

Supplementary Materials

Analysis of the fermentation kinetics and gut microbiota modulatory effect of dried chicory root reveals the impact of the plant-cell matrix rationalizing its conversion in the distal colon

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The aim of the study was to assess the effect of the plant cell matrix in dried chicory root on its upper gastrointestinal (GI), lower gut fermentation and the potential effect thereof on gut barrier integrity by means of *in vitro* and *ex vivo* models. Here we present first a more detailed description of materials and methods. This is followed by additional data on micro- and macroscopic changes as well as pectin and inulin release during upper gastro-intestinal *in vitro* digestion. Subsequently we show the additional analyses of the gut microbiota composition during lower gastro-intestinal fermentation of dried chicory root powder and cubes versus inulin. Here we include visualizations of β -diversity and the details on the statistical analysis of changes in α -diversity, common genera and fermentation outcomes (pH, gas, short-chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs), lactate and ammonium). Finally, for the *ex vivo* assessment of the effect of fermentation supernatants on human colonic biopsies in an Ussing chamber experiment, we summarize additional details. These include gut microbiota composition changes during the fermentation to produced fermentation supernatants as well as changes in fermentation outcomes. Furthermore, we provide more information on electrophysiological and gut permeability markers assessed during the Ussing experiment.

Supplementary Materials and Methods

Dried chicory root powder

To study the effect of particle size, dried chicory root cubes were ground for upper gastrointestinal tract digestion using a cryogenic grinder (60 seconds, SPEX SamplePrep, 6875D Freezer/Mill®) and a 40 mesh sieve (Retsch, Waalwijk, The Netherlands) to obtain particles of <0.5 mm and for lower GI tract fermentation dried chicory root powder (mean particle size <0.5 mm) was provided by WholeFiber BV.

Adapted Infogest model

To study upper gastrointestinal changes we used the INFOGEST model ^[1,2]. The INFOGEST protocol is an international consensus providing standardized guidelines for the use of electrolyte and enzyme concentrations as well as duration and pH to simulate the upper gastrointestinal tract. In short, the food of interest is incubated for 2 min at pH 7 to mimic the oral phase in simulated salivary fluid, followed by an incubation for 2 hours at pH 3 of the oral bolus in 1:1 simulated gastric fluids and a small intestinal phase for 2 hours at pH 7 at a ratio of 1:1 in small intestinal fluids. All chemicals were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands), except for HCl (37%) and ethanol (96%) from VWR (Amsterdam, The Netherlands). Digestive fluids were prepared according to the INFOGEST protocol, as well as pepsin stocks and a pancreatin stock based on trypsin activity ^[1,2]. The protocol was further adapted to the research question as recommended by the INFOGEST protocol. 1) For the oral phase an additional 1:1 (w/v) dilution of the product was done in water prior to the addition of simulated salivary fluid, to mimic the intake of the product with a liquid/semi-liquid food and achieve a consistency as recommended by INFOGEST. To this mix simulated salivary fluid was added at a ratio of 1:1 based on the dry weight of the product. Salivary amylase was omitted due to the absence of glucose-polymers in the product. 2) For the gastric phase the pH was lowered in two steps to mimic potentially harsher conditions of gastric pH close to the fasting state or end of the gastric digestion (pH 2). The product was first incubated for 1 hour at pH 3 followed by lowering to pH 2 and further incubation for another hour. No phospholipids were used due to the absence of fat in the product. 3) For the small intestinal phase pancreatin was chosen over individual enzymes to assess the overall effect of the small intestinal phase

rather than focusing solely on the specific enzymatic activity targeting a single compound in the plant cell matrix. Additionally, bile acids were omitted due to the absence of fat in the product. For each particle size (powder and cubes) 4 g were used as starting material. The experiment was executed in 50 mL Falcon tubes attached to a rotary wheel set to 20 rpm (Stuart, Tube Rotator SB3) and incubated in an oven (Thermo Scientific Heraeus®, UB 12) set to keep the temperature of the digestive liquid at 37 °C. Separate experiments were executed in triplicate to sample from each phase as the physical structure of the product did not allow for continuous sampling. Samples were taken at the end of the oral phase (2 min) and after 30, 60 and 120 min of both the gastric and the small intestinal phases. After each respective phase the experiment was stopped by separating the liquid digesta from the solid part using a sieve. Liquid digesta were heat treated (5 min at 100 °C) to inactivate remaining enzymatic activity and snap frozen in liquid nitrogen and solid parts were fixated in 70% ethanol for immediate image processing.

Scanning Electron and Light Microscopy

To assess possible changes in the plant cellular matrix of the chicory root, samples of the dried chicory root (rehydrated cubes before start of the oral phase and samples at the end of the gastric and small intestinal phase) and for comparison fresh chicory root were visualized using scanning electron microscopy (SEM) and light microscopy. For SEM fresh chicory root was cut into piece of similar size as the dried chicory root. Digested dried chicory samples were prepared on the morning of the analysis. Samples were first dehydrated in 70% ethanol and then cut into smaller pieces to visualize the inner structure of the plant cell matrix. Subsequently samples were dried in 100% ethanol with CO₂ using a Leica EM CPD300 critical point dryer (Leica Camera, Germany), mounted onto aluminum stubs and coated with a layer of 12 nm tungsten. Samples were visualized using a FEI Magellan 400 Scanning Electron Microscope (Field Electron and Ion Company, USA). For light microscopy, fresh and rehydrated as well as digested chicory root were first cut into thin slices using a razor blade and treated with a drop of 100% ethanol or methanol to precipitate inulin dissolved inside the vacuoles. Ruthium red (0.05% w/v) in distilled water) was added to increase the contrast between plant cell wall fibers and intracellular inulin. Then, the plant cell structure was visualized using a Olympus BX41 light microscope equipped with a

Olympus SC30 Color Camera and cellSens software (Olympus, Hoofddorp, The Netherlands).

Pectin analysis as a measure of pectin leakage

To estimate possible pectin leakage from the plant cell matrix, uronic acid concentration as a measure of pectin content was determined in the liquid digesta obtained from the gastric and small intestinal phase. Samples were first centrifuged for 5 min at 16100 x g and the respective supernatants were diluted 12.5 x for the gastric phase and 7.5 x for the small intestinal phase. Uronic acid analysis was performed with an automated colorimetric *m*-hydroxydiphenyl assay ^[3] using a Skalar San++ continuous flow analyzer (Skalar Holding B.V., Breda, The Netherlands). Neglecting other pectic sugars, the amount of pectin was then estimated based on the detected uronic acid concentration and expressed as percentage of an expected total uronic acid content (5 % w/w) in chicory root as determined according to Ramasamy et al. ^[4].

Inulin analysis as a measure of inulin leakage

To assess potential differences between inulin from dried chicory root powder versus cubes leaking into digestive fluids in the upper GI tract we used High Performance Anion Exchange Chromatography (HPAEC). Liquid digesta were first centrifuged for 5 min at 16100 x g and the respective supernatants were diluted 100 x for samples from the gastric phase and 50x for the samples from the small intestinal phase. Samples were then injected into a Dionex ICS-5000+ Ion Chromatography System (Dionex, Sunnyvale, CA, USA) equipped with a Dionex CarboPac PA-1 column (250 mm × 2 mm ID), a CarboPac PA-1 guard column (25 mm × 2 mm ID) and a ISC5000 ED detector (Dionex) in the pulsed amperometric detector (PAD) mode and measured according to established methods ^[5]. Chromatograms revealing fructan-molecules of different chain lengths (degree of polymerization, DP) of inulin including the mono/disaccharides and fructan-oligomers (DP2 – 10) as well as longer-chain fructan-polymers (>DP10) present in the digesta were analyzed using Chromeleon™ Chromatography Data System software version 7 (ThermoFischer Scientific).

Batch incubation and fermentation model for assessing particle size effects

A fecal *in vitro* batch fermentation experiment was performed at ProDigest (Gent, Belgium). A 26-year-old female donor was selected, meeting the criteria of a normal BMI (18.5 – 24.9 kg/m²), with no history of diseases related to disturbances in the gut microbiome (e.g., irritable bowel syndrome, Parkinson's, or diabetes mellitus), and no antibiotic usage within four months prior to fecal donation. All chemicals were purchased from Sigma Aldrich (Overijse, Belgium). A simplified upper GI tract incubation was performed, by incubating 350 mg of dried chicory root cubes, powder or inulin versus control (no product) in 20 mL sterile water for 2 h under shaking (90 rpm) at 37°C in 100mL Schott bottles. To this 43 mL of buffered minimal medium was added containing 7.6 g/L K₂HPO₄, 23.9 g/L KH₂PO₄, 2.9 g/L NaHCO₃, 2.9 g/L yeast extract, 2.9 g/L peptone, 0.7 g/L cysteine and 2.9 mL/L Tween80 as well as five mucin-covered beads prepared as specified elsewhere [6]. The bottles were sealed and flushed with nitrogen. For the inoculum, a fecal slurry was prepared by mixing fresh feces at 1:13 (m/v) in an anaerobic phosphate buffer containing 8.8 g/L K₂HPO₄, 6.8 g/L KH₂PO₄, 0.1 g/L sodium thioglycolate, 0.015 g/L sodium dithionite, homogenizing the mix for 10 min in a lab blender (Bag Mixer 400, Interscience, Louvain-La-Neuve, Belgium) and removing big particle by centrifugation at 500 x g for 2 min. All bottles, were inoculated with 7 mL fecal slurry and incubated at 37 °C at 90 rpm for 48 h. Experiments were performed in triplicate and samples were taken at baseline and after 6, 24 and 48 h and frozen immediately for bacterial metabolite analysis. For DNA extraction 1 mL of sample was centrifuged at 5000 x g for 10 min and separated into pellet and supernatant before storing at -80 °C. Metabolic microbial activity was assessed by measuring in the fermentation samples pH using a Senseline F410 pH meter (ProSense, Oosterhout, The Netherlands), and fecal (un-/branched) short-chain fatty acids (SCFAs) as described elsewhere [7] as well as lactate and ammonia [8]. Gas pressure in the fermentation bottles was measured using a hand-held pressure indicator (CPH620, Wika, Echt, The Netherlands) and recorded before and after sampling at each timepoint. The change in gas pressure between timepoints was calculated based on the respective timepoint's gas pressure levels before sampling minus the gas pressure levels of the previous timepoint after sampling.

Human donors for Ussing chamber

Four healthy adult subjects with a mean age of 42 years (range 27-62 years) were recruited to donate fecal material and colonic biopsies and all subjects had signed an informed consent. The study was approved by the Research Ethics Committee of Örebro University (Dnr 2022-01814-02) and was registered at ClinicalTrials.gov (NCT05421793) and conducted in accordance with the Helsinki Declaration. Subjects did not consume any pre- or probiotics during the previous month, no antibiotics, laxatives, anti-diarrheal/-cholinergic drugs during the previous three months, and no Non Steroidal Anti Inflammatory Drugs during the past two months. Furthermore, they had no gastrointestinal disorder, previous surgery, psychiatric disorder expected to affect study outcomes or obesity. Subjects provided a fecal sample in the same or preceding week of biopsy collection.

Batch fermentation model for Ussing chamber

All chemicals were purchased from Sigma Aldrich (Stockholm Sweden) unless stated otherwise. A batch fermentation experiment was performed for each donor to produce fermentation supernatants from dried chicory root cubes. Fecal slurries were prepared from fresh feces (within 2 h after feces collection) by mixing 20 g of feces in 100 mL anaerobic phosphate buffer (Phosphate Buffered Saline containing 0.14 M NaCl, 0.0027 KCl and 0.010 M phosphate buffer pH 7.4, Medicago, Uppsala Sweden) with 0.5 g/L L-cysteine, homogenizing for 2 min by hand in a Stomacher® Circulator Strainer Bag with a pore size of 0.5 mm (Seward, Worthing, UK). Of this fecal slurry 10 mL were transferred into sterile 500 mL Schott bottles containing 90 mL of buffered colonic background medium containing 2.8 g/L K₂HPO₄, 15.4 g/L KH₂PO₄, 2.0 g/L NaHCO₃, 2.0 g/L yeast extract (Oxoid, Malmö, Sweden), 2.0 g/L peptone water (Oxoid, Malmö, Sweden), 0.1 g/L NaCl, 0.01 g/L MgSO₄·7H₂O, 0.01 g/L CaCl₂·6H₂O, 0.05 g/L hemin, 10 µL/L vitamin K1, 0.5 g/L bile salts, 1.0 g/L cysteine and 2.0 mL/L Tween80. Prior to inoculation, 5 g/L of dried chicory root cubes were incubated for approximately 2 h in the medium in the bottles, which were flushed with nitrogen and placed in a water bath for incubation at 37 °C. Per donor a single fermentation of dried chicory root cubes and a single negative control (only inoculum) fermentation were executed. Samples were taken at baseline, 6, 24 and 48h (except for donor 1 for which samples

were taken at 42h) and stored at -80 °C. Fermentation supernatants were produced by centrifuging 1 mL of sample at 31,514 x g for 10 min at 4 °C. Supernatants were aliquoted for SCFA measurements and Ussing chamber experiments and the pellets were stored for DNA extraction at -80 °C. Changes in pH were assessed in fermentation supernatant using a FiveGo pH meter F2 equipped with a Sensor InLab® micro Pro pH probe (Mettler Toledo, Stockholm, Sweden) and gas pressure was recorded using the Automatic Gas Flow Measuring System Gas Endeavour (BPC Instruments, Lund, Sweden). Short-chain fatty acids were analyzed at Wageningen University using high-performance liquid chromatography (HPLC). First a Carrez clarification was performed to remove proteins and carbohydrates in the fermentation supernatant that could interfere with the HPLC measurement. For this first two parts of fermentation supernatant were mixed with one part of Carrez reagent I (0.1 M $K_4Fe(CN)_6 \cdot 3H_2O$) followed by adding one part of Carrez reagent II (0.2 M $ZnSO_4 \cdot 7H_2O$) and centrifugation for 5 min at 21,000 x g. The clear supernatant was then acidified by mixing 1:1 with 0.2 N H_2SO_4 containing 10 mM DMSO as internal standard. A standard of acetic, propionic, butyric, lactic and formic acid was prepared at 1 mM, 5 mM, 10 mM, 20 mM and 30 mM. SCFA were quantified on a Shimadzu LC_2030C equipped with a refractive index detector and a Hi-Plex H column (Shimadzu Europe Company, 's-Hertogenbosch, The Netherlands). Ten μ L of the sample was injected at an oven temperature of 45 °C with a flow rate of 1.00 mL/min using 0.01 N H_2SO_4 as eluent. Obtained chromatograms were further processed using Chromeleon™ Chromatography Data System software version 7 (ThermoFischer Scientific).

Ussing chamber experiment and gut permeability analysis

Four Ussing chamber experiments were conducted using colonic biopsies, with each colonic biopsy paired to the fermentation supernatant derived from its respective donor's fecal material. Collection of colonic biopsies as well as set-up and execution of the Ussing chamber experiment were performed according to previously described methods [9]. In short, colonic biopsies were collected by endoscopic procedure from unprepared sigmoid colon after an overnight fast, immediately put in ice-cold oxygenated Krebs-Ringer bicarbonate transport buffer hereafter called Krebs buffer) containing 116 mM NaCl, 1.27 mM $CaCl_2$, 3.65 mM KCl, 1.37 mM KH_2PO_4 , 23 mM $NaHCO_3$, 1.20 mM $MgCl_2$ at pH of 7.2 (Merck, Solna, Sweden) and brought to the

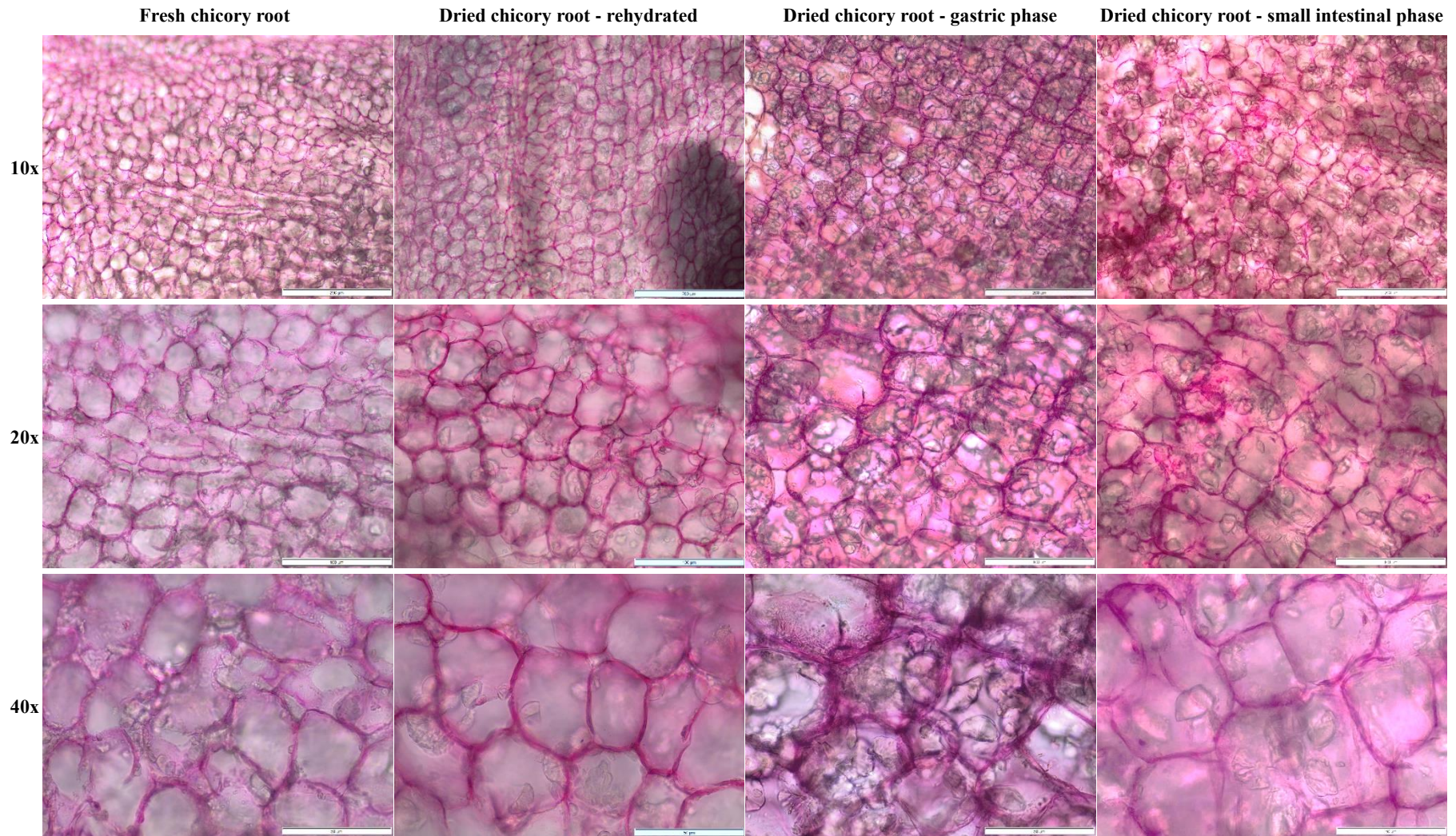
facility within 10 min. Biopsies were mounted into an Ussing chamber of 1.5 mL and each condition was run in duplicate. Throughout the whole experiment the Ussing chambers were held at 37 °C, continuously oxygenated and electrophysiological measurements were taken every 30 s (transepithelial electrical resistance (TER), potential difference (PD), short-circuit current (Isc)). Biopsies with low chance of viability (among which PD > 0.5) were excluded from the experiments. Krebs experiment buffer (containing additional 5.7 mM sodium pyruvate and 5.7 mM sodium glutamate) with 0.01 M glucose was used for the serosal side and 0.01 M mannitol was used for the mucosal side of the biopsies. After a 25 min adaption period cold Krebs buffer was replaced with warm Krebs buffer, which was again replaced after another 10 min. Then baseline samples were taken from the serosal side for permeability assessment. After this 150 µL of diluted fermentation supernatant (2% (v/v) in mannitol Krebs buffer) was added to the mucosal side of the treatment biopsies, while the control biopsies were kept in only mannitol Krebs buffer. After 20 min incubation, the experiment was started by first adding 300 µL of the stressor (1 mM sodium deoxycholate (SDC) in mannitol-Krebs buffer) followed by the addition 150 µL of a permeability marker solution prepared in mannitol Krebs, which included 2.5 nM Fluorescein isothiocyanate–dextran FD4 (FITC-dextran) as paracellular permeability marker. Samples were taken after 60 and 90 min and paracellular permeability was assessed by fluorometrically measuring passage of FITC-dextran 3000–5000 [9].

Gut microbiota analysis

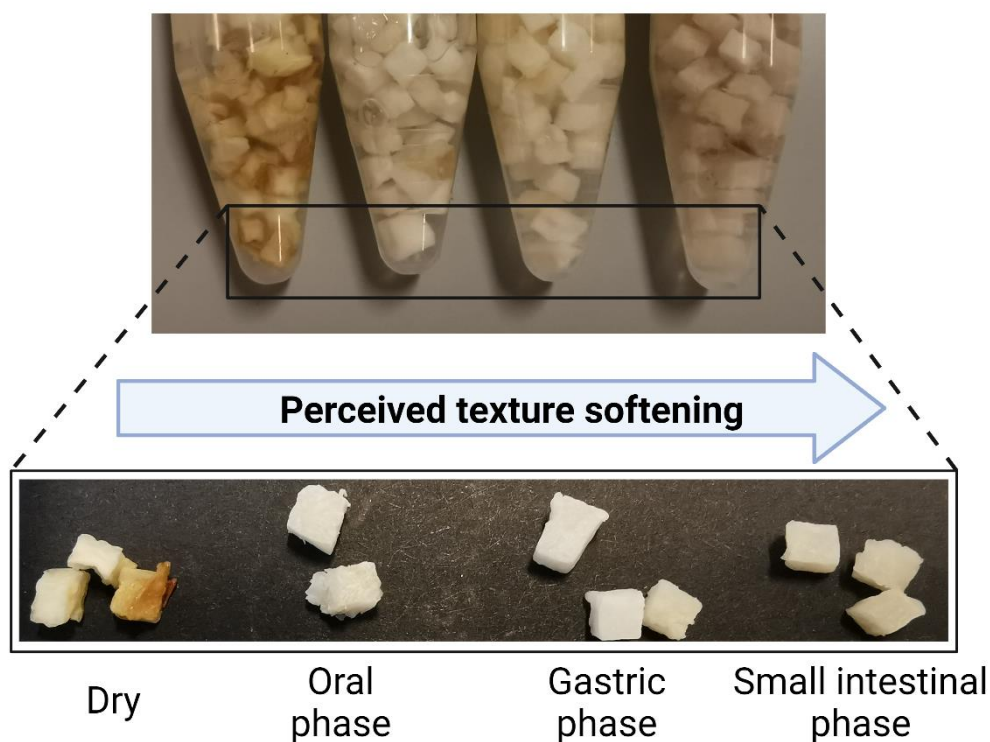
Gut microbiota composition was determined in both the fermentation pellets of the single donor *in vitro* fermentation experiment as well as fermentations with multiple donors for the Ussing chamber experiment. Bacterial DNA was extracted by resuspending the pellets in 300 µL Stool Transport and Recovery (STAR) buffer (Roche Diagnostics, Almere, The Netherlands), then transferring into sterile screw-cap tubes filled with 0.25 g of 0.1 mm zirconia bead and 3 glass beads of 2.5 mm diameter. Samples were subjected to repeated (3 x 1 min at 5.5 ms) bead-beating in a FastPrep-24™ 5G Instrument (MP Biomedicals, The Netherlands) followed by heating for 15 min at 95 °C at 300 rpm and centrifuging for 5 min at 4 °C at 16100 x g. The supernatant was transferred into a sterile Eppendorf tubes, the pellet was resuspended in 200 µL STAR and the cycle of bead-beating, heating and centrifuging was repeated.

Supernatants were pooled and DNA was purified using a customized Maxwell® 16 Tissue LEV Total RNA purification Kit (XAS1220) on the Maxwell® 16 LEV Instrument (Promega, The Netherlands) and eluted in 50 µL nuclease free water (Qiagen, Hilden, Germany). DNA concentration was measured using a Qubit™ dsDNA Quantification BR Assay Kit on a Qubit Fluorometer (ThermoFisher Scientific, The Netherlands) and adjusted to 20 ng/µL with nuclease-free water. The V4 region of the 16S rRNA gene was amplified in duplicate using the barcoded primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3')^[10] and 806R (5'-GGACTACNVGGGTWTCTAAT-3')^[11]. Each 50 µL PCR reaction contained 10 µL 5x Phusion Green HF buffer (ThermoFisher Scientific), 1 µL 10mM dNTP's (Promega, Madison, WI, United States), 0.5 µL Phusion Hot start II DNA 2 U/µL polymerase (ThermoFisher Scientific, The Netherlands), 1 µL of each barcoded forward and reverse 10 µM primer, 1 µL of 20 ng/µL DNA template and 36.5 µL nuclease-free water. The PCR program consisted of an initial 30 s denaturation at 98 °C for 10 min, followed by 25 cycles of 10 s denaturation at 98 °C, 10 s annealing at 50 °C, 10 s elongation at 72 °C, and final extension for 420 s at 72 °C. To verify the presence and size of each PCR product 2 µL were loaded onto a 2.2% agarose gel (Lonza Benelux B.V., Breda, The Netherlands) and run for 5 min at 200 V. The PCR products were pooled, further purified using the CleanPCR kit (CleanNA, Waddinxveen, The Netherlands) and the DNA concentration was again measured using Qubit. A library with an equimolar mix of purified PCR product, negative PCR and DNA extraction controls as well as positive mock communities was prepared and sent for Illumina Hiseq sequencing to Novogene (Novogene, The Netherlands). Raw amplicon sequences were processed using NG-Tax 2.0 with default settings^[12] and resulting amplicon sequence variants (ASV) were taxonomically annotated using the SILVA 138.1 database^[13].

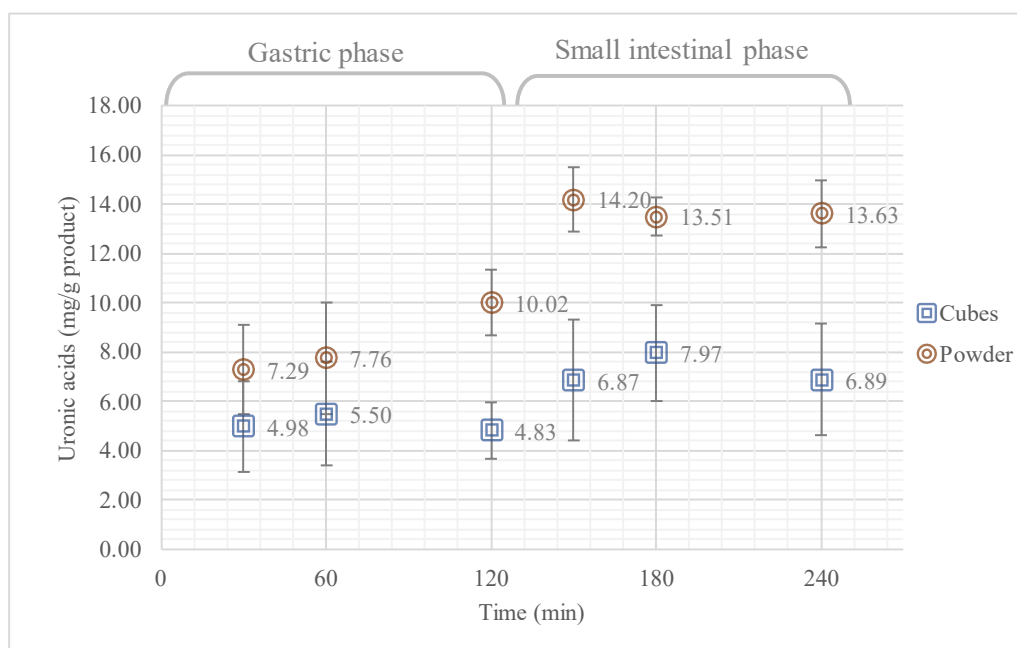
Upper gastro-intestinal *in vitro* digestion: changes in micro- and macrostructure of dried chicory root and pectin and inulin leakage.



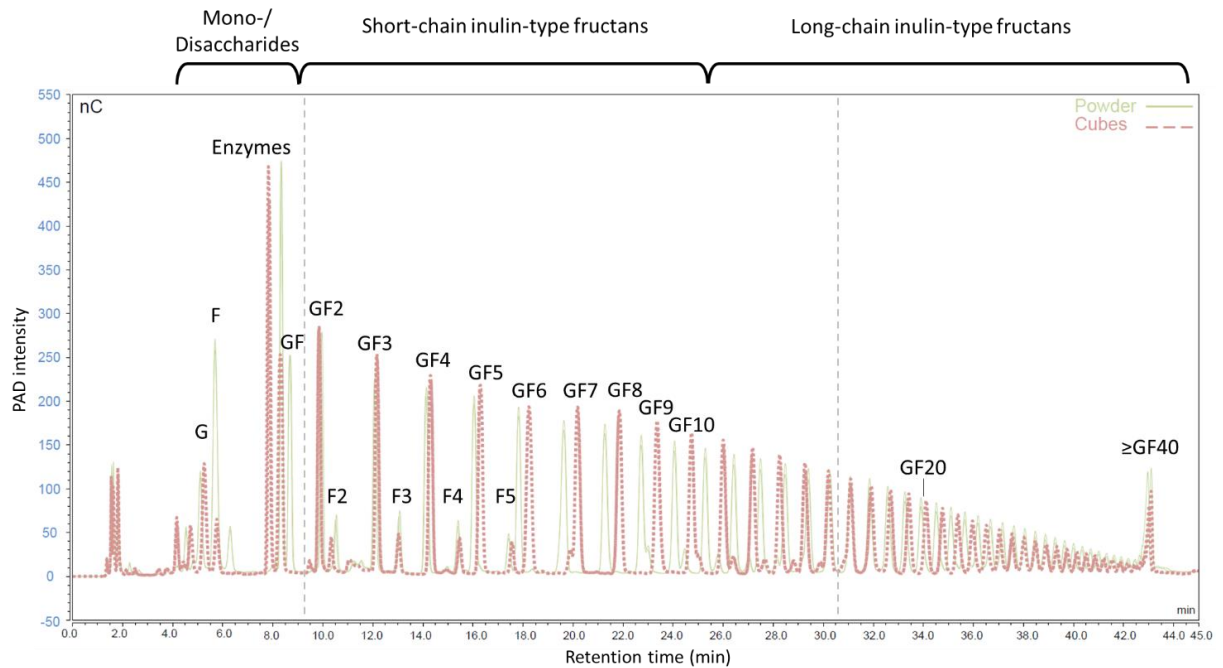
Supplementary Figure 1. Light Microscopy micrographs of the plant cell matrix of fresh and *in vitro* digested dried chicory root cubes before the oral phase (rehydrated), at the end of the gastric (120 min) and small intestinal phase (240 min). Ruthium red (0.05 %w/v) was added to increase the contrast between plant cell and intracellular components. Each picture is shown at three magnification with the scale bar representing 200 μm for 10x magnification, 100 μm for 20x and 50 μm for 40x magnification.



Supplementary Figure 2. Macrostructure of dried chicory root cubes before and during upper gastrointestinal *in vitro* digestion. Created with Biorender.com



Supplementary Figure 3. Release of uronic acids as a measure of pectin leakage from dried chicory roots cubes (□) and powder (○) during *in vitro* digestion in the gastric phase (at t = 30 min, 60 min and 120 min (end)) and small intestinal phase (30 min at t = 150 min, 60 min at t = 180 min and 60 min at t = 240 min (end)). For each data point the corresponding value is given on its respective right side.

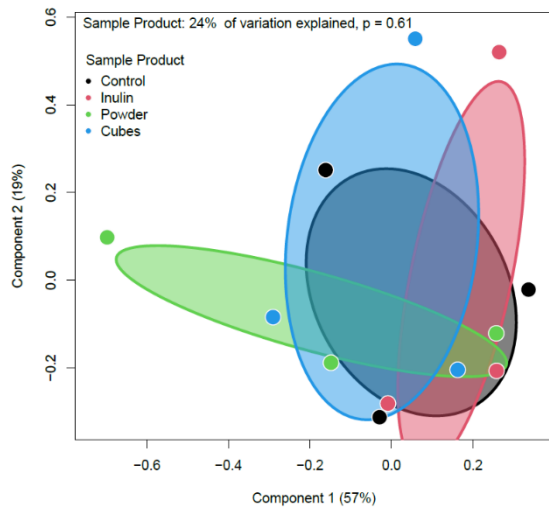


Supplementary Figure 4. High Performance Anion Exchange Chromatography (HPAEC) chromatograms of the liquid digesta retrieved at the end of the upper gastrointestinal *in vitro* digestion (end of the small intestinal phase) for dried chicory root powder (green) compared to cubes (red). Native inulin contains so-called inulin-type fructans, which are fructose-polymers linked by $\beta(2\text{—}1)$ -bonds and have varying chain lengths up to 60, which are described as degree of polymerization. Native inulin contains oligomers with and without glucose as end unit as well as glucose and fructose monomers and sucrose (di-saccharide of glucose and fructose). Different chain lengths are represented by individual peaks in the chromatogram obtained from the signal of each detected mono-, oligo- and polymer. G: glucose; F: fructose; Enzymes: signal obtained from the added pancreatin (containing digestive enzymes) in the digesta; GF: Sucrose (containing a glucose and a fructose monomer); GF2-GF40: fructose-chains of increasing chain lengths with glucose as end unit (DP is equal to the amount of fructose monomers); F2-5: fructose-chains of increasing chain lengths without glucose as end unit (DP is equal to the amount of fructose monomers).

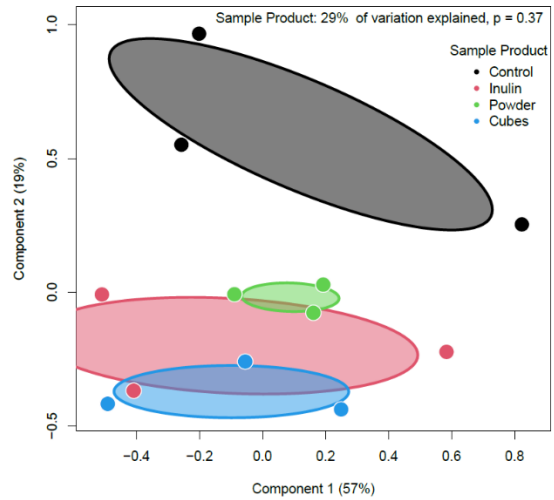
Native inulin as present in the chicory root plant cells contains fructans with a wide range of molecular size (DP 2 – 60; average of DP 12), that decrease in solubility with increasing chain length ^[14]. Consequently, we expected that longer fructan-polymers (DP>10) leak less easily from the intact plant cells compared to smaller fructo-oligosaccharides (DP 2-10), but that damage might advance their release. We observed indeed higher amounts of fructan-oligomers as well as some longer-chain fructose polymers for dried chicory root powder, which we hypothesize to represent and relate to the higher content of plant cell damage. Thus, it is likely that dried chicory

root cubes transport inulin mainly encapsulated inside the intact plant cells into the lower (distal) gut.

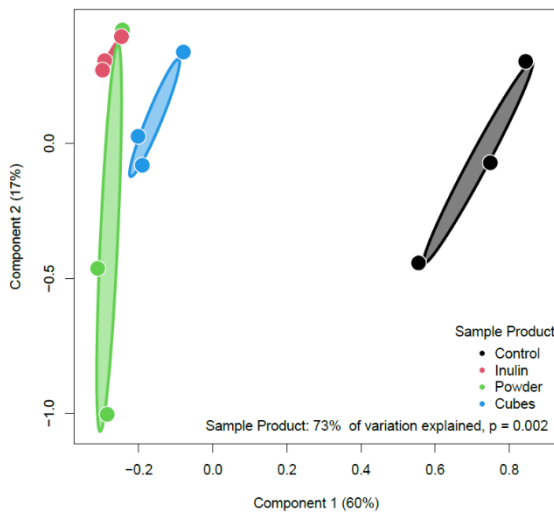
Lower gastro-intestinal *in vitro* fermentation: changes in overall gut microbiota composition and individual genera over time as well as between genera per timepoint



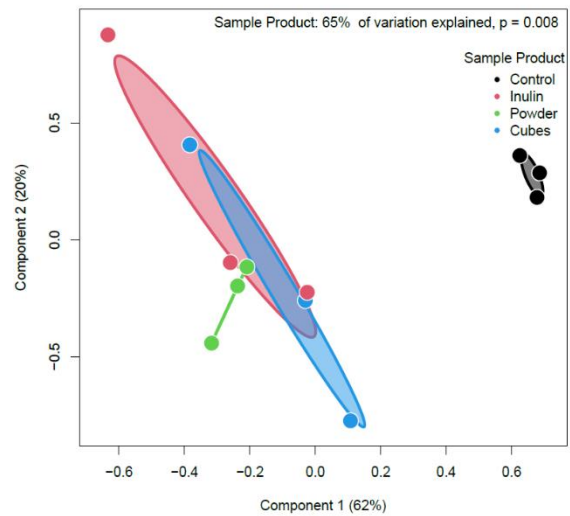
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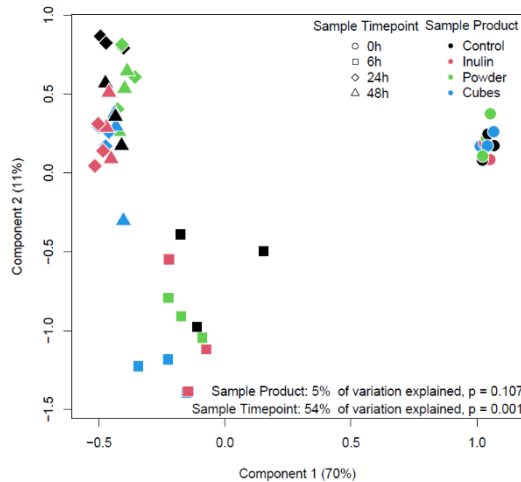
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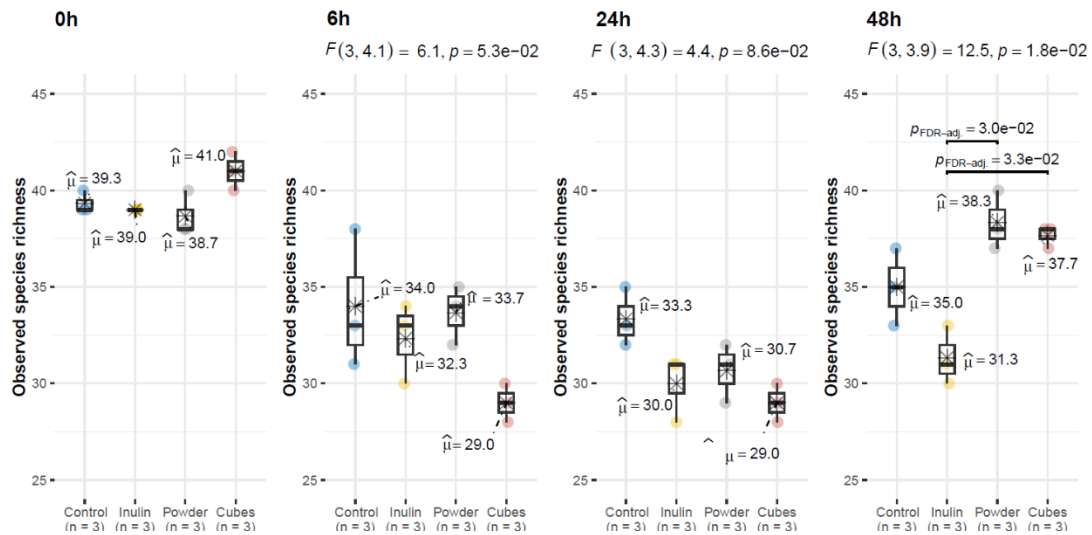


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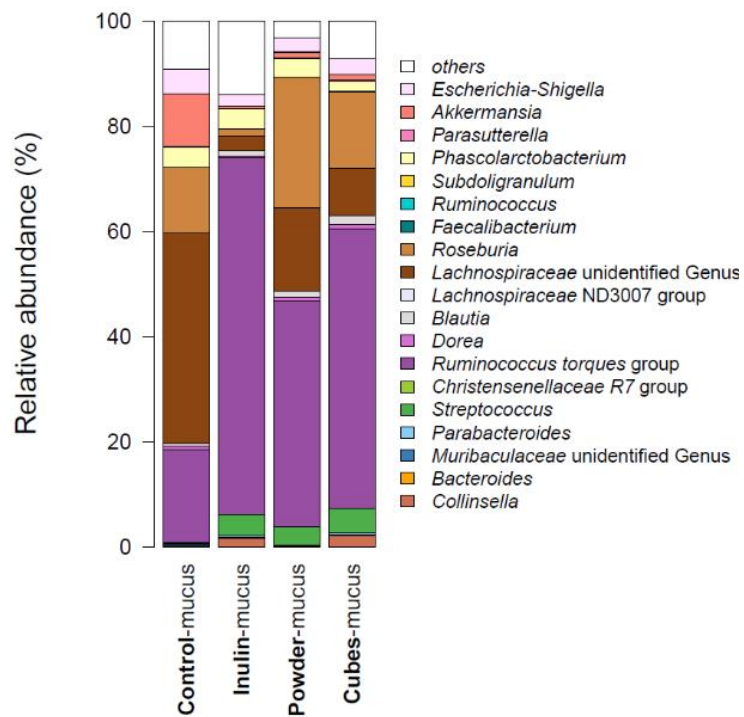
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Supplementary Figure 5. Principal Coordinate Analysis of gut microbiota composition. A-C: Principal coordinate analysis based on Bray-Curtis dissimilarity to assess differences in overall gut microbiota composition at genus level (β -diversity representing between sample differences) between control fermentation (Control, only inoculum) or *in vitro* fermentation of inulin (Inulin), or dried chicory root powder (Powder) or cubes (Cubes). A: baseline (t = 0 h); B: after 6 h of *in vitro* fermentation; C: after 24 h of *in vitro* fermentation; and D: after 48 h of *in vitro* fermentation. E: at all time points (0h, 6 h, 24 and 48 h)

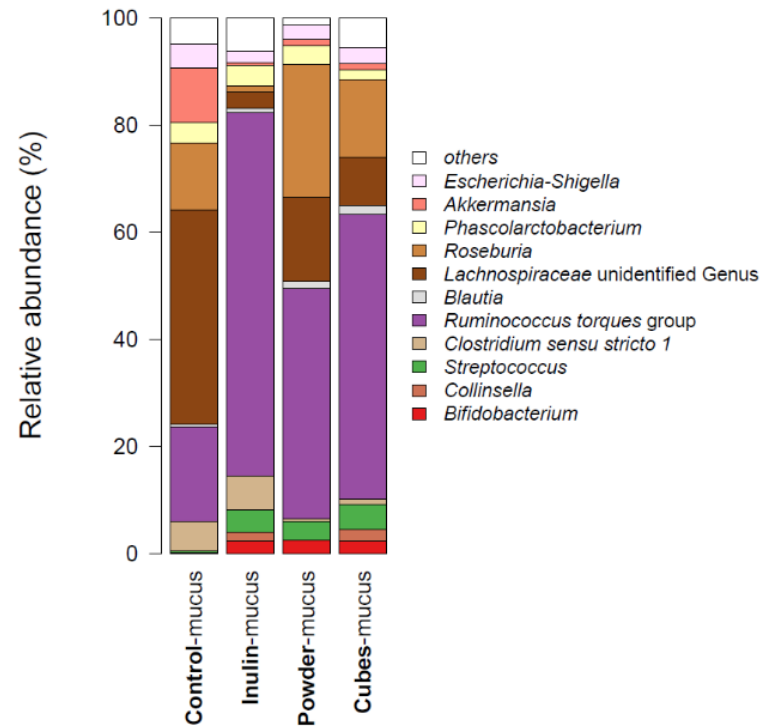


Supplementary figure 6. Alpha-diversity assessed by observed gut bacteria richness (mean richness within each sample) at baseline ($t = 0$ h), 6 h, 24 h and 48 h *in vitro* fermentation of the control (Control; only inoculum), inulin (Inulin) and dried chicory root powder (Powder) and cubes (Cubes). Statistical details for robust ANOVA (F-statistics and corresponding p-value) are given above the plot. No statistically significant differences were observed at 0 h, 6 h and 24 h of fermentation between the conditions (pairwise comparison).

Concomitantly with the changes in β -diversity we also observed a rapid change in α -diversity (within sample diversity; Supplementary Figure 6). Overall gut bacteria richness as a measure of α -diversity decreased in all conditions within 6 h and this decrease was largest for dried chicory root cubes. Gut microbiota richness of dried chicory root cubes remained at the lowest level between 6 h and 24 h while inulin and dried chicory root powder further decreased from 6 h to 24 h. However, after 24 h of fermentation gut bacteria richness increased again in dried chicory root powder and cubes reaching statistically significantly higher levels compared to inulin whose gut bacteria richness remained low. In contrast, the richness of the control samples remained decreased throughout the rest of the fermentation.



A



B

Supplementary Figure 7. Mean relative abundances of genera present in mucus recovered from mucin-covered beads for each *in vitro* fermentation (Control, Inulin, dried chicory root Powder or Cubes) at t = 48 h. A: relative abundance of genera present in the mucus samples based on the mean relative abundance of 1% and prevalence of 50% of these taxa in both the fermentation medium and mucin-covered beads. Note that no *Bifidobacterium* spp. is present here. B: relative abundance of genera present in the mucus samples based on the mean relative abundance of 1% and prevalence of 50% of these taxa on only the mucin-covered beads. Note that *Bifidobacterium* spp. is present here.

Supplementary Table 1. Changes in mean relative abundances of common genera (mean relative abundance of at least 1% and mean prevalence of 50% in the whole dataset) during the fermentation of dried chicory root cubes (Cubes) or powder (Powder) vs inulin (Inulin) or negative control (Control; only inoculum) from baseline (t = 0 h) to 6 h, 24 h and 48 h. Respective fold-changes (fold- Δ) over time within each group and the p-values and fdr-corrected q-values are given per taxon for each fermentation.

Taxon	Product	relative abundance (%)				fold- Δ			p-value			q-value		
		0h	6h	24h	48h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h
<i>Adlercreutzia</i>	Control	0.38%	0.76%	0.56%	0.28%	2.02	1.49	0.73	<0.001	0.001	0.009	<0.001	0.001	0.012
	Inulin	0.30%	0.58%	0.47%	0.29%	1.977	1.604	0.975	0.311	0.482	0.971	0.324	0.503	1.000
	Powder	0.35%	0.51%	0.38%	0.44%	1.443	1.081	1.236	0.025	0.287	0.075	0.030	0.342	0.097
	Cubes	0.34%	0.40%	0.52%	0.47%	1.172	1.528	1.361	0.406	0.026	0.108	0.448	0.029	0.119
<i>Akkermansia</i>	Control	1.01%	3.49%	29.12%	20.40%	3.46	28.83	20.20	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	1.05%	2.12%	3.66%	4.51%	2.029	3.503	4.312	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	0.93%	1.83%	5.61%	7.16%	1.972	6.038	7.702	0.011	<0.001	<0.001	0.013	<0.001	<0.001
	Cubes	1.13%	1.51%	6.69%	7.72%	1.338	5.938	6.855	0.219	<0.001	<0.001	0.251	<0.001	<0.001
<i>Bacteroides</i>	Control	0.08%	1.25%	1.51%	0.73%	15.53	18.75	8.99	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
	Inulin	0.31%	2.06%	2.62%	2.32%	6.600	8.362	7.408	0.001	<0.001	0.001	0.001	<0.001	0.002
	Powder	0.08%	1.20%	2.47%	2.62%	14.203	29.325	30.993	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	0.13%	1.61%	3.19%	1.82%	12.438	24.587	13.997	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
<i>Blautia</i>	Control	7.47%	2.58%	0.66%	0.73%	0.34	0.09	0.10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	7.05%	2.19%	0.82%	3.83%	0.310	0.116	0.544	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	8.50%	2.57%	4.18%	4.42%	0.303	0.491	0.520	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	8.18%	2.22%	3.63%	4.35%	0.272	0.444	0.532	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Christensenellaceae</i> R7 group	Control	8.35%	1.57%	0.66%	0.97%	0.19	0.08	0.12	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	7.32%	1.17%	0.40%	0.43%	0.159	0.054	0.059	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	8.61%	1.59%	0.37%	0.41%	0.185	0.043	0.047	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	8.24%	0.82%	0.39%	0.49%	0.100	0.048	0.060	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Taxon	Product	relative abundance (%)				fold-Δ			p-value			q-value		
		0h	6h	24h	48h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h
<i>Dorea</i>	Control	0.72%	0.28%	2.96%	3.28%	0.39	4.08	4.52	0.450	<0.001	<0.001	0.519	<0.001	<0.001
	Inulin	0.65%	0.25%	10.97%	3.07%	0.388	16.960	4.754	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	0.75%	0.46%	10.72%	1.54%	0.610	14.215	2.047	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	0.82%	0.35%	6.65%	1.43%	0.424	8.120	1.745	0.162	0.002	0.006	0.192	0.002	0.007
<i>Enterobacteriaceae</i> unidentified Genus	Control	0.00%	1.04%	0.36%	0.18%	NA	NA	NA	1.000	1.000	1.000	1.000	1.000	1.000
	Inulin	0.00%	0.62%	0.29%	0.49%	NA	NA	NA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	0.00%	0.28%	0.22%	0.26%	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Cubes	0.00%	0.97%	0.54%	0.35%	NA	NA	NA	1.000	1.000	1.000	1.000	1.000	1.000
<i>Erysipelotrichaceae</i> UCG-003	Control	3.50%	1.22%	0.33%	0.21%	0.35	0.10	0.06	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	3.04%	0.57%	0.25%	0.17%	0.188	0.083	0.054	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	2.88%	0.50%	0.23%	0.19%	0.172	0.079	0.067	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	3.19%	0.44%	0.25%	0.17%	0.139	0.079	0.053	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Escherichia-Shigella</i>	Control	0.00%	46.09%	26.06%	33.52%	NA	NA	NA	0.999	0.999	0.999	1.000	1.000	1.000
	Inulin	0.00%	46.23%	31.01%	36.35%	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Powder	0.00%	51.40%	27.27%	32.84%	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Cubes	0.00%	53.84%	30.62%	39.08%	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Faecalibacterium</i>	Control	15.73%	4.65%	0.60%	0.39%	0.30	0.04	0.02	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	15.70%	3.83%	0.65%	0.55%	0.244	0.042	0.035	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	18.66%	4.42%	0.56%	0.47%	0.237	0.030	0.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	17.21%	3.30%	0.77%	0.61%	0.192	0.045	0.035	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Fusicatenibacter</i>	Control	2.24%	0.46%	0.00%	0.00%	0.21	0.00	0.00	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	1.94%	0.46%	0.05%	0.00%	0.237	0.024	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	2.51%	0.38%	0.00%	0.00%	0.153	<0.01	<0.01	<0.001	1.000	1.000	<0.001	1.000	1.000
	Cubes	2.39%	0.35%	0.00%	0.00%	0.145	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Taxon	Product	relative abundance (%)				fold-Δ			p-value			q-value		
		0h	6h	24h	48h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h
<i>Haemophilus</i>	Control	0.00%	0.31%	0.23%	0.08%	NA	NA	NA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	0.08%	0.31%	0.43%	0.31%	4.053	5.652	4.011	0.011	0.002	0.012	0.014	0.002	0.014
	Powder	0.00%	0.37%	0.74%	0.48%	NA	NA	NA	1.000	1.000	1.000	1.000	1.000	1.000
	Cubes	0.09%	0.28%	0.60%	0.39%	3.124	6.579	4.324	0.043	0.001	0.009	0.053	0.001	0.011
<i>Lachnoclostridium</i>	Control	0.10%	0.00%	0.94%	0.92%	0.00	9.12	8.89	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	0.00%	0.00%	0.92%	1.15%	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Powder	0.08%	0.00%	1.26%	0.92%	<0.01	15.726	11.453	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	0.08%	0.00%	1.18%	0.81%	<0.01	14.282	9.801	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Lachnospira</i>	Control	0.92%	0.36%	0.09%	0.00%	0.39	0.10	0.00	0.133	<0.001	<0.001	0.174	<0.001	<0.001
	Inulin	0.85%	0.36%	0.07%	0.00%	0.425	0.085	<0.01	0.231	0.001	1.000	0.252	0.001	1.000
	Powder	1.02%	0.29%	0.00%	0.00%	0.282	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	1.09%	0.25%	0.00%	0.30%	0.232	<0.01	0.275	0.003	0.001	0.003	0.004	0.001	0.004
<i>Lachnospiraceae</i> ND3007 group	Control	3.54%	1.21%	0.46%	0.30%	0.34	0.13	0.08	0.001	<0.001	<0.001	0.002	<0.001	<0.001
	Inulin	3.92%	1.16%	0.54%	0.38%	0.296	0.137	0.098	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	3.74%	1.10%	0.29%	0.32%	0.295	0.078	0.085	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	4.00%	1.15%	0.43%	0.28%	0.287	0.107	0.069	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Lachnospiraceae</i> UCG-004	Control	0.00%	0.00%	0.38%	0.39%	NA	NA	NA	0.765	<0.001	<0.001	0.881	<0.001	<0.001
	Inulin	0.00%	0.00%	0.50%	0.56%	NA	NA	NA	0.618	<0.001	<0.001	0.660	<0.001	<0.001
	Powder	0.00%	0.00%	0.26%	0.30%	NA	NA	NA	1.000	<0.001	<0.001	1.000	<0.001	<0.001
	Cubes	0.08%	0.00%	0.34%	0.35%	<0.01	4.193	4.282	1.000	0.038	0.035	1.000	0.040	0.040
<i>Lachnospiraceae</i> unidentified Genus	Control	13.68%	2.53%	0.69%	1.77%	0.19	0.05	0.13	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	12.73%	2.02%	2.34%	0.72%	0.159	0.184	0.057	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	16.41%	1.95%	2.00%	0.66%	0.119	0.122	0.040	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	14.75%	1.49%	1.30%	0.50%	0.101	0.088	0.034	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Taxon	Product	relative abundance (%)				fold-Δ			p-value			q-value		
		0h	6h	24h	48h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h
<i>Monoglobus</i>	Control	0.51%	0.16%	0.00%	0.00%	0.32	0.00	0.00	0.016	0.001	0.001	0.024	0.001	0.001
	Inulin	0.44%	0.18%	0.00%	0.00%	0.404	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	0.50%	0.16%	0.00%	0.49%	0.319	<0.01	0.974	<0.001	0.999	0.624	<0.001	1.000	0.716
	Cubes	0.53%	0.09%	0.00%	0.32%	0.167	<0.01	0.608	0.012	1.000	0.484	0.016	1.000	0.500
<i>Muribaculaceae</i> unidentified Genus	Control	6.21%	2.73%	1.91%	1.73%	0.44	0.31	0.28	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	7.11%	1.60%	1.23%	1.60%	0.225	0.173	0.225	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	5.82%	2.34%	1.40%	1.20%	0.402	0.241	0.206	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	5.51%	1.64%	1.34%	1.59%	0.296	0.243	0.289	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Parabacteroides</i>	Control	0.37%	1.82%	2.78%	3.57%	4.90	7.48	9.59	0.034	0.003	<0.001	0.048	0.004	<0.001
	Inulin	0.44%	1.78%	4.69%	4.78%	4.013	10.568	10.788	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	0.39%	1.82%	4.12%	4.32%	4.689	10.640	11.143	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	0.41%	1.59%	2.92%	4.10%	3.842	7.042	9.882	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Paraprevotella</i>	Control	1.05%	0.43%	0.17%	0.04%	0.41	0.16	0.04	0.212	<0.001	<0.001	0.255	<0.001	<0.001
	Inulin	0.67%	0.25%	0.12%	0.09%	0.375	0.173	0.138	0.114	0.005	0.001	0.136	0.005	0.002
	Powder	1.01%	0.70%	0.24%	0.27%	0.697	0.238	0.265	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	1.21%	0.26%	0.19%	0.20%	0.217	0.161	0.166	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Parasutterella</i>	Control	1.06%	2.46%	2.49%	3.43%	2.31	2.34	3.22	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	1.10%	1.60%	1.28%	1.37%	1.450	1.165	1.243	<0.001	0.003	<0.001	<0.001	0.004	<0.001
	Powder	1.12%	3.06%	2.90%	2.73%	2.727	2.584	2.432	0.010	0.009	0.009	0.013	0.012	0.012
	Cubes	1.06%	3.26%	2.54%	3.20%	3.089	2.407	3.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Phascolarctobacterium</i>	Control	1.51%	5.05%	5.03%	3.34%	3.35	3.33	2.21	<0.001	<0.001	0.005	<0.001	<0.001	0.007
	Inulin	1.52%	4.09%	11.74%	14.50%	2.680	7.701	9.509	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	2.35%	5.66%	12.54%	15.02%	2.410	5.339	6.393	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	1.94%	3.56%	12.29%	11.44%	1.838	6.349	5.913	0.001	<0.001	<0.001	0.001	<0.001	<0.001

Taxon	Product	relative abundance (%)				fold-Δ			p-value			q-value		
		0h	6h	24h	48h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h
<i>Roseburia</i>	Control	1.89%	0.20%	0.00%	0.00%	0.11	0.00	0.00	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	1.71%	0.13%	0.00%	0.00%	0.078	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	2.17%	0.14%	0.00%	0.19%	0.064	<0.01	0.086	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	2.15%	0.04%	0.00%	0.25%	0.020	<0.01	0.116	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Ruminococcus</i>	Control	7.60%	2.40%	1.04%	1.34%	0.32	0.14	0.18	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	7.38%	2.10%	1.96%	1.61%	0.285	0.265	0.218	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	7.20%	3.90%	3.27%	3.64%	0.541	0.454	0.505	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Cubes	10.67%	1.86%	1.74%	1.86%	0.174	0.163	0.174	0.010	0.009	0.010	0.014	0.010	0.011
<i>Streptococcus</i>	Control	0.00%	0.35%	0.20%	0.16%	NA	NA	NA	1.000	1.000	1.000	1.000	1.000	1.000
	Inulin	0.00%	11.83%	9.19%	3.94%	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Powder	0.00%	8.83%	6.17%	4.37%	NA	NA	NA	1.000	1.000	1.000	1.000	1.000	1.000
	Cubes	0.00%	14.88%	11.17%	5.38%	NA	NA	NA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Subdoligranulum</i>	Control	9.11%	4.48%	1.29%	1.13%	0.49	0.14	0.12	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	9.01%	2.62%	0.96%	1.01%	0.291	0.107	0.113	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	10.90%	2.62%	0.86%	0.89%	0.240	0.079	0.082	0.005	0.002	0.002	0.007	0.002	0.002
	Cubes	10.44%	2.15%	1.04%	1.06%	0.206	0.099	0.101	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Oscillospiraceae</i> UCG-002	Control	0.81%	0.12%	0.13%	0.64%	0.15	0.16	0.79	0.012	0.011	0.535	0.018	0.014	0.595
	Inulin	0.73%	0.07%	0.00%	0.00%	0.098	<0.01	<0.01	NA	NA	NA	NA	NA	NA
	Powder	0.85%	0.09%	0.00%	0.28%	0.101	<0.01	0.330	0.001	1.000	0.119	0.002	1.000	0.148
	Cubes	1.01%	0.00%	0.00%	0.00%	<0.01	<0.01	<0.01	NA	NA	NA	NA	NA	NA
<i>Eubacterium eligens</i> group	Control	1.01%	0.61%	0.15%	0.00%	0.61	0.15	0.00	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Inulin	1.07%	0.53%	0.00%	0.00%	0.492	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Powder	1.23%	0.35%	0.00%	1.11%	0.284	<0.01	0.904	<0.001	<0.001	0.401	<0.001	<0.001	0.478
	Cubes	1.36%	0.34%	0.00%	1.15%	0.248	<0.01	0.852	<0.001	<0.001	0.450	<0.001	<0.001	0.480

Taxon	Product	relative abundance (%)				fold-Δ			p-value			q-value		
		0h	6h	24h	48h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h	0-6 h	0-24 h	0-48 h
<i>Eubacterium hallii</i> group	Control	0.24%	0.09%	0.00%	0.14%	0.37	0.00	0.56	0.148	1.000	0.404	0.185	1.000	0.466
	Inulin	0.20%	0.04%	0.00%	0.86%	0.195	<0.01	4.359	0.124	0.026	0.026	0.141	0.028	0.030
	Powder	0.27%	0.05%	0.04%	0.48%	0.169	0.152	1.765	0.227	0.201	0.699	0.261	0.249	0.774
	Cubes	0.26%	0.00%	0.00%	0.56%	<0.01	<0.01	2.166	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Ruminococcus torques</i> group	Control	1.14%	0.79%	10.18%	15.12%	0.69	8.90	13.21	0.001	<0.001	<0.001	0.002	<0.001	<0.001
	Inulin	1.04%	0.90%	6.77%	7.27%	0.858	6.484	6.969	0.008	<0.001	<0.001	0.011	<0.001	<0.001
	Powder	1.31%	1.31%	7.45%	8.80%	1.002	5.695	6.723	0.927	<0.001	<0.001	1.000	<0.001	<0.001
	Cubes	1.30%	1.02%	8.43%	9.50%	0.782	6.467	7.288	0.021	<0.001	<0.001	0.027	<0.001	<0.001

fold-Δ: fold-change in mean relative abundances over baseline for each condition (control or inulin or dried chicory root powder or cube fermentation); NA: represents p- and q-values that could not be reliably estimated due to violation of the assumption underlying the statistical models for calculating statistical inference ^[15]

The time-dependent development in overall and within sample gut microbiota composition was reflected in changes of individual genera over time. At the genus level we observed numerous taxa in all conditions to decrease rapidly within 6 h and plateauing at 24 h of fermentation (Supplementary Table 1). The most abundant taxa (mean ± SE relative abundance across all conditions) that decreased were *Faecalibacterium* spp. (16.82 ± 0.71%), an unidentified genus of *Lachnospiraceae* (14.39 ± 0.79%), *Subdoligranulum* spp. (9.87 ± 0.48%) and *Christensenellaceae* R7 group (8.13 ± 0.28%), all decreasing to 1% or less relative abundance over the course of 48 h. For *Faecalibacterium* spp. these decreases were consistent across all conditions and levels did not differ between conditions (Supplementary Table 2). In contrast to the rapid decreases between 0 to 6 h of fermentation, taxa increasing over time underwent the largest fold-changes between 6 to 24 h of fermentation. The highest fold-changes (fold-change ± SE) were observed for *Bacteroides* spp. (24 h: 20.3 ± 4.5 fold), *Lachnoclostridium* spp. (24 h: 13.0 ± 0.7 fold), *Parabacteroides* spp. (48 h: 10.4 ± 0.4 fold) as well as *Akkermansia* spp. (24 h: 11.1 ± 6.0 fold) and bacteria of the *Ruminococcus*

torques group (48h: 8.55 ± 1.56 fold) - for the two latter increasing the most in the control incubations. A number of taxa peaked at 6 h but then decreased again, which included mainly *Streptococcus* spp., with the highest levels at 6 h observed for dried chicory root cubes and inulin, as well as *Parasutterella* spp. with the highest levels in dried chicory root cubes and powder (Supplementary Table 1). We also observed a number of genera to first decrease between 0 to 6 h but then increase again between 6 to 24 and 48 h. The abundant *Blautia* spp. decreased in all conditions at 6 h but increased back to half its baseline levels for inulin and dried chicory root powder and cubes at 48 h. Similarly, bacteria from the *Eubacterium hallii* group decreased in all conditions and were not detected at 6 or 24 h, but then increased again above baseline levels at 48 h in inulin (4.5-fold) and dried chicory root powder (1.8-fold) and cubes (2.2-fold).

Supplementary Table 2. Mean relative abundances of common genera (mean relative abundance of at least 1% and mean prevalence of 50% in the whole dataset) at each timepoint (baseline 0 h, 6 h, 24 h, and 48 h) in the negative control fermentation (Control; only inoculum) or fermentation of inulin (Inulin) vs dried chicory root powder (Powder) or cubes (Cubes) and the respective fold-difference (fold- Δ) between the conditions as well as respective p-values and fdr-corrected q-values.

Taxon	relative abundance (%)				fold- Δ compared to Inulin			<i>p</i> -value compared to Inulin			<i>q</i> -value compared to Inulin			
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	
	Time 0 h													
<i>Adlercreutzia</i>	0.42%	0.34%	0.35%	0.34%	1.24	1.05	1.01	0.599	0.825	0.943	0.599	0.891	0.979	
<i>Akkermansia</i>	1.12%	1.19%	0.93%	1.13%	0.94	0.78	0.95	0.909	0.510	0.873	0.909	0.751	0.943	
<i>Bacteroides</i>	0.09%	0.38%	0.08%	0.13%	0.24	0.22	0.34	0.599	0.256	0.337	0.599	0.540	0.660	
<i>Blautia</i>	8.24%	8.08%	8.50%	8.18%	1.02	1.05	1.01	0.909	0.471	0.857	0.909	0.749	0.943	
<i>Christensenellaceae</i> R7 group	9.20%	8.26%	8.61%	8.24%	1.11	1.04	1.00	0.625	0.743	0.986	0.625	0.848	0.986	
<i>Dorea</i>	0.80%	0.73%	0.75%	0.82%	1.09	1.03	1.12	0.599	0.741	0.178	0.599	0.848	0.497	
<i>Erysipelotrichaceae</i> UCG 003	3.85%	3.46%	2.88%	3.19%	1.11	0.83	0.92	0.599	0.045	0.297	0.599	0.221	0.660	
<i>Faecalibacterium</i>	17.31%	17.79%	18.66%	17.21%	0.97	1.05	0.97	0.909	0.560	0.795	0.909	0.756	0.943	
<i>Fusicatenibacter</i>	2.47%	2.21%	2.51%	2.39%	1.12	1.14	1.08	0.599	0.139	0.342	0.599	0.382	0.660	
<i>Haemophilus</i>	0.00%	0.09%	0.00%	0.09%	<0.01	<0.01	1.03	NA	NA	NA	NA	NA	NA	
<i>Lachnoclostridium</i>	0.11%	0.00%	0.08%	0.08%	NA	NA	NA	<0.001	0.092	0.088	<0.001	0.310	0.395	
<i>Lachnospira</i>	1.02%	0.96%	1.02%	1.09%	1.05	1.06	1.13	0.757	0.528	0.203	0.757	0.751	0.497	
<i>Lachnospiraceae</i> ND3007 group	3.91%	4.54%	3.74%	4.00%	0.86	0.82	0.88	0.659	0.347	0.540	0.659	0.625	0.810	
<i>Lachnospiraceae</i> unidentified Genus	15.09%	14.53%	16.41%	14.75%	1.04	1.13	1.01	0.659	0.026	0.762	0.659	0.221	0.943	
<i>Monoglobus</i>	0.56%	0.50%	0.50%	0.53%	1.13	1.00	1.06	0.599	0.997	0.667	0.599	0.997	0.943	
<i>Muribaculaceae</i> unidentified Genus	6.85%	8.20%	5.82%	5.51%	0.84	0.71	0.67	0.599	0.047	0.024	0.599	0.221	0.217	
<i>Parabacteroides</i>	0.41%	0.51%	0.39%	0.41%	0.80	0.76	0.81	0.599	0.051	0.190	0.599	0.221	0.497	
<i>Paraprevotella</i>	1.16%	0.78%	1.01%	1.21%	1.48	1.29	1.54	0.524	0.230	0.083	0.524	0.540	0.395	
<i>Parasutterella</i>	1.17%	1.26%	1.12%	1.06%	0.93	0.89	0.84	0.599	0.057	0.010	0.599	0.221	0.217	

Taxon	relative abundance (%)				fold-Δ compared to Inulin			p-value compared to Inulin			q-value compared to Inulin		
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes
<i>Phascolarctobacterium</i>	1.67%	1.73%	2.35%	1.94%	0.96	1.36	1.12	0.911	0.289	0.717	0.911	0.557	0.943
<i>Roseburia</i>	2.09%	1.94%	2.17%	2.15%	1.07	1.11	1.11	0.599	0.056	0.071	0.599	0.221	0.395
<i>Ruminococcus</i>	8.37%	8.48%	7.20%	10.67%	0.99	0.85	1.26	0.909	0.045	0.373	0.909	0.221	0.665
<i>Subdoligranulum</i>	10.03%	10.23%	10.90%	10.44%	0.98	1.07	1.02	0.909	0.454	0.805	0.909	0.749	0.943
<i>UCG 002</i>	0.89%	0.83%	0.85%	1.01%	1.08	1.03	1.22	0.599	0.716	0.022	0.599	0.848	0.217
<i>Eubacterium eligens</i> group	1.11%	1.23%	1.23%	1.36%	0.90	1.00	1.10	0.707	0.980	0.503	0.707	0.997	0.798
<i>Eubacterium hallii</i> group	0.27%	0.22%	0.27%	0.26%	1.20	1.22	1.17	0.599	0.260	0.394	0.599	0.540	0.665
<i>Eubacterium ventriosum</i> group	0.48%	0.33%	0.35%	0.43%	1.45	1.05	1.28	0.365	0.754	0.125	0.365	0.848	0.482
<i>Ruminococcus torques</i> group	1.26%	1.19%	1.31%	1.30%	1.06	1.10	1.09	0.599	0.142	0.156	0.599	0.382	0.497
Time 6 h													
<i>Adlercreutzia</i>	0.84%	0.63%	0.51%	0.40%	1.34	0.81	0.64	0.089	0.312	0.161	0.258	0.476	0.312
<i>Akkermansia</i>	3.82%	2.26%	1.83%	1.51%	1.70	0.81	0.67	0.086	0.402	0.217	0.258	0.545	0.370
<i>Bacteroides</i>	1.38%	2.23%	1.20%	1.61%	0.62	0.54	0.72	0.386	0.245	0.546	0.746	0.394	0.688
<i>Blautia</i>	2.83%	2.33%	2.57%	2.22%	1.21	1.11	0.96	0.050	<0.001	0.625	0.200	0.001	0.713
<i>Christensenellaceae</i> R7 group	1.73%	1.24%	1.59%	0.82%	1.40	1.29	0.66	0.301	0.453	0.380	0.624	0.571	0.500
<i>Collinsella</i>	1.40%	2.65%	0.00%	0.33%	0.53	<0.01	0.13	0.991	0.005	0.178	0.991	0.017	0.323
<i>Dorea</i>	0.30%	0.27%	0.46%	0.35%	1.14	1.72	1.30	0.696	0.002	0.064	0.916	0.010	0.211
<i>Eggerthella</i>	0.15%	0.03%	0.12%	0.00%	4.51	3.63	<0.01	0.055	0.068	0.330	0.200	0.165	0.500
<i>Enterobacteriaceae</i> unidentified Genus	1.19%	0.67%	0.28%	0.97%	1.78	0.41	1.45	0.420	0.109	0.664	0.760	0.244	0.713
<i>Erysipelotrichaceae</i> UCG-003	1.33%	0.61%	0.50%	0.44%	2.18	0.81	0.73	<0.001	0.168	0.038	0.004	0.304	0.211
<i>Escherichia Shigella</i>	50.81%	48.96%	51.40%	53.84%	1.04	1.05	1.10	0.790	0.564	0.348	0.916	0.654	0.500
<i>Faecalibacterium</i>	5.12%	4.08%	4.42%	3.30%	1.26	1.08	0.81	0.156	0.621	0.274	0.376	0.687	0.442
<i>Fusicatenibacter</i>	0.50%	0.49%	0.38%	0.35%	1.03	0.78	0.71	0.859	0.164	0.076	0.922	0.304	0.211
<i>Haemophilus</i>	0.35%	0.33%	0.37%	0.28%	1.05	1.11	0.86	0.777	0.413	0.104	0.916	0.545	0.243

Taxon	relative abundance (%)				fold-Δ compared to Inulin			p-value compared to Inulin			q-value compared to Inulin		
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes
<i>Lachnospira</i>	0.39%	0.39%	0.29%	0.25%	1.01	0.75	0.66	0.971	0.182	0.080	0.991	0.310	0.211
<i>Lachnospiraceae</i> ND3007 group	1.35%	1.24%	1.10%	1.15%	1.09	0.89	0.92	0.758	0.689	0.782	0.916	0.713	0.810
<i>Lachnospiraceae</i> unidentified Genus	2.77%	2.15%	1.95%	1.49%	1.29	0.91	0.69	0.033	0.044	0.001	0.189	0.116	0.005
<i>Monoglobus</i>	0.18%	0.19%	0.16%	0.09%	0.94	0.84	0.46	0.848	0.008	0.050	0.922	0.027	0.211
<i>Muribaculaceae</i> unidentified Genus	3.01%	1.70%	2.34%	1.64%	1.77	1.38	0.96	0.045	<0.001	0.642	0.200	0.002	0.713
<i>Parabacteroides</i>	2.01%	1.89%	1.82%	1.59%	1.06	0.96	0.84	0.715	0.521	0.031	0.916	0.630	0.211
<i>Paraprevotella</i>	0.47%	0.27%	0.70%	0.26%	1.76	2.61	0.97	0.177	<0.001	0.861	0.395	0.002	0.861
<i>Parasutterella</i>	2.72%	1.70%	3.06%	3.26%	1.60	1.80	1.92	0.001	<0.001	<0.001	0.004	0.001	0.001
<i>Phascolarctobacterium</i>	5.44%	4.33%	5.66%	3.56%	1.26	1.31	0.82	0.625	0.391	0.372	0.916	0.545	0.500
<i>Roseburia</i>	0.22%	0.14%	0.14%	0.04%	1.56	0.98	0.30	0.130	0.898	0.059	0.344	0.898	0.211
<i>Ruminococcus</i>	2.66%	2.24%	3.90%	1.86%	1.19	1.74	0.83	0.696	0.001	0.066	0.916	0.002	0.211
<i>Streptococcus</i>	0.37%	12.56%	8.83%	14.88%	0.03	0.70	1.18	<0.001	<0.001	<0.001	<0.001	0.002	0.004
<i>Subdoligranulum</i>	4.90%	2.79%	2.62%	2.15%	1.76	0.94	0.77	<0.001	0.640	0.109	0.004	0.687	0.243
<i>Oscillospiraceae</i> UCG-002	0.13%	0.08%	0.09%	0.00%	1.72	1.11	<0.01	NA	NA	NA	NA	NA	NA
<i>Eubacterium eligens</i> group	0.67%	0.56%	0.35%	0.34%	1.18	0.62	0.60	0.567	0.162	0.152	0.914	0.304	0.312
<i>Eubacterium hallii</i> group	0.10%	0.04%	0.05%	0.00%	2.38	1.15	<0.01	NA	NA	NA	NA	NA	NA
<i>Ruminococcus torques</i> group	0.86%	0.96%	1.31%	1.02%	0.90	1.37	1.07	0.528	0.035	0.664	0.900	0.103	0.713
Time 24 h													
<i>Adlercreutzia</i>	0.60%	0.50%	0.38%	0.52%	1.21	0.77	1.05	0.171	0.132	0.706	0.231	0.274	0.901
<i>Akkermansia</i>	30.94%	3.84%	5.61%	6.69%	8.06	1.46	1.74	<0.001	0.442	0.143	0.001	0.550	0.317
<i>Bacteroides</i>	1.62%	2.74%	2.47%	3.19%	0.59	0.90	1.16	0.058	0.521	0.788	0.093	0.612	0.901
<i>Blautia</i>	0.70%	0.86%	4.18%	3.63%	0.81	4.88	4.24	0.232	<0.001	<0.001	0.299	<0.001	0.001
<i>Christensenellaceae</i> R7 group	0.70%	0.42%	0.37%	0.39%	1.67	0.89	0.94	0.034	0.677	0.834	0.068	0.703	0.901
<i>Collinsella</i>	2.88%	1.44%	4.05%	0.88%	2.00	2.82	0.61	0.939	0.628	0.319	0.939	0.703	0.575

Taxon	relative abundance (%)				fold-Δ compared to Inulin			p-value compared to Inulin			q-value compared to Inulin		
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes
<i>Dorea</i>	3.15%	11.48%	10.72%	6.65%	0.27	0.93	0.58	<0.001	0.202	0.002	<0.001	0.389	0.007
<i>Eggerthella</i>	0.14%	0.13%	0.11%	0.13%	1.07	0.86	1.02	0.682	0.276	0.893	0.736	0.447	0.927
<i>Enterobacteriaceae</i> unidentified Genus	0.38%	0.30%	0.22%	0.54%	1.28	0.75	1.80	0.569	0.448	0.389	0.673	0.550	0.617
<i>Erysipelotrichaceae</i> UCG-003	0.35%	0.26%	0.23%	0.25%	1.35	0.86	0.96	0.148	0.245	0.745	0.210	0.441	0.901
<i>Escherichia-Shigella</i>	27.74%	32.46%	27.27%	30.62%	0.85	0.84	0.94	0.105	0.080	0.497	0.158	0.179	0.745
<i>Faecalibacterium</i>	0.63%	0.68%	0.56%	0.77%	0.93	0.82	1.12	0.714	0.384	0.550	0.742	0.544	0.781
<i>Haemophilus</i>	0.25%	0.45%	0.74%	0.60%	0.54	1.64	1.32	<0.001	0.006	0.017	<0.001	0.021	0.052
<i>Lachnoclostridium</i>	1.00%	0.96%	1.26%	1.18%	1.05	1.31	1.24	0.616	0.281	0.298	0.693	0.447	0.575
<i>Lachnospira</i>	0.10%	0.08%	0.00%	0.00%	1.28	<0.01	<0.01	NA	NA	NA	NA	NA	NA
<i>Lachnospiraceae</i> ND3007 group	0.49%	0.56%	0.29%	0.43%	0.87	0.52	0.76	0.573	0.066	0.371	0.673	0.179	0.617
<i>Lachnospiraceae</i> UCG-004	0.41%	0.52%	0.26%	0.34%	0.78	0.49	0.65	0.003	<0.001	<0.001	0.008	<0.001	0.002
<i>Lachnospiraceae</i> unidentified Genus	0.73%	2.45%	2.00%	1.30%	0.30	0.82	0.53	<0.001	0.005	0.001	<0.001	0.019	0.005
<i>Muribaculaceae</i> unidentified Genus	2.04%	1.29%	1.40%	1.34%	1.58	1.09	1.04	<0.001	0.403	0.720	0.002	0.544	0.901
<i>Parabacteroides</i>	2.96%	4.91%	4.12%	2.92%	0.60	0.84	0.59	0.001	0.076	0.001	0.003	0.179	0.005
<i>Paraprevotella</i>	0.18%	0.12%	0.24%	0.19%	1.44	1.96	1.60	0.055	0.001	0.016	0.092	0.006	0.052
<i>Parasutterella</i>	2.65%	1.34%	2.90%	2.54%	1.97	2.16	1.89	<0.001	0.003	<0.001	<0.001	0.014	0.003
<i>Phascolarctobacterium</i>	5.35%	12.30%	12.54%	12.29%	0.43	1.02	1.00	0.014	0.916	0.995	0.032	0.916	0.995
<i>Ruminococcus</i>	1.11%	2.05%	3.27%	1.74%	0.54	1.60	0.85	0.001	0.001	0.108	0.002	0.006	0.266
<i>Streptococcus</i>	0.21%	9.62%	6.17%	11.17%	0.02	0.64	1.16	<0.001	<0.001	<0.001	<0.001	0.004	0.002
<i>Subdoligranulum</i>	1.37%	1.01%	0.86%	1.04%	1.36	0.86	1.03	0.035	0.338	0.830	0.068	0.508	0.901
<i>Eubacterium ventriosum</i> group	0.21%	0.10%	0.25%	0.21%	2.16	2.55	2.14	0.039	0.011	0.042	0.070	0.033	0.114
<i>Ruminococcus torques</i> group	10.84%	7.08%	7.45%	8.43%	1.53	1.05	1.19	0.002	0.677	0.152	0.006	0.703	0.317

Taxon	relative abundance (%)				fold-Δ compared to Inulin			p-value compared to Inulin			q-value compared to Inulin			
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	
	Time 48 h													
<i>Adlercreutzia</i>	0.29%	0.31%	0.44%	0.47%	0.93	1.39	1.49	0.894	0.470	0.383	0.894	0.616	0.547	
<i>Akkermansia</i>	21.42%	4.89%	7.16%	7.72%	4.38	1.46	1.58	<0.001	0.031	0.314	<0.001	0.071	0.470	
<i>Bacteroides</i>	0.76%	2.51%	2.62%	1.82%	0.30	1.04	0.72	0.005	0.815	0.165	0.011	0.844	0.309	
<i>Blautia</i>	0.77%	4.16%	4.42%	4.35%	0.18	1.06	1.05	<0.001	0.606	0.774	0.001	0.699	0.862	
<i>Christensenellaceae</i> R7 group	1.02%	0.46%	0.41%	0.49%	2.20	0.87	1.06	0.001	0.493	0.596	0.002	0.616	0.716	
<i>Collinsella</i>	0.26%	0.07%	3.06%	0.12%	3.80	44.57	1.72	NA	NA	NA	NA	NA	NA	
<i>Dorea</i>	3.44%	3.32%	1.54%	1.43%	1.04	0.47	0.43	0.811	0.006	0.004	0.839	0.016	0.021	
<i>Eggerthella</i>	0.19%	0.13%	0.12%	0.16%	1.43	0.94	1.24	<0.001	0.415	0.032	<0.001	0.593	0.107	
<i>Enterobacteriaceae</i> unidentified Genus	0.19%	0.53%	0.26%	0.35%	0.36	0.49	0.65	0.037	0.080	0.211	0.073	0.161	0.373	
<i>Erysipelotrichaceae</i> UCG-003	0.22%	0.18%	0.19%	0.17%	1.23	1.08	0.94	0.436	0.720	0.799	0.524	0.800	0.862	
<i>Escherichia Shigella</i>	35.15%	39.36%	32.84%	39.08%	0.89	0.83	0.99	0.250	0.091	0.935	0.326	0.170	0.935	
<i>Faecalibacterium</i>	0.41%	0.59%	0.47%	0.61%	0.69	0.79	1.03	0.153	0.324	0.891	0.230	0.486	0.921	
<i>Haemophilus</i>	0.09%	0.33%	0.48%	0.39%	0.26	1.44	1.18	0.002	0.004	0.107	0.004	0.013	0.214	
<i>Lachnoclostridium</i>	0.97%	1.24%	0.92%	0.81%	0.78	0.74	0.65	0.250	0.181	0.091	0.326	0.320	0.210	
<i>Lachnospiraceae</i> ND3007 group	0.32%	0.42%	0.32%	0.28%	0.76	0.76	0.66	0.569	0.574	0.427	0.628	0.688	0.577	
<i>Lachnospiraceae</i> UCG 004	0.41%	0.61%	0.30%	0.35%	0.67	0.50	0.58	0.001	<0.001	<0.001	0.002	<0.001	0.001	
<i>Lachnospiraceae</i> unidentified Genus	1.85%	0.78%	0.66%	0.50%	2.38	0.85	0.64	0.170	0.302	0.011	0.242	0.478	0.042	
<i>Monoglobus</i>	0.00%	0.00%	0.49%	0.32%	NA	NA	NA	0.113	<0.001	<0.001	0.178	<0.001	<0.001	
<i>Muribaculaceae</i> unidentified Genus	1.82%	1.73%	1.20%	1.59%	1.05	0.69	0.92	0.458	0.002	0.274	0.529	0.006	0.432	
<i>Parabacteroides</i>	3.74%	5.18%	4.32%	4.10%	0.72	0.83	0.79	0.010	0.081	0.036	0.022	0.161	0.108	
<i>Paraprevotella</i>	0.04%	0.10%	0.27%	0.20%	0.40	2.68	2.01	0.369	0.016	0.105	0.461	0.040	0.214	
<i>Parasutterella</i>	3.60%	1.48%	2.73%	3.20%	2.43	1.84	2.15	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	
<i>Phascolarctobacterium</i>	3.50%	15.72%	15.02%	11.44%	0.22	0.96	0.73	<0.001	0.776	0.061	0.001	0.832	0.154	

Taxon	relative abundance (%)				fold-Δ compared to Inulin			p-value compared to Inulin			q-value compared to Inulin		
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes
<i>Roseburia</i>	0.00%	0.00%	0.19%	0.25%	NA	NA	NA	0.105	<0.001	<0.001	0.175	<0.001	<0.001
<i>Ruminococcus</i>	1.41%	1.74%	3.64%	1.86%	0.81	2.10	1.07	0.101	<0.001	0.447	0.175	<0.001	0.577
<i>Streptococcus</i>	0.17%	4.27%	4.37%	5.38%	0.04	1.02	1.26	<0.001	0.846	0.049	<0.001	0.846	0.134
<i>Subdoligranulum</i>	1.19%	1.10%	0.89%	1.06%	1.08	0.81	0.96	0.586	0.242	0.805	0.628	0.403	0.862
<i>Oscillospiraceae</i> UCG-002	0.67%	0.00%	0.28%	0.00%	NA	NA	NA	<0.001	<0.001	0.461	<0.001	<0.001	0.577
<i>Eubacterium eligens</i> group	0.00%	0.00%	1.11%	1.15%	NA	NA	NA	0.104	<0.001	<0.001	0.175	<0.001	<0.001
<i>Eubacterium hallii</i> group	0.14%	0.93%	0.48%	0.56%	0.16	0.52	0.61	<0.001	0.002	0.008	<0.001	0.007	0.032
<i>Ruminococcus torques</i> group	15.85%	7.87%	8.80%	9.50%	2.02	1.12	1.21	<0.001	0.487	0.237	0.001	0.616	0.395

48 h mucus

<i>Akkermansia</i>	10.04%	0.50%	1.11%	1.12%	20.02	2.21	2.23	<0.001	0.124	0.050	0.002	0.234	0.106
<i>Bifidobacterium</i>	0.06%	2.32%	2.43%	2.32%	0.02	1.05	1.00	0.009	0.916	1.000	0.022	0.974	1.000
<i>Blautia</i>	0.46%	0.87%	1.30%	1.62%	0.53	1.49	1.86	0.113	0.101	0.011	0.160	0.234	0.048
<i>Clostridium sensu stricto 1</i>	5.57%	6.45%	0.50%	1.07%	0.86	0.08	0.17	0.368	<0.001	<0.001	0.447	<0.001	0.001
<i>Dorea</i>	0.64%	0.39%	0.67%	0.86%	1.65	1.73	2.22	0.009	0.116	0.038	0.022	0.234	0.091
<i>Eisenbergiella</i>	0.28%	0.29%	0.18%	0.24%	0.97	0.61	0.81	0.857	0.040	0.273	0.919	0.113	0.387
<i>Escherichia.Shigella</i>	4.63%	2.14%	2.68%	2.92%	2.17	1.25	1.37	0.008	0.470	0.305	0.022	0.665	0.399
<i>Lachnoclostridium</i>	0.16%	0.41%	0.00%	0.16%	0.38	0.00	0.40	0.024	<0.001	0.030	0.049	<0.001	0.086
<i>Lachnospiraceae</i> unidentified Genus	40.04%	2.97%	15.80%	9.06%	13.46	5.31	3.05	<0.001	<0.001	0.002	<0.001	0.001	0.013
<i>Muribaculaceae</i> unidentified Genus	0.28%	0.10%	0.06%	0.19%	2.91	0.59	1.96	0.026	0.575	0.208	0.049	0.752	0.322
<i>Parabacteroides</i>	0.12%	0.29%	0.17%	0.20%	0.40	0.57	0.71	0.153	0.294	0.466	0.200	0.454	0.566
<i>Parasutterella</i>	0.04%	0.06%	0.16%	0.23%	0.74	2.78	4.21	0.865	0.267	0.064	0.919	0.454	0.122
<i>Phascolarctobacterium</i>	3.86%	3.82%	3.53%	1.93%	1.01	0.93	0.50	0.973	0.827	0.142	0.973	0.937	0.242
<i>Roseburia</i>	12.58%	1.20%	24.77%	14.44%	10.47	20.61	12.02	<0.001	0.011	<0.001	<0.001	0.049	0.001

Taxon	relative abundance (%)				fold- Δ compared to Inulin			<i>p</i> -value compared to Inulin			<i>q</i> -value compared to Inulin		
	Control	Inulin	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes	Control	Powder	Cubes
<i>Streptococcus</i>	0.28%	4.05%	3.48%	4.76%	0.07	0.86	1.17	0.029	0.751	0.721	0.049	0.912	0.817
<i>Eubacterium hallii</i> group	0.00%	0.19%	0.19%	0.16%	0.00	0.98	0.84	0.106	0.978	0.781	0.160	0.978	0.830
<i>Ruminococcus torques</i> group	17.60%	67.81%	42.99%	53.12%	0.26	0.63	0.78	<0.001	0.015	0.023	<0.001	0.050	0.078
<i>Akkermansia</i>	10.04%	0.50%	1.11%	1.12%	20.02	2.21	2.23	<0.001	0.124	0.050	0.002	0.234	0.106

fold- Δ : fold-difference in mean relative abundances per timepoint of control or dried chicory root powder or cube fermentation compared to inulin fermentation; NA: represents p- and q-values that could not be reliably estimated due to violation of the assumption underlying the statistical models for calculating statistical inference ^[15]

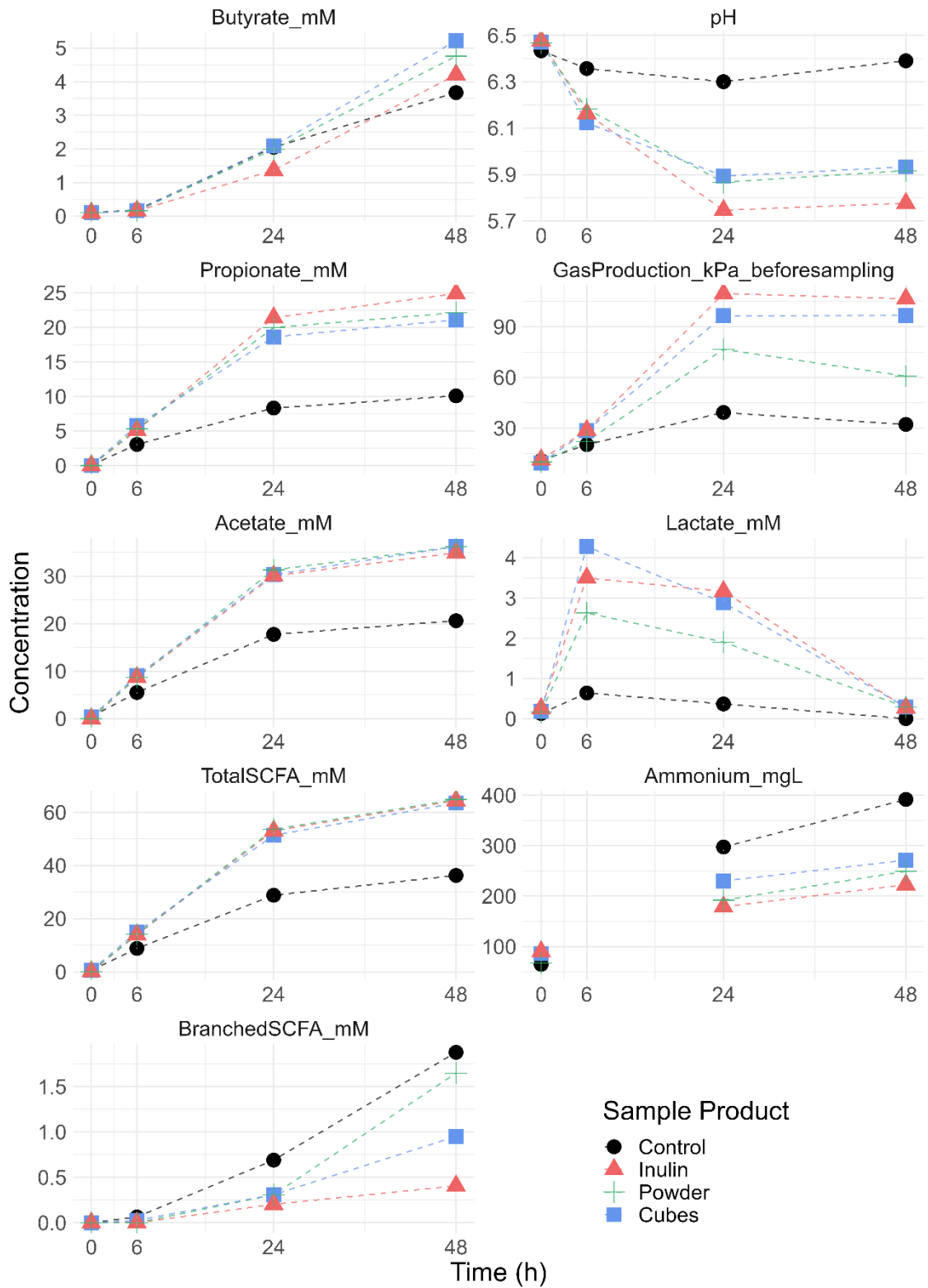
Comparing differences in relative abundance of genera at each timepoint between inulin and dried chicory root cubes or powder or control (Supplementary Table 2), we observed that the number of taxa statistically significantly differing from inulin increased over time. At 6 h, 12 genera were found to differ in dried chicory root cubes or powder or control from inulin, while at 24 h these were 15 genera and at 48 h 22 genera. The majority of taxa identified to differ statistically significantly from inulin at each time point were contributed by dried chicory root powder, which had the following amounts of statistically significantly different taxa: at 6h nine out of 12 genera, at 24 h nine out of 15 genera and at 48h 12 out 22 genera. For dried chicory root cubes these were at 6 h three of the 12 genera, at 24 h seven out of the 15 genera and at 48 h eight out of the 22 taxa. The taxa with statistically significantly largest positive fold-differences (>1.2-fold) between dried chicory root powder and inulin were *Paraprevotella* spp., *Parasutterella* spp, and *Ruminococcus* spp. at 6 h, 24 and 48 h, *Blautia* spp. at 6 and *Haemophilus* spp. at 24 h and *Eubacterium ventrosium* group and *Colinsella* spp. at 24h, as well as *Dorea* spp. at 6 h and 48 h. Besides *Parasutterella* spp and *Streptococcus* spp for dried chicory root cubes at 6 h and 24 h, *Blautia* spp. and *Dorea* spp. underwent the largest fold-changes statistically significantly different from inulin at 24 h. Both dried chicory root powder and cubes had statistically significantly higher levels of *Monoglobus*

spp., bacteria from the *Eubacterium eligens* group and *Roseburia* spp at 48 h, which were virtually not present in inulin or control. In the respective mucus samples collected at 48 h of fermentation *Bifidobacterium* spp became apparent at low relative levels for all three fibers (Supplementary Figure 7) in contrast to no detected presence in the pellets at any timepoint of the fermentation. Besides higher levels in *Roseburia* spp and *Bifidobacterium* spp, dried chicory root powder and cubes fermentation also resulted in significantly lower levels of the potential pathogen containing genus *Clostridium sensu stricto 1* compared to the control and inulin. The mucin-covered beads were also dominated by bacteria from the known mucin-degrading *Ruminococcus torques* group ^[16] and we suspect that due to absence of other fiber substrate to feed on *Akkermansia* spp. and bacteria from the *Ruminococcus torques* group were released from the mucin-covered beads explaining their highest level in the control incubation pellets.

Supplementary Table 3. Number of genera found to significantly increase after 48 h of *in vitro* fermentation in the control (Control) or inulin (Inulin), dried chicory root powder (Powder) or cubes (Cubes).

<i>Taxon</i>	Control				Inulin				Powder				Cubes			
	q-value	(%)	foldΔ	Δ%	q-value	(%)	foldΔ	Δ%	q-value	(%)	foldΔ	Δ%	q-value	(%)	foldΔ	Δ%
1. Akkermansia	<0.001	20.40%	20.20	19.39%	<0.001	4.51%	4.31	3.47%	<0.001	6.73%	8.01	5.89%	<0.001	7.17%	7.02	6.15%
2. Bacteroides	0.001	0.73%	8.99	0.65%	0.002	2.32%	7.41	2.00%	<0.001	2.46%	31.95	2.38%	<0.001	1.69%	14.73	1.58%
3. Coprococcus	-	-	-	-	-	-	-	-	-	-	-	-	<0.001	0.57%	1.24	0.11%
4. Dorea	<0.001	3.28%	4.52	2.55%	<0.001	3.07%	4.75	2.43%	<0.001	1.45%	2.15	0.78%	0.007	1.34%	1.81	0.60%
5. Eggerthella	<0.001	0.18%	NA	0.18%	-	-	-	-	<0.001	0.12%	NA	0.12%	-	-	-	-
6. Enterobacteriaceae unidentified Genus	-	-	-	-	<0.001	0.49%	NA	0.49%	-	-	-	-	<0.001	0.32%	NA	0.32%
7. Eubacterium hallii	-	-	-	-	0.030	0.86%	4.36	0.66%	-	-	-	-	<0.001	0.53%	2.24	0.29%
8. Haemophilus	<0.001	0.08%	NA	0.08%	0.014	0.31%	4.01	0.23%	-	-	-	-	0.009	0.37%	4.54	0.29%
9. Lachnoclostridium	<0.001	0.92%	8.89	0.82%	-	-	-	-	<0.001	0.86%	11.79	0.79%	<0.001	0.76%	9.96	0.68%
10. Lachnospiraceae UCG-004	<0.001	0.39%	NA	0.39%	<0.001	0.56%	NA	0.56%	<0.001	0.28%	NA	0.28%	0.012	0.33%	4.36	0.25%
11. Parabacteroides	<0.001	3.57%	9.59	3.19%	<0.001	4.78%	10.79	4.34%	<0.001	4.06%	11.67	3.71%	<0.001	3.82%	10.20	3.45%
12. Parasutterella	<0.001	3.43%	3.22	2.37%	<0.001	1.37%	1.24	0.27%	0.012	2.57%	2.55	1.56%	<0.001	2.99%	3.13	2.04%
13. Peptostreptococcus	-	-	-	-	<0.001	0.38%	NA	0.38%	-	-	-	-	<0.001	0.61%	NA	0.61%
14. Phascolarcto- bacterium	0.006	3.34%	2.21	1.83%	<0.001	14.50%	9.51	12.97%	<0.001	14.12%	6.71	12.01%	<0.001	10.69%	6.11	8.94%
15. Ruminococcus torques	<0.001	15.12%	13.21	13.97%	<0.001	7.27%	6.97	6.23%	<0.001	8.28%	7.06	7.11%	<0.001	8.91%	7.57	7.73%
16. Streptococcus	-	-	-	-	-	-	-	-	-	-	-	-	0.041	5.03%	NA	5.03%
17. Veillonella	-	-	-	-	-	-	-	-	<0.001	0.56%	NA	0.56%	-	-	-	-

fold-Δ: fold-difference in mean relative abundances at 48 h over baseline per condition; Δ%: absolute change in mean relative abundance at 48 h over baseline



Supplementary Figure 8. Concentrations of pH, gas production levels and fermentation metabolites over time (T = 0h, 6 h, 24 h and 48 h) for control (Control; only inoculum) *in vitro* fermentation and i fermentation of inulin (Inulin) and dried chicory root powder (Powder) and cubes (Cubes).

Supplementary Table 4. Differences in pH, gas production and levels of fermentation metabolites at baseline (T = 0h), 6 h, 24 h and 48 h between control *in vitro* fermentation (Control; only inoculum) and *in vitro* fermentation of inulin (Inulin) and dried chicory root powder (Powder) and cubes (Cubes) and their changes over baseline. Statistical testing was done using by robust ANOVA followed by pairwise comparison using the corresponding Yuen's trimmed means test and fdr-correction (p_{adj}). Data are presented as mean \pm SE.

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)					
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes
pH	0	6.43 \pm 0	6.48 \pm 0	6.47 \pm 0.01	6.47 \pm 0.02	F(3, 4.13) = 21.87 p = 0.006	p_{adj} = 0.005	p_{adj} = 0.148	p_{adj} = 0.329	p_{adj} = 0.568	p_{adj} = 0.875	p_{adj} = 0.875
	6	6.36 \pm 0	6.16 \pm 0	6.18 \pm 0.01	6.12 \pm 0.01	F(3, 4.1) = 453.57 p = 0.001	p_{adj} < 0.001	p_{adj} = 0.006	p_{adj} = 0.006	p_{adj} = 0.233	p_{adj} = 0.125	p_{adj} = 0.053
	Δ 0-6	-0.08 \pm 0	-0.31 \pm 0	-0.28 \pm 0.01	-0.35 \pm 0.03	F(3, 4.06) = 647.97 p = 0.001	p_{adj} < 0.001	p_{adj} = 0.01	p_{adj} = 0.02	p_{adj} = 0.203	p_{adj} = 0.362	p_{adj} = 0.203
	24	6.3 \pm 0.01	5.75 \pm 0.01	5.87 \pm 0	5.89 \pm 0	F(3, 4.3) = 1355.53 p = 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} = 0.001	p_{adj} < 0.001	p_{adj} = 0.005
	Δ 0-24	-0.13 \pm 0	-0.73 \pm 0.01	-0.6 \pm 0.01	-0.58 \pm 0.02	F(3, 4.13) = 2658.83 p = 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} = 0.002	p_{adj} < 0.001	p_{adj} = 0.014	p_{adj} = 0.37
	Δ 6-24	-0.06 \pm 0	-0.42 \pm 0.01	-0.32 \pm 0.01	-0.23 \pm 0.01	F(3, 3.82) = 444.84 p = 0.001	p_{adj} = 0.001	p_{adj} = 0.003	p_{adj} = 0.004	p_{adj} = 0.006	p_{adj} = 0.001	p_{adj} = 0.008
	48	6.39 \pm 0.01	5.78 \pm 0.01	5.92 \pm 0.01	5.93 \pm 0.01	F(3, 4.41) = 1454.29 p = 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} < 0.001	p_{adj} = 0.211
	Δ 0-48	-0.04 \pm 0	-0.70 \pm 0.01	-0.55 \pm 0	-0.54 \pm 0.02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Δ 24-48	0.09 \pm 0	0.03 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)						
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes	
Gas production (kPa)	0	10.87 ± 0.8	11.33 ± 2.07	10.03 ± 0.56	9.3 ± 0.76	F(3, 4.26) = 0.61 p = 0.643	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	6	20.27 ± 0.89	28.8 ± 1.22	22.23 ± 0.47	28.47 ± 0.94	F(3, 4.15) = 16.95 p = 0.009	p _{adj} = 0.019	p _{adj} = 0.173	p _{adj} = 0.019	p _{adj} = 0.033	p _{adj} = 0.84	p _{adj} = 0.02	
	6*	15.07 ± 0.89	26.27 ± 1.07	17.8 ± 0.62	27.33 ± 0.97	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	Δ0-6	9.4 ± 0.12	17.47 ± 0.88	12.2 ± 0.15	19.17 ± 0.19	F(3, 4.21) = 514.41 p = 0.001	p _{adj} = 0.016	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.029	p _{adj} = 0.19	p _{adj} < 0.001	
	24	39.3 ± 1.01	109.6 ± 1.21	76.67 ± 1.31	96.53 ± 0.72	F(3, 4.33) = 690.65 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.002	p _{adj} = 0.001	
	24*	32.37 ± 0.7	98.4 ± 0.78	67.1 ± 1.26	86 ± 1.04	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	Δ6-24	24.23 ± 0.14	83.33 ± 0.14	58.87 ± 1.15	69.2 ± 0.26	F(3, 4.19) = 21660.24 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} = 0.002	p _{adj} < 0.001	p _{adj} = 0.009	
	Δ0-24	33.63 ± 0.22	100.8 ± 0.79	71.07 ± 1.27	88.37 ± 0.13	F(3, 3.89) = 11631.29 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.004	p _{adj} = 0.005	
	48	32.23 ± 0.79	106.53 ± 0.58	60.77 ± 1.19	96.67 ± 1.16	F(3, 4.27) = 1591.21 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.005	p _{adj} < 0.001	
	Δ24-48	-0.13 ± 0.18	8.13 ± 0.52	-6.33 ± 0.38	10.67 ± 0.12	F(3, 4.05) = 937.9 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.034	p _{adj} < 0.001	
Δ0-48	33.5 ± 0.36	108.93 ± 1	64.73 ± 1.13	99.03 ± 0.2	F(3, 3.89) = 6457.69 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.008	p _{adj} = 0.001		

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)						
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes	
Butyrate (mM)	0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	F(3, 4.08) = 0.61 p = 0.648	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	6	0.18 ± 0.02	0.16 ± 0	0.16 ± 0	0.16 ± 0	F(3, 4.02) = 0.78 p = 0.566	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Δ0-6	0.08 ± 0.02	0.06 ± 0	0.06 ± 0	0.06 ± 0	F(3, 4.24) = 0.58 p = 0.662	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	24	2.05 ± 0.01	1.37 ± 0.01	1.99 ± 0.04	2.09 ± 0.05	F(3, 3.9) = 491.9 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.346	p _{adj} = 0.52	p _{adj} = 0.008	p _{adj} = 0.008	p _{adj} = 0.299	
	Δ6-24	1.87 ± 0.03	1.21 ± 0.01	1.83 ± 0.05	1.93 ± 0.05	F(3, 3.66) = 169.13 p = 0.001	p _{adj} = 0.007	p _{adj} = 0.523	p _{adj} = 0.435	p _{adj} = 0.008	p _{adj} = 0.008	p _{adj} = 0.31	
	Δ0-24	1.95 ± 0.01	1.26 ± 0.01	1.88 ± 0.04	1.99 ± 0.05	F(3, 3.88) = 640.4 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.353	p _{adj} = 0.493	p _{adj} = 0.009	p _{adj} = 0.009	p _{adj} = 0.287	
	48	3.67 ± 0.18	4.21 ± 0.11	4.76 ± 0.14	5.22 ± 0.21	F(3, 4.32) = 10.45 p = 0.02	p _{adj} = 0.094	p _{adj} = 0.031	p _{adj} = 0.031	p _{adj} = 0.057	p _{adj} = 0.049	p _{adj} = 0.155	
	Δ24-48	1.62 ± 0.17	2.84 ± 0.1	2.77 ± 0.12	3.13 ± 0.16	F(3, 4.35) = 12.17 p = 0.015	p _{adj} = 0.016	p _{adj} = 0.016	p _{adj} = 0.016	p _{adj} = 0.67	p _{adj} = 0.265	p _{adj} = 0.233	
	Δ0-48	3.57 ± 0.18	4.11 ± 0.11	4.66 ± 0.14	5.12 ± 0.21	F(3, 4.32) = 10.58 p = 0.02	p _{adj} = 0.09	p _{adj} = 0.03	p _{adj} = 0.03	p _{adj} = 0.058	p _{adj} = 0.048	p _{adj} = 0.153	

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)						
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes	
Propionate (mM)	0	0.05 ± 0.05	0 ± 0	0 ± 0	0 ± 0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	6	3.06 ± 0.01	5.09 ± 0.07	5.31 ± 0.06	5.79 ± 0.09	F(3, 3.52) = 635.94 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.002	p _{adj} = 0.002	p _{adj} = 0.089	p _{adj} = 0.007	p _{adj} = 0.02	
	Δ0-6	3.01 ± 0.04	5.09 ± 0.07	5.31 ± 0.06	5.79 ± 0.09	F(3, 4.19) = 430.7 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.089	p _{adj} = 0.007	p _{adj} = 0.02	
	24	8.33 ± 0.09	21.41 ± 0.16	19.97 ± 0.23	18.61 ± 0.48	F(3, 4.03) = 1617.34 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.003	p _{adj} = 0.015	p _{adj} = 0.025	p _{adj} = 0.09	
	Δ6-24	5.27 ± 0.1	16.32 ± 0.16	14.66 ± 0.29	12.82 ± 0.44	F(3, 4.05) = 979.06 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} = 0.005	p _{adj} = 0.017	p _{adj} = 0.013	p _{adj} = 0.032	
	Δ0-24	8.28 ± 0.14	21.41 ± 0.16	19.97 ± 0.23	18.61 ± 0.48	F(3, 4.25) = 1155.61 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.002	p _{adj} = 0.015	p _{adj} = 0.025	p _{adj} = 0.09	
	48	10.09 ± 0.51	24.89 ± 0.95	22.14 ± 0.63	21.08 ± 1.06	F(3, 4.3) = 84.63 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.001	p _{adj} = 0.006	p _{adj} = 0.099	p _{adj} = 0.084	p _{adj} = 0.451	
	Δ24-48	1.76 ± 0.42	3.48 ± 0.91	2.16 ± 0.64	2.47 ± 0.58	F(3, 4.32) = 0.83 p = 0.542	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	Δ0-48	10.04 ± 0.55	24.89 ± 0.95	22.14 ± 0.63	21.08 ± 1.06	F(3, 4.32) = 79.45 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.001	p _{adj} = 0.005	p _{adj} = 0.099	p _{adj} = 0.084	p _{adj} = 0.451	

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)						
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes	
Acetate (mM)	0	0.41 ± 0.21	0 ± 0	0 ± 0	0.43 ± 0.21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	6	5.52 ± 0.04	8.71 ± 0.09	8.7 ± 0.05	9.08 ± 0.07	F(3, 4.27) = 971 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.944	p _{adj} = 0.049	p _{adj} = 0.027	
	Δ0-6	5.11 ± 0.22	8.71 ± 0.09	8.7 ± 0.05	8.65 ± 0.15	F(3, 3.92) = 66.29 p = 0.001	p _{adj} = 0.003	p _{adj} = 0.005	p _{adj} = 0.002	p _{adj} = 0.944	p _{adj} = 0.918	p _{adj} = 0.918	
	24	17.75 ± 0.48	30.07 ± 0.21	31.32 ± 0.5	30.32 ± 0.9	F(3, 3.95) = 146.27 p = 0.001	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} = 0.002	p _{adj} = 0.176	p _{adj} = 0.804	p _{adj} = 0.484	
	Δ6-24	12.22 ± 0.44	21.35 ± 0.17	22.61 ± 0.48	21.25 ± 0.86	F(3, 3.87) = 96.72 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.001	p _{adj} = 0.005	p _{adj} = 0.163	p _{adj} = 0.912	p _{adj} = 0.305	
	Δ0-24	17.33 ± 0.55	30.07 ± 0.21	31.32 ± 0.5	29.9 ± 0.72	F(3, 3.95) = 123.96 p = 0.001	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} = 0.176	p _{adj} = 0.841	p _{adj} = 0.229	
	48	20.62 ± 0.17	34.9 ± 1.56	36.2 ± 0.97	36.24 ± 1.77	F(3, 3.45) = 96.3 p = 0.001	p _{adj} = 0.024	p _{adj} = 0.018	p _{adj} = 0.024	p _{adj} = 0.72	p _{adj} = 0.72	p _{adj} = 0.985	
	Δ24-48	2.88 ± 0.58	4.83 ± 1.49	4.88 ± 0.96	5.91 ± 1.04	F(3, 4.22) = 2.1 p = 0.239	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	Δ0-48	20.21 ± 0.08	34.9 ± 1.56	36.2 ± 0.97	35.81 ± 1.66	F(3, 3.36) = 106.35 p = 0.001	p _{adj} = 0.022	p _{adj} = 0.021	p _{adj} = 0.022	p _{adj} = 0.787	p _{adj} = 0.849	p _{adj} = 0.853	

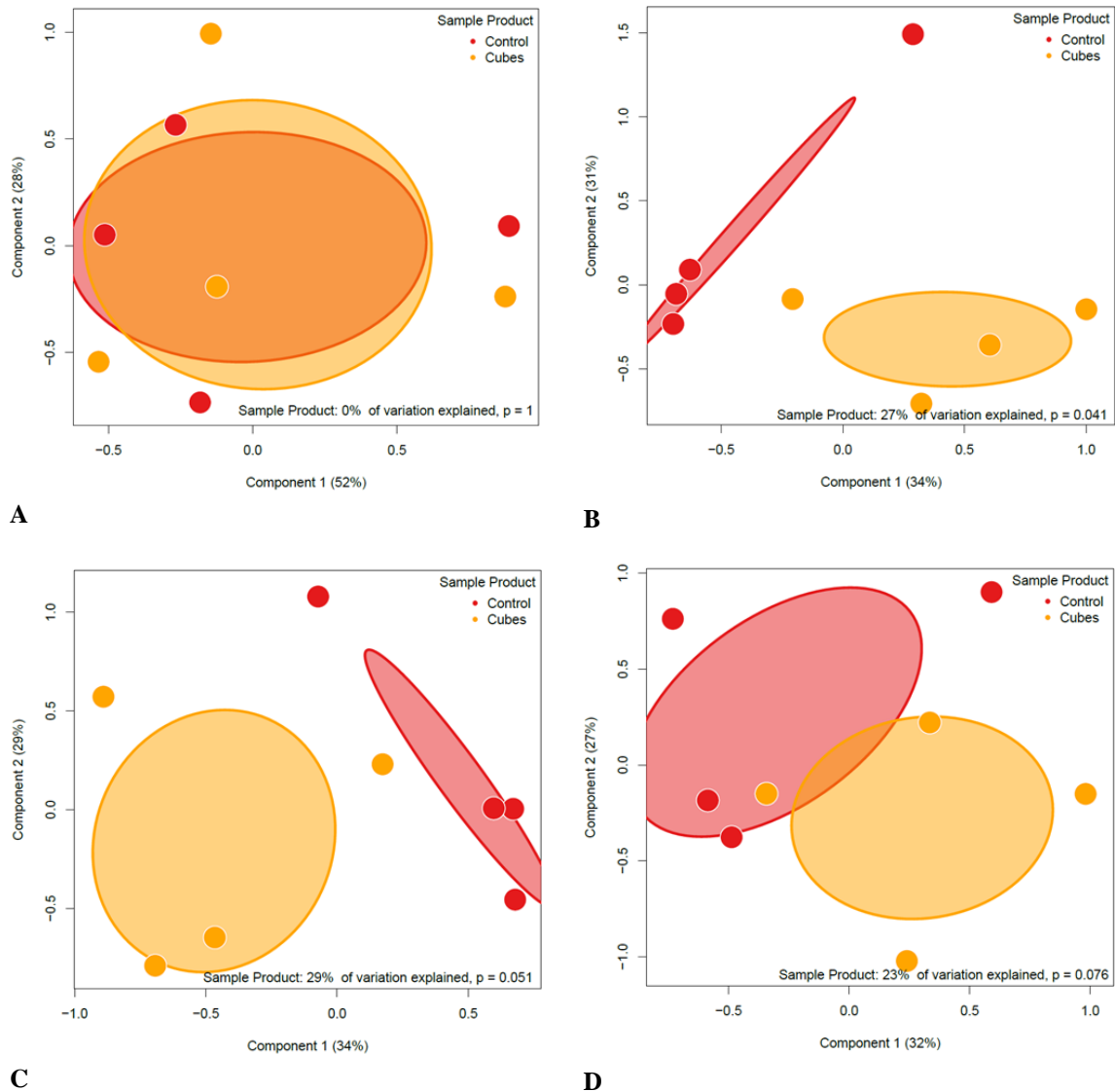
	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)					
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes
Total SCFA (mM)	0	0.57 ± 0.23	0.1 ± 0	0.1 ± 0	0.53 ± 0.21	F(3, 3.65) = 2.39 p = 0.222	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	6	8.83 ± 0.02	13.96 ± 0.16	14.18 ± 0.08	15.06 ± 0.19	F(3, 3.51) = 1408.13 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.001	p _{adj} = 0.002	p _{adj} = 0.33	p _{adj} = 0.018	p _{adj} = 0.033
	Δ0-6	8.26 ± 0.22	13.86 ± 0.17	14.08 ± 0.08	14.53 ± 0.07	F(3, 4.21) = 181.47 p = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} = 0.001	p _{adj} = 0.337	p _{adj} = 0.048	p _{adj} = 0.024
	24	28.81 ± 0.62	53.05 ± 0.39	53.58 ± 0.79	51.34 ± 1.41	F(3, 4.14) = 300.87 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.002	p _{adj} = 0.588	p _{adj} = 0.418	p _{adj} = 0.381
	Δ6-24	19.99 ± 0.6	39.08 ± 0.35	39.4 ± 0.83	36.28 ± 1.31	F(3, 4.08) = 197.72 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.004	p _{adj} = 0.747	p _{adj} = 0.191	p _{adj} = 0.191
	Δ0-24	28.25 ± 0.72	52.95 ± 0.39	53.48 ± 0.79	50.81 ± 1.24	F(3, 4.11) = 244.14 p = 0.001	p _{adj} < 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} = 0.589	p _{adj} = 0.266	p _{adj} = 0.235
	48	36.26 ± 1.41	64.4 ± 2.63	64.84 ± 1.68	63.49 ± 3.02	F(3, 4.29) = 55.99 p = 0.001	p _{adj} = 0.007	p _{adj} = 0.001	p _{adj} = 0.009	p _{adj} = 0.896	p _{adj} = 0.896	p _{adj} = 0.896
	Δ24-48	7.45 ± 0.95	11.35 ± 2.53	11.26 ± 1.68	12.15 ± 1.76	F(3, 4.18) = 2.13 p = 0.235	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Δ0-48	35.7 ± 1.36	64.3 ± 2.63	64.74 ± 1.68	62.96 ± 2.9	F(3, 4.28) = 59.91 p = 0.001	p _{adj} = 0.007	p _{adj} = 0.001	p _{adj} = 0.008	p _{adj} = 0.896	p _{adj} = 0.896	p _{adj} = 0.896

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)						
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes	
Branched SCFA (mM)	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	6	0.06 ± 0	0 ± 0	0 ± 0	0.03 ± 0.03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Δ0-6	0.06 ± 0	0 ± 0	0 ± 0	0.03 ± 0.03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	24	0.69 ± 0.03	0.2 ± 0.03	0.31 ± 0.01	0.31 ± 0.02	F(3, 3.85) = 36.93 p = 0.003	p _{adj} = 0.002	p _{adj} = 0.009	p _{adj} = 0.004	p _{adj} = 0.069	p _{adj} = 0.069	p _{adj} = 0.997	
	Δ6-24	0.63 ± 0.03	0.2 ± 0.03	0.31 ± 0.01	0.28 ± 0.01	F(3, 4) = 34.64 p = 0.003	p _{adj} = 0.003	p _{adj} = 0.012	p _{adj} = 0.012	p _{adj} = 0.081	p _{adj} = 0.106	p _{adj} = 0.081	
	Δ0-24	0.69 ± 0.03	0.2 ± 0.03	0.31 ± 0.01	0.31 ± 0.02	F(3, 3.85) = 36.93 p = 0.003	p _{adj} = 0.002	p _{adj} = 0.009	p _{adj} = 0.004	p _{adj} = 0.069	p _{adj} = 0.069	p _{adj} = 0.997	
	48	1.88 ± 0.71	0.41 ± 0.01	1.65 ± 0.06	0.95 ± 0.09	F(3, 3.42) = 116.46 p = 0.001	p _{adj} = 0.263	p _{adj} = 0.779	p _{adj} = 0.387	p _{adj} = 0.01	p _{adj} = 0.055	p _{adj} = 0.018	
	Δ24-48	1.19 ± 0.68	0.2 ± 0.03	1.34 ± 0.06	0.64 ± 0.11	F(3, 3.83) = 67 p = 0.001	p _{adj} = 0.429	p _{adj} = 0.844	p _{adj} = 0.611	p _{adj} = 0.004	p _{adj} = 0.101	p _{adj} = 0.032	
	Δ0-48	1.88 ± 0.71	0.41 ± 0.01	1.65 ± 0.06	0.95 ± 0.09	F(3, 3.42) = 116.46 p = 0.001	p _{adj} = 0.263	p _{adj} = 0.779	p _{adj} = 0.387	p _{adj} = 0.01	p _{adj} = 0.055	p _{adj} = 0.018	

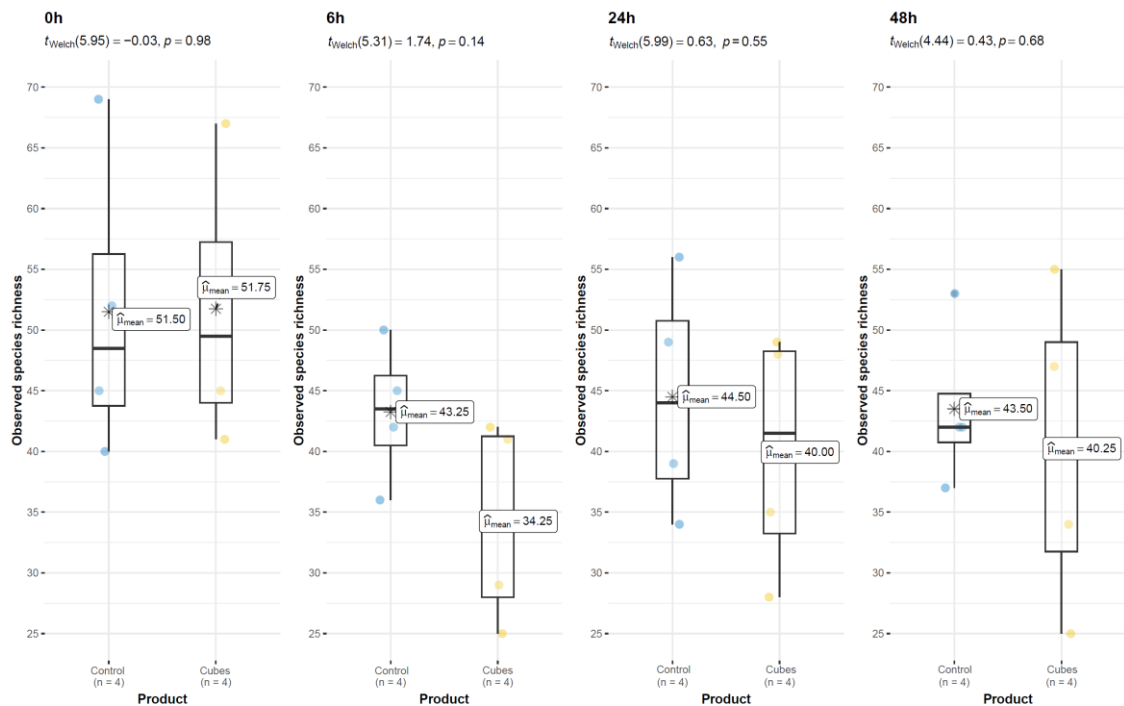
	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)					
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes
Lactate (mM)	0	0.13 ± 0.02	0.26 ± 0.01	0.17 ± 0	0.18 ± 0	F(3, 3.67) = 18.18 p = 0.012	p _{adj} = 0.032	p _{adj} = 0.194	p _{adj} = 0.164	p _{adj} = 0.025	p _{adj} = 0.032	p _{adj} = 0.164
	6	0.64 ± 0.01	3.5 ± 0.1	2.63 ± 0.04	4.28 ± 0.09	F(3, 3.49) = 996.82 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.001	p _{adj} = 0.001	p _{adj} = 0.007	p _{adj} = 0.006	p _{adj} = 0.001
	Δ0-6	0.51 ± 0.03	3.24 ± 0.1	2.46 ± 0.05	4.1 ± 0.09	F(3, 4.07) = 650.6 p = 0.001	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} = 0.001	p _{adj} = 0.008	p _{adj} = 0.004	p _{adj} = 0.001
	24	0.37 ± 0.01	3.16 ± 0.12	1.91 ± 0.06	2.89 ± 0.1	F(3, 3.45) = 401.28 p = 0.001	p _{adj} = 0.004	p _{adj} = 0.004	p _{adj} = 0.004	p _{adj} = 0.004	p _{adj} = 0.164	p _{adj} = 0.004
	Δ6-24	-0.27 ± 0.01	-0.34 ± 0.18	-0.72 ± 0.02	-1.39 ± 0.18	F(3, 3.56) = 164.22 p = 0.001	p _{adj} = 0.758	p _{adj} = 0.003	p _{adj} = 0.049	p _{adj} = 0.186	p _{adj} = 0.041	p _{adj} = 0.096
	Δ0-24	0.24 ± 0.03	2.91 ± 0.12	1.73 ± 0.06	2.7 ± 0.1	F(3, 3.85) = 320.26 p = 0.001	p _{adj} = 0.002	p _{adj} = 0.002	p _{adj} = 0.002	p _{adj} = 0.004	p _{adj} = 0.258	p _{adj} = 0.004
	48	0 ± 0	0.28 ± 0.02	0.29 ± 0.01	0.29 ± 0	F(3, 3.55) = 1473.72 p = 0.001	p _{adj} = 0.007	p _{adj} = 0.001	p _{adj} < 0.001	p _{adj} = 0.587	p _{adj} = 0.587	p _{adj} = 0.709
	Δ24-48	-0.36 ± 0.01	-2.89 ± 0.12	-1.61 ± 0.05	-2.6 ± 0.1	F(3, 3.44) = 335.32 p = 0.001	p _{adj} = 0.004	p _{adj} = 0.004	p _{adj} = 0.004	p _{adj} = 0.004	p _{adj} = 0.137	p _{adj} = 0.004
	Δ0-48	-0.13 ± 0.02	0.02 ± 0.01	0.12 ± 0.01	0.11 ± 0	F(3, 3.88) = 39.13 p = 0.003	p _{adj} = 0.011	p _{adj} = 0.011	p _{adj} = 0.011	p _{adj} = 0.011	p _{adj} = 0.019	p _{adj} = 0.23

	Time (h)	Control	Inulin	Powder	Cubes	robust ANOVA	pairwise comparisons (<i>adjusted p-value</i>)					
							Control vs Inulin	Control vs Powder	Control vs Cubes	Inulin vs Powder	Inulin vs Cubes	Powder vs Cubes
Ammonium (mg/L)	0	64.99 ± 8.37	90.3 ± 2.52	67.61 ± 1.89	86.38 ± 1.26	F(3, 4.12) = 21.37 p = 0.006	P _{adj} = 0.167	P _{adj} = 0.787	P _{adj} = 0.183	P _{adj} = 0.008	P _{adj} = 0.311	P _{adj} = 0.008
	24	297.32 ± 3.64	179.01 ± 8.19	192.08 ± 6.88	229.99 ± 4.9	F(3, 4.27) = 83.8 p = 0.001	P _{adj} = 0.003	P _{adj} = 0.002	P _{adj} = 0.002	P _{adj} = 0.291	P _{adj} = 0.015	P _{adj} = 0.017
	Δ0-24	232.32 ± 10.07	88.71 ± 8.95	124.47 ± 8.04	143.61 ± 5.94	F(3, 4.36) = 30.81 p = 0.003	P _{adj} = 0.003	P _{adj} = 0.004	P _{adj} = 0.007	P _{adj} = 0.05	P _{adj} = 0.015	P _{adj} = 0.134
	48	391.53 ± 5.29	222.61 ± 6.31	249.22 ± 12.75	271.17 ± 6.6	F(3, 4.34) = 123.22 p = 0.001	P _{adj} < 0.001	P _{adj} = 0.006	P _{adj} = 0.001	P _{adj} = 0.193	P _{adj} = 0.009	P _{adj} = 0.224
	Δ0-48	326.54 ± 10.16	132.32 ± 7.96	181.62 ± 12.62	184.79 ± 7.38	F(3, 4.38) = 59.42 p = 0.001	P _{adj} = 0.001	P _{adj} = 0.002	P _{adj} = 0.002	P _{adj} = 0.046	P _{adj} = 0.013	P _{adj} = 0.841
	Δ24-48	94.22 ± 8.79	43.6 ± 3.48	57.15 ± 8.67	41.18 ± 8.42	F(3, 4.02) = 7.63 p = 0.04	P _{adj} = 0.054	P _{adj} = 0.08	P _{adj} = 0.054	P _{adj} = 0.308	P _{adj} = 0.809	P _{adj} = 0.308

Ex vivo gut permeability assessment: changes in gut microbiota composition, *in vitro* fermentation metabolites and gut integrity and permeability



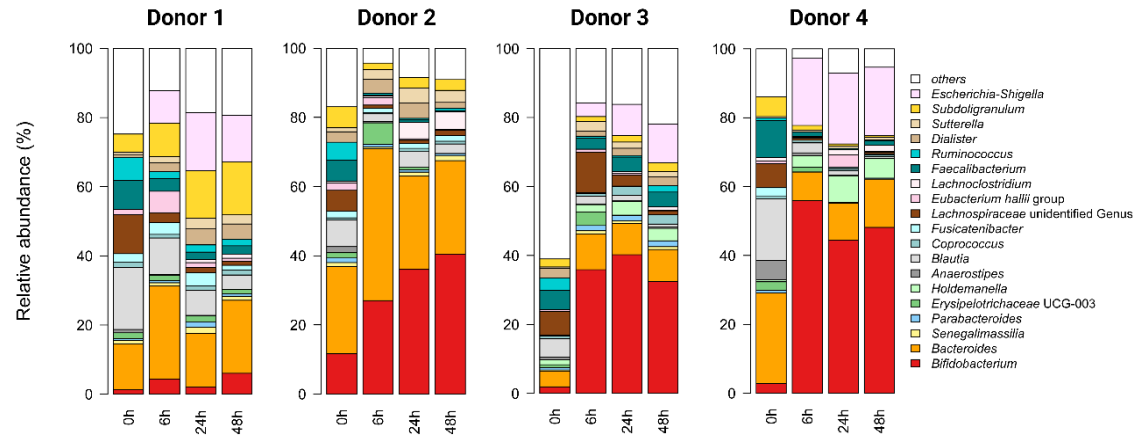
Supplementary Figure 9. Analysis of gut microbiota composition. A-C: Principal coordinate analysis based on Bray-Curtis dissimilarity to assess differences in overall gut microbiota composition (β -diversity representing between sample differences) at each timepoint (0 h, 6 h, 24 h and 48 h) between control fermentation (Control; only inoculum) or *in vitro* fermentation of dried chicory root cubes (Cubes). A: baseline (t = 0 h); B: after 6 h of *in vitro* fermentation; C: after 24 h of *in vitro* fermentation; and D: after 48 h of *in vitro* fermentation.



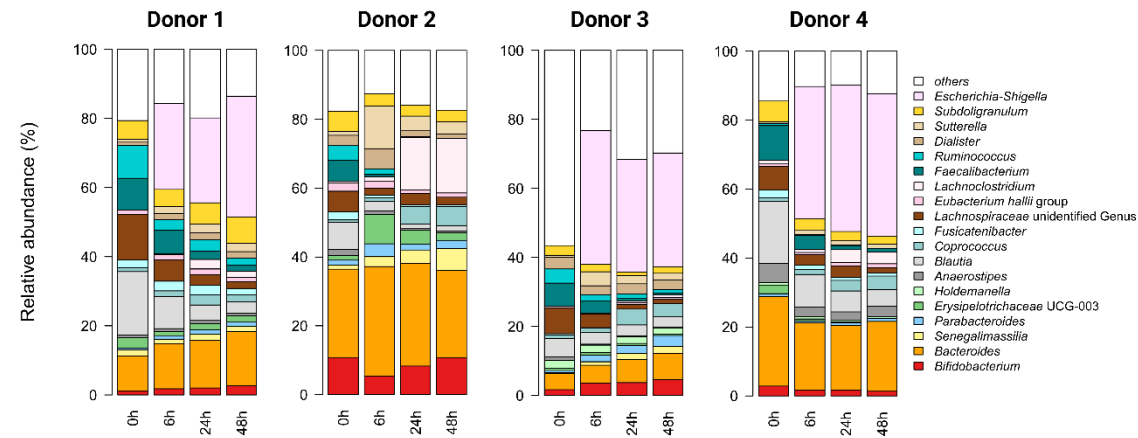
Supplementary Figure 10. Alpha-diversity assessed by observed gut bacteria richness (mean richness within each sample) at baseline (t = 0 h), 6 h, 24 h and 48 h *in vitro* fermentation of the control (Control; only inoculum) and dried chicory root cubes (Cubes). Statistical details for unpaired t-test with unequal variances and corresponding p-value are given above the plot.

Besides statistically significantly different changes between control and dried chicory root cube fermentations in *Bifidobacterium* spp., we also observed differences for changes in *Butyricicoccus* spp levels at 24 h (p = 0.074, q = 0.356) and 48h (p = 0.034, q = 0.181) and *Coprococcus* spp. at 6 h (p = 0.003, q = 0.076), 24 h (p = 0.021, q = 0.356) and 48 h (p = 0.048, q = 0.216). We observed a number of other genera changing over time and (Supplementary Table 5) and differing between timepoints (Supplementary Table 6) for control versus dried chicory root cube fermentation. *Lachnospira* spp. decreased at 6 h in both groups but re-increased for dried chicory root cubes at 24 and 48 h. Also, changes in an unidentified genus of *Oscillospiraceae* differed, which decreased in both groups, but more pronounced in for dried chicory root cubes. Taxa that decrease in both conditions were (ordered by decreasing mean relative levels) *Blautia* spp, an unidentified *Lachnospiraceae*, *Faecalibacterium* spp., *Anaerostipes* spp, and *Lachnospiraceae* ND3007 group, while *Sutterella* spp. levels increased in both

conditions. Both these decreases and increases occurred rapidly within 6 h and plateaued at 24 h. Several other genera decreased only in one of the conditions. For dried chicory root cubes these were decreases in an unidentified *Clostridia* UCG-014 genus, *Dorea* spp, *Coriobacteriales* Incertae Sedis, *Romboutsia* spp., *Ruminococcus gauvreauii* group (Supplementary Table X). For the control condition decreasing genera were *Roseburia* spp. and *Subdoligranulum* spp. and increasing genera *Lachnospiraceae* UCG-004, *Parabacteroides* spp. and *Senegalimassilia* spp. Comparing mean relative levels at each timepoint, we observed at 24 h in dried chicory root cubes compared to control fermentations statistically significantly higher *Butyricoccus* spp. and lower *Coproccoccus* spp., lower *Lachnospiraceae* UCG-004 and lower *Erysipelotrichaceae*_UCG.003. At 48 h we observed in dried chicory root cubes compared to control fermentations statistically significantly higher *Bifidobacterium* spp. and higher *Lachnospira* spp., lower *Coprococcus* spp., lower unidentified genus of *Oscillospiraceae* and lower *Eubacterium hallii* group.



A



B

Supplementary Figure 11. Analysis of individual gut microbiota composition. Common genera present with a mean relative abundance of at least 1% and mean prevalence of 50% in the whole dataset at each timepoint in each of the *in vitro* fermentation. A: genera present in each donor during *in vitro* fermentation of dried chicory root cubes. B: genera present in each donor during in the control fermentation.

Supplementary Table 5. Changes in mean relative abundances of common genera (mean relative abundance of at least 1% and mean prevalence of 50% in the whole dataset) during the fermentation of dried chicory root cubes (CUBES) or negative control (CONTROL; only inoculum) from baseline (t = 0 h) to 6 h, 24 h and 48 h (used for Ussing experiment). Respective fold-changes (fold- Δ) over time within each group and the p-values and fdr-corrected q-values are given per taxon for each fermentation.

Taxon	Product	0h	6h	24h	48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h
		Relative abundance (%)				Fold- Δ over time			p-value			q-value		
<i>Alistipes</i>	CUBES	1.02%	0.30%	0.73%	0.94%	0.30	0.71	0.92	0.070	0.616	0.901	0.166	0.849	0.987
	CONTROL	1.06%	1.10%	1.06%	1.37%	1.04	1.00	1.29	0.299	0.299	0.487	0.722	0.559	0.640
<i>Anaerostipes</i>	CUBES	2.39%	0.45%	0.15%	0.10%	0.19	0.06	0.04	0.196	0.031	0.013	0.313	0.112	0.049
	CONTROL	2.52%	1.30%	1.08%	1.21%	0.52	0.43	0.48	< 0.001	< 0.001	< 0.001	0.005	< 0.001	< 0.001
<i>Bacteroides</i>	CUBES	18.69%	22.97%	16.58%	19.01%	1.23	0.89	1.02	0.591	0.515	0.979	0.739	0.736	1.000
	CONTROL	17.76%	18.15%	18.66%	18.63%	1.02	1.05	1.05	0.958	0.508	0.265	1.000	0.743	0.486
<i>Bifidobacterium</i>	CUBES	4.90%	32.45%	33.55%	34.44%	6.62	6.84	7.03	0.019	0.092	0.033	0.051	0.283	0.094
	CONTROL	4.46%	3.34%	4.38%	5.41%	0.75	0.98	1.21	0.297	0.716	0.805	0.722	0.860	0.917
<i>Bilophila</i>	CUBES	0.10%	0.03%	0.09%	0.10%	0.29	0.86	1.03	0.310	0.992	0.355	0.427	1.000	0.546
	CONTROL	0.15%	0.16%	0.35%	0.44%	1.08	2.26	2.86	0.715	0.279	0.138	0.890	0.559	0.334
<i>Blautia</i>	CUBES	13.59%	4.62%	3.86%	2.26%	0.34	0.28	0.17	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	CONTROL	13.49%	6.53%	4.03%	3.40%	0.48	0.30	0.25	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Taxon	Product	0h	6h	24h	48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h
		Relative abundance (%)				Fold-Δ over time			p-value			q-value		
<i>Butyricicoccus</i>	CUBES	0.22%	0.03%	0.26%	0.17%	0.15	1.18	0.77	0.114	0.888	0.829	0.217	0.946	0.987
	CONTROL	0.17%	0.10%	0.09%	0.00%	0.56	0.53	0.00	0.786	0.763	1.000	0.920	0.860	1.000
<i>Lachnospiraceae</i> CAG.56	CUBES	0.79%	0.33%	0.27%	0.11%	0.42	0.34	0.14	0.590	0.197	0.093	0.739	0.414	0.233
	CONTROL	0.80%	0.41%	0.05%	0.00%	0.51	0.07	0.00	0.761	0.224	0.996	0.918	0.509	1.000
<i>Clostridia</i> UCG-014 unidentified Genus	CUBES	0.98%	0.09%	0.13%	0.21%	0.09	0.14	0.21	0.005	0.008	0.014	0.018	0.034	0.049
	CONTROL	1.21%	0.45%	0.53%	0.50%	0.37	0.44	0.41	0.582	0.240	0.179	0.851	0.518	0.367
<i>Collinsella</i>	CUBES	0.80%	0.79%	0.86%	0.47%	0.99	1.07	0.59	0.918	0.892	0.664	0.993	0.946	0.885
	CONTROL	0.07%	0.52%	0.25%	0.53%	7.31	3.53	7.48	0.429	0.616	0.424	0.752	0.815	0.621
<i>Coprococcus</i>	CUBES	1.15%	0.66%	1.47%	1.48%	0.58	1.28	1.29	<0.001	0.381	0.633	0.001	0.588	0.882
	CONTROL	1.13%	1.42%	4.36%	4.32%	1.26	3.85	3.82	0.272	<0.001	<0.001	0.722	<0.001	<0.001
<i>Dialister</i>	CUBES	2.22%	2.13%	2.94%	2.45%	0.96	1.33	1.11	0.976	0.827	0.938	1.000	0.946	0.987
	CONTROL	2.33%	2.76%	1.96%	1.69%	1.19	0.84	0.73	0.397	0.955	0.632	0.752	1.000	0.762
<i>Dorea</i>	CUBES	1.29%	0.75%	1.13%	0.52%	0.59	0.88	0.41	0.006	0.284	<0.001	0.023	0.473	<0.001
	CONTROL	1.32%	1.16%	1.85%	0.71%	0.88	1.40	0.54	0.846	0.616	0.361	0.937	0.815	0.600
<i>Erysipelotrichaceae</i>	CUBES	1.82%	3.41%	0.69%	0.32%	1.87	0.38	0.18	0.654	0.489	0.217	0.793	0.725	0.394

Taxon	Product	0h	6h	24h	48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h
		Relative abundance (%)				Fold-Δ over time			p-value			q-value		
UCG-003	CONTROL	2.02%	2.87%	1.86%	1.22%	1.42	0.92	0.60	0.999	0.675	0.384	1.000	0.858	0.600
<i>Escherichia-Shigella</i>	CUBES	0.00%	8.59%	12.64%	12.21%	Inf	Inf	Inf	1.000	1.000	1.000	1.000	1.000	1.000
	CONTROL	0.00%	27.89%	27.53%	29.90%	Inf	Inf	Inf	1.000	1.000	1.000	1.000	1.000	1.000
<i>Faecalibacterium</i>	CUBES	8.97%	2.20%	2.03%	2.34%	0.25	0.23	0.26	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	CONTROL	0.00	76.10	117.00	117.00	0.00	76.10	0.10	0.383	0.032	0.017	0.752	0.110	0.059
<i>Fusicatenibacter</i>	CUBES	1.84%	1.35%	1.50%	0.86%	0.73	0.82	0.47	0.236	0.269	0.060	0.363	0.468	0.159
	CONTROL	1.93%	1.31%	1.08%	0.83%	0.68	0.56	0.43	0.481	0.314	0.219	0.752	0.560	0.428
<i>Coriobacteriales Incertae Sedis</i>	CUBES	0.45%	0.10%	0.10%	0.15%	0.22	0.22	0.32	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	CONTROL	0.49%	0.22%	0.19%	0.29%	0.45	0.39	0.58	0.717	0.690	0.148	0.890	0.858	0.337
<i>Lachnoclostridium</i>	CUBES	0.40%	0.19%	2.05%	2.35%	0.48	5.19	5.93	0.591	0.229	0.193	0.739	0.457	0.386
	CONTROL	0.43%	0.67%	6.01%	5.99%	1.57	14.10	14.07	0.623	0.025	0.029	0.851	0.095	0.084
<i>Lachnospira</i>	CUBES	0.52%	0.04%	0.39%	0.92%	0.07	0.75	1.75	0.010	0.775	0.583	0.033	0.940	0.863
	CONTROL	0.49%	0.18%	0.14%	0.14%	0.37	0.29	0.29	0.488	0.387	0.395	0.752	0.618	0.600
<i>Lachnospiraceae</i>	CUBES	9.31%	4.35%	1.57%	1.15%	0.47	0.17	0.12	0.002	<0.001	<0.001	0.011	<0.001	<0.001
unidentified Genus	CONTROL	9.64%	4.04%	2.98%	1.83%	0.42	0.31	0.19	<0.001	<0.001	<0.001	0.002	<0.001	<0.001

Taxon	Product	0h	6h	24h	48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h
		Relative abundance (%)				Fold-Δ over time			p-value			q-value		
<i>Lachnospiraceae</i> FCS020 group	CUBES	0.30%	0.00%	0.00%	0.00%	0.00	0.00	0.00	NA	NA	NA	NA	NA	NA
	CONTROL	0.13%	0.04%	0.00%	0.00%	0.29	0.00	0.00	NA	NA	NA	NA	NA	NA
<i>Lachnospiraceae</i> ND3007 group	CUBES	2.03%	0.36%	0.40%	0.42%	0.18	0.20	0.21	0.012	0.017	0.023	0.037	0.068	0.072
	CONTROL	1.62%	0.89%	0.72%	0.61%	0.55	0.44	0.37	0.002	<0.001	<0.001	0.013	<0.001	<0.001
<i>Lachnospiraceae</i> NK4A136 group	CUBES	1.49%	0.29%	0.42%	0.76%	0.19	0.28	0.51	0.256	0.382	0.640	0.373	0.588	0.882
	CONTROL	1.38%	0.64%	0.45%	0.43%	0.46	0.33	0.31	0.367	0.200	0.381	0.752	0.483	0.600
<i>Lachnospiraceae</i> UCG-004	CUBES	0.18%	0.00%	0.56%	0.50%	0.00	3.19	2.85	1.000	0.256	0.305	1.000	0.468	0.515
	CONTROL	0.18%	0.16%	1.47%	1.23%	0.88	7.99	6.68	0.833	0.001	0.003	0.937	0.006	0.015
<i>Monoglobus</i>	CUBES	0.36%	0.03%	0.25%	0.45%	0.09	0.70	1.26	0.159	0.834	0.889	0.277	0.946	0.987
	CONTROL	0.32%	0.14%	0.08%	0.09%	0.44	0.25	0.27	0.488	0.179	0.168	0.752	0.483	0.362
<i>Oscillospiraceae</i> unidentified Genus	CUBES	0.29%	0.03%	0.18%	0.22%	0.09	0.63	0.77	0.095	0.743	0.855	0.190	0.940	0.987
	CONTROL	0.42%	0.17%	0.74%	0.62%	0.41	1.74	1.47	0.172	0.392	0.555	0.641	0.618	0.690
<i>Parabacteroides</i>	CUBES	0.97%	0.78%	1.07%	0.90%	0.80	1.10	0.92	0.760	0.899	0.916	0.869	0.946	0.987
	CONTROL	0.97%	1.93%	1.69%	2.13%	1.99	1.74	2.19	0.022	0.025	0.002	0.131	0.095	0.013
<i>Romboutsia</i>	CUBES	0.91%	0.18%	0.24%	0.29%	0.20	0.26	0.31	<0.001	<0.001	<0.001	0.001	0.001	0.001

Taxon	Product	0h	6h	24h	48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h
		Relative abundance (%)				Fold- Δ over time			p-value			q-value		
<i>Roseburia</i>	CONTROL	1.03%	0.78%	0.82%	0.89%	0.76	0.80	0.86	0.408	0.756	0.731	0.752	0.860	0.857
	CUBES	1.81%	0.13%	0.14%	0.38%	0.07	0.08	0.21	0.088	0.102	0.314	0.190	0.291	0.515
<i>Ruminococcus</i>	CONTROL	1.80%	0.15%	0.00%	0.04%	0.08	0.00	0.02	0.093	1.000	0.012	0.424	1.000	0.049
	CUBES	4.82%	0.81%	0.81%	1.18%	0.17	0.17	0.24	0.130	0.128	0.217	0.236	0.327	0.394
<i>Senegalimassilia</i>	CONTROL	5.38%	1.86%	1.33%	0.82%	0.35	0.25	0.15	0.286	0.087	0.027	0.722	0.275	0.084
	CUBES	0.69%	0.62%	0.97%	0.94%	0.90	1.40	1.36	0.836	0.131	0.131	0.929	0.327	0.290
<i>Streptococcus</i>	CONTROL	0.87%	1.41%	2.08%	2.77%	1.62	2.39	3.18	0.225	0.017	0.004	0.722	0.078	0.016
	CUBES	1.03%	0.29%	0.29%	0.31%	0.28	0.28	0.30	0.173	0.170	0.192	0.289	0.386	0.386
<i>Subdoligranulum</i>	CONTROL	1.10%	0.75%	0.67%	0.65%	0.68	0.61	0.59	0.389	0.161	0.272	0.752	0.472	0.486
	CUBES	5.40%	3.67%	5.10%	5.68%	0.68	0.94	1.05	0.019	0.079	0.128	0.051	0.262	0.290
<i>Sutterella</i>	CONTROL	5.52%	3.66%	3.42%	4.06%	0.66	0.62	0.74	0.011	0.001	0.017	0.075	0.004	0.059
	CUBES	0.76%	2.07%	2.62%	2.23%	2.72	3.45	2.93	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Oscillospiraceae</i> UCG-002	CONTROL	0.89%	5.20%	2.88%	2.54%	5.82	3.22	2.84	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	CUBES	1.89%	0.30%	0.43%	0.72%	0.16	0.23	0.38	0.092	0.174	0.322	0.190	0.386	0.515
	CONTROL	2.17%	0.83%	1.31%	1.60%	0.38	0.60	0.74	0.167	0.200	0.499	0.641	0.483	0.640

Taxon	Product	0h	6h	24h	48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h	0-6h	0-24h	0-48h
		Relative abundance (%)				Fold-Δ over time			p-value			q-value		
<i>Erysipelotrichaceae</i> UCG-003	CUBES	0.15%	0.12%	0.15%	0.12%	0.12	0.34	0.27	0.001	0.003	0.002	0.006	0.017	0.010
	CONTROL	0.27%	0.11%	0.27%	0.11%	0.35	0.53	0.21	0.644	0.776	0.489	0.852	0.860	0.640
<i>Eubacterium</i> <i>coprostanoligenes</i> group	CUBES	0.00%	0.00%	0.00%	0.00%	0.00	0.00	0.00	NA	NA	NA	NA	NA	NA
	CONTROL	0.04%	0.00%	0.04%	0.00%	0.09	0.03	0.00	NA	NA	NA	NA	NA	NA
<i>Eubacterium eligens</i> group	CUBES	0.17%	0.56%	0.17%	0.56%	0.17	0.33	1.06	0.063	0.265	0.923	0.157	0.468	0.987
	CONTROL	0.22%	0.10%	0.22%	0.10%	0.45	0.47	0.21	0.074	0.436	0.051	0.378	0.662	0.139
<i>Eubacterium hallii</i> group	CUBES	1.31%	2.55%	1.58%	0.46%	1.95	1.21	0.35	0.261	0.741	0.020	0.373	0.940	0.066
	CONTROL	1.36%	1.18%	1.17%	1.12%	0.86	0.86	0.82	0.253	0.616	0.481	0.722	0.815	0.640
<i>Eubacterium ventriosum</i> group	CUBES	0.00%	0.00%	0.00%	0.00%	0.37	0.00	0.00	NA	NA	NA	NA	NA	NA
	CONTROL	0.09%	0.00%	0.09%	0.00%	0.90	0.43	0.00	0.907	0.330	0.960	0.978	0.564	1.000
<i>Ruminococcus gauvreauii</i> group	CUBES	0.08%	0.08%	0.08%	0.08%	0.16	0.13	0.15	0.001	0.001	0.001	0.004	0.003	0.003
	CONTROL	0.26%	0.12%	0.26%	0.12%	0.53	0.44	0.21	0.495	0.300	0.089	0.752	0.559	0.229
<i>Ruminococcus torques</i> group	CUBES	1.52%	1.22%	1.52%	1.22%	1.86	1.59	1.27	0.676	0.756	0.872	0.795	0.940	0.987
	CONTROL	0.72%	0.76%	0.72%	0.76%	0.55	0.75	0.79	0.605	0.799	0.835	0.851	0.862	0.925

fold-Δ: fold-change over baseline in mean relative abundances for each condition (dried chicory root cubes fermentation versus control); NA: represents p- and q-values that could not be reliably estimated due to violation of the assumption underlying the statistical models for calculating statistical inference ^[15]

Supplementary Table 6. Mean relative abundances of common genera (mean relative abundance of at least 1% and mean prevalence of 50% in the whole dataset) at each timepoint (baseline 0 h, 6 h, 24 h, and 48 h) in the negative control fermentation (Control; only inoculum) or fermentation of dried chicory root cubes (Cubes) and the respective fold-difference (fold- Δ) between both conditions as well as respective p-values and fdr-corrected q-values.

taxon	0h					6h					24h					48h				
	Control (%)	Cubes (%)	Fold- Δ	p-value	q-value	Control (%)	Cubes (%)	Fold- Δ	p-value	q-value	Control (%)	Cubes (%)	Fold- Δ	p-value	q-value	Control (%)	Cubes (%)	Fold- Δ	p-value	q-value
<i>Alistipes</i>	1.06%	1.02%	0.96	0.783	1.000	1.10%	0.30%	0.28	0.241	0.557	1.06%	0.73%	0.69	0.529	0.731	1.37%	0.94%	0.69	0.952	0.957
<i>Anaerostipes</i>	2.52%	2.49%	0.99	0.998	1.000	1.30%	0.45%	0.35	0.136	0.557	1.08%	0.15%	0.14	0.093	0.452	1.21%	0.10%	0.08	NA	NA
<i>Bacteroides</i>	17.76%	18.69%	1.05	0.885	1.000	18.15%	22.97%	1.27	0.647	0.778	18.66%	16.58%	0.89	0.703	0.886	18.63%	19.01%	1.02	0.946	0.957
<i>Bifidobacterium</i>	4.46%	4.90%	1.099	0.851	1.000	3.34%	32.45%	9.71	0.018	0.216	4.38%	33.55%	7.66	0.081	0.452	5.41%	34.44%	6.36	0.029	0.152
<i>Bilophila</i>	0.15%	0.10%	0.66	NA	NA	0.16%	0.03%	0.17	NA	NA	0.35%	0.09%	0.25	NA	NA	0.44%	0.10%	0.24	0.623	0.810
<i>Blautia</i>	13.49%	13.59%	1.01	0.982	1.000	6.53%	4.62%	0.71	0.380	0.608	4.03%	3.86%	0.96	0.931	0.964	3.40%	2.26%	0.67	0.294	0.637
<i>Butyricoccus</i>	0.17%	0.22%	1.28	0.568	1.000	0.10%	0.03%	0.30	NA	NA	0.09%	0.26%	2.89	0.039	0.280	0.00%	0.17%	Inf	NA	NA
<i>Lachnospiraceae</i> CAG.56	0.79%	0.80%	0.997	0.971	1.000	0.41%	0.33%	0.82	NA	NA	0.05%	0.27%	4.94	NA	NA	0.00%	0.11%	Inf	NA	NA
<i>Clostridia UCG-014</i> unidentified Genus	1.23%	1.04%	0.85	0.947	1.000	0.45%	0.09%	0.19	NA	NA	0.53%	0.13%	0.26	NA	NA	0.50%	0.21%	0.42	NA	NA
<i>Collinsella</i>	0.07%	0.80%	11.26	NA	NA	0.52%	0.79%	1.52	NA	NA	0.25%	0.86%	3.43	NA	NA	0.53%	0.47%	0.88	NA	NA
<i>Coprococcus</i>	1.13%	1.15%	1.02	0.958	1.000	1.42%	0.66%	0.47	0.015	0.216	4.36%	1.47%	0.34	<0.001	0.007	4.32%	1.48%	0.34	0.005	0.060
<i>Dialister</i>	2.33%	2.22%	0.95	0.950	1.000	2.76%	2.13%	0.77	0.705	0.806	1.96%	2.94%	1.50	0.495	0.717	1.69%	2.45%	1.46	0.541	0.810
<i>Dorea</i>	1.32%	1.29%	0.97	0.723	1.000	1.16%	0.75%	0.65	0.359	0.608	1.85%	1.13%	0.61	0.338	0.637	0.71%	0.52%	0.73	0.579	0.810
<i>Erysipelotrichaceae</i> UCG-003	2.02%	1.82%	0.898	0.698	1.000	2.89%	3.41%	1.18	0.438	0.658	1.86%	0.69%	0.37	<0.001	<0.001	1.22%	0.32%	0.26	NA	NA
<i>Escherichia-Shigella</i>	0.00%	0.00%	NA	NA	NA	27.89%	8.59%	0.31	0.135	0.557	27.53%	12.64%	0.46	0.230	0.607	29.90%	12.21%	0.41	0.160	0.461
<i>Faecalibacterium</i>	9.18%	8.97%	0.98	0.884	1.000	3.93%	2.20%	0.56	0.302	0.557	1.15%	2.03%	1.77	0.373	0.637	0.90%	2.34%	2.59	0.223	0.579

taxon	0h					6h					24h					48h				
	Control	Cubes	Fold-Δ	p-value	q-value	Control	Cubes	Fold-Δ	p-value	q-value	Control	Cubes	Fold-Δ	p-value	q-value	Control	Cubes	Fold-Δ	p-value	q-value
	(%)	(%)				(%)	(%)				(%)	(%)				(%)	(%)			
<i>Fusicatenibacter</i>	1.93%	1.84%	0.96	0.901	1.000	1.31%	1.35%	1.03	0.886	0.928	1.08%	1.50%	1.39	0.481	0.717	0.83%	0.86%	1.04	0.957	0.957
<i>Coriobacteriales</i> Incertae Sedis	0.50%	0.49%	0.99	NA	NA	0.22%	0.10%	0.46	NA	NA	0.19%	0.10%	0.53	NA	NA	0.29%	0.15%	0.51	NA	NA
<i>Lachnoclostridium</i>	0.43%	0.40%	0.93	NA	NA	0.67%	0.19%	0.28	NA	NA	6.01%	2.05%	0.34	0.362	0.637	5.99%	2.35%	0.39	0.458	0.794
<i>Lachnospira</i>	0.49%	0.52%	1.08	0.798	1.000	0.18%	0.04%	0.21	NA	NA	0.14%	0.39%	2.82	0.125	0.456	0.14%	0.92%	6.46	0.007	0.060
<i>Lachnospiraceae</i> unidentified Genus	9.64%	9.31%	0.97	0.874	1.000	4.04%	4.35%	1.08	0.483	0.682	2.98%	1.57%	0.53	0.154	0.497	1.83%	1.15%	0.63	0.064	0.278
<i>Lachnospiraceae</i> ND3007 group	1.62%	2.05%	1.26	0.637	1.000	0.89%	0.36%	0.41	0.124	0.557	0.72%	0.40%	0.55	0.312	0.637	0.61%	0.42%	0.70	0.602	0.810
<i>Lachnospiraceae</i> NK4A136 group	1.44%	1.50%	1.04	0.995	1.000	0.64%	0.29%	0.45	0.648	0.778	0.45%	0.42%	0.94	NA	NA	0.43%	0.76%	1.78	0.556	0.810
<i>Lachnospiraceae</i> UCG-004	0.18%	0.18%	0.96	0.950	1.000	0.16%	0.00%	0.00	NA	NA	1.47%	0.56%	0.38	0.005	0.046	1.23%	0.50%	0.41	0.265	0.625
<i>Monoglobus</i>	0.32%	0.36%	1.12	0.828	1.000	0.14%	0.03%	0.24	NA	NA	0.08%	0.25%	3.16	NA	NA	0.09%	0.45%	5.17	NA	NA
<i>Oscillospiraceae</i> unidentified Genus	0.42%	0.29%	0.69	0.232	1.000	0.17%	0.03%	0.15	NA	NA	0.74%	0.18%	0.25	0.182	0.529	0.62%	0.22%	0.36	0.022	0.144
<i>Parabacteroides</i>	0.97%	0.97%	1.00	0.992	1.000	1.93%	0.78%	0.40	0.283	0.557	1.69%	1.07%	0.63	0.310	0.637	2.13%	0.90%	0.42	0.119	0.443
<i>Romboutsia</i>	1.03%	0.94%	0.92	0.975	1.000	0.78%	0.18%	0.23	NA	NA	0.82%	0.24%	0.29	0.126	0.456	0.89%	0.29%	0.32	0.141	0.459
<i>Roseburia</i>	1.85%	1.84%	0.996	0.981	1.000	0.15%	0.13%	0.88	NA	NA	0.00%	0.14%	Inf	NA	NA	0.04%	0.38%	8.92	NA	NA
<i>Ruminococcus</i>	5.38%	4.82%	0.90	0.824	1.000	1.86%	0.81%	0.43	0.214	0.557	1.33%	0.81%	0.61	0.461	0.717	0.82%	1.18%	1.44	NA	NA
<i>Senegalimassilia</i>	0.87%	0.69%	0.80	0.737	1.000	1.41%	0.62%	0.44	0.277	0.557	2.08%	0.97%	0.47	0.290	0.637	2.77%	0.94%	0.34	0.817	0.923
<i>Streptococcus</i>	1.10%	1.03%	0.94	0.884	1.000	0.75%	0.29%	0.38	0.276	0.557	0.67%	0.29%	0.43	0.853	0.916	0.65%	0.31%	0.48	0.322	0.643
<i>Subdoligranulum</i>	5.52%	5.40%	0.98	0.831	1.000	3.66%	3.67%	1.00	0.996	0.996	3.42%	5.10%	1.49	0.994	0.994	4.06%	5.68%	1.40	0.789	0.923

taxon	0h					6h					24h					48h				
	Control (%)	Cubes (%)	Fold-Δ	p-value	q-value	Control (%)	Cubes (%)	Fold-Δ	p-value	q-value	Control (%)	Cubes (%)	Fold-Δ	p-value	q-value	Control (%)	Cubes (%)	Fold-Δ	p-value	q-value
<i>Sutterella</i>	0.89%	0.76%	0.85	0.507	1.000	5.36%	2.07%	0.39	0.266	0.557	2.88%	2.62%	0.91	0.797	0.916	2.54%	2.23%	0.88	0.692	0.857
<i>Oscillospiraceae</i> UCG-002	2.17%	2.00%	0.92	0.967	1.000	0.83%	0.30%	0.36	0.254	0.557	1.31%	0.43%	0.33	0.850	0.916	1.60%	0.72%	0.45	0.367	0.682
<i>Erysipelotrichaceae</i> UCG-003	0.52%	0.45%	0.87	1.000	1.000	0.18%	0.05%	0.30	NA	NA	NA	NA	NA	NA	NA	0.11%	0.12%	1.10	NA	NA
<i>Eubacterium eligens</i> group	0.46%	0.53%	1.15	0.956	1.000	0.20%	0.09%	0.45	NA	NA	0.22%	0.17%	0.80	NA	NA	0.10%	0.56%	5.75	NA	NA
<i>Eubacterium hallii</i> group	1.36%	1.31%	0.96	0.898	1.000	1.18%	2.49%	2.12	0.534	0.712	1.17%	1.58%	1.35	0.640	0.843	1.12%	0.46%	0.41	0.004	0.060
<i>Eubacterium ventriosum</i> group	0.20%	0.23%	1.13	0.854	1.000	0.18%	0.08%	0.46	NA	NA	0.09%	0.00%	NA	NA	NA	0.00%	0.00%	NA	NA	NA
<i>Ruminococcus gauvreauii</i> group	0.59%	0.56%	0.96	0.944	1.000	0.31%	0.09%	0.29	NA	NA	0.26%	0.08%	0.29	NA	NA	0.12%	0.08%	0.67	NA	NA
<i>Ruminococcus torques</i> group	0.97%	0.96%	0.99	0.976	1.000	0.54%	1.78%	3.32	0.890	0.928	0.72%	1.52%	2.10	0.839	0.916	0.76%	1.22%	1.60	NA	NA

fold-Δ: fold-difference in mean relative abundances per timepoint between conditions (dried chicory root cubes fermentation versus control); NA: represents p- and q-values that could not be reliably estimated due to violation of the assumption underlying the statistical models for calculating statistical inference ^[15]

Supplementary Table 7. pH, gas production and fermentation metabolites measured for each donor and expressed as mean \pm SE at baseline (t = 0 h), 6 h, 24 h and 48 h of *in vitro* fermentation of dried chicory root cubes and negative control (only inoculum) to produce fermentation supernatant used in the *ex vivo* Ussing chamber experiment. Fermentation supernatants at t = 48 h were used for the Ussing experiment.

		Control				Dried chicory root cubes			
		0h	6h	24h	48h	0h	6h	24h	48h
pH	Donor 1	6.39	6.35	6.36	6.4	6.39	5.82	5.75	5.86
	Donor 2	6.39	6.38	6.35	6.41	6.39	5.73	5.68	5.78
	Donor 3	6.44	6.38	6.44	6.45	6.42	5.97	5.9	5.85
	Donor 4	6.35	6.25	6.28	6.33	6.38	5.62	5.87	5.92
	Mean \pm SE	6.39 \pm (0.02)	6.34 \pm (0.03)	6.36 \pm (0.03)	6.4 \pm (0.02)	6.4 \pm (0.01)	5.79 \pm (0.07)	5.8 \pm (0.05)	5.85 \pm (0.03)
Gas production (mL)	Donor 1	0.00	27.60	36.40	36.40	0.00	76.10	117.00	117.00
	Donor 2	0.00	13.10	36.40	36.40	0.00	36.50	81.10	81.10
	Donor 3	0.00	11.60	27.30	27.30	0.00	34.90	90.00	90.00
	Donor 4	0.00	27.30	27.30	27.30	0.00	106.90	117.00	117.00
	Mean \pm SE	0 \pm (0)	19.9 \pm (4.37)	31.85 \pm (2.63)	31.85 \pm (2.63)	0 \pm (0)	63.6 \pm (17.29)	101.28 \pm (9.26)	101.28 \pm (9.26)
Butyrate (mM)	Donor 1	0.00	2.28	3.33	3.74	0.00	6.68	11.21	11.43
	Donor 2	0.00	2.14	3.40	3.84	1.78	3.20	9.29	9.89
	Donor 3	2.07	2.54	4.10	5.26	0.00	3.35	12.71	12.05
	Donor 4	0.00	2.38	3.47	4.44	1.91	2.30	14.51	16.86
	Mean \pm SE	0.52 \pm (0.52)	2.33 \pm (0.08)	3.57 \pm (0.18)	4.32 \pm (0.35)	0.92 \pm (0.53)	3.88 \pm (0.96)	11.93 \pm (1.11)	12.56 \pm (1.5)
Propionate (mM)	Donor 1	0.00	0.00	3.76	4.02	0.00	10.65	13.60	13.56
	Donor 2	0.00	1.65	3.64	4.01	0.00	9.08	15.45	16.98
	Donor 3	0.00	3.26	3.85	3.55	0.00	4.36	8.13	8.28
	Donor 4	0.00	0.00	3.60	4.99	0.00	0.00	5.96	9.64
	Mean \pm SE	0 \pm (0)	1.23 \pm (0.78)	3.71 \pm (0.06)	4.14 \pm (0.3)	0 \pm (0)	6.02 \pm (2.41)	10.79 \pm (2.24)	12.12 \pm (1.97)

		Control				Dried chicory root cubes			
		0h	6h	24h	48h	0h	6h	24h	48h
Acetate (mM)	Donor 1	0.00	11.77	17.82	17.85	0.00	26.21	37.35	36.81
	Donor 2	0.00	5.20	17.23	17.60	0.00	21.10	42.08	40.85
	Donor 3	0.00	9.40	17.66	15.75	0.00	15.82	34.30	33.86
	Donor 4	0.00	10.15	17.12	21.31	0.00	24.55	34.77	42.90
	Mean ± SE	0 ± (0)	9.13 ± (1.4)	17.46 ± (0.17)	18.13 ± (1.16)	0 ± (0)	21.92 ± (2.29)	37.12 ± (1.78)	38.6 ± (2.03)
Lactate (mM)	Donor 1	0.00	0.00	0.00	0.00	0.00	3.39	0.00	0.00
	Donor 2	0.00	0.00	0.00	0.00	0.00	4.34	0.00	0.00
	Donor 3	0.00	0.00	0.00	0.00	0.00	4.74	0.00	0.00
	Donor 4	0.00	0.00	0.00	0.00	0.00	10.85	0.00	0.00
	Mean ± SE	0 ± (0)	0 ± (0)	0 ± (0)	0 ± (0)	0 ± (0)	5.83 ± (1.7)	0 ± (0)	0 ± (0)
Iso-butyrate (mM)	Donor 1	0.00	0.00	0.00	0.00	0.00	2.94	2.81	1.98
	Donor 2	0.00	0.00	0.00	0.00	0.00	0.00	2.98	2.23
	Donor 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Donor 4	0.00	0.00	0.00	0.00	0.00	1.67	2.46	2.71
	Mean ± SE	0 ± (0)	0 ± (0)	0 ± (0)	0 ± (0)	0 ± (0)	1.15 ± (0.71)	2.06 ± (0.7)	1.73 ± (0.6)
Formate (mM)	Donor 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Donor 2	0.00	0.00	0.00	0.00	0.00	2.28	0.00	0.00
	Donor 3	0.00	0.00	0.00	0.00	0.00	2.25	0.00	0.00
	Donor 4	0.00	0.00	0.00	0.00	0.00	6.54	0.00	0.00
	Mean ± SE	0 ± (0)	0 ± (0)	0 ± (0)	0 ± (0)	0 ± (0)	2.77 ± (1.37)	0 ± (0)	0 ± (0)

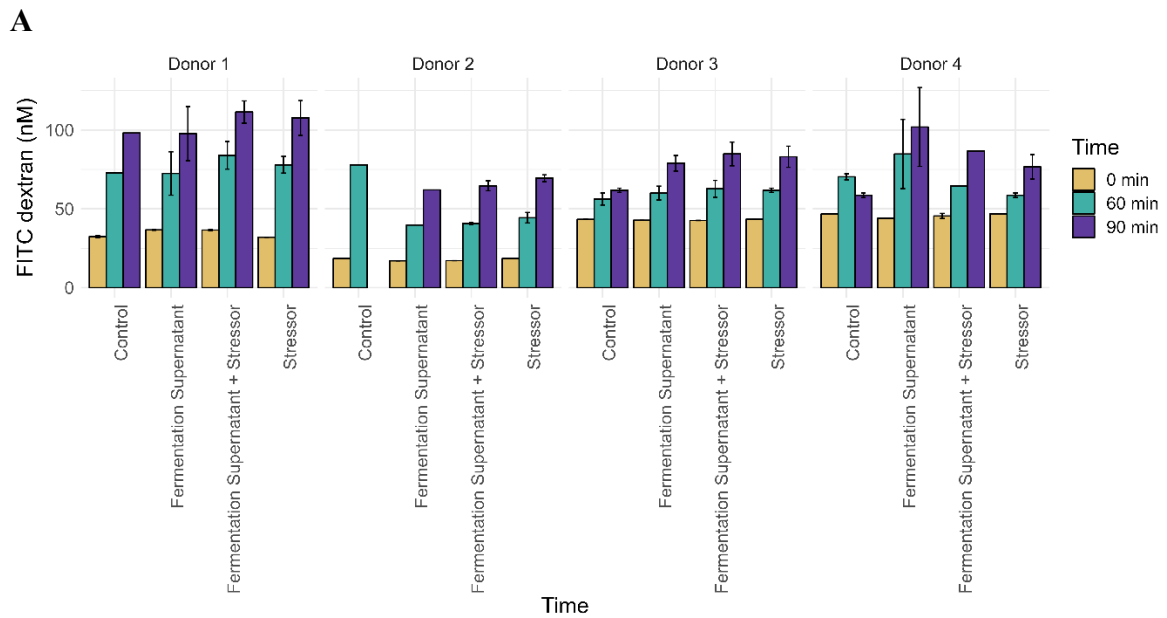
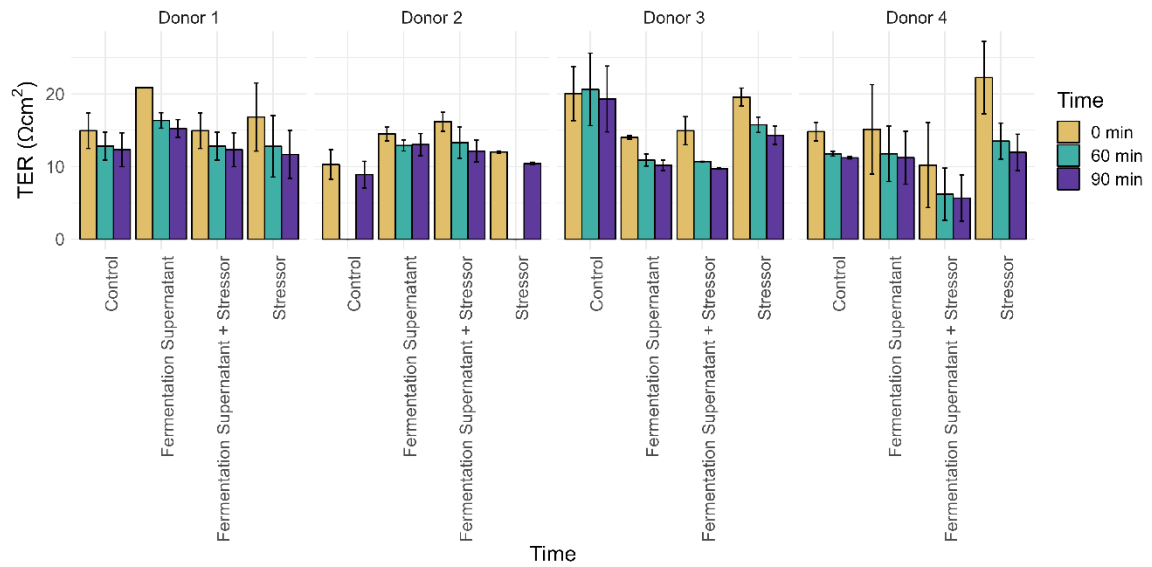
Note: Gas production was measured as long as changes in volume (mL) were detected. Values at respective timepoints are therefor maximum levels reached either at this or a previous timepoint (if stagnation in gas production occurred).

Supplementary Table 8. Electrophysiology and gut permeability measures of the Ussing chamber experiment. Electrophysiological and permeability measurements assessed at t = 0 min, 60 min and 90 min assessed during the Ussing experiment in either control or stimulated biopsies using either the stressor (sodium deoxycholate), the fermentation supernatant or their combination. The electrophysiological parameter TER represents the overall gut integrity and is expressed as absolute value or percentage of its baseline. Concentration of FITC at the serosal side of the biopsy is used as a measure of paracellular permeability (between cells) and presented as concentration (nM) at each respective baseline, absolute change (nM) over baseline or relative change (concentration at t = 60 or 90 min divided by each biopsies' respective baseline value).

Measurement	Stimulation	t = 0 min	t = 60 min	t = 90 min
TER ($\Omega \cdot \text{cm}^2$)	Control	15.03 \pm 2	13.41 \pm 1	12.94 \pm 2.24
	Stressor	17.68 \pm 2.19	14.02 \pm 0.78	12.09 \pm 0.81
	Fermentation supernatant	16.14 \pm 1.60	12.98 \pm 1.20	12.43 \pm 1.11
	Fermentation supernatant + Stressor	14.07 \pm 1.32	10.75 \pm 1.62	9.96 \pm 1.55
	<i>p</i> -value (between-conditions)	<i>p</i> = 0.64	<i>p</i> = 0.42	<i>p</i> = 0.35
TER absolute Δ ($\Omega \cdot \text{cm}^2$)	Control	<i>n.a.</i>	-1.54 \pm 1.09	-2.09 \pm 0.64
	Stressor	<i>n.a.</i>	- 5.54 \pm 1.61	- 5.58 \pm 1.79
	Fermentation supernatant	<i>n.a.</i>	-3.15 \pm 0.60	-3.71 \pm 0.85
	Fermentation supernatant + Stressor	<i>n.a.</i>	-3.32 \pm 0.50	-4.11 \pm 0.55
	<i>p</i> -value (between-conditions)	<i>n.a.</i>	<i>p</i> = 0.05	<i>p</i> = 0.17
TER%	Control	100 \pm 0	89.16 \pm 6.53	85.02 \pm 4.05
	Stressor	100 \pm 0	72.18 \pm 5.69	70.72 \pm 6.77
	Fermentation supernatant	100 \pm 0	81.68 \pm 2.74	78.47 \pm 4.26
	Fermentation supernatant + Stressor	100 \pm 0	75.16 \pm 5.67	69.72 \pm 5.63
	<i>p</i> -value (between-conditions)	<i>n.a.</i>	<i>p</i> = 0.07	<i>p</i> = 0.10
FITC (nM)	Control	35.25 \pm 6.35	69.35 \pm 4.63	72.83 \pm 10.99
	Stressor	35.17 \pm 6.4	60.66 \pm 6.89	84.17 \pm 8.31
	Fermentation supernatant	35.09 \pm 6.21	64.24 \pm 9.61	92.87 \pm 6.1
	Fermentation supernatant + Stressor	35.46 \pm 6.38	62.96 \pm 8.84	86.9 \pm 9.59
	<i>p</i> -value (between-conditions)	<i>p</i> = 0.88	<i>p</i> = 0.68	<i>p</i> = 0.12
FITC absolute Δ (nM)	Control	<i>n.a.</i>	34.1 \pm 10.15	32.01 \pm 14.69
	Stressor	<i>n.a.</i>	25.49 \pm 7.39	49 \pm 9.88
	Fermentation supernatant	<i>n.a.</i>	29.15 \pm 5.53	51.77 \pm 6.76
	Fermentation supernatant + Stressor	<i>n.a.</i>	27.5 \pm 6.7	52.79 \pm 9.56

Measurement	Stimulation	t = 0 min	t = 60 min	t = 90 min
	<i>p</i> -value (between-conditions)	<i>n.a</i>	<i>p</i> = 0.70	<i>p</i> = 0.13
FITC fold- Δ	Control	<i>n.a</i>	2.31 \pm 0.66	1.9 \pm 0.49
	Stressor	<i>n.a</i>	1.88 \pm 0.31	2.67 \pm 0.53
	Fermentation supernatant	<i>n.a</i>	1.91 \pm 0.19	2.28 \pm 0.21
	Fermentation supernatant + Stressor	<i>n.a</i>	1.89 \pm 0.26	2.68 \pm 0.45
	<i>p</i> -value (between-conditions)	<i>n.a</i>	<i>p</i> = 0.50	<i>p</i> = 0.44

TER: Transepithelial resistance; TER%: percentage change in TER over each biopsies respective baseline; FITC: Fluorescein isothiocyanate–dextran; absolute Δ : total change in nM over each biopsies respective baseline; fold- Δ : relative change over each biopsies respective baseline (respective level expressed as factor of its baseline)



B

Supplementary Figure 12. Individual changes over time in electrophysiology and gut permeability measures of the Ussing chamber experiment. A: Overall gut integrity measured by transepithelial resistance (TER) at t = 0 min, 60 min and 90 min in each donors' biopsies. B: Paracellular permeability measured by FITC-dextran (Fluorescein isothiocyanate–dextran) at the serosal side at t = 0 min, 60 min and 90 min in each donors' biopsies.

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