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High fiber, whole foods dietary intervention alters the human gut microbiome but not fecal short-chain fatty acids — Source link

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2 **fecal short-chain fatty acids**
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28 **ABSTRACT (250 words, 250 LIMIT)**
29
30 Dietary shifts can have a direct impact on the gut microbiome by preferentially selecting for
31 microbes capable of utilizing the various dietary nutrients. Intake of dietary fiber has decreased
32 precipitously in the last century, while consumption of processed foods has increased. Fiber, or
33 microbiota-accessible carbohydrates (MACs), persist in the digestive tract and can be
34 metabolized by specific bacteria encoding fiber degrading enzymes. Digestion of MACs results
35 in the accumulation of short-chain fatty acids (SCFAs) and other metabolic byproducts that are
36 critical to human health. Here, we implemented a two-week dietary fiber intervention aiming for
37 40-50 grams of fiber per day within the context of a course-based undergraduate research
38 experience (CURE) (n = 20). By coupling shotgun metagenomic sequencing and targeted gas-
39 chromatography mass spectrometry (GC/MS), we found that the dietary intervention
40 significantly altered the composition of individual gut microbiomes, accounting for 8.3% of the
41 longitudinal variability within subjects. Notably, microbial taxa that increased in relative
42 abundance as a result of the diet change included known MAC degraders (i.e., *Bifidobacterium*
43 and *Lactobacillus*). We further assessed the genetic diversity within *Bifidobacterium*, assayed by
44 amplification of the *groEL* gene. Concomitant with microbial composition changes, we show an
45 increase in the abundance of genes involved in inositol degradation. Despite these changes in gut
46 microbiome composition, we did not detect a consistent shift in SCFA abundance. Collectively,
47 our results demonstrate that on a short-term timescale of two weeks, increased fiber intake can
48 induce compositional changes of the gut microbiome, including an increase in MAC degrading
49 bacteria.
50
51 **IMPORTANCE (145 words, 150 LIMIT)**
52
53 A profound decrease in the consumption of dietary fiber in many parts of the world in the last
54 century may be associated with the increasing prevalence of Type II diabetes, colon cancer, and
55 other health problems. A typical U.S. diet includes about ~15 grams of fiber per day, far less
56 fiber than daily recommended allowance. Changes in dietary fiber intake affect human health not
57 only through the uptake of nutrients directly, but also indirectly through changes in the microbial
58 community and their associated metabolism. Here we conducted a two-week diet intervention in
59 healthy young adults to investigate the impact of fiber consumption on the gut microbiome.
60 Participants increased their average fiber consumption by 25 grams/day on average for two
61 weeks. The high fiber diet intervention altered the gut microbiome of the study participants,
62 including increases in known fiber degrading microbes such as *Bifidobacterium* and
63 *Lactobacillus*.
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INTRODUCTION

Consumption of dietary fiber has declined dramatically in the last century as processed foods have become a larger part of diets in the industrialized world. Pre-industrial and modern-day rural societies consume between 60-120 grams (g)/day of fiber, while individuals in the United States consume about half of the daily recommended allowance of 38 g/day for men and 25 g/day for women (1, 2). Declines in fiber intake over the past century have contributed to complications for human health. For example, chronic low fiber intake has been associated with Type 2 diabetes mellitus, heart disease, and colon cancer (3–5). Indeed, a reciprocal diet intervention exchanging African Americans low-fiber western diet with rural Africans high-fiber diet (increasing on average 40g per day) led to significant decreases in pre-cancerous biomarkers, further providing a link between fiber and human health (6). Furthermore, dietary fiber has been shown to protect against influenza infection (7), and may influence vaccine efficacy (8).

Dietary fiber is a mixture of polysaccharides that resist rapid digestion in the small intestine by endogenous enzymes and persists through the digestive tract into the colon. Once in the colon, fiber can be digested by the resident microbes (1, 9). This is due, in part, to the human genome encoding only 17 enzymes (i.e., glycoside hydrolases) that are capable of digesting carbohydrates (10). Conversely, the resident gut microbial communities collectively encode thousands of diverse enzymes from 152 gene families that can break down dietary fiber (11). In the colon, specialized microbes metabolize recalcitrant carbohydrates and produce fermented byproducts, including short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (12). SCFAs are capable of being absorbed across the human intestinal epithelial cells, and have direct impacts on human health (reviewed in (13)) such as stimulating and maintaining the mucus layer for the gut epithelium (14) and providing an energy source for butyrate-consuming colonocytes (15). SCFAs have also been shown to have immunomodulatory effects, including increased viral protection through altered T-cell metabolism (7), and inhibitory effects on pathogenic bacteria (e.g. *Clostridioides difficile*) (16).

Understanding the role of dietary fiber in structuring the gut microbiota could provide insights into managing chronic diseases associated with the gut microbiome. Typical diet intervention studies assessing the impact of fiber on gut microbial communities and the production of SCFAs have relied on single fiber supplements (17–19). Fiber supplements such as psyllium husks, inulin, wheat bran, resistant potato starch, and resistant corn starch vary in their efficacy for each individual (17, 20). Individuals might be more or less susceptible to the intervention depending on their initial resident microbial community and its ability to digest a particular fiber supplement. For example, one group investigating the impact of three fermentable fibers on gut microbiome composition and SCFA abundance found no significant effect when study participants consumed 20-24g resistant maize starch per day for two weeks (17). However, in addition to the quantity, the variety of dietary fibers may be important. Studies that have increased dietary fiber have previously observed changes in microbiome composition (3, 17, 18), yet results remain mixed on SCFA production (6, 21, 22). Further, the American Gut Project found that individuals who eat more than 30 types of plants in a week have a more diverse gut microbiome (23). Thus, the consumption of a diversity of fiber sources through

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122 whole foods may provide more opportunities for an individual's gut microbiome to respond to
123 the dietary changes and result in more dramatic changes in fiber degrader abundance and activity
124 in the gut microbiome. The increase of fiber from a diverse set of dietary foods, rather than
125 single fiber supplements, may also contribute to increased consumption of other micronutrients
126 and vitamins that affect the microbiome as well (24).
127 In this study, we sought to answer three questions: 1) does a diet rich in fiber from whole
128 foods alter the overall microbiome? 2) does the intervention alter the abundance and diversity of
129 known fiber-degraders (e.g., *Bifidobacterium*)? and 3) if we observe compositional shifts in the
130 microbiome, do these correspond with metabolic changes in the production of short-chain fatty
131 acids? To address these questions, we developed and employed a course-based undergraduate
132 research experience (CURE) at UC Irvine to assess individual responses to a high-fiber diet (25).
133 Integrating authentic research experiences within lab courses in order to facilitate a deeper
134 understanding of academic and industrial research continues to be a priority for both national
135 education reform and the American Society for Microbiology (25–28). During the intervention,
136 participants were given ten meals each week from a food service that specializes in providing
137 high fiber, unprocessed meals. Individuals tracked dietary information of macronutrients for
138 every meal for three weeks, with the goal of increasing dietary fiber intake to 50 grams/day
139 during a two-week intervention period. We then compared overall bacterial composition using
140 metagenomic sequencing and assessed the production of volatile SCFAs using mass
141 spectrometry. In addition to the shotgun metagenomic sequencing, we targeted a known-fiber
142 degrader, *Bifidobacterium*, by analyzing its diversity using amplicon sequencing of the *groEL*
143 marker gene, enabling a unique high-resolution view of the impact of a dietary fiber intervention
144 on a key taxon.
145
146 **METHODS**
147
148 *Study Design*
149 Twenty-six UC Irvine students and instructors volunteered for a three-week high fiber diet
150 intervention study (**Figure 1A**); only 22 individuals elected to provide stool samples for
151 microbiome analyses (20 of whom we recovered enough sequence data for analysis, see
152 **Supplemental Table 1**). The dietary intervention was approved by UC Irvine IRB # 2018-4297.
153 For the first week of the study, all participants consumed their normal diets, tracking all
154 nutritional information using the smartphone application MyFitnessPal (MyFitnessPal, Inc.).
155 Prior to the end of week one, each subject provided three fecal samples from three days within
156 the first week. The intervention commenced in week two, when participants were instructed to
157 raise their dietary fiber intake to approximately 40 grams per day. To assist with the dietary
158 shifts, we provided 10 meals per week with ~15 grams of fiber ~5.8 unique fruits or vegetables
159 per meal from the food delivery service Thistle (https://www.thistle.co/ San Francisco,
160 California, USA). During week three, subjects were encouraged to further increase fiber intake to
161 ~50 grams of fiber per day. Subjects provided three fecal samples from three days during week
162 three, concluding the intervention period. As part of the CURE course, students were educated
163 on human health, dietary information on high-fiber meals, the human gut microbiome, and the
164 quantitative methods for microbiome analyses (from DNA extraction and library preparation to
165 metagenome and statistical analyses), as previously described (25).
166
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168 *Sample collection*
169 Subjects were given materials to collect fecal samples at home. Each stool sample was split into
170 three 2ml tubes by the individual and immediately stored in the freezer. When convenient,
171 students transported their anonymized and coded samples using cold packs and insulated boxes
172 to a common lab freezer. Upon the conclusion of the intervention period (week 1 or 3), all
173 samples were transported to a -20 °C freezer.
174
175 *DNA extraction and metagenomic library preparation*
176 To characterize the bacterial community composition of the samples, DNA was extracted with
177 the ZymoBIOMICS 96 DNA Kit (Product D4309) from Zymo Research using the
178 manufacturer's suggested protocol. Sequencing libraries were prepared using the Illumina
179 Nextera kit and methods described in Baym et al. (29). Briefly, DNA was diluted to 0.5ng/µl and
180 added to 0.25µl of Nextera enzyme and 1.25 µl of Tagmentation Buffer. This mixture was
181 incubated at 55 °C for 10 minutes and then placed on ice for the remainder of the protocol.
182 Barcodes were added using the Phusion polymerase (New England Biolabs) and excess adaptors
183 were cleaned using AMPure XP (Beckman Coulter Life Sciences) magnetic beads. Quality and
184 concentration were assessed using a Picogreen assay (ThermoFisher) and the distribution of
185 fragment sizes was determined using a Bioanalyzer. These libraries were loaded onto the
186 Illumina Next-Seq 500 at 1.8 picomolar concentrations and sequenced using Illumina's mid-
187 output kit for 75 bp paired-end sequencing, resulting in a total of 144,023,583 reads and an
188 average of 1,425,976 reads / sample (max: 5,902,966; min: 7) (**Supplemental Table 1**).
189
190 *Amplicon library preparation*
191 To characterize the genetic diversity of *Bifidobacterium* at a finer-genetic scale than could be
192 assayed by metagenomics, we used genus-specific primers to target this group for sequencing
193 (30). Sequencing libraries were prepared by setting up an initial 25 µl PCR reactions with
194 AccuStart II PCR ToughMix (2x), the *groEL* forward primer (5'-
195 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCGATTACGAYCGYGAGAAGCT-
196 3', 20 µM), and the *groEL* reverse primer (5'-
197 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCSGCYTCGGTSGTCAGGAACAG-
198 3', 20 µM). The initial PCR ran for 28 cycles 95°C for 30 sec, 60 °C 30 sec, 72 °C 50 sec
199 followed by the addition of 0.5 µl of dual Nextera XT index (Illumina) to each sample
200 proceeding with an additional 8 cycles 95 °C 30 sec, 60 °C 30 sec and 72 °C 50 sec. Amplicons
201 were pooled based on visual quantification of the bands on an agarose gel and purified using
202 magnetic Speed Beads The pool was run on a MiSeq PE 300 at University of California Irvine's
203 Genetic High Throughput Facility resulting in a total of 20,052,935 reads and an average of
204 185,675 reads/sample (max: 6,815,601; min: 155).
205
206 *SCFA extraction and measurements*
207 SCFA extractions were done following the methods by Zhao et al. (2005) (31). One-hundred mg
208 of fecal material was added to 1ml of HPLC grade water and vortexed for two minutes. Ten
209 microliters of 6N HCl was added to the fecal slurry and vortexed briefly. This mixture was
210 incubated at room temperature for 10 minutes with occasional shaking. Afterwards the mixture
211 was centrifuged at 14,000g for 1 minute, and 400 µl of the supernatant was transferred to a new
212 tube, which was then filtered through a 0.22 µm filter. An aliquot (200 µl) of this suspension was
213 then transferred to a glass vial with a 0.2 ml vial insert and stored at -20 °C. When running the

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214 sample, 10 μl of an internal standard of 10mM ethyl butyrate was added to the extraction prior to
215 the run. Before running each sample, the instrument was calibrated using a standard comprising
216 100 mg/l of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and ethyl butyrate.
217 Six samples were run on an Agilent 7890A gas chromatograph with dual column FID detectors.
218 Two microliters per extracted sample were hand-injected on a stainless-steel column (2 meters x
219 3.2 mm) containing 10% SP-1000 and 1% H3PO4 on 100/120 Chromosorb W AW (Supelco,
220 Inc., Bellefonte, PA, USA). The flow rate of the N2 carrier gas was 26.14 ml/min. Between sets
221 of six samples the instrument was washed using water and phosphoric acid. Peaks were auto
222 integrated using ChemStation v1.0 on a PC running Windows 2000 (Microsoft). A subset of
223 samples (n = 44 from 8 individuals) were run in duplicate to examine technical variation (see
224 coefficient of variation (CV) in **Table 1**), and the average CV was 55%.
225
226 *Metagenomic sequence analysis*
227 Raw shotgun metagenome sequences were filtered using Prinseq v0.20.4 (32) to remove
228 sequences that had a mean quality score of 30 or less. Reads from human DNA were also
229 removed by aligning the filtered reads to the human genome (hg38), using Bowtie2 v2.2.7 (33),
230 and keeping the reads that failed to align. A total of 130,755,383 paired-end reads (average
231 1,294,607 non-human reads/sample) were retained and passed through MIDAS, which assigns
232 taxonomy to short read data using a marker gene approach (34). Species counts per sample
233 represent the average of 100 subsamples, rarefied to 900 sequences per sample using the
234 EcolUtils (v0.1) package in R. Taxonomy was also assessed using IGGsearch (35). To analyze
235 functional differences related to SCFA metabolism between high and low fiber treatment groups,
236 HUMAnN3 (36) was used with default parameters. All pathways within the MetaCyc pathway
237 class "Fermentation to Short-Chain Fatty Acids" were searched for within the HUMANnN
238 pathway output, which resulted in nine pathways used for analysis (37). For genes related to
239 carbohydrate breakdown, we translated reads using Prodigal (38) to predict open reading frames
240 (ORFs) and searched all ORFs against the Pfam database (39) with hmmer/3.1b2 (40). Resulting
241 PFAM annotations were then screened against the CAZyDB.07202017 (41) with Blast/2.8.1 (42)
242 using alignments >70% amino acid identity and 30% coverage. Alpha diversity and
243 PERMANOVA analyses were performed using the Vegan v2.5-6 (43) package in R (44). Non-
244 metric multidimensional analysis was done using the metaMDS function in Vegan on Bray-
245 Curtis distances. StrainPhlAn (45), under default parameters, was used to analyze strain-level
246 variation within the metagenomes. To root the phylogenetic tree, *Prosthecochloris aestuarii*
247 (accession: GCA_000020625) was used, and two reference genomes of *Eubacterium rectale*
248 (accession: GCA_000209935 and GCA_001404855).
249
250
251 *GroEL amplicon analysis*
252 We downloaded 780 genomes from the genus *Bifidobacterium* on the PATRIC database (46). All
253 genomes were screened for completeness by searching for 21 single-copy ribosomal marker
254 genes using Prodigal (38) and HMMer v3.1b2 (40) with an E value of 1×10^{-10}. The remaining
255 578 genomes were used to create a multi-locus, concatenated phylogeny of the ribosomal marker
256 genes with ClustalO v1.2.0 (47) to produce a 4272 amino acid alignment for phylogenetic
257 analysis using RAxML v8.0.0 (48) under the PROTGAMMABLOSUM62 model for 100
258 replicates. Next, we parsed the filtered genomes for the *groEL* gene sequences by using 260 non-
259 redundant gene sequences to build a *groEL* phylogeny under identical parameters to the whole-

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260 genome analysis. The *groEL* amino acid sequences, alignment, and phylogeny were used to
261 construct BLASTp, HMMer, and pplacer reference databases for metagenomic analyses.
262 For each *groEL* amplicon library, sequences were quality trimmed and adapters were removed
263 with BBDuk (49) (qtrim=rl trimq=10 ktrim=r k=25). Paired end sequences were merged together
264 with BBMerge (49) and, if paired reads did not overlap, only the forward read was retained. The
265 reads were then searched against the *groEL* reference databases using BLAT (50) and
266 hmmsearch, respectively. Passed reads were aligned with ClustalO to the pplacer reference
267 package and placed onto the *groEL* reference phylogeny using pplacer v1.1.alpha17 (51).
268 Relative abundance was calculated from the single branch assignments and aggregated at the
269 species level to be normalized by the total number of extracted *groEL* gene sequences. We show
270 that the phylogenetic relationship between species of *Bifidobacterium* based on the *groEL* gene
271 closely reflects a phylogeny based on 21 single copy marker genes from 578 *Bifidobacterium*
272 genomes (**Supplemental Figure 4**).
273
274 *Statistical analysis*
275 Permutational analysis of variance (PERMANOVA) was conducted on Bray-Curtis
276 dissimilarities at the genus level with 999 permutations using the Adonis test in the Vegan
277 package in R (see Data availability and GitHub). We tested the effect of the intervention (pre-
278 versus post-fiber increase), the effect of the individual, and the interaction between these two
279 factors. Genus contributions to significant results from the PERMANOVA model were
280 determined by passing the resulting PERMANOVA object through the coefficients function
281 found in the base Stats package R. A similar procedure was used to analyze compositional
282 differences between CAZy enzymes and HUMAnN gene predictions in the metagenomes, with
283 permutations on Euclidean distances. Linear mixed effects models, using the nLME package (44)
284 in R, were also conducted for comparison because they take repeated measures into account.
285 Specifically, to support the PERMANOVA analysis of beta diversity, an LME was performed on
286 the rank-transformed first principal coordinate of a principal coordinates analysis on the Bray
287 Curtis community dissimilarity matrix. Individual was used as the random effect and the model
288 used the default autoregressive (Lag 1) structure (AR1) for regression across a time-series. For
289 the functional analyses, reads analyzed using HUMAnN3 were normalized by copies per million;
290 CAZy were normalized to the total number of reads per metagenome and compared using
291 Wilcoxon rank sum test. Gene features for HUMAnN were reduced by analyzing only
292 unstratified data, for which 70% of samples had non-zero reads mapping to each feature.
293 HUMAnN pathway abundances were analyzed in their entirety with stratification and without
294 feature reduction. Lefse (52) was used to determine pathways which may differentiate pre- vs
295 post-intervention samples. Wilcoxon rank sum tests were also used to compare nutritional and
296 gene differences between intervention periods when residuals were not normally distributed and
297 reads or macronutrients were averaged out within individuals (by treatment) to account for
298 repeated measures. When normality assumptions of residuals were met (tested using the Shapiro-
299 Wilk test) ANOVAs were used. To assess which taxa were correlated with changing amounts of
300 fiber, all species within each sample (the rarefied species abundance matrix) and fiber were
301 correlated using the Corrr package v0.4.2 (53) in R. To analyze which genera co-correlate with
302 the genus *Bifidobacterium*, Spearman correlations were used and, where appropriate, p-values
303 were corrected (q-value) for multiple comparisons using a false discovery rate cutoff of 0.05. To
304 assess significance of strains between individuals, cophenetic distances were calculated on the
305 RAxML tree output from StrainPhlAn and passed into the above PERMANOVA model.

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306
307 *Data availability*
308 All scripts are stored on GitHub (https://github.com/aoliver44/Fiber-Analysis). All metagenomic
309 and amplicon sequences are available on NCBI under the Bioproject PRJNA647720. Metadata
310 linking the shotgun metagenomes and *groEL* sequences with the appropriate sample ID and
311 intervention can be found in Supplementary Table 1.
312
313
314 **RESULTS**
315
316 *Dietary intervention within the CURE course*
317 Twenty-six individuals participated in a CURE course at UC Irvine, designed to tandemly
318 investigate pedagogical methods (25) and the role of fiber on the microbiome. We collected
319 nutritional data from all 26 individuals who initially began the intervention, over three weeks
320 (one week prior to, and two during, the dietary intervention) (**Figure 1A**). We collated the total
321 amount of macronutrients consumed per day, including fiber, protein, carbohydrates, fats, as well
322 as overall calories (**Figure 1B-F**). Additionally, we informally surveyed food items the study
323 participants frequently used to supplement their meal plans, beyond the meals supplied from
324 Thistle, and found that items such as fiber fortified cereals, lentils or beans, and berries were
325 common (25). For the intervention, subjects increased their average fiber consumption from 21.0
326 g/day (± 14.2 g/day) before the intervention to 46.4 g/day (± 12.5 g/day) during the intervention
327 (**Figure 1B**; Wilcoxon rank sum test, $p < 0.0001$). While these dietary shifts increased
328 carbohydrate intake by an average of 84% (36 g) during the intervention ($p = 0.013$), other
329 macronutrients measured, such as calories, fat, and proteins, did not significantly change ($p >$
330 0.05) (**Figure 1C-F**).
331
332 *Diet intervention altered gut microbial community composition within individuals*
333 To evaluate whether increased fiber consumption contributed to shifts in the gut
334 microbiome, we characterized the microbial communities from 20 individuals using 86 shotgun
335 metagenomic libraries collected before and after the fiber intervention (**Figure 2A**). Alpha-
336 diversity of microbial taxa decreased during the high fiber diet intervention as measured by the
337 Shannon diversity index (**Figure 2B**)(Wilcoxon rank sum test, $p < 0.05$). Using alternative
338 approaches to assess taxonomy and diversity (see methods) showed either no change or
339 supported the decreasing trend of diversity during the intervention period (**Supplemental Figure**
340 **1).**
341 Despite little difference in alpha-diversity, beta-diversity changed significantly in
342 response to a high fiber diet. Multivariate analysis of marker gene abundances showed that most
343 of the variation in microbiome composition could be explained by the individual
344 (PERMANOVA: main individual effect: $R^2 = 0.78$, $p < 0.001$, **Supplemental Table 2**). The diet
345 intervention shifted the microbial composition of the entire study cohort significantly (main
346 intervention effect: $R^2 = 0.014$, $p < 0.001$). Within samples from each individual, the pre- and
347 post- diet intervention samples explain significant variation in the community composition
348 (intervention-by-individual effect: $R^2 = 0.083$, $p < 0.001$). A linear mixed-effects (LME) model
349 confirmed these results, which identified diet as a significant determinant of an individual's
350 microbiome composition (LME, $p < 0.01$). Individual gut microbiome samples grouped together
351 in nonmetric multidimensional space (nMDS; **Figure 2C),** further providing support that each

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352 individual is associated with a unique microbiome. Some individuals (i.e., Individual 13) gut
353 microbiomes were more distinct from others (**Figure 2C inset)**. Additionally, we used
354 *Eubacterium rectale* (due to its high coverage in our data) to ask whether the diet intervention
355 had an impact at the strain level. Strains were highly individual specific (PERMANOVA: main
356 individual effect: $R^2 = 0.99$, $p < 0.001$) and did not change in response to increased fiber intake
357 ($p > 0.05$; Figure 2D).
358 We next parsed the taxonomic data to assess which microbial taxa increased or decreased
359 in response to the diet intervention. One species in the family *Lachnospiraceae* was significantly
360 negatively associated with increasing fiber intake (Spearman, r = -0.43, q = 0.01) (**Supplemental**
361 **Figure 2A, B**). *Coprococcus sp.* and *Anaerostipes hadrus* were both positively associated with
362 increasing fiber intake, but this association was not significant when p-values were FDR-
363 corrected for multiple comparisons (r = 0.32, q = 0.33 both species) (**Supplemental Figure 2A**).
364 Furthermore, positive linear coefficients of a PERMANOVA model, which detect differences
365 between community composition due to the diet intervention, included genera such as
366 *Bifidobacterium*, *Bacteroides*, and *Prevotella* (**Figure 3A**). Conversely, *Blautia* and
367 *Ruminococcus* contributed negative linear coefficients to the PERMANOVA model (**Figure**
368 **3A**).
369
370 Bifidobacterium *species were enriched by the diet intervention*
371 Of the 105 microbial genera detected in this study, *Bifidobacterium* was the strongest
372 predictor genus for the post-intervention microbiomes (**Figure 3A).** Indeed, taxonomic analysis
373 of the metagenomic samples identified *Bifidobacterium* abundances increasing, on average, 1.4-
374 fold between the pre- and post-intervention periods (**Supplemental Figure 3A)**. Further, we
375 identified several species of *Bifidobacterium* present within and across individuals, with *B.*
376 *adolescentis* being the most abundant species on average (**Figure 3B).** When we investigated the
377 taxonomic profiles at the species level, we found that *B. adolescentis*, *B. biavatii*, *B. breve*, *B.*
378 *longum*, and *B. ruminantium* all increased in mean abundance on a high fiber diet whereas the
379 other, lesser abundant species exhibited no change or decreased in abundance (**Supplemental**
380 **Figure 3B**).
381 Given that *Bifidobacterium* was the strongest predictor genus in the post-fiber gut
382 microbiomes, we employed a targeted analysis into the diversity within *Bifidobacterium* to
383 examine species-level patterns. Specifically, we applied targeted amplicon approaches to amplify
384 the *groEL* gene, a conserved phylogenetic marker gene to track *Bifidobacterium* diversity
385 (**Supplemental Figure 4)**. Using phylogenetic inference of the *groEL* gene, we compared the
386 observed *Bifidobacterium* diversity observed at the community level to our targeted analysis of
387 the *groEL* gene. Similar to the metagenomic analysis, we found that individuals were largely
388 comprised of *B. adolescentis* and *B. longum*, with six other abundant species of *Bifidobacterium*
389 (**Figure 3C).** This analysis also revealed extensive *Bifidobacterium* diversity within the human
390 gut, detecting 22 species across all individuals.
391 Since *Bifidobacterium* species are known to participate in cross-feeding with other gut
392 microbes (reviewed in (54)), we next assessed the co-occurrence of *Bifidobacterium* with other
393 genera. *Bifidobacterium* was positively correlated (r = 0.43, q = 0.001) with an increasing
394 abundance of *Lactobacillus* and negatively correlated with *Roseburia* (r = -0.49, q = 0.0002) and
395 *Ruminococcus* (r = -0.38, q = 0.007) (**Supplemental Figure 5)** suggesting possible species
396 interactions between these taxa.
397

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398 *Genes involved in inositol degradation increase on high fiber diet*
399 Our results demonstrate that a shift in dietary fiber consumption influenced
400 compositional changes in the gut microbial community. As such, we sought to correlate the
401 observed taxonomic shifts to functional shifts, particularly the enrichment of genes related to
402 carbohydrate degradation. Despite taxonomic shifts at the individual level, we observed no
403 changes in in the overall abundance (average number of normalized reads) mapping to gene
404 families for glycoside hydrolases (GH) (Wilcoxon, $p = 0.42$), carbohydrate esterases ($p = 0.58$),
405 glycoside transferases ($p = 0.73$), and polysaccharide lyases ($p = 0.77$) as a result of the
406 intervention (**Supplemental Figure 6A**). No individual families GH and polysaccharide lyase
407 CAZy classes changed in abundance during the intervention when corrected for multiple
408 comparisons (Wilcoxon, $p > 0.05$, **Supplemental Figure 6B**). Further, the diversity (**Figure 4A,**
409 n = 106 families, ANOVA, $p > 0.05$) and composition (PERMANOVA, $p > 0.05$) of GHs
410 detected were indistinguishable between the pre- and post-intervention samples. Compositional
411 analysis all genes identified by HUMAnN revealed no individual signature (PERMANOVA:
412 main individual effect: $R^2 = 0.017$, $p > 0.05$, **Figure 4B**), and no shifts in response to a high fiber
413 diet (intervention-by-individual effect: $R^2 = 0.015$, $p > 0.05$). We performed a linear discriminant
414 analysis to determine if there were pathways that were differentially abundant due to the diet
415 intervention, and found inositol degradation (in addition to several unintegrated pathways) to be
416 increased in abundance on a high fiber diet (**Figure 4C, Supplemental Figure 7**). For the
417 pathways involved in SCFA metabolism, we found no significant (Wilcoxon, $p > 0.05$) changes
418 as a result of the high fiber diet (**Figure 4D**).
419
420 *Fecal short-chain fatty acid concentrations were unaltered by the diet intervention*
421 While the presence of genes related to SCFA production provide insights into the functional
422 changes of the microbiome, these genes only reflect the genomic potential to process these
423 pathways. Therefore, we applied a targeted GC/MS analysis on 149 samples from 18 individuals
424 for the presence of SCFA molecules. Across the intervention period, the average abundance of
425 acetate, propionate, butyrate, and valerate increased (Table 1); however, these increases were not
426 statistically significant (LME, $p > 0.05$) (**Figure 5**). For the eight individuals with samples run in
427 duplicate, three had biological differences that were greater than the technical variation seen in
428 the duplicates (**Supplemental Figure 8**). Acetate had the least technical variation (mean CV =
429 45%), followed by propionate (mean CV = 51%) (**Table 1, Supplemental Figure 8**).
430
431

432 DISCUSSION

433 We examined the impact of dietary foods, rich in their diversity of fiber, on the human
434 gut microbiome. We expected that an increase in fiber consumption through whole foods
435 consumed would lead to a more generalizable shift of the microbiome in contrast to previous
436 studies that utilize a single fiber supplement. For instance, a recent meta-analysis (55) found
437 mixed results in how fiber may impact the gut microbiome richness and composition. Among
438 papers published prior to October 2017, only 18% (12 out of 64) studies (56, 57, 66, 67, 58–65)
439 contained food-based fiber interventions, and most of these studies only modified one aspect of
440 diet (e.g., addition of whole grain breakfast cereal). One study in particular increased dietary
441 fiber by 40g from a diverse set of foods during a five day period (57). The authors similarly
442 found microbiome composition changes within individuals when they accounted for differences
443 in the subjects' starting microbiomes. Despite the variation in implementing a fiber intervention,

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444 it is becoming increasingly clear that fiber alters the composition of the gut microbiome (17) and
445 the associated microbial changes affect human health (i.e., type 2 diabetes mellitus (3)). A
446 common observation in fiber intervention studies (55) is the specific involvement of the genus
447 *Bifidobacterium* in response to fiber interventions. However, to our knowledge, no study has
448 documented how fiber impacts the genus at the strain-level in the human gut.
449
450 *Does a diet intervention rich in fiber alter the microbiome?*
451 Past studies have shown that an increase in the diversity of dietary foods could lead to an
452 increase in microbial diversity (24). Moreover, individuals living in rural societies often harbor
453 far greater gut microbial diversity than individuals from western societies (68–70), which may in
454 part be linked to a greater proportion of plant-based polysaccharide intake. However, we did not
455 measure an increase in species diversity (alpha diversity) after subjects consumed >40g of fiber
456 from a diverse set of foods (**Figure 2B**). These results could be attributed to the brevity of the
457 intervention as the rapid change in dietary composition may result in the loss of microbes poorly
458 adapted to recalcitrant carbohydrates. Similarly, other studies have reported finding no increases
459 in alpha diversity as a result of a fiber intake (57, 71–74), which may indicate a trade-off where
460 fiber-degraders increased while other taxa decreased. Although alpha diversity was unaffected,
461 we did observe a significant impact of the high-fiber diet on microbial community composition
462 (beta diversity) (**Figure 2**). The composition of microbial communities within individuals shifted
463 ~8% during the intervention period. We found changes in communities to be at broader
464 taxonomic levels than strain-level. We were able to examine strains of *E. rectale* due to its high
465 coverage in our data, and showed these strains stayed constant and individual specific during the
466 intervention (**Figure 2D**). Future work should determine if this pattern holds up for other species.
467 While we suspect the high fiber diet treatment played an instrumental role in shifting the
468 microbial composition, we cannot rule out other factors such as host genetics or non-dietary
469 behaviors. As discussed, many food-based fiber interventions have shown mixed results on
470 changing the microbial communities (6, 75). The drastic increase in fiber from a variety of foods
471 may lead to rapid shifts in community composition over the two-week period. Changes in
472 community composition pre- and post- intervention were largely driven by shifts in known-fiber
473 degraders, such as *Bifidobacterium*, *Bacteroides*, and *Prevotella* (**Figure 3A**).
474 We expected the taxonomic shifts in the microbiota would be associated with changes in
475 the functional potential of the microbial communities (**Figure 4**). While we initially
476 hypothesized that a high fiber diet would increase the abundance or diversity of carbohydrate
477 active enzymes, we did not detect changes associated with the intervention (**Supplemental**
478 **figure 6**). Our findings support a similar result showing no difference in CAZy abundance due to
479 increased fiber intake (74). We acknowledge that sequencing depth is an important consideration
480 in the detection of genes; increasing reads beyond our ~1.3 million paired-end reads (avg per
481 sample, **Supplemental table 1**) may allow for greater detection. However, we did find a notable
482 increase in the abundance of genes mapping to the inositol pathway (**Figure 4C**). We suspect
483 that the increased consumption of fiber-fortified cereals and legumes, which contain higher
484 levels of inositol, during the diet intervention allowed for an expansion in organisms capable of
485 breaking down this sugar. There is substantial interest in the role of inositol (specifically phytic
486 acid) in its protective role against colon cancer and other metabolic disorders (76, 77). Next, we
487 assessed whether genes involved in SCFA metabolism changed in abundance during the
488 intervention. Although appreciable cross-feeding between lactate-producing *Bifidobacterium*
489 spp. and butyrogenic bacteria has been shown (78) we did not find significant increases in genes

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490 involved in various SCFA metabolic pathways (**Figure 4D**). This further supports our results
491 showing no clear correlations between *Bifidobacterium* spp. and butyrate-producers within our
492 diet intervention (**Supplemental figure 5**). Indeed, we would not be the first to suggest that
493 perhaps these complex trophic interactions require more time to establish (17). Rather, our
494 results suggest that while broad taxonomic shifts occur, these do not correspond to changes in
495 functional potential and fine-scale (intraspecies) shifts are less susceptible to dietary shifts on
496 short-term timescales.
497
498 *Does the intervention alter the abundance and diversity of* Bifidobacterium*, a known fiber-*
499 *degrader?*
500 Many studies have indicated that bifidobacteria (often identified as the genus
501 *Bifidobacterium* by FISH probes, PCR, or DNA-sequencing) are highly abundant in the gut
502 following increased fiber intake (meta-analysis of 51 studies (55)). Increased abundance of
503 *Bifidobacterium* is somewhat unsurprising, as they harbor numerous genetic components, such as
504 carbohydrate active enzymes, that make them especially adapted to a fiber-rich diet (79). In one
505 study, both resistant potato starch and inulin increased the relative abundance of *Bifidobacterium*
506 spp.; however, the 16S amplicon sequencing in this study did not have the resolving power to
507 identify which species of *Bifidobacterium* were increasing (17). Using a targeted amplicon
508 approach, the *groEL* gene, has been shown to delineate species of *Bifidobacterium* that otherwise
509 share >99% sequence identity in the 16S rRNA gene, making it a robust marker gene for
510 analyzing within-genus species diversity (80). In our study, the most abundant species of
511 *Bifidobacterium* were *B. adolescentis* and *B. longum*, both of which are efficient degraders of
512 plant-based fructo-oligosaccharides (FOS) and produce acetate and lactate in the process (81).
513 Mirroring our results, other studies have found selective increases in certain species of
514 *Bifidobacterium* as a result of carbohydrate intake; for example, in one study, intake of inulin
515 resulted in a greater increase of *B. adolescentis* (82). We speculate that on a high fiber diet,
516 bifidobacteria are the initial members of the community accessing fiber substrates, easily adapted
517 to utilize various FOS, and pivotal to the creation of the initial metabolic cross-feeding networks.
518 Future studies should extend the intervention period to examine the dynamics of longer-term
519 trophic interactions in response to increased dietary fiber intake.
520
521 *Can we detect diet-induced changes in the abundance of fecal short-chain fatty acids?*
522 While SCFAs did generally increase during the diet intervention, trending toward their
523 naturally occurring gut ratio of 3:1:1 (acetate:propionate:butyrate) (12, 83, 84), we did not
524 observe a statistically significant increase in SCFAs post-intervention. Static fecal concentrations
525 of SCFAs may not reflect the total pool of molecules fluxing through a given individual, as the
526 molecules are preferred substrates of the cells lining the gut epithelia (15). It is also possible that
527 the intervention period was too short to observe increases in SCFA abundances.
528 It should be noted that accurate SCFA measurements are notoriously difficult. Our
529 examination of technical variability within 44 samples from eight individuals showed that
530 technical variation between pre-intervention replicates or post-intervention replicates was greater
531 than the average difference between pre- and post-intervention for any given SCFA. One study
532 reported high intra-fecal variability of butyrate quantification (coefficient of variation = 38%),
533 prior to optimizing a freeze-drying method (85). Numerous studies have indicated the benefit of
534 SCFAs to human health (5, 86); yet the heterogeneity in reported acetate, propionate, and
535 butyrate abundances remains high. In one meta-analysis of fiber studies, only butyrate was

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536 generally found to increase with fiber intake, yet the heterogeneity of reported results was 70%
537 (I^2), similar to other SCFAs analyzed (55). Outside of technical limitations, shifts in microbial
538 community structure are not predictive of changes in static measurements of fecal SCFA
539 abundances (87). The difficulty of finding meaningful correlations between microbiome
540 composition and SCFA abundances likely reflects a failure to measure both circulating and fecal
541 SCFAs across time in conjunction with microbial abundances. Indeed, it has been observed that
542 fecal levels of acetate are inversely related to the rate of its absorption (88). Future studies are
543 needed to confirm whether correlation analysis between fecal SCFAs and microbiome
544 composition is a useful tool to understand the interplay between microbiome, SCFAs, and health.
545 In sum, our results indicate that gut microbial communities are malleable to an influx in
546 recalcitrant carbohydrates, contributing to significant community and functional shifts in certain
547 metabolic pathways. However, these compositional changes did not correspond to broad
548 functional changes, at least over the short-term timescales for this intervention. Further studies
549 exploring the impact of timing and composition of dietary fiber interventions, particularly while
550 taking into account the starting composition of the gut microbiomes of study participants, are
551 critical for understanding the generalizability of fiber interventions for engineering microbiomes.
552 Increasing fiber intake could have the most impact in contexts where low gut microbial diversity
553 increases risk of *C. difficile* infection, such as for nursing home residents, cancer patients or after
554 antibiotic treatment.
555
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557
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562 thoughtful edits to the manuscript. We would like to give an enormous thank you to the students
563 from the course M130L at UC Irvine Spring in 2018.
564
565
566 **Table 1: Abundances of four SCFAs in samples pre- and post- intervention**

	PRE (MEAN)	PRE (SD)	PRE CV	POST (MEAN)	POST (SD)	POST CV
ACETATE	480 mg/L	208 mg/L	43 %	525 mg/L	244 mg/L	47 %
PROPIONATE	277 mg/L	112 mg/L	41 %	288 mg/L	175 mg/L	61 %
BUTYRATE	244 mg/L	107 mg/L	44 %	257 mg/L	176 mg/L	68 %
VALERATE	49 mg/L	31 mg/L	65 %	43 mg/L	31 mg/L	72 %

567
568
569 **TABLE LEGENDS**
570
571 **Table 1:** Abundances of four SCFAs in samples pre- and post- intervention
572
573 **Supplemental Table 1:** Sequence statistics, accession IDs and experimental metadata.
574
575 **Supplemental Table 2:** PERMANOVA model and term results.

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576
577 ## FIGURE LEGENDS
578
579 **Figure 1: Intervention timeline and sample collection.** The study subjects began eating their
580 normal diet for one week, tracking all their food intake using the MyFitnessPal app. At the end
581 of the week, each individual provided a daily fecal sample on three different days. At the start of
582 week two, subjects started a wholesome high fiber diet, getting at least 40 grams of fiber per day.
583 During week three, subjects were encouraged to get 50 or more grams of fiber per day. At the
584 end of week three, each subject provided a fecal sample on three different days. B-F) Self-
585 reported macronutrients from individuals using the MyFitnessPal app. Change in macronutrients
586 across the 3-week diet intervention for B) fiber, C) carbohydrates, D) protein, E) fat, and F)
587 calories. Fiber changed the most in magnitude between pre-intervention intake and during the
588 diet intervention (linear mixed-effects model, $p < 0.001$). There were modest, but significant,
589 changes in carbohydrate, protein, and caloric intake, but not fat intake, across the same time
590 interval.
591
592 **Figure 2: Microbiome community composition through a dietary fiber intervention.** A)
593 Relative abundances of genera detected in microbiomes from individuals throughout the diet
594 intervention study. B) Alpha diversity, measured using the Shannon index, changed significantly
595 during the intervention period (Wilcoxon, $p < 0.05$). C) NMDS ordination showed that samples
596 from individuals mostly group together. Dotted lines connect the same individual and point
597 towards the final post-fiber intervention sample. Samples in this study were highly personalized:
598 the individual explained 78% of the variation in the data. The inset shows an extended version of
599 the NMDS plot that includes Individual 13. D) A phylogeny of *Eubacterium rectale* strains
600 found in individuals (denoted by color) during the intervention.
601
602 **Figure 3: GroEL amplicon analysis of *Bifidobacterium* during the fiber intervention.** A)
603 Model coefficients of the PERMANOVA analysis (model: species ~ Individual*Intervention).
604 Species with high coefficients (positive or negative) were best able to distinguish the pre vs post
605 diet intervention groups. Only the top 20 genera are shown. The genus Bifidobacterium had the
606 largest positive coefficient, indicating that it was important to the model for disguising
607 microbiomes before and after the diet intervention. Relative abundances of 12 detected species of
608 Bifidobacterium from B) shotgun metagenomics and C) groEL amplicon sequencing.
609
610 **Figure 4: Genes involved in carbohydrate degradation and SCFA metabolism within**
611 **metagenomes.** A) Number of distinct glycoside hydrolase families within individual
612 metagenomes (different colored circles), separated by pre-intervention (mean = 83) (grey) and
613 post intervention (mean = 84) (red). B) NMDS ordination of Euclidean distance matrix based on
614 19680 gene features, shape denotes intervention (triangle = pre-, circles = post-) and individuals
615 are separated based on color. C) Lefse analysis of pathways that differentiate samples by
616 intervention. D) Log abundance (copies per million) of pathways involved in SCFA production.
617
618 **Figure 5: GC-FID measurements of fecal volatile SCFAs during intervention.** Fecal SCFA
619 abundances, averaged across replicates where applicable, before and after the intervention.
620

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621 **Supplemental Figure 1:** Comparisons of diversity measures obtained using different databases
622 for taxonomic assignments. IGG_rich and MIDAS were run using default parameters. IGG-
623 Lenient was run at 25% species quality and 15% marker genes.
624
625 **Supplemental Fig 2:** A) Correlations above a r > 0.2 cutoff of microbial abundance and fiber
626 intake. Only *Lachnospiraceae* bacterium 51870 was significantly negatively correlated at an
627 FDR cutoff of 0.05. B) Raw spearman correlation of a species of *Lachnospiraceae* with fiber
628 intake.
629
630 **Supplemental Figure 3:** A) Mean abundance (MIDAS read counts) of the genus
631 Bifidobacterium during the diet intervention. Points are colored by individual. B) Changes in
632 mean abundance of each species of Bifidobacterium, detected by MIDAS, during the diet
633 intervention period.
634
635 **Supplemental Figure 4:** *Bifidobacterium* phylogenetic analyses. A) Multi-locus phylogenetic
636 analysis of conserved ribosomal marker genes. B) Phylogenetic analysis of the *groEL* gene
637 sequences used for amplicon analyses. The top 8 species observed are shown.
638
639 **Supplemental Figure 5:** Significant (FDR = 0.05) correlations from comparing abundances of
640 99 different genera with *Bifidobacterium.*
641
642 **Supplementary Figure 6**: A) Average abundances (normalized reads) of carbohydrate active
643 enzymes within individuals during the diet intervention period. Each different color point
644 represents an individual, and lines connect the same individual. B) Log2 transformed GH and
645 polysaccharide lyase gene abundances during the intervention.
646
647
648 **Supplemental Figure 7:** Inositol degradation abundance (normalized copies per million), for
649 metagenomes before and after the intervention.
650
651 **Supplemental Figure 8:** Technical variation of SCFAs seen in a subset of samples run in
652 duplicate. A) Amount of SCFAs by individual, with color denoting if it was measured during
653 replicate 1 or 2. B) Normalized (by mean) difference between absolute difference between
654 treatment, subtracted by absolute difference between technical replicates. Larger negative values
655 suggest differences between technical replicates were larger than the differences detected
656 between pre- and post- intervention arms.
657
658
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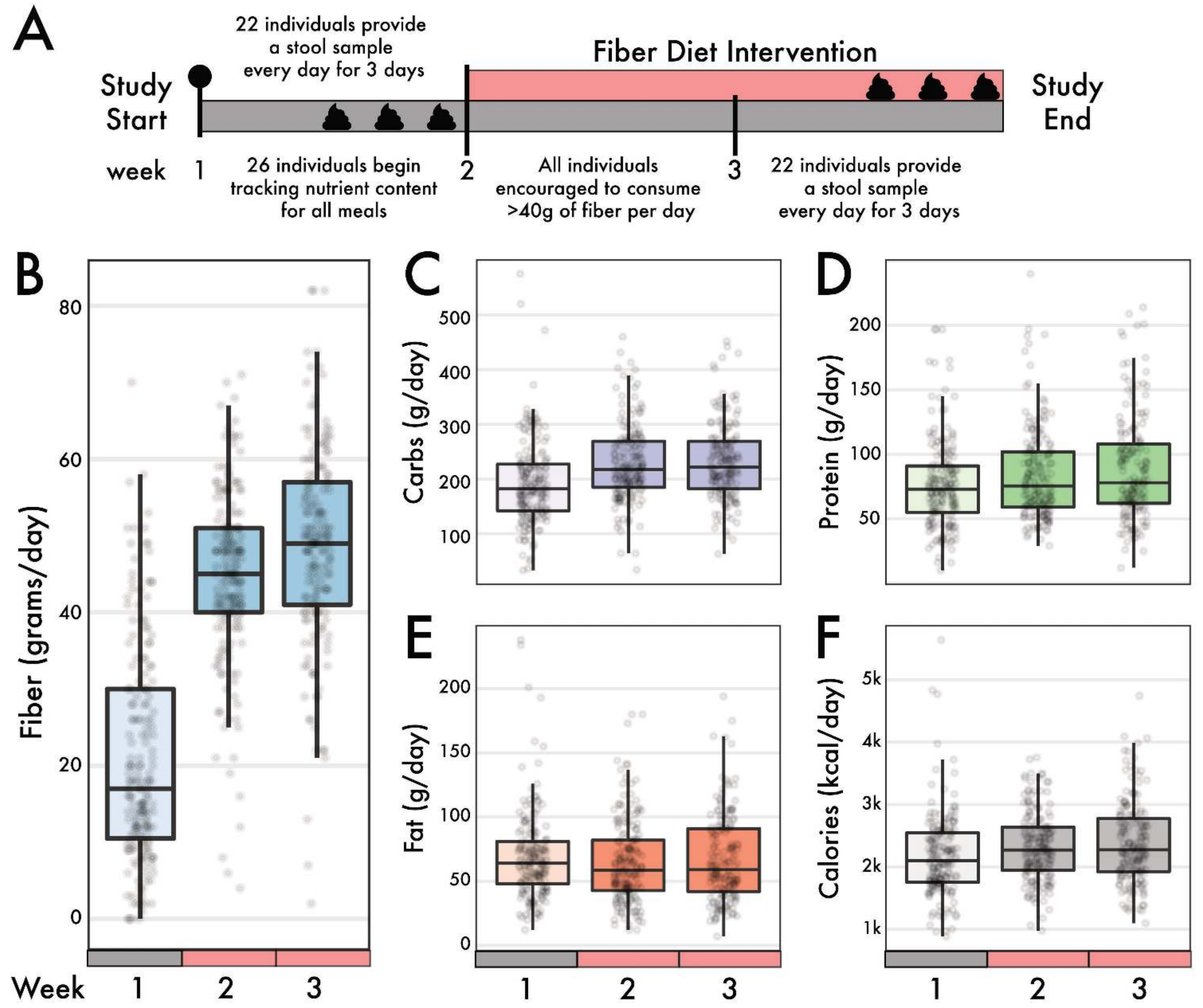
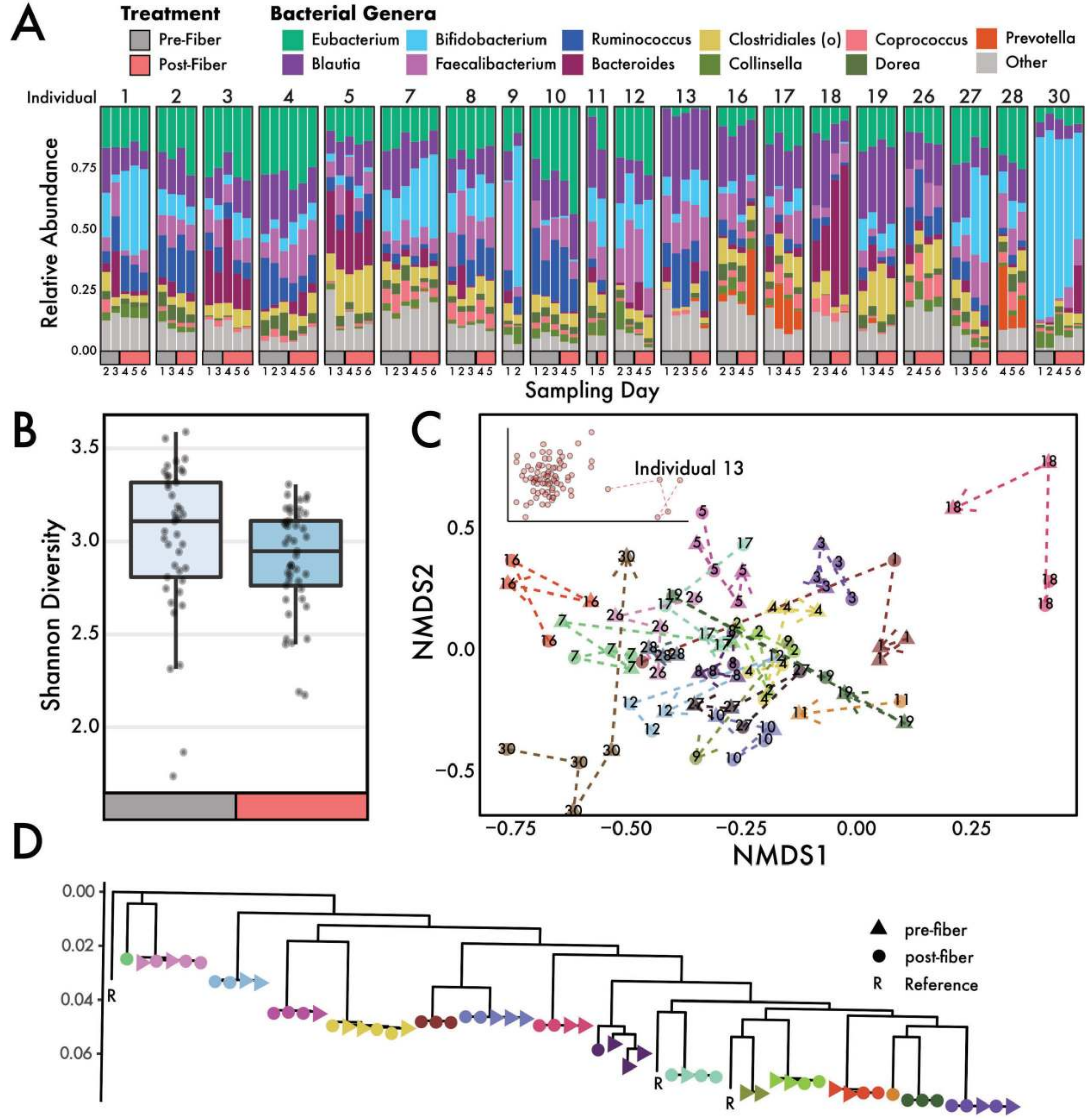
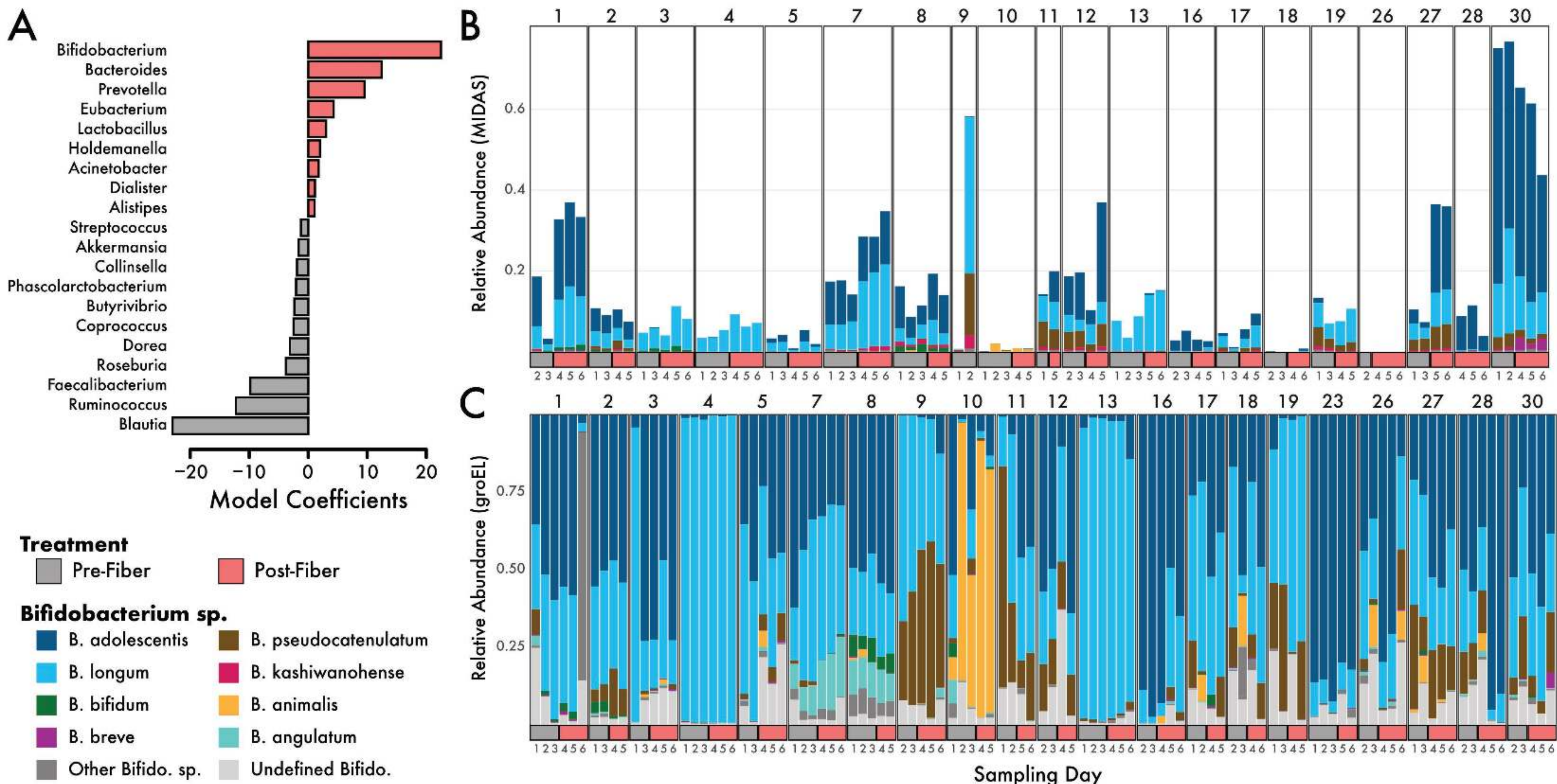

A
Fiber Diet Intervention
22 individuals provide
a stool sample
every day for 3 days
Study
Start
Study
End
week
1
2
3
26 individuals begin
tracking nutrient content
for all meals
All individuals
encouraged to consume
>40g of fiber per day
22 individuals provide
a stool sample
every day for 3 days
B
Fiber (grams/day)
C
Carbs (g/day)
D
Protein (g/day)
E
Fat (g/day)
F
Calories (kcal/day)
Week
1
2
3


A
Treatment
Pre-Fiber
Post-Fiber
Bacterial Genera
Eubacterium
Blautia
Bifidobacterium
Faecalibacterium
Ruminococcus
Bacteroides
Clostridiales (o)
Collinsella
Coprococcus
Dorea
Prevotella
Other
Individual
Relative Abundance
Sampling Day
B
Shannon Diversity
C
Individual 13
NMDS1
NMDS2
D
pre-fiber
post-fiber
R Reference


A
Bifidobacterium
Bacteroides
Prevotella
Eubacterium
Lactobacillus
Holdemanella
Acinetobacter
Dialister
Alistipes
Streptococcus
Akkermansia
Collinsella
Phascolarctobacterium
Butyrivibrio
Coprococcus
Dorea
Roseburia
Faecalibacterium
Ruminococcus
Blautia
−20 −10 0 10 20
Model Coefficients
Treatment
Pre-Fiber
Post-Fiber
Bifidobacterium sp.
B. adolescentis
B. longum
B. bifidum
B. breve
Other Bifido. sp.
B. pseudocatenulatum
B. kashiwanohense
B. animalis
B. angulatum
Undefined Bifido.
B
1 2 3 4 5 7 8 9 10 11 12 13 16 17 18 19 26 27 28 30
Relative Abundance (MIDAS)
0.2
0.4
0.6
C
1 2 3 4 5 7 8 9 10 11 12 13 16 17 18 19 23 26 27 28 30
Relative Abundance (groEL)
0.25
0.50
0.75
Sampling Day

A

GH Richness

90

80

B

NMDS1

0.5

0.0

0.0

0.5

NMDS 1

C

pre-intervention

post-intervention

Unintegrated: Eubacterium siraeum

PWY_7237: inositol degradation

Unintegrated: Megamonas funiformis

Unintegrated: Gemmiger formicilis

Unintegrated: Anaerostipes hadrus

0 1 2 3 4

LDA Score (log10)

D

log10(HUMAnN cpm counts)

2.5

2.0

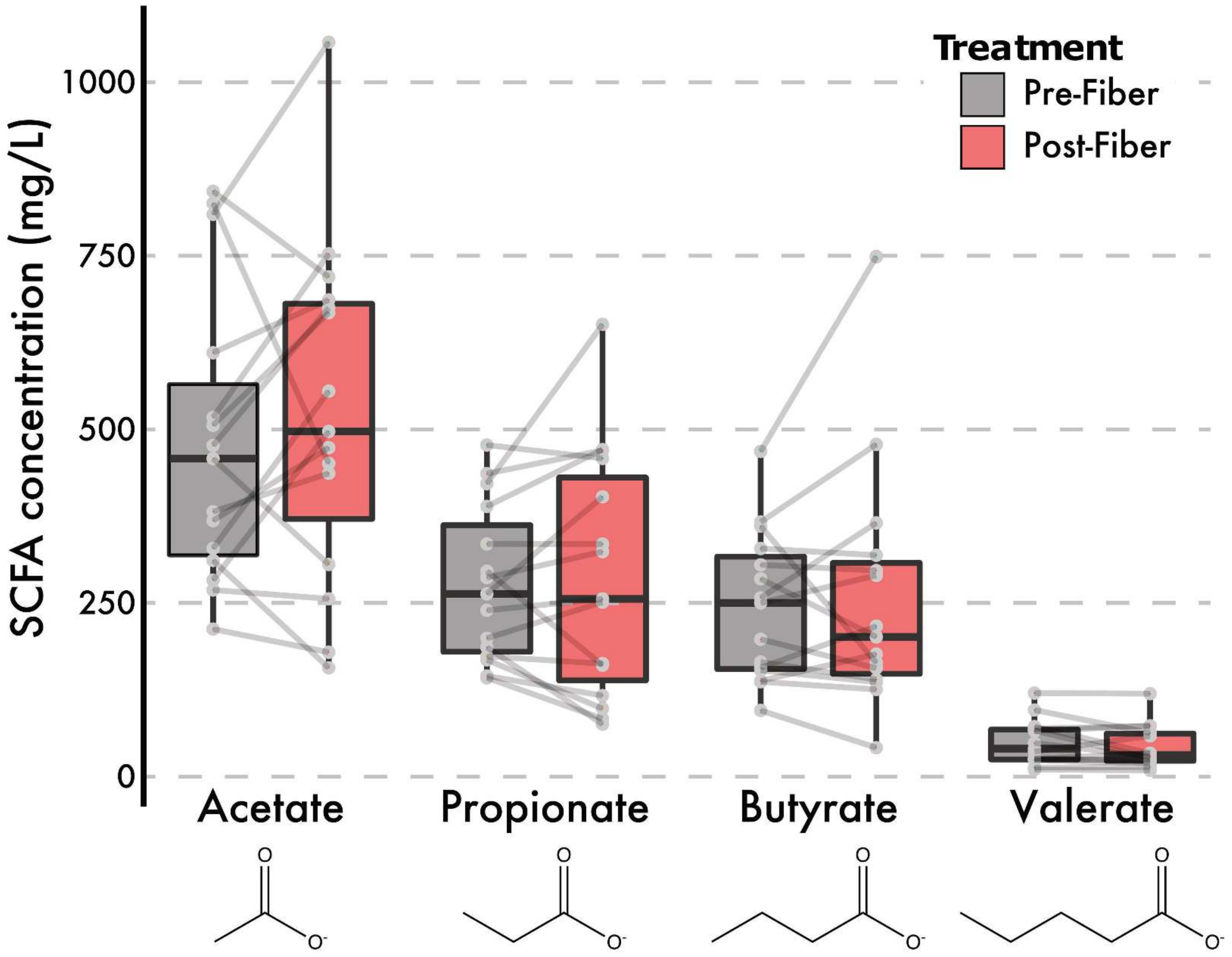
1.5

1.0

Acetate	Butyrate			Lactate		Propionate	Multiple Pathways	
acetylene degradation (anaerobic)	4-amino-butanoate degradation V	acetyl-CoA fermentation to butanoate II	pyruvate fermentation to butanoate	heterolactic fermentation	homolactic fermentation	(S)-propane-1,2-diol degradation	Bifido shunt	hexitol fermentation

Number of metagenomes in analysis:

Acetylene degradation		4-aminobutanoate degradation V		Acetyl-CoA fermentation		Pyruvate fermentation		Heterolactic	Homolactic		Propanediol degradation		Bifido shunt		Hexitol fermentation	
4	4	3	5	30	39	11	13	1	36	41	26	31	22	29	25	38


Treatment
Pre-Fiber
Post-Fiber
SCFA concentration (mg/L)
1000
750
500
250
0
Acetate
Propionate
Butyrate
Valerate
O
O-
O
O-
O
O-
O
O-