

1 **Specific substrate-driven changes in human faecal microbiota composition contrast with**
2 **functional redundancy in short-chain fatty acid production**

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20 **Conflict of interest.**

21 The authors declare that they have no competing interests.

22

23 **Abstract**

24

25 The diet provides carbohydrates that are non-digestible in the upper gut and are major carbon and
26 energy sources for the microbial community in the lower intestine, supporting a complex metabolic
27 network. Fermentation produces the short-chain fatty acids acetate, propionate and butyrate, which
28 have health-promoting effects for the human host. Here we investigated microbial community changes
29 and short-chain fatty acid production during *in vitro* batch incubations of 15 different non-digestible
30 carbohydrates, at two initial pH values with faecal microbiota from three different human donors. To
31 investigate temporal stability and reproducibility, a further experiment was performed one year later
32 with four of the carbohydrates. The lower pH (5.5) led to higher butyrate and the higher pH (6.5) to
33 more propionate production. The strongest propionigenic effect was found with rhamnose, followed
34 by galactomannans, whereas fructans and several α - and β -glucans led to higher butyrate production.
35 16S rRNA gene-based qPCR analysis of 22 different microbial groups together with 454 sequencing
36 revealed significant stimulation of specific bacteria in response to particular carbohydrates. Some
37 changes were ascribed to metabolite cross-feeding, eg. utilization by *Eubacterium hallii* of 1,2-
38 propanediol produced from fermentation of rhamnose by *Blautia* spp. Despite marked inter-individual
39 differences in microbiota composition, short-chain fatty acid production was surprisingly reproducible
40 for different carbohydrates, indicating a level of functional redundancy. Interestingly, butyrate
41 formation was influenced not only by the overall % butyrate-producing bacteria in the community but
42 also by the initial pH, consistent with a pH-dependent shift in the stoichiometry of butyrate production.

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47 **Introduction**

48

49 The relationship between the gut microbiota and host health is well established. The highest
50 concentration and diversity of gut microbes is found in the colon, which acts as a fermentor system for
51 dietary compounds that escape the digestive system of the host. Quantitatively, non-digestible dietary
52 carbohydrates (NDCs) are the main energy sources for bacterial growth in the colon. It is estimated
53 that between 20 and 60 g of NDCs, including plant cell wall polysaccharides, resistant starches (RS),
54 oligosaccharides and sugar alcohols, escape the digestive enzymatic breakdown and reach the human
55 colon each day (Cummings and Macfarlane, 1991). Over the last years research has established that
56 gut bacteria possess an enormous variety of carbohydrate-degrading enzyme activities which allow
57 them to access NDCs (Flint *et al.*, 2012). Microbial fermentation of NDCs mainly leads to the
58 production of the short chain fatty acids (SCFA) acetate, propionate and butyrate, and of lactate,
59 succinate, ethanol, methane, carbon dioxide and hydrogen (Cummings and Macfarlane, 1991). SCFAs
60 are of particular interest for maintaining host health as they are known not only to contribute directly
61 to energy metabolism, but also have positive effects on the host's physiology. Butyrate is mainly
62 metabolised by colonic cells (Hamer *et al.*, 2008), whereas acetate and propionate are absorbed and
63 metabolised by the liver and peripheral organs (Den Besten *et al.*, 2013). Besides serving as an energy
64 source, SCFA are associated with a number of health benefits for the host. Whereas butyrate and
65 propionate have been shown to modulate cell differentiation and to exert anti-carcinogenic and anti-
66 inflammatory effects (Hamer *et al.*, 2008; Louis *et al.*, 2014), acetate and propionate are of interest
67 because of their potential to enhance satiety and suppress appetite either through receptor-mediated or
68 other central mechanisms (Frost *et al.*, 2014; Arora *et al.*, 2011).

69 The intake of NDCs can have direct (primary) and secondary effects on the microbial community
70 in the large intestine, and therefore on the host's physiology. Particular NDCs can lead to the
71 stimulation of specialised groups of microorganisms that possess the carbohydrate active enzymes

72 necessary for their utilization (Flint *et al.*, 2012). Through cross feeding, NDC breakdown
73 intermediates or fermentation products from primary degraders can serve as substrates for secondary
74 degraders, which are not directly capable of degrading a certain carbohydrate. This has been
75 demonstrated *in vitro* (Belenguer *et al.*, 2006; Rogowski *et al.*, 2015) and reflects the complex nature
76 of the intestinal ecosystem. The decrease of pH in the colon due to the production of SCFAs can also
77 lead to selective effects on the microbial community as has been demonstrated *in vitro* (Walker *et al.*,
78 2005; Duncan *et al.*, 2009).

79 The human gut microbiota is composed of several phyla, with the Firmicutes and Bacteroidetes
80 being the most abundant. Firmicutes not only include the major butyrate producing species (Louis *et al.*
81 *et al.*, 2010), but also include propionate producers and acetogens (Louis *et al.*, 2014; Reichardt *et al.*,
82 2014). Bacteroidetes possess genes encoding for the succinate pathway and therefore represent the
83 main propionate producers in the gut (Reichardt *et al.*, 2014). Many *Bacteroides* species are able to
84 degrade a wide range of soluble plant cell wall polysaccharides (Flint *et al.*, 2012; Martens *et al.*,
85 2014). Firmicutes, on the other hand, tend to have fewer genes involved in carbohydrate breakdown
86 (Flint *et al.*, 2012), but specific members appear to play key roles in insoluble polysaccharide
87 degradation (Ze *et al.*, 2013). For example, *Ruminococcus bromii* is of key importance for the
88 degradation of resistant starch (Ze *et al.*, 2015).

89 It is important to obtain a good understanding of how different NDCs are degraded and how this
90 affects the gut microbiota and its fermentation products in order to reach conclusions on their effects
91 upon the host's health. This study investigated the degradation of 15 different NDCs by human faecal
92 bacteria during *in vitro* fermentations. They included α - and β -glucans, pectins, galactomannans,
93 arabinoxylan and fructans to achieve a good representation of different dietary NDCs. Fermentations
94 were run at two different initial pH values to simulate proximal and distal colon conditions. The aim
95 was to gain a comprehensive overview of the microbial changes and SCFA production in a complex
96 community of human faecal microbiota in response to NDC breakdown.

97

98 **Methods**

99

100 *In vitro fermentations*

101 Anaerobic *in vitro* incubations were carried out in a total volume of 10 ml in triplicate in Hungate
102 tubes sealed with butyl rubber stoppers and screw caps (Bellco Glass, Shrewsbury, UK). The medium
103 (details provided in supplementary methods) contained minerals, bile salts, volatile fatty acids,
104 vitamins and 0.2% (wt/vol) of the test NDC. Cysteine was added to the medium following boiling and
105 dispensed into Hungate tubes while they were flushed with CO₂. The vitamin solution and the NDCs
106 were added from stock solutions after autoclaving of the medium, directly before inoculation with the
107 faecal suspension. NDC stock solutions were prepared anaerobically by flushing with CO₂ at 1% in
108 water and boiled for 1 min. 15 different NDCs (Table 1) were used in fermentation 1 and four in
109 fermentation 2 to assess reproducibility. The supplier of one of the NDCs used in fermentation 2 was
110 changed (Table 1), as this study formed part of a larger project that investigated carbohydrate effects
111 *in vivo* (to be reported elsewhere). Ethical approval for the study was granted by the Rowett Institute
112 Ethical review panel (number 09/005).

113 Fresh faecal samples were obtained from 4 different donors (fermentation 1, donors 1, 2, 3;
114 fermentation 2, approximately 12 months later, donors 2b, 3b, 4) with no history of gastrointestinal
115 disorders or antibiotic treatment for at least 3 months prior to the study. Faecal samples were processed
116 within 2 h after defecation. Eight ml of pre-reduced phosphate buffered saline were added to 2 g of
117 faecal sample and then homogenised in a Dispomix Drive (Medic Tools, Lussiwag, Switzerland) and
118 0.5 ml of the homogenised faecal suspension was used as an inoculum for the fermentation tubes (final
119 faecal concentration: 1%). Incubations for each NDC were carried out in triplicate at pH 5.5, and 6.5
120 respectively, on a rotator (Stuart SB3, Bibby Scientific, Stone, UK) at 25 rpm for 24 h at 37 °C. A no-
121 NDC control was run in triplicate with every fermentation experiment. At 0 h, 6 h and 24 h an aliquot

122 of 2 ml was taken from the fermentation vessels and centrifuged at 10000 x g for 10 min at 4°C. The
123 supernatant was stored at -20°C for analysis of SCFA. The cell pellet was re-suspended in 800 µl of
124 sodium phosphate buffer and 122 µl of MT buffer, transferred to a Lysing Matrix E tube (all part of
125 the FastDNA® spin kit for soil, MP Biomedicals, Illkirch, France) and stored at -70°C until DNA
126 extraction. Six h samples were processed if the 24 h sample was not available, as growth had taken
127 place by then. Only primary data of those samples were included (Tables S1A and S2B/C, shown in
128 grey font), and they were excluded for any statistical analyses.

129

130 *DNA extractions*

131 DNA from the faecal inoculates and the cell pellets from the fermentation experiments (resuspended
132 in buffer and stored as described above) was extracted using the FastDNA® spin kit for soil (MP
133 Biomedicals, Illkirch, France). For the DNA extraction of the faecal inoculates an aliquot of 500 µl of
134 the homogenised faecal suspension was transferred to a Lysing Matrix E tube and 300 µl of sodium
135 phosphate buffer and 122 µl of MT buffer was added. The samples were stored at -70 °C until DNA
136 extraction.

137

138 *SCFA analysis*

139 SCFA concentrations were measured in culture supernatants (0.5 ml) using gas chromatography as
140 described previously (Richardson *et al.*, 1989). After derivatisation, 1 µl of sample was analysed using
141 a Hewlett-Packard gas chromatograph fitted with a fused silica capillary column with helium as a
142 carrier gas. The SCFA concentrations were calculated from the relative response factor with respect to
143 the internal standard 2-ethylbutyrate.

144

145 *Quantitative PCR*

146 The pooled triplicate DNA samples from the *in vitro* fermentations 1 and 2 were analysed by
147 quantitative real time PCR as described previously (Fuller *et al.*, 2007; Ramirez-Farias *et al.*, 2009)
148 with the following modifications. Reactions were performed in duplicate with iTaqTM Universal
149 SYBR® Green Supermix (Bio-Rad, Hemel Hempstead, UK) in a total volume of 10 µl with primers
150 at 500 nM and 5 ng of DNA in optical-grade 384-well plates sealed with optical sealing tape in the
151 presence of 1 µg/ml herring sperm DNA (Promega, Madison, WI, USA). Amplification was performed
152 with a CFX384TM Real-time System (Bio-Rad, Hemel Hempstead, UK) with the following protocol:
153 one cycle of 95 °C for 3 min, 40 cycles of 95 °C for 5 s and annealing temperature as per Table S3 for
154 30 s, 1 cycle of 95 °C for 10 s and a stepwise increase of the temperature from 65 °C to 95 °C (at 5 s
155 per 0.5 °C) to obtain melt curve data. Primers used for the quantification of the specific bacterial groups
156 are given in Table S3. Standard curves consisted of dilution series of amplified bacterial 16S rRNA
157 genes from reference strains. The abundance of 16S rRNA gene was determined from standard curves
158 and bacterial groups were either expressed as a percentage of total bacteria determined by universal
159 primers or as 16S rRNA gene copies per ml culture. The detection limit was determined with negative
160 controls containing only herring sperm DNA.

161

162 *454 sequencing*

163 Amplicon sequencing of the V1-V3 region of the 16S rRNA genes was performed on GS FLX 454
164 platform by the Centre of Genomic Research of the University of Liverpool and Bioinformatics were
165 conducted in-house using Mothur v. 1.34.4. software platform (Schloss *et al.*, 2009) on the University
166 of Aberdeen's HPC cluster (Maxwell). Full details are given in supplementary methods. Reads per
167 sample varied from 426 to 82 791 (average 9069.1 ± 11044.5). Good's coverage was over 95% for all
168 but one sample (Table S2B) and rarefaction and collectors curves (Fig. S1) showed that a good
169 coverage was achieved for all samples. Exclusion of samples with Good's coverage of less than 97%
170 resulted in very similar results (Table 2) and subsampling to 426 sequence reads per sample revealed

171 that the relative abundance of the top 50 OTUs (representing >88% of all reads) was very similar to
172 the full dataset (Fig. S2). It was therefore decided to work with the full dataset to preserve as much of
173 the data as possible. OTUs were generated at $\geq 97\%$ sequence identity, which resulted in 1552 OTUs
174 (Table S2C), and the relative abundance was calculated. OTUs with an overall abundance of >100
175 reads (201 OTUs, 95.6-99.8% of sequence reads per sample) were analysed using the BLAST
176 algorithm (Altschul *et al.*, 1990) and compared to the taxonomy from the SILVA database (Quast *et*
177 *al.*, 2013). OTUs were then assigned to their corresponding qPCR assays if possible as detailed in
178 supplementary methods and Table S2A.

179

180 *Polysaccharide analysis*

181 All analyses were performed in duplicate. To evaluate authenticity and purity of the NDCs used in this
182 study, their monosaccharide composition was analysed by HPAEC-PAD after acid hydrolysis as
183 described previously (Wefers and Bunzel, 2015). Details of the hydrolysis conditions for the different
184 NDCs are given in supplementary methods.

185

186 *Statistical analysis*

187 Data from each fermentation study were analysed by ANOVA with random effect for Donor and fixed
188 effects for NDC, pH, and their interaction, followed by post-hoc t-test. Principal Components Analysis
189 and Partial Least Squares were used to investigate associations between bacteria and SCFA production.
190 Associations of interest were quantified by random effects regression with Donor as random effect and
191 with pH, microbial abundance and their interaction as fixed effects. The agreement between the 454
192 sequencing and qPCR methods between corresponding bacterial groups (expressed as percentage of
193 total bacteria) was investigated by linear regression. Full details are given in Supplementary methods.

194 For the regression analyses $P < 0.05$ was regarded significant. For the ANOVA analyses and
195 subsequent post-hoc comparisons, however, to reduce the reporting of false positives due to the large
196 number of comparisons, an effect was considered significant only when $P < 0.001$.

197

198

199 **Results**

200

201 *SCFA from in vitro batch culture incubations*

202 Anaerobic incubations were conducted with 15 different NDCs as energy sources (Table 1; 0.2% w/v)
203 in the presence of human faecal slurries from three healthy donors (fermentation 1) at initial pH values
204 of 5.5 and 6.5. For four NDCs, another fermentation was carried out approximately one year later to
205 assess reproducibility of the responses (fermentation 2, two of the three donors were the same as in
206 fermentation 1). Profiles of net SCFA production after 24 hours of incubation differed between NDCs,
207 especially for those from different NDC classes. Analysis of the chemical composition of NDCs used
208 (Table S4) showed that their composition was consistent with their description. For NDCs used in both
209 fermentations the SCFA profiles were very reproducible (Fig. 1).

210 After 24 hours the cumulative amount of fermentation acids produced was significantly higher at
211 pH 6.5 compared to pH 5.5 in both fermentations (Table S5, $P < 0.001$). The main SCFAs produced
212 were acetate, propionate and butyrate, with pH 6.5 leading to higher acetate and propionate and pH
213 5.5 to higher butyrate formation for most NDCs (Fig. 1, $P < 0.001$). The branched-chain fatty acids iso-
214 butyrate and iso-valerate as well as formate, valerate, and lactate were only detected in minor amounts
215 (< 1.13 mM each), while succinate was not detected in any of the incubations.

216 When individual NDCs were compared to the no-NDC control, starch, β -glucan and
217 inulin/oligofructose gave rise to significantly ($P < 0.001$) increased butyrate concentrations when the
218 initial pH was 5.5. At an initial pH of 6.5, this butyrogenic effect was seen for inulin/oligofructose but

219 not for the starches (Fig. 1). Pyrodextrin, laminarin, rhamnose, rhamnogalacturonan and the two
220 galactomannans all gave rise to increased propionate at initial pH 5.5. These same NDCs increased
221 propionate when the initial pH was 6.5, but in addition pullulan, β -glucan and some of the inulin-type
222 NDCs also promoted propionate significantly at the higher pH (all $P < 0.001$). The NDC that stands out
223 with regard to propionate production however is rhamnose. The absolute amount produced was
224 between 1.5- and 5.5-fold higher than in the presence of the other NDCs, and the molar ratio (40-42%
225 of total SCFA) was similar to the percentage of acetate in both fermentations and independent of the
226 pH (Fig. 1, Table S5).

227

228 *Microbial population changes detected by qPCR*

229 The microbial composition of the *in vitro* incubations from both fermentation experiments was
230 analysed using qPCR against 21 different bacterial species and groups, in addition to total bacteria and
231 methanogenic Archaea (Table S3). Analysis of the inocula revealed high inter-individual variability
232 of bacterial composition as well as intra-individual differences for the donors used in both
233 fermentations (donor 2 and 3). In addition to quantitative differences, certain microbial groups were
234 only found in some faecal samples (some ruminococci, *Coprococcus eutactus*, *Eubacterium eligens*,
235 certain bifidobacteria and methanogenic Archaea), and none of the donors had detectable levels of
236 *Prevotella* spp. (Table S1A). After 24 hours of incubation the total amount of bacteria had increased
237 in all incubations, including the no-NDC control (average fold change of total 16S rRNA gene copies
238 8.0 ± 2.5). In order to investigate bacterial changes specific to the different NDCs, the data were
239 expressed as the ratio of the absolute 16S rRNA gene copies per ml culture between each carbohydrate
240 incubation and the no-NDC control after 24 hours of incubation (Fig. 2, Table S1B). For several
241 bacterial groups and NDCs, responses were similar in the different donors. Thus, *R. bromii*
242 significantly ($P < 0.001$) increased on both types of resistant starch at both pH values, with highest
243 levels reached at pH 5.5 (Fig. 2). Bifidobacteria and several other Firmicutes, especially the *Roseburia*

244 group, also increased on resistant starches and pullulan, in particular at the lower pH value, but due to
245 inter-individual variability this mostly did not reach significance. Barley β -glucan resulted in
246 significant increases in the *Roseburia* group at both pH values in fermentation 2, whereas *R.*
247 *inulinivorans* showed a significant increase on laminarin only at pH 6.5 (Fig. 2, all $P < 0.001$). At the
248 lower pH value, *Blautia* spp. increased significantly on barley β -glucan in fermentation 1, whereas at
249 the higher pH value, *Bacteroides* spp. increased on two of the three β -glucan-type incubations (Fig. 2).
250 *Coprococcus eutactus*, which was only detected in one donor, increased dramatically on barley β -
251 glucan in both fermentations at both pH tested, but not with laminarin (Fig. 2).

252 Rhamnose led to a significant ($P < 0.001$) increase of both *Eubacterium hallii* and *Blautia* spp. at
253 both pH values in fermentation 2, whereas this response was weaker and only observed for *Blautia*
254 spp. at pH 5.5 in fermentation 1 (Fig. 2). NDCs of the pectin class resulted in the highest fold changes
255 relative to the no-NDC control for *F. prausnitzii*, *E. hallii* and *B. bifidum* at pH 5.5, but this did not
256 reach significance. At pH 6.5, *F. prausnitzii* and *E. hallii* showed a significant response on apple pectin
257 during fermentation 2, whereas *Bacteroides* spp. increased significantly ($P < 0.001$) on
258 rhamnogalacturonan. *Eubacterium eligens*, which was not detected in all donors (Table S1), competed
259 poorly on most of the NDCs tested (fold changes relative to no-NDC control < 1 ; Fig. 2), but showed
260 a numerical increase for the pectin-type NDCs (Fig. 2), especially at the higher pH value. For the two
261 galactomannans, *C. eutactus* (present in only one donor, Table S1) increased relative to no-NDC
262 control, and *Bacteroides* spp. had significantly ($P < 0.001$) higher levels on guar galactomannan at pH
263 6.5. Bifidobacteria showed the strongest response to arabinoxylan, at pH 5.5, which reached
264 significance for *B. longum*, whereas *Roseburia* spp. increased significantly on arabinoxylan at pH 6.5
265 only (Fig. 2).

266 The inulin-type fructans tested resulted in significant ($P < 0.001$) increases of the *Roseburia* group
267 and *A. hadrus* at pH 5.5. Increases were also seen for several other groups, including bifidobacteria,
268 *R. bromii* (pH 5.5 only) and *Blautia* spp., but were mostly not significant ($P > 0.001$, Fig. 2, Table S1B).

269 Interestingly *Bifidobacterium* spp. showed mostly higher increases on oligofructose than on medium-
270 or long-chain inulin (Fig. 2). Individual *Bifidobacterium* species were subject to large inter-individual
271 differences (Fig. 3). For example, *B. adolescentis* and *B. longum*, detected in all donors, responded
272 with higher increases on oligofructose in donor 1 and 3, whereas for donor 2 stronger responses were
273 seen with the longer chain inulin-type fructans. *B. adolescentis* showed a much stronger stimulation in
274 donor 2 compared to *B. longum*, regardless of the type of fructan, whereas *B. longum* achieved high
275 levels of stimulation on various NDCs in the other donors. The *B. catenulatum* group responded
276 strongly to several NDCs in donor 1, whereas *B. bifidum* showed the strongest response to fructan-
277 type NDCs in donor 3 (Fig. 3).

278 Interestingly, the *R. flavefaciens* group, *Oscillibacter* group, *Dorea* spp. and Negativicutes group
279 were not significantly stimulated by any of the NDCs tested ($P>0.001$, Fig. 2). Methanogenic Archaea
280 did not exhibit big increases compared to no-NDC control for most incubations they were detected in
281 (Table S1). Regression analysis of SCFA and bacterial groups showed a significant ($P<0.05$) positive
282 correlation of *Bacteroides* spp. and propionate production and of *A. hadrus*, *F. prausnitzii* and
283 *Roseburia* group, respectively, and butyrate production (Fig. S3).

284
285 *Relationship between microbiota composition and SCFA formation*

286 In addition to qPCR, the influence of the different NDCs on the microbiota of fermentation 1 was
287 analysed using 454 sequencing. Like qPCR, operational taxonomic unit (OTU) analysis revealed large
288 inter-individual differences in the inocula. In the sample of donor 1 and donor 3 we detected 116 and
289 114 different OTUs, respectively, with an abundance of >100 reads, whereas the inoculum of donor 2
290 contained 162. OTU 1 (*Blautia obeum*) was the most abundant OTU in donors 2 and 3 and the second
291 most abundant in donor 1 after OTU 8 (*A. hadrus*) (Table S2). Statistical analysis of all OTUs that
292 were detected in at least two thirds of all samples after 24 h of incubation revealed that 17 OTUs,

293 covering a range of different Bacteroidetes and Firmicutes species, were significantly ($P < 0.001$)
294 increased compared to the inoculum on at least one NDC (Table 2).

295 In order to compare the qPCR results with sequencing results, the OTUs were assigned to groups
296 that would be targeted by the qPCR primers used (Fig. S4, Table S2). This led to an assignment rate
297 of 29.2-83.2% per sample (average 59.1%) of all sequences. The results in Fig. S4 show that the
298 changes detected by 454 sequencing agree well with those detected by qPCR. This applies for example
299 to the increase in *Blautia* spp. with rhamnose, in *R. bromii* with RS and in *A. hadrus* with fructans.
300 Correlations were calculated between relative data from qPCR and the sum of OTUs assigned to the
301 corresponding primer set. Significant ($P < 0.05$) correlations were found for all OTU groups which
302 could be assigned to corresponding qPCR primers, except for the *Oscillibacter* group (Fig. S5).
303 Weaker correlations likely reflect technical differences arising from either qPCR or sequencing
304 methodology, or a limited understanding of the groups under study, which may affect the accuracy of
305 assigning sequence OTUs to the corresponding qPCR group. *Bifidobacteria* were not included in this
306 comparison as they are underestimated by 454 sequencing with the primers used here (Walker *et al.*,
307 2015).

308 Heat map analysis of relative OTU abundance revealed high inter-individual variation (Fig. S6, 100
309 most abundant OTUs). Propionate- and butyrate-producing status was assigned to all classified OTUs
310 (Table S2A; 39-87% of sequence data per sample assigned to fermentation product formation based
311 on at least 97% sequence identity to known species) and heat maps for propionate- and butyrate-
312 producing OTUs, respectively, were generated (Fig. 4). This shows that different OTUs contribute to
313 SCFA production in different donors. However, regression analyses of the sum of all propionate- or
314 butyrate-producing bacteria (as percentage of total sequences) to percentage propionate or butyrate
315 produced over 24 h of incubation showed a strong correlation (Fig. 5, $P < 0.001$ for both SCFAs). For
316 propionate the initial pH of the incubations had no effect on this relationship, but for butyrate a strong
317 effect of initial pH was found (Fig. 5, $P < 0.001$). Partial least squares regression was carried out on all

318 classified OTUs to reveal any associations with acetate, propionate or butyrate, which revealed some
319 strong associations that mostly were individual-specific (Table S2A). These may reflect not just a
320 direct conversion of NDCs to SCFA, but could also include cross-feeding effects.

321 Relative qPCR and SCFA data obtained from NDCs that were examined in years one and two after
322 24 h of incubation were further analysed by principal component analysis. This revealed some
323 clustering by donor, but the samples originating from the same donor in different years showed little
324 overlap, showing a relatively large intra-individual variation (Fig. S7A). Rhamnose incubations in
325 particular clustered separately and were associated with propionate production, *Blautia* spp. and *E.*
326 *hallii* (Fig. S7B&D). Long-chain inulin also tended to result in a bigger difference to no-NDC control
327 than the other NDCs examined (Fig. S7B). A separation by pH could be observed especially for the
328 NDCs other than rhamnose, which was associated with butyrate formation at pH 5.5 and propionate
329 formation at pH 6.5 (Fig. S7C&D).

330

331 **Discussion**

332

333 This study investigated the impact of 15 different NDCs upon microbiota composition in anaerobic
334 batch cultures inoculated with human faecal samples. The *in vitro* batch culture system provided a fast
335 and cost effective way to study the effects of an extensive set of NDCs on the microbial community
336 from four donors. Because the pH of batch cultures cannot be controlled precisely as acids are
337 produced during incubation, we set the initial pH at two different values (5.5 and 6.5) to simulate
338 prevailing conditions in the healthy proximal and distal colon. Using 0.2% carbohydrate, we anticipate
339 that acid production during incubation will have reduced the pH further by 0.5-1 unit by 24 hours so
340 that growth will have occurred largely under mildly acidic conditions. When pH is controlled at 6.5
341 using a continuous flow fermentor system, we have shown that *Bacteroides* spp. outcompete
342 Firmicutes and Actinobacteria within the human colonic microbiota for soluble carbohydrate

343 substrates (Walker *et al.*, 2005; Duncan *et al.*, 2003; Chung *et al.*, 2016). By contrast in the present
344 study, this dominance of *Bacteroides* spp. was curtailed by the lower pH conditions, and probably also
345 by the reduction in the peptide content of the medium (to 0.1% casitone and 0.1% yeast extract). This
346 has helped to reveal the response of Firmicutes and Actinobacteria to different carbohydrates.
347 Lowering of gut pH due to increased fermentation may also contribute to the reduced abundance of
348 *Bacteroides* species often observed in human dietary trials with NDC (Martinez *et al.*, 2013; Duncan
349 *et al.* 2009).

350 An overview of the major microbiota responses on the different carbohydrate classes and
351 corresponding pathways for SCFA formation is provided in Fig. 6. The NDC that promoted by far the
352 highest SCFA proportion of propionate was rhamnose. This can be explained by the fact that rhamnose
353 is fermented via the propanediol pathway in some anaerobic bacteria, yielding propionate and
354 sometimes also propanol (Reichardt *et al.*, 2014; Scott *et al.*, 2006; Louis and Flint, 2017). The
355 distribution of the propanediol pathway of propionate formation from deoxy sugars is however quite
356 limited, being found so far in *Roseburia inulinivorans* and in *Blautia* spp. (Reichardt *et al.*, 2014). This
357 fits very well with the observed highly specific enrichment of *Blautia* spp. on rhamnose that was
358 detected both by 16S rRNA-based qPCR and sequence analysis. In other bacteria that can utilise deoxy
359 sugars, such as *Bacteroides* spp. (Rodionova *et al.*, 2013) and *Anaerostipes rhamnosivorans* (Bui *et*
360 *al.*, 2014), propionate is not formed and 1,2-propanediol is an end product. *E. hallii* also showed a high
361 qPCR ratio for rhamnose relative to the no-NDC control. Existing *E. hallii* strains are not known to
362 grow on rhamnose (Holdeman and Moore, 1974), but its stimulation is likely to be indirect, due to
363 cross-feeding of 1,2-propanediol formed from rhamnose by *Bacteroides* spp. and *A. rhamnosivorans*,
364 since a recent study demonstrated the ability of *E. hallii* to metabolise 1,2-propanediol (Engels *et al.*,
365 2016). *E. hallii* also has the ability to utilize lactate (Duncan *et al.*, 2004), which is a major fermentation
366 product of *Blautia faecis* (Park *et al.*, 2013), the *Blautia* species that was most strongly stimulated by
367 rhamnose in these experiments. The propanediol pathway may also contribute significantly to

368 propionate formation from rhamnose residues on rhamnogalacturonan and pectin (25.3 and 4.8% of
369 monosaccharide composition, Table S4), but for the remaining polysaccharides it is expected that
370 propionate will originate mainly via the succinate pathway found in the Bacteroidetes (Reichardt *et*
371 *al.*, 2014). Consistent with this, the level of propionate produced showed a significant correlation with
372 the abundance of *Bacteroides* spp. based on qPCR results (Fig. S3). Based on sequencing data, relative
373 propionate production correlated more strongly with the sum of all propionate producers (Fig. 5) than
374 with propionate producers that employ either the succinate or propanediol pathway (data not shown),
375 confirming that both pathways contribute to propionate formation. The percentage of butyrate among
376 SCFA was highest for fructans at both initial pHs, and for pullulan, resistant starch type II and III and
377 β -glucan at pH 5.5. This appeared to reflect the stimulation of known butyrate-producing species, in
378 particular the *Roseburia* group, *F. prausnitzii*, *A. hadrus*, and *C. eutactus*, depending on the NDC.

379 A number of responses to particular NDCs agreed well with previous reports from *in vivo* and *in*
380 *vitro* studies. They include stimulation of *R. bromii* on resistant starch (Ze *et al.*, 2013; Ze *et al.*, 2015;
381 Walker *et al.*, 2011; Abell *et al.*, 2008; Martínez *et al.*, 2010), of bifidobacteria (Ramirez-Farias *et al.*,
382 2009; Scott *et al.*, 2014; Selak *et al.*, 2016; McLaughlin *et al.*, 2015), butyrate-producing
383 *Roseburia/Eubacterium rectale* group and *A. hadrus* (Louis *et al.*, 2010; Scott *et al.*, 2014; van den
384 Abbeele *et al.*, 2011) and *Dorea longicatena* (Taras *et al.*, 2002) on inulin type fructans, and of
385 *Bacteroides* spp., *F. prausnitzii* and *E. eligens* on pectin-type NDCs (Chung *et al.*, 2016; Lopez-Siles
386 *et al.*, 2012; Salyers *et al.*, 1977). Arabinoxylan increased bifidobacteria at the lower pH, which
387 reached significance for *B. longum* based on qPCR results. This is in agreement with another *in vitro*
388 study that investigated pure culture growth of different *Bifidobacterium* species and found good growth
389 on arabinoxylan only for strains belonging to *B. longum* (McLaughlin *et al.*, 2015).

390 SCFA production was surprisingly reproducible for the different NDCs investigated here compared
391 to the high microbiota variation between donors, which indicated that different OTUs contributed to
392 NDC breakdown and SCFA formation in the different donors. For example, OTU 11 (closest relative

393 *R. bromii*, 94% identity) responded strongly to resistant starch in donor one, whereas it was low in
394 donor 3 and OTU 9 (*R. bromii*, 99%) responded strongly to resistant starch in this donor (Fig. S6).
395 When looking specifically at propionate- or butyrate-producing bacteria, it becomes clear that they
396 show a heterogeneous response to different carbohydrates (Fig. 4), but their combined response
397 correlates very strongly with the corresponding SCFA output (Fig. 5), revealing functional redundancy
398 in the microbiota. The activities of each microbiota member will be dependent not only on their genetic
399 potential to degrade certain NDCs and produce certain SCFA, but also on their interaction with other
400 microbes and their competitive fitness. This likely underlies the different response seen for some OTUs
401 in different donors (for example, OTU 8, *A. hadrus* (100%) showing an increase on arabinoxylan in
402 donor 1 and 3, but not donor 2). The poor response of *R. inulinivorans* on inulin and rhamnose likely
403 also reflects its poor ability to compete effectively in the complete microbiota, despite the fact that it
404 can grow on those NDCs in pure culture (Reichardt *et al.*, 2014; Scott *et al.*, 2014; Duncan *et al.*,
405 2006). This agrees with a human intervention study, which also failed to see an increase in this species
406 after inulin supplementation in subjects with high baseline levels of this species (Louis *et al.*, 2010).

407 Our data also reveal that while the % butyrate among SCFA products was related to the proportion
408 of butyrate-producing bacteria, the initial pH altered this relationship considerably (Fig. 5). It is known
409 that species such as *F. prausnitzii* and *Roseburia* spp. that use the butyryl-CoA:acetate CoA-transferase
410 route for butyrate formation, exhibit a shift in fermentation stoichiometry in pure culture at lower pH
411 (5.5) in favour of greater butyrate production and greater acetate consumption per mol of carbohydrate
412 consumed (Louis and Flint, 2017; Kettle *et al.*, 2015). The relationships seen in Fig. 5 indicate that
413 this shift in stoichiometry applies also to butyrate production by the mixed community, while
414 propionate production was simply related to the % propionate-producing bacteria regardless of the
415 initial pH.

416 In conclusion, the work presented here is one of few *in vitro* studies that compares the impact of a
417 large variety of NDCs on the composition and metabolic activity of the human faecal microbiota. Some

418 of the NDCs investigated here are currently classed as prebiotics, but this study does not reveal a clear
419 distinction between those and NDCs currently classed as dietary fibre, in terms of a selective
420 stimulation of specific bacteria. Prebiotic NDCs are considered to have consequences for health mainly
421 through their impact upon the gut microbiota. These impacts can be ascribed to two types of
422 mechanism. First, as shown here, prebiotics can promote the growth of a limited number of bacterial
423 species, boosting their populations and their representation within the gut microbiota, although the
424 pattern of stimulation can vary between microbiota from different individuals. Some of these bacteria
425 may interact with the host's immune system, but we can expect that inter-individual variability in the
426 microbiota and the selective effects of different NDCs may result in wide variation in health
427 consequences. Second, we know that the metabolites produced by the microbial community have
428 important consequences for health. Here our results suggest, at least for short chain fatty acids, that
429 the consequences of NDC fermentation are likely to be more consistent between individuals. This is
430 explained by the large number of gut anaerobes capable of producing the major SCFA, resulting in
431 functional redundancy that tends to mask inter-individual variation at the species level. Thus we have
432 shown that, in spite of inter-individual differences in microbiota composition, SCFA profiles were
433 very similar for each individual and for a given NDC. We should also note however that for metabolites
434 whose production is limited to a smaller number of species, individual variability is likely to be
435 correspondingly greater. In addition, if keystone species are absent, the capacity of the microbiota to
436 ferment NDC can be greatly reduced, as shown for individuals lacking *R. bromii* on diets high in RS
437 (Walker *et al.*, 2011). There is currently much debate on the prebiotic concept, and the stipulation that
438 they have to selectively stimulate certain microbes is increasingly challenged (Louis *et al.*, 2016;
439 Bindels *et al.*, 2015; Steinert *et al.*, 2016). The data presented here are in support of a more general
440 definition with regard to the modulation of the gut microbiota in order to achieve a beneficial effect
441 on the host.

442

443

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450

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452

453

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584

585

586 **Figure legends**

587

588 **Figure 1:** Net SCFA production after 24 h incubation of human faecal samples with different NDCs.
589 Average of three donors in 2 fermentations (f1 and f2) at pH 5.5 and 6.5 (t=24 h minus t=0, standard
590 error of the difference and percentages given in Table S5). Faecal donors were donor 1, 2 and 3 in f1;
591 and 2, 3 and 4 in f2. Analysed by ANOVA with donor as random effect and with NDC, pH and their
592 interaction as fixed effects. NDCs that differ ($P<0.001$) from the no-NDC control for each of the two
593 pH levels are indicated by *.

594

595 **Figure 2:** Increase of bacterial groups analysed by qPCR after 24 h incubation of human faecal samples
596 with different NDCs. Average of three donors in 2 fermentations (f1 and f2) at pH 5.5 and pH 6.5 in
597 relation to the increases with no NDC (given as relative fold change). Faecal donors were donor 1, 2
598 and 3 in f1 (for pH 5.5 only donors 1 and 3 were included, as no-NDC control was available at 24 h
599 for donor 2; data for all donors are shown in Table S1) and 2, 3 and 4 in f2. Bacterial 16S rRNA gene
600 copies/ml culture were expressed relative to the no-NDC control for each donor and pH. The log-
601 transformed ratios were analysed by ANOVA with donor as random effect and with NDC, pH and
602 their interaction as fixed effects. Presented here are the back-transformed mean log ratios. Test NDCs
603 that differ ($P<0.001$) from the no-NDC control for each of the two pH levels are shown in bold and
604 with a border. Rhamnogal.ur., rhamnogalacturonan; gal.man., galactomannan; MC, medium-chain;
605 LC, long-chain. †, bacterial group not detected in all donors.

606

607 **Figure 3:** Growth response of individual *Bifidobacterium* species on α -glucans, arabinoxyln and
608 fructans. Data shown are from individual faecal incubations (fermentation 1 donor 1, 2, 3; fermentation
609 2 donor 2b, 3b, 4) analysed by qPCR after 24 h incubation at pH 5.5. At 0 h, *Bifidobacterium* species
610 levels in all donors ranged from 6.1×10^4 to 9.7×10^6 /ml (see Table S1).

611

612 **Figure 4:** Relative abundance of propionate- and butyrate-producing OTUs with at least 97% sequence
613 identity to known bacterial species after 24 h incubation of human faecal samples from fermentation
614 1 with different NDCs (white – black: panel A, Propionate producing OTUs 0 – 34%; panel B, Butyrate
615 producing OTUs 0 – 24%). Rationale for assignment of SCFA production capacity is given in Table
616 S2A. Relative production of the corresponding SCFA is given at the top of each heat map (white –
617 black: panel A, Propionate % 0 - 43%; panel B, Butyrate % 0 – 47%). OTUs showing a significant
618 ($P < 0.001$) increase under certain conditions, compared to inoculum, are indicated by * (for details see
619 Table 2). AP, apple pectin; AX, arabinoxylan; BG, β -glucan; Ca, carob galactomannan; Gu, guar
620 galactomannan; i, inoculum; I-GP, medium-chain inulin; I-HP, long-chain inulin; La, laminarin; no,
621 no-NDC; OF, oligofructose; Pu, pullulan; Py, pyrodextrin; RG, rhamnogalacturonan; Rh, rhamnose;
622 RSII, type II resistant starch; RSIII, type III resistant starch. Panel A: Acr, acrylate pathway; Pdu,
623 propanediol pathway.

624

625 **Figure 5:** Relationship between sum of all propionate- or butyrate-producing OTUs with at least 97%
626 sequence identity to known bacterial species after 24 h of incubation of human faecal samples from
627 fermentation 1, expressed as percentage of total sequences, and SCFA production. Relative propionate
628 or butyrate production (percentage of total SCFA produced) was regressed on percentage of propionate
629 or butyrate producers, using mixed effect models with donor as random effect and with fixed effects
630 for pH, percentage producers, and their interaction. pH 5.5, crosses; pH 6.5, triangles. Lines correspond
631 to the fit for each pH where a significant effect of pH was observed (B, solid line pH 5.5, dashed line
632 pH 6.5).

633

634 **Figure 6:** Overview of known fermentation pathways for SCFA formation in human gut bacteria. NDC
635 class colour is based on whether they mainly stimulated propionate (red) or butyrate (blue) production

636 or both (purple) (see Fig. 1). Responses of gut bacteria to different NDCs detected in this study are
637 shown above each NDC class; significant responses detected by qPCR (see Fig. 2, compared to no-
638 NDC) and 454 sequencing (see Table 2, compared to inoculum, only OTUs with >97% identity to
639 known species given) are shown in black, non-significant qPCR-based responses with a fold change
640 >10 are shown in grey.

641

642 **Supplementary Information**

643

644 **Fig. S1:** Rarefaction curves of 454 sequencing data. The inset shows collectors curves of observed
645 OTUs for the two samples with the lowest number of sequences.

646

647 **Fig. S2:** Relative abundance of 50 most abundant OTUs after 24 h incubation of human faecal samples
648 from fermentation 1 with different NDC from full sequence dataset (A) compared to corresponding
649 OTUs after subsampling to 426 sequence reads (B).

650

651 **Fig. S3:** Relationship between log bacterial 16S rRNA gene copies/ml culture and propionate or
652 butyrate production. Combined qPCR data from fermentations f1 and f2 for *Bacteroides* spp. (A), *A.*
653 *hadrus* (B), *F. prausnitzii* (C) and the *Roseburia* groups (D). SCFA production was regressed on log
654 bacterial 16S rRNA gene copies/ml culture observed at 24 h using a mixed effects model with donor
655 and year within donor regarded as random effects, and with fermentation, pH, log bacterial 16S rRNA
656 gene copies/ml culture, and their interactions as fixed effects. Significant effects ($P < 0.05$) are listed
657 above each plot. pH 5.5: blue, pH 6.5: red, fermentation 1: circles, fermentation 2: +. Lines correspond
658 to the fit for each year and pH combination (solid lines: fermentation 1, dashed lines: fermentation 2).

659

660 **Fig. S4:** Relative abundance of OTUs after 24 h incubation of human faecal samples with different
661 NDCs. Average data from three donors from fermentation 1 analysed by 454 sequencing. OTUs are
662 grouped together per corresponding qPCR assay. Assignment of individual OTUs to corresponding
663 qPCR groups are given in Table S2A. This figure also provides a comparison of the microbiota
664 composition between the inoculum and no-NDC (24 h incubation) control. Certain groups, notably
665 *Blautia* spp., *Roseburia* and *E. hallii*, appear to have decreased in relative abundance from the
666 inoculum to the 24 h incubation (no-NDC) at both pH values. Possibly these groups may be less able
667 to replicate and/or become more prone to cell lysis in the absence of an added carbohydrate energy
668 source; this effect may therefore have amplified some of the changes shown in Fig. 2, which compares
669 24 h incubations with and without added NDCs.

670

671 **Fig. S5:** Linear regression analysis between microbial composition as determined by qPCR and 454
672 sequencing. The analysis was performed for bacterial groups that were detected by both methods (*C.*
673 *eutactus* was only found in D3 by qPCR, corresponding OTU-137 was found in 6 and 3 of 31 samples,
674 respectively, in D1 and D2, see Table S2). Blue, donor 1; green, donor 2; purple, donor 3; lighter
675 colours pH 5.5; darker colours pH 6.5.

676

677 **Fig. S6:** Relative abundance of 100 most abundant OTUs after 24 h incubation of human faecal
678 samples from fermentation 1 with different NDCs (white - black: 0 - 51%). Relative production of the
679 corresponding SCFA is given at the top of each heat map (white - black: 0 - 47%). OTUs showing a
680 significant increase ($P < 0.001$) under certain conditions, compared to inoculum, are indicated by * (for
681 details see Table 2). NDC abbreviations as per Fig. 4.

682

683 **Fig. S7:** Principal component analysis of relative SCFA and qPCR data of NDCs included in both
684 fermentation years. A: scores plot colour-coded by donor and year. B: scores plot colour-coded by

685 NDC. C: scores plot colour-coded by pH. NDC abbreviations as per Fig. 4; pH 6.5, italics. D: loading
686 plot of variables. Ac, acetate; But, butyrate; Prop, propionate; Ahad, *A. hadrus*; Bact, *Bacteroides* spp;
687 Bif, *Bifidobacterium* spp.; Blaut, *Blautia* spp.; Dorea, *Dorea* spp.; Ehal, *E. hallii*; Fprau, *F. prausnitzii*;
688 Neg, Negativicutes; Osc, *Oscillibacter* group; Rbro, *R. bromii*; Rfla, *R. flavefaciens* group; Rinul, *R.*
689 *inulinivorans*; Ros, *Roseburia* group.

690

691 **Table S1:** qPCR analysis of faecal incubations. A: 16S rRNA gene copies per ml culture for each
692 donor in the inoculum and after 24 h of incubation (grey font: 6 h of incubation as 24 h sample was
693 not available). Key for bacterial groups given to right of table; colour shading by conditional formatting
694 per bacterial group (yellow low - green high values); nd: not detected. B: Average fold change and
695 confidence interval (for groups present in all donors) after growth on different NDCs compared to no-
696 NDC control after 24 h of incubation (6 h samples were excluded).

697

698 **Table S2:** 454 sequencing analysis of faecal incubations of fermentation experiment 1. A: Average
699 relative abundance of operational taxonomic units (OTUs, 97% sequence identity; abundance > 100
700 reads; complete dataset given in C below, row 839) after 24 h of incubation of human faecal samples
701 (n=3, fermentation 1, 6 h samples were excluded; individual donor data are given under B below, row
702 213) with different NDCs at pH 5.5 and 6.5. Assignment to butyrate or propionate producing status
703 based on closest known species is given in columns AR-AY; Partial least squares regression of
704 association with acetate, propionate or butyrate in columns AX-BJ. B: Relative abundance of
705 operational taxonomic units per individual donor after 24 h incubation (grey font: 6 h of incubation as
706 24 h sample was not available; empty cells: not enough sequences obtained for those samples). C:
707 Number of sequence reads obtained per sample for all 1552 OTUs (97% identity; grey font: 6 h of
708 incubation as 24 h sample was not available).

709

710 **Table S3:** Quantitative PCR primers and annealing temperatures used in this study.

711

712 **Table S4:** Monosaccharide composition (mol%) of the NDCs used in this study. Monosaccharides
713 were analysed by HPAEC-PAD after methanolysis followed by TFA hydrolysis (apple pectin and
714 rhamnogalacturonan I), mild TFA hydrolysis (oligofructose, medium-chain inulin, and long-chain
715 inulin), and sulfuric acid hydrolysis (all other samples).

716

717 **Table S5:** Net SCFA production (mM) and proportions after 24 h incubation of human faecal samples
718 with different NDCs. Average and individual data from 2 fermentations (f1: d1, 2, 3 and f2: d2, 3, 4)
719 at pH 5.5 and 6.5.

720

Table 1: NDCs used for anaerobic *in vitro* incubations with human faecal samples in fermentation (f) 1 and 2 and their suppliers.

Class	NDC	Commercial name and supplier	f1	f2
α -glucans	Pyrodextrin	Fibersol-2; gifted by Matsutani, Itami-City, Japan	x	
	Pullulan	Megazyme, Bray, Ireland (Cat No P-PULLN)	x	
	Resistant starch type II	Hylon VII, National Starch & Chemical Comp., Bridgewater, USA	x	
	Resistant starch type III	Novelose330, National Starch & Chemical Comp., Bridgewater, USA	x	
β -glucans	β -Glucan from barley	Megazyme, Bray, Ireland (Cat No P-BGBL)	x	
	β -Glucan from barley	Glucagel, PolyCell Technologies, Crookston, USA		x
	Laminarin	Sigma Aldrich, UK (Cat No L9634)	x	
Methyl-pentose	Rhamnose	Sigma Aldrich, UK (Cat No W373011)	x	x
Pectins	Rhamnogalacturonan from potato	Megazyme, Bray, Ireland (Cat No P-RHAM1)	x	
	Apple pectin	Sigma Aldrich, UK (Cat No 76282)	x	x
Galactomannans	Carob galactomannan	Megazyme, Bray, Ireland (Cat No P-GALML)	x	
	Guar galactomannan	Megazyme, Bray, Ireland (Cat No P-GGMMV)	x	
Hemicellulose	Arabinoxylan	Megazyme, Bray, Ireland (Cat No P-WAXYL)	x	
Inulin-type fructans	Oligofructose, DP=2-8	Orafti P95, gifted by Beneo, Tienen, Belgium	x	
	Medium-chain inulin, average DP \geq 10	Orafti GR, Beneo, Tienen, Belgium	x	

Long-chain inulin, average DP ≥ 23

Orafti HP, gifted by Beneo, Tienen, Belgium

x x

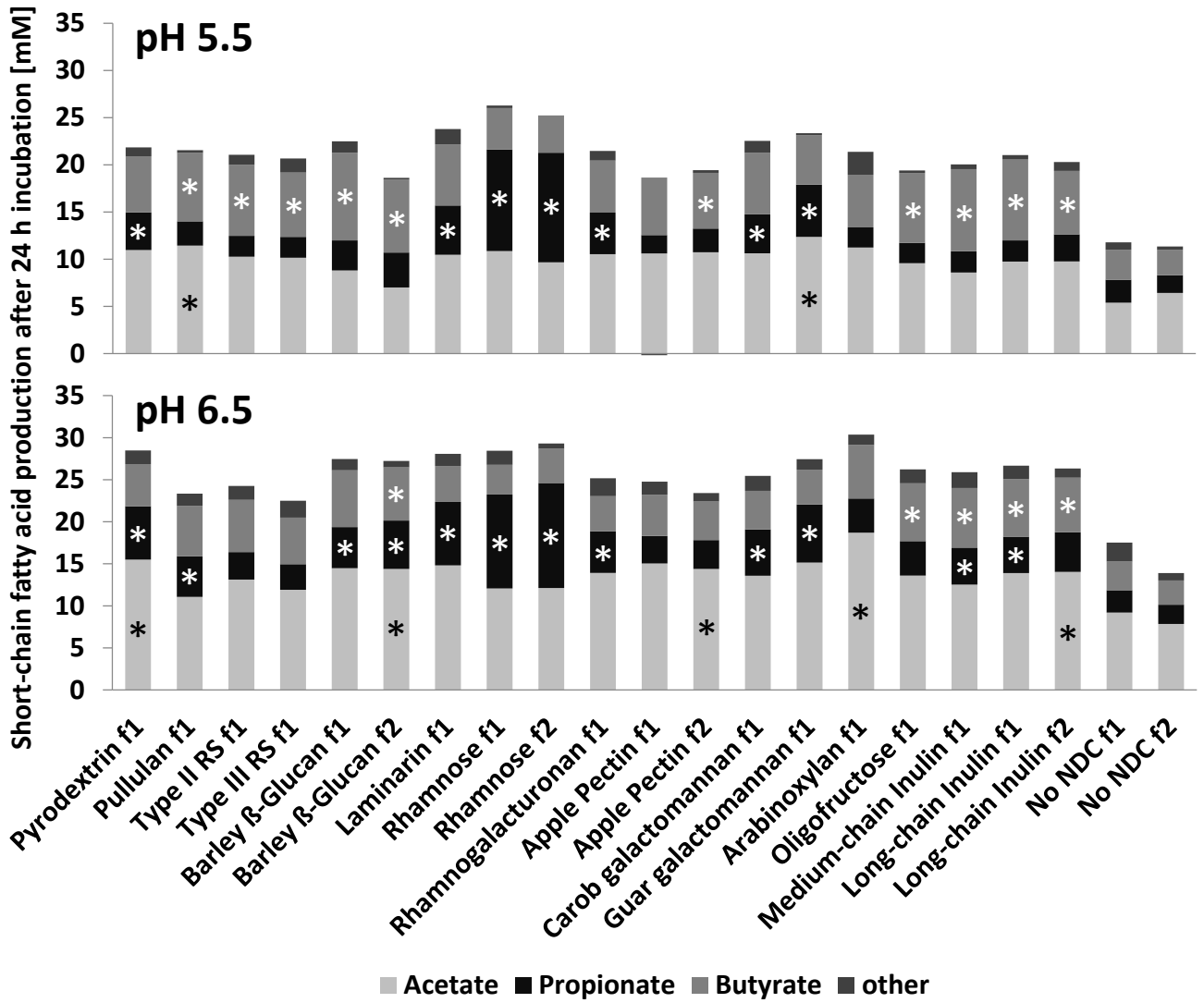
Table 2: Operational taxonomic units (OTUs) from 454 sequencing analysis of fermentation 1 exhibiting a significant increase on specific NDCs and pH values (P<0.001, see Table S2).

OTU No.	closest relative bacterial species (BLAST)	Identity	Non-digestible carbohydrate	
			pH 5.5	pH 6.5
Otu0002	<i>Faecalibacterium prausnitzii</i>	99%	apple pectin ^{1,2}	apple pectin ^{1,2} carob galactomannan ^{1,2}
Otu0003	<i>Clostridium spiroforme</i>	93%	laminarin ^{1,2}	
Otu0005	<i>Bacteroides uniformis</i>	100%		pyrodextrin ^{1,2} laminarin ^{1,2} guar galactomannan ^{1,2}
Otu0006	<i>Blautia faecis</i>	99%	rhamnose ^{1,2}	rhamnose ^{1,2}
Otu0010	<i>Fusicatenibacter saccharivorans</i>	99%	laminarin ¹ carob galactomannan ^{1,2}	
Otu0013	<i>Subdoligranulum variabile</i>	99%	arabinoxylan ^{1,2}	
Otu0017	<i>Oscillibacter ruminantium</i>	96%		rhamnogalacturonan ¹
Otu0018	<i>Dorea longicatena</i>	99%	pullulan guar galactomannan	

			medium-chain inulin ^{1,2}	
Otu0024	<i>Lactobacillus rogosae</i>	96%	rhamnogalacturonan ¹	
			guar galactomannan	
Otu0026	<i>Parabacteroides merdae</i>	98%		guar galactomannan ¹
Otu0027	<i>Bacteroides thetaiotaomicron</i>	99%		barley β -glucan ¹
Otu0031	unclassified		carob galactomannan ¹	
			guar galactomannan	guar galactomannan ¹
Otu0037	unclassified		rhamnogalacturonan ¹	
Otu0041	<i>Flavonifractor plautii</i>	96%	pullulan ¹	rhamnogalacturonan ¹
Otu0043	<i>Eubacterium ventriosum</i>	98%	pyrodextrin	
			pullulan ¹	
			laminarin ¹	
Otu0045	<i>Bacteroides ovatus</i>	100%		barley β -glucan ¹
Otu0055	<i>Clostridium bolteae</i>	97%	rhamnogalacturonan	

1 ¹also significant after removal of samples with <97% Good's coverage

2 ²also significant after subsampling to lowest coverage (426 sequence reads)



NDC	f	Bacteroides spp. F. prausnitzii R. bromii R. albus gr.† R. flavefaciens gr. Oscillibacter gr. Roseburia gr. R. inulinivorans E. hallii A. hadrus Blautia spp. Dorea spp. C. eutactust† Negativicutes Bifidobacterium spp. B. adolescentis B. longum B. catenulatum gr.† B. bifidum†																				
		0.9	1.5	1.2	0.7	1.1	0.5	1.6	1.0	0.8	1.4	1.7	0.7	1.2	0.7	0.8	1.5	1.2	1.0	1.9	3.4	
pH 5.5	Pyrodextrin	1	0.9	1.5	1.2	0.7	1.1	0.5	1.6	1.0	0.8	1.4	1.7	0.7	1.2	0.7	0.8	1.5	1.2	1.0	1.9	3.4
	Pullulan	1	0.6	1.9	3.0	0.7	0.7	0.3	3.1	1.6	1.3	2.1	1.8	0.8	4.3	0.5	0.9	3.5	5.7	4.5	1.9	2.4
	Type II RS	1	0.8	1.9	12.8	1.1	1.1	0.5	3.6	1.3	2.1	2.5	1.8	1.1	5.5	0.7	1.0	8.3	14.9	3.4	7.3	3.8
	Type III RS	1	0.8	2.5	13.8	1.1	1.1	0.4	2.6	1.5	1.6	1.3	1.2	1.2	3.2	0.8	0.8	3.5	6.7	1.7	2.8	2.6
	β-Glucan	1	0.6	2.5	1.2	0.8	1.5	0.3	2.4	2.0	1.4	3.3	4.2	0.8	18.4	0.6	1.1	1.2	0.8	0.8	1.6	2.9
	β-Glucan	2	1.6	2.2	1.5	nd	0.6	0.6	5.7	2.7	1.7	2.2	2.6	0.7	10.6	0.5	0.7	3.1	3.0	2.3	2.8	1.1
	Laminarin	1	1.2	1.6	1.6	1.2	0.7	0.7	1.6	2.3	1.7	2.1	1.3	2.3	2.0	0.6	0.8	0.7	0.8	0.8	1.0	2.6
	Rhamnose	1	1.0	1.2	1.2	1.2	0.9	0.4	1.3	1.5	3.2	0.7	5.7	0.6	2.3	0.6	0.7	0.7	0.6	0.8	1.0	2.2
	Rhamnose	2	1.7	1.0	1.3	nd	0.3	0.8	1.1	1.1	12.8	0.6	13.3	0.8	2.0	0.4	0.4	1.1	1.1	1.0	1.0	0.9
	Rhamnagal.ur.	1	1.5	2.9	1.2	0.8	1.2	0.5	1.5	0.9	2.6	1.0	1.4	1.1	1.1	1.5	0.6	1.0	1.1	1.2	1.5	3.6
	Apple Pectin	1	0.9	3.4	2.3	0.7	0.9	0.4	1.4	1.2	1.5	1.9	1.3	1.1	2.7	1.5	1.1	2.0	2.1	1.9	2.6	5.3
	Apple Pectin	2	1.3	2.8	1.4	nd	0.3	1.1	1.2	1.2	2.8	0.9	1.6	1.6	1.4	1.7	0.5	2.3	2.2	1.6	1.9	1.4
	Carob gal.man.	1	0.4	0.8	0.4	0.7	0.3	0.1	0.9	0.5	0.4	0.4	0.5	0.5	12.8	0.2	0.3	0.5	0.3	0.2	1.2	2.2
	Guar gal.man.	1	1.2	1.2	1.7	4.6	0.7	0.8	1.8	1.1	0.7	0.6	1.4	1.7	3.7	0.6	0.7	0.7	0.7	0.7	0.8	1.9
	Arabinoxylan	1	0.6	0.9	1.2	0.8	0.9	0.3	1.8	1.4	1.1	2.6	1.2	0.7	1.9	0.5	0.7	5.3	5.5	12.0	4.5	2.9
	Oligofructose	1	0.8	1.8	4.4	1.0	1.3	0.5	3.5	1.6	2.4	4.7	1.9	1.7	4.1	0.7	1.2	7.0	8.0	5.5	7.3	11.5
	MC Inulin	1	0.5	2.4	4.2	0.6	1.2	0.3	4.2	1.6	1.8	5.2	2.2	1.2	4.2	0.5	1.2	1.7	1.8	1.6	2.2	6.7
LC Inulin	1	0.5	2.2	4.2	0.7	1.0	0.2	3.7	1.4	1.8	5.2	2.8	1.2	4.7	0.5	0.9	1.5	1.5	1.4	1.9	5.9	
LC Inulin	2	0.9	1.9	2.3	1.1	1.3	0.3	3.5	1.1	1.5	7.4	3.1	0.8	2.7	0.4	0.5	5.3	5.3	2.0	1.5	2.1	
pH 6.5	Pyrodextrin	1	2.2	0.3	0.5	0.8	0.2	0.4	1.1	0.6	0.1	0.1	0.2	1.4	1.0	0.4	0.3	0.1	0.1	0.7	0.1	nd
	Pullulan	1	2.1	1.2	1.8	1.3	0.8	0.3	2.4	1.2	2.0	1.2	1.6	1.0	2.7	0.8	1.2	2.1	8.3	2.5	0.0	0.8
	Type II RS	1	1.0	1.4	6.3	1.0	0.9	0.4	4.1	1.9	2.3	1.3	2.3	1.2	1.0	0.6	0.6	3.6	4.6	1.7	4.7	0.9
	Type III RS	1	1.2	0.7	7.0	0.9	0.3	0.5	2.0	0.9	0.4	0.2	0.3	1.1	2.1	0.4	0.3	0.4	0.6	0.6	0.1	0.0
	β-Glucan	1	1.5	0.7	0.4	1.2	0.5	0.3	3.2	3.2	0.3	0.3	0.5	0.7	9.8	0.4	0.4	0.2	0.1	0.4	0.1	nd
	β-Glucan	2	2.6	2.2	0.9	nd	0.3	0.8	4.6	4.5	3.2	1.7	1.8	0.8	9.5	0.7	1.2	2.5	2.5	2.4	1.8	1.1
	Laminarin	1	2.8	1.0	1.0	0.9	0.8	0.5	1.0	9.0	1.9	1.2	1.5	1.3	0.8	0.6	1.8	1.2	0.8	1.7	1.1	0.7
	Rhamnose	1	1.6	0.6	0.4	0.9	0.3	0.3	0.7	0.6	0.8	0.2	0.7	0.2	1.5	0.3	0.3	0.2	0.2	0.4	0.1	0.0
	Rhamnose	2	1.5	1.3	2.0	nd	0.3	1.2	1.0	0.9	17.9	0.8	12.4	1.0	0.9	0.8	0.7	1.4	1.3	1.1	1.0	0.8
	Rhamnagal.ur.	1	2.6	0.7	0.3	0.7	0.2	0.7	0.5	0.6	0.5	0.2	0.2	1.2	1.4	4.1	0.4	0.1	0.1	0.5	0.2	nd
	Apple Pectin	1	1.2	1.9	0.9	0.9	0.9	0.5	1.1	1.1	1.7	1.1	1.5	1.2	0.9	2.7	0.8	1.6	1.4	1.4	1.5	1.7
	Apple Pectin	2	1.7	5.9	1.3	nd	0.1	1.5	1.2	0.9	7.4	1.0	1.5	1.5	1.1	1.9	0.7	2.4	2.3	1.9	1.7	1.3
	Carob gal.man.	1	1.6	0.6	0.5	1.3	0.3	0.3	1.5	0.7	0.3	0.4	0.4	1.0	5.4	0.3	0.6	0.4	0.4	0.9	0.3	nd
	Guar gal.man.	1	2.2	1.0	1.0	2.2	0.6	0.5	2.5	0.8	1.0	0.9	1.3	1.0	4.7	0.7	1.4	0.4	1.0	1.8	0.8	0.9
	Arabinoxylan	1	1.2	0.3	0.3	0.7	0.1	0.3	5.0	1.2	0.2	0.7	0.2	1.4	1.3	0.3	0.5	0.2	0.1	1.7	0.6	nd
	Oligofructose	1	1.4	1.7	1.8	1.5	1.1	0.4	2.4	1.2	3.7	2.5	2.2	1.5	2.2	0.9	1.0	2.8	9.1	3.2	8.0	3.0
	MC Inulin	1	1.3	1.9	1.6	1.3	1.3	0.4	3.1	1.3	2.9	2.6	2.8	1.4	2.6	0.8	1.2	1.6	7.0	2.3	0.1	2.8
LC Inulin	1	1.0	1.1	0.8	1.3	0.4	0.3	2.1	1.1	0.4	0.8	0.7	1.3	2.2	0.4	0.5	0.6	1.1	0.9	0.1	nd	
LC Inulin	2	1.6	3.1	1.4	nd	1.1	0.8	2.1	0.9	4.3	3.1	3.3	1.0	1.0	0.6	1.2	5.8	4.9	2.3	1.1	1.5	

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- ≤1
- >1
- >2.5
- >5
- >10

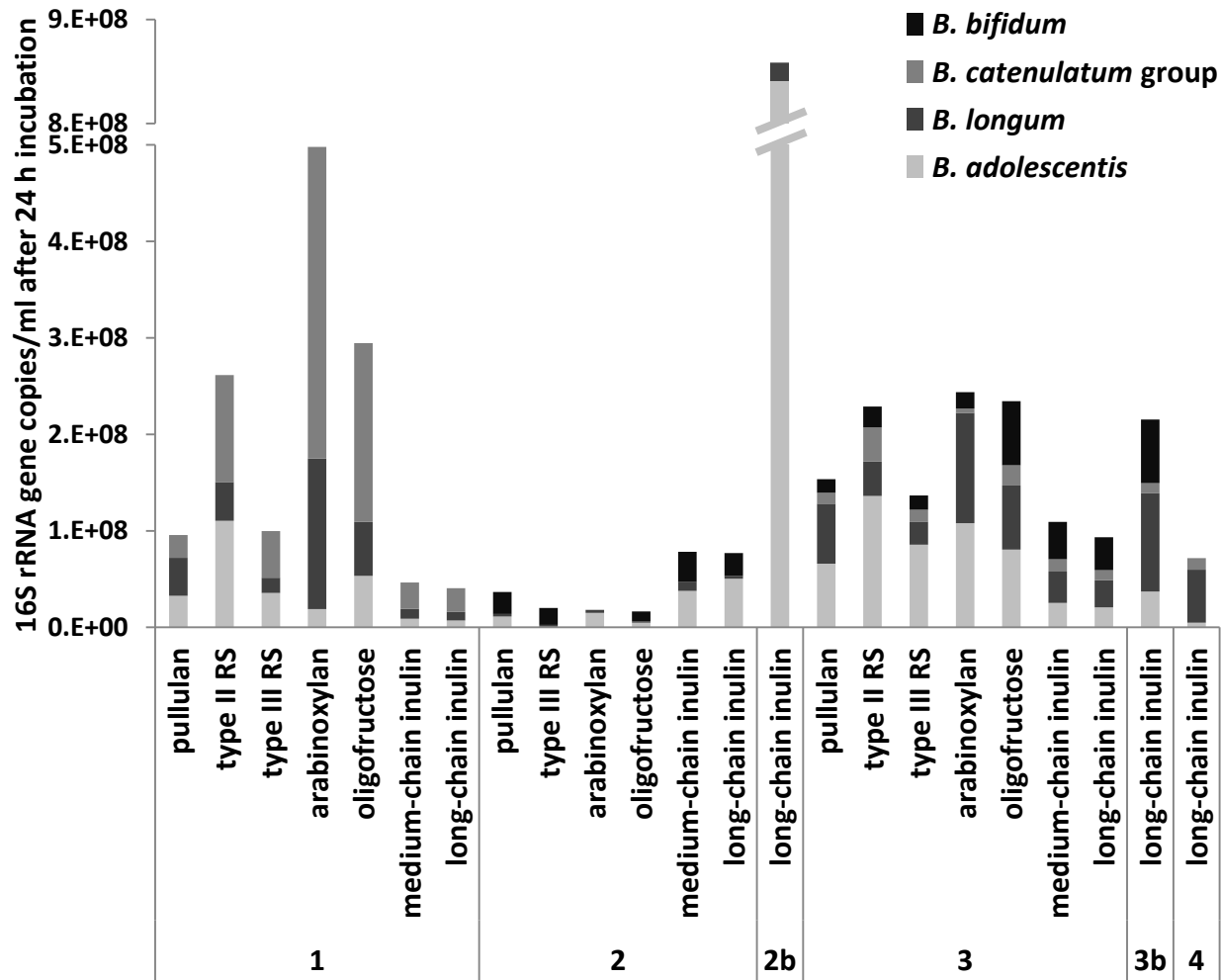


Fig. 4

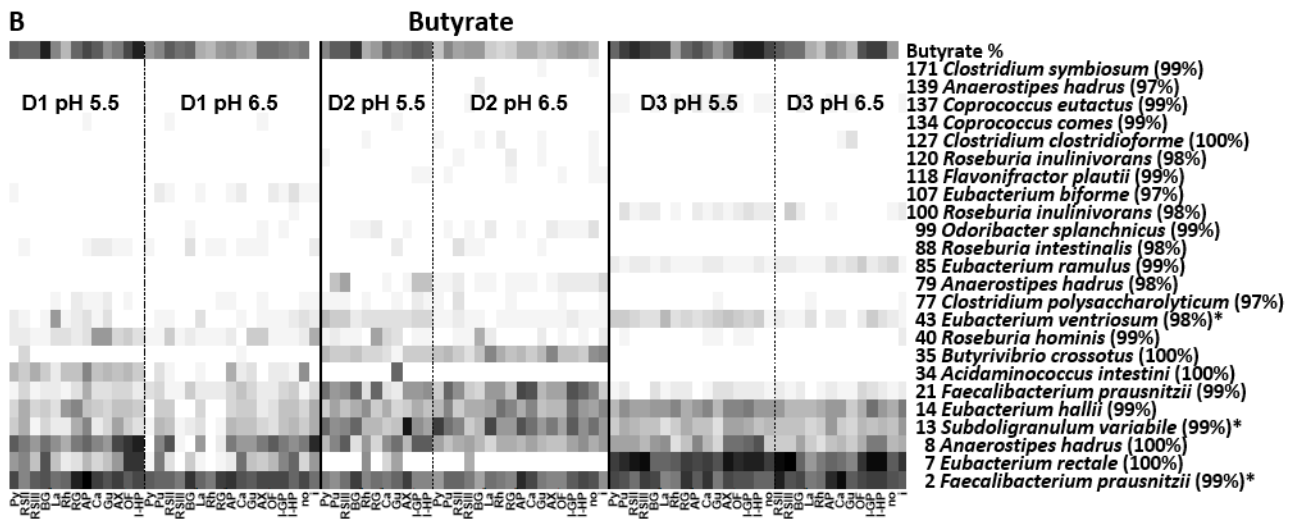
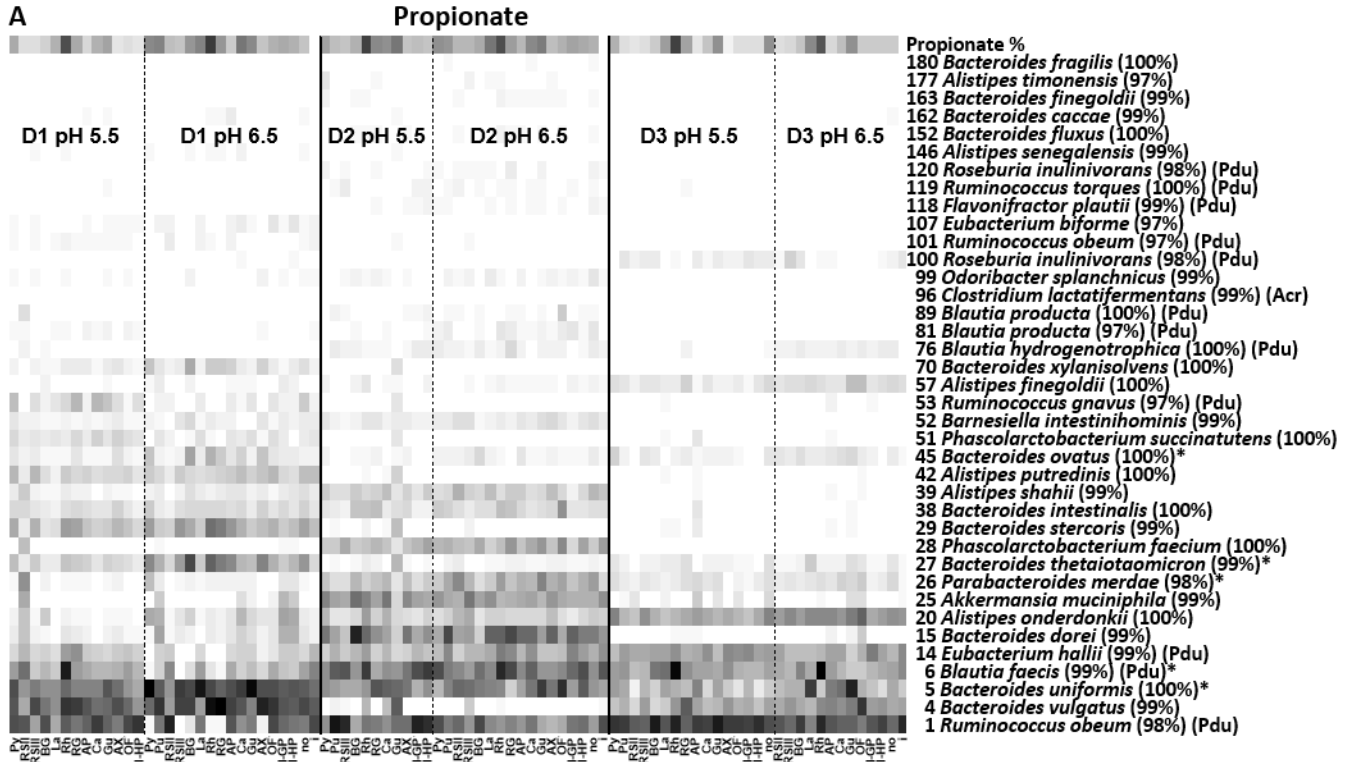


Fig. 5

