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1 Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions
2 with three fermentable fibers
3
4 Nielson T. Baxter,[a*] Alexander W. Schmidt,[a] Arvind Venkataraman,[a†] Kwi S. Kim,[a] Clive Waldron,[a]
5 Thomas M. Schmidt[ab]#
6
7 [a] Department of Internal Medicine, University of Michigan, Ann Arbor, MI.
8 [b] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI
9
10 Running Head: Different Responses to Fermentable Fibers
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12 #Address correspondence to Thomas M. Schmidt, schmidti@umich.edu
13 *Present address: Nielson T. Baxter, Elanco Animal Health, Greenfield, Indiana
14 [†]Present address: Arvind Venkataraman, CF Biosciences, The Procter & Gamble Company,
15 Cincinnati, Ohio
16
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18 **ABSTRACT**

19 Production of short-chain fatty acids (SCFAs), especially butyrate, in the gut microbiome is
20 required for optimal health but is frequently limited by the lack of fermentable fiber in the diet.
21 We attempted to increase butyrate production by supplementing the diets of 174 healthy young
22 adults for two weeks with resistant starch from potatoes (RPS), resistant starch from maize (RMS),
23 inulin from chicory root, or an accessible corn starch control. RPS resulted in the greatest increase
24 in total SCFAs, including butyrate. Although the majority of microbiomes responded to RPS with
25 increases in the relative abundance of bifidobacteria, those that responded with an increase in
26 *Ruminococcus bromii* or *Clostridium chartatabidum* were more likely to yield higher butyrate
27 concentrations, especially when their microbiota were replete with populations of the butyrate-
28 producing species *Eubacterium rectale*. RMS and inulin induced different changes in fecal
29 communities, but they did not generate significant increases in fecal butyrate levels.
30
31 IMPORTANCE These results reveal that not all fermentable fibers are equally capable of
32 stimulating SCFA production, and they highlight the importance of the composition of an
33 individual's microbiota in determining whether or not they respond to a specific dietary
34 supplement. In particular, *R. bromii or C. chartatabidum* may be required for enhanced butyrate
35 production in response to RS. Bifidobacteria, though proficient at degrading RS and inulin, may not
36 contribute to the butyrogenic effect of those fermentable fibers in the short term.

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37 **INTRODUCTION**

38 Short-chain fatty acids (SCFAs) are major end products of bacterial fermentation in the human
39 colon and are known to have wide-ranging impacts on host physiology. Butyrate in particular is
40 important for maintaining health via regulation of the immune system (1), maintenance of the
41 epithelial barrier (2, 3), and promoting satiety following meals (4). It may be protective against
42 several diseases, including colorectal cancer (5), inflammatory bowel disease (6), graft-versus-host
43 disease (7), diabetes (8), and obesity (8, 9). Therefore, stimulating butyrate production by the
44 colonic microbiome could be useful for sustaining health and treating diseases.

45

46 One strategy for stimulating butyrate production is to supplement the diet with carbohydrates
47 that are resistant to degradation by human enzymes but can be metabolized by select bacteria in
48 the colon. We previously demonstrated that one such resistant starch (RS) prepared from
49 potatoes (RPS) could increase average fecal butyrate in healthy, young adults (10). Others have
50 reported increased butyrate in response to inulin in humans and resistant starch from maize (RMS)
51 in mice (11, 12). A critical challenge to these potential therapies is the variable responses between
52 individuals, likely influenced by differences in the composition of their gut microbiota. To capture
53 this variability large numbers of subjects are required. We analyzed samples from 174 university
54 students who consented to participate in and then successfully completed this short-term
55 interventional study. This young cohort provided a wide diversity of gut communities without the
56 additional complications of chronic health conditions such as obesity, Type 2 diabetes or
57 cardiovascular disease that are related to altered microbiome structure and function (13, 14). We
58 did not ask the participants to make changes to their diets other than taking the supplements
59 provided, even though we recognize different diets also have a profound impact on microbiome

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60 structure and function. Our objective was to determine how these different resistant
61 polysaccharides affected the concentrations of SCFAs when added to "normal" diets, not just
62 under one dietary regimen. With this number of participants we were able to evaluate three
63 different resistant polysaccharides and an amylase-sensitive polysaccharide as a negative control.
64
65 Understanding the butyrogenic effect of these supplements and specific gut bacteria is important
66 for designing more broadly effective therapies and predicting which individuals are likely to
67 benefit from them. More generally, defining metabolic interactions among gut microbes enhances
68 our understanding of the assembly, maintenance and outputs from the gut microbiome.
69
70 Identifying butyrogenic configurations of the microbiome is challenging because several different
71 bacteria (or combinations of bacteria) may be involved in the multi-step process. Many bacteria in
72 the colon are involved in the degradation of dietary fiber, the complex mixture of plant
73 polysaccharides that is not susceptible to host enzymes (15, 16). However, many of these bacteria
74 are specialists, attacking specific bonds in specific types of polymers (17-19). Only a limited
75 number of gut bacteria may be able to degrade any given resistant polysaccharide that is used as a
76 dietary supplement. Primary degraders depolymerize specific polysaccharides to mono-, di- and
77 oligo-saccharides that they can take-up and ferment themselves to acidic end products such as
78 acetate or lactate (20). Their selective growth on the dietary supplement should result in a higher
79 relative abundance in fecal communities. However, most resistant starch degraders are not
80 among known butyrate producers (19, 21). Thus, for these supplements to stimulate butyrate
81 production, the activities of additional organisms would be required. These secondary fermenters
82 capture degradation and fermentation products from primary degraders and metabolize them into

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83 new molecules including butyrate (Fig. 1). However, if primary degraders use the supplements
84 efficiently, only a fraction of the carbon and energy they contain may become available to the
85 secondary fermenters. Therefore, increases in the relative abundance of butyrate producers may
86 be more difficult to detect, but their metabolic activity could still be evidenced by an increase in
87 fecal butyrate.
88
89 Candidates for performing one or more of the steps in Fig. 1 *in vivo* have already been identified
90 by their metabolic capabilities *in vitro*. For example, *Ruminococcus bromii* and *Bifidobacterium*
91 *adolescentis* have been shown to degrade resistant starches (22). There have been claims that
92 other species degrade resistant starches, but they are less compelling because they either involved
93 starch preparations that could include sensitive as well as resistant fractions (23) or because the
94 evidence was indirect (e.g. binding to starch granules (24)) or the presence of DNA sequences
95 encoding amylase-like GH13 domains (25). The ability to degrade inulin *in vitro* has been
96 demonstrated for some species of *Bifidobacterium* (though not all strains) (26) and by several
97 members of the *Lachnospiraceae* and *Ruminococcaceae* families in the phylum Firmicutes (27).
98 These two families include most of the known butyrate producers (21). The most abundant of
99 them in the human gut are *Eubacterium rectale* and *Faecalibacterium prausnitzii,* both of which
100 are capable of degrading inulin and producing butyrate from it (27). *E. rectale* has been shown
101 incapable of degrading resistant starches unless they are heat-treated to denature some of the
102 crystal structure (22). Such rigorous tests have not been reported to our knowledge for *F.*
103 *prausnitzii*. *In vitro* studies have also demonstrated that combinations of primary degraders and
104 secondary bacteria can produce butyrate from resistant polysaccharides by cross feeding. For
105 example, *Eubacterium rectale* can grow on degradation products of RS released by *R. bromii*, and

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106 several species of *Roseburia* and *F. prausnitzii* have been cross-fed by *Bifidobacterium* spp. (20, 22,
107 28, 29). Bifidobacteria also promote butyrate production by another select group of bacteria
108 because they produce both acetate and lactate via a unique fermentation pathway known as the
109 Bifid shunt (30). This combination of end products can be converted into butyrate by *Eubacterium*
110 *hallii, Anaerostipes caccae* or *Anaerostipes hadrus* (31, 32).

111

112 These known degraders and butyrate producers were targeted for evaluation in this dietary
113 intervention. But we also analyzed the entire community of fecal bacteria to identify any
114 organisms that had not previously been associated with the metabolism of these fermentable
115 fibers. We attempted to address four major issues:

116 1. Do the three resistant polysaccharides stimulate butyrate production in this population of
117 healthy, young individuals? If so, do they have similar impacts on butyrate production?
118 2. Which gut bacteria respond to these dietary additions by increasing in relative abundance?
119 Can we identify any species that were unexpectedly affected? Are the same bacteria affected
120 by all three supplements?
121 3. Can we find any evidence of selectivity, either in the substrates used by primary degraders or
122 in the butyrate producers they cross feed?
123 4. Do changes in the relative abundance of primary degraders and butyrate producers explain
124 differences in individuals' butyrate concentrations?

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125

126 **RESULTS**

127 **Effects on Short Chain Fatty Acids**

128 We first examined the impact of each supplement on the concentration of SCFAs in the feces. Both
129 RPS and inulin significantly increased total SCFA concentrations, by 32% and 12% respectively
130 (both p<0.001). Supplementation with RPS increased butyrate concentrations by an average of
131 29% (p<0.001) and acetate by an average of 21% (p=0.0012, Table 1). However, the response was
132 highly variable between individuals: the median concentration of butyrate increased in 63% of
133 individuals and was either unchanged or decreased in the remaining 37%. Although total SCFA
134 concentrations increased with inulin supplementation, there were no statistically significant
135 changes in individual SCFAs. Nor were there significant changes in the concentration of any of the
136 SCFAs in the groups whose diet was supplemented with either RMS or accessible starch (Table 1).
137 Furthermore, there were no significant differences in SCFA concentrations between the control
138 group that consumed 20 g of accessible starch compared to the group that consumed 40 g of
139 accessible starch.

140

141 **Effects on Bacterial Communities**

142 We characterized changes to the gut microbiota using 16S rRNA gene sequencing. We chose not to
143 cluster sequences into operational taxonomic units (OTUs) after discovering that several taxa of
144 interest, with very different responses to the dietary supplements, would be clustered into a single
145 OTU even at 99% identity. For example, *Bifidobacterium longum* and *B. faecale* have varying
146 abilities to degrade RS, but the V4 region of the 16S rRNA gene in these species is 99.6% identical.
147 Combining sequences corresponding to these species masks a biological pattern that is readily

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148 apparent when considering the unique sequences. Unlike OTUs that are calculated *de novo* with
149 each new data set, unique sequences also have the benefit of being directly comparable across
150 datasets. Nevertheless, the small size of the V4 region of *Bifidobacterium* makes it impossible to
151 resolve all species within this genus. *B. adolescentis, B. faecale* and *B. stercoris* have identical V4
152 regions, as do *B. longum* and *B. breve.* A third group, *B. catenulatum, B. pseudocatenulatum,* and
153 *B. kashiwanohense,* also share identical V4 regions. For all other species of interest a single
154 sequence was identified that was specific to each species. To avoid analysis of spurious sequences,
155 we limited our analysis to the 500 most abundant unique sequences, which accounted for 71% of
156 the approximately 70 million curated sequencing reads. Using this approach we determined that
157 both the RPS and inulin significantly altered the overall structure of the community (PERMANOVA,
158 p=0.001 and p=0.002, respectively), while the accessible starch and RMS did not (p=1.0 and
159 p=0.65, respectively). None of the supplements significantly changed the alpha diversity, as
160 measured by the inverse Simpson index (p>0.05).
161
162 **The Most Affected Bacterial Populations**
163 The sequences that changed the most were identified by the ratio of their relative abundance
164 during supplementation to their relative abundance before (Fig. 2). Most of the sequences that
165 significantly increased in relative abundance were from species already known to degrade
166 resistant polysaccharides. RPS increased the relative abudance of *B. faecale/adolescentis/stercoris*
167 sequences 6.5-fold (p<0.001), but there were no significant changes in any of the other sequences
168 classified in the genus *Bifidobacterium* (Fig. 2). RMS resulted in a 2.5-fold increase in the relative
169 abundance of sequences classified as *R. bromii* (p<0.001), but no significant changes in any of
170 those classified as *Bifidobacterium* (Fig. 2, Supplemental Table 2). Inulin significantly increased the

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171 relative abundance of each of the four most abundant sequences assigned to a *Bifidobacterium*
172 species (Fig. 2, all p<0.05) and sequences classified as *Anaerostipes hadrus* (Fig. 2, Supplemental
173 Table 3). No bacterial populations significantly changed in response to the accessible starch
174 supplement (Fig. 2, Supplemental Table 4).

175

176 We also noted increases in sequences that were not significant for the population as a whole, but
177 which were dramatic within a subset of individuals (Supplementary Table 1). RPS increased the
178 relative abundance of *R. bromii* sequences in a subset of individuals (Fig. 3A), but not for the group
179 as whole (p=0.72). One of the most striking increases in a smaller subset was in Seq100, classified
180 as a member of the *Clostridium* cluster IV within the family *Ruminococcaceae*. *Clostridium leptum*
181 is the closest cultured relative, but their 16S rRNA V4 regions are only 95% identical. This
182 *Clostridium leptum*-related sequence was detected in 11 of the 50 individuals who consumed RPS,
183 increasing by an average of 10-fold and exceeding 10% relative abundance in several individuals
184 (Fig. 3A). Another unanticipated increase was in Seq176 whose V4 region is 98.8% identical to
185 *Clostridium chartatabidum*, a rumen isolate shown to degrade a variety of dietary fibers (33, 34).
186 It was rarely detected before supplementation, but its relative abundance increased up to 4%
187 relative abundance in 11 of the individuals consuming RPS (Fig. 3A). Although a large fold change
188 for Seq176 was observed in the accessible starch and Hi-Maize groups (Fig 3A), it was limited to
189 one individual in each group. Seq176 reached only 0.03% and 0.14% relative abundance in those
190 two individuals after starting below the limit of detection.

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191

192 **Associations between Butyrate Changes and Community Changes**

193 In individuals consuming RPS, increases in the relative abundance of sequences attributed to
194 either *R. bromii* or *C. charatabidum* were each associated with an increase in butyrate (p=0.025
195 and p=0.0024, Fig. 3B). On average, there was a 9.1 mmol/kg increase in fecal butyrate when
196 either *R. bromii* or *C. charatabidum* sequences increased in relative abundance in response to RPS.
197 There was a decrease of 2.1 mmol/kg in individuals in whom neither sequence increased (Fig. 3C).
198 Furthermore, the presence or absence *R. bromii* prior to RPS supplementation was indicative of
199 whether an individual would have higher fecal butyrate in response to RPS. Of the 29 individuals
200 with detectable *R. bromii* at baseline, 22 (76%) had higher fecal butyrate during RPS
201 supplementation, compared to 5 (36%) of the 14 individuals without detectable *R. bromii*. The
202 baseline abundance of *C. charatabidum* was not associated with a butyrate response because it
203 was below the limit of detection (<0.008% abundance) in all but five individuals prior to
204 supplementation. With greater sequencing depth or a more sensitive assay it may be possible to
205 use the presence of these two organisms to predict whether an individual will respond to RPS with
206 increased butyrate. Increases in either *B. faecalis/adolescentis/stercoris* or *Clostridium leptum*-like
207 Seq100 were not associated with an increase in butyrate (Fig. 3B).

208

209 **Partnerships Converting Polysaccharides to Butyrate**

210 Increasing *R. bromii* was associated with higher butyrate levels, but butyrate is not a major end
211 product of *R. bromii* metabolism (35). Therefore, an increase in *R. bromii* is not sufficient in itself
212 to explain the association with higher butyrate concentrations. Based on our working model (Fig.

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213 1), we expected the increase in primary degraders to lead to an increase in the abundance of
214 butyrate producers (though to a lesser extent because the primary degraders were presumed to
215 extract most of the nutritional value of the supplements for themselves). To test this expectation,
216 we correlated the change in abundance of sequences associated with each of the putative primary
217 degraders with changes in the abundance of the most common known butyrate producers in our
218 cohort (Fig. 4). Consistent with our model, change in *R. bromii* sequences was positively
219 correlated with the change in abundance of *E. rectale* sequences (Fig 4). This observation is also
220 consistent with previous reports that populations of *R. bromii* and *E. rectale* are associated with
221 each other, both physically and metabolically (22, 24). The relative abundance of *E. rectale* was
222 also correlated with the concentration of butyrate in the RPS group (Spearman rho=0.42, $p<0.001$,
223 Fig. 5), which would explain the higher butyrate in individuals where *R. bromii* increased.
224

225 **DISCUSSION**

226 We tested the effects of fermentable fiber supplements on the structure and function of bacterial
227 communities in the human colon. The three supplements we studied include fractions that are
228 resistant to degradation by host enzymes and therefore pass through the small intestine to the
229 colon. There they can be metabolized by specialized bacteria (which distinguishes them from
230 “bulking fibers” that promote regularity but pass through both small and large intestines without
231 degradation). Two of the supplements we tested were Type 2 Resistant Starches: unmodified
232 starches extracted from potato tubers (RPS) or hi-amylose maize seeds (RMS). However, they had
233 not been pre-treated with α-amylase so they included some accessible starch. Only 50%-70% of
234 such flours is in the crystalline form that is resistant to mammalian α-amylase (23); the rest is
235 “sensitive” or “accessible” to host enzymes. As a negative control we therefore included a maize

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236 starch that is completely digestible by human α-amylase so it should be broken down in the small
237 intestine and not affect colonic communities. It was added at a low dose (equivalent to the
238 sensitive portion of the RMS) or a high dose (equivalent to all the glucose monomers in that
239 resistant supplement). In neither case did this placebo produce any statistically significant changes
240 in the microbiota composition or SCFA production during consumption. The third supplement was
241 the fructose polymer, inulin, that is entirely resistant to digestion in the small intestine.
242
243 Consuming resistant RPS led to an increase in the average concentration of fecal butyrate (Table
244 1). Neither inulin nor RMS produced a significant change in butyrate. An important caveat is that
245 the amount of amylose-resistant polysaccharide consumed was not equal across treatment
246 groups. The inulin and Hi-Maize groups consumed approximately 20 and 20-24 grams,
247 respectively, while the RPS group consumed approximately 28-34 grams. However based on
248 preliminary data, the discrepancy is not sufficient to explain the lack of a butyrogenic effect from
249 inulin and Hi-Maize. In a pilot study, we observed a significant increase in fecal butyrate in
250 individuals consuming half the dose of RPS (24 g total, 14-17 g resistant; data not shown). The
251 butyrogenic response to RPS appears due to the nature of the supplement, not just the amount of
252 RS it contains. So the answer to our first research question is that the fiber supplements are not
253 equally effective at stimulating levels of this health-promoting metabolite.
254
255 All the fermentable fiber supplements had some effect on the fecal community (Fig. 2). In
256 individuals consuming RPS, the largest and most common change in V4 sequences was an increase
257 in sequences attributed to *B. faecale/adolescentis/stercoris*. This contrasts with an earlier study of
258 dietary supplementation with resistant starch Type 3, where *R. bromii* was the dominant

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259 responder (22, 36) and was therefore proposed to be a keystone species for the degradation of
260 resistant starch (18). The different responses in our study could be due to differences in the
261 supplements, as has been observed previously when Type 2 or Type 4 resistant starches are
262 consumed (37). The higher abundance of bifidobacteria in our study is consistent with the known
263 ability of *B. adolescentis* to degrade and ferment RPS *in vitro* (20), but the increase was not
264 associated with a change in fecal butyrate (Fig. 3B), suggesting that these organisms are not
265 effectively cross-feeding butyrate producers *in vivo* – even though they can *in vitro* (20, 28, 29). It
266 is conceivable that *in vivo* production of butyrate from such cross-feeding requires additional time
267 for interactions to be established in the gut microbiome. Longer-term studies are currently
268 underway to assess this possibility. The microbiomes in a subset of individuals consuming RPS did
269 respond with an increase in *R. bromii*-associated sequences. In this group there was an associated
270 increase in fecal concentrations of butyrate so *R. bromii* may be considered a keystone degrader
271 with regard to butyrate production in our cohort (Fig. 3B). The major fermentation products of *R.*
272 *bromii* are acetate, H_2 and CO_2, but not butyrate (35) so there must be cross-feeding of a
273 butyrogenic microbe. The most likely candidate is *Eubacterium rectale* because its abundance
274 increased most consistently with that of *R. bromii* (Fig. 4). Two less-well characterized bacterial
275 populations also increased in relative abundance in a few individuals consuming RPS. The increase
276 in the *Clostridium leptum*-related Seq100 was not associated with an increase in butyrate (Fig. 3B),
277 suggesting that it too may be a degrader but not a cross-feeder. The Seq176 increase was
278 associated with a butyrate increase (Fig. 3B), which may be because it is both a degrader and a
279 butyrate producer. The activity of its cultured relative *Clostridium chartatabidum* against RPS is
280 not documented, but it is a rumen bacterium capable of degrading a variety of plant fibers and, it
281 produces butyrate (33, 34). Attempts to culture the human strain are underway.

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282

283 Individuals consuming RMS did not respond with an increase in the abundance of *B.*
284 *faecale/adolescentis/stercoris* sequences. With this supplement, like previous studies (36), an
285 increase in *R. bromii*-associated sequences was the most common response (Fig. 2). Unlike the
286 RPS-induced *R. bromii* increase, the increase in *R. bromii* on RMS was not associated with a
287 significant increase in fecal butyrate. The lack of a butyrogenic response to RMS was unexpected
288 because the supplement has led to increased fecal butyrate in animal models, though over four
289 weeks (12). We speculate that the crystal structures of the resistant starches from the two plants
290 are different because the RPS is phosphorylated (once every 200 glycosyl residues (38)). They may
291 therefore be degraded with different efficiency or by different strains. Consequently more time
292 may be required to develop cross-feeding interactions that generate measurable differences in
293 fecal butyrate.

294

295 Inulin increased the relative abundance of four species of *Bifidobacterium*, consistent with the
296 widespread occurrence of this degradative capability within the genus (26). There were also
297 increases in the abundance of the butyrate producers *Anaerostipes hadrus* and *E. rectal*e (Fig. 2),
298 but they did not result in increased fecal butyrate. The *A. hadrus* may be feeding on the lactate
299 and acetate produced by the more abundant Bifidobacteria on this supplement (31, 32) or it may
300 be able to metabolize inulin itself. We note that the low pKa of lactic acid (pKA = 3.86) produced
301 by the Bifid shunt (30) could reduce fecal pH and inhibit butyrogenic microbes that are sensitive to
302 the lower pH. Subsequent utilization of lactic acid would restore pH but may extend the time
303 required to see an effect. Or it could alter the distribution of microbial populations in the GI tract
304 such that the balance of butyrate consumption and excretion by the host is affected (39). It may

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305 also be that lactate produced by *Bifidobacterium* species was converted to fermentation products
306 other than butyrate. Notably propionate increased by 27% on average with inulin consumption,
307 though the change was not statistically significant due to high variability. Other than
308 *Bifidobacterium spp.* and *A. hadrus*, there were no significant increases in any other sequences
309 (Supplementary Table 3), surprisingly including *F. prausnitzii* (which has the metabolic capacity to
310 ferment inulin to butyrate (27)).
311
312 Thus we observed that all the fermentable fiber supplements we tested increased the relative
313 abundances of some members of the fecal microbiota. Most of the affected sequences are
314 associated with known degraders of resistant polysaccharides or producers of butyrate but we did
315 uncover two Firmicutes not previously associated with fiber supplements. The organisms
316 responding depended both on the individual and the supplement (research question 2).
317
318 Together our observations on SCFA and community composition changes suggest that the working
319 model for stimulating butyrate production with fiber supplements (Fig. 1) is an over-simplification,
320 in that fiber degradation does not always lead to butyrate production. The requirement for fiber
321 breakdown by specialized primary degraders appears to hold, with lack of an appropriate degrader
322 preventing butyrate increase. However, not all degraders lead to a butyrogenic response. The lack
323 of enhanced butyrate production in *Bifidobacterium*-responding microbiomes suggests that while
324 bifidobacteria are particularly effective at using some fiber supplements, they do not establish
325 cross-feeding reactions with butyrogenic populations as readily as *Ruminococcus*-responsive
326 microbiomes. Furthermore, the concept of a wide variety of butyrate-producing organisms having
327 ready access to the products of degradation and fermentation is not supported. Even though many

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328 of these organisms can be cross-fed by primary degraders *in vitro* (20, 22, 28, 29), the *in vivo*
329 scenario seems much more restricted. The only butyrate producer whose abundance increased
330 with a primary degrader (Fig. 4) and was associated with higher fecal butyrate (Fig. 5) is *E. rectale*.
331 This organism has a multi-protein, cell-wall attached system for degrading sensitive starch (40). It
332 includes carbohydrate binding domains that enable it to attach to RS granules (but not degrade
333 them), as well as ABC transporter proteins that capture oligosaccharide degradation products of
334 the same size as those preferred for growth of *R. bromii* (22). It has been shown to co-localize
335 with *R. bromii* on starch granules (24) and to grow on the products of RS degradation by *R. bromii*
336 in vitro (22). It thus has the characteristics of a preferred partner in converting RPS to butyrate
337 (research question 3). A single organism, such as *C. chartatabidum*, may be an alternative route,
338 but it occurs in fewer individuals. If the *C. chartatabidum*-related organism from this cohort is
339 indeed a primary degrader of RPS that produces butyrate, it would be an appealing probiotic to
340 give in combination with RPS to enhance butyrate production in a larger percentage of individuals.
341
342 To improve the efficacy of dietary supplements like these, it may be necessary to personalize them
343 according to an individual's gut microbiota (41). The presence of *R. bromii* or *C. chartatabidum*
344 suggests whether a gut microbiome would yield increased butyrate concentrations following
345 short-term (2-week) supplementation with RPS (research question 4). Individuals without *R. bromii*
346 in their gut microbiome may benefit from a probiotic supplementation with *R. bromii* to increase
347 the likelihood of a butyrogenic response to RPS. There may also be a synergistic effect of
348 combining RPS with both *R. bromii* and *E. rectale* to maximize the butyrogenic effect of the
349 supplement. In contrast, microbiomes with high levels of bifidobacteria are less likely to increase
350 butyrate production in response to RPS (or inulin), at least in the short term. In these

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351 microbiomes, a different supplement or combination of supplements may be needed, or a longer
352 period of time may be required for the microbiome to respond to the supplement. Such
353 considerations are necessary when attempting to effect a particular change in the highly variable
354 structures of human gut communities.
355

356 **MATERIALS AND METHODS**

357 **Study Participants**

358 Study participants were recruited through the Authentic Research Sections of the introductory
359 biology laboratory course at the University of Michigan (BIO173). Individuals with self-reported
360 history of inflammatory bowel syndrome, inflammatory bowel disease, or colorectal cancer were
361 excluded from the study, as were individuals who had taken antibiotics within the last 6 months.
362 Pre- or probiotic usage was not an exclusion criterion for the study. Nor was the amount of fiber
363 already being consumed. All participants gave written, informed consent prior to participating in
364 the study. Participants under the age of 18 were granted permission by a parent or legal guardian.
365 Participants ranged in age from 17 to 29, with a median age of 19. This study was approved by the
366 Institutional Review Board of the University of Michigan Medical School (HUM00094242 and
367 HUM00118951) and was conducted in compliance with the Helsinki Declaration.
368

369 The participants were randomly assigned to study groups. During the first semester they were
370 blinded to the supplement they were taking; in subsequent studies they were informed of its
371 identity. Researchers analyzing samples were always blinded to the supplement associated with
372 the samples. Some participants were excluded from the analysis because fewer than three
373 samples were collected or successfully analyzed either before or during dietary supplementation.

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374

375 **Study Design**

376 This replicated intervention study was conducted during four separate academic semesters from
377 the fall of 2015 to the spring of 2017. It was a parallel design with different but similar groups
378 taking experimental or control supplements. Replicated baseline data were collected for each
379 individual during the first week of each study. Each participant collected 3 to 4 fecal samples on
380 separate days during this period. During the second week, participants underwent a 4- to 7-day
381 transition phase, that began with consumption of a half dose of the supplement before taking the
382 full dose. No fecal samples were collected during the transition phase. In the third week of the
383 study participants continued taking the full dose of their assigned supplement until they had
384 collected 3 to 4 fecal samples on separate days.

385

386 **Dietary Supplements**

387 Four different supplements were tested in this study; resistant potato starch (RPS, Bob's Red Mill,
388 Milwaukee OR), Hi-Maize 260 resistant corn starch (RMS, manufactured by Ingredion Inc.,
389 Westchester, IL and distributed by myworldhut.com), inulin isolated from chicory root (Swanson
390 Health Products, Fargo, ND), and amylase-accessible corn starch (Amioca powder, Skidmore Sales
391 and Distribution, West Chester OH). Preliminary studies suggested that 40-48 g of supplement
392 could be comfortably consumed per day. This amount was therefore used for the studies with RPS
393 and RMS. However, it should be noted that these supplements do not contain the same
394 proportion of amylase-resistant starch: RPS contains approximately 70% RS (Type 2) by weight and
395 RMS approximately 50% (23). Subjects therefore consumed 28 – 34 grams of RPS or 20 – 24 grams
396 of RMS per day. The inulin supplement, which is resistant to human enzymes (42), was consumed

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397 at 20 g/day to provide a similar amount of resistant polysaccharide. The accessible starch
398 supplement was given at either 40 g/day (n=15) to provide a similar amount of total carbohydrates
399 or at 20 g/day (n=24) to provide approximately the same amount of host-accessible
400 carbohydrates. Consumption of the supplements was split into two half doses per day and logged
401 on the study website. Participants were provided with a shaker bottle to aid in mixing the
402 supplement with water, however they were permitted to consume the supplement with any type
403 of food or beverage. Participants were instructed not to add the supplement to any warm food or
404 beverage. Aside from the supplement, participants maintained their normal diet throughout the
405 study.

406

407 **Fecal Collection**

408 Fecal samples were collected by participants as described previously (10). Participants collected
409 approximately half a gram of fecal material into an OMNIgene-Gut® (DNA Genotek) collection kit,
410 following manufacturer instructions. Collection tubes were transferred to a -20˚C freezer within 24
411 hours of collection and stored at -20˚C until thawed for DNA and metabolite extractions. Collection
412 tubes were weighed before and after fecal collection, to determine the weight of the fecal
413 material collected.

414

415 **SCFA Quantification**

416 To extract SCFAs, 1 ml of fecal suspension was transferred into a 2 ml 96-well V-bottom collection
417 plate and centrifuged at 4,500 x G for 15 minutes at 4˚C. 200 µl of supernatant fractions were
418 successively filtered through 1.20, 0.65, and 0.22 µm 96-well filter plates at 4˚C. Filtrates were
419 transferred into 1.5 ml screw cap vials containing 100 µl inserts in preparation for analysis by high-

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420 performance liquid chromatography (HPLC). Samples were analyzed in a randomized order.
421 Quantification of SCFAs was performed using a Shimadzu HPLC system (Shimadzu Scientific
422 Instruments, Columbia, MD) that included an LC-10AD vp pump A, LC-10AD vp pump B, degasser
423 DGU-14A, CBM-20A, autosampler SIL-10AD HT, Column heater CTO- 10A(C) vp, UV detector SPD-
424 10A(V) vp, and an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA). We used a
425 mobile phase of 0.01 N H_2SO_4 at a total flow rate of 0.6 ml per minute with the column oven
426 temperature at 50˚C. The sample injection volume was 10 µl and each sample eluted for 40
427 minutes. Concentrations were calculated using a cocktail of short chain organic acids standards at
428 concentrations of 20, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 mM. After correcting the baseline of
429 chromatographs, the quality of peaks was assessed using peak width, relative retention time, and
430 5% width. Peaks that fell outside predetermined cutoffs for relative retention time and peak width
431 were removed. Concentrations in experimental samples were normalized to the wet weight of
432 fecal material.

433

434 **16S rRNA gene sequencing**

435 DNA was extracted from 250 µl of fecal suspension using the 96-well MagAttract
436 PowerMicrobiome DNA Isolation kit (Qiagen) and EpMotion liquid handling systems (Eppendorf).
437 The V4 region of the bacterial 16S rRNA gene was amplified and sequenced as described
438 previously using 2 x 250 base pair paired end kits on the Illumina MiSeq sequencing platform (43).
439 Samples were assigned randomly to different runs each semester, with 8 separate DNA
440 sequencing runs in total. Sequences were curated using the mothur software package as described
441 previously (43, 44). Briefly, paired end reads were merged into contigs, screened for sequencing
442 errors, and aligned to the SILVA bacterial SSU reference database. Aligned sequences were

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443 screened for chimeras and classified using the Ribosomal Database Project database. Sequences
444 classified as mitochondria, chloroplasts, or archaea were removed. Sequences of interest were
445 further identified using BLAST to align against the 16S rRNA gene sequences database. Unless
446 stated otherwise, species designations indicate 100% identity to a single species in the database.
447 The number of sequences per sample was rarified to 3000 to prevent biases from uneven
448 sampling.

449

450 **Statistical analyses**

451 All statistical analyses were performed using R (version 3.2.4) via RStudio (version 1.0.136). To
452 determine whether there was a significant change in SCFA concentration from before to during
453 fiber supplementation, we performed repeated measures ANOVAs on SCFA concentrations in all
454 fecal samples from individuals consuming each supplement. For other analyses, we used the
455 median SCFA concentration within each individual at each time point. Changes in the overall
456 community structure in response to supplements were assessed using a within-subjects
457 PERMANOVA on Bray-Curtis distances. Diversity was compared using repeated measures ANOVA
458 on the inverse Simpson index for each sample. For other comparisons of the relative abundance of
459 microbiota we used the average relative abundance within each individual at each time point,
460 yielding a single average community structure for each individual before and during
461 supplementation. Organisms that increased the most in response to each supplement were
462 identified using one-tailed paired Wilcoxon-tests on the average abundances in each subject
463 before and during supplementation with Benjamini-Hochberg correction for multiple comparisons
464 (45). P-values in Figure 2 were corrected for the multiple tests applied to those 14 species, which
465 were *a priori* expected to important for fiber degradation and/or butyrate production, while p-

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466 values in Supplemental Tables 1-4 were corrected for test across all 500 sequences. Correlation
467 between the changes in abundance of primary degraders and butyrate producers (Fig. 4) were
468 calculated using Spearman correlation with Benjamini-Hochberg correction for multiple
469 comparisons. Raw sequencing reads and metadata, including SCFA concentrations, are available
470 through the NCBI Short Read Archive under accession number SRP128128.

471

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476

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479 preparation and sequencing.

480 **REFERENCES**

481 1. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C,
482 Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K,
483 Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita
484 T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. 2013. Commensal microbe-derived
485 butyrate induces the differentiation of colonic regulatory T cells. Nature 504:446-50.

bioRxiv preprint doi: https://doi.org/10.1101/487900; this version posted December 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

486 2. Peng L, Li ZR, Green RS, Holzman IR, Lin J. 2009. Butyrate enhances the intestinal barrier by
487 facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2
488 cell monolayers. J Nutr 139:1619-25.
489 3. Ruemmele FM, Schwartz S, Seidman EG, Dionne S, Levy E, Lentze MJ. 2003. Butyrate
490 induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. Gut 52:94-100.
491 4. Mikkelsen KH, Frost M, Bahl MI, Licht TR, Jensen US, Rosenberg J, Pedersen O, Hansen T,
492 Rehfeld JF, Holst JJ, Vilsboll T, Knop FK. 2015. Effect of Antibiotics on Gut Microbiota, Gut
493 Hormones and Glucose Metabolism. PLoS One 10:e0142352.
494 5. Wang L, Luo HS, Xia H. 2009. Sodium butyrate induces human colon carcinoma HT-29 cell
495 apoptosis through a mitochondrial pathway. J Int Med Res 37:803-11.
496 6. Segain JP, de la Bletiere DR, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C,
497 Blottiere HM, Galmiche JP. 2000. Butyrate inhibits inflammatory responses through NF
498 kappa B inhibition: implications for Crohn's disease. Gut 47:397-403.
499 7. Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, Oravecz-
500 Wilson K, Wu SR, Sun Y, Rossi C, Fujiwara H, Byun J, Shono Y, Lindemans C, Calafiore M,
501 Schmidt TC, Honda K, Young VB, Pennathur S, van den Brink M, Reddy P. 2016. Gut
502 microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate
503 graft-versus-host disease. Nat Immunol 17:505-13.
504 8. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. 2009. Butyrate
505 improves insulin sensitivity and increases energy expenditure in mice. Diabetes 58:1509-
506 17.
507 9. Lin HV, Frassetto A, Kowalik EJ, Jr., Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D,
508 Yao X, Forrest G, Marsh DJ. 2012. Butyrate and propionate protect against diet-induced

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509 obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms.
510 PLoS One 7:e35240.
511 10. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. 2016. Variable
512 responses of human microbiomes to dietary supplementation with resistant starch.
513 Microbiome 4:33.
514 11. Welters CF, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG. 2002.
515 Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with
516 an ileal pouch-anal anastomosis. Dis Colon Rectum 45:621-7.
517 12. Le Leu RK, Hu Y, Brown IL, Young GP. 2009. Effect of high amylose maize starches on
518 colonic fermentation and apoptotic response to DNA-damage in the colon of rats. Nutr
519 Metab (Lond) 6:11.
520 13. Harsch IA, Konturek PC. 2018. The Role of Gut Microbiota in Obesity and Type 2 and Type 1
521 Diabetes Mellitus: New Insights into "Old" Diseases. Med Sci (Basel) 6.
522 14. Brown JM, Hazen SL. 2018. Microbial modulation of cardiovascular disease. Nat Rev
523 Microbiol 16:171-181.
524 15. Makki K, Deehan EC, Walter J, Backhed F. 2018. The Impact of Dietary Fiber on Gut
525 Microbiota in Host Health and Disease. Cell Host Microbe 23:705-715.
526 16. Deehan EC, Duar RM, Armet AM, Perez-Munoz ME, Jin M, Walter J. 2017. Modulation of
527 the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To
528 Improve Human Health. Microbiol Spectr 5.
529 17. Cockburn DW, Koropatkin NM. 2016. Polysaccharide Degradation by the Intestinal
530 Microbiota and Its Influence on Human Health and Disease. J Mol Biol 428:3230-3252.

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531 18. Watson D, O'Connell Motherway M, Schoterman MH, van Neerven RJ, Nauta A, van
532 Sinderen D. 2013. Selective carbohydrate utilization by lactobacilli and bifidobacteria. J
533 Appl Microbiol 114:1132-46.
534 19. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. 2012. Microbial degradation of complex
535 carbohydrates in the gut. Gut Microbes 3:289-306.
536 20. Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, Flint HJ. 2006. Two
537 routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-
538 producing anaerobes from the human gut. Appl Environ Microbiol 72:3593-9.
539 21. Louis P, Flint HJ. 2017. Formation of propionate and butyrate by the human colonic
540 microbiota. Environ Microbiol 19:29-41.
541 22. Ze X, Duncan SH, Louis P, Flint HJ. 2012. Ruminococcus bromii is a keystone species for the
542 degradation of resistant starch in the human colon. ISME J 6:1535-43.
543 23. Bednar GE, Patil AR, Murray SM, Grieshop CM, Merchen NR, Fahey GC, Jr. 2001. Starch and
544 fiber fractions in selected food and feed ingredients affect their small intestinal digestibility
545 and fermentability and their large bowel fermentability in vitro in a canine model. J Nutr
546 131:276-86.
547 24. Leitch EC, Walker AW, Duncan SH, Holtrop G, Flint HJ. 2007. Selective colonization of
548 insoluble substrates by human faecal bacteria. Environ Microbiol 9:667-79.
549 25. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. 2013. The abundance and
550 variety of carbohydrate-active enzymes in the human gut microbiota. Nat Rev Microbiol
551 11:497-504.

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552 26. Rossi M, Corradini C, Amaretti A, Nicolini M, Pompei A, Zanoni S, Matteuzzi D. 2005.
553 Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of
554 pure and fecal cultures. Applied and Environmental Microbiology 71:6150-6158.
555 27. Moens F, De Vuyst L. 2017. Inulin-type fructan degradation capacity of Clostridium cluster
556 IV and XIVa butyrate-producing colon bacteria and their associated metabolic outcomes.
557 Benef Microbes 8:473-490.
558 28. Falony G, Vlachou A, Verbrugghe K, De Vuyst L. 2006. Cross-feeding between
559 Bifidobacterium longum BB536 and acetate-converting, butyrate-producing colon bacteria
560 during growth on oligofructose. Appl Environ Microbiol 72:7835-41.
561 29. Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, de los Reyes-Gavilan CG. 2015.
562 Enhanced butyrate formation by cross-feeding between Faecalibacterium prausnitzii and
563 Bifidobacterium adolescentis. FEMS Microbiol Lett 362.
564 30. Scardovi V, Trovatelli LD. 1965. The fructose-6-phosphate shunt as a peculiar pattern of
565 hexose degradation in the genus Bifidobacterium. Annals of Microbiology 15:19-29.
566 31. Allen-Vercoe E, Daigneault M, White A, Panaccione R, Duncan SH, Flint HJ, O'Neal L,
567 Lawson PA. 2012. Anaerostipes hadrus comb. nov., a dominant species within the human
568 colonic microbiota; reclassification of Eubacterium hadrum Moore et al. 1976. Anaerobe
569 18:523-9.
570 32. Duncan SH, Louis P, Flint HJ. 2004. Lactate-utilizing bacteria, isolated from human feces,
571 that produce butyrate as a major fermentation product. Appl Environ Microbiol 70:5810-7.
572 33. Kelly WJ, Asmundson RV, Hopcroft DH. 1987. Isolation and characterization of a strictly
573 anaerobic, cellulolytic spore former: Clostridium chartatabidum sp. nov. Arch Microbiol
574 147:169-73.

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575 34. Yang JC, Chynoweth DP, Williams DS, Li A. 1990. Clostridium-Aldrichii Sp-Nov, a Cellulolytic
576 Mesophile Inhabiting a Wood-Fermenting Anaerobic Digester. International Journal of
577 Systematic Bacteriology 40:268-272.
578 35. (ed). 2009. Bergey's manual of systematic bacteriology. Volume three, The firmicutes.
579 Springer, Dordrecht ; New York. Accessed
580 36. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, Scott P,
581 Bergerat A, Louis P, McIntosh F, Johnstone AM, Lobley GE, Parkhill J, Flint HJ. 2011.
582 Dominant and diet-responsive groups of bacteria within the human colonic microbiota.
583 ISME J 5:220-30.
584 37. Martinez I, Kim J, Duffy PR, Schlegel VL, Walter J. 2010. Resistant starches types 2 and 4
585 have differential effects on the composition of the fecal microbiota in human subjects.
586 PLoS One 5:e15046.
587 38. Nielsen TH, Wischmann B, Enevoldsen K, Moller BL. 1994. Starch Phosphorylation in Potato
588 Tubers Proceeds Concurrently with de Novo Biosynthesis of Starch. Plant Physiol 105:111-
589 117.
590 39. Donaldson GP, Lee SM, Mazmanian SK. 2016. Gut biogeography of the bacterial microbiota.
591 Nat Rev Microbiol 14:20-32.
592 40. Cockburn DW, Suh C, Medina KP, Duvall RM, Wawrzak Z, Henrissat B, Koropatkin NM.
593 2018. Novel carbohydrate binding modules in the surface anchored alpha-amylase of
594 Eubacterium rectale provide a molecular rationale for the range of starches used by this
595 organism in the human gut. Mol Microbiol 107:249-264.
596 41. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D,
597 Avnit-Sagi T, Lotan-Pompan M, Suez J, Mahdi JA, Matot E, Malka G, Kosower N, Rein M,

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598 Zilberman-Schapira G, Dohnalova L, Pevsner-Fischer M, Bikovsky R, Halpern Z, Elinav E,
599 Segal E. 2015. Personalized Nutrition by Prediction of Glycemic Responses. Cell 163:1079-
600 1094.

601 42. Niness KR. 1999. Inulin and oligofructose: what are they? J Nutr 129:1402S-6S.

602 43. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-
603 index sequencing strategy and curation pipeline for analyzing amplicon sequence data on
604 the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112-20.

605 44. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley
606 BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009.
607 Introducing mothur: open-source, platform-independent, community-supported software
608 for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-41.

609 45. Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and
610 powerful approach to multiple testing. Journal of the royal statistical society Series B
611 (Methodological):289-300.

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613

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614 **FIGURE LEGENDS**

615

616 **Figure 1.** Proposed model of metabolites and microbes that catalyze the flow of carbon from
617 resistant polysaccharides to butyrate. There are cultivated strains from the gut microbiome that
618 possess the metabolic activities proposed for the species listed.

619

620 **Figure 2.** Average fold changes in the relative abundance of sequences representing selected
621 primary degraders of resistant polysaccharides and secondary butyrate fermenters in response to
622 dietary supplements (* $p<0.05$, paired Wilcoxon test). Seq100 represents an unknown species in
623 the family Ruminococcaceae while seq176 is 98.8% identical to *Clostridium chartatabidum.* Both
624 are inferred to be primary degraders (dashed bracket) based on the dynamics of their response to
625 dietary supplements. Bar plot to the right shows the average relative abundance of each species
626 prior to fiber supplementation.

627

628 **Figure 3.** Associations between primary degraders and changes in fecal butyrate concentrations in
629 response to dietary supplementation with resistant potato starch (RPS). For all panels, darker
630 shades indicate an increase in abundance or concentration and lighter shades indicate a decrease
631 or no change. (A) Average relative abundance of putative primary degraders in each individual
632 before (Bef) and during (Dur) RPS supplementation. (B) Change in fecal butyrate in individuals
633 grouped on whether a primary degrader increased ($\Delta > 0$) or did not increase ($\Delta \leq 0$) in relative
634 abundance in response to RPS supplementation (* $p<0.05$, t-test). (C) Butyrate concentrations for
635 each individual before (circles) and during (triangles) RPS supplementation. Subjects are sorted by
636 initial butyrate concentration.

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637

638 **Figure 4**. Pairs of microbes that consistently responded in concert either positively (red) or
639 negatively (blue) to dietary supplementation. Correlations between changes in the abundance of
640 primary degraders and butyrate producers were calculated using the combined dataset that
641 includes responses to all supplements.

642

643 **Figure 5.** Positive relationship between fecal butyrate concentrations and the relative abundance
644 of sequences characterized as *E. rectale* both before and during dietary supplementation with RPS.

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645 **TABLES**

646

647 **Table 1.**

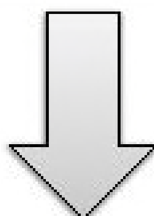
	Butyrate				Acetate				Propionate			
Substrate	before (mean ± SD)	during (mean ± SD)	Change	p-value	before (mean ± SD)	during (mean ± SD)	Change	p-value	before (mean ± SD)	during (mean ± SD)	Change	p-value
Accessible (n=39)	13 ± 6.1	15 ± 8.3	+13%	0.18	41 ± 17	41 ± 16	0%	0.89	9.9 ± 6.0	9.3 ± 6.5	-6%	0.47
Hi-Maize (n=43)	9.3 ± 4.1	9.7 ± 5.6	+5%	0.81	37 ± 17	33 ± 15	-10%	0.20	12 ± 15	12 ± 13	+0.3%	0.81
Potato (n=43)	13 ± 6.0	16 ± 7.5	+29%	**<0.001**	48 ± 22	58 ± 26	+21%	**0.0012**	10 ± 7.7	8.6 ± 5.3	-16%	0.39
Inulin (n=49)	11 ± 6.0	13 ± 7.0	+17%	0.14	38 ± 18	41 ± 20	+8%	0.077	11 ± 10	13 ± 15	+27%	0.31

648 The concentrations of fecal SCFAs (in mmoles/kg) before and during dietary supplements. All p-

649 values are based on repeated measures ANOVA.

Resistant Polysaccharides

- Hi-Maize
- Potato Starch
- Inulin



1° Degraders

- *Bifidobacterium spp.*
- *Ruminococcus bromii*
- Others

Intermediate Products

- Acetate
- Lactate
- Mono/Oligosaccharides

Butyrate producers

- *Faecalibacterium prausnitzii*
- *Eubacterium rectale*
- *Roseburia faecis*
- *Roseburia intestinalis*
- *Roseburia inulinivorans*
- *Eubacterium halii*
- *Anaerostipes hadrus*

Butyrate

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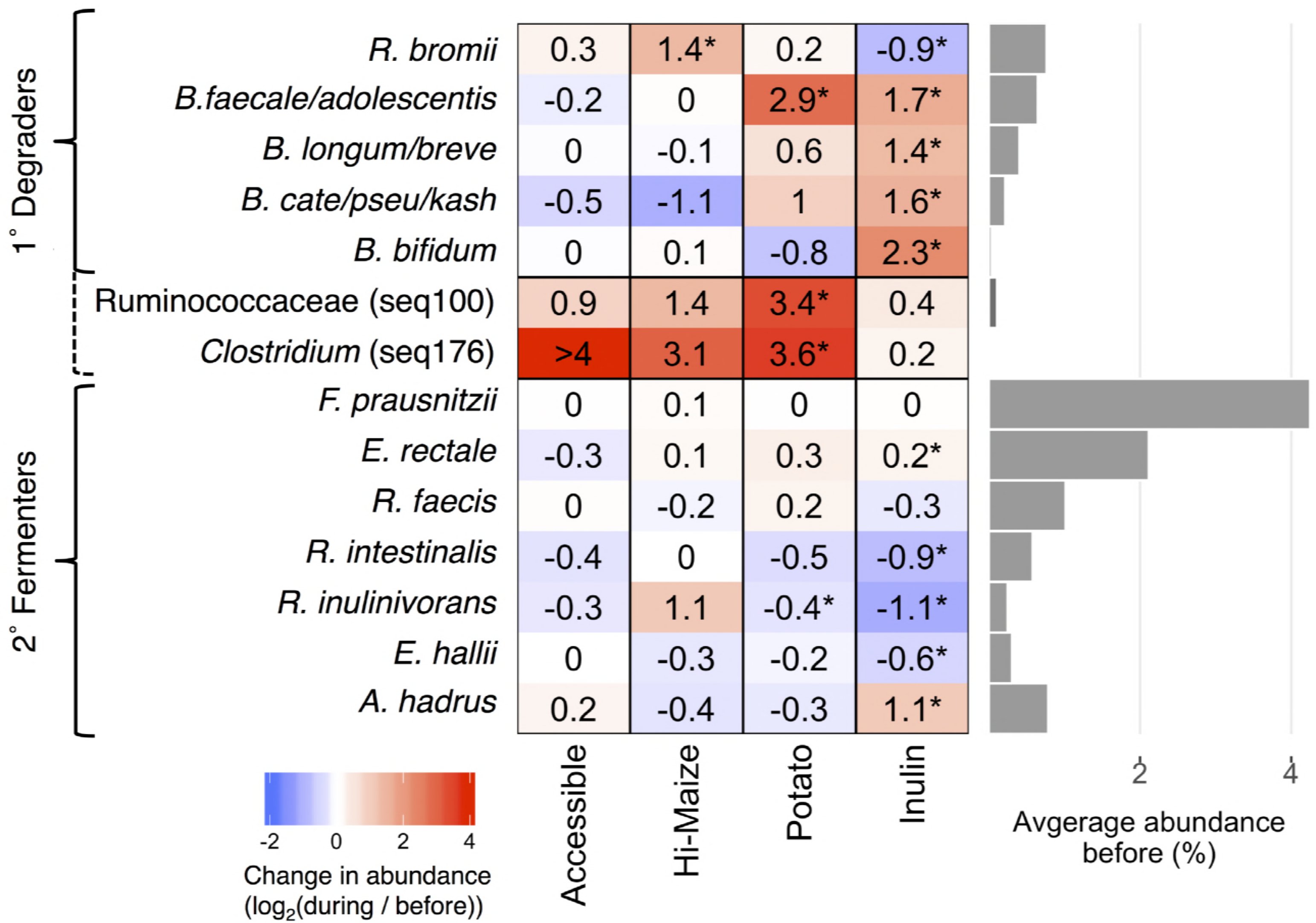
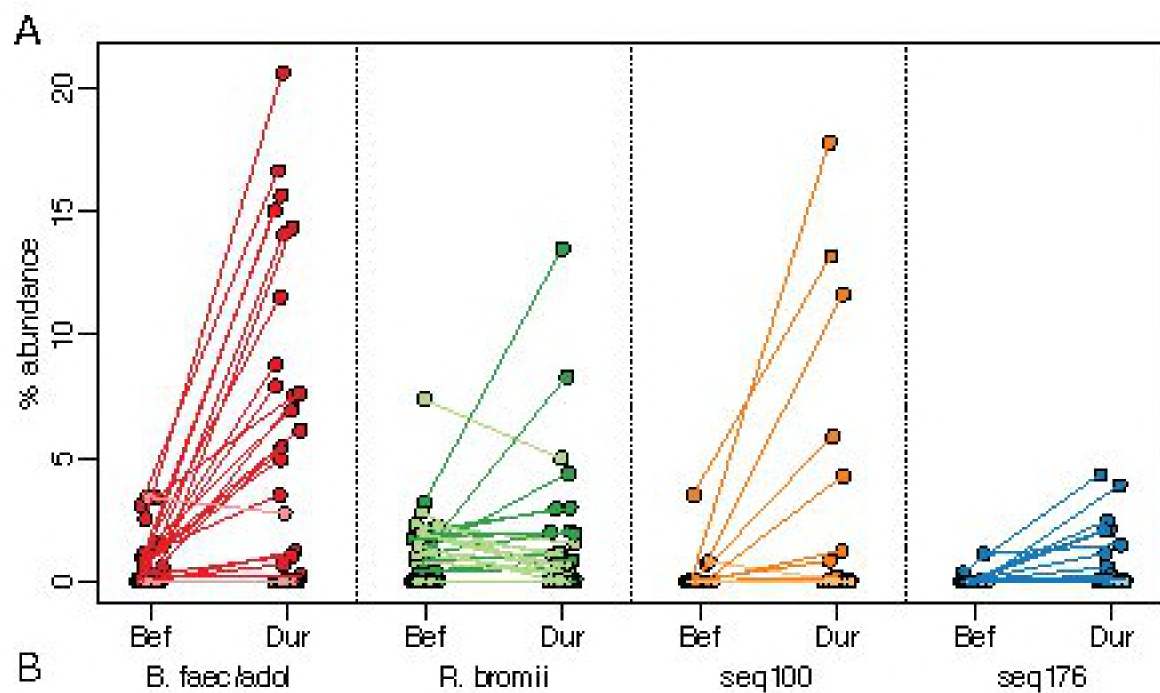
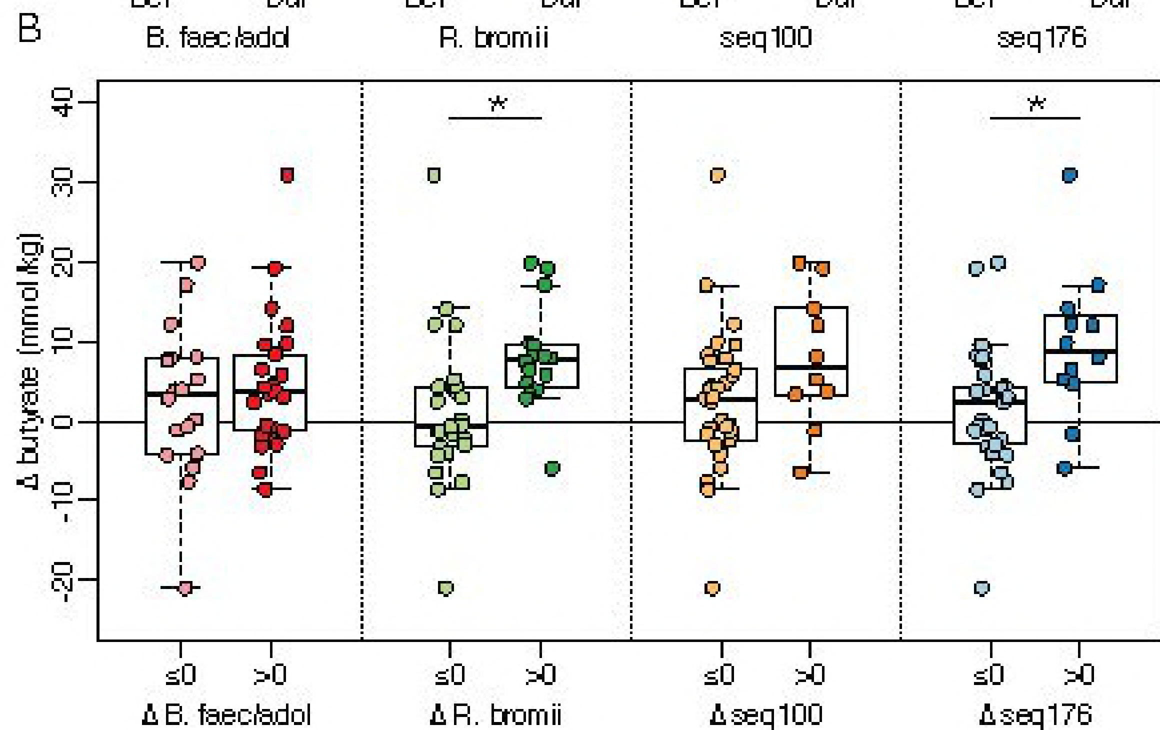
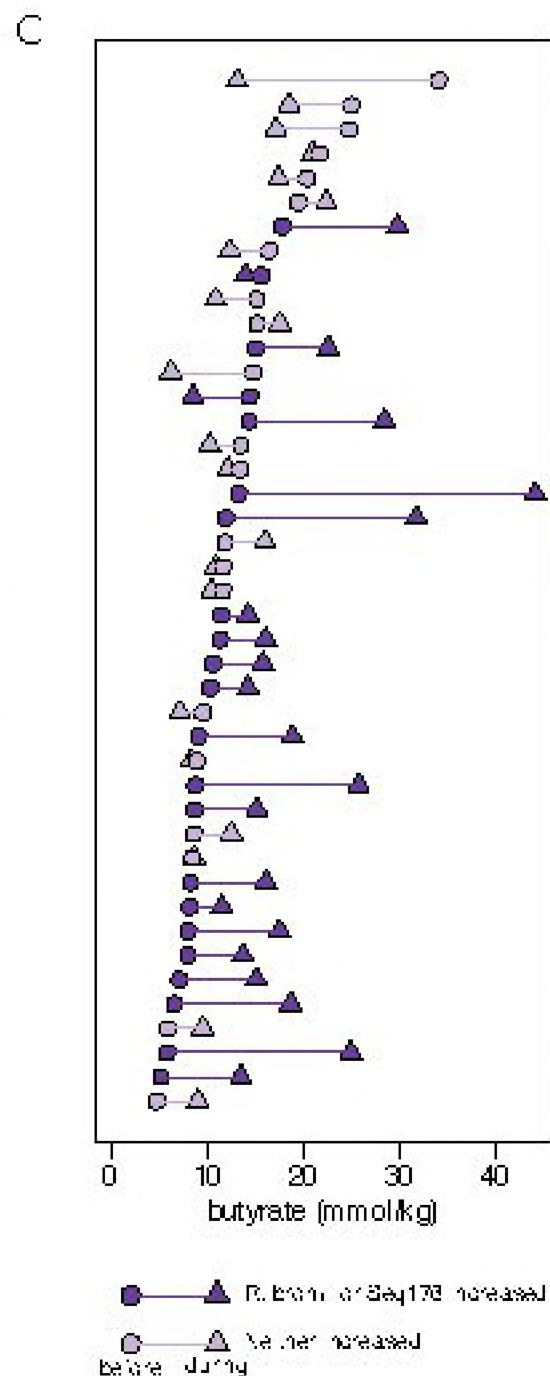




A
% abundance
0
5
10
15
20
Bef
Dur
Bef
Dur
Bef
Dur
Bef
Dur
B. faec/adol
R. bromii
seq100
seq176



B
Δ butyrate (mmol/kg)
-20
-10
0
10
20
30
40
*
*
≤0
>0
≤0
>0
≤0
>0
≤0
>0
Δ B. faec/adol
Δ R. bromii
Δ seq100
Δ seq176



C
0
10
20
30
40
butyrate (mmol/kg)

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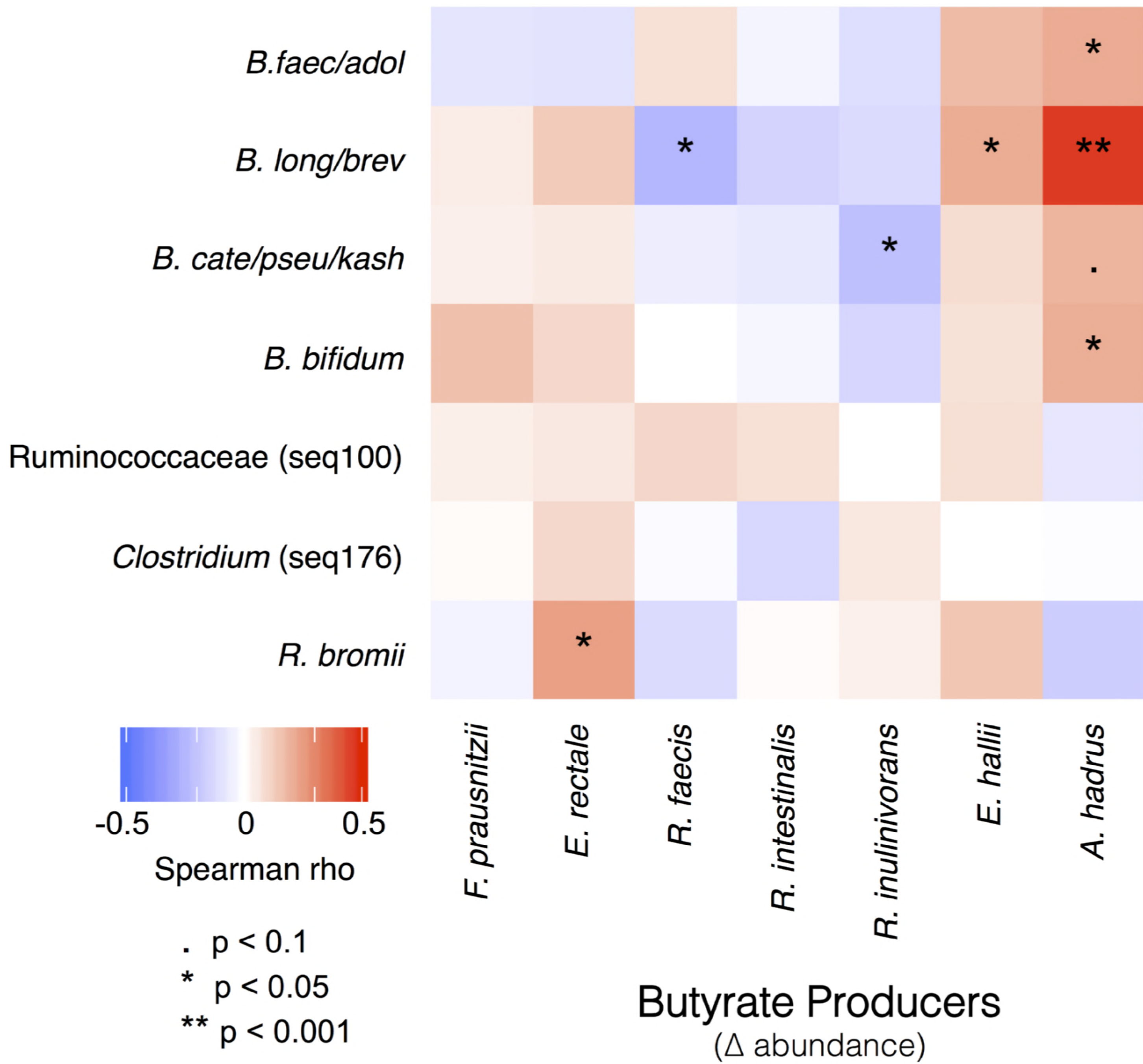

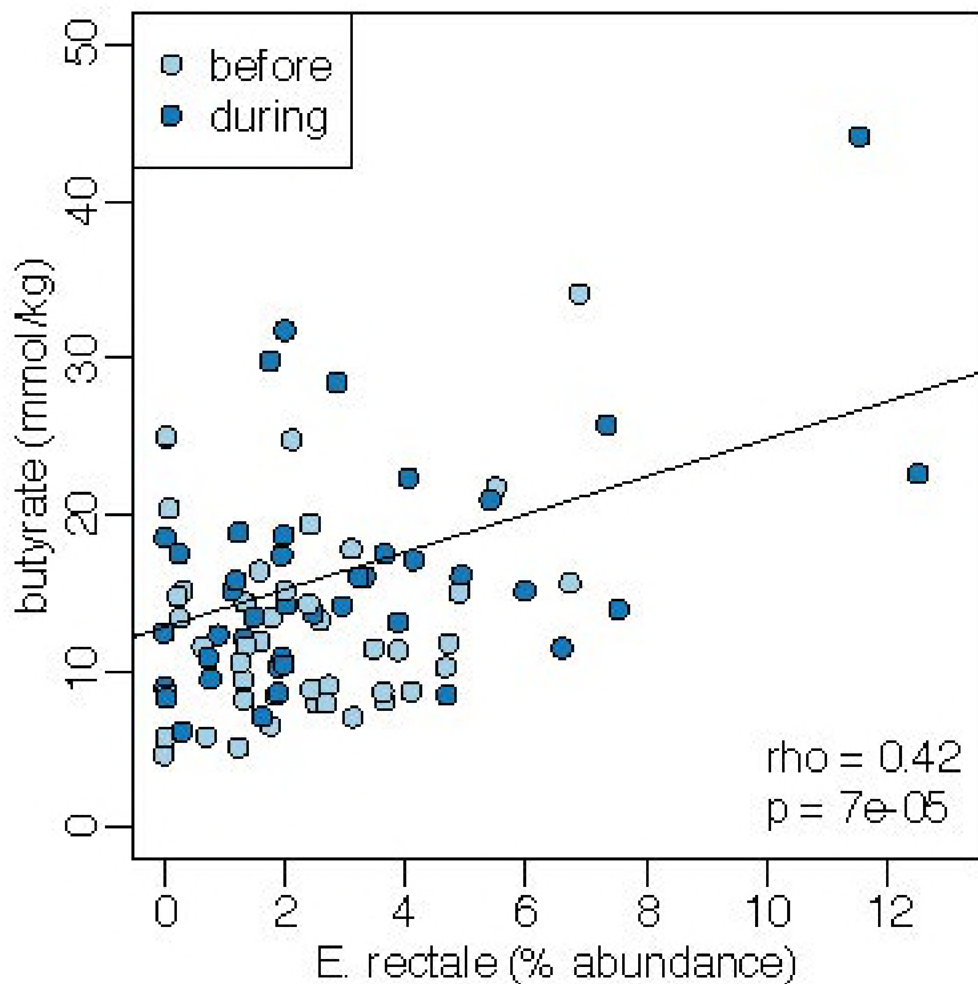

before
during
butyrate (mmol/kg)
0
10
20
30
40
50
0
2
4
6
8
10
12
E. rectale (% abundance)
rho = 0.42
p = 7e-05