*, but not *Ruminococcus*. Although enterotype assignments from the three approaches were very different (Supp. Fig. 11), community subjects were more frequently of the *Prevotella* enterotype.

To identify patterns in the microbiota, we established co-abundance associations of genera (Supplementary Fig. 12A), and then clustered correlated genera into six co-abundance groups (CAGs) (Supplementary Fig. 12B). These are not alternatives to enterotypes, which are subject-driven and poorly

supported in this elderly cohort, but they describe the microbiota structures found across the subject groups in statistically significant co-abundance groups (Supplementary Notes). The dominant genera in these CAGs were *Bacteroides*, *Prevotella*, *Ruminococcus*, *Oscillibacter*, *Alistipes*, and the central *Odoribacter* CAG. These CAG relationships are termed Wiggum plots, in which genus abundance can be represented as discs proportional to abundance (Supplementary Fig. 12), to normalized over-abundance (Fig. 4), or to differential over-abundance (Supp. Fig. 13). In the Wiggum plot corresponding to the whole cohort (Supplementary Fig. 12), the path away from the *Ruminococcus* CAG towards the *Oscillibacter* CAG shows a reduced number of genera that make butyrate, and an increased number able to metabolize fermentation products.

To simplify the microbiota data for health correlation, we utilized the eight subject divisions identified by OTU clustering (Fig. 1C). These eight divisions were superimposed on a UniFrac PCoA analysis of the data in Fig 1A, defining 8 subject groups (Fig. 4, Groups 1a through 4b). These are separation points within a microbiota composition spectrum that represent groups of individuals who have significantly different microbiota as defined by the permutation MANOVA test on un-weighted Unifrac data. We then constructed individual Wiggum plots for the microbiota in these 8 groups (Fig. 4). The transition from healthy community-dwelling subjects, to frail long-term care residents, is accompanied by distinctive CAG dominance, most significantly in abundances of *Prevotella* and *Ruminococcus* CAGs (community associated) and *Alistipes* and *Oscillibacter* CAGs (long-stay associated).

Our analysis of Figure 4 suggested two paths from community-associated health to long-stay associated frailty (plot 1a through 4a, and 1b through 4b), which were examined with reference to health correlations in Table 1, plus separate PCoAs for the community-only, and long-stay only subjects. The community and whole-cohort analyses identified an association of depression with axis 2 – subjects in the lower path had higher GDT scores. IL-6 and IL-8 levels were higher in the upper path by whole-cohort analysis (Fig. 4; Supplementary figure 14), while CRP levels were higher in the lower path in long-stay-only analysis. Furthermore, subjects in the lower path had higher systolic and diastolic BP, except in the community-only analysis. This apparent inconsistency is explained by a highly significant change in diastolic BP along the primary PCoA axis in the long-stay subjects, emphasizing the value of a stratified cohort. The subjects in the upper path were older but displayed higher Barthel and FIM scores than subjects of a similar age in the lower path (Supp. Fig. 14), consistent with healthier aging. Movement along PCoA axis 1 of the whole cohort (i.e. from community to

long-stay left to right, Fig. 4) is associated with a reduction in abundance of *Ruminococcus* and *Prevotella*, and increased abundance of the *Oscillibacter* CAG, accompanied by calf circumference decrease and weight decrease (Table 1), and increase in IL-6 levels. Moving along axis 1 of the long-stay PCA (i.e. between the two right-ward arms) is accompanied by a reduction in the *Oscillibacter* CAG, increase in abundance of the *Bacteroides* CAG, reduced FIM and Barthel indices, and increased levels of CRP (Fig. 4). Consideration of the microbiota-health correlations in the long-stay cohort (Fig. 4), upwards along axis 2, highlights the association with increased frailty, reduced muscle mass, and poorer mental activity moving away from community type microbiota.

Health-microbiota associations were statistically significant, even when regression models were adjusted for location. Although other factors undoubtedly contribute to health decline, which are difficult to completely adjust for in retrospective studies, the most plausible interpretation of our data is that diet shapes the microbiota, which then impacts on health in older persons. Diet-determined differences in microbiota composition may have subtle impacts in young adults in developed countries which would be difficult to correlate with health parameters, but become far more evident in the elderly who are immunophysiologically compromised. This is supported by the stronger microbiota-health associations evident in the long-stay cohort, where there is now a reasonable case for microbiota-related acceleration of aging-related health deterioration. An ageing population is now a generalized feature of western countries[35,36] and an emerging phenomenon even among developing countries. The association of the intestinal microbiota of older persons with inflammation[12] and the clear association between diet and microbiota outlined in this and previous studies[20,21,37,38], argue in favour of an approach of modulating the microbiota with dietary interventions designed to promote healthier aging. Dietary supplements with defined food ingredients that promote particular components of the microbiota may prove useful for maintaining health in older persons. On a community basis, microbiota profiling, potentially coupled with metabolomics, offers the potential for biomarker-based identification of individuals at risk for, or undergoing, less healthy aging.

METHODS SUMMARY

Amplicons of the 16S rRNA gene V4 region were sequenced on a 454 Genome Sequencer FLX Titanium platform. Sequencing reads were quality-filtered, OTU-clustered, chimera-filtered and further analyzed using the QIIME pipeline[39] and RDP-classifier[40]. Statistical analysis was performed using Stata and R software packages. Nuclear magnetic resonance (NMR) spectroscopy was performed on a 600 MHz Varian NMR Spectrometer as previously described[41].

Habitual dietary intake was assessed using a validated, semi-quantitative, food frequency questionnaire (FFQ), administered by personnel who received standardised training in dietary assessment. FFQ coding, data cleaning and data checks were conducted by a single, trained individual to ensure consistency of data.

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Author contribution statement All authors are members of the ELDERMET consortium (http://eldermet.ucc.ie). PWOT, EOC, SC[1] and RPR managed the project; DVS, GFF, CS, JRM, FS, CH, RPR and PWOT designed the analyses; MJC, IBJ, SC[3], EOC, HH, MC, BL, OOS, APF, MW and LB performed the analyses; JD performed DNA extraction and PCR; MW and LB performed NMR metabolomics; MOC, NH, KOC, and DOM performed clinical analyses; MJC, IBJ, SC[3], EOC, LB, JRM, APF, RPR, CH, FS, and PWOT wrote the manuscript.

Author Information Amplicon sequence data, and shotgun sequence data, contigs, genes and annotations, have been deposited in MG-RAST under the Project ID 154. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to PWOT (pwotoole@ucc.ie).

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Figure legends

Figure 1. Microbiota analysis separates elderly subjects based upon where they live in the community. A) unweighted and B) weighted UniFrac PCoA of faecal microbiota from 191 subjects. Subject colour coding: Community (green), Day Hospital (yellow), Rehabilitation (orange), Long-stay (red), and Young healthy control subjects (purple). C) Hierarchical Ward-linkage clustering based on the Spearman correlation coefficients of the proportion of OTUs, filtered for OTU subject prevalence of at least 20%. Subjects colour coding as in A. Labelled clusters in top of panel C (basis for the eight groups in Figure 4) are highlighted by black squares. OTUs are clustered by the vertical tree, colour coded by Family assignments. *Bacteroidetes* phylum: blue gradient, *Firmicutes* - red, *Proteobacteria* - green, and *Actinobacteria* - yellow. Only 774 OTUs confidently classified to Family level are visualised. The bottom panel shows relative abundance of Family-classified microbiota.

Figure 2. Dietary patterns in community location correlate with separations based upon microbiota composition. A) Food correspondence analysis. Top panel, FFQ PCA; bottom panel, driving food types. B) Procrustes analysis combining unweighted and weighted UniFrac PCoA of microbiota (non-circle end of lines) with Food Type PCA (circle-end of lines). C) Four dietary groups revealed through complete linkage clustering using Euclidean distances applied to first eigenvector in correspondence analysis. Colour codes in A, and horizontal clustering in B and C, are community location, as per Figure 1. Food labelling in lower panel in A, and vertical clustering in C: fruit and vegetables (green), grains such as potatoes/cereals/bread (orange), meat (brown), fish (cyan), dairy products (yellow), sweets/cakes/alcohol (blue), vitamins/minerals/tea . (grey). Only peripheral and most driving foods are labelled; for complete list see Suppl. Table 2.

Figure 3. PLS-DA plots of ^{1}H NMR spectra of faecal water from A) community subjects (green) versus long-stay subjects (red); R^2=0.517, Q^2=0.409, 2 component model,B) community subjects (green) versus rehabilitation subjects (orange).R^2=0.427, Q^2=0.163, 2 component model. The ellipses represent the Hotellings T2 with 95% confidence. To confirm the validation of the model, permutation tests (n=1,000) were performed. For model A, the 95% CI for the misclassification error rate (MER) was (0.43, 0.57). Using the PLS-DA model

on the data resulted in an MER of 0.2 which is outside the 95% CI obtained for random permutation tests thus validating the model. For model B, using permutation testing the 95% CI for the MER was (0.45, 0.55). Using the PLS-DA model on the data resulted in an MER of 0.16 which is outside the 95% CI obtained for random permutation tests.

Figure 4. Transition in microbiota composition across residence location is mirrored by changes in health indices. The PCoA plots show 8 groups of subjects defined by unweighted UniFrac microbiota analysis of community subjects (left), the whole cohort (centre), and long-stay subjects (right). The main circle shows the Wiggum plots corresponding to the 8 groups from whole-cohort analysis, in which disc sizes indicate genus over-abundance relative to background. The pie charts show residence location proportions (colour coded as in Fig 1C) and number of subjects per subject group. Curved arrows indicate transition from health (green) to frailty (red).

Figure 1

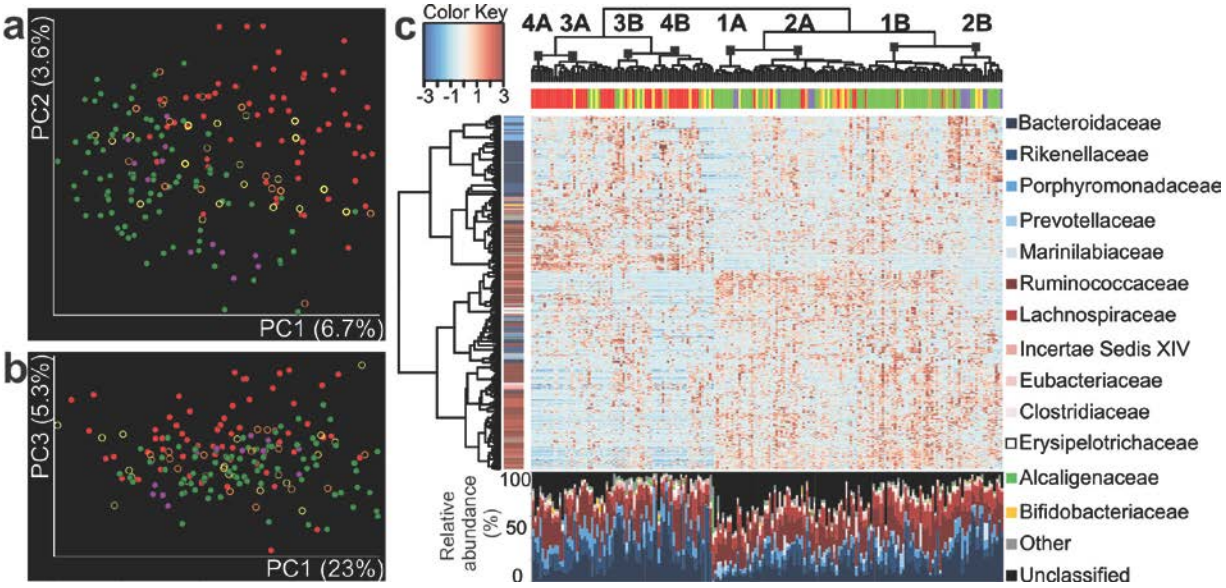

a
PC2 (3.6%)
PC1 (6.7%)
b
PC3 (5.3%)
PC1 (23%)
c
Color Key
-3 -1 1 3
4A 3A 3B 4B 1A 2A 1B 2B
Relative abundance (%)
100
50
0
Bacteroidaceae
Rikenellaceae
Porphyromonadaceae
Prevotellaceae
Marinilabiaceae
Ruminococcaceae
Lachnospiraceae
Incertae Sedis XIV
Eubacteriaceae
Clostridiaceae
Erysipelotrichaceae
Alcaligenaceae
Bifidobacteriaceae
Other
Unclassified

Figure 2

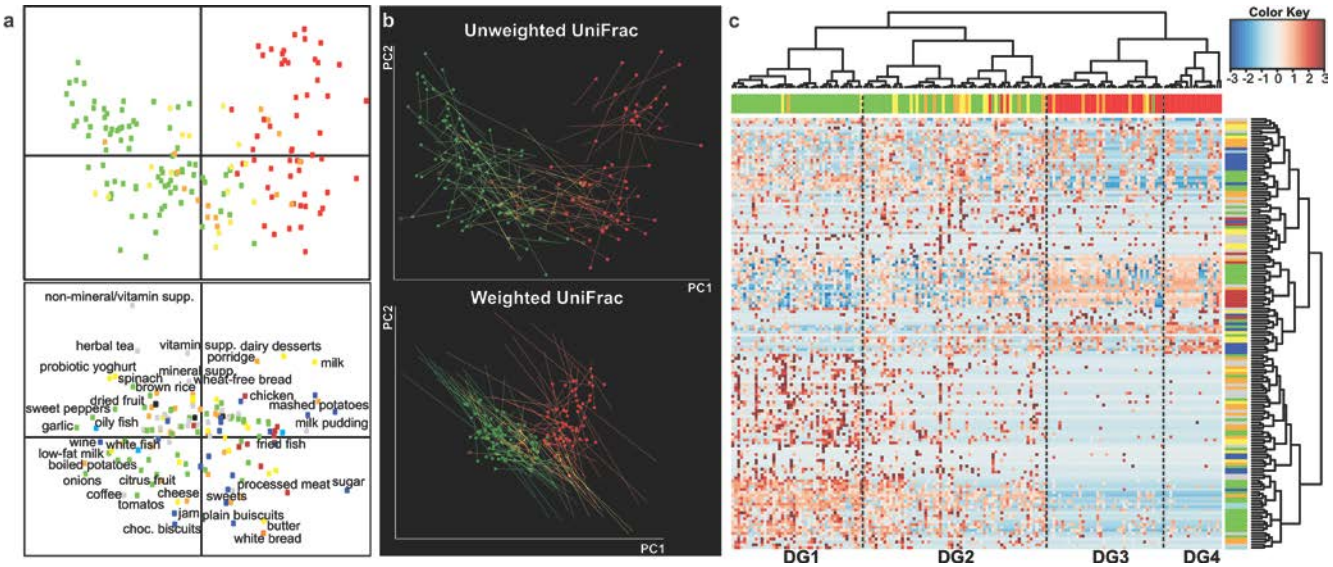

a
non-mineral/vitamin supp.
herbal tea
vitamin supp.
dairy desserts
porridge
milk
probiotic yoghurt
mineral supp.
spinach
wheat-free bread
brown rice
chicken
dried fruit
mashed potatoes
sweet peppers
garlic
oily fish
milk pudding
wine
white fish
fried fish
low-fat milk
boiled potatoes
onions
citrus fruit
processed meat
sugar
coffee
cheese
sweets
tomatos
jam
plain buiscuits
butter
choc. biscuits
white bread
b
Unweighted UniFrac
PC2
PC1
Weighted UniFrac
PC2
PC1
c
Color Key
-3 -2 -1 0 1 2 3
DG1
DG2
DG3
DG4

Figure 3

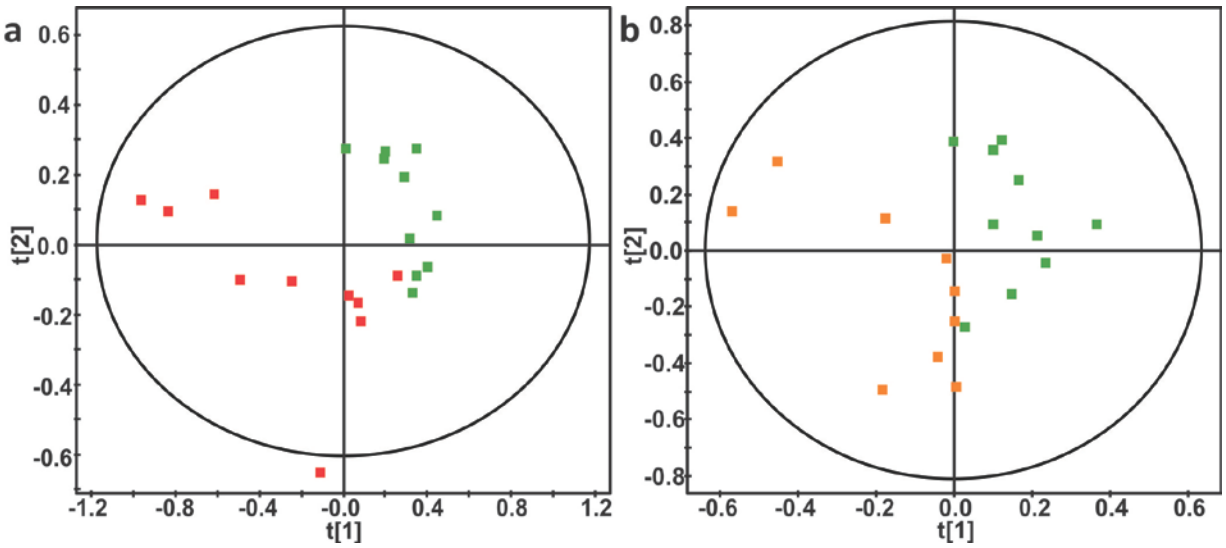
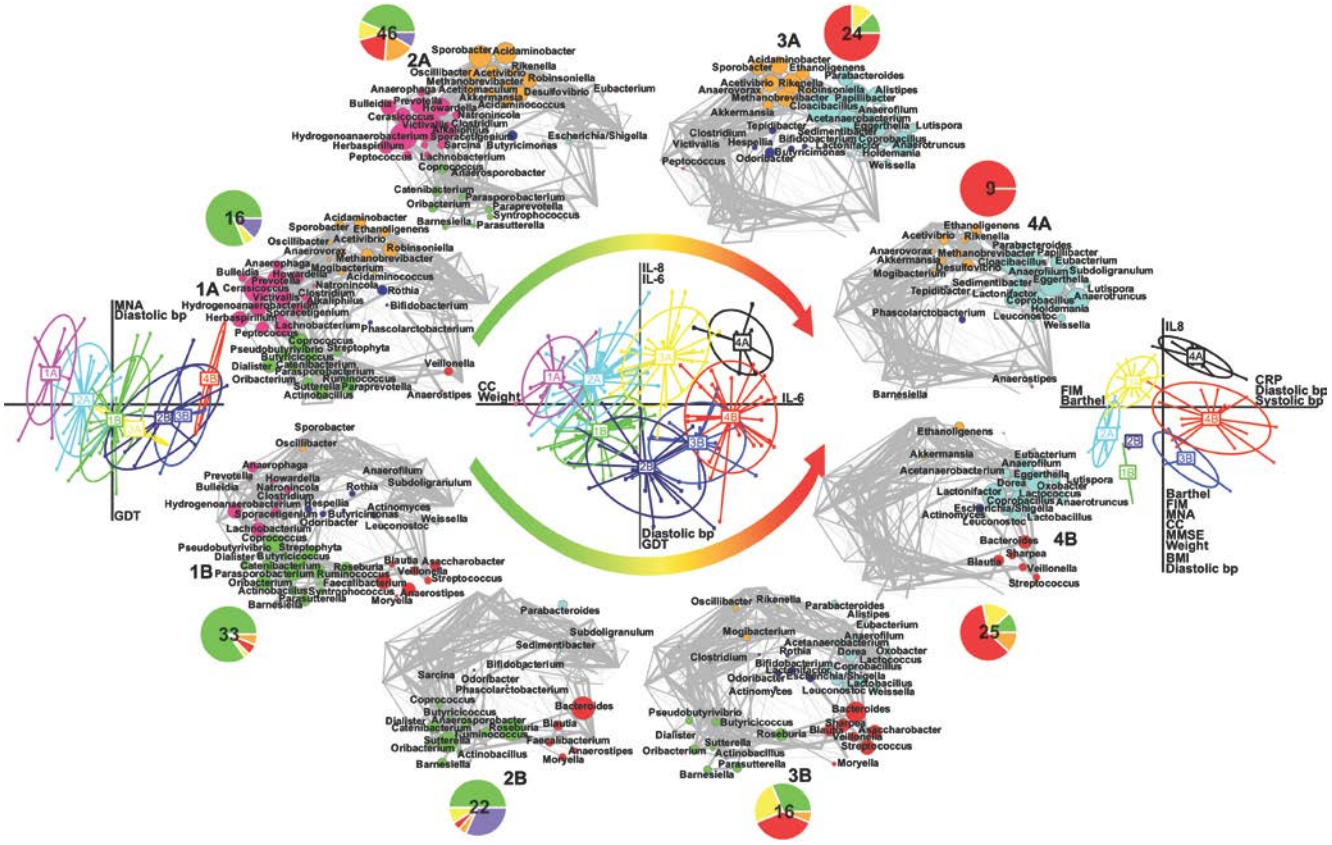

a
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
t[2]
-1.2
-0.8
-0.4
-0.0
0.4
0.8
1.2
t[1]
b
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
t[2]
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
t[1]

Figure 4


46
2A
Sporobacter
Acidaminobacter
Oscillibacter
Acetivibrio
Rikenella
Robinsoniella
Methanobrevibacter
Anaerophaga
Acetitomaculum
Desulfovibrio
Eubacterium
Akkermansia
Bulleidia
Prevotella
Howardella
Acidaminococcus
Natronincola
Cerasicoccus
Clostridium
Victivallis
Alkaliphilus
Hydrogenoanaerobacterium
Sporacetigenium
Escherichia/Shigella
Herbaspirillum
Sarcina
Butyricimonas
Peptococcus
Lachnobacterium
Coprococcus
Anaerosporobacter
Catenibacterium
Parasporobacterium
Oribacterium
Paraprevotella
Syntrophococcus
Barnesiella
Parasutterella
3A
24
Acidaminobacter
Sporobacter
Ethanoligenens
Parabacteroides
Acetivibrio
Rikenella
Anaerovorax
Robinsoniella
Alistipes
Methanobrevibacter
Papillibacter
Cloacibacillus
Akkermansia
Anaerofilum
Acetanaerobacterium
Tepidibacter
Sedimentibacter
Eggerthella
Lutispora
Clostridium
Victivallis
Hespellia
Bifidobacterium
Coprobacillus
Lactonifactor
Anaerotruncus
Butyricimonas
Holdemania
Peptococcus
Odoribacter
Weissella
16
1A
Acidaminobacter
Sporobacter
Ethanoligenens
Oscillibacter
Acetivibrio
Anaerovorax
Robinsoniella
Methanobrevibacter
Anaerophaga
Mogibacterium
Bulleidia
Prevotella
Howardella
Acidaminococcus
Natronincola
Cerasicoccus
Clostridium
Victivallis
Alkaliphilus
Rothia
Bifidobacterium
Hydrogenoanaerobacterium
Sporacetigenium
Herbaspirillum
Lachnobacterium
Phascolarctobacterium
Peptococcus
Coprococcus
Pseudobutyrivibrio
Streptophyta
Butyricicoccus
Dialister
Parasporobacterium
Ruminococcus
Oribacterium
Sutterella
Paraprevotella
Actinobacillus
Veillonella
Anaerostipes
9
4A
Ethanoligenens
Acetivibrio
Rikenella
Parabacteroides
Anaerovorax
Methanobrevibacter
Papillibacter
Akkermansia
Cloacibacillus
Eubacterium
Desulfovibrio
Mogibacterium
Anaerofilum
Subdoligranulum
Sedimentibacter
Eggerthella
Tepidibacter
Lactonifactor
Lutispora
Coprobacillus
Anaerotruncus
Holdemania
Phascolarctobacterium
Leuconostoc
Weissella
Barnesiella
Anaerostipes
MNA
Diastolic bp
GDT
CC
Weight
IL-8
IL-6
IL-6
Diastolic bp
GDT
FIM
Barthel
IL8
CRP
Diastolic bp
Systolic bp
Barthel
FIM
MNA
CC
MMSE
Weight
BMI
Diastolic bp
1B
Sporobacter
Oscillibacter
Anaerophaga
Anaerofilum
Prevotella
Howardella
Subdoligranulum
Bulleidia
Natronincola
Rothia
Clostridium
Hydrogenoanaerobacterium
Hespellia
Actinomyces
Sporacetigenium
Butyricimonas
Odoribacter
Leuconostoc
Weissella
Lachnobacterium
Coprococcus
Pseudobutyrivibrio
Streptophyta
Dialister
Butyricicoccus
Blautia
Asaccharobacter
Catenibacterium
Roseburia
Veillonella
Parasporobacterium
Ruminococcus
Streptococcus
Oribacterium
Faecalibacterium
Actinobacillus
Syntrophococcus
Anaerostipes
Parasutterella
Barnesiella
Moryella
4B
Ethanoligenens
Akkermansia
Eubacterium
Anaerofilum
Acetanaerobacterium
Eggerthella
Lutispora
Dorea
Oxobacter
Lactonifactor
Lactococcus
Coprobacillus
Anaerotruncus
Escherichia/Shigella
Actinomyces
Lactobacillus
Leuconostoc
Bacteroides
Sharpea
Blautia
Veillonella
Streptococcus
33
Parabacteroides
Subdoligranulum
Sedimentibacter
Bifidobacterium
Sarcina
Odoribacter
Phascolarctobacterium
Coprococcus
Bacteroides
Butyricicoccus
Dialister
Anaerosporobacter
Catenibacterium
Roseburia
Blautia
Ruminococcus
Sutterella
Faecalibacterium
Oribacterium
Actinobacillus
Anaerostipes
Barnesiella
Moryella
2B
22
25
Oscillibacter
Rikenella
Parabacteroides
Alistipes
Mogibacterium
Eubacterium
Anaerofilum
Acetanaerobacterium
Rothia
Dorea
Oxobacter
Clostridium
Bifidobacterium
Lactococcus
Coprobacillus
Escherichia/Shigella
Odoribacter
Lactobacillus
Actinomyces
Leuconostoc
Weissella
Bacteroides
Pseudobutyrivibrio
Sharpea
Butyricicoccus
Blautia
Asaccharobacter
Dialister
Roseburia
Veillonella
Sutterella
Oribacterium
Actinobacillus
Streptococcus
Parasutterella
Moryella
Barnesiella
3B
16