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ORIGINAL CONTRIBUTION

High-level dietary fibre up-regulates colonic fermentation and relative abundance of saccharolytic bacteria within the human faecal microbiota in vitro

Qing Shen · Lu Zhao · Kieran M. Tuohy

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Abstract
Background Health authorities around the world advise citizens to increase their intake of foods rich in dietary fibre because of its inverse association with chronic disease. However, a few studies have measured the impact of increasing mixed dietary fibres directly on the composition of the human gut microbiota.
Aims of the study We studied the impact of high-level mixed dietary fibre intake on the human faecal microbiota using an in vitro three-stage colonic model.
Methods The colonic model was maintained on three levels of fibre, a basal level of dietary fibre, typical of a Western-style diet, a threefold increased level and back to normal level. Bacterial profiles and short chain fatty acids concentrations were measured.
Results High-level dietary fibre treatment significantly stimulated the growth of *Bifidobacterium*, *Lactobacillus-Enterococcus* group, and *Ruminococcus* group ($p < 0.05$) and significantly increased clostridial cluster XIVa and *Faecalibacterium prausnitzii* in vessel 1 mimicking the proximal colon ($p < 0.05$). Total short chain fatty acids concentrations increased significantly upon increased fibre fermentation, with acetate and butyrate increasing significantly in vessel 1 only ($p < 0.05$). Bacterial species richness changed upon increased fibre supplementation. The microbial community and fermentation output returned to initial levels once supplementation with high fibre ceased.
Conclusions This study shows that high-level mixed dietary fibre intake can up-regulate both colonic fermentation and the relative abundance of saccharolytic bacteria within the human colonic microbiota. Considering the important role of short chain fatty acids in regulating human energy metabolism, this study has implications for the health-promoting potential of foods rich in dietary fibres.

Keywords Dietary fibre · Faecal microbiota · Fermentation · Short chain fatty acids

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Introduction

Epidemiological studies have revealed an inverse relationship between dietary fibre intake and the risk of developing chronic diseases such as cardiovascular disease (CVD) [1–3] and cancer [4]. Studies on ancient diets, such as the Palaeolithic diet, as well as studies in extant hunter gatherers or traditional rural diets in the Mediterranean, Asia, Africa and Australia have suggested that our ancestors consumed, on a daily basis, three to five times the level of dietary fibre typically consumed by individuals following the modern Western-style diet [5]. The emergence of autoimmune diseases such as inflammatory bowel disease and increased incidence of CVD, certain cancers and obesity, over the past 50 years suggests that modern humans may be ill-adapted to the energy-rich nutritional environment in which they now find themselves [5]. Dietary fibre is a heterogenous group of food components

including non-starch polysaccharides (NSP), resistant oligosaccharides, resistant starch and lignin associated with the dietary fibre polysaccharides, according to the latest definition from European Food Safety Authority [6]. Dietary fibre has been suggested to impact on human energy metabolism in a number of ways including regulation of intestinal transit time and digestive function through bulking and gel-forming activities, reducing the energy density of foods and acting as a substrate for carbohydrate fermentation by the colonic microbiota [7, 8]. Carbohydrate fermentation in the colon results in production of short chain fatty acids (SCFA), mainly acetate, propionate and butyrate. These biologically active organic acids have been shown in molecular studies to impact on many different physiological processes including regulation of de novo lipogenesis and cholesterol biogenesis in the liver, acting as an energy source for human cells in the intestine and in other organs including muscle, brain, heart and liver, regulating production of gut hormones involved in satiety, impacting on fat storage and adipocyte leptin and adipokine production, and possibly impacting on thermogenesis in muscle [9–14]. Although the molecular basis of these activities mediated through G-coupled receptors (GPR-41 and GPR-43) or histone deacetylase inhibition has been demonstrated in animals, only few studies have examined the ability of increased intake of mixed dietary fibres to modulate microbial populations within the gut, up-regulate colonic fermentation and impact on SCFA production by the gut microbiota.

The intestinal microbiota, which has closely co-evolved with humans under nutritional selective pressure largely determined by our diet, has recently been linked to the aetiology or maintenance of many of chronic diseases, particularly inflammatory bowel disease, colon cancer, obesity and the diseases of obesity (type 2 diabetes and non-alcoholic fatty liver disease) [15, 16]. Fermentation of certain types of dietary fibre particularly prebiotic oligosaccharides, e.g. inulin, fructo-oligosaccharides and galacto-oligosaccharides, induce significant bifidogenic effects within the human faecal microbiota [17, 18]. Increment of beneficial bacteria like *Bifidobacterium* and *Lactobacillus* may provide further health-promoting effects such as enhancing immunity [19, 20], producing vitamins [21, 22] and inhibiting potential pathogens [23]. A recent ecological study characterising the gut microbiota of children in a rural African community in Berkino Fasa found that children following a traditional, largely plant-based African diet rich in different types of fibre, displayed a surprisingly distinct microbiota composition compared to children in Italy following a typical Western-style diet [24]. The African children had a microbiota dominated by saccharolytic bacteria, some never before associated with the human intestinal microbiota such as *Xylanibacter*. This was reflected in fermentation output, with three to four times the concentration of short chain fatty acids, especially acetate, being measured in the faeces of the African children compared to the faeces of the Italian children. Although genetics may have accounted for some of the differences in microbiota composition, the major contributor to these differences in microbiota composition and fermentation output appeared to be diet, particularly the concentration of dietary fibre, which was double in the African children compared to the urban European children. EFSA report that the average dietary fibre intake in adults across European countries is 15–30 g per day with the lowest at 6–9 g per day and the highest at 39–51 g per day [6]. The major sources of dietary fibre are from bread, fruits and vegetables according to the report; thus, an increase insuch fibre-rich food in daily diet should provide sufficient amount of healthy dietary fibre. As evidenced from the "high-fibre" intake group identified in the EFSA statistics, Europeans can intake levels of dietary fibre approaching those found in African or more traditional diets without ill effect.

Here we investigated the impact of increasing total dietary fibre by threefold on the composition and metabolic activities of human gut microbiota using an in vitro three-stage continuous culture system. The total dietary fibre used was a mixture of starch, pectin, guar gum, xylan, arabinogalactan and inulin, which are the major forms of dietary fibre commonly found plant foods present in human diets. The high-fibre treatment brought the total dietary fibre input into the system from as low as 8 g per 24 h to 24 g per 24 h, which mirrored the recommendation amount from EFSA report of 25 g per day, and about three times higher than the "low fibre" consumers identifiable from the EFSA statistics.

Materials and methods

Fermentations using the three-stage continuous culture system (Colonic Model)

The continuous culture fermentations ($n = 3$) were carried out with a three-stage continuous culture system that had been previously validated against colonic contents of sudden death victims [25]. The system comprised three glass vessels aligned in series. The first vessel in the system had an operating volume of 280 mL with growth medium being introduced into it. The second vessel had an operating volume of 300 mL and was fed from the overflow of the first vessel. The third vessel had a volume of 300 mL and was fed from the overflow of the second vessel. Culture fluid from the final vessel was vented into a waste bottle. Each vessel was continuously stirred and maintained at

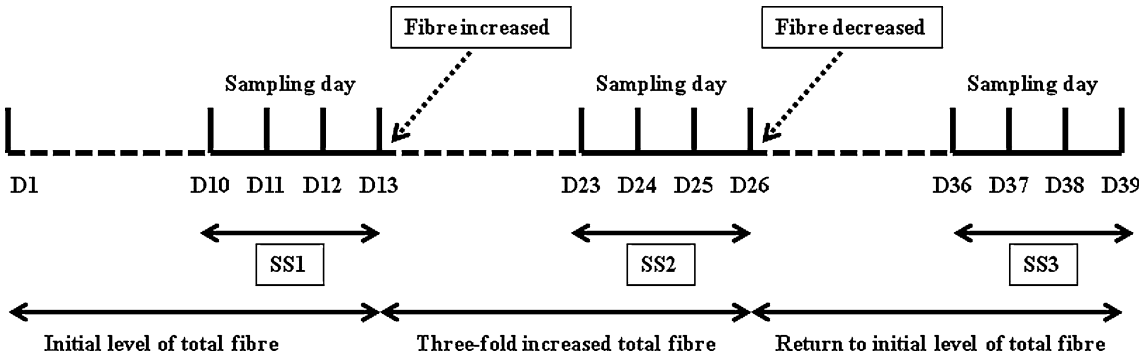

Fig. 1 A diagram of the fermentation course and the high-fibre treatment. Samples were taken on four consecutive days over each steady state, analysed and averaged. *SS1* initial level of total fibre, *SS2* threefold increased total fibre, *SS3* return to initial level of total fibre

37 °C by using a circulating water jacket. The pH of the vessels was maintained at 5.5, 6.2 and 6.8 for vessels 1, 2 and 3, respectively, by using FerMac 260 pH Controller (Electrolab, Tewkesbury, UK) pumping in 0.5 M HCl/NaOH solutions as appropriate. The entire system (medium reservoir included) was operated under anaerobic conditions by continuous sparging with oxygen-free nitrogen through the liquid (approximately 15 mL/min) in all the vessels. Vessel 1 simulated microbial conditions found in the proximal colon, vessel 2 modelled the transverse colon and vessel 3 mimicked the distal region of the colon. Vessels were initially filled with culture medium of 180, 200 and 200 mL in vessel 1, vessel 2 and vessel 3, respectively. This culture medium, originally designed by Macfarlane et al. [25] as a mimic of the prevailing Western-style diet in the UK, consisted of the following compounds (in grams per litre): starch (starch from potatoes, Sigma), 5.0; peptone water (Oxoid), 5.0; tryptone (Oxoid), 5.0; yeast extract (Oxoid), 4.5; NaCl (Fisher Scientific), 4.5; KCl (BDH), 4.5; pectin (citrus, Sigma), 2.0; casein (Sigma), 3.0; xylan (oat spelt, Sigma), 2.0; arabinogalactan (larch wood, Fluka), 2.0; $NaHCO_3$ (Fisher Scientific), 1.5; $MgSO_4{\cdot}7H_2O$ (BDH), 1.25; guar gum (Sigma), 1.0; inulin (Orafti), 1.0; cysteine–HCl (Sigma), 0.8; KH_2PO_4 (BDH), 0.5; K_2HPO_4 (BDH), 0.5; bile salts no. 3 (Oxoid), 0.4; $CaCl_2{\cdot}6H_2O$ (BDH), 0.15; hemin (Sigma), 0.05; vitamin K (Sigma), 0.01; and $FeSO_4{\cdot}7H_2O$ (BDH), 0.005. Tween 80 (Sigma) was used at 1 mL/L. A 4-mL/L concentration of a 0.025% (w/v) solution of resazurin was added to the growth medium to act as an indicator of anaerobicity. Faecal inocula, one for each model system, were provided by three healthy human volunteers (omnivores, 2 men: age 37 and 25; 1 woman: age 25), who had not taken any antibiotics prior to the study for at least 6 months. Each vessel was inoculated with 100 mL of 20% fresh faecal slurry (w/v) prepared with pre-reduced PBS (pH 7.2, Oxoid).

The fermentation period was divided into three phases (Steady State, SS) as pictured in Fig. 1. In phase 1, gut models were fed with a standard medium with initial level of dietary fibre content as described above to reach the first steady state (SS1). In phase 2, the level of total dietary fibre was increased to threefold including starch (from 5.0 to 15.0 g L^{-1}), pectin (from 2.0 to 6.0 g L^{-1}), guar gum (from 1.0 to 3.0 g L^{-1}), xylan (from 2.0 to 6.0 g L^{-1}), arabinogalactan (from 2.0 to 6.0 g L^{-1}) and inulin (from 1.0 to 3.0 g L^{-1}) to reach the second steady state (SS2). In phase 3, the dietary fibre content returned to the initial level to reach the third steady state (SS3). The retention time in each phase was kept at approximately 36 h. When the fibre content was increased, 1 M HCl-NaOH solutions were used to adjust the pH in order to keep the same retention time. Each phase was run for at least seven full turnovers of the total volume to achieve a steady state (SS) during which samples of culture fluid were taken on four consecutive days for bacterial and short chain fatty acids analysis. Steady states were checked by analysing each type of SCFA concentrations over four consecutive sampling days with no significant differences detected.

Bacterial enumeration using fluorescent in situ hybridisation (FISH)

FISH analysis was conducted according to Martin-Pelaez [26]. The Cy3-labelled oligonucleotide probes used in this study were the following: Bif164 (targeting most bifidobacteria and *Parascardovia denticolens*) [27], Bac303 (most *Bacteroides* and *Prevotella* spp., *Barnesiella* spp. and *Odoribacter splanchnicus*) [28], Chis150 (most members of clostridial cluster I and all members of clostridial cluster II) [29], Erec482 (most members of clostridial cluster XIVa) [29], Lab158 (most *Lactobacillus*, *Leuconostoc* and *Weissella* spp., all *Enterococcus*, *Vagococcus*, *Melisococcus*, *Tetragenococcus*, *Paralactobacillus*, *Pediococcus*,

Oenococcus and *Catellicoccus* spp. and *Lactococcus lactis*) [30], Ato291 (all *Cryptobacterium*, *Collinsella*, *Atopobium*, *Eggerthella* and *Olsenella* spp.) [31], Fpra655 (*Faecalibacterium prausnitzii*) [32], Rbro730 (*Ruminococcus bromii*-like; *Clostridium sporosphaeroides* and *Clostridium leptum*) and Rfla729 (*Ruminococcus albus* and *Ruminococcus flavefaciens*) [33]. A probe mixture of Eub338, Eub338 II and Eub338 III at equal concentration was used for detecting the total bacteria [34]. Counts of the bacterial numbers were presented as $\log_{10}$ (no. of cells/ml of sample).

SCFA analysis using GC

SCFA analysis was performed as described by Zhao et al. with minor modifications [35]. One millilitre of the culture sample was centrifuged at 10,000*g* for 10 min, and the supernatant was filtered through 0.2-μm filters (Millipore, Cork, UK). Ethyl-butyrate (10 mM) was used as internal standard, and external standards contained acetic acid, propionic acid, *i*-butyric acid, *n*-butyric acid, *i*-valeric acid, *n*-valeric acid and *n*-caproic acid at a concentration of 25 mM, respectively. Dilutions of the external standards were prepared and added to the internal standard to give a final concentration for the internal standard of 2 mM ethyl butyric, and a final concentration of external standards as 20 mM,10 mM, 5 mM, 1 mM and 0.5 mM. A 1-μl aliquot of each sample was injected into a 5890 Series II GC system (HP, Crawley, West Sussex, UK) fitted with a free fatty acid phase (FFAP) column (25 m × 0.32 mm, diameter = 0.25 μm; J&W Scientific, Folsom, CA, USA) and a flame ionisation detector. The carrier gas was helium at a column flow rate of 2.5 mL/min. The injector was maintained at 220 °C and the detector at 250 °C. The column was maintained at 100 °C for 1 min, then raised at 8 °C/min to 200 °C, and at 20 °C/min to 250 °C and maintained for 3 min. Peaks were integrated using Atlas Lab managing software (Thermo Lab Systems, Mainz, Germany). The concentration of SCFAs in the samples was calculated against the standards and expressed in mM.

Bacterial DNA extraction and PCR-DGGE

Bacterial cell pellets were collected by centrifugation of 1 mL of the culture sample and immediately frozen at −20 °C until DNA extraction. Samples were thawed on ice, and bacterial DNA was extracted using the FastDNA Spin Kit (Qbiogene, Cambridge, UK) according to the manufacturer's instructions. Approximately 5 ng of DNA was loaded into each PCR using universal bacterial primers P2 and P3 according to Muyzer [36]. Five microlitres of each PCR product was applied to DGGE using a VWR CTV400-DGGE Unit (VWR, UK). The polyacrylamide gel was composed of 8% (v/v) polyacrylamide (acrylamide-bisacrylamide, 37.5:1, Bio-Rad, UK) with a linear denaturing gradient of 30–70% (100% denaturant was defined as 7 M urea and 40% [v/v] deionised formamide). Electrophoresis was run in 0.5× TAE buffer (made from 50× concentrate; Fisher, UK) at 100 V and 60 °C for 16 h. Gels were silver stained according to Sanguinetti's method with minor modifications [37].

Analysis of DGGE profiles

DGGE gels were scanned with a Canon scanner (Lide 50; Canon, Surrey, UK) and analysed by Gelcompar 6.00 (Applied Maths, Sint-Martens-Latem, Belgium). One PCR sample was selected as the standard and run across gels in order to normalise banding patterns. Similarities between sample lanes were determined by calculating similarity indices based on the Dice similarity coefficient and the unweighted-pair group method using arithmetic averages (UPGMA). Composite data sets for group-specific DGGE profiles were generated and numerical band matching character tables were produced for biodiversity analysis. Simpson's, Shannon–Weaver and Fisher's alpha ecological indices of diversity were generated for statistical analysis. Principal component analysis (PCA) by Simca P+ (Umetrics Ltd, Crewe, UK) was performed on the pooled binary data matrix in which all bands were divided into classes of common bands; for each pattern, a particular band class could have one of two states: present (termed 1) or absent (termed 0).

Statistical analysis

Statistical analysis of bacterial counts, SCFA data and diversity indices of DGGE banding patterns were performed by one-way ANOVA (Genstat 11th; VSN International Ltd, Hemel Hempstead, UK) with the block structure for samples from each gut model. When differences were found, least significant differences of means were used for multiple comparisons at 5% for significant level.

Results

Bacterial enumeration

Table 1 shows the mean changes ($n = 3$) in different bacterial populations measured by FISH in the three vessels at three steady states (SS1: initial level of total fibre; SS2: threefold increased total fibre; SS3: return to initial level of total fibre). Significant increases in numbers of *Bifidobacterium* spp., *Ruminococcus* spp. and *Lactobacillus-Enterococcus* group were observed at SS2, after high-

Table 1 Bacterial enumeration using FISH in the three-stage continuous system at three steady states (SS)

FISH probes	Vessel1			Vessel2			Vessel3		
	SS1	SS2	SS3	SS1	SS2	SS3	SS1	SS2	SS3
Bif164	8.57 ± 0.23	9.21 ± 0.58*	8.75 ± 0.42	8.64 ± 0.38	9.35 ± 0.34*	8.75 ± 0.46	8.57 ± 0.46	9.19 ± 0.43*	8.68 ± 0.53
Ato291	8.49 ± 0.38	8.55 ± 0.46	8.50 ± 0.49	8.41 ± 0.39	8.45 ± 0.45	8.54 ± 0.58	8.47 ± 0.37	8.51 ± 0.41	8.46 ± 0.45
Erec482	9.12 ± 0.32	9.29 ± 0.31*	9.02 ± 0.33	9.19 ± 0.19	9.26 ± 0.08	9.21 ± 0.25	9.12 ± 0.21	9.19 ± 0.06	9.08 ± 0.22
Fpra655	8.41 ± 0.21	8.92 ± 0.07*	8.58 ± 0.22	8.34 ± 0.07	8.50 ± 0.04	8.43 ± 0.10	8.11 ± 0.11	8.24 ± 0.13	8.10 ± 0.15
Rbro730/Rfla729	8.53 ± 0.41	8.97 ± 0.46*	8.42 ± 0.27	8.20 ± 0.25	8.82 ± 0.41*	8.39 ± 0.20	8.48 ± 0.44	8.66 ± 0.42*	8.46 ± 0.35
Lab158	7.13 ± 0.17	7.52 ± 0.19*	7.11 ± 0.15	7.17 ± 0.21	7.52 ± 0.12*	7.10 ± 0.19	7.12 ± 0.05	7.44 ± 0.24*	7.10 ± 0.07
Chis150	8.43 ± 0.17	8.16 ± 0.16	8.31 ± 0.06	8.43 ± 0.21	8.28 ± 0.12	8.32 ± 0.14	8.46 ± 0.26	8.32 ± 0.22	8.21 ± 0.23
Bac303	8.69 ± 0.80	8.80 ± 0.79	9.02 ± 0.65	9.29 ± 0.31	9.40 ± 0.30	9.26 ± 0.34	9.09 ± 0.29	9.24 ± 0.11	8.99 ± 0.20
EUB I, II, III	10.00 ± 0.21	10.10 ± 0.15*	10.02 ± 0.16	10.04 ± 0.04	10.15 ± 0.03*	10.00 ± 0.08	9.80 ± 0.24	10.00 ± 0.08	9.91 ± 0.15

Bacterial numbers were measured as Log_{10} cells/ml. Measurements performed on four consecutive days during each steady state were averaged. Data from three gut models were then averaged and represented mean ± SD ($n = 3$)

SS1 initial level of total fibre; *SS2* threefold increased total fibre; *SS3* return to initial level of total fibre

* Significant change compared to SS1 and SS3 (ANOVA, $p < 0.05$ and LSD, $p < 0.05$). No significant difference between SS1 and SS3 was detected (LSD, $p < 0.05$)

fibre dosing, in all three vessels while *Faecalibacterium prausnitzii* and *Eubacterium rectale-Clostridium coccoides* groups were significantly increased in vessel 1 at SS2.

SCFA analysis

Concentrations of SCFA present in gut models also changed upon high-mixed fibre supplementation (Table 2). The concentration of acetate was increased most strikingly in all three vessels upon high-fibre dosing while the concentration of butyrate was increased significantly in vessel 1mimicking the proximal colon.

PCR-DGGE

Table 3 shows the comparison of diversity indices of each sample from the DGGE gel based on the banding pattern (presence/absence of certain bands). Increasing the level of total mixed fibre induced a relatively more diverse microbial community with statistically significant changes in diversity indices observed in vessel 2.

According to the cluster analysis, each gut model, from three different human donors, shows a distinct DGGE banding pattern as expected (Figs. 2, 3, 4). Within each gut model, a clear separation of samples from the increased total fibre treatment (SS2) to the initial fibre level (SS1) and the return to initial fibre level (SS3) indicated significant modulating effect of the increased total mixed fibre on the bacterial profiles (Figs. 2, 3, 4). The neighbourhood of sample lanes from SS1 and SS3 indicated the returning of bacterial profiles once the increased total fibre treatment was ceased.

The total binary data matrix generated by the presence (represented as 1) or absence (represented as 0) of the DGGE bands of three gut models was pooled and normalised by running a sample lane across the gels. This data matrix was further analysed by principal component analysis (PCA). Figure 5 shows the PCA score plot of the first two significant components (PC) which accounted for 14.8% (PC1) and 11.3% (PC2) of the variability in the data set. The most striking difference was based on the different gut models that implied a significant inter-gut model variation existed likely due to the unique faecal inocula from three different human donors. However, a clear trend of separation based on the high-fibre treatment (SS2) was also observed (shown in different colours). Therefore, another score plot was generated by using the second and third significant components as shown in Fig. 6. Here, PC2 and PC3 account for 11.3% (PC2) and 8.8% (PC3) of the variability within the data, respectively. Samples were clearly separated into three groups according to the steady states with mild overlapping in the centre. Samples from

Table 2 SCFA analysis using GC in the three-stage continuous system at three steady states (SS)

SCFA	Vessel1			Vessel2			Vessel3		
	SS1	SS2	SS3	SS1	SS2	SS3	SS1	SS2	SS3
Acetate	32.04 ± 4.14	63.10 ± 16.73*	40.76 ± 6.76	37.83 ± 6.33	75.03 ± 16.66*	46.65 ± 10.67	41.43 ± 9.47	78.02 ± 15.71*	50.26 ± 7.93
Propionate	33.05 ± 2.85	28.55 ± 3.01	27.64 ± 2.28	38.41 ± 4.50	39.90 ± 6.29	36.66 ± 4.72	38.52 ± 5.00	41.37 ± 7.55	38.32 ± 2.19
n-Butyrate	24.84 ± 3.29	50.87 ± 14.84*	22.99 ± 4.51	30.82 ± 1.46	48.69 ± 22.41	28.01 ± 5.08	30.81 ± 1.96	48.74 ± 25.68	30.06 ± 5.80
Total SCFA	93.32 ± 8.69	143.33 ± 3.46*	95.08 ± 9.78	115.39 ± 6.12	166.92 ± 23.37*	118.90 ± 13.99	119.66 ± 11.16	173.00 ± 27.72*	127.74 ± 3.60

SCFA concentration was measured as mM. Measurements performed on four consecutive days during each steady state were averaged. Data from three gut models were then averaged and represented mean ± SD ($n = 3$)

SS1 initial level of total fibre, *SS2* threefold increased total fibre, *SS3* return to initial level of total fibre

* Significant change compared to SS1 and SS3 (ANOVA, $p < 0.05$ and LSD, $p < 0.05$). No significant difference between SS1 and SS3 was detected (LSD, $p < 0.05$)

Table 3 Diversity values of the bacterial community calculated from the DGGE profiles

Vessels and steady states (SS)	Diversity index value (mean ± SD)								
	Simpson's (*D*)			Shannon–Weaver (*H*′)			Fisher's alpha (α)		
	SS1	SS2	SS3	SS1	SS2	SS3	SS1	SS2	SS3
Vessel1	0.066 ± 0.010	0.062 ± 0.002	0.058 ± 0.006	2.717 ± 0.150	2.791 ± 0.024	2.853 ± 0.095	2.161 ± 0.310	2.358 ± 0.055	2.512 ± 0.232
Vessel2	0.055 ± 0.001	0.047 ± 0.005*	0.056 ± 0.003	2.893 ± 0.015	3.067 ± 0.095*	2.884 ± 0.049	2.571 ± 0.015	3.101 ± 0.273*	2.585 ± 0.125
Vessel3	0.055 ± 0.002	0.048 ± 0.006	0.054 ± 0.005	2.913 ± 0.054	3.057 ± 0.129	2.934 ± 0.079	2.662 ± 0.187	3.057 ± 0.360	2.734 ± 0.216

Diversity indices were calculated firstly on four consecutive days during each steady state and averaged. Data from three gut models were then averaged and represented mean ± SD ($n = 3$)

SS1 initial level of total fibre, *SS2* threefold increased total fibre, *SS3* return to initial level of total fibre

* Significant difference compared to SS1 and SS3 (ANOVA, $p < 0.05$ and LSD, $p < 0.05$). No significant difference between SS1 and SS3 was detected (LSD, $p < 0.05$)

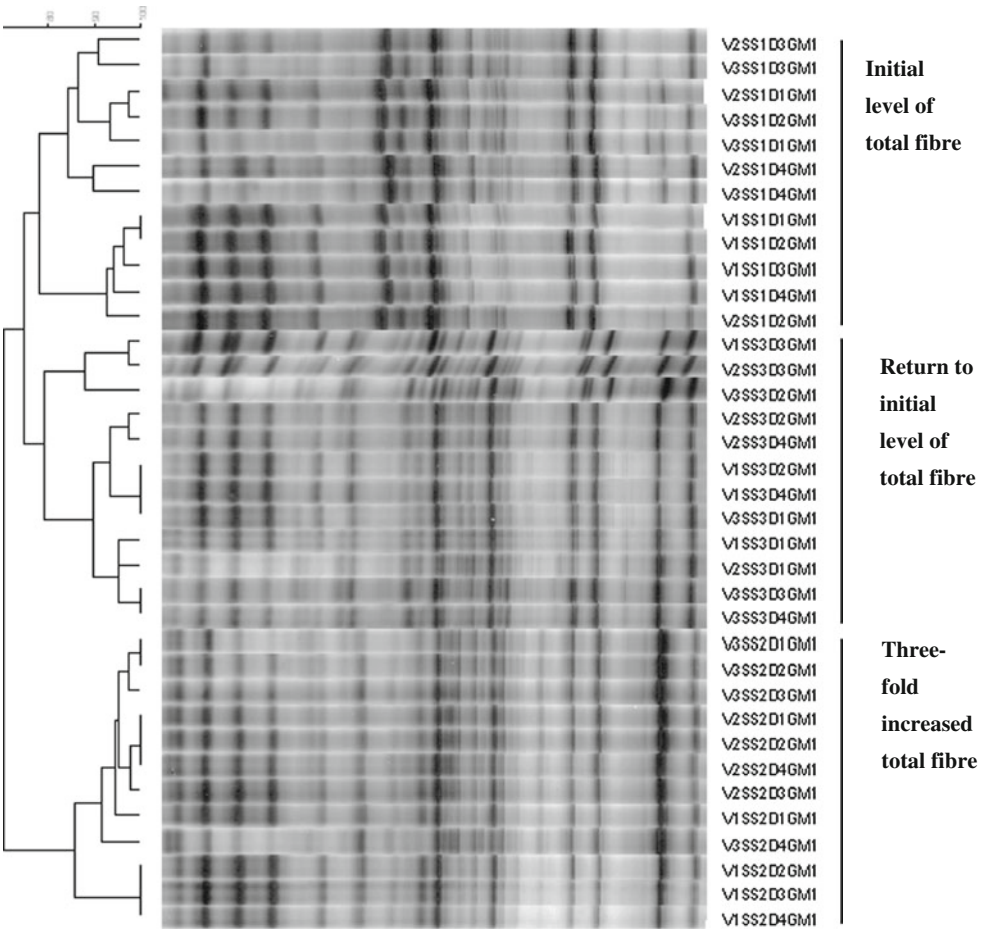

Fig. 2 DGGE clustering profile of samples from gut model 1 (GM1). Samples are denoted by the vessel number, the steady state and sampling day number as well as the gut model number; for example, V1SS1D1GM1 represents a sample taken from vessel 1 (V1) at first steady state (SS1) and first day (D1) from gut model 1 (GM1). The dendrogram was constructed using the Dice similarity coefficient and the unweighted-pair group method using arithmetic averages (UPGMA) cluster method. The *image* shows three clear clusters based on the steady states, which represent the fibre-increasing/decreasing treatment

SS2 were grouped together while samples from SS1 and SS3 appeared to be more similar.

Discussion

Dietary fibre is considered an important part of a healthy human diet, and fibre consumption is inversely related to many chronic disease states including CVD and colon cancer [1–4]. Dietary fibres are a chemically diverse collection of mainly plant-based non-digestible polysaccharides and oligosaccharides, which may affect human health by different physiological mechanisms. Bacterial fermentation of certain dietary fibres results in increased SCFA production and a stimulated growth of saccharolytic bacteria although a few studies have measured the impact of mixed fibres on the microbial populations responsible for colonic carbohydrate fermentation within the gut microbiota. Here we used a three-stage continuous culture system mimicking the microbial composition and fermentative activity of the human colon to determine the impact of threefold increased total mixed fibre intake on the human faecal microbiota. Like any in vitro model, this continuous culture system does have its limitations, particularly a lack of human cell or immune interactions, no capacity to mimic absorption of organic acids and other microbiota metabolites or conversely, host secretions and water absorption. However, it does represent a useful model for studying microbe–microbe interactions within the gut microbiota [38] and diet–microbe interactions, and has been shown, at least for prebiotic fibre fermentations, to give similar results to human prebiotic dietary intervention [39, 40].

Upon high-fibre supplementation, the relative abundance of *Bifidobacterium*, *Eubacterium rectale-Clostridium coccoides*, *Faecalibacterium prausnitzii, Lactobacillus/Enterococcus* and *Ruminococcus* was stimulated to different extents in the three different vessels of the in vitro colon model. *Bifidobacterium* showed a larger increment in population (>0.5 $\log_{10}$ unit in all three vessels) compared to other bacterial groups. Prebiotic dietary fibres such as inulin and fructo-oligosaccharides have repeatedly been shown to increase colonic bifidobacterial numbers in vitro and in vivo [41, 42], while other dietary fibres have also been reported to induce bifidogenic effects in faecal microbiota including whole grain cereals [43], resistant starch [44], pectins and pectic-oligosaccharides [17]. A wide range of carbohydrase genes encoded by bifidobacterial genomes have indicated

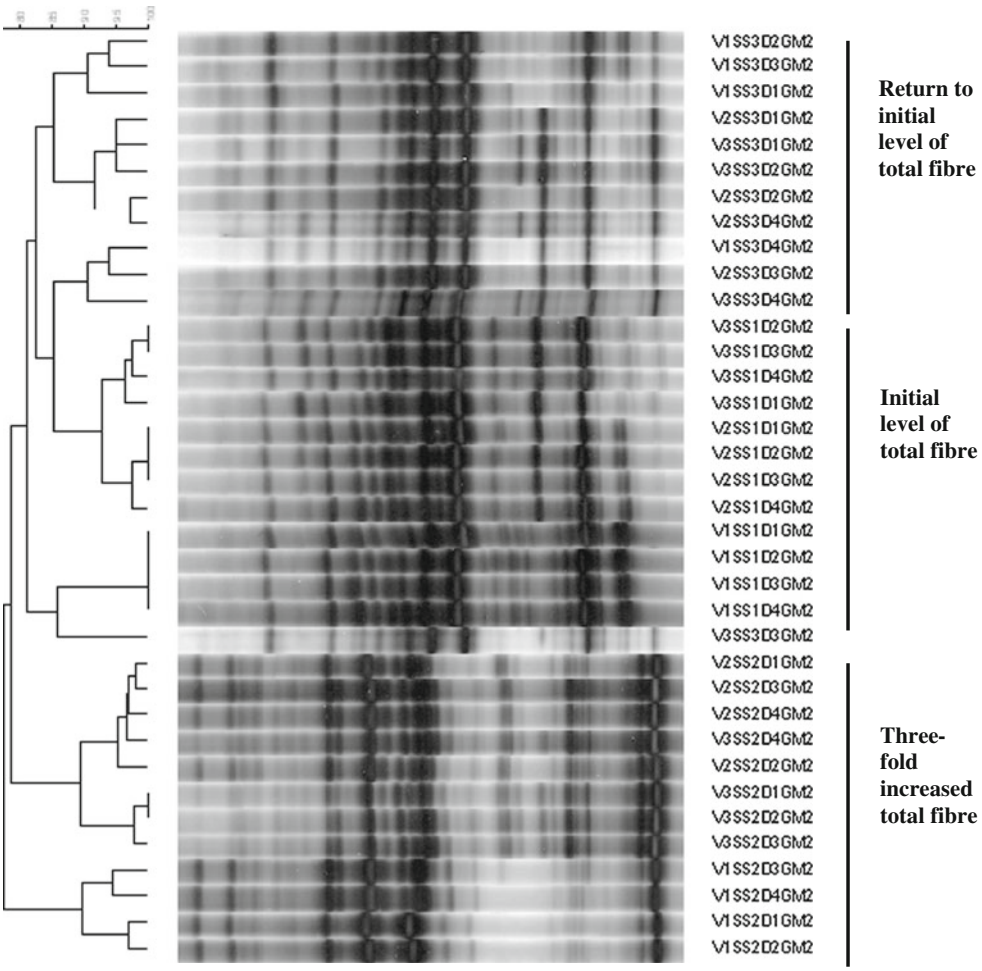

Fig. 3 DGGE clustering profile of samples from gut model 2 (GM2). Samples are denoted by the vessel number, the steady state and sampling day number as well as the gut model number; for example, V1SS1D1GM2 represents a sample taken from vessel 1 (V1) at first steady state (SS1) and first day (D1) from gut model 2 (GM2). The dendrogram was constructed using the Dice similarity coefficient and the unweighted-pair group method using arithmetic averages (UPGMA) cluster method. The *image* shows three clear clusters based on the steady states, which represent the fibre-increasing/decreasing treatment

the bacterial genus may play an important role in colonic saccharolytic activity [45]. Therefore, generally increasing dietary intake of certain fibres and prebiotics may significantly stimulate this bacterium to grow. Recent intervention studies have shown that intake of *Bifidobacterium*-containing probiotics or synbiotics is associated with improved biomarkers of CVD [46] and colon cancer risk [47, 48], which indicates a potential therapeutic role of the dietary fibre-*Bifidobacterium* effect in these chronic diseases.

Ruminococcus is another major fibre degrader in the human colon, which may preferentially adhere to starch particles and thrive [49]. In this study, the carbohydrate-enriched environment obviously enhanced the growth of this bacterium with the increment of bacterial numbers about 0.5 log unit in vessel 1 and 2, and 0.2 log unit in vessel 3. A recent in vivo study from Walker further demonstrated relatives of *Ruminococcus bromii* (R-ruminococci) as well as relatives of *Eubacterium rectale* increased in people following a resistant starch-rich diet compared to a non-starch polysaccharides-rich diet and a weight loss diet using a 16S rRNA clone library analysis, while no significant changes were detected in most other bacterial groups. This implies a sensitivity of this bacterial group to modulation by starch/carbohydrate-rich diet [50].

Faecalibacterium prausnitzii detected by Fpra655 and *Eubacterium* group detected by Erec482 produce the majority of butyrate in the human colon [51, 52]. A concurrent increase in butyrate production and the numbers of *Faecalibacterium prausnitzii* and *Eubacterium* group observed in vessel 1 in this study verified the "butyrogenic effect" by increased mixed fibre supplementation. Such carbohydrate fermentation is usually more profound in the proximal colon [53]. Recent studies have highlighted the beneficial role of *Faecalibacterium prausnitzii* in maintaining colonic health. People with inflammatory bowel diseases tend to have a lower number of this bacterium [54, 55]. The anti-inflammatory effect of *Faecalibacterium prausnitzii* has been confirmed in vitro and in vivo, and proposed to be partially due to secreted metabolites able to block NF-κB activation and IL-8 production [56]. The reason why increased numbers of this microorganism were not detected in vessel 2 and 3 upon increased fibre supplementation may be due to a pH-dependent effect (pH 6.2 and 6.8 in vessel 2 and 3, respectively). Walker et al.

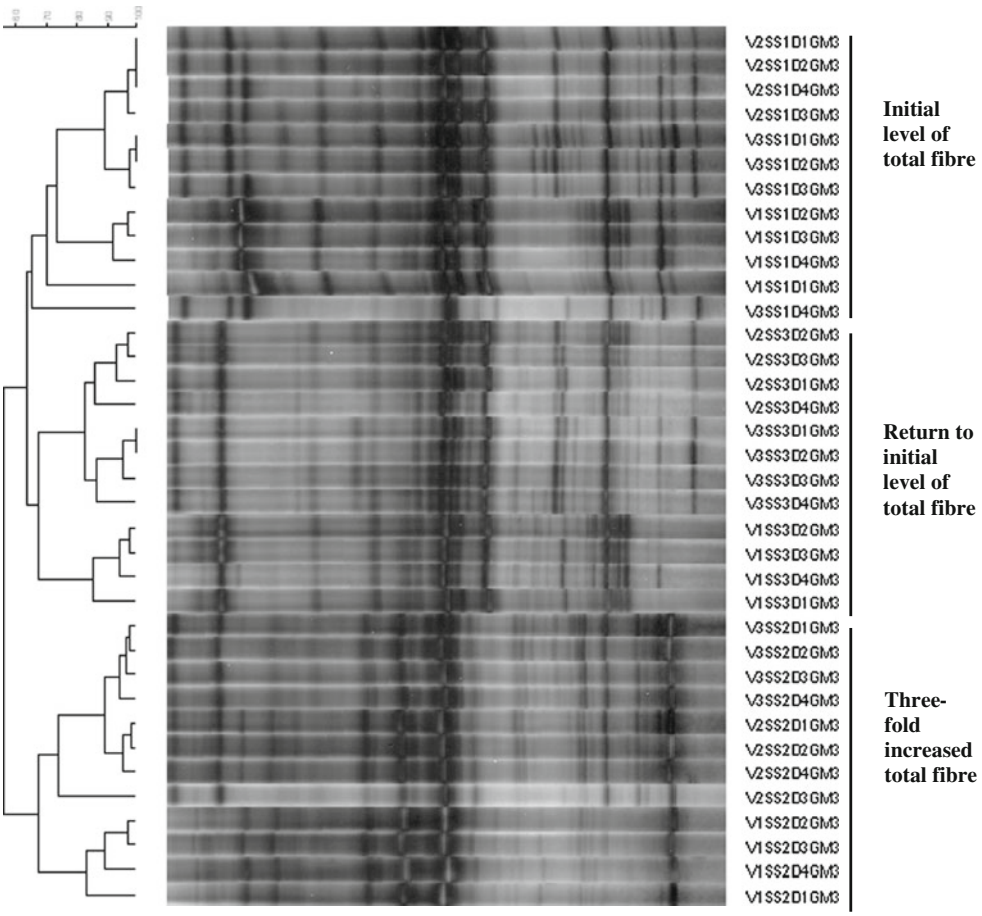

Fig. 4 DGGE clustering profile of samples from gut model 3 (GM3). Samples are denoted by the vessel number, the steady state and sampling day number as well as the gut model number; for example, V1SS1D1GM3 represents a sample taken from vessel 1 (V1) at first steady state (SS1) and first day (D1) from gut model 3 (GM3). The dendrogram was constructed using the Dice similarity coefficient and the unweighted-pair group method using arithmetic averages (UPGMA) cluster method. The *image* shows three clear clusters based on the steady states, which represent the fibre-increasing/decreasing treatment

reported that *Roseburia* (covered by probe Erec482), another major butyrate producer, decreased in number together with a decrease in butyrate production when pH increased from 5.5 to 6.5 in a continuous culture system, while *Bacteroides* populations were found to grow preferentially at pH 6.5 [57]. Our results supported their findings showing that butyrate was produced in higher levels in vessel 1 with pH of 5.5 and *Bacteroides* grew better in vessels 2 (pH 6.2) and 3 (pH 6.8) accounting for 12–19% of the total bacteria while they comprised less than 10% in vessel 1 (pH 5.5). Since the fermentation system was pH controlled, the natural decrease in pH due to the increased intake of fermentable fibre was not allowed to overly impact on the microbiota. A similar buffering effect is likely in the colon too where pH is regulated by SCFA and ammonium absorption and bicarbonate secretion.

Bacterial numbers of *Lactobacillus/Enterococcus* group were also increased in step with numbers of *Bifidobacterium* in this study and such a concurrent increase has been observed previously in oligofructose fermentations [58], and upon faecal microbiota fermentation of an enzymatically treated durum wheat dietary fibre [59] using a similar in vitro fermentation systems. However, the Lab158 probe could not differentiate lactobacilli from enterococci [30]. The increased numbers of this bacterial group could be due to the flourish of enterococci, although the bacteria enumerated with this probe in this study were predominantly rod shaped. The *Clostridium* spp. detected by FISH probe Chis150 contain proteolytic and potentially harmful bacteria. The stability of this bacterial population during the fermentation indicated that this bacterial group was less competitive for utilising the mixed fibre carbohydrate substrates in the fermentation system and might also be inhibited by other bacterial groups and the acidified environment.

Despite inter-individual variations, DGGE analysis showed a significant change in the community profiles due to the high-fibre treatment. For two of the model systems, modulated profiles gradually changed back when the fibre content returned to the initial level, indicating that increasing and decreasing dietary fibre content may significantly affect the microbial community in the colon but only transiently. This backed up the findings from the FISH data that significant changes in bacterial populations were detected after running high-fibre treatment while such changes returned to the initial level after switching back to

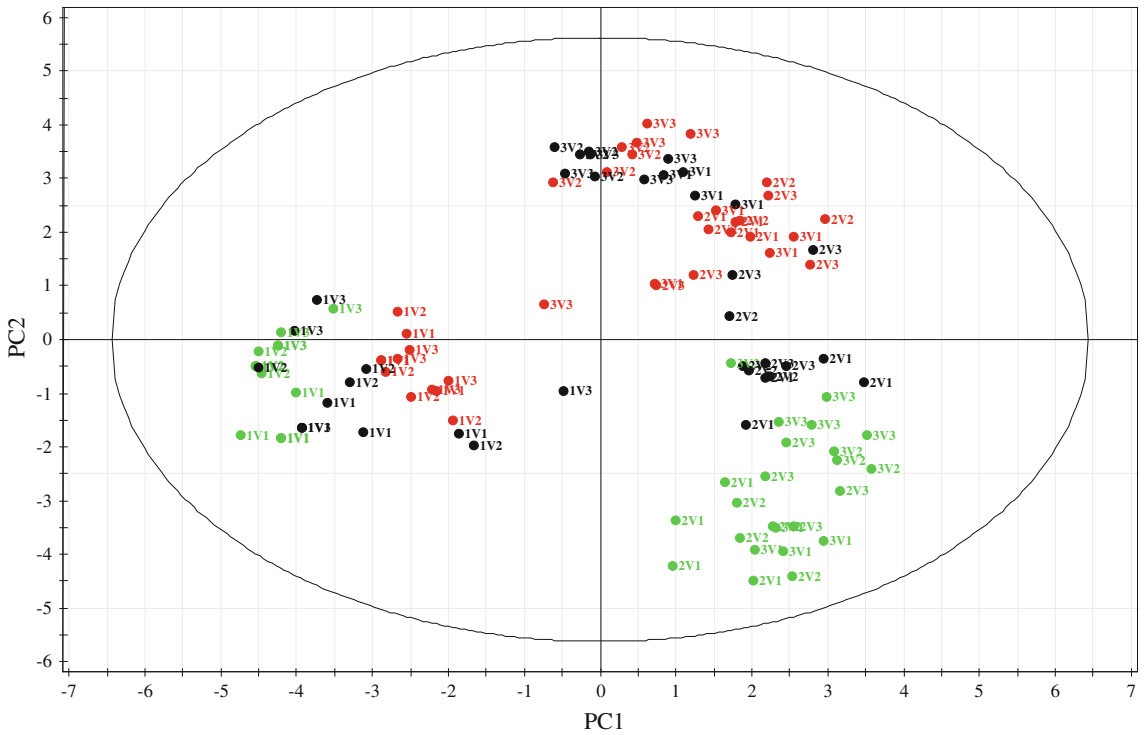

Fig. 5 PCA score plot of the DGGE image by using the first and second significant components. Samples are shown in *different colours* as: ● steady state 1, ● steady state 2, ● steady state 3. Samples are denoted by the gut model number and the vessel number; for example, 1V1 represents a sample taken from gut model 1, vessel 1. Four samples shown in the *same colour* and labelling represent repeated samples taken on four consecutive days during each steady state. PC1 accounts for 14.8% of the variability and PC2 accounts for 11.3% of the variability

the low mixed fibre medium. In the third model system, although there was a dramatic modulation of the microbiota in response to high-fibre intervention, observed in both FISH and DGGE data, these alterations tended to persist after low-level fibre feed was reinstated. This may reflect differences in response to high-level fibre intake between the gut microbiota of different human hosts and their background diet. Indeed, recent high-resolution metagenomics-based sequencing studies have highlighted the existence of distinct "enterotypes" within the human microbiome, which may respond different to diet or drugs [60]. How these different enterotypes respond to high fermentable fibre availability remains to be determined.

SCFA production is a major colonic microbial activity, and three major SCFAs regulate energy metabolism in different ways with butyrate being an important energy source for the colonic mucosa, acetate acting as a substrate for hepatic de novo lipogenesis via acetyl-coA and fatty acid synthase (FAS), while propionate down-regulates lipogenesis-reduced expression of fatty acid synthase (FAS) [9, 14]. Butyrate may enhance adaptive thermogenesis thus increases energy expenditure to control body weight and markers of the metabolic syndrome [10]. SCFA produced upon carbohydrate fermentation in the colon also affect gut hormone production including peptide YY (PYY) and glucagon-like peptide 1 and 2 (GLP), which in turn regulate production and release of digestive enzymes and satiety, our feeling of fullness, thus reduces food intake [12, 56]. Moreover, oligofructose, which leads to increased caecal butyrate production, upregulated GLP-2 production from intestinal L cells thereby reducing mucosal permeability and restoring mucosal barrier function compromised by high-fat diets and to control low-grade chronic systemic inflammation caused by leakage of inflammatory molecules, especially lipopolysaccharides, from gut lumen to blood stream [61].

The significance of diet, particularly traditional diets high in fiber, in modulating the composition and activity of the gut microbiome in children from Africa and Europe has been shown in a recent prospective study [24], while our in vitro work further demonstrated a significant growth of several saccharolytic bacterial groups coupled with enhanced SCFA production due to the increased total dietary fibre intake. The flourish of bifidobacteria and lactobacilli in our results support PASSCLAIM that "A healthy, or balanced, flora is (therefore), one that is predominantly saccharolytic and comprises significant numbers of bifidobacteria and lactobacilli" [62].

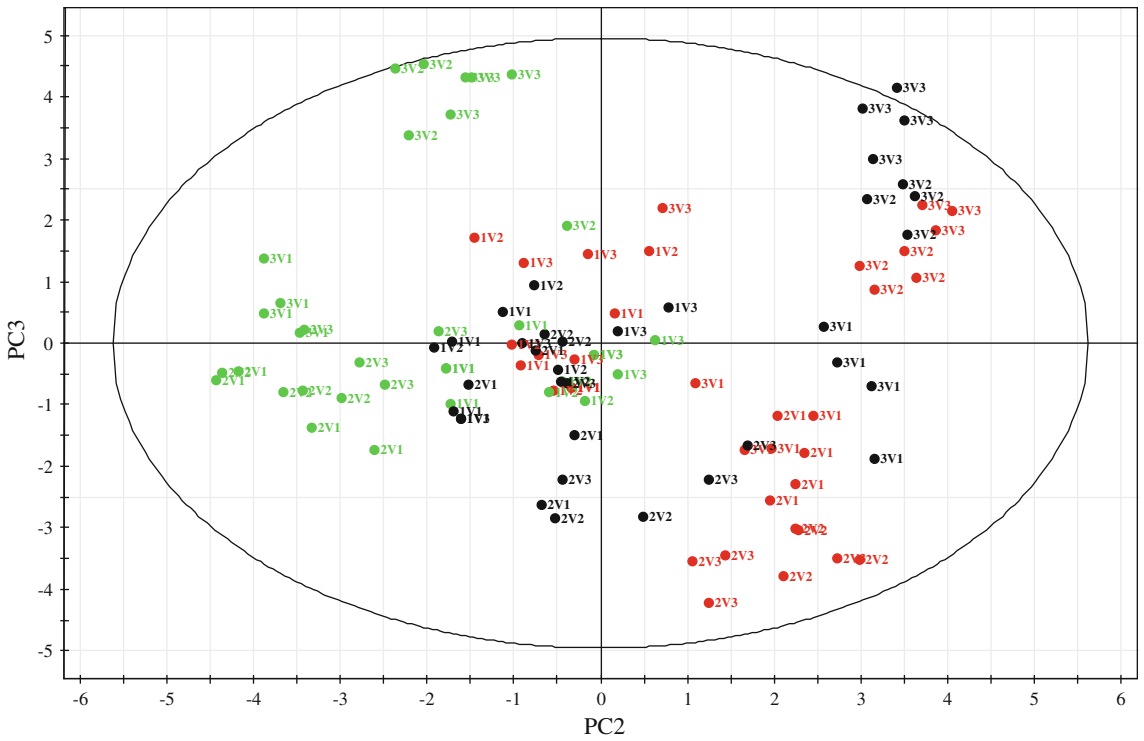

Fig. 6 PCA score plot of the DGGE image by using the second and third significant components. Samples are shown in *different colours* as: ● steady state 1, ● steady state 2, ● steady state 3. Samples are denoted by the gut model number and the vessel number; for example, 1V1 represents a sample taken from gut model 1, vessel 1. Four samples shown in the *same colour* and labelling represent repeated samples taken on four consecutive days during each steady state. PC2 accounts for 11.3% of the variability and PC3 accounts for 8.8% of the variability

Conclusion

This in vitro work provides insights into beneficial effects of increased total dietary mixed fibre intake on the composition and activity of the human faecal microbiota implying mechanistic health effects of whole plant foods rich in dietary fibre of diverse chemical structure, such as whole grain cereals, fruit and vegetables, could be due to their impact on the human gut microbiota composition and their metabolites. However, such effects may abate once the high-fibre intake ceases. Therefore, this study supports the notion that dietary habits that include a continuous consumption of large quantities of high-fibre foods improve colonic health and by extension human health at the whole body level.

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