Original research

Alterations to the duodenal microbiota are linked to gastric emptying and symptoms in functional dyspepsia

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ABSTRACT

Objective Functional dyspepsia (FD) is a complex disorder, with debilitating epigastric symptoms. Evidence suggests alterations in gastrointestinal (GI) motility, visceral hypersensitivity, permeability and low-level immune activation in the duodenum may play a role. However, we still have a relatively poor understanding of how these factors interact to precipitate the onset of FD symptoms which are frequently meal related. The duodenal microbiota, in combination with specific dietary substrates, may be important mediators in disease pathophysiology; however, these interlinked factors have not been thoroughly investigated in FD.

Design Eighty-six individuals (56 FD, 30 controls) undergoing endoscopy were consecutively recruited and underwent detailed clinical assessment, including upper GI symptoms, gastric emptying and dietary assessment. Duodenal biopsies were obtained aseptically, and the mucosa-associated microbiota (MAM) analysed via 16S rRNA gene amplicon sequencing.

Results The relative abundances of predominant members of the Firmicutes, Bacteroidota and Fusobacteriota phyla were linked to symptom burden in FD. Inverse relationships between the relative abundances of *Streptococcus* and *Prevotella,* and the relative abundance of *Veillonella* spp with gastric emptying time, were also observed. No significant differences in long-term nutrient intake or diet quality were found between FD and controls, and there appeared to be limited association between habitual diet and duodenal MAM profiles.

Conclusion This study suggests a link between the duodenal MAM, gastric emptying and FD symptoms, and this is largely independent of long-term dietary intake.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Duodenal inflammation, altered gastroduodenal motility, visceral hypersensitivity and epigastric symptoms are all characteristic of functional dyspepsia. Although symptoms may be meal related, it remains unclear how dietary substrates and the duodenal mucosa-associated microbiota may interact to contribute to symptoms in this condition.

WHAT THIS STUDY ADDS

⇒ Differences in relative abundances of *Firmicutes* and *Bacteroidota* phyla were expressed as an inverse relationship between *Streptococcus* and *Prevotella,* which in addition to *Fusobacterium* could also be linked with functional dyspepsia symptom burden. Here, the relative abundance of *Veillonella* spp was also inversely related with gastric emptying, while diet and duodenal mucosa-associated microbiota profiles were not associated.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The duodenal mucosa-associated microbiota is dynamic and site-specific, which has the potential to impact symptom generation in functional dyspepsia and thus should be the target of further investigation and management.

INTRODUCTION

Functional dyspepsia (FD) is a common chronic condition manifested by recurrent upper gastrointestinal (GI) symptoms that include early satiety, postprandial fullness and/or epigastric pain, all without a readily identifiable organic cause. A number of functional disturbances are observed in FD including alterations of gastroduodenal motility,[1] along with hypersensitivity to distension of the stomach.[2] In addition, functional brain imaging shows altered gut-brain axis signalling.[3] FD represents a considerable disease burden for both patients and the healthcare system, resulting in reduced quality of life and economic loss. Current treatment approaches aim to improve symptoms but are effective only in a modest proportion of patients.[4 5]

More recently, evidence for potential mechanisms that may underlie functional changes and symptoms in FD have emerged. Increased permeability of the duodenal mucosa has been observed,[6] along with low level inflammation, characterised by the presence of eosinophils and in some cases mast cells.[7] These cells are capable of releasing mediators

that may alter epithelial permeability and intestinal sensorimotor function.[8] An increase in circulating small bowel homing T lymphocytes has also been observed in patients with FD, correlated with symptom severity and delayed gastric emptying.[9]

In many GI disorders, the microbiota may also be a key factor in the inflammation-permeability-symptom axis. Microbes colonising the GI mucosa can aid in promoting gut health and immune homoeostasis, but changes to the community composition and/or density of these microbes are implicated in a variety of disease states.[10 11] The microbiota has also been implicated in control of GI motility,[12] visceral pain[13] and homoeostasis of gut–brain signalling.[14] A contributory or precipitating role for the gut microbiota has been suggested in postinfectious FD, where dyspepsia following acute gastroenteritis is likely to continue long term.[15] More recently, the microbiota present in duodenal aspirates has been suggested to contribute to functional GI symptoms,[16] and a clinical trial of rifaximin treatment has demonstrated that antibiotic therapy can result in a significant reduction in FD symptoms.[17] We have also reported the results from a pilot study that showed differences in the profile of the duodenal mucosa-associated microbiota (MAM) in FD patients, and specifically, established correlations between bacterial density and clinical measurements of meal-related symptoms.[18]

Understanding the role of the microbiota in GI disease is complicated by various external and environmental factors. While a substantial body of literature now emphasises diet as a major driver of both the form and function of the luminal microbiota present in the large bowel and stool,[19] our understanding of these interactions in the small intestine are limited, despite their relevance for functional GI disorders such as FD. Patients with FD often present with meal-related symptoms, and evidence suggests specific foods are associated with symptom generation,[20–22] and particularly, dietary fat.[23 24] Observational data also suggests FODMAP restriction may improve symptoms in this patient group.[25] However, there are limited studies investigating the habitual dietary intakes, or diet quality, of patients with FD. In summary, investigations of the interrelationships between the duodenal MAM, GI function, and habitual diet in FD are scant.

Here, we present our findings that examine the duodenal MAM in patients with FD and integrate these data with detailed measurements of upper GI function, symptoms and habitual dietary intake. This study reveals an inverse relationship in the relative abundances of *Streptococcus* and *Prevotella* lineages within the duodenal MAM and differences between these taxa and Fusobacteria, in relation to symptom burden in FD subjects. Intriguingly, the relative abundance of *Veillonella* is negatively associated with gastric emptying time. All these associations also appear to be independent of long-term dietary intakes.

METHODS

Patient recruitment

Patients were recruited at the Outpatients clinic of the Department of Gastroenterology and Hepatology at the Princess Alexandra Hospital, Brisbane, Australia. We included patients presenting with FD based on Rome IV,[26] or FD with additional irritable bowel syndrome-like symptoms. Control subjects were symptom-free (non-FD), with either documented iron-deficiency (ID) with and without anaemia, or individuals undergoing screening following a positive faecal-occult blood test (online supplemental table 1). Both groups underwent upper endoscopy and colonoscopy as routine clinical care. Only those who did not show any evidence of gastric/duodenal mucosal abnormalities, lesions, or structural changes (based on endoscopic and clinical histology findings) were included in the study. Patients who reported onset of GI symptoms after an acute infection have not been included into the study. The FD group includes data from 8/9 FD subjects reported in Zhong et al.[18] None of the subjects recruited in this study had current clinical *Helicobacter pylori* infection based on routine testing, although eight subjects (three patients with FD and five controls) had had previous *H. pylori* infection and underwent *H. pylori* eradication more than 3 months prior to study inclusion. Additionally, the genus *Helicobacter* was not identified in the microbiota data for any of the subjects included here.

Patient characterisation

Participants completed a set of validated questionnaires, specifically the Structured Analysis of Gastrointestinal Symptoms (SAGIS)[27] and the Nepean Dyspepsia Index[28] to achieve a global assessment of symptoms and the impact on quality of life. A medical interview was conducted to collect information about participants' current symptoms, past medical and surgical history, and medications. In consented participants, a ^{13}C-octanoic acid breath test was performed to assess gastric emptying rate (online supplemental methods).

Dietary intake

Habitual intake of foods and fluids over the preceding 12 months was evaluated. A validated Food Frequency Questionnaire (FFQ)[29] was completed by each participant estimating mean daily intake of energy, macronutrients, fibre, 13 micronutrients, total and individual FODMAP carbohydrates (fructans, galacto-oligosaccharides, lactose, fructose in excess of glucose, polyols; Foodworks 7; Xyris Software, Australia). Overall diet quality, a recognised measure of the overall healthfulness of the diet, was assessed using the Alternate Healthy Eating Index-2010.[30] Statistical analysis was performed using IBM SPSS Statistics for Windows V.24.0. Refer to the online supplemental methods for further details.

Biopsy collection

Duodenal biopsies were taken from the second part of the duodenum utilising the Brisbane Aseptic Biopsy device (MTW, Germany),[31] which enables specific sampling of the MAM, through collection of mucosal samples with exclusion of contamination from luminal contents or other regions of the GI tract. Biopsy samples were immediately placed under aseptic conditions into a sterile tube containing RNAlater (Qiagen). Samples were allowed to incubate at room temperature for 30 min, then frozen and stored at −80°C.

Microbiota analysis

Total DNA was extracted from biopsies, and sample free reagent controls, using a repeated bead-beating based method as described previously.[32] The MAM present on duodenal tissue (d-MAM) were characterised by 16S rRNA gene amplicon (V6-V8) sequencing with dual-index barcoding using the Illumina MiSeq platform, as described previously.[32] The libraries were sequenced on an Illumina MiSeq using the MiSeq Reagent Kit v3 (2×300 bp), using facilities provided by the Australian Centre for Ecogenomics. Sequence data was processed using the Quantitative Insights into Microbial Ecology 2 pipeline[33] (V.2021.4) and DADA2,[34] with amplicon sequence variants (ASVs) assigned using default settings and

Table 1 Patient cohort characteristics

	FD (n=56)	Controls (n=30)	P value
Female gender: n (%)	29 (52)	15 (50)	NS*
Age (year): median (range)	47 (17–77)	59 (22–74)	**0.02**†
BMI (kg/m^2): mean (SD)	25.2 (5.6)	27.5 (6.1)	NS‡
Current PPI use: n (%)	33 (59)	6 (20)	**0.0006***
Smoking status (current/previous/never): n (%)§	11/19/24 (20/34/43)	7/5/18 (23/17/60)	NS¶
Epigastric symptom domain—total score: median (range)**	8 (1–20)	0 (0–3)	<**0.0001**†
NDI-QOL: mean (SD)§	57.1 (27.0)	96.9 (4.2)	<**0.0001**‡
Atrophic gastritis on histology: n (%)	2 (3.6)	3 (10)	

Values shown in **bold** are statistically significant.

*Fisher's exact test.
†Mann-Whitney U test.
‡T-test.
§Patient numbers (where different from overall study cohort): smoking status (FD n=54); (FD n=30; controls n=10);
¶χ^2.
**Structured assessment of GI symptoms. The total possible upper GI symptom score is 20.
BMI, body mass index; GI, gastrointestinal; NDI-QoL, Nepean Dyspepsia Index Quality of Life; NS, not significant; PPI, Proton Pump Inhibitor.

the SILVA_138 database.[35] Further details of the laboratory and bioinformatics workflows utilised are provided in online supplemental methods.

RESULTS

Patient cohort

A total of 86 individuals were recruited into the study, with 56 diagnosed with upper functional GI symptoms (FD), and the remainder considered non-FD, symptom-free controls (n=30) presenting with ID anaemia. There were no differences in gender, body mass index (BMI) or smoking status across the two groups, however, the FD patients were significantly younger and had greater Proton Pump Inhibitor (PPI) use compared with controls (table 1). The FD patients experienced epigastric symptoms and reduced quality of life compared with controls (table 1). Many also experienced overlapping lower GI symptoms (online supplemental table 1).

Habitual dietary intake does not differ between FD and controls

Long-term dietary intake surveys were completed by 39 participants (FD patients n=28, non-FD controls n=11; online supplemental table 2). There were no differences between the FD patients and control groups in macronutrient intake (table 2) and overall diet quality scores (56 (48–62)) vs 51 (41–64), p=0.412). Only resistant starch intake in the FD patients was lower (p=0.03), while the proportion of patients meeting gender and age-specific national dietary recommendations was similar between patients and controls for all nutrients analysed (online supplemental table 3). There were no associations between epigastric domain symptom scores (SAGIS) and daily energy, macronutrient or FODMAP intake (online supplemental table 4).

Table 2 Mean energy and macronutrient intake in FD patients and controls

	FD (n=28)	Controls (n=11)	P value
Energy (kJ/day)	7476 (5768–9485)	7847 (6239–9421)	0.652
Carbohydrate (g/day)	202 (153–261)	209 (171–293)	0.632
Starch (g/day)	97 (66–129)	102 (54–158)	0.592
Sugars (g/day)	106 (67–159)	119 (62–155)	0.693
Protein (g/day)	84 (64–111)	91 (68–121)	0.350
Fat (g/day)	58 (46–94)	62 (44–81)	0.592
Saturated fat (g/day)	24 (16–32)	24 (15–35)	0.933
Monounsaturated fat (g/day)	27 (20–41)	24 (15–32)	0.463
Polyunsaturated fat (g/day)	10 (6–13)	8 (5–14)	0.693
Dietary fibre (g/day)	28 (14–34)	26 (14–39)	0.572

Values are median (IQR); Mann-Whitney U test comparing FD with controls.
FD, functional dyspepsia.

Alterations to the duodenal MAM in FD compared with non-FD control subjects

Microbiota profiles from duodenal mucosal biopsies were obtained for 80 participants (FD n=51, non-FD controls n=29) who showed essentially the same characteristics as the entire cohort (table 2, online supplemental table 5). A total of 58 genus-level taxonomic classifications, comprised of 271 bacterial ASVs were detected from the duodenal MAM profiles for all the subjects (online supplemental table 6 and 7). The most abundant phylum overall was the Firmicutes, although its representation was significantly less in the FD subject group (p=0.033, figure 1A). The Fusobacteriota (p=0.057) and Patescibacteria (p=0.068) were observed to trend higher in relative abundance in FD subjects. Multiple regression controlling for patient age, gender, smoking status, BMI and PPI use showed the effect size between groups for the Fusobacteriota and Firmicutes remained significant with low to moderate FDR values (p=0.002, q=0.075 and p=0.045, q=0.31 for Fusobacteriota and Firmicutes, respectively). While the Shannon diversity measure of richness and evenness was not different between the two subject groups, the difference in the Chao1 measure of richness was greater for the FD group and approached statistical significance (p=0.076, figure 1B).

The twenty most abundant bacterial genera observed across the FD and non-FD control groups and their distribution across individual subjects, along with the representation of other bacterial genera are shown in figure 2; and shown normalised to the top 20 genera only in online supplemental figure 1. Similar to our previous pilot study,[18] the most abundant bacterial genera across the case and control groups were *Streptococcus*, *Prevotella* and *Veillonella* and proportionally represented as much as ~75% of the duodenal MAM communities. Interestingly and across all subjects, a strong inverse relationship between the relative abundance of *Streptococcus* affiliated ASVs and those from the genus *Prevotella* was observed (r=−0.348, p=0.0015, figure 3). The relative abundances of the other dominant genera were similarly compared, with these analyses revealing positive correlations between *Prevotella* and *Fusobacterium* in both the FD (r=0.432, p=0.019) and non-FD control group (r=0.399, p=0.0038) and between *Veillonella* and *Fusobacterium* in both the FD (r=0.303, p=0.031) and non-FD control group (r=0.548, p=0.0021, online supplemental figure 2).

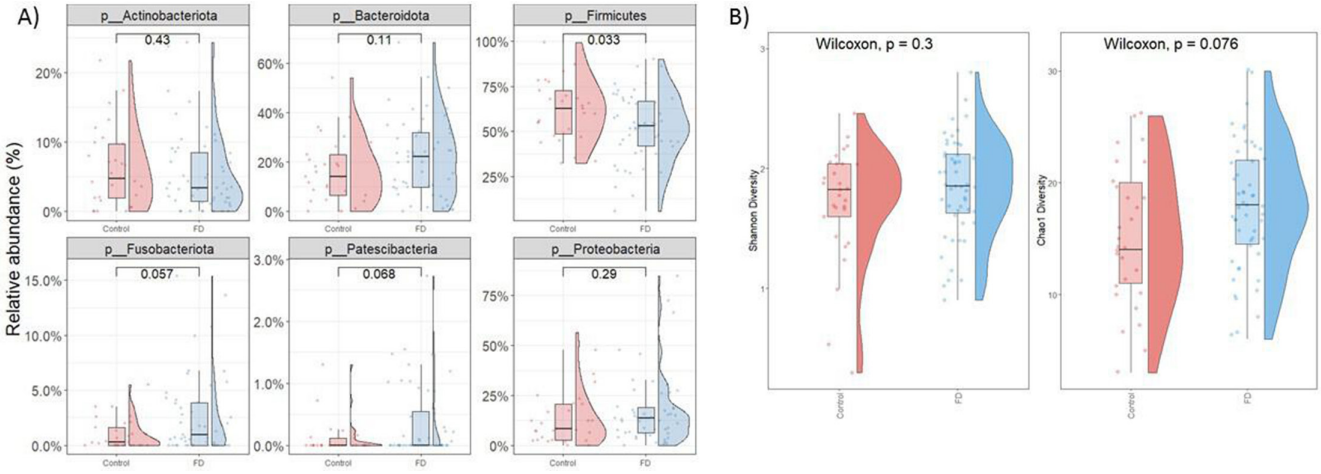

Figure 1 A) Differences in the relative abundances of the Bacterial phyla represented in the duodenal mucosa-associated microbiota (MAM) communities from functional dyspepsia (FD) patients and non-FD control subjects. The data were normalised via total sum scaling and subjected to the Wilcoxon test of differences between groups. (B) The Shannon (richness and evenness) and Chao1 (richness) measures for the FD and non-FD control subjects were not significantly different (Wilcoxon test) although the duodenal MAM for the FD group does trend to possess greater bacterial richness.

The unsupervised principal coordinates analysis of the weighted UniFrac distance metrics did not reveal any distinct clustering of the duodenal MAM profiles between the case and control groups (figure 4A). However, our constrained (supervised) model using sparse partial least squares discriminant analysis[36] did reveal a separation between the case and control groups (figure 4B) with a number of bacterial taxa discriminatory for each group (figure 4C). Here and in order of strength of contribution, taxa (ASVs) affiliated with the genera *Fusobacterium*, *Alloprevotella*, *Prevotella*, *Leptotrichia*, *Atopobium*, *Neisseria*, *Actinobacillus* and *Granulicatella*, were discriminatory of the FD group; whereas *Rothia*, *Streptococcus*, *Haemophilus*, *Actinomyces*, *Stomatobaculum*, *Faecalibaculum* and *Gemella*, were discriminatory of the non-FD control group.

We also examined the effects of PPI use on the Shannon index and Bray Curtis dissimilarity metrics as measures of alpha (within sample) and beta (between sample) diversity, respectively, for the FD, non-FD, and combined groups. Interestingly, only the differences in the Bray Curtis dissimilarity metrics between PPI users and non-users in the non-FD group was statistically significant

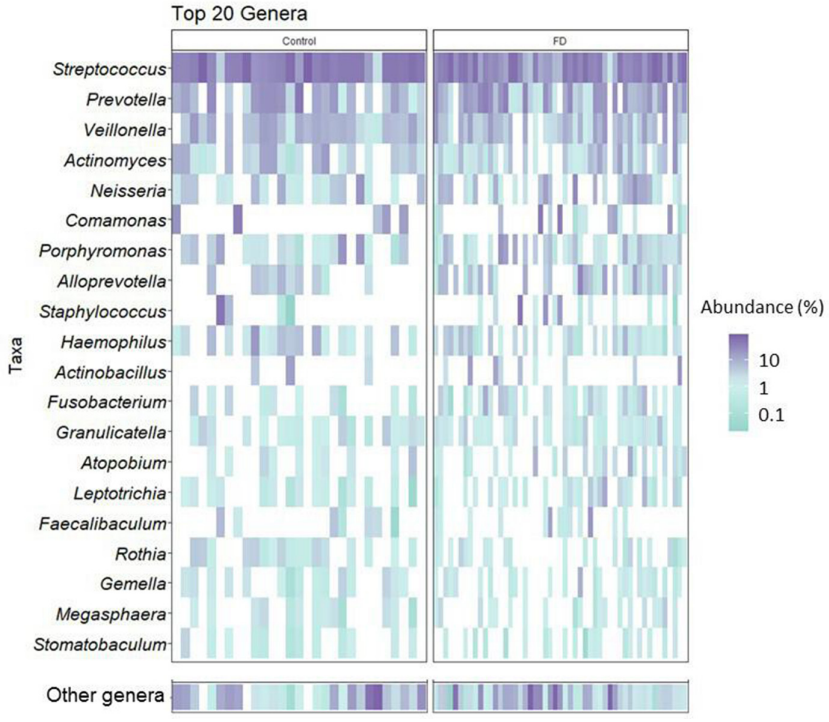

Figure 2 Heat map showing the relative abundances of the top 20 most abundant genera detected from the duodenal mucosa-associated microbiota (MAM) profiles of all 80 study participants. Each column represents an individual subject assigned to either the functional dyspepsia (FD) or non-FD control group and shows the relative abundance of each genus-level taxon representing the individual top 20 genera across the study group, and the cumulative relative abundance of the other genera (n=38) present in the individual samples.

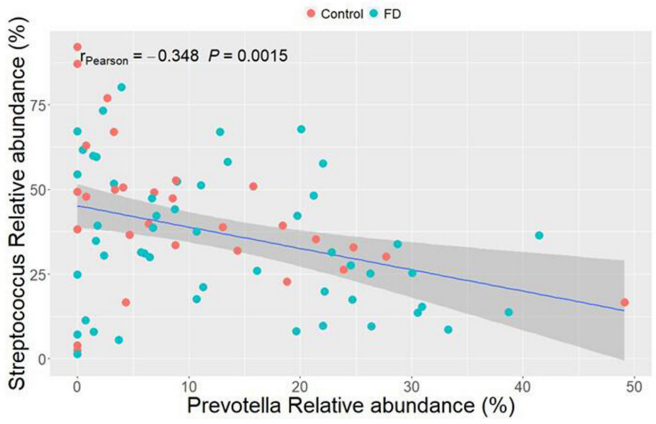

Figure 3 The relative abundances of *Streptococcus* and *Prevotella* (expressed relative to all genera in each subject) are inversely correlated in duodenal mucosa-associated microbiota (MAM) communities for both functional dyspepsia (FD, blue) and non-FD control subjects (red). Pearson correlation shows there is a strong inverse relationship between these two genera on duodenal mucosa (r=–0.348, p=0.0015).

(p=0.02, online supplemental figure 3). On that basis, our comparisons of these data here remain based on clinical phenotype (ie, FD vs non-FD controls).

We next assessed the relative abundances of key Bacterial Phyla (figure 5A) and genera (figure 5B) between non-FD control subjects, and the FD subjects stratified according to their reported symptom burden (low, medium, and high). At the Phylum level, the differences between the non-FD control group and FD subjects with low symptom burden were significant for the Firmicutes and Bacteroidota (p≤0.05, figure 4A). Interestingly, the relative abundance of taxa affiliated with the *Firmicutes* increased with FD symptom burden (p<0.05), whereas taxa affiliated Bacteroidota and Fusobacteria showed some decrease, or no change, respectively. These trends were retained when the predominant lineage from each Phylum was examined (ie, *Streptococcus, Prevotella* and *Fusobacterium,* figure 5B) and the difference between the control group and FD subgroup with low symptom burden was statistically significant for *Prevotella* and *Fusobacterium* (p<0.05).

Gastric emptying time is associated with *Veillonella* relative abundance

A subset of the participants in this study also consented to have gastric emptying time measured via ^{13}C-octanoic acid breath test (Controls=5, FD=24) and there was no significant difference between these groups in estimated gastric emptying time (online supplemental figure 4). We then assessed associations between gastric emptying time and genera within the duodenal MAM. Using the top 20 bacterial genera represented across all study participants, a negative correlation was observed between the relative abundance of the genus *Veillonella* in the duodenal MAM and gastric emptying $t^{1/2}$ times, both in the FD subjects alone (figure 6A, r=−0.494, p=0.014,) and for all subjects tested (figure 6B, r=−0.447, p=0.015) and the FDR values calculated for these univariate models were also small (q=0.104 and 0.093 for FD subjects only, and all subjects, respectively). Similar effect sizes were observed when these data were applied to a multivariate model controlling for patient age, gender, smoking status, BMI and PPI use, although the FDR values for both models were attenuated (p=0.025, q=0.349; and p=0.073, q=0.533 for FD subjects and all subjects, respectively).

The duodenal MAM is not significantly impacted by habitual diet

A subset of patients with both habitual diet and duodenal MAM data available was assessed (FD n=18; non-FD controls n=8; online supplemental table 8). Overall, habitual diet did not appear to significantly associate with the composition of the duodenal MAM, with the only association identified being a positive correlation between total carbohydrate intake and the relative abundance of the genus *Neisseria* in those subjects where it was detected (r=0.501, p=0.0092, q=0.36, online supplemental figure 5). No links to bacterial diversity or any other taxa were observed, in either the FD or non-FD control groups, for any other dietary parameter examined (total energy, fat, FODMAP intake or diet quality).

DISCUSSION

FD is a complex disorder, hypothesised to involve a variety of pathophysiologic mechanisms including disordered motility, visceral hypersensitivity, alterations to the gut–brain axis,

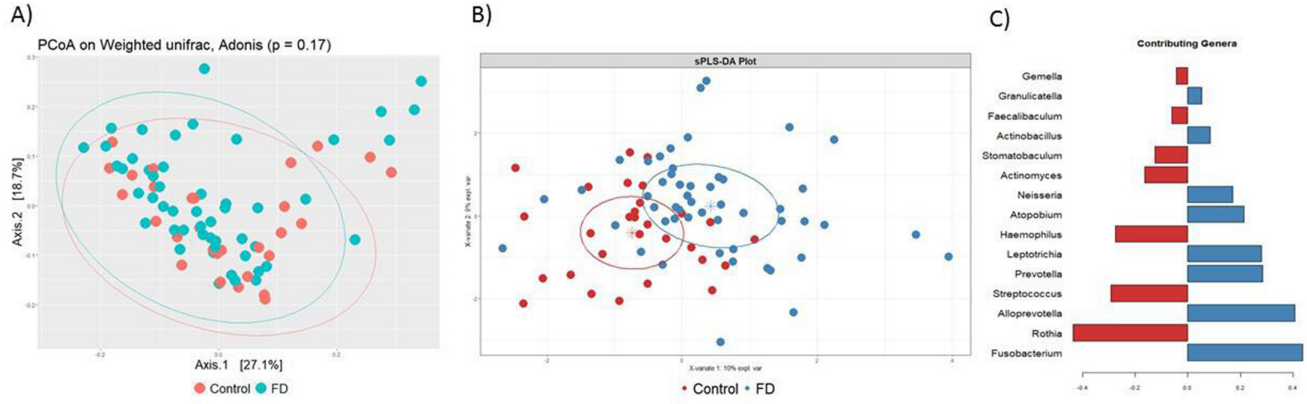

Figure 4 (A) Unsupervised principal co-ordinates analysis plot of the weighted UniFrac distance metrics for functional dyspepsia (FD, blue) and non-FD control (red) subjects. The data were subjected to ADONIS testing (p=0.17), controlling for patient age, gender, body mass index, proton pump inhibitor use, and smoking status. (B) Spatial plot of the sparse partial least squares discriminant analysis (sPLS-DA) of the normalised (by total sum scaling) genus-level relative abundances of the FD (blue) and non-FD control subjects results in a distinct separation of the two groups. (C) Bacterial taxa (genus level) that from sPLS-DA were identified as being discriminatory of the duodenal MAM communities of FD (blue) and non-FD control subjects (red).

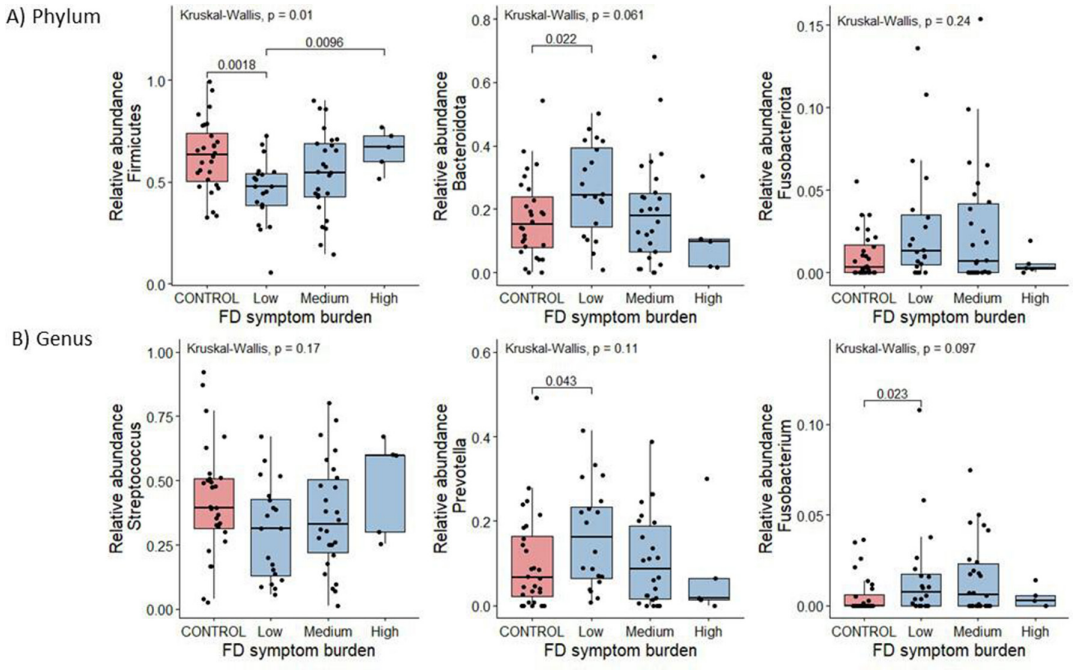

Figure 5 (A) Relative abundance of the Firmicutes, Bacteroidota and Fusobacteriota phyla, and (B) the predominant genus within each phylum (ie, *Streptococcus*, *Prevotella* and *Fusobacterium*, respectively) in the duodenal mucosa-associated microbiota (MAM) of functional dyspepsia (FD) and non-FD control subjects. The FD subjects were stratified into tertiles based on their symptom burden using epigastric domain scores from the structured analysis of gastrointenstinal symptoms (SAGIS) questionnaire. These data were normalised via total sum scaling and are expressed as relative abundance. Error bars represent SD. Significance was tested via Kruskal-Wallis and Wilcoxon tests for comparison (via ggpubr package in R).

low level inflammation, and intestinal permeability, which contribute to the generation of often debilitating meal-related symptoms.[5 37] However, the interactions between these factors are poorly understood, as is any potential involvement of the duodenal microbiota as a trigger or suppressor of these symptoms. In this study, the relative abundances of *Prevotella*, *Streptococcus* and *Fusobacterium* spp were discriminatory between the FD and control groups and linked to symptom burden. Further, an inverse interrelationship between the *Streptococcus* and *Prevotella* genera was evident across the entire cohort; and positive correlations between the relative abundances of *Prevotella* and *Fusobacterium*, as well as *Fusobacterium* and *Veillonella* were also observed. While members of the genus *Streptococcus* are capable of microaerophilic growth, both *Prevotella* and *Veillonella* spp, as well as the Fusobacteriota, are widely recognised as fastidious anaerobes. Moreover, most of the other bacterial taxa discriminatory of the control group are also capable of microaerophilic growth, whereas those discriminatory of the FD group are primarily fastidious/obligate anaerobes. As such, the interrelationships revealed as part of this study

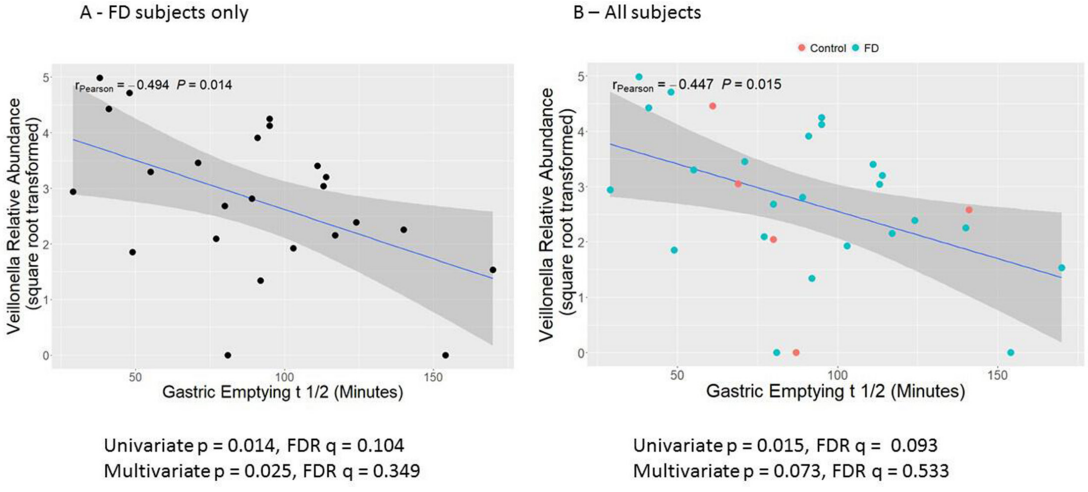

Figure 6 Gastric emptying t1/2 time was negatively correlated with (A) the relative abundance of the genus *Veillonella* in the duodenal mucosa of functional dyspepsia (FD) subjects alone (n=24), and (B) the FD (blue) and non-FD subjects (red) combined (n=29). Pearson correlations were performed with the relative abundance of *Veillonella* normalised to the top 20 genera across all study participants and subjected to square root transformation. False discovery rate (FDR) corrections were calculated with the MaAsLin2 package for both the univariate analysis, as well as a multivariate linear regression in which patient age, gender, smoking status, body mass index, and proton pump inhibitor use were also included.

are consistent with the notion that variations in partial pressure of oxygen (pO_2) within the duodenal mucosal environment has a strong deterministic effect on the resident microbiota at the duodenum. Indeed, changes in pO_2, leading to hypo- and hyperoxia, have been associated with the inflammatory tenor and mucosal epithelial barrier function in the gut.[38 39]

Interestingly, similar observations have been made for the oesophageal microbiota. For instance, Gall *et al*[40] reported that the ratio of *Streptococcus:Prevotella* within the oesophageal microbiota recovered from a cohort of Barrett's oesophagus subjects was inversely correlated with risk factors for oesophageal adenocarcinoma (hiatal hernia length). Similarly, Lopetuso *et al*[41] reported that the oesophageal MAM showed reductions in the relative abundance of *Streptococcus* (as well as *Granulicatella* and *Propionibacterium*) and commensurate increases in *Prevotella, Veillonella,* and *Leptotrichia* (a member of the *Fusobacteriota*) spp for Barrett's oesophagus subjects with metaplasia, and these shifts were more pronounced in samples from patients with oesophageal adenocarcinoma. While members of the phylum Fusobacteriota are considered 'commensal' in terms of their interaction with the host, *Fusobacterium nucleatum* is recognised as a periodontal pathogen and has more recently been implicated in the pathogenesis of colon cancer and inflammatory bowel disease, via mechanisms that include invasion into epithelial cells and disruption to immune homoeostasis.[42 43] In a rat model, *Fusobacterium* spp have also been linked to visceral hypersensitivity.[44] Our observation that these taxa are discriminatory of the duodenal MAM in FD patients suggests a potential role for specific lineages of this bacterium in the generation of epigastric symptoms. For instance, *Fusobacterium* spp (and *Veillonella* spp) can produce hydrogen sulphide,[45] which can contribute to pain and visceral hypersensitivity.[13] They are also considered important 'bridge' organisms in the formation of oral biofilms[46] and our previous studies suggest that bacterial load on duodenal tissue is greater in FD subjects than healthy controls.[47] Indeed, for the cohort of patients included in this study, bacterial load in FD patients was significantly greater as compared with the control group, (0.16 ± 0.26 vs 0.054 ± 0.07, $p=0.01$). This is well aligned with the observation that small intestinal bacterial overgrowth (SIBO) is associated not only with the irritable bowel syndrome[48] but also FD.[49]

The microbiota has been implicated in control of intestinal motility via mechanisms including control of hormonal mediators, interaction with the immune system and regulation of bile metabolism, as well as microbial metabolites such as methane, short chain fatty acids and lipopolysaccharide playing a role.[12 50 51] Furthermore, antimicrobial therapy in FD patients improves symptoms and visceral sensory function.[52] However, less is known about the potential for upper GI microbes to regulate motility. Here, we have used the ^{13}C-octanoic acid breath test for gastric emptying of solids, which is as reproducible as scintigraphy provided the sampling time is sufficiently long. Despite the gastric emptying kinetics measured in our subjects falling within the calculated 'normal' range, the observed inverse relationship between gastric emptying half time and the relative abundance of *Veillonella* spp in the duodenum is intriguing. In general terms, *Veillonella* spp. are commonly prevalent throughout the alimentary tract, and recognised mainly for their asaccharolytic growth, using lactic and other short-chain organic acids for growth. As such, these bacteria can develop cooperative associations and biofilms with carbohydrate-utilising and/or lactic acid producing bacteria such as *Streptococcus, Actinomyces* and *Fusobacterium*. These syntrophic associations have been best characterised for communities within the oral cavity.[46] Additionally, like *Fusobacterium* spp, *Veillonella* spp. can produce sulfide from both sulfur- containing amino acids and other reduced S-containing metabolites (e.g., thiosulfate)[53] but this metabolism is both dependent on (neutral) pH and lactate availability.[54] As such, both the pH and lactate availability in the duodenal bulb would be permissive to microbial sulfide production by *Veillonella* spp but is unlikely within the stomach. Studies with rat models suggest a dual effect from sulfide in terms of gastric emptying and motility, via promoting contractions at low tissue concentrations, but relaxation at high concentrations.[55] However, the impacts and implications of sulfide production by endogenous (tissue-based) mechanisms, as compared with that derived from GI microbes on GI function, tissue hypoxia and motility remains enigmatic. In summation, the results presented here highlight the need to consider how the MAM influences the presence of various metabolites in the small intestine, and how these might impact GI motility, gut homoeostasis, and the pathophysiology of functional GI disorders.

The impacts from PPI use (or non-use) on the within sample (alpha) diversity of the duodenal MAM appeared minimal for both subject groups alone or when compared in combination. Similarly, the between sample (beta diversity) dissimilatory measures were no different between the PPI users and non-users with FD, which further suggests there are no drastic impacts from the use of this medication on the duodenal MAM. In contrast, the measure of dissimilarity among the PPI users in the control group were significantly greater than the non-users, but most likely reflects the difference in the sample sizes for the respective subgroups. Here, the nature of PPI use would be considered long term for the FD and control subjects, with no subjects likely deemed in either group to be PPI 'starters' or 'stoppers' as described by Wauters *et al*.[56] Although the experimental design, sampling methods and nature of the comparison made differs from that used here, there are complementary findings of clinical relevance between the two studies. For instance, Wauters *et al*[56] suggested increased *Streptococcus* occurs with both short term use of PPI in FD subjects, and in FD subjects after long-term PPI use, and in the latter group, might ultimately be associated with increased eosinophil infiltration. Indeed, previous studies have linked density of eosinophil or mast cell infiltration into the duodenal mucosa,[7] along with cytokine levels and circulating gut-homing T lymphocytes,[9] with FD symptoms. Here, we have shown that the differences in relative abundances of Firmicutes (*Streptococcus*), Bacteroidota (*Prevotella*) and Fusobacteriota (*Fusobacterium*) could be linked to symptom burden in FD subjects, with those who reported the highest symptom burden also having the greatest proportions of *Streptococcus*. In that context, there is a large amount of phenotypic variation inherent to *Streptococcus* spp. from the small intestine[57] and Fukui *et al*[58] reported from a small number of FD subjects that symptom scores were positively correlated with the relative abundance of a specific bacterial lineage most closely affiliated with *Streptococcus infantis*. Taken together our data, along with these studies suggest that the relationship between the duodenal MAM and symptoms in FD likely involves a feedback loop in which a dysregulated immune reaction occurs in response to specific lineages of key bacterial taxa, which can result in symptoms. As such, changes to the duodenal MAM composition could be both a trigger and a consequence of these immune responses. Furthermore, given FD is a disorder that is associated with temporal variations in symptom burden in an individual, these data suggest that the point in time at which patients are assessed, and whether this coincides with a period of higher or

lower symptom burden, will influence findings related to the duodenal MAM.

FD is often associated with meal-related symptoms, particularly the postprandial distress subtype, and some associations between specific dietary components, such as fat, and symptoms, have been observed previously.[22 23] In our study, we did not find any link between symptom exacerbation and specific foods. Although it could be speculated that patients self-restrict foods that they perceive to contribute to symptoms, in this cohort we did not observe any significant differences in dietary intake in FD patients as compared with controls. However, our measures of dietary intake reported here are primarily restricted to macronutrient components, and in the future both methods to identify and quantify lesser components, such as food additives, are warranted. Although a prospectively recorded food diary would measure actual short-term dietary intake, both habitual long-term diet and recent dietary intake are relevant when examining the microbiome at one point in time, and the retrospective nature of the FFQ used here was chosen to limit the burden on the enrolled subjects in advance of their diagnostic procedures.

Overall, there has been very little investigation of the impact of habitual diet on the upper GI microbiota, including the duodenum. Saffouri *et al*[16] used a short-term dietary intervention of low fibre and high simple sugars to induce GI symptoms and increase gut permeability, with commensurate reductions in microbial diversity in duodenal aspirates and stool. However, our findings and those of others, e.g., [56] suggest that, in contrast to the large bowel, there are no striking relationship(s) between long-term dietary pattern and the composition of the duodenal MAM. Thus, the strongest selective pressures that operate in the duodenal niche appear to be largely independent of diet and rather are host related. Relatively rapid transit times, when combined with the competition from endogenous (host-derived) nutrient uptake systems, likely limits the fermentative capacity of the microbiota in this niche.[54] The duodenum is also subject to relatively rapid fluctuations in conditions, with variations in pH and bile concentration also having the potential to influence the microbiota composition. Additionally, recent studies suggest that goblet cell, and thus mucus, homoeostasis is disrupted in the duodenum of FD patients, with reduced villous tip goblets cells in FD patients over controls. Such changes are likely to have profound effects on MAMs.[59]

Our results provide new insights into host-microbe and microbe-microbe interactions that appear relevant to both GI motility in general and/or symptoms associated with FD more specifically. These results, when placed in context with previous literature regarding antibiotic use,[17] the duodenal microbiota[18] and potential routes of immune activation in FD[60], all suggest that the duodenum possesses a microbiome that is dynamic and site-specific rather than opportunistic and transient, which has the potential to impact symptom generation in FD. There are also several limitations associated with the current study. We acknowledge that the cross-sectional nature of this study may not be as powerful as other study designs, but we have attempted to minimise the impact of potential confounding factors and multiplicity on the results. Second, although our findings suggest there are likely to be intra-genus changes in the duodenal microbiota in FD and/or driving symptom burden, 16S rRNA gene amplicon sequencing often lacks the resolution to definitively validate all these microbial alterations. However, deep functional characterisation of the MAM by DNA sequencing remains constrained by the overwhelming amounts of host DNA coextracted from tissue and will remain so until alternative methods of microbial (DNA) enrichment are realised. Despite these limitations, the findings from this study are both novel and provide the rationale and justification for more demanding intervention-based and/or longitudinal studies. The duodenal microbiome should be the target of further investigation, if a more complete understanding of the pathophysiology of FD is to be realised and translated into more targeted treatments for this disorder.

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Supplementary Tables

ALTERATIONS TO THE DUODENAL MICROBIOTA ARE LINKED TO GASTRIC EMPTYING AND SYMPTOMS IN FUNCTIONAL DYSPEPSIA

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Supplementary Table S1. Additional participant demographic data.

	Functional (n=56)	**Controls (n=30)**	**p-value**
IBS symptoms (clinical diagnosis): n (%)	43 (77)	NA	NA
Iron deficiency/FOBT: n (%)	NA	11 (36.6) / 19 (63.3)	NA
SAGIS: Median (range)			
Reflux symptoms[a]	1 (0-4)	0 (0-3)	#
Upper GI symptom domain – total score[b]	8 (1-20)	0 (0-3)	**< 0.0001**[e]
Fullness[a]	2 (0-4)	0 (0-1)	#
Early satiety[a]	2 (0-4)	0 (0-2)	#
Post-prandial pain[a]	2 (0-4)	0 (0-2)	#
Epigastric pain[a]	2 (0-4)	0 (0-3)	#
Retrosternal discomfort[a]	1 (0-4)	0 (0-3)	#
Lower GI diarrhoea symptom domain – total score[c]	7 (0-23)	2 (0-4)	**< 0.0001**[e]
Lower GI constipation symptom domain – total score[d]	5 (0-12)	0 (0-2)	**< 0.0001**[e]
Current NSAID use: n (%)	16 (29)	1 (3.3)	**0.013**[f]
Asthma: n (%)	8 (14.3)	3 (10)	NS[f]
Diabetes (Type 2): n (%)	7 (12.5)	3 (10)	NS[f]
HADS Anxiety: median (range)	5 (0-18)	1 (0-12)	0.05[e]
HADS Depression: median (range)	6 (0-17)	5 (0-17)	0.44[e]

a Total possible score is 4. b Total possible score is 20. c Total possible score is 24. d Total possible score is 12. e Mann-Whitney. f Fisher's Exact Test. # Significance not calculated due to high frequency of zero values in controls.

Supplementary Table S2. Diet Cohort (n=39) – Demographics

	FD (n=28)	**Controls (n=11)**	***p*-value**
Female gender: n (%)	14 (50)	5 (45)	1.00[a]
Age (yr): median (range)	50 (17-76)	52 (22-71)	0.99[b]
Body Mass Index (kg/m^2): mean (SD)	25.3 (6.9)	29.0 (7.5)	0.23[c]
Current PPI use: n (%)	20 (71)	4 (36)	0.07[a]
Smoking status (Current/Previous/Never): n (%)	6/9/13 (21/32/47)	3/1/7 (27/9/64)	0.33[d]
Upper GI Symptom Domain – total score: Median (range)[#]	9 (1-20)	0 (0-3)	**<0.0001**[b]
NDI-QOL: mean (SD)*	55.9 (30.5)	97.3 (4.3)	**0.0039**[c]
Meal related symptoms: median (range)*	337 (0-2065)	174 (0-904)	0.37[b]

* Patient numbers (where different from overall study cohort): NDI-QOL (FD n=16; Controls n=6); Meal related symptoms (FD n=25; Controls n=9). [#] The total possible upper GI symptom score is 20. [a] Fisher's Exact Test. [b] Mann-Whitney. [c] T-test. [d] Chi-squared.

Supplementary Table S3. A Mean energy, nutrient and FODMAP intake in patients with functional dyspepsia and controls. **B** National dietary recommendations for protein, fibre and micronutrients analysed in this study, and proportion of patients with FD and controls meeting recommendations.

A		**FD (n=28)**		**Controls (n=11)**		***p*-value**	**General population****
Energy	(kJ/d)	7476	(5768-9485)	7847	(6239-9421)	0.652	9345
Carbohydrate	(g/d)	202	(153-161)	209	(171-293)	0.632	235
Starch	(g/d)	97	(66-129)	102	(54-158)	0.592	126
Sugars	(g/d)	106	(67-159)	119	(62-155)	0.693	102
Protein	(g/d)	84	(64-111)	91	(68-121)	0.35	98
Fat	(g/d)	58	(46-94)	62	(44-81)	0.592	78
Saturated fat	(g/d)	24	(16-32)	24	(15-35)	0.933	29
Monounsaturated fat	(g/d)	27	(20-41)	24	(15-32)	0.463	30
Polyunsaturated fat	(g/d)	10	(6-13)	8	(5-14)	0.693	12
Dietary fibre	(g/d)	28	(14-34)	26	(14-39)	0.572	25
Resistant starch	(g/d)	3	(2-4)	5	(3-7)	0.03	-
Vitamin A^	(µg/d)	1304	(738-1738)	1386	(868-1471)	0.866	875
Thiamin	(mg/d)	2	(1-3)	2	(1-3)	0.592	2
Riboflavin	(mg/d)	2	(1-4)	3	(2-4)	0.257	2
Niacin	(mg/d)	25	(17-33)	21	(18-33)	0.672	45
Folate	(µg/d)	347	(199-450)	466	(299-674)	0.131	305
Vitamin C	(mg/d)	174	(75-245)	178	(122-230)	0.933	113
Sodium	(mg/d)	2295	(1259-2995)	2018	(1803-2495)	0.910	2540
Potassium	(mg/d)	3284	(2314-4463)	3704	(3093-5021)	0.201	3172
Magnesium	(mg/d)	275	(192-374)	344	(276-440)	0.191	366
Calcium	(mg/d)	733	(451-1038)	845	(741-1200)	0.131	781
Phosphorous	(mg/d)	1372	(973-1732)	1631	(1287-1771)	0.146	1574
Iron	(mg/d)	12	(9-15)	14	(10-20)	0.181	12
Zinc	(mg/d)	12	(9-16)	12	(10-15)	0.800	12
Total FODMAPs	(g/d)	22.8	(9.5-33.1)	25.1	(20.9-39.8)	0.269	-

Fructo-oligosaccharides	(g/d)	2.2	(1.2-3.8)	2.6	(1.1-3.8)	0.910	-
Galacto-oligosaccharides	(g/d)	0.6	(0.3-0.9)	0.7	(0.4-1.9)	0.245	-
Lactose	(g/d)	12.7	(3.0-17.5)	16.1	(5.3-23.8)	0.412	-
Excess fructose	(g/d)	2.6	(0.9-5.5)	2.1	(0.6-5.6)	0.632	-
Total polyols	(g/d)	2	(0.6-4.0)	2.5	(0.2-2.3)	0.612	-

Values are median (IQR) unless stated; Mann Whitney U test comparing functional dyspepsia with controls **Data are mean values for 51-70 year old males from the Australian Health Survey 2011-2012, Australian Bureau of Statistics 2015. ^retinol equivalents.

B		**National dietary recommendations**		**FD**		**Controls**		**p-value**
		Males	Females	n	%	n	%	
Protein	g/d	64-81	46-57	25	89	9	82	1
Dietary fibre	g/d	30	25	14	50	5	46	0.629
Vitamin A^	µg/d	900	700	23	82	8	73	1
Thiamin	mg/d	1.2	1.1	21	75	8	73	1
Riboflavin	mg/d	1.3-1.6	1.1-1.3	25	89	11	100	0.172
Niacin	mg/d	16	14	28	100	11	100	1
Folate	µg/d	400	400	11	39	6	55	0.305
Vitamin C	mg/d	45	45	28	100	10	91	0.723
Sodium	mg/d	2000	2000	13	46	5	46	0.557
Potassium	mg/d	3800	2800	15	54	5	46	1
Magnesium	mg/d	400-420	310-320	9	32	4	36	0.713
Calcium	mg/d	1000-1300	1000-1300	8	29	3	27	1
Phosphorous	mg/d	1000	1000	23	82	11	100	0.086
Iron	mg/d	8	28	20	71	9	82	0.453
Zinc	mg/d	14	8	19	68	7	64	1

Nutrient reference values for Australia and New Zealand, age-specific recommendations apply where ranges are specified; Fisher's exact test (proportion of patients meeting national recommendations; ^retinol equivalents.

Supplementary Table S4. Assessment of epigastric domain symptom scores (SAGIS) and daily energy, macronutrient, or FODMAP intakes in FD patients.

	Spearman Correlation	***p*-value**
Energy (kJ/d)	-0.082	0.659
Carbohydrate (g/d)	0.014	0.942
Protein (g/d)	-0.045	0.808
Fat (g/d)	0.211	0.255
Dietary fibre (g/d)	0.133	0.475
Total FODMAPs (g/d)	0.090	0.632

Supplementary Table S5. Microbiota Cohort (n=80) – Demographics.

	Functional (n=51)	**Controls** (n=29)	***p*-value**
Female: n (%)	27 (53)	14 (48)	NS[a]
Age: median (range)	47.4 (17-76)	60 (21-74)	0.01[b]
BMI: mean (range)	25.1 (15.3-41.8)	27.5 (18.7-40.2)	NS[c]
Current PPI use: n (%)*	30 (60)	6 (21)	< 0.0005[a]
Smoking status (Current/Previous/Never): n (%)*	9/19/21 (18/39/43)	6/5/18 (21/17/62)	NS[d]
Upper GI Symptom Domain – total score: Median (range)[#]	8 (1-20)	0 (0-3)	< 0.0001[b]
NDI-QOL: mean (SD)*	58.9 (25.3)	97 (4.2)	< 0.005[c]

*Number of subjects with data available (where different from overall microbiota cohort): Smoking status (Functional n=49); Current PPI use (Functional n=50; Controls n=28); NDI-QOL (Functional n=29; Controls n=9). [#] The total possible upper GI symptom score is 20. [a] Fisher's Exact Test. [b] Mann-Whitney. [c] T-test. [d] Chi-squared. NS – not significant.

Supplementary Table S6. Taxonomic affiliation and mean relative abundances (%) of the bacterial ASVs recovered from the duodenal mucosa-associated microbiota of FD (n=51) and non-FD control subjects (n=29).

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
74b27e89cd9c9ef97c763370921752bf; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.594	0.312	1.903
6823dbf5cd57e2a8006073ac311d8c84; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.023	0.072	0.324
9edb4a4aa86f51103b3351b0f3b847cc; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.637	0.350	1.819
38dab7479889db638ce832e53721c529; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.000	0.133	0.000
dad71ea3230954b6042aa24ff90b89f8; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.168	0.254	0.661
1056e0b2c2c32a57349a618478805b1f; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.373	0.147	2.545
8e71b7869760ce1b22be8550d15340a5; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.842	0.698	1.206
a76f4bfd933c5079816778a1629e5dd0; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.005	0.137	0.037
32e5bb7a0ec8845ae07de693f1b266d0; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.366	0.472	0.776
9983c3f66e6c7c41e6f116b98113c625; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.118	0.045	2.632
9a357f2c5b7093e12cdfbf78ef7a9178; p__Actinobacteriota; g__Actinomyces; s__Schaalia_odontolytica	0.276	0.027	10.301
d6168aae7a5cacd29bcab2ff2333996a; p__Actinobacteriota; g__Actinomyces; s__Actinomyces_graevenitzii	0.082	0.136	0.604
e467715a67ce6ea88f3ad3c295e483dc; p__Actinobacteriota; g__Actinomyces; s__Actinomyces_graevenitzii	0.391	0.205	1.907
013ee41b8ff587d1bdf1887fe6eaf555; p__Actinobacteriota; g__Actinomyces; s__Actinomyces_graevenitzii	0.127	0.139	0.913
d712ab1c70f18c134126cb995b11b3b3; p__Actinobacteriota; g__Actinomyces;	0.183	0.169	1.077
f05e503e7194ed8e46159daaf5298cd0; p__Actinobacteriota; g__Rothia;	1.160	0.564	2.058
a09947c14d8385f61d82319d966bb99c; p__Actinobacteriota; g__Corynebacterium; s__Corynebacterium_sp.	0.000	0.333	0.000
b48f2abfde36ed7d46d47ea5482018e7; p__Actinobacteriota; g__Atopobium;	0.675	1.111	0.607
b39370284ec9a2c80f5d8ff87212efd1; p__Actinobacteriota; g__Coriobacteriaceae_UCG-002; s__uncultured_bacterium	0.231	0.162	1.426
fb10b2e1e8c83ce40986d5ed799df201; p__Patescibacteria; g__TM7x; s__uncultured_bacterium	0.093	0.299	0.311
31d4be96fd859e1eaf66b84dd0dd1541; p__Patescibacteria; g__Candidatus_Saccharimonas; s__uncultured_bacterium	0.015	0.058	0.253

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
6704e4978f6f5da9879ea18cebfcd974; p__Bacteroidota; g__Capnocytophaga; s__Capnocytophaga_sputigena	0.000	0.027	0.000
cdb05e31708e9b8d83b4d4b0681f1b02; p__Bacteroidota; g__Capnocytophaga; s__Capnocytophaga_leadbetteri	0.002	0.038	0.047
bf86bd401f319ddf1cea03466ab3d645; p__Proteobacteria; g__Acidovorax;	0.142	0.935	0.151
1b6e009cbfae8224d8cac5cf0af92871; p__Proteobacteria; g__Comamonas;	0.294	0.949	0.309
7244e3c017ce480036880d30250dd471; p__Proteobacteria; g__Comamonas; s__Comamonas_testosteroni	1.715	1.248	1.374
534fffa65be1aa048d257bd5e1f491e5; p__Proteobacteria; g__Delftia;	0.449	0.347	1.293
216fd7c203973b2829b657582a6f9157; p__Proteobacteria; g__Comamonas; s__Ottowia_sp.	0.000	0.037	0.000
36565e9de9fff682b3b18a43bf282762; p__Proteobacteria; g__Diaphorobacter;	0.279	1.146	0.244
a890f5bedd05f3b2282ad20324166345; p__Proteobacteria; g__Comamonas;	1.501	0.787	1.908
9d098ec81517a92f9502f1e593ea37ea; p__Proteobacteria; g__Stenotrophomonas;	1.563	0.467	3.349
b5be60693308a27fa2b10cfbf5134052; p__Proteobacteria; g__Acinetobacter;	0.641	0.656	0.977
273a2a4a194f67df671b3217e889b009; p__Proteobacteria; g__Pseudomonas;	0.000	2.826	0.000
df338f0037e7d53188efb8063011d228; p__Proteobacteria; g__Neisseria; s__Neisseria_elongata	0.000	0.114	0.000
3e50e48935df8d94b77c951e601e7049; p__Proteobacteria; g__Neisseria; s__Neisseria_elongata	0.000	0.127	0.000
e74d89b465edafa3cfa0f8b1e3118972; p__Proteobacteria; g__Neisseria;	0.000	0.389	0.000
d0f87608e153fe98b7a1c3e5da0c79b0; p__Proteobacteria; g__Neisseria;	0.428	1.054	0.406
39df02979923e57e9cbdf1997d834e55; p__Proteobacteria;;	0.000	0.075	0.000
38bb7974d2b96b54dea0c27714084869; p__Proteobacteria; g__Neisseria;	0.000	0.095	0.000
69128e028ddf561a6fc474e63a31acb4; p__Proteobacteria; g__Neisseria;	1.189	0.418	2.845
b7942f2ea4bd5e163819fe5ec8633532; p__Proteobacteria; g__Neisseria;	0.487	0.860	0.565
345f76b27b534508282f2662faba423a; p__Proteobacteria; g__Neisseria;	0.586	0.198	2.963
1e799f1500e5efedbb303d7929f632fa; p__Proteobacteria; g__Neisseria;	0.000	0.464	0.000
da8e4038f48d5919bbd850ac3e91cfec; p__Proteobacteria; g__Neisseria;	0.000	0.261	0.000
6fa5c599de9f95c29406541318a123a5; p__Proteobacteria; g__Aquabacterium;	0.385	0.113	3.390

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
216c5f0cc84e6903a32a0a1dd063ba64; p__Proteobacteria; g__Ralstonia;	0.159	0.778	0.204
6830d1a55335a0ba31ef244f2a97759c; p__Proteobacteria; g__Methylophilus;	0.119	0.076	1.566
0f9eac676ac9bfc1ea4036e1adcef734; p__Proteobacteria; g__Afipia;	0.146	0.115	1.268
a18eac3fb6b0f3480d349b5d28499e97; p__Proteobacteria; g__Paracoccus;	0.419	0.038	10.947
261828f18fdc48a610ec8e4811f8fbf3; p__Proteobacteria; g__Haemophilus;	0.431	0.308	1.400
cf5896c307a5dd9b932abf6255ae4a14; p__Proteobacteria; g__Haemophilus;	0.148	0.041	3.624
c2f0e05b681333bb4b516bff1065056b; p__Proteobacteria; g__Haemophilus;	0.617	0.212	2.914
055c6437480a0f4362de6cd6ec2a92a4; p__Proteobacteria; g__Haemophilus;	0.103	0.200	0.514
4054fcf6774e8ccc973443811d91234b; p__Proteobacteria; g__Haemophilus;	0.041	0.018	2.250
2fa3bac17a218b41bfc341c42d9c5c8d; p__Proteobacteria; g__Haemophilus; s__uncultured_organism	0.000	0.113	0.000
ec68e6c1df047eb9b402661726ff12b2; p__Proteobacteria; g__Haemophilus;	0.434	0.209	2.076
3f864fa71befcba1299d936b1f6406a2; p__Proteobacteria; g__Haemophilus;	0.078	0.060	1.306
b1188dd6b5f949e0f0e3ecc1b413ac46; p__Proteobacteria; g__Haemophilus;	0.209	0.049	4.284
aa2d1436aedfb0481c1e4779956f7e22; p__Proteobacteria; g__Haemophilus;	0.313	0.309	1.011
79772f9be3899db9b3c2c35b842f70a3; p__Proteobacteria; g__Haemophilus;	0.218	0.046	4.706
2ecc52aa64027655f581b6439a76cdad; p__Proteobacteria; g__Haemophilus; s__Haemophilus_haemolyticus	0.056	0.092	0.615
732fe78b4a7af11d7797adfed713f071; p__Proteobacteria; g__Actinobacillus;	0.107	0.759	0.141
f408fe002d42ffb853bebd5a9f85e823; p__Proteobacteria; g__Actinobacillus;	0.090	0.000	N/A
45ed6baf57440bddd0a76cfcc9944b81; p__Proteobacteria; g__Actinobacillus;	0.000	0.030	0.000
11f57e8f7561390e1aec80db07467063; p__Proteobacteria; g__Actinobacillus;	0.000	0.041	0.000
bd5cc870aec1e982da648826a9d5e448; p__Proteobacteria; g__Actinobacillus;	0.109	0.000	N/A
67afd64199384d1dd87dfeb0ee9b7347; p__Proteobacteria; g__Actinobacillus; s__uncultured_bacterium	0.076	0.095	0.800
51ca26d1881b6837b55171c350d54cd0; p__Proteobacteria; g__Actinobacillus;	0.088	0.334	0.265
b4d6b100f8f7c49a137c6057c440fa38; p__Proteobacteria; g__Actinobacillus;	0.319	0.110	2.908

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
f35170b975fa6e2d5c937cf9f1f8ec97; p__Bacteroidota; g__Cloacibacterium;	0.190	1.235	0.154
d3f48c8073a4dcab7e45eec3aadaa925; p__Bacteroidota; g__Cloacibacterium;	0.000	0.298	0.000
1ec036f8894f3092d0e25691548dcaed; p__Bacteroidota; g__Alloprevotella; s__uncultured_Bacteroidetes	0.126	0.481	0.262
b8b4f40b8d5210a9b4da2e2105763429; p__Bacteroidota; g__Alloprevotella; s__uncultured_Bacteroidetes	1.284	1.667	0.771
082f37db298be4647a3e0cc4e1a768b0; p__Bacteroidota; g__Alloprevotella; s__uncultured_Bacteroidetes	0.000	0.026	0.000
4644c533937043ef7eabf01e8bcf3816; p__Bacteroidota; g__Alloprevotella; s__Alloprevotella_tannerae	0.000	0.241	0.000
11a23073a41fdc3e4e9a052b01ab466d; p__Bacteroidota; g__Alloprevotella; s__Alloprevotella_tannerae	0.000	0.117	0.000
1d819270289c698c9fb4fd9446e6bd86; p__Bacteroidota; g__Alloprevotella; s__Prevotellaceae_bacterium	0.000	0.668	0.000
62ff085ca7529e07e787c14c36d58b3f; p__Bacteroidota; g__Alloprevotella; s__Prevotellaceae_bacterium	0.000	0.091	0.000
9010663bb0610f77ef0f3a8223d707aa; p__Bacteroidota; g__Porphyromonas; s__uncultured_bacterium	1.897	0.975	1.945
074e527f490051ccc9a80deea3083f69; p__Bacteroidota; g__Porphyromonas; s__uncultured_bacterium	0.090	0.240	0.376
6318e8f2dcd091326bbf6f13ba9c969c; p__Bacteroidota; g__Porphyromonas;	0.000	0.031	0.000
6825338ddc6325f93236d02aa3c33dc1; p__Bacteroidota; g__Porphyromonas;	0.978	0.833	1.174
7e85a3b08d91247b9a8355c014b6dc56; p__Bacteroidota; g__Porphyromonas; s__uncultured_bacterium	0.005	0.038	0.120
b65f47549a79cb100b8ef96aef092f7a; p__Bacteroidota; g__Porphyromonas; s__uncultured_bacterium	0.123	0.098	1.250
e103c140d199986183d6fcdf0494f156; p__Bacteroidota; g__Porphyromonas; s__Porphyromonas_endodontalis	0.000	0.765	0.000
8b6251d613bf1800ca3979fe90a7c1dc; p__Bacteroidota; g__Prevotella; s__Prevotella_baroniae	0.000	0.588	0.000
93b68f9d67cdb7c5554d3c7dbc2d3922; p__Bacteroidota; g__Prevotella; s__Prevotella_nigrescens	0.000	0.105	0.000
765b26409608b5a9fd6b359183b2564a; p__Bacteroidota; g__Prevotella; s__Prevotella_nigrescens	0.027	0.415	0.065
79e05d4f80fe8b724edd211d345b67af; p__Bacteroidota; g__Prevotella; s__Prevotella_nigrescens	0.083	0.100	0.822
7b6d5cc7a9975b3b6e5e55bc2c3c4eb8; p__Bacteroidota; g__Prevotella; s__Prevotella_nigrescens	0.640	0.091	7.054
fbb860e2745d3d52e657cee87c6a7647; p__Bacteroidota; g__Prevotella; s__Prevotella_scopos	0.123	0.031	3.964
552f877efc1cea5779afa39eaf357977; p__Bacteroidota; g__Prevotella; s__Prevotella_salivae	0.340	0.394	0.863
34b97e62101d653c52768424a5b10b87; p__Bacteroidota; g__Prevotella; s__Prevotella_shahii	0.342	0.060	5.686

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
d92af488c13f0f1f704d5ff2a3a026f8; p__Bacteroidota; g__Prevotella; s__Prevotella_loescheii	0.000	0.119	0.000
d632100c471e43d1600d05d0026503f5; p__Bacteroidota; g__Prevotella; s__Prevotella_nanceiensis	0.198	0.342	0.580
078bacdaa1c491bf1c8f80d7f30af1f6; p__Bacteroidota; g__Prevotella; s__Prevotella_nanceiensis	0.158	0.067	2.374
55449126e2d260bd8198fdc29631c60e; p__Bacteroidota; g__Prevotella; s__Prevotella_nanceiensis	0.000	0.096	0.000
89ec430d6b38001259a72ff6a267a535; p__Bacteroidota; g__Prevotella; s__Prevotella_jejuni	0.052	0.496	0.105
eb30dd8073dbebf6ee9b01cbfa387452; p__Bacteroidota; g__Prevotella;	0.000	0.101	0.000
107ae5198cc68bddf50697f4a323185d; p__Bacteroidota; g__Prevotella; s__Prevotella_jejuni	0.087	0.123	0.713
41f9fbdfcc12ec6a7f200f906cc14d50; p__Bacteroidota; g__Prevotella; s__Prevotella_jejuni	0.000	0.220	0.000
8aa414567e722892028b26dcc92c1d11; p__Bacteroidota; g__Prevotella;	0.364	0.094	3.851
949c20c2b15a9f6a1e7fd10da046b6b0; p__Bacteroidota; g__Prevotella; s__Prevotella_jejuni	0.187	0.574	0.326
3abe880aac275be5e579062a4750645a; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.161	0.171	0.946
f49023a86bd0eab30012f9cafe3e7cd2; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.691	0.627	1.102
2dc06f56fbdf5cbdbcb53df1b1846c43; p__Bacteroidota; g__Prevotella;	0.075	0.552	0.135
35f86afd983ea1d38246d01f117eb785; p__Bacteroidota; g__Prevotella;	0.160	0.490	0.328
d24f782732ea28532bcb63f173a819d0; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.000	0.065	0.000
65f1d4de6268d71f35c4e539ce0a824d; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.063	0.323	0.196
85d6bd68d26a231b7e82fdb453dc812a; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	2.823	1.515	1.864
22bec8320f7bed692cae2892cebd467a; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.110	0.559	0.197
0fa00fc8f1b53675a72e5405adc62601; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.371	0.048	7.651
e764dde5b209115b95b6fa2df1116d0b; p__Bacteroidota; g__Prevotella;	0.015	0.280	0.053
793f54fd6d62ace6253ec30f3c11b50b; p__Bacteroidota; g__Prevotella;	0.501	0.446	1.124
e88567e430179939l8eebd7453fa4564; p__Bacteroidota; g__Prevotella; s__Prevotella_jejuni	0.196	0.000	N/A
c169e98a91a5fcf1e39ace4b8596bd4b; p__Bacteroidota; g__Prevotella; s__Prevotella_pallens	0.045	0.014	3.291
3bf6f7017749064e7d0771cb33a1d52e; p__Bacteroidota; g__Prevotella; s__Prevotella_pallens	0.161	0.150	1.071

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
f463c00fd4088dfde4c2a45c6ba26d20; p__Bacteroidota; g__Prevotella; s__Prevotella_pallens	0.030	0.107	0.277
a96964d0d89a4e14db01b3a24d439be4; p__Bacteroidota; g__Prevotella; s__Prevotella_pallens	0.014	0.373	0.036
f3de9a6ada63d91187b3c91e0cbb5a20; p__Bacteroidota; g__Prevotella; s__Prevotella_pallens	0.000	0.099	0.000
953d2b7ab237c0a98b5e0e4edc80e3c2; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.007	0.098	0.069
04796c4392df70a31e10600dd189fd50; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.051	0.021	2.414
fe99ce101795b454cdb2ed063049df99; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.000	0.217	0.000
cde929f348040c6d464559d38e115445; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.000	0.085	0.000
48e321ced03e3340a151745f86ce3bf2; p__Bacteroidota; g__Prevotella; s__Prevotella_melaninogenica	0.132	0.110	1.198
db70d55955fadf6fc6889cf933a490eb; p__Bacteroidota; g__Prevotella; s__Prevotella_intermedia	0.184	0.000	N/A
8cadd601eaadfbe38caffb6bda9a7d5c; p__Bacteroidota; g__Prevotella; s__Prevotella_intermedia	0.000	0.163	0.000
78144992b8548ebf73dcb1196683f87c; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.022	0.307	0.072
e551c62a3d247c36fda236e9dcebf7a9; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.000	0.290	0.000
c01178c193078c3548e19e99ca94f914; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.000	0.372	0.000
0268fb988c133ae1a55e77e0bd5afb46; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.179	0.350	0.510
2494e1895d6257fed945a545a3c398ee; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.171	0.201	0.855
d1e2af01b49f154c82093d2816a63725; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.246	0.000	N/A
13386368981462b97b342c4ce6bde6d5; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.471	0.279	1.691
b607582b2eee61b470b0f12eaa4f395f; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.184	0.455	0.405
e068f133c3dc246942163421a5f767b2; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.325	0.000	N/A
3ddbe83ea243363ee107a5de3e92e420; p__Bacteroidota; g__Prevotella; s__Prevotella_histicola	0.039	0.394	0.098
f0617bd438dc1d9a92961a12e14a853c; p__Firmicutes; g__Faecalibaculum; s__Faecalibaculum_rodentium	0.180	0.263	0.685
ccd205f63287aed136fea8d9509abed5; p__Firmicutes; g__Faecalibaculum; s__Faecalibaculum_rodentium	0.455	0.531	0.857
20c2d546d65037204201bc65eb2fc3ae; p__Firmicutes; g__Faecalibaculum; s__Faecalibaculum_rodentium	0.052	0.149	0.346
247e97af9f94955c547d8d0a97c89ff5; p__Firmicutes; g__Allobaculum; s__uncultured_bacterium	0.350	0.109	3.222

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
efa3acb308144a1bdfc1c6b1f577b695; p__Firmicutes; g__Allobaculum; s__uncultured_bacterium	0.523	0.194	2.700
47f5f71889d57f4ab3e0fc8bbc9e327d; p__Firmicutes; g__Megasphaera; s__Megasphaera_micronuciformis	0.344	0.213	1.617
a0a6092dc4f628a9fc60971edfa0c0d8; p__Firmicutes; g__Megasphaera; s__Megasphaera_micronuciformis	0.047	0.125	0.374
5d52394402f13f4ff1947b2b97a31e52; p__Firmicutes; g__Megasphaera; s__Megasphaera_micronuciformis	0.205	0.239	0.857
3818f843ed70a1138ddce42dc41ed532; p__Firmicutes; g__Veillonella;	0.878	0.830	1.058
adc60ce7efed0c09242cb03ee0646177; p__Firmicutes; g__Veillonella; s__Veillonella_atypica	0.254	0.655	0.388
b8076f562c8b692f8f10a197a688d39d; p__Firmicutes; g__Veillonella; s__Veillonella_parvula	0.189	0.082	2.301
cf06db36e238c68ee808d24dd64ec5a8; p__Firmicutes; g__Veillonella;	0.586	0.342	1.717
d2c48dc3337f713e413ceeb821a8fcf4; p__Firmicutes; g__Veillonella;	0.052	0.004	12.664
deba6c7e0e182cd5d09d5b1c56698951; p__Firmicutes; g__Veillonella;	0.000	0.049	0.000
c90158d88522b2a86cb653cad1b95b77; p__Firmicutes; g__Veillonella;	1.933	2.449	0.789
a27bc1df37ec34ae3a95c71a7f3cbb89; p__Firmicutes; g__Veillonella;	0.516	0.505	1.022
434db050089f57a1122421834fb0442f; p__Firmicutes; g__Veillonella;	0.070	0.048	1.473
ec58012752b27403e7cf3772f7ed2f24; p__Firmicutes; g__Veillonella;	0.195	0.090	2.162
166d2b31136f74d18b024ac1e83d201f; p__Firmicutes; g__Veillonella;	1.710	1.075	1.591
359aeb26bb0448ab7179fe427dade8b2; p__Firmicutes; g__Veillonella;	0.427	0.168	2.534
ffd8aad6ec54d39d148d210e02b5db8c; p__Firmicutes; g__Veillonella;	0.041	0.138	0.298
0fd33217d14567b3988e506f406eabf2; p__Firmicutes; g__Veillonella; s__uncultured_methanogenic	0.073	0.044	1.637
fefafcba6f1058e66956b68aef69d9bf; p__Firmicutes; g__Lachnospiraceae_UCG-006; s__uncultured_bacterium	0.055	0.152	0.365
b72e2d6ac1351225e96464117180c3d1; p__Firmicutes; g__Oribacterium; s__uncultured_organism	0.407	0.163	2.502
5a81665a7ded6a4064201a8c7293ce1d; p__Firmicutes; g__Oribacterium; s__uncultured_organism	0.046	0.049	0.935
546b84c00601b65ddc080005162ada37; p__Firmicutes; g__GCA-900066575; s__uncultured_bacterium	0.127	0.055	2.298
76403f7757dbb8005c67832599ad3a2b; p__Firmicutes; g__Lachnospiraceae_NK4A136_group;	2.044	1.101	1.857
f04bf296b24b6557a60a4dbdcaa59d12; p__Firmicutes; g__Stomatobaculum; s__uncultured_bacterium	0.011	0.051	0.210

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
9e1ab87cb49db0f30677c1a038a53ed4; p__Firmicutes; g__Stomatobaculum; s__uncultured_bacterium	0.034	0.070	0.477
fa2207d42d8712f846d2e9c79eae5e3c; p__Firmicutes; g__Stomatobaculum; s__uncultured_bacterium	0.332	0.174	1.903
f6ed27efc8778310cc8a680d7027278f; p__Firmicutes; g__Blautia;	0.222	0.180	1.236
bdcc5b12d9ee2dbd173c8b71d07114ad; p__Firmicutes;;	0.000	0.099	0.000
b3431c11060d2f6f4f4275e80ceb621d; p__Firmicutes; g__Staphylococcus; s__Staphylococcus_cohnii	0.000	0.112	0.000
826346891c486c88e2d53d9b4cb7cd7f; p__Firmicutes; g__Staphylococcus;	0.009	0.221	0.041
ebfff416dc75df5552cd10af37921d64; p__Firmicutes; g__Staphylococcus;	0.000	0.230	0.000
6304e1d5dbe219667557f5bd13d69c8e; p__Firmicutes; g__Staphylococcus; s__Staphylococcus_hominis	2.146	1.530	1.403
54af7fec8668ba2a7524da131b279966; p__Firmicutes; g__Gemella; s__uncultured_organism	0.299	0.158	1.892
edaa08e8505a58b597c12e0e898861ca; p__Firmicutes; g__Gemella; s__uncultured_organism	0.387	0.150	2.585
d7d0aa669fe8abb027ac002bec1848c4; p__Firmicutes; g__Gemella; s__uncultured_organism	0.070	0.055	1.279
95545f5404b49e56590de31ccd67fa48; p__Firmicutes; g__Gemella;	0.054	0.077	0.708
792c4e177533c2a95ada1a2887cb9c79; p__Firmicutes; g__Gemella; s__Gemella_haemolysans	0.000	0.199	0.000
614b119ca498e1a7474e191690c09664; p__Firmicutes; g__Dubosiella; s__Dubosiella_newyorkensis	0.497	0.366	1.359
40f703b4e99ace7ede1bacfc6859a0e9; p__Firmicutes; g__Turicibacter; s__Turicibacter_sp.	0.328	0.088	3.727
8507ddd08c521d44317256fe4c9ae75d; p__Firmicutes; g__Turicibacter;	0.503	0.107	4.697
f887075289522ed7eb4d7deee98231a1; p__Firmicutes; g__Enterococcus; s__Enterococcus_faecalis	0.465	0.239	1.941
af863a6a5b3c1bc66717aa82931830d3; p__Firmicutes; g__Granulicatella;	0.174	0.123	1.423
5027b9bf859e720ac1120ecd5b54570b; p__Firmicutes; g__Granulicatella;	0.874	1.031	0.848
58ba8ac3e486937c27761fd0281b1f24; p__Firmicutes; g__Lactococcus; s__Lactococcus_lactis	0.000	0.040	0.000
e7b41c913607eebdcb5bb61ed9f36fe7; p__Firmicutes; g__Lactobacillus; s__Lactobacillus_johnsonii	0.504	0.271	1.858
531595cd55461c97908d65f621b1e0a1; p__Firmicutes; g__Streptococcus; s__Streptococcus_cristatus	0.201	0.167	1.203
c5a6d757b82f8d91c24d5d98589fda91; p__Firmicutes; g__Streptococcus; s__Streptococcus_cristatus	0.151	0.060	2.519
e7d58b53a8e6e240f08bade4448c2abb; p__Firmicutes; g__Streptococcus; s__Streptococcus_cristatus	0.104	0.080	1.296

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
984c198b023760ee1382dca3f0e39ac1; p__Firmicutes; g__Streptococcus; s__Streptococcus_salivarius	6.524	4.732	1.379
da7ea4b6d50e8468c610f62f8a5dc552; p__Firmicutes; g__Streptococcus;	0.000	0.483	0.000
7eace213225d1cccd1b856f63ac500d7; p__Firmicutes; g__Streptococcus;	1.532	0.064	23.771
d88e1d0697a4db2989162cdf9241edb9; p__Firmicutes; g__Streptococcus;	0.000	0.173	0.000
172327fea19d917f7633c96e14edba14; p__Firmicutes; g__Streptococcus; s__Streptococcus_intermedius	0.002	0.105	0.015
458ab1ab32f26b61de42d8563b27f85f; p__Firmicutes; g__Streptococcus; s__Streptococcus_gordonii	0.074	0.089	0.827
d7bdd43f84f696ec913fd557fca7ecf7; p__Firmicutes; g__Clostridia_UCG-014; s__uncultured_bacterium	0.179	0.000	N/A
6462e149e36488325633921d7ce6d94a; p__Firmicutes; g__Clostridium_sensu_stricto_12;	0.000	0.504	0.000
82daaddc518f3d49a1f1cee6f31d9cd5; p__Firmicutes; g__Clostridium_sensu_stricto_1; s__Clostridium_perfringens	0.000	0.110	0.000
740531b95866f753a9520ae045f1bf7e; p__Firmicutes; g__Parvimonas; s__Parvimonas_micra	0.145	0.203	0.714
ac58854ed918549221120 45b1e6e0a2d; p__Firmicutes; g__[Eubacterium]_nodatum_group; s__Eubacterium_sulci	0.005	0.155	0.030
c9056fc4da49d9f22c923fa6b8924d30; p__Firmicutes; g__Peptostreptococcus; s__uncultured_organism	0.215	0.337	0.638
fde9b9970f32f7ac0f86df14ee2ac193; p__Firmicutes; g__Mogibacterium; s__Mogibacterium_diversum	0.155	0.250	0.619
5d0feb23fb25cddb122122ea56f7a061; p__Firmicutes; g__Solobacterium; s__Solobacterium_moorei	0.025	0.121	0.210
f04e69ac93845b33bed463d967565168; p__Firmicutes; g__Solobacterium; s__Solobacterium_moorei	0.324	0.174	1.863
575db35010d497f952f0841659198f00; p__Firmicutes; g__Solobacterium; s__Solobacterium_moorei	0.126	0.089	1.414
d84dd888254731d2a2766855e2d00c5e; p__Firmicutes; g__Streptococcus;	0.494	1.446	0.341
b2d257ec69fd6ac14a7348ed364b8174; p__Firmicutes; g__Streptococcus;	0.195	0.158	1.241
3442caa1a5b23598c105d026b2ac68e3; p__Firmicutes; g__Streptococcus;	0.000	0.280	0.000
2af0d508377b01a8363e78709199abc0; p__Firmicutes; g__Streptococcus;	0.383	0.493	0.776
ff8f15165ec9bbe3e1ef77cff6adc698; p__Firmicutes; g__Streptococcus;	0.046	0.127	0.365
632f9b31fd42e2954817bdc8e0dd5667; p__Firmicutes; g__Streptococcus; s__Streptococcus_constellatus	0.022	0.068	0.331
e9f53e15f4b669d92aa0b706879cdc54; p__Firmicutes; g__Streptococcus;	0.349	1.447	0.241

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
8e9c8406ab6a82df44f6aa8e2f6f6ac2; p__Firmicutes; g__Streptococcus;	0.007	0.095	0.072
5ae793b584b92950ddf57ab374b9608e; p__Firmicutes; g__Streptococcus; s__Streptococcus_mitis	0.032	0.048	0.660
efa90301eedd0949fffb6aaaf565a38c; p__Firmicutes; g__Streptococcus;	0.016	0.087	0.181
35bd083980632ad969b2c767fc9b7691; p__Firmicutes; g__Streptococcus;	0.194	0.057	3.414
02a20a8cce03a755158ebef4dba5802b; p__Firmicutes; g__Streptococcus;	1.484	1.563	0.950
936e692b28471a90d081685799cb323e; p__Firmicutes; g__Streptococcus;	0.640	0.642	0.998
eead0fcfdbb69d070257cf9ecb135f9c; p__Firmicutes; g__Streptococcus;	0.866	0.338	2.560
5d6cf2e436b1b1b6c30edd683bda6cd0; p__Firmicutes; g__Streptococcus;	0.295	0.137	2.146
96c7d7a304737c45f375a51e3d44fc35; p__Firmicutes; g__Streptococcus;	0.000	0.039	0.000
1f00e06ea2bc667962d50390828e4fab; p__Firmicutes; g__Streptococcus;	0.004	0.070	0.056
a0b2a4c8d61a90433f0b8d4573d1ec74; p__Firmicutes; g__Streptococcus;	0.192	0.110	1.753
9f4682d3c8456eeb6bc9d436218a19e7; p__Firmicutes; g__Streptococcus;	0.000	0.050	0.006
a8d3a1ece9b641c21ee87796664fc79f; p__Firmicutes; g__Streptococcus;	0.051	0.256	0.198
65e56808220f955f3b9976b04569a1d7; p__Firmicutes; g__Streptococcus;	0.012	0.251	0.047
b6a4e8aaacea482592a528bc3aba39cf; p__Firmicutes; g__Streptococcus;	0.164	0.228	0.717
7b83d663721eacb41551a26ca349bdec; p__Firmicutes; g__Streptococcus;	0.167	0.181	0.922
92f5bc86020b5b6e847111f03a4c89e3; p__Firmicutes; g__Streptococcus;	0.484	0.100	4.855
4e4aa8d03b38dff0cd9574b4e254a0fe; p__Firmicutes; g__Streptococcus;	0.131	0.000	N/A
73c4416bfc7a374b662bd00f81857a71; p__Firmicutes; g__Streptococcus;	0.000	0.042	0.000
1952f0b93fc60606e69a3b446851b479; p__Firmicutes; g__Streptococcus;	0.000	0.032	0.000
921f9d654a9c823915f4490eeb1db1c3; p__Firmicutes; g__Streptococcus;	14.085	10.828	1.301
c2c5df98d10a38c9f0ebc1d4b32ea2f9; p__Firmicutes; g__Streptococcus;	3.314	3.292	1.007
4af8da50d63906eba3758ade5d0437c4; p__Firmicutes; g__Streptococcus;	2.516	1.402	1.795
fac4d58dbd6a188a2b95b0a9ddf7f548; p__Firmicutes; g__Streptococcus;	0.457	0.727	0.629

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
4c0aebad661be77fea6b98b88fca3c1e; p__Firmicutes; g__Streptococcus;	0.080	0.251	0.318
90d953b920b9c33f8351dad805bd751b; p__Firmicutes; g__Streptococcus;	0.241	0.164	1.469
83186b64088f61937a9f4b5e84c73932; p__Firmicutes; g__Streptococcus; s__Streptococcus_sanguinis	0.092	0.051	1.824
071f38a5c0df309bebaa445f44377d79; p__Firmicutes; g__Streptococcus;	0.170	0.102	1.659
2db016bdba077f15641feb0f8fdf8251; p__Firmicutes; g__Streptococcus;	0.477	0.343	1.391
a6a3067da6810395d88190aeb5e24dfc; p__Firmicutes; g__Streptococcus;	0.867	0.321	2.697
7b8c888db4948d7358f7e11d6c776d58; p__Firmicutes; g__Streptococcus;	1.880	1.163	1.616
7b907bbf30efe2f954d6b91411b0cbc3; p__Firmicutes; g__Streptococcus;	0.083	0.046	1.826
0d4134ac7d1731ec1e60d1287053a2d7; p__Firmicutes; g__Streptococcus;	2.662	1.343	1.982
de2982f59340143d2fc5ce74ee403305; p__Firmicutes; g__Streptococcus;	0.568	0.479	1.184
b0b914bc3b00ec240164493be136c432; p__Firmicutes; g__Streptococcus;	0.101	0.022	4.607
b69b76ac20f626b8fe4214b7230aacbd; p__Fusobacteriota; g__Fusobacterium; s__Fusobacterium_periodonticum	0.000	0.122	0.000
5771ff924b3bb4f5159910e721cd90d4; p__Fusobacteriota; g__Fusobacterium; s__Fusobacterium_periodonticum	0.332	0.037	8.935
d33334cdabfcc8c64a2b10da3888f5a2; p__Fusobacteriota; g__Fusobacterium; s__Fusobacterium_nucleatum	0.018	0.573	0.031
be771b2b63aeb7a15e756f3ae816e80a; p__Fusobacteriota; g__Fusobacterium; s__Fusobacterium_nucleatum	0.013	0.091	0.143
707b0875725b27ff7dc23900f000c0b8; p__Fusobacteriota; g__Fusobacterium; s__Fusobacterium_periodonticum	0.041	0.377	0.110
120f88f2e049fe2c6b7d071166f438be; p__Fusobacteriota; g__Fusobacterium; s__Fusobacterium_periodonticum	0.027	0.165	0.167
e49b6c97531c72a28c13bcead26f5154; p__Fusobacteriota; g__Fusobacterium;	0.000	0.039	0.000
13430d6ed2e6efb4f5d282f57c9b2b6a; p__Fusobacteriota; g__Fusobacterium;	0.126	0.081	1.554
ea0c17ecc7b047d223e26131098adb82; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.000	0.243	0.000
8cf6beeab72398247801670b664bdcc4; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.052	0.077	0.669
0afe1f8e216a26f23a3b382b192866ed; p__Fusobacteriota; g__Leptotrichia;	0.000	0.104	0.000
55e0f26c4bc750f37b2e13008ddc01b9; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.130	0.113	1.151
f3e183dbb4037254046cfded43388dc7; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.000	0.080	0.000

ASVs; Phylum (p_) Genus (g_) Species (s_)	Control	FD	Fold change
faa8c808d2ed7d8bf6be3e1e1c3b46f7; p__Fusobacteriota; g__Leptotrichia; s__Leptotrichia_sp.	0.047	0.083	0.568
cbbf419d3594398ebcf61afd2af355bd; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.077	0.043	1.812
c7ed76f7e9d0e7b36b9485594cbc21fe; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.121	0.290	0.419
4923ac233afb8a6977ef74f4999b72ab; p__Fusobacteriota; g__Leptotrichia; s__uncultured_organism	0.030	0.065	0.461
b64b12a756faaeaa2b22152724a2ea2b; p__Bacteroidota; g__Alloprevotella; s__uncultured_bacterium	0.000	0.170	0.000
089974521c2f2b4bbabfe7628b428429; p__Bacteroidota; g__Alloprevotella;	0.000	0.054	0.000
cfbc4fbe48a179931088a3f657f04a30; p__Bacteroidota; g__Alloprevotella; s__Alloprevotella_rava	0.000	0.038	0.000
e32010c34a6309135dc489a24bfcb254; p__Bacteroidota; g__Alloprevotella; s__Alloprevotella_rava	0.080	0.080	1.010
597f3be0f7955d638958dccf5dfaf206; p__Bacteroidota; g__Muribaculaceae; s__mouse_gut	0.257	0.036	7.049
b6f413120fff128ccfc188b5fa548845; p__Bacteroidota; g__Muribaculaceae;	1.382	0.000	N/A
9e5a99fe040ee9db147049158c9f037d; p__Bacteroidota; g__Paludibacter; s__uncultured_bacterium	0.000	0.410	0.000

Supplementary Table S7. Mean differences (log scale) of bacterial genera between groups (Non-FD control and FD) were calculated based on the ANCOM-BC function. ns – not significant. ** significantly different based on ANCOM-BC.

Genus	Beta[a]	p_val	q_val	ANCOM-BC[b]
Fusobacterium	**1.408**	**0.001**	**0.023**	**
Alloprevotella	1.369	0.010	0.136	ns
Acidovorax	0.980	0.007	0.136	ns
Prevotella	0.854	0.075	0.425	ns
Ralstonia	0.629	0.102	0.465	ns
TM7x	0.608	0.045	0.371	ns
Porphyromonas	0.543	0.283	0.828	ns
Cloacibacterium	0.420	0.263	0.828	ns
Diaphorobacter	0.405	0.312	0.829	ns
Granulicatella	0.403	0.344	0.829	ns
Neisseria	0.317	0.558	0.869	ns
Staphylococcus	0.313	0.465	0.829	ns
Parvimonas	0.262	0.418	0.829	ns
Comamonas	0.261	0.657	0.888	ns
Leptotrichia	0.259	0.500	0.829	ns
Atopobium	0.247	0.600	0.869	ns
Acinetobacter	0.231	0.583	0.869	ns
Peptostreptococcus	0.216	0.506	0.829	ns
Oribacterium	0.173	0.615	0.869	ns
Methylophilus	0.094	0.740	0.949	ns
Actinobacillus	0.073	0.869	0.962	ns
Streptococcus	0.000	0.999	0.999	ns
Mogibacterium	-0.028	0.929	0.962	ns
Faecalibaculum	-0.032	0.939	0.962	ns
Actinomyces	-0.046	0.920	0.962	ns
Veillonella	-0.053	0.896	0.962	ns
Enterococcus	-0.068	0.839	0.962	ns

Genus	Beta[a]	p_val	q_val	ANCOM-BC[b]
Solobacterium	-0.098	0.789	0.951	ns
Coriobacteriaceae_UCG-002	-0.127	0.671	0.888	ns
Haemophilus	-0.135	0.778	0.951	ns
Dubosiella	-0.246	0.461	0.829	ns
Stomatobaculum	-0.305	0.417	0.829	ns
Gemella	-0.311	0.467	0.829	ns
Megasphaera	-0.333	0.417	0.829	ns
Delftia	-0.335	0.407	0.829	ns
Allobaculum	-0.540	0.173	0.642	ns
Lactobacillus	-0.602	0.114	0.467	ns
Stenotrophomonas	-0.649	0.188	0.642	ns
Turicibacter	-0.669	0.064	0.425	ns
Rothia	-0.797	0.083	0.425	ns
Lachnospiraceae_NK4A136_group	-0.946	0.024	0.249	ns

[a]Beta-coefficients obtained from the ANalysis of COmpositions of Microbiomes with Bias Correction (ANCOM-BC)[1]. Beta values that are positive represent a mean greater abundance of the taxon in the FD group, and negative values represent a mean greater abundance in the Non-FD Control group. [b]Significance tested via ANCOM-BC on non-transformed sequence reads.

Supplementary Table S8. Cohort data for patients with both diet and microbiota data (n=26)

	FD patients (n=18)	**Controls (n=8)**	**P***
Male n (%)	10 (56)	4 (50)	0.615
Age (yr)	52 (43-63)	56 (42-65)	1
Body mass index (kg/m^2)	27 (22-34)	27 (25-36)	0.306
Symptom score (SAGIS)	27 (15-43)	3 (1-9)	<0.001
Energy intake (kj/d)	6475 (5160-9068)	7263 (5526-8438)	0.856
Carbohydrate intake (g/d)	180 (149-1256)	189 (140-235)	0.979
FODMAP intake (g/d)	20 (16-30)	24 (16-34)	0.696
Diet quality (points)	54 (43-62)	55 (44-68)	0.696

Figure S1

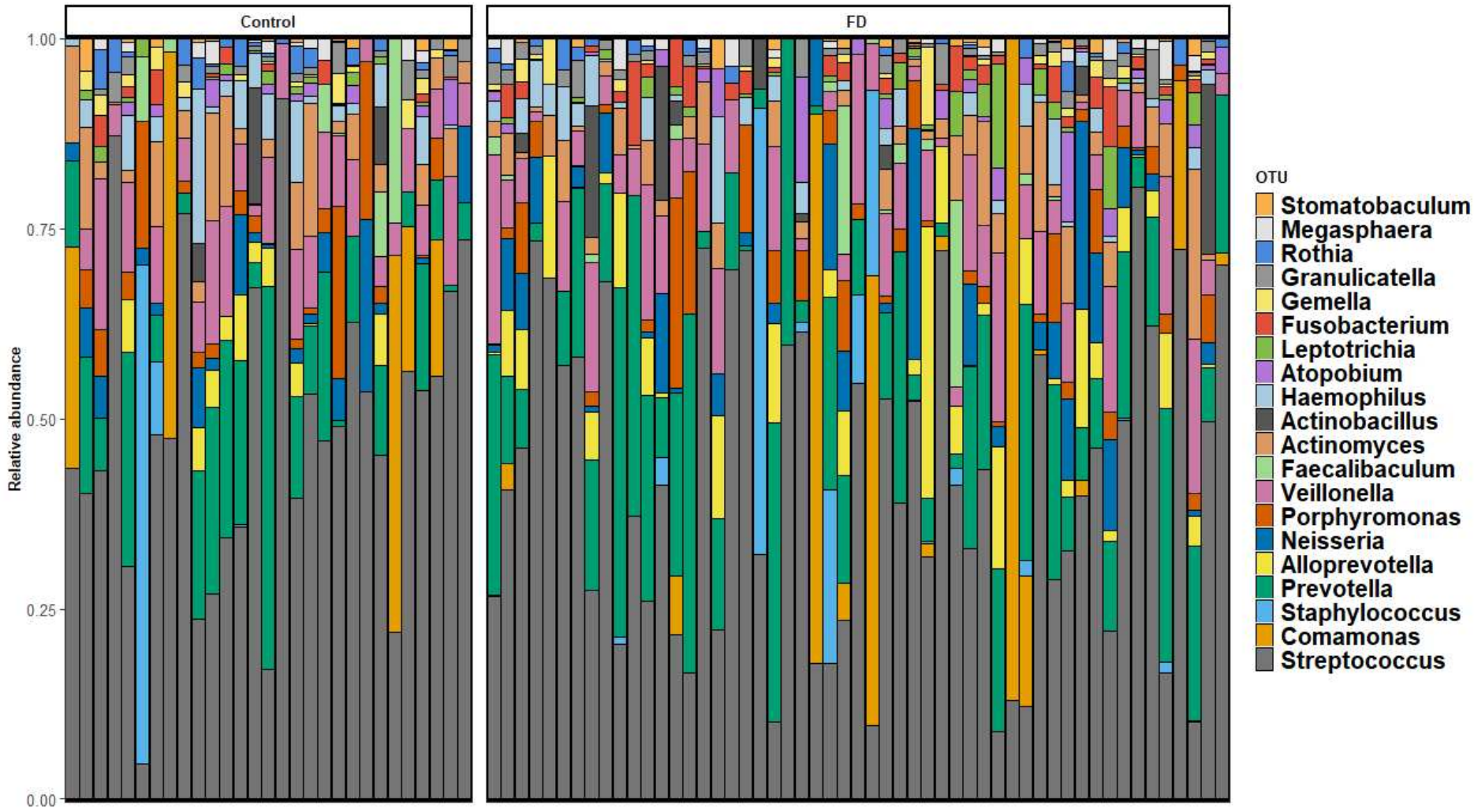

Control
FD
1.00
0.75
0.50
0.25
0.00
Relative abundance
OTU
Stomatobaculum
Megasphaera
Rothia
Granulicatella
Gemella
Fusobacterium
Leptotrichia
Atopobium
Haemophilus
Actinobacillus
Actinomyces
Faecalibaculum
Veillonella
Porphyromonas
Neisseria
Alloprevotella
Prevotella
Staphylococcus
Comamonas
Streptococcus

Figure S2

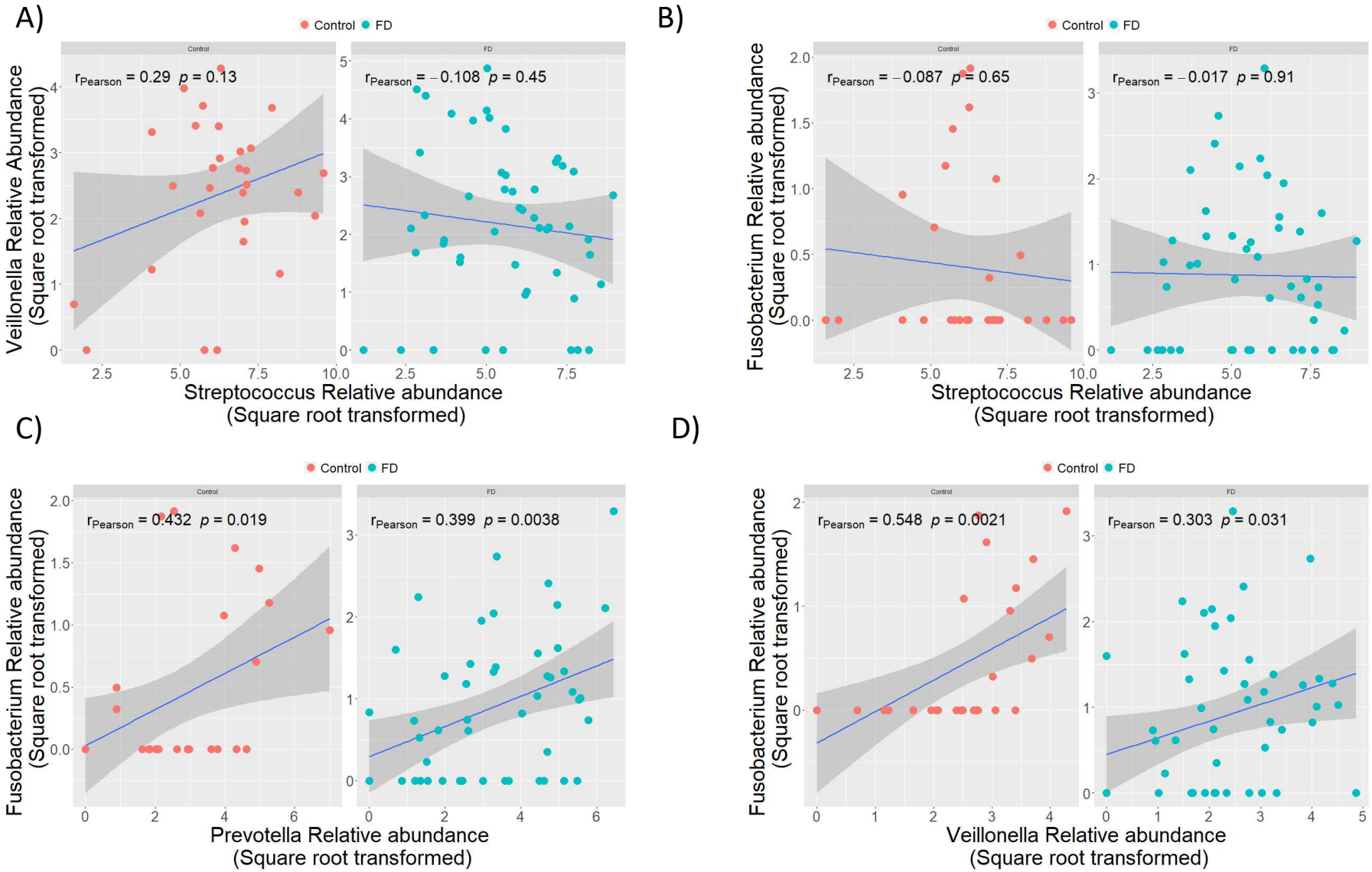
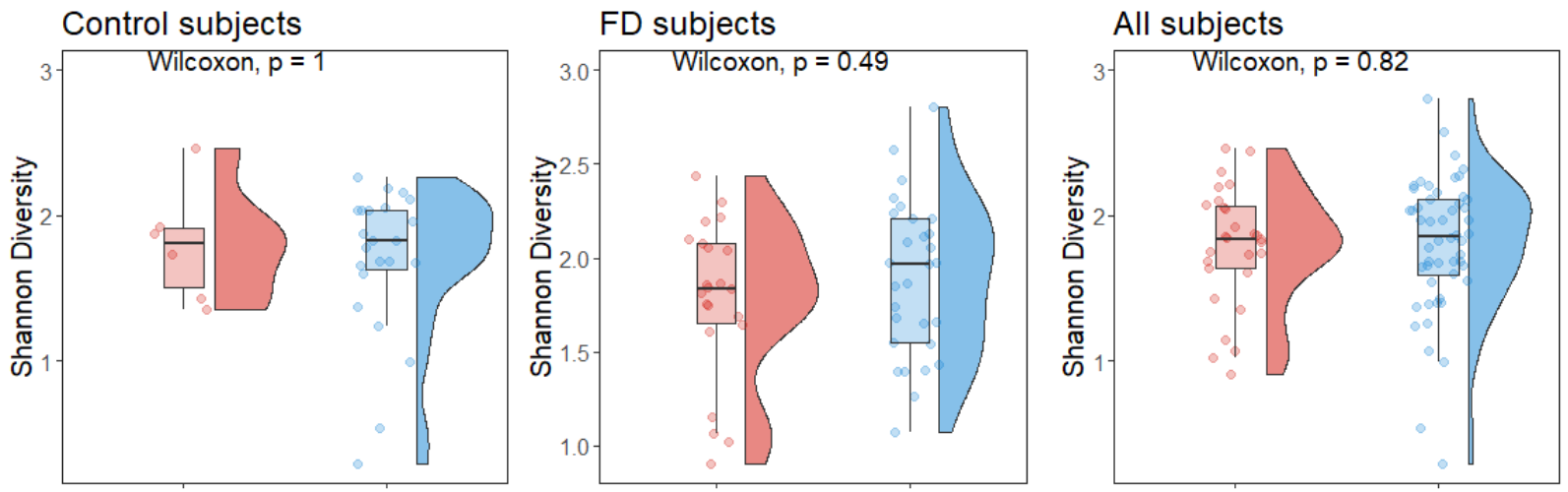

A)
Control FD
Control
FD
$r_{Pearson}$ = 0.29 p = 0.13
$r_{Pearson}$ = −0.108 p = 0.45
Veillonella Relative Abundance (Square root transformed)
Streptococcus Relative abundance (Square root transformed)
B)
Control FD
$r_{Pearson}$ = −0.087 p = 0.65
$r_{Pearson}$ = −0.017 p = 0.91
Fusobacterium Relative abundance (Square root transformed)
Streptococcus Relative abundance (Square root transformed)
C)
Control FD
$r_{Pearson}$ = 0.432 p = 0.019
$r_{Pearson}$ = 0.399 p = 0.0038
Fusobacterium Relative abundance (Square root transformed)
Prevotella Relative abundance (Square root transformed)
D)
Control FD
$r_{Pearson}$ = 0.548 p = 0.0021
$r_{Pearson}$ = 0.303 p = 0.031
Fusobacterium Relative abundance (Square root transformed)
Veillonella Relative abundance (Square root transformed)

Figure S3

A)


Control subjects
Wilcoxon, p = 1
FD subjects
Wilcoxon, p = 0.49
All subjects
Wilcoxon, p = 0.82
Shannon Diversity

B)

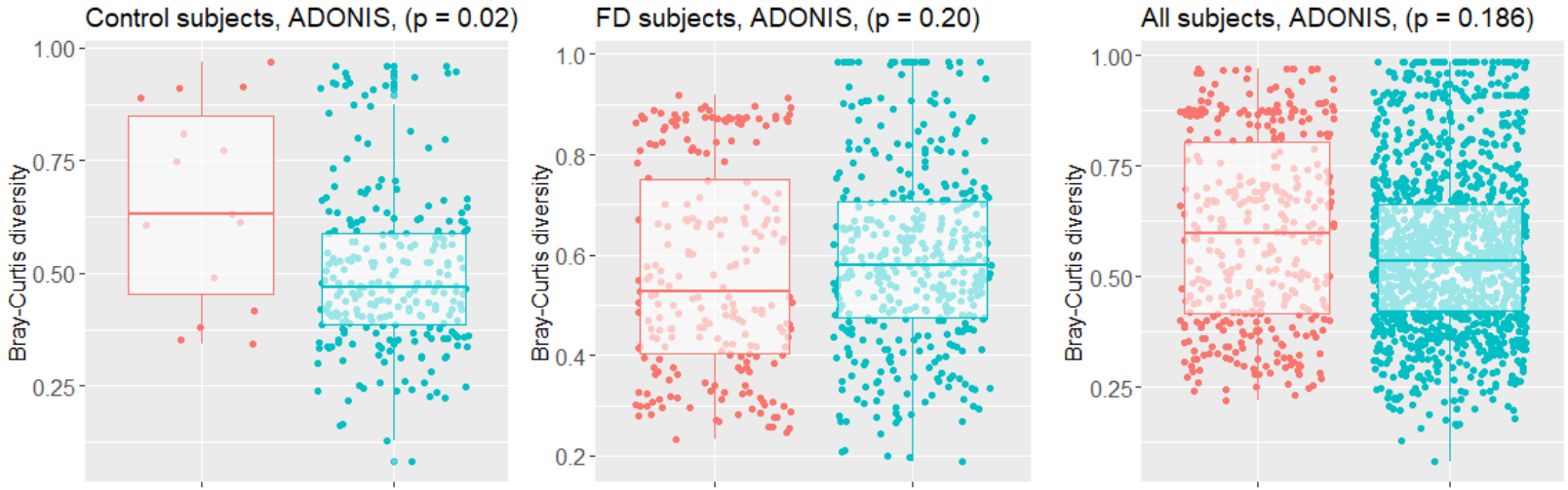

Control subjects, ADONIS, (p = 0.02)
FD subjects, ADONIS, (p = 0.20)
All subjects, ADONIS, (p = 0.186)
Bray-Curtis diversity

Figure S4

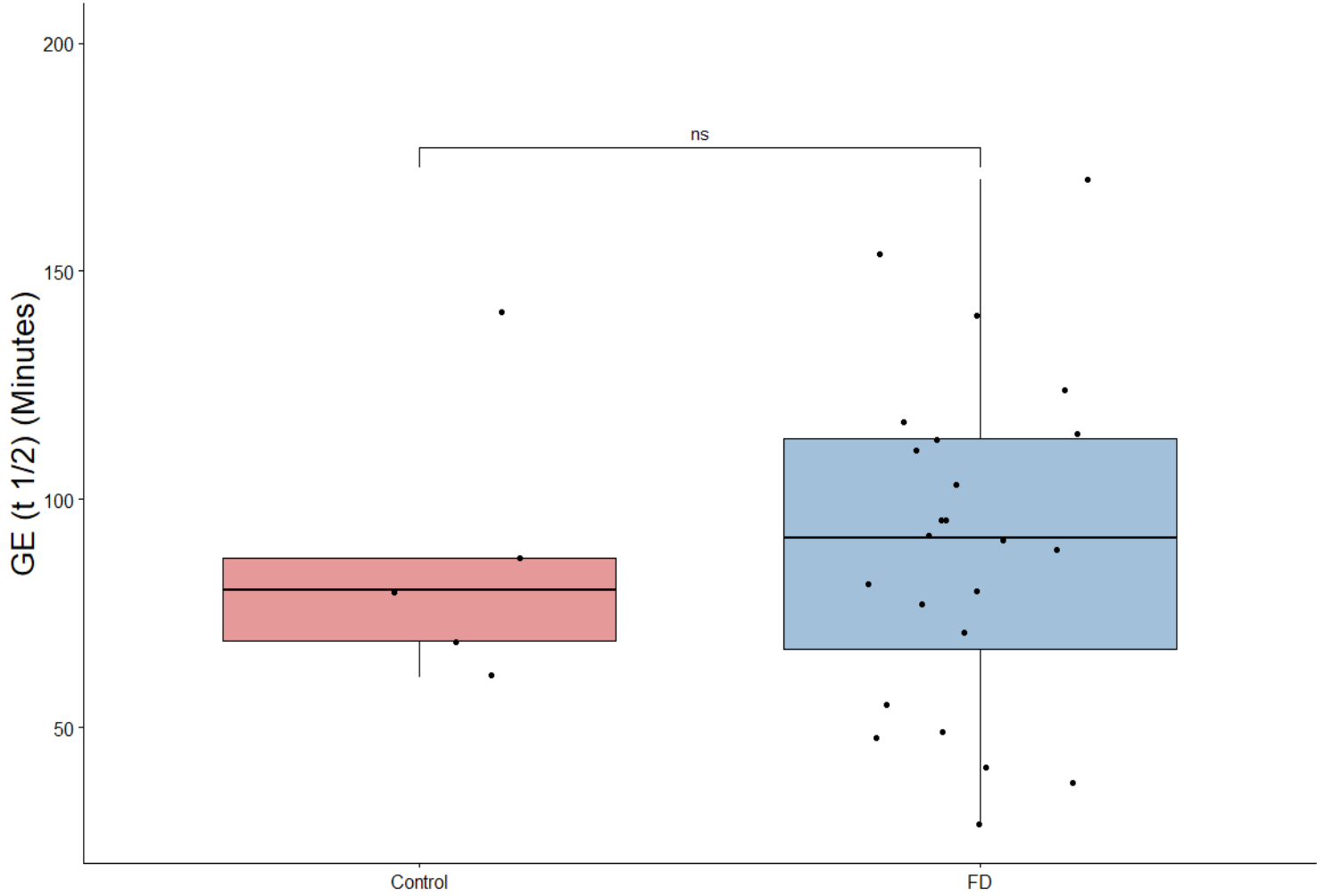
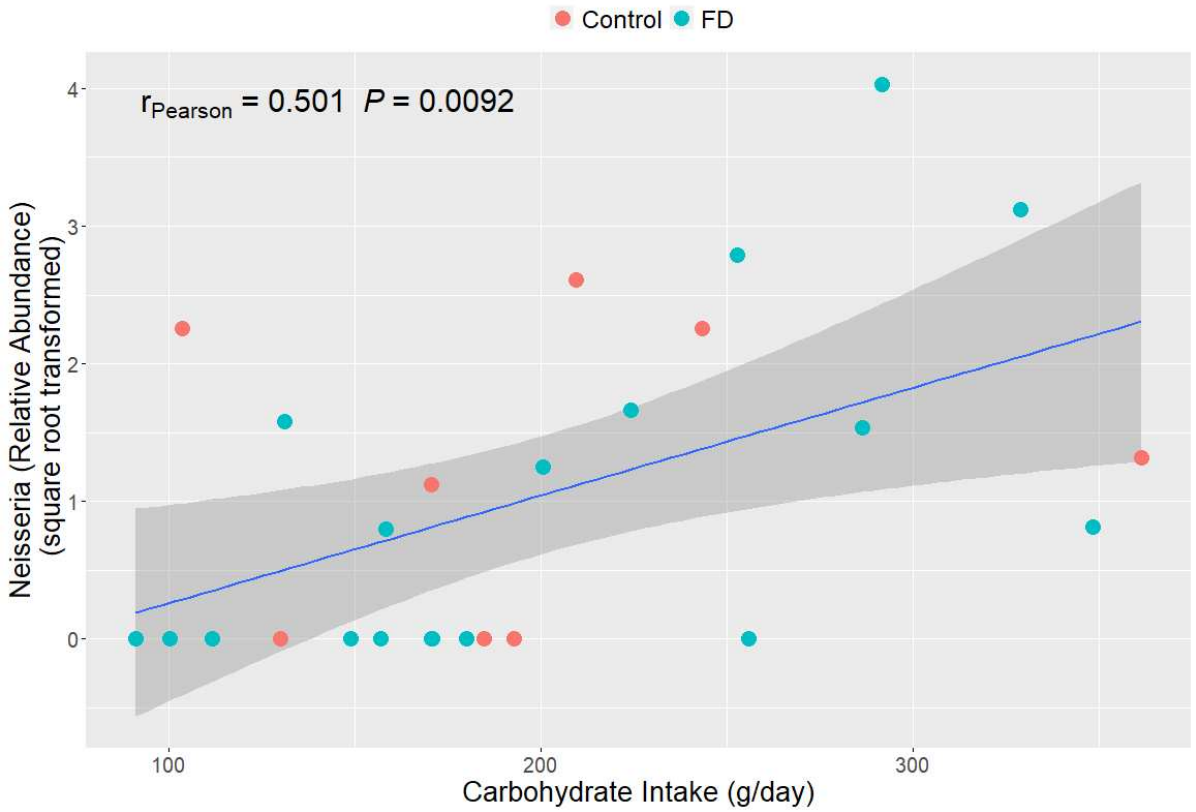


Figure S5


Control
FD
$r_{Pearson}$ = 0.501 *P* = 0.0092
Neisseria (Relative Abundance)
(square root transformed)
0
1
2
3
4
100
200
300
Carbohydrate Intake (g/day)

Supplementary Material

ALTERATIONS TO THE DUODENAL MICROBIOTA ARE LINKED TO SYMPTOMS IN FUNCTIONAL DYSPEPSIA

Authors

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Supplementary Table S1. Additional participant demographic data.

	Functional (n=56)	**Controls (n=30)**	**p-value**
IBS symptoms (clinical diagnosis): n (%)	43 (77)	NA	NA
Iron deficiency/FOBT: n (%)	NA	11 (36.6) / 19 (63.3)	NA
SAGIS: Median (range)			
Reflux symptoms[a]	1 (0-4)	0 (0-3)	#
Upper GI symptom domain – total score[b]	8 (1-20)	0 (0-3)	**< 0.0001**[e]
Fullness[a]	2 (0-4)	0 (0-1)	#
Early satiety[a]	2 (0-4)	0 (0-2)	#
Post-prandial pain[a]	2 (0-4)	0 (0-2)	#
Epigastric pain[a]	2 (0-4)	0 (0-3)	#
Retrosternal discomfort[a]	1 (0-4)	0 (0-3)	#
Lower GI diarrhoea symptom domain – total score[c]	7 (0-23)	2 (0-4)	**< 0.0001**[e]
Lower GI constipation symptom domain – total score[d]	5 (0-12)	0 (0-2)	**< 0.0001**[e]
Current NSAID use: n (%)	16 (29)	1 (3.3)	**0.013**[f]
Asthma: n (%)	8 (14.3)	3 (10)	NS[f]
Diabetes (Type 2): n (%)	7 (12.5)	3 (10)	NS[f]
HADS Anxiety: median (range)	5 (0-18)	1 (0-12)	0.05[e]
HADS Depression: median (range)	6 (0-17)	5 (0-17)	0.44[e]

a Total possible score is 4. b Total possible score is 20. c Total possible score is 24. d Total possible score is 12. e Mann-Whitney. f Fisher's Exact Test. # Significance not calculated due to high frequency of zero values in controls.

Supplementary Table S2. Diet Cohort – Demographics

	FD (n=28)	**Controls (n=11)**	***p*-value**
Female gender: n (%)	14 (50)	5 (45)	1.00[a]
Age (yr): median (range)	50 (17-76)	52 (22-71)	0.99[b]
Body Mass Index (kg/m^2): mean (SD)	25.3 (6.9)	29.0 (7.5)	0.23[c]
Current PPI use: n (%)	20 (71)	4 (36)	0.07[a]
Smoking status (Current/Previous/Never): n (%)	6/9/13 (21/32/47)	3/1/7 (27/9/64)	0.33[d]
Upper GI Symptom Domain – total score: Median (range)[#]	9 (1-20)	0 (0-3)	**<0.0001**[b]
NDI-QOL: mean (SD)*	55.9 (30.5)	97.3 (4.3)	**0.0039**[c]
Meal related symptoms: median (range)*	337 (0-2065)	174 (0-904)	0.37[b]

* Patient numbers (where different from overall study cohort): NDI-QOL (FD n=16; Controls n=6);
Meal related symptoms (FD n=25; Controls n=9). [#] The total possible upper GI symptom score is 20. [a] Fisher's Exact Test.
[b] Mann-Whitney. [c] T-test. [d] Chi-squared.

Supplementary Table S3. A Mean energy, nutrient and FODMAP intake in patients with functional dyspepsia and controls. **B** National dietary recommendations for protein, fibre and micronutrients analysed in this study, and proportion of patients with FD and controls meeting recommendations.

A		**FD (n=28)**		**Controls (n=11)**		***p*-value**	**General population****
Energy	(kJ/d)	7476	(5768-9485)	7847	(6239-9421)	0.652	9345
Carbohydrate	(g/d)	202	(153-161)	209	(171-293)	0.632	235
Starch	(g/d)	97	(66-129)	102	(54-158)	0.592	126
Sugars	(g/d)	106	(67-159)	119	(62-155)	0.693	102
Protein	(g/d)	84	(64-111)	91	(68-121)	0.35	98
Fat	(g/d)	58	(46-94)	62	(44-81)	0.592	78
Saturated fat	(g/d)	24	(16-32)	24	(15-35)	0.933	29
Monounsaturated fat	(g/d)	27	(20-41)	24	(15-32)	0.463	30
Polyunsaturated fat	(g/d)	10	(6-13)	8	(5-14)	0.693	12
Dietary fibre	(g/d)	28	(14-34)	26	(14-39)	0.572	25
Resistant starch	(g/d)	3	(2-4)	5	(3-7)	0.03	-
Vitamin A^	(µg/d)	1304	(738-1738)	1386	(868-1471)	0.866	875
Thiamin	(mg/d)	2	(1-3)	2	(1-3)	0.592	2
Riboflavin	(mg/d)	2	(1-4)	3	(2-4)	0.257	2
Niacin	(mg/d)	25	(17-33)	21	(18-33)	0.672	45
Folate	(µg/d)	347	(199-450)	466	(299-674)	0.131	305
Vitamin C	(mg/d)	174	(75-245)	178	(122-230)	0.933	113
Sodium	(mg/d)	2295	(1259-2995)	2018	(1803-2495)	0.910	2540
Potassium	(mg/d)	3284	(2314-4463)	3704	(3093-5021)	0.201	3172
Magnesium	(mg/d)	275	(192-374)	344	(276-440)	0.191	366
Calcium	(mg/d)	733	(451-1038)	845	(741-1200)	0.131	781
Phosphorous	(mg/d)	1372	(973-1732)	1631	(1287-1771)	0.146	1574
Iron	(mg/d)	12	(9-15)	14	(10-20)	0.181	12
Zinc	(mg/d)	12	(9-16)	12	(10-15)	0.800	12
Total FODMAPs	(g/d)	22.8	(9.5-33.1)	25.1	(20.9-39.8)	0.269	-

Fructo-oligosaccharides	(g/d)	2.2	(1.2-3.8)	2.6	(1.1-3.8)	0.910	-
Galacto-oligosaccharides	(g/d)	0.6	(0.3-0.9)	0.7	(0.4-1.9)	0.245	-
Lactose	(g/d)	12.7	(3.0-17.5)	16.1	(5.3-23.8)	0.412	-
Excess fructose	(g/d)	2.6	(0.9-5.5)	2.1	(0.6-5.6)	0.632	-
Total polyols	(g/d)	2	(0.6-4.0)	2.5	(0.2-2.3)	0.612	-

Values are median (IQR) unless stated; Mann Whitney U test comparing functional dyspepsia with controls **Data are mean values for 51-70 year old males from the Australian Health Survey 2011-2012, Australian Bureau of Statistics 2015. ^retinol equivalents.

B		**National dietary recommendations**		**FD**		**Controls**		**p-value**
		Males	Females	n	%	n	%	
Protein	g/d	64-81	46-57	25	89	9	82	1
Dietary fibre	g/d	30	25	14	50	5	46	0.629
Vitamin A^	µg/d	900	700	23	82	8	73	1
Thiamin	mg/d	1.2	1.1	21	75	8	73	1
Riboflavin	mg/d	1.3-1.6	1.1-1.3	25	89	11	100	0.172
Niacin	mg/d	16	14	28	100	11	100	1
Folate	µg/d	400	400	11	39	6	55	0.305
Vitamin C	mg/d	45	45	28	100	10	91	0.723
Sodium	mg/d	2000	2000	13	46	5	46	0.557
Potassium	mg/d	3800	2800	15	54	5	46	1
Magnesium	mg/d	400-420	310-320	9	32	4	36	0.713
Calcium	mg/d	1000-1300	1000-1300	8	29	3	27	1
Phosphorous	mg/d	1000	1000	23	82	11	100	0.086
Iron	mg/d	8	28	20	71	9	82	0.453
Zinc	mg/d	14	8	19	68	7	64	1

Nutrient reference values for Australia and New Zealand, age-specific recommendations apply where ranges are specified; Fisher's exact test (proportion of patients meeting national recommendations; ^retinol equivalents.

Supplementary Table S4. Assessment of epigastric domain symptom scores (SAGIS) and daily energy, macronutrient, or FODMAP intakes in FD patients.

	Spearman Correlation	***p*-value**
Energy (kJ/d)	-0.082	0.659
Carbohydrate (g/d)	0.014	0.942
Protein (g/d)	-0.045	0.808
Fat (g/d)	0.211	0.255
Dietary fibre (g/d)	0.133	0.475
Total FODMAPs (g/d)	0.090	0.632

Supplementary Table S5. Microbiota Cohort – Demographics.

	Functional (n=47)	**Controls** (n=27)	***p*-value**
Female: n (%)	24 (51)	14 (52)	NS[a]
Age: median (range)	48.2 (20-76)	60.6 (21-74)	0.011[b]
BMI: mean (range)	26.0 (15.8-41.8)	28.0 (19.5-40.2)	NS[c]
Current PPI use: n (%)*	28 (60)	4 (15)	0.0002[a]
Smoking status (Current/Previous/Never): n (%)*	9/18/18 (19/38/38)	6/5/16 (22/19/59)	NS[d]
Upper GI Symptom Domain – total score: Median (range)[#]	8 (1-20)	0 (0-3)	< 0.0001[b]
NDI-QOL: mean (SD)*	60.6 (25.8)	95.6 (4.4)	0.0014[c]

* Patient numbers (where different from overall study cohort): Smoking status (Functional n=45); Current PPI use (Functional n=46; Controls n=26); NDI-QOL (Functional n=25; Controls n=7). [#] The total possible upper GI symptom score is 20. [a] Fisher's Exact Test. [b] Mann-Whitney. [c] T-test. [d] Chi-squared. NS – not significant.

Supplementary Table S6. Mean relative abundance (%) of bacterial OTUs observed in the duodenal mucosa-associated microbiota of FD patients and symptom free controls. OTU – Operational taxonomic unit. Number following the taxonomic assignment - Greengenes reference.

OTU Relative Abundance (%)	Control	Functional	Fold Change
p__Actinobacteria__g__Actinomyces_4350499	1.9	1.1	1.771
p__Actinobacteria__g__Actinomyces_526682	0.41	0.34	1.205
p__Actinobacteria__g__Actinomyces_565136	2.3	1.7	1.336
p__Actinobacteria__g__Atopobium_2163609	0.54	0.91	-1.677
p__Actinobacteria__g__Rothia_532388	1.2	0.61	1.977
p__Bacteroidetes__g__Porphyromonas__s__endodontalis_573034	0.4	1.2	-2.907
p__Bacteroidetes__g__Porphyromonas_970138	2.7	2.5	1.116
p__Bacteroidetes__g__Prevotella__s__intermedia_246785	0.46	0.94	-2.019
p__Bacteroidetes__g__Prevotella__s__melaninogenica_535359	10	13	-1.253
p__Bacteroidetes__g__Prevotella__s__nanceiensis_536492	0.22	0.31	-1.412
p__Bacteroidetes__g__Prevotella__s__nanceiensis_New.ReferenceOTU26428	0.26	0.22	1.154
p__Bacteroidetes__g__Prevotella__s__nigrescens_2195	0.4	1	-2.6
p__Bacteroidetes__g__Prevotella__s__pallens_2222	1.2	1.5	-1.275
p__Bacteroidetes__g__Prevotella__s__tannerae_38227	0.47	0.85	-1.799
p__Bacteroidetes__g__Prevotella_2469654	0.23	0.32	-1.361
p__Bacteroidetes__g__Prevotella_269907	0.48	1.1	-2.196
p__Bacteroidetes__g__Prevotella_4295238	1.4	2.5	-1.733
p__Bacteroidetes__g__Prevotella_851822	0.77	1.3	-1.652
p__Firmicutes__g__Bulleidia_851938	0.52	0.45	1.141
p__Firmicutes__g__Enterococcus_New.ReferenceOTU10017	2.6	2.5	1.061
p__Firmicutes__g__Gemella_1694541	0.37	0.23	1.604
p__Firmicutes__g__Gemella_4384936	0.51	0.38	1.341
p__Firmicutes__g__Gemella_529233	0.39	1.3	-3.368
p__Firmicutes__g__Granulicatella_New.CleanUp.ReferenceOTU26441	0.54	0.41	1.297
p__Firmicutes__g__Granulicatella_New.ReferenceOTU28293	0.81	0.84	-1.032
p__Firmicutes__g__Megasphaera_518686	0.91	0.97	-1.072
p__Firmicutes__g__Moryella_714766	0.53	0.5	1.057
p__Firmicutes__g__Oribacterium_527630	0.62	0.57	1.078

p__Firmicutes__g__Peptostreptococcus_532521	0.26	0.4	-1.517
p__Firmicutes__g__Selenomonas_295019	0.25	0.17	1.495
p__Firmicutes__g__Streptococcus__s__infantis_517754	2.7	3.6	-1.309
p__Firmicutes__g__Streptococcus_349024	7.2	7.1	1.012
p__Firmicutes__g__Streptococcus_561636	1.1	0.92	1.219
p__Firmicutes__g__Streptococcus_797560	0.99	0.6	1.643
p__Firmicutes__g__Streptococcus_New.CleanUp.ReferenceOTU182950	16	13	1.258
p__Firmicutes__g__Streptococcus_New.CleanUp.ReferenceOTU44699	0.14	0.31	-2.163
p__Firmicutes__g__Streptococcus_New.ReferenceOTU10193	14	9.9	1.422
p__Firmicutes__g__Streptococcus_New.ReferenceOTU10767	1.6	2	-1.254
p__Firmicutes__g__Streptococcus_New.ReferenceOTU11209	3.2	1.9	1.701
p__Firmicutes__g__Streptococcus_New.ReferenceOTU11482	1.7	1.5	1.143
p__Firmicutes__g__Veillonella__s__dispar_518743	5.6	5.8	-1.028
p__Firmicutes__g__Veillonella__s__dispar_561537	2.8	2.4	1.168
p__Fusobacteria__g__Fusobacterium_34791	0.27	0.95	-3.529
p__Fusobacteria__g__Fusobacterium_545299	0.93	1.6	-1.676
p__Fusobacteria__g__Leptotrichia_2480553	0.2	0.8	-4.023
p__Fusobacteria__g__Leptotrichia_4430826	0.34	0.44	-1.294
p__Proteobacteria__f__Enterobacteriaceae_1109844	0.014	0.02	-1.393
p__Proteobacteria__f__Enterobacteriaceae_797229	0.09	0.06	1.502
p__Proteobacteria__f__Enterobacteriaceae_823118	0.026	0.028	-1.08
p__Proteobacteria__g__Actinobacillus__s__parahaemolyticus_9610	0.74	1.4	-1.849
p__Proteobacteria__g__Haemophilus__s__parainfluenzae_New.CleanUp.ReferenceOTU45741	1.1	0.92	1.18
p__Proteobacteria__g__Haemophilus__s__parainfluenzae_New.ReferenceOTU10143	1.2	0.59	2.005
p__Proteobacteria__g__Haemophilus_92231	0.84	0.34	2.45
p__Proteobacteria__g__Helicobacter__s__pylori_132837	0.022	0.01	2
p__Proteobacteria__g__Neisseria__s__subflava_New.ReferenceOTU18917	0.49	0.75	-1.541
p__Proteobacteria__g__Neisseria_589124	0.085	0.43	-5.065
p__Proteobacteria__g__Neisseria_New.ReferenceOTU11192	0.46	0.67	-1.467
p__Proteobacteria__g__Neisseria_New.ReferenceOTU11390	0.58	0.84	-1.448
p__Proteobacteria__g__Neisseria_New.ReferenceOTU13233	1.3	1.3	1.01
p__TM7__c__TM73_799024	0.26	0.56	-2.113

Supplementary Table S7. Mean relative abundance (%) of bacterial phyla observed in the duodenal mucosa-associated microbiota of functional patients and symptom free controls. No significant differences were observed on analysis with ANCOM.

Phylum	**Functional Patients (%)**	**Controls (%)**	***p*-value[a]**	**FDR *q*-value**
TM7	0.56	0.26	0.81	0.81
Proteobacteria	7.3	6.9	0.151	0.302
Fusobacteria	3.8	1.7	**0.001**	**0.006**
Firmicutes	58	66	0.42	0.63
Bacteroidetes	26	19	0.766	0.81
Actinobacteria	4.6	6.3	0.096	0.288

a – Significance was tested via multiple regression controlling for patient age, gender, smoking status, BMI and PPI use. Statistical analysis was performed on relative abundance data that was square root transformed.

Supplementary Table S8. Mean relative abundance (%) of bacterial genera observed in the duodenal mucosa-associated microbiota of functional patients and symptom free controls. ns – not significant. ** significantly different based on ANCOM.

Genus	Functional Patients (%)	Controls (%)	*p*-value[a]	FDR *q*-value[a]	ANCOM[b]
Actinobacillus	1.4	0.74	0.934	0.983	ns
Actinomyces	3.1	4.6	0.119	0.5712	ns
Atopobium	0.91	0.54	0.833	0.983	ns
Bulleidia	0.45	0.52	0.091	0.546	ns
Enterobacteriaceae (family)	0.11	0.13	0.295	0.89538	ns
Enterococcus	2.5	2.6	0.485	0.89538	ns
Fusobacterium	2.5	1.2	0.005	0.12	**
Gemella	1.9	1.3	0.476	0.89538	ns
Granulicatella	1.2	1.3	0.299	0.89538	ns
Haemophilus	1.8	3.1	0.975	0.983	ns
Helicobacter	0.01	0.022	0.446	0.89538	ns
Leptotrichia	1.2	0.53	0.014	0.168	ns
Megasphaera	0.97	0.91	0.955	0.983	ns
Moryella	0.5	0.53	0.932	0.983	ns
Neisseria	4	2.9	0.041	0.328	ns
Oribacterium	0.57	0.62	0.262	0.89538	ns
Peptostreptococcus	0.4	0.26	0.983	0.983	ns
Porphyromonas	3.6	3.1	0.949	0.983	ns
Prevotella	23	16	0.722	0.983	ns
Rothia	0.61	1.2	0.35	0.89538	ns
Selenomonas	0.17	0.25	0.426	0.89538	ns
Streptococcus	41	49	0.578	0.983	ns
TM7 (phylum)	0.56	0.26	0.81	0.983	ns
Veillonella	8.2	8.4	0.746	0.983	ns

a – Significance tested via multiple regression controlling for patient age, gender, smoking status, BMI and PPI use. Analysis was performed on relative abundance data that was square root transformed. b – Significance tested via ANCOM on non-transformed sequence reads.

Supplementary Table S9. Cohort data for patients with both diet and microbiota data

	FD patients (n=19)	Controls (n=8)	P*
Male n (%)	10 (53)	4 (50)	0.615
Age (yr)	52 (43-63)	56 (42-65)	1
Body mass index (kg/m^2)	27 (22-34)	27 (25-36)	0.306
Symptom score (SAGIS)	27 (15-43)	3 (1-9)	<0.001
Energy intake (kj/d)	6475 (5160-9068)	7263 (5526-8438)	0.856
Carbohydrate intake (g/d)	180 (149-1256)	189 (140-235)	0.979
FODMAP intake (g/d)	20 (16-30)	24 (16-34)	0.696
Diet quality (points)	54 (43-62)	55 (44-68)	0.696

ALTERATIONS TO THE DUODENAL MICROBIOTA ARE LINKED TO GASTRIC EMPTYING AND SYMPTOMS IN FUNCTIONAL DYSPEPSIA

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SUPPLEMENTARY METHODS

Gastric Emptying

In participants who consented, a ^{13}C-octanoic acid breath test was performed to assess gastric emptying rate. After an overnight fast, participants were asked to eat one scrambled egg containing 91.4 mg ^{13}C-octanoic acid, two slices of white bread, 8 g butter and drink 150 ml of water. Patients' breath was collected at baseline before eating, at fifteen-minute intervals for the first two hours after eating then at thirty-minute intervals for the next two hours. The breath samples were analysed by isotope ratio infrared spectrometry and the ratio of ^{13}C to ^{12}C was used to calculate the gastric emptying half-time ($t_{1/2}$).

Statistical Analysis – Clinical Parameters

Statistical analysis of cohort demographics and patient characteristics were performed in GraphPad Prism Version 7. The Fisher's Exact Test, Mann-Whitney/Kruskal Wallis, T-test and Chi-squared tests were used as appropriate.

Dietary Intake

Habitual intake of foods and fluids over the preceding 12 months was evaluated. A validated food frequency questionnaire (FFQ)[1] was completed by each participant estimating mean daily intake of energy, macronutrients, fiber, 13 micronutrients, total and individual FODMAP carbohydrates (fructans, galacto-oligosaccharides, lactose, fructose in excess of glucose, polyols) (Foodworks 7; Xyris Software, Australia). Contribution from vitamin and mineral supplements was not included. Implausible intakes were identified using published cut-offs <500 kcal/d (2,100 kJ/d) or >3,500 kcal/d (14,700 kJ/d)[2] and were excluded from the final analysis. The proportion of patients meeting age- and gender-specific national dietary recommendations (i.e. nutrient reference values) for protein, fiber and micronutrients[3, 4] was calculated.

Diet quality was assessed using the Alternate Healthy Eating Index (AHEI-2010)[5]. Diet quality scores were calculated using published scoring methods for each participant for 11 dietary components (vegetables, fruit, whole grains, sweetened beverages and fruit juice, nuts and legumes, red/processed meat, trans fats, long chain fats, polyunsaturated fats, sodium, alcohol)[5] and summed to produce a total diet quality score, with a possible maximum score of 110. Trans fats were estimated using 0.5% of total energy intake to calculate a trans fats subscore[6].

Normality of data was evaluated by visual inspection of histograms. Where data was missing, food frequency was recorded as 'never' and individual symptom severity was recorded as 'no problem'. Mann-Whitney U tests were used to compare groups for continuous variables as data was not normally distributed. Chi-squared and Fisher's exact tests were used to compare categorical data. Spearman's correlations were used to examine associations between symptom scores, dietary components and diet quality. Data are presented as median (interquartile range) for continuous and number (%) for categorical variables. Differences were considered significant where $p \leq 0.05$. Statistical analysis was performed using IBM SPSS Statistics for Windows Version 24.0.

DNA Extraction from Duodenal Biopsies

Samples were lysed using a protocol optimised for extraction of microbial DNA for community analyses[7]. Frozen samples were thawed on ice and individual tissue biopsy samples (approx. 1-2 mm^3) were utilised for gDNA extraction. Each sample was placed in a screw-cap tube containing 300 µL lysis buffer (NaCl 0.5M, Tris-HCl 50mM,pH 8.0, EDTA 50mM and SDS 4% w/v) and 0.4 g sterile zirconia beads (1:1, 0.1 mm and 1mm; Daintree Scientific). Homogenization was undertaken in a tissue homogenizer (Precelleys) for 3 min followed by incubation at 70°C for 10 min. The lysate was collected and the homogenization procedure repeated with the addition of further lysis buffer, providing 500 µL of pooled lysate for each

sample to be used for DNA extraction. The DNA was extracted using an automated system (Maxwell® 16) with the Maxwell® 16 Tissue DNA Purification Kit (Promega), following the manufacturer's instructions. Extracted gDNA was quantified (Nanodrop) and stored at -80°C. Recent studies have demonstrated that samples with relatively small amounts of microbial biomass can produce spurious results, due in part to DNA contamination of extraction reagents used[8]. To assess the possible impact of this on our results we also prepared a set of reagent controls, to which no additional tissue or DNA was added. These reagent only mixtures were processed in an identical manner to the tissue samples, commencing at the lysis step.

Microbiota Profiling - Library Preparation and Sequencing

The small intestinal and control samples were profiled by high-throughput amplicon sequencing with dual-index barcoding using the Illumina MiSeq platform. The V6-V8 region of the gene encoding 16S ribosomal RNA was amplified using the primers 917-Forward (GAATTGRCGGGGRCC; bacterial domain specific) and 1392-Reverse (ACGGGCGGTGWGTRC; universal), which also contained Illumina adapter sequences. Amplification was undertaken using the Q5 DNA polymerase (NEB) as per the manufacturer's instructions. PCR products were purified using AMPure XP beads (Beckman Coulter). The PCR libraries were then barcoded using the Illumina dual-index system (Nextera XT v2 Index Kit). Following a second round of purification (AMPure XP beads), libraries were quantified (Quantus) and pooled to 4nM. The libraries were sequenced on an Illumina MiSeq using the MiSeq Reagent Kit v3 (2x 300bp), using facilities provided by the Australian Centre for Ecogenomics.

Bioinformatics

Sequence data was processed using the Quantitative Insights into Microbial Ecology 2 pipeline (QIIME2 ver. 2021.4)[9]. After removing adapter sequences using the Cutadapt [10], we merged

the overlapping paired-end reads and trimmed low-quality bases from the de-multiplexed reads. Sequence data was denoised by filtering and correcting Illumina amplicon sequencing errors using the Divisive Amplicon Denoising Algorithm 2 (DADA2) [11] plugin incorporated in QIIME2. Amplicon sequence variants (ASVs) were taxonomically assigned using the feature classifier in QIIME2 against version 138 of the SILVA database (updated December 2019) based on 99% similarity. The reagent control samples were concurrently processed to generate a list of specific "contaminant" ASVs. These specific ASVs were then subtracted from the duodenal dataset subsequently filtered to remove contaminant ASVs to generate a contaminant-free ASVs table. ASVs with a relative abundance of less than 0.05% and all samples with a final read count of less than 500 sequence reads were also excluded from the ASVs table.

Statistical Analyses – Microbiota

Shannon and Chao1 alpha (within sample) diversity metrics were generated at the genus level using the microbiome package in R [12]. Significance was tested using the wilcoxon rank sum test. For beta (between sample) diversity analysis, a weighted UniFrac distance matrix was constructed. Principal coordinate plots were generated from the weighted UniFrac distance matrix using the first two coordinates and coded based on patient diagnosis. To test compositional differences between samples, we analyzed the permutational multivariate analysis of variance (PERMANOVA) test using phyloseq [13] and the "adonis2" function in the R package vegan [14].

To generate a constrained multivariate model to differentiate FD patients from non-FD controls, the sparse partial least squares discriminant analysis method mixOmics was used [15]. Here, the ASVs table was normalised by total sum scaling followed by centred-log ratio transformation, and these transformed data were used to generate a model in which patients

and controls could be differentiated based on their microbiota profiles. A plot based on the first two components of the model was also generated.

To assess differences between the relative abundances of specific bacterial taxa, the ASVs table was normalised via total sum scaling (TSS) or followed by square root transformation. Average relative abundances were then calculated and significant differences assessed using Kruskal-Wallis (KW) with Wilcoxon test for multiple comparisons. Correlations with clinical variables were performed via Pearson correlation and/or multivariate linear regression. FDR values were computed using MaAslin2 R package[16] (with an FDR value of < 0.05 considered significant).

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Supplementary Figures:

Figure S1: Relative abundances of the top 20 bacterial genera represented across the entire study group, and present among the individuals assigned to the FD and non-FD control groups. Each column represents an individual subject and the relative abundance for each taxon is colour coded according to the key.

Figure S2: Pearson correlation analyses of the relative abundances of A) *Streptococcus* and *Veillonella*, B) *Streptococcus* and *Fusobacterium*, C) *Prevotella* and *Fusobacterium* and D) *Veillonella* and *Fusobacterium*. Separate analyses for the FD subjects (blue) and non-FD control subjects (red) are shown, with the respective calculated r and *p* values shown.

Figure S3: Measures of A) within-sample (alpha) diversity (Shannon index) and B) between-sample (beta) diversity (Bray Curtis) between the PPI users (red) and PPI non-users (blue/teal) for the FD and non-FD control groups, as well as for all participants. While the Bray-Curtis dissimilatory measures for the PPI users and PPI non-users within the control group reached statistical significance, there was no differences between the two subgroups in the combined analysis, suggesting there can be variable, but overall limited, impacts from PPI use on the duodenal MAM.

Figure S4: Gastric emptying t-half time (minutes), as measured via ^{13}C-octanoic acid breath test in FD patients (blue, n = 24) and non-FD control subjects (red, n = 5). No significant difference was observed between the two groups (Wilcoxon test p =0.69).

Figure S5: Pearson correlation analysis of the relative abundance of the genus *Neisseria* in the duodenal MAM and total carbohydrate intake (g/day) for the subgroup of subjects for which both dietary and microbiota data was available (n = 26, FD subjects in blue, non-FD control subjects in red).