Spatial and Temporal Analysis of the Stomach and Small Intestinal Microbiota in Fasted Healthy Humans

Authors: Anna M. Seekatz, PhD[1*], Matthew K. Schnizlein[2*], Mark J. Koenigsknecht, PhD[1,3*], Jason R. Baker, PhD[4], William L. Hasler, MD[4], Barry E. Bleske, PharmD[5], Vincent B. Young, MD, PhD[1,2†], Duxin Sun, PhD[3†]

[1]*Department of Internal Medicine, Division of Infectious Disease, University of Michigan, Ann Arbor, MI 48109, USA*
[2]*Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109, USA*
[3] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA*
[4]*Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI 48109, USA*
[5]*Department of Pharmacy Practice and Administrative Sciences, University of New Mexico, Albuquerque, NM 87131*

[*]These authors contributed equally
[†]Corresponding authors

Contact information:
Duxin Sun, PhD
Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA, duxins@umich.edu

Vincent B. Young, MD, PhD
Department of Internal Medicine, Division of Infectious Disease, University of Michigan, Ann Arbor, MI 48109, USA, youngvi@umich.edu

Manuscript word count: 3105

Patient consent: Informed consent was obtained from individuals prior to the time of sampling. IRB approved on 02/04/2015.

Conflicts of Interest: None

Abstract

Although the microbiota in the proximal gastrointestinal (GI) tract has been implicated in health and disease, much of these microbes remains understudied compared to the distal GI tract. This study characterized the microbiota across multiple proximal GI sites over time in healthy individuals.

As part of a study of the pharmacokinetics of oral mesalamine administration, healthy, fasted volunteers (N=8; 10 observation periods total) were orally intubated with a four-lumen catheter with multiple aspiration ports. Samples were taken from stomach, duodenal, and multiple jejunal sites, sampling hourly (≤7 hours) to measure mesalamine (administered at t=0), pH, and 16S rRNA gene-based composition.

We observed a predominance of Firmicutes across proximal GI sites, with significant variation compared to stool. The microbiota was more similar within individuals over time than between subjects, with the fecal microbiota being unique from that of the small intestine. The stomach and duodenal microbiota displayed highest intra-individual variability compared to jejunal sites, which were more stable across time. We observed significant correlations in the duodenal microbial composition with changes in pH; linear mixed models identified positive correlations with multiple *Streptococcus* operational taxonomic units (OTU) and negative correlations with multiple *Prevotella* and *Pasteurellaceae* OTUs. Few OTUs correlated with mesalamine concentration.

The stomach and duodenal microbiota exhibited greater compositional dynamics compared to the jejunum. Short-term fluctuations in the duodenal

24 microbiota was correlated with pH. Given the unique characteristics and dynamics
25 of the proximal GI tract microbiota, it is important to consider these local
26 environments in health and disease states.

27

28

INTRODUCTION

The microbiota of the proximal gastrointestinal tract in humans represent an understudied yet highly relevant microbial community.[1] Physiological processes such as gastric emptying, bile acid secretion, and the transit of food can influence the proximal gastrointestinal (GI) tract and disease development.[2-5] However, our current understanding of how the processes and microbiota in different regions of the proximal GI tract relate to health and disease remains limited compared to other areas of the GI tract.

Much of our knowledge about the involvement of the human GI microbiota in health and disease has relied on fecal sampling, a non-invasive sampling method that is largely representative of the large intestine.[6,7] Although it is known that the microbiota across the GI tract varies in composition and density,[8-10] studying the microbiota at these sites is difficult, limiting our knowledge to invasive procedures, specific patient populations, or single time points.[1] Analyses of mucosal samples from autopsies, endoscopies, and colonoscopies have revealed that *Streptococci* and *Lactobacilli,* both members of the oral and esophageal microbiota, are abundant members of the jejunal and ileal microbiota.[11-17] Studies using naso-ileal catheters and ileostoma effluent, which allow collection over time, have supported these conclusions and revealed that the small intestinal microbiota is highly dynamic over short time courses, likely reflective of physiological processes at the stomach-small intestine interface.[18-21]

Understanding how these microbiota along the GI tract are related is of physiological relevance, particularly in relation to intestinal homeostasis and

disease. Recent evidence suggests that the drug mesalamine, designed to reach high concentrations in the GI tract as treatment for irritable bowel disease (IBD), may directly target the microbiota in addition to host effectors.[22,23] It is possible that some of the effectiveness of mesalamine treatment for IBD is mediated by the microbiota, potentiating the need to characterize these microbial communities to a fuller extent in the context of mesalamine administration.

This study investigated the bacterial composition across the intact upper GI tract in the same healthy, fasted adults over time. We used a multi-lumen tube designed to sample multiple sites along the upper GI tract. As part of a previously published study aimed at measuring mesalamine dissolution, subjects were given a dose of mesalamine and the proximal GI tract lumen was sampled over time.[24] We used these samples to 1) characterize and compare microbial community dynamics over time at multiple upper GI sites within an individual and 2) identify how environmental factors, such as pH and the acute effect of mesalamine, shaped the microbiota. To the best of our knowledge, this is the first study to characterize the luminal microbiota across multiple upper GI sites over time within the same individual.

METHODS

Study recruitment

Healthy individuals (age 18-55) were included who were free of medications for the past two weeks, passed routine health screening, had a BMI 18.5-35, and had no significant clinical illness within three weeks. Health screening included a review

of medical history and a physical examination (checking vital signs, electrocardiography, and clinical laboratory tests) described in Yu et al.[24]

Catheter design and sterilization

A customized multi-channel catheter was constructed by Arndorfer Inc. (Greendale, WI), consisting of independent aspiration ports located 50 cm apart. The catheter had a channel to fit a (0.035 in x 450 cm) guidewire (Boston Scientific, Marlborough, MA), a channel connected to a balloon that could be filled with 7 ml of water to assist tube placement, and an end that was weighted with 7.75 grams of tungsten. Each single-use catheter was sterilized according to guidelines set by the American Society for Gastrointestinal Endoscopy at the University of Michigan prior to insertion (Supplemental Methods).[25]

Collection of GI fluid samples

The full details of catheter placement have been previously described.[24] Briefly, catheter placement occurred approximately 12 hours before sample collection. The catheter was orally inserted into the GI tract with aspiration ports located in the stomach, duodenum, and the proximal, mid and distal jejunum, confirmed by fluoroscopy. Subjects were given a light liquid snack approximately 11 hours before sample collection and fasted overnight for 10 hours prior to sample collection. At 0 hours, a mesalamine formulation was administered to each subject (Table 1). Luminal GI fluid samples (approximately 1.0 ml) were collected from up to four sites of the upper GI tract hourly up to 7 hours. Samples were collected by syringe, transferred to sterile tubes, and placed at -80°C until sample processing. A

paired sample was collected to detect pH using a calibrated micro pH electrode (Thermo Scientific (Waltham, MA) Orion pH probe catalog no. 9810BN).

DNA extraction and Illumina MiSeq sequencing

The detailed protocol for DNA extraction and Illumina MiSeq sequencing was followed as previously described with modifications (Supplemental Methods).[26] Briefly, 0.2 ml of GI fluid or 20 mg of stool was used for DNA isolation using a Qiagen (Germantown, MD) MagAttract Powermag microbiome DNA isolation kit (catalog no. 27500-4-EP). Barcoded dual-index primers specific to the V4 region of the 16S rRNA gene were used to amplify the DNA,[27] using a "touchdown PCR" protocol (Supplemental Methods). Multiple negative controls were run parallel to each PCR reaction. PCR reactions were normalized, pooled and quantified.[28] Libraries were prepared and sequenced using the 500 cycle MiSeq V2 Reagent kit (Illumina, San Diego, CA, catalog no. MS-102-2003). Raw FASTQ files, including those for negative controls, were deposited in the Sequence Read Archive database (BioProjectID: PRJNA495320; BioSampleIDs: SAMN10224451-SAMN10224634).

Data processing and microbiota analysis

Analysis of the V4 region of the 16S rRNA gene was done using mothur (v1.39.3).[27,29] Full methods, including detailed processing steps, raw processed data, and code for each analysis, are described in: https://github.com/aseekatz/SI_mesalamine. Briefly, following assembly, quality filtering, and trimming, reads were aligned to the SILVA 16S rRNA sequence database (v128).[30] Chimeric sequences were removed using UCHIME.[31] Prior to analysis, both mock and negative control samples (water) were assessed for

120 potential contamination; samples with < 2500 sequences were excluded (Table S1).
121 Sequences were binned into operational taxonomic units (OTUs), 97% similarity,
122 using the opticlust algorithm.[32] The Ribosomal Database Project (v16) was used to
123 classify OTUs or sequences directly for compositional analyses (> 80% confidence
124 score).[33] Alpha and beta diversity measures (inverse Simpson index; the Yue &
125 Clayton dissimilarity index, θ_{YC})[34] were calculated from unfiltered OTU data. Basic R
126 commands were used to visualize results, calculate % OTUs shared between
127 samples, and conduct statistics, using packages plyr, dplyr, gplots, tidyr, and
128 tidyverse. The nonparametric Kruskal-Wallis test, using Dunn's test for multiple
129 comparisons and adjusting *p*-values with the Benjamini-Hochberg method when
130 indicated, was used for multi-group comparisons. The R packages lme4[35] and
131 lmerTest[36] were used for mixed linear models between OTU relative abundance
132 (filtered to include OTUs present in at least half of samples collected from a subject,
133 per site) and pH or mesalamine.
134
135 **RESULTS**
136 **Study population**
137 Using a multi-channel catheter with multiple aspiration points,[24] samples
138 collected from the upper GI tract of 8 healthy subjects during 10 different study
139 visits were processed for 16S microbial community analysis (Supplemental
140 Methods, Table 1, Table S1). Samples were collected hourly over the course of 7
141 hours primarily from the proximal GI tract in the following possible locations: the
142 stomach (n=44), duodenum (n=64), proximal/mid/distal jejunum (n=46), and stool

143 (n=3). At the beginning of the study, subjects were given one form of mesalamine
144 (Table 1). One of the seven subjects was studied three times over the course of 10
145 months; for most analyses, each study visit from this subject was considered
146 independently.
147
148 **The proximal GI microbiota is dominated by Firmicutes and is distinct from**
149 **the fecal microbiota**
150 Analysis of the relative abundances of 16S rRNA-encoding genes from the GI
151 tract across all timepoints and individuals demonstrated that the small intestinal
152 microbiota was compositionally unique compared to stool (Fig. 1A). At all four sites
153 in the proximal GI tract, Firmicutes composed the most abundant phyla (i.e.
154 *Streptococcus*, *Veillonella*, and *Gemella* sp.). Higher levels of Bacteroidetes species
155 (*Prevotella*) were detected in the stomach and duodenum. Proteobacteria and
156 Actinobacteria predominated the remainder of the community at all sites. Diversity
157 of the microbiota (inverse Simpson index) was decreased in sites of the upper GI
158 tract compared to stool, which were enriched in Firmicutes (*Blautia*,
159 Ruminococcaceae sp., and *Faecalibacterium*) and depleted in Bacteroidetes in these
160 individuals (n=3) (Fig. 1B).
161
162 **The proximal GI microbiota is individualized and variable over time**
163 To compare the microbiota across the proximal GI tract within and across
164 individuals, we assessed pairwise community dissimilarity using the Yue & Clayton
165 dissimilarity index, θ_{YC}, which takes into account relative abundance of OTU

166 compositional data. Both across (inter-individual) and within (intra-individual)
167 subjects, stool was highly dissimilar to any proximal GI site (Figure 2A, 2B). Across
168 proximal GI sites, subjects were more similar to their own samples than samples
169 across other individuals (Figure 2A-D). The stomach microbiota was highly
170 dissimilar across individuals compared to the duodenum or any part of the jejunum,
171 which exhibited the least amount of dissimilarity (Figure 2C). A similar degree of
172 dissimilarity was observed within an individual in the stomach, duodenum, and
173 combined parts of the jejunum (Figure 2D).

174 Using a dissimilarity measure such as θ_{YC} allows us to assess stability based
175 on changes in the relative abundance of OTUs. It is possible that certain GI sites
176 fluctuate more in total OTUs. To measure whether any site had a higher rate of flux
177 in their community, i.e. a higher rate of OTU turnover, we calculated the % OTUs
178 detected at a given timepoint from the total number of OTUs detected within that
179 individual at a given site. We observed that for each proximal GI site, a mean of
180 36.6% of the OTUs ever detected in that subject at a given site (mean number of
181 total OTUs ever detected per subject per site = 135; range 78-212) were detectable
182 at a given timepoint (Figure 3A). Similarly, we calculated the number of OTUs that
183 were consistently present in all samples collected at that site within an individual
184 (mean number of consistently detected OTUs per subject per site = 14.1; range 2-
185 45). Overall, only 28.7% of the total OTUs ever detected at a given time point within
186 an individual at a given site were represented by these consistently prevalent OTUs
187 (Figure 3B). However, these prevalent OTUs explained an average of 72.0% of the
188 relative abundance observed in the samples (Figure 3C). Of all sites, the relative

abundance explained by the individual's most prevalent OTUs in the stomach was lowest, followed by the duodenum, suggesting more variation at these sites compared to the jejunum (Kruskal-Wallis, $p < 0.05$).

One subject (M046) returned three times over the course of 10 months, allowing us to compare long-term changes. Across the sites that were sampled during multiple visits (the duodenum and mid-jejunum), prevalent OTUs were still detected during all three visits, explaining 74.4% and 66.1% OTUs in the duodenum and mid-jejunum, respectively (Fig. S1).

Large fluctuations in the duodenal microbiota are associated with pH but not mesalamine

We next investigated how these compositional trends changed over time across the subjects. We focused on the duodenum and stomach since these sites were highly sampled across and within individuals and demonstrated variable pH. In the duodenum, we observed large fluctuations in genus-level composition across hourly timepoints within individuals (Figure 4, Figure S2, S3). Specifically, the relative abundance of *Streptococcus*, *Prevotella*, and an unclassified Pasteurellaceae species fluctuated in all individuals. We hypothesized that these fluctuations could be driven by mesalamine, administered in different forms to each subject at study onset. However, no visible pattern was observed with mesalamine levels. Interestingly, we observed that these compositional changes tracked with pH fluctuations (Figure 4). These patterns were less apparent in the stomach, where individuals displayed variable dynamics and highly individualized compositional

patterns independent of mesalamine levels or pH, or in the jejunum of the subject with three different admissions, where pH fluctuated less (Figure S1, S2).

To identify whether any singular OTUs correlated with changes in pH, we applied a generalized linear mixed model approach that takes into account subject-specificity.[37-39] Within duodenal samples (n=56), we observed 15 OTUs that significantly correlated with pH changes. Linear regression of pH and relative abundance of these OTUs was significant across all samples (Figure 5; Table S2). Of the negatively correlated OTUs, six OTUs were classified as Bacteroidetes, mainly *Prevotella*, and two OTUs were classified as Pasteurellaceae sp. (Proteobacteria). The majority of the OTUs that were positively correlated with pH were Firmicutes, mainly *Streptococcus*, alongside an *Actinomyces* OTU (Actinobacteria). Only one OTU in the duodenum was significantly correlated to mesalamine (Table S2). We identified 17 OTUs that correlated with pH or mesalamine in the stomach; however, these were not representative at all sites (Table S2).

DISCUSSION

Our results demonstrate that the microbial communities inhabiting the GI tract are distinct and dynamic across different sites within the proximal GI tract. Our sampling procedure provided us with an opportunity to longitudinally characterize such microbial populations in conjunction with the administration of a commonly used drug, mesalamine. We observed high stability of the microbiota in the jejunum compared to the stomach or duodenum, indicating that the indigenous microbiota residing in more proximal regions of the GI tract may experience greater changes.

While we did not observe strong correlations between mesalamine concentration and particular microbiota members at any site, we did observe a strong correlation between the microbiota composition and pH, particularly in the duodenum.

In this report, we describe the use of a multi-lumen catheter design with unique aspiration ports that enabled sampling of small intestinal content over the course of seven hours.[24] Many studies aimed at investigating the microbiota of the proximal GI have overcome sampling difficulty in this region by using ileostoma effluent, samples from newly deceased individuals, or naso-ileal tubes. Although easy to access, ileostoma effluent does not fully recapitulate the distal small intestine, as it more closely resembles the colon than the small intestine due to increased oxygen concentrations near the stoma.[40-43] Single lumen naso-ileal tubes are unable to sample multiple sites simultaneously.[18,20,21,44] GI fluid collected with our methodology was sufficient for determining mesalamine concentration, assaying fluid pH, and isolating microbial DNA across time and GI sites, which has not been previously described.[24]

Our results support previous observations that the small intestine is dynamic with higher inter-individual than intra-individual variability.[18,21,45] However, the mid-distal small intestine also contains a resilient microbial community composed of several highly abundant OTUs. This resilience is demonstrated by the shift from an altered to a normal ileal microbiota following the resolution of an ileostoma.[46] This mirrors the colonic microbiota, which also has a small community which is stable over long periods of time.[42,47,48]

257 This and other studies have shown that the jejunal and proximal ileal
258 microbiota are distinct from the colonic microbiota.[10,49] Despite changes in overall
259 community structure and an overall decrease in microbial diversity across the
260 stomach and small intestine compared stool, many of the same organisms
261 commonly observed in stool were also present in the upper GI tract, albeit at very
262 different abundances.[10] Interestingly, colonic resection and ileal pouch-anal
263 anastomosis has been shown to shift the terminal ileum microbiota to a state similar
264 to the colon, suggesting that a colonic community structure can develop at these
265 sites given the right conditions.[21,43,49-51]

266 Many of the abundant microbes observed in our study, *Streptococcus*,
267 *Veillonella*, *Gemella*, and Pasteurellaceae species, are also common residents of the
268 oral cavity, which reflects the proximity of these locations in the GI tract.
269 Populations of Proteobacteria, such as Pasteurellaceae, have also been observed
270 consistently in the small intestinal microbiota in other studies, particularly in
271 patients with IBD.[14,52-54] In our study, *Streptococcus* and *Veillonella* were correlated
272 with pH in duodenal samples. It is possible that growth of these organisms drives a
273 decrease in pH via metabolism of short-chain fatty acids, an observed functional
274 capacity of these genera.[21,55] Conversely, large fluctuations in environmental pH
275 may select for genera like *Streptococcus*, which have evolved a variety of
276 mechanisms to control pH intracellularly.[56-59] In any case, our data suggests a
277 relationship between microbial dynamics and environmental physiology of the
278 duodenum, which is an important observation to consider when comparing this site
279 across individuals.

We observed little association between mesalamine concentration and changes in microbial relative abundance in our cohort. Several studies have reported differences in the fecal microbiota of patients with or without IBD, in particular Crohn's disease, which can affect the small intestine.[52] Compositional shifts in the small intestine have been reported during IBD, specifically increased levels of Enterobacteriaceae species, such as *Enterococcus, Fusobacterium*, or *Haemophilus*.[14,53,54] It has been hypothesized that mesalamine's ability to reduce inflammation in patients with ulcerative colitis could be by altering the microbiota.[22,23] While acute effects of mesalamine on the microbiota have not previously been reported, earlier work has demonstrated that mesalamine decreases bacterial polyphosphate accumulation and pathogen fitness, suggesting an influence on the microbiota.[23] We did not observe strong correlations between mesalamine concentration and the microbiota here. However, our study was small, used different doses of mesalamine that may be metabolized differently across GI sites, and was conducted in healthy individuals.[24] It is possible that mesalamine is less likely to impact the small intestinal microbiota, which historically has lower efficacy in treating active Crohn's Disease, which manifests in the small intestine, compared to ulcerative colitis, which manifests in the large intestine.[22,60,61] As indicated by the variability of mesalamine in the subjects in this study, the effects of mesalamine on the small intestinal microbiota may be highly individualized.[24,62-64] Furthermore, individuals with disease may harbor a distinct microbiota that responds to mesalamine differently.

Despite the opportunity provided by our method to describe the microbiota across the GI tract, our study has some lingering questions. Movement by the subject during the study can result in movement of each sampling port, particularly between the distal stomach and antrum. This may explain the inconsistent pH values and severe fluctuations of the microbiota observed in the stomach. Similarly, the shorter length of the sampling device, as compared to a naso-ileal catheter, prevented reliable collection of fluid from the distal small intestine, limiting our sampling to the proximal region. We also were limited to three concurrent fecal samples, each of which was low in Bacteroidetes, a profile generally observed in individuals on low fat-high fiber, non-Western diets.[65]

The use of a novel catheter allowed us to assess the microbiota across several proximal GI sites overtime, representing a powerful clinical and/or investigative tool for studying the small intestinal microbiota. Future studies on the upper GI microbiota should collect concurrent oral swab/sputum and fecal samples to strengthen the ability to "track" microbial populations across the GI tract, potentiating our ability to correlate the microbiota from fecal sampling, a more convenient method to study the microbiota, to other sites of the GI tract.

Declarations:

Acknowledgements:

This research was funded by FDA grant HHSF223201000082C. Clinical samples collected with help from Michigan Institute for Clinical & Health Research (MICHR) NIH grant UL1TR000433. The authors would also like to thank the Host Microbiome Initiative and the Microbial Systems Molecular Biology Laboratory at

the University of Michigan for their support with the 16s rRNA sequencing, and Krishna Rao and Rose Putler for their assistance with the modelling and statistical analyses. This research was funded by the FDA(HHSF223201000082C) and the NIH (5U01AI124255-03).

Availability of data and material:

SRA: BioProjectID: PRJNA495320; BioSampleIDs: SAMN10224451-SAMN10224634

GitHub: https://github.com/aseekatz/SI_mesalamine

Authors' contributions:

MJK, DS, BEB, AMS, VBY, MKS - Conception or design of the work

MJK, BEB, WLH, JRB - Data collection

AMS, MJK, MKS - Data analysis and interpretation

MKS, AMS - Drafting the article

MKS, AMS, DS, VBY - Critical revision of the article

AMS, MKS, MJK, JRB, WLH, BEB, VBY, DS - Final approval of the version to be published

Ethics approval and consent to participate:

Samples collected in this study were part of clinical trial NCT01999400. The institutional review boards at the University of Michigan (IRBMED) and the Department of Health and Human Services, Food and Drug Administration

(Research Involving Human Subjects Committee/RIHSC) both approved the study protocol. All subjects provided written informed consent in order to participate.

Consent for publication:

Not applicable

Competing interests:

None

Table 1: Subject Recruitment. Selected metadata and sample collections for 10 admissions (subject M046 was admitted for three visits).

Figure 1: Bacterial community relative abundance and diversity in the upper GI tract. A) The mean relative abundance of genera at each GI site (sample *n* indicated). **B)** Boxplots of the inverse Simpson Index measuring community diversity across the GI tract (median, with first and third inter-quartile ranges). Statistical analysis: Kruskal-Wallis test (ns).

Figure 2: Dissimilarity of the proximal GI tract within and across individuals. Heatmap of the Yue & Clayton dissimilarity index, θ_{YC}, comparing different proximal GI sites and stool **A)** across individuals (inter-individual pairwise comparisons) and **C)** within individuals (intra-individual pairwise comparisons). C) Inter-individual and D) intra-individual dissimilarity in the stomach, duodenum, and jejunum (sites combined). Statistical analysis: Kruskal-Wallis test (will add p values to graph). Statistical analysis: Dunn's test for multiple comparisons with a Benjamini-Hochberg p-value adjustment (*p < 0.01; **p < 0.001; ***p < 0.0001).

Figure 3: Fluctuations in prevalent OTUs observed within an individual across the proximal GI tract. A) Boxplots of the percentage of OTUs detected in a given sample out of all OTUs detected (all OTUs possible for that individual) at a subject-site. **B)** Boxplots of the percentage of OTUs that were consistently detected at a subject-site out of the total OTUs detected in a given sample. **C)** The percent of

relative abundance explained by prevalent OTUs at a subject-site in a given sample. Statistical analysis: Kruskal-Wallis test.

Figure 4: Longitudinal compositional dynamics, mesalamine levels, and pH in the duodenum. Streamplots of genus-level composition over time in the duodenum of six individuals (%, left y-axis; genera coded in legend). White lines indicate pH measurements (black y-axis labels on right) and red lines indicate mesalamine concentration (red y-axis labels on right).

Figure 5: Relative abundance of significant OTUs vs. pH. Log relative abundance (log(RA)) as a function of pH of OTUs found to be significantly correlated with pH using linear mixed models (all samples with measurable pH). Lines represent linear fit per OTU. OTUs classified as **A)** Firmicutes, **B)** Bacteroidetes, **C)** Proteobacteria, and **D)** Actinobacteria are depicted (genus-level OTU classification coded by legends).

REFERENCES

1 El Aidy, S., van den Bogert, B. & Kleerebezem, M. The small intestine microbiota, nutritional modulation and relevance for health. *Current Opinion in Biotechnology* **32**, 14-20, doi:10.1016/j.copbio.2014.09.005 (2015).

2 Poulakos, L. & Kent, T. H. Gastric Emptying and Small Intestinal Propulsion in Fed and Fasted Rats. *Gastroenterology* **64**, 962-967, doi:10.1016/S0016-5085(73)80008-3 (1973).

3 Ridlon, J. M., Kang, D. J., Hylemon, P. B. & Bajaj, J. S. Bile acids and the gut microbiome. *Current opinion in gastroenterology* **30**, 332-338, doi:10.1097/mog.0000000000000057 (2014).

4 Araújo, J. R., Tomas, J., Brenner, C. & Sansonetti, P. J. Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity. *Biochimie* **141**, 97-106, doi:10.1016/j.biochi.2017.05.019 (2017).

5 Martinez-Guryn, K. *et al.* Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host & Microbe* **23**, 458-469.e455, doi:10.1016/j.chom.2018.03.011 (2018).

6 Flynn, K. J., Ruffin, M. T., Turgeon, D. K. & Schloss, P. D. Spatial Variation of the Native Colon Microbiota in Healthy Adults. *Cancer Prevention Research* **11**, 393-402, doi:10.1158/1940-6207.Capr-17-0370 (2018).

7 Falony, G., Vieira-Silva, S. & Raes, J. Richness and ecosystem development across faecal snapshots of the gut microbiota. *Nature microbiology* **3**, 526-528, doi:10.1038/s41564-018-0143-5 (2018).

8 Sekirov, I., Russell, S. L., Antunes, L. C. M. & Finlay, B. B. Gut Microbiota in Health and Disease. *Physiological Reviews* **90**, 859-904, doi:10.1152/physrev.00045.2009 (2010).

9 Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology* **14**, 20, doi:10.1038/nrmicro3552 (2015).

10 Zmora, N. *et al.* Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell* **174**, 1388-1405.e1321, doi:10.1016/j.cell.2018.08.041 (2018).

11 Hayashi, H., Takahashi, R., Nishi, T., Sakamoto, M. & Benno, Y. Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. *Journal of medical microbiology* **54**, 1093-1101, doi:10.1099/jmm.0.45935-0 (2005).

12 Wang, M., Ahrné, S., Jeppsson, B. & Molin, G. Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. *FEMS Microbiology Ecology* **54**, 219-231, doi:10.1016/j.femsec.2005.03.012 (2005).

13 Wang, X., Heazlewood, S. P., Krause, D. O. & Florin, T. H. J. Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. *Journal of Applied Microbiology* **95**, 508-520, doi:10.1046/j.1365-2672.2003.02005.x (2003).

14 Dey, N., Soergel, D. A., Repo, S. & Brenner, S. E. Association of gut microbiota with post-operative clinical course in Crohn's disease. *BMC gastroenterology* **13**, 131, doi:10.1186/1471-230x-13-131 (2013).

15 Barrett, E. *et al.* Microbiota diversity and stability of the preterm neonatal ileum and colon of two infants. *MicrobiologyOpen* **2**, 215-225, doi:10.1002/mbo3.64 (2013).

16 Di Pilato, V. *et al.* The esophageal microbiota in health and disease. *Annals of the New York Academy of Sciences* **1381**, 21-33, doi:10.1111/nyas.13127 (2016).

17 Verma, D., Garg, P. K. & Dubey, A. K. Insights into the human oral microbiome. *Archives of Microbiology* **200**, 525-540, doi:10.1007/s00203-018-1505-3 (2018).

18 Booijink, C. C. G. M. *et al.* High temporal and inter‐individual variation detected in the human ileal microbiota. *Environmental Microbiology* **12**, 3213-3227, doi:10.1111/j.1462-2920.2010.02294.x (2010).

19 den Bogert, B. v. *et al.* Diversity of human small intestinal Streptococcus and Veillonella populations. *FEMS Microbiology Ecology* **85**, 376-388, doi:10.1111/1574-6941.12127 (2013).

20 Angelakis, E. *et al.* A Metagenomic Investigation of the Duodenal Microbiota Reveals Links with Obesity. *PLOS ONE* **10**, e0137784, doi:10.1371/journal.pone.0137784 (2015).

21 Zoetendal, E. G. *et al.* The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *The Isme Journal* **6**, 1415, doi:10.1038/ismej.2011.212 (2012).

22 Hauso, Ø., Martinsen, T. C. & Waldum, H. 5-Aminosalicylic acid, a specific drug for ulcerative colitis. *Scandinavian journal of gastroenterology* **50**, 933-941, doi:10.3109/00365521.2015.1018937 (2015).

23 Dahl, J.-U. *et al.* The anti-inflammatory drug mesalamine targets bacterial polyphosphate accumulation. *Nature microbiology* **2**, 16267, doi:10.1038/nmicrobiol.2016.267 (2017).

24 Yu, A. *et al.* Measurement of in vivo Gastrointestinal Release and Dissolution of Three Locally Acting Mesalamine Formulations in Regions of the Human Gastrointestinal Tract. *Molecular Pharmaceutics* **14**, 345-358, doi:10.1021/acs.molpharmaceut.6b00641 (2017).

25 Committee, A. Q. A. I. E. *et al.* Multisociety guideline on reprocessing flexible gastrointestinal endoscopes: 2011. *Gastrointest Endosc* **73**, 1075-1084, doi:10.1016/j.gie.2011.03.1183 (2011).

26 Seekatz, A. M. *et al.* Fecal Microbiota Transplantation Eliminates Clostridium difficile in a Murine Model of Relapsing Disease. *Infection and immunity* **83**, 3838-3846, doi:10.1128/IAI.00459-15 (2015).

27 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**, 5112-5120, doi:10.1128/AEM.01043-13 (2013).

28 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**, doi:10.1128/aem.01043-13 (2013).

29 Schloss, P. D. *et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**, 7537-7541, doi:10.1128/AEM.01541-09 (2009).

30 Pruesse, E. *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**, 7188-7196, doi:10.1093/nar/gkm864 (2007).

31 Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. & Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194-2200, doi:10.1093/bioinformatics/btr381 (2011).

32 Westcott, S. L. & Schloss, P. D. OptiClust, an Improved Method for Assigning Amplicon-Based Sequence Data to Operational Taxonomic Units. *mSphere* **2**, doi:10.1128/mSphereDirect.00073-17 (2017).

33 Cole, J. R. *et al.* The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37**, D141-145, doi:10.1093/nar/gkn879 (2009).

34 Yue, J. C. & Clayton, M. K. A Similarity Measure Based on Species Proportions. *Communications in Statistics - Theory and Methods* **34**, 2123-2131, doi:10.1080/STA-200066418 (2005).

35 Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software; Vol 1, Issue 1 (2015)* (2015).

36 Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software; Vol 1, Issue 13 (2017)* (2017).

37 Xia, Y. & Sun, J. Hypothesis testing and statistical analysis of microbiome. *Genes & Diseases* **4**, 138-148, doi:10.1016/j.gendis.2017.06.001 (2017).

38 Gajer, P. *et al.* Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* **4**, 132ra152, doi:10.1126/scitranslmed.3003605 (2012).

39 Mehta, S. D. *et al.* The vaginal microbiota over an 8- to 10-year period in a cohort of HIV-infected and HIV-uninfected women. *PLoS One* **10**, e0116894, doi:10.1371/journal.pone.0116894 (2015).

40 Heimesaat, M. M. *et al.* Comprehensive Postmortem Analyses of Intestinal Microbiota Changes and Bacterial Translocation in Human Flora Associated Mice. *PLOS ONE* **7**, e40758, doi:10.1371/journal.pone.0040758 (2012).

41 DeBruyn, J. M. & Hauther, K. A. Postmortem succession of gut microbial communities in deceased human subjects. *PeerJ* **5**, e3437, doi:10.7717/peerj.3437 (2017).

42 Fukuyama, J. *et al.* Multidomain analyses of a longitudinal human microbiome intestinal cleanout perturbation experiment. *PLOS Computational Biology* **13**, e1005706, doi:10.1371/journal.pcbi.1005706 (2017).

43 Young, V. B. *et al.* Multiphasic analysis of the temporal development of the distal gut microbiota in patients following ileal pouch anal anastomosis. *Microbiome* **1**, 9, doi:10.1186/2049-2618-1-9 (2013).

44 Muller-Lissner, S. A. *et al.* Effect of gastric and transpyloric tubes on gastric emptying and duodenogastric reflux. *Gastroenterology* **83**, 1276 (1982).

45 Onishi, J. C. *et al.* Bacterial communities in the small intestine respond differently to those in the caecum and colon in mice fed low- and high-fat diets. *Microbiology (Reading, England)* **163**, 1189-1197, doi:10.1099/mic.0.000496 (2017).

46 Hartman, A. L. *et al.* Human gut microbiome adopts an alternative state following small bowel transplantation. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 17187-17192, doi:10.1073/pnas.0904847106 (2009).

47 Caporaso, J. G. *et al.* Moving pictures of the human microbiome. *Genome Biology* **12**, R50, doi:10.1186/gb-2011-12-5-r50 (2011).

48 Dethlefsen, L. & Relman, D. A. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Sciences* **108**, 4554-4561, doi:10.1073/pnas.1000087107 (2011).

49 Villmones, H. C. *et al.* Species Level Description of the Human Ileal Bacterial Microbiota. *Scientific Reports* **8**, 4736, doi:10.1038/s41598-018-23198-5 (2018).

50 Hinata, M. *et al.* A Shift from Colon- to Ileum-Predominant Bacteria in Ileal-Pouch Feces Following Total Proctocolectomy. *Digestive diseases and sciences* **57**, 2965-2974, doi:10.1007/s10620-012-2165-9 (2012).

51 Maharshak, N. *et al.* Alterations of Enteric Microbiota in Patients with a Normal Ileal Pouch Are Predictive of Pouchitis. *Journal of Crohn's and Colitis* **11**, 314-320, doi:10.1093/ecco-jcc/jjw157 (2017).

52 Harris, K. G. & Chang, E. B. The intestinal microbiota in the pathogenesis of inflammatory bowel diseases: new insights into complex disease. *Clinical science (London, England : 1979)* **132**, 2013-2028, doi:10.1042/cs20171110 (2018).

53 De Cruz, P. *et al.* Association between specific mucosa-associated microbiota in Crohn's disease at the time of resection and subsequent disease recurrence: a pilot study. *J Gastroenterol Hepatol* **30**, 268-278, doi:10.1111/jgh.12694 (2015).

54 Gevers, D. *et al.* The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* **15**, 382-392, doi:10.1016/j.chom.2014.02.005 (2014).

55 Pancholi, V. & Caparon, C. in *Streptococcus pyogenes : Basic Biology to Clinical Manifestations* (eds J. J. Ferretti, D. L. Stevens, & V. A. Fischetti) Ch. Streptococcus pyogenes Metabolism, (University of Oklahoma Health Sciences Center

(c) The University of Oklahoma Health Sciences Center., 2016).

56 Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J. & Duncan, S. H. The influence of diet on the gut microbiota. *Pharmacological Research* **69**, 52-60, doi:10.1016/j.phrs.2012.10.020 (2013).

57 Abuhelwa, A. Y., Williams, D. B., Upton, R. N. & Foster, D. J. R. Food, gastrointestinal pH, and models of oral drug absorption. *European Journal of Pharmaceutics and Biopharmaceutics* **112**, 234-248, doi:10.1016/j.ejpb.2016.11.034 (2017).

58 Lund, P., Tramonti, A. & De Biase, D. Coping with low pH: molecular strategies in neutralophilic bacteria. *FEMS Microbiology Reviews* **38**, 1091-1125, doi:10.1111/1574-6976.12076 (2014).

59 Bradshaw, D. & Marsh, P. D. Analysis of pH–Driven Disruption of Oral Microbial Communities in vitro. *Caries Research* **32**, 456-462 (1998).

60 Wright, E. K. *et al.* Recent Advances in Characterizing the Gastrointestinal Microbiome in Crohn's Disease: A Systematic Review. *Inflammatory bowel diseases* **21**, 1219-1228, doi:10.1097/MIB.0000000000000382 (2015).

61 Lim, W. C., Wang, Y., MacDonald, J. K. & Hanauer, S. Aminosalicylates for induction of remission or response in Crohn's disease. *The Cochrane database of systematic reviews* **7**, Cd008870, doi:10.1002/14651858.CD008870.pub2 (2016).

62 Allocca, M. *et al.* Effectiveness of Mesalazine, Thiopurines and Tumour Necrosis Factor Antagonists in Preventing Post-Operative Crohn's Disease Recurrence in a Real-Life Setting. *Digestion* **96**, 166-172 (2017).

63 Hanauer, S. B. *et al.* Postoperative maintenance of Crohn's disease remission with 6-mercaptopurine, mesalamine, or placebo: A 2-year trial. *Gastroenterology* **127**, 723-729, doi:10.1053/j.gastro.2004.06.002 (2004).

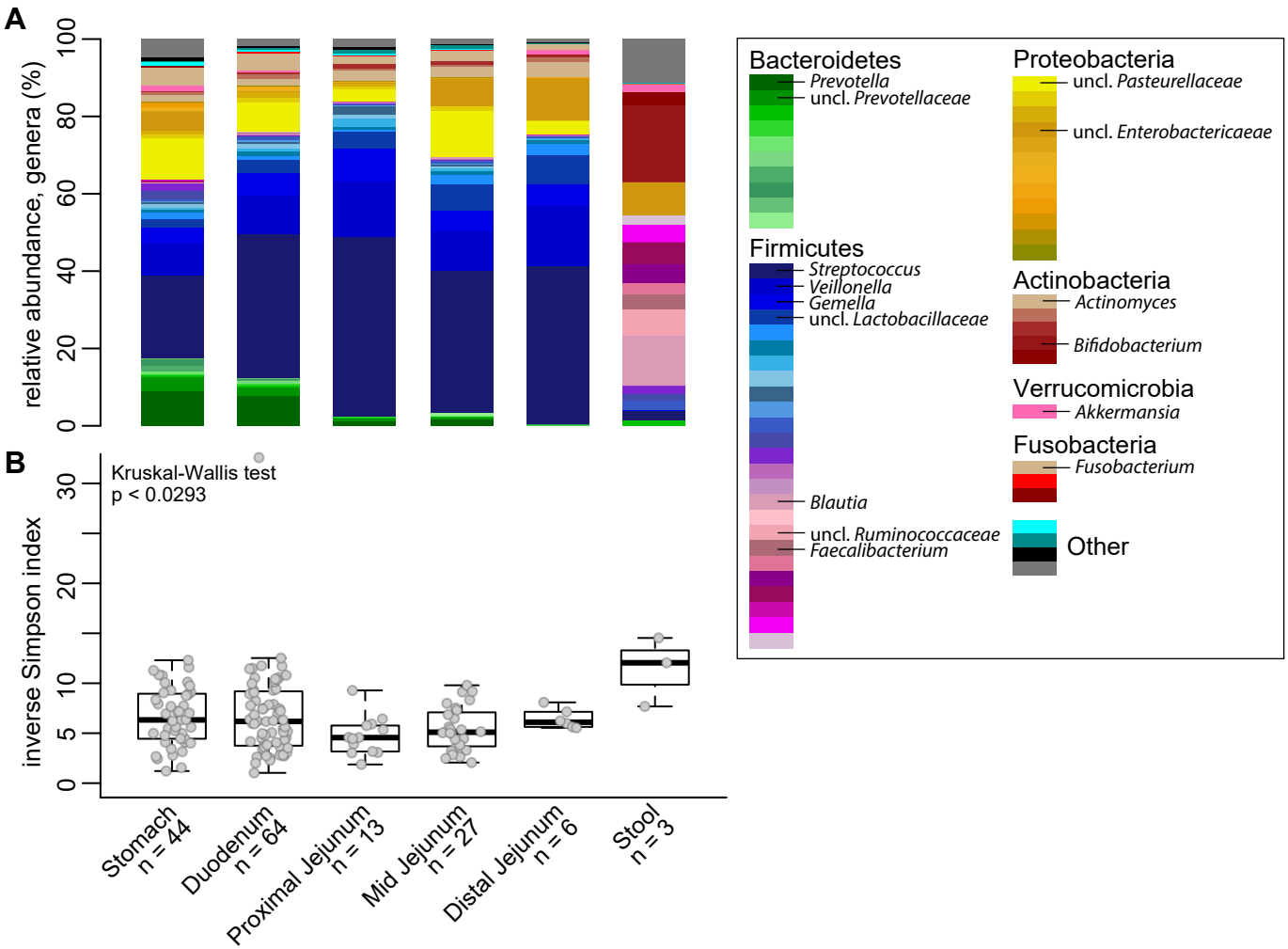
64 Singh, S. & Nguyen, G. C. Management of Crohn's Disease After Surgical Resection. *Gastroenterology clinics of North America* **46**, 563-575, doi:10.1016/j.gtc.2017.05.011 (2017).

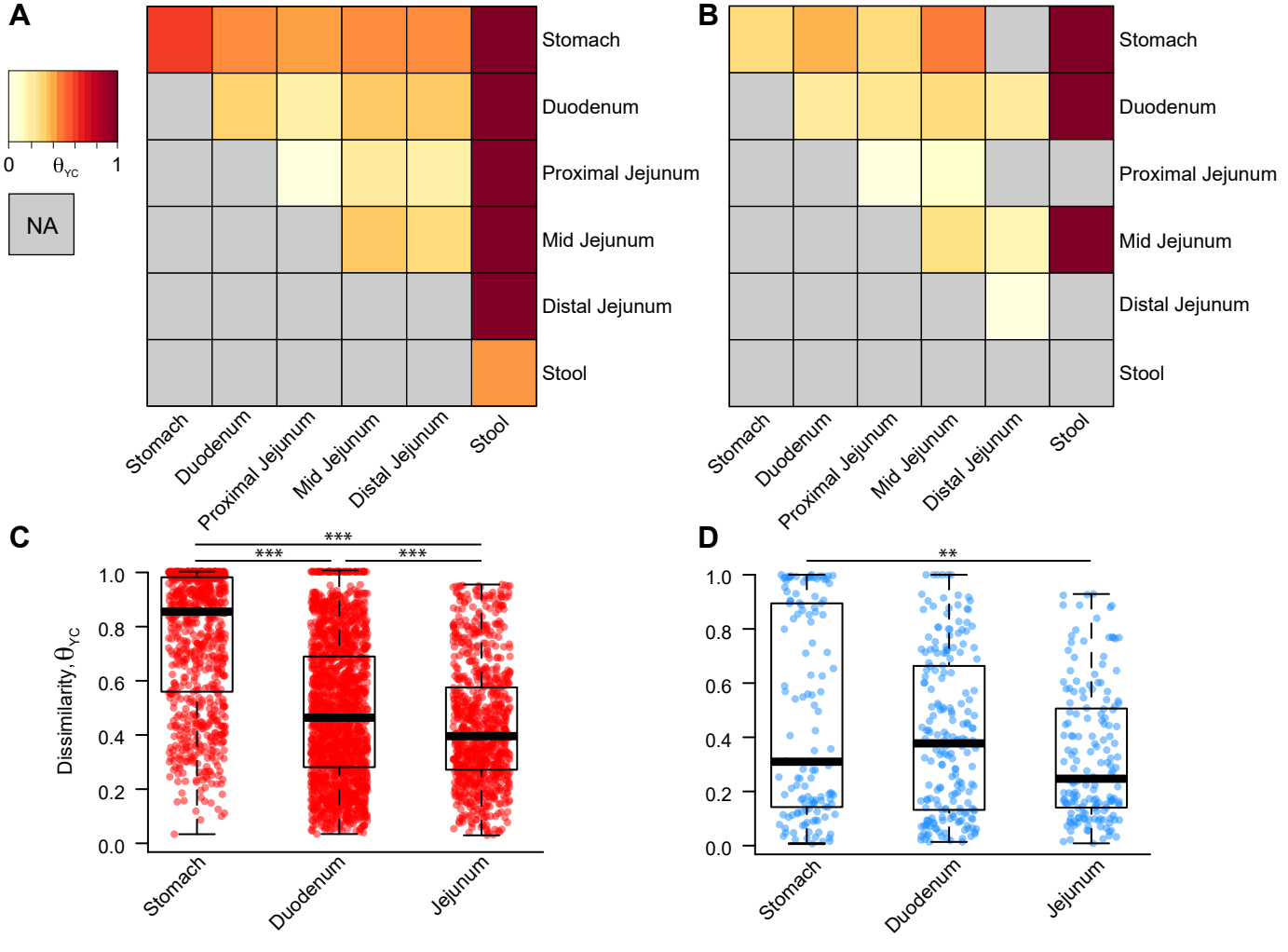
65 Smits, S. A. *et al.* Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* **357**, 802-806, doi:10.1126/science.aan4834 (2017).

Subject ID*	Mesalamine Formulation†	Age	BMI	Sex	Stomach	Duodenum	Jejunum			Stool	Total
							Proximal	*Mid*	*Distal*		
M046-A	Pentasa	38	21.2	M	1	8	-	7	-	1	17
M046-B	Apriso	38	21.3	M	-	8	-	5	6	-	19
M046-C	Lialda	38	21.7	M	8	6	-	7	-	1	22
M047	Pentasa	36	21.1	M	-	8	6	-	-	-	14
M048	Apriso	51	34.3	F	5	7	-	-	-	-	12
M053	Apriso	34	25.2	F	1	-	7	3	-	-	11
M061	Pentasa	51	21.6	M	7	8	-	-	-	-	15
M062	Pentasa	37	27.3	M	7	7	-	-	-	1	15
M063	Lialda	26	28.6	M	7	5	-	5	-	-	17
M064	Lialda	25	27.5	F	8	7	-	-	-	-	15
Summary	**40% P, 30% A, 30% L**	**37 ±8.6**	**25 ±4.4**	**70% M**	**44**	**64**	**13**	**27**	**6**	**3**	**157**

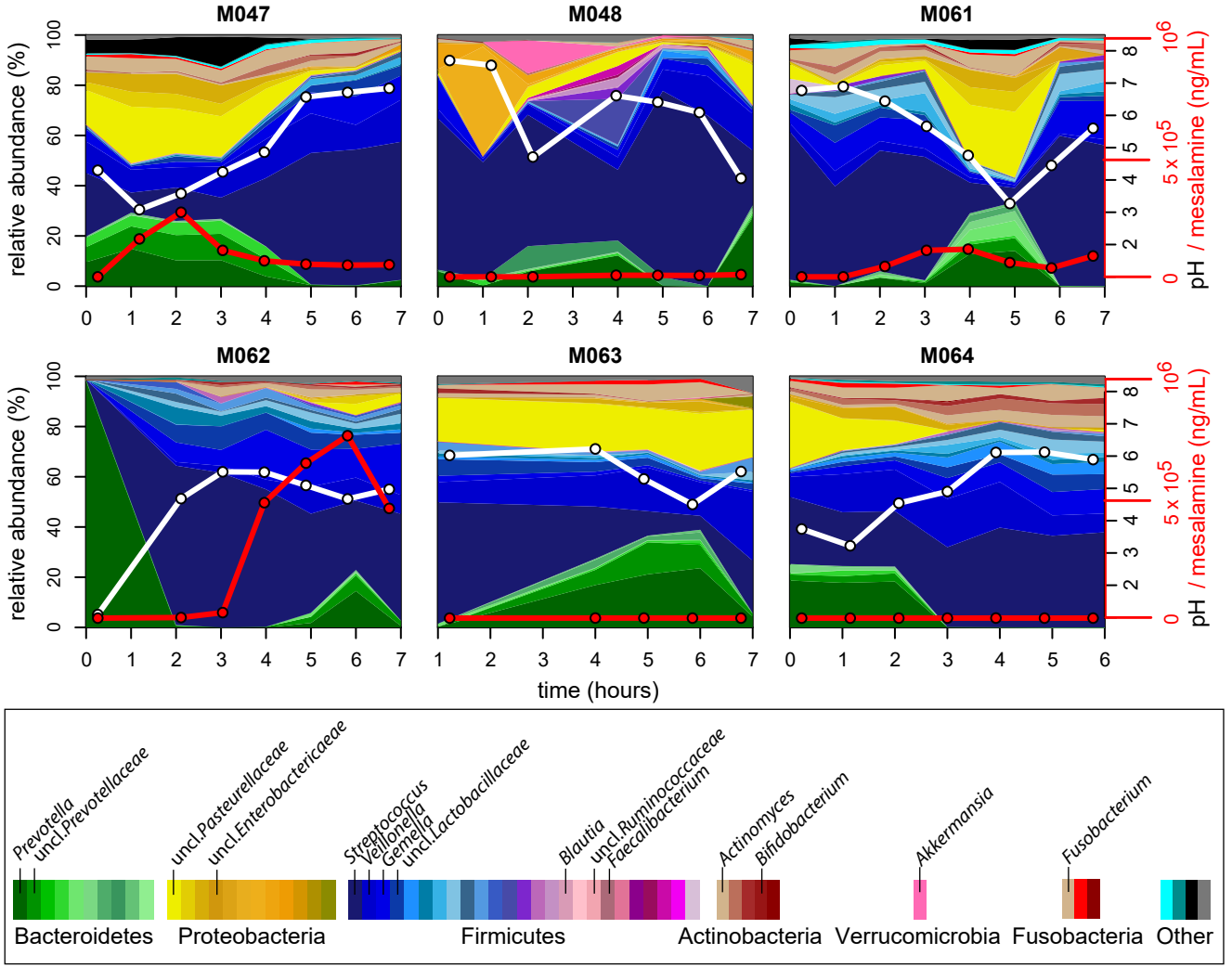
*All subjects were caucasian and none identified as hispanic/latinx.

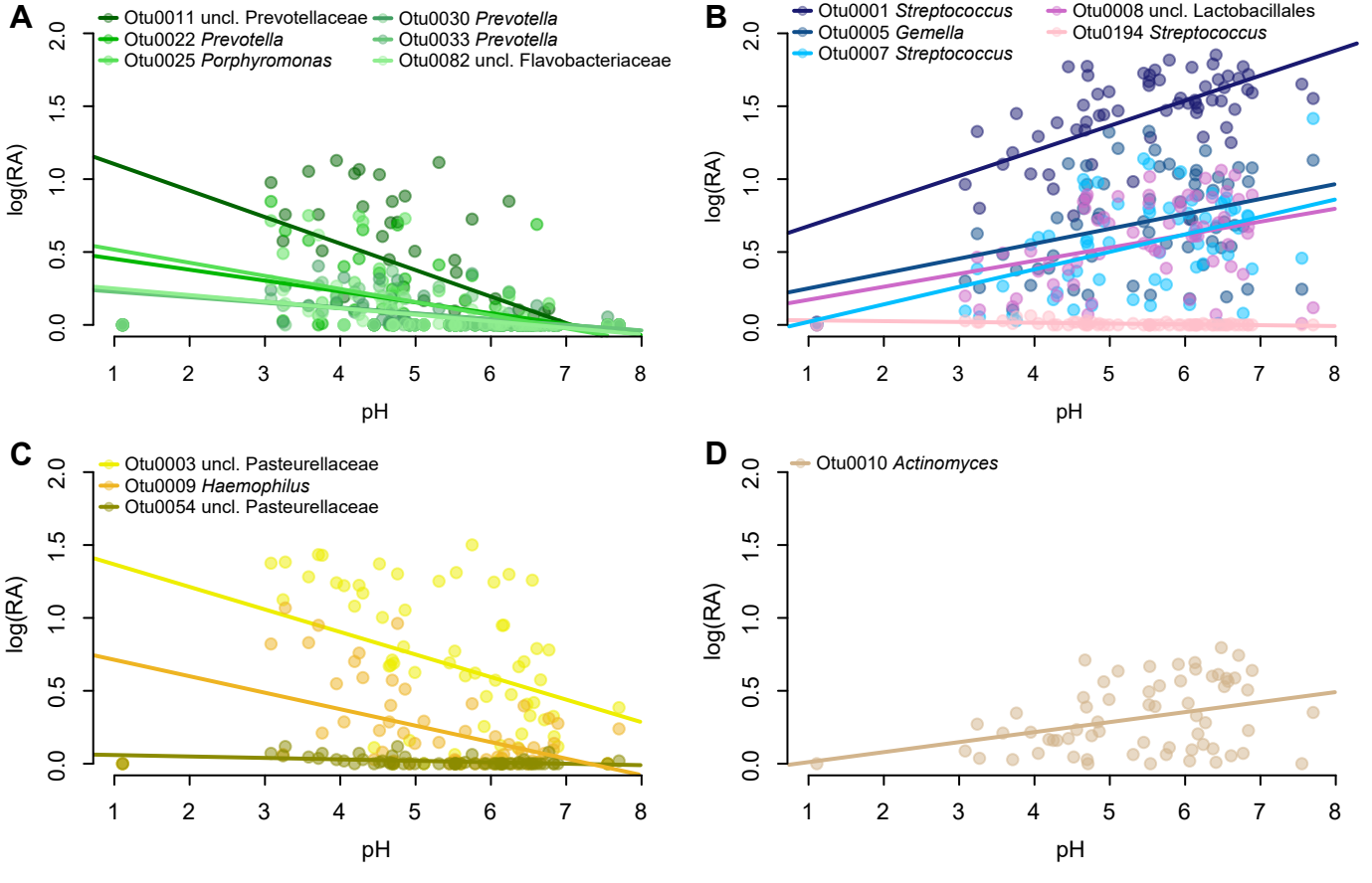
†Pentasa = Immediate release in stomach acid; Apriso = Extended release at pH > 6; Lialda = Extended release at pH > 7


A
relative abundance, genera (%)
0
20
40
60
80
100
B
inverse Simpson index
Kruskal-Wallis test
p < 0.0293
0
5
10
20
30
Stomach n = 44
Duodenum n = 64
Proximal Jejunum n = 13
Mid Jejunum n = 27
Distal Jejunum n = 6
Stool n = 3
Bacteroidetes
Prevotella
uncl. Prevotellaceae
Firmicutes
Streptococcus
Veillonella
Gemella
uncl. Lactobacillaceae
Blautia
uncl. Ruminococcaceae
Faecalibacterium
Proteobacteria
uncl. Pasteurellaceae
uncl. Enterobactericaceae
Actinobacteria
Actinomyces
Bifidobacterium
Verrucomicrobia
Akkermansia
Fusobacteria
Fusobacterium
Other


A
0
θYC
1
NA
Stomach
Duodenum
Proximal Jejunum
Mid Jejunum
Distal Jejunum
Stool
B
C
Dissimilarity, θYC
***
***
***
1.0
0.8
0.6
0.4
0.2
0.0
Jejunum
D
**

A
%OTUs detected of total OTUs per patient
0
20
40
60
80
100
p < 0.652
B
% prevalent OTUs detected in all OTUs at a given timepoint
0
20
40
60
80
100
p < 0.109
C
% of relative abundance occupied by prevalent OTUs
0
20
40
60
80
100
p < 0.0241
Stomach n = 42
Duodenum n = 64
Proximal Jejunum n = 13
Mid Jejunum n = 27
Distal Jejunum n = 6


M047
M048
M061
M062
M063
M064
relative abundance (%)
pH / mesalamine (ng/mL)
time (hours)
Prevotella
uncl.Prevotellaceae
uncl.Pasteurellaceae
uncl.Enterobacteriaceae
Streptococcus
Veillonella
Gemella
uncl.Lactobacillaceae
Blautia
uncl.Ruminococcaceae
Faecalibacterium
Actinomyces
Bifidobacterium
Akkermansia
Fusobacterium
Bacteroidetes
Proteobacteria
Firmicutes
Actinobacteria
Verrucomicrobia
Fusobacteria
Other


A
Otu0011 uncl. Prevotellaceae
Otu0022 Prevotella
Otu0025 Porphyromonas
Otu0030 Prevotella
Otu0033 Prevotella
Otu0082 uncl. Flavobacteriaceae
log(RA)
pH
B
Otu0001 Streptococcus
Otu0005 Gemella
Otu0007 Streptococcus
Otu0008 uncl. Lactobacillales
Otu0194 Streptococcus
log(RA)
pH
C
Otu0003 uncl. Pasteurellaceae
Otu0009 Haemophilus
Otu0054 uncl. Pasteurellaceae
log(RA)
pH
D
Otu0010 Actinomyces
log(RA)
pH