

Supplementary Information on Gut Microbiome

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Methods**Feces parameters**

Fecal samples were collected independently at home, where they were frozen and stored at -15 to -20°C. Samples were delivered to the institute in isolated boxes until they were stored at -80°C. Samples were aliquoted in frozen state. For microbial measurements, DNA was extracted and further analysis was done by GENEWIZ Germany GmbH, Leipzig, Germany. For metabolomics (SCFA measurements), samples were analyzed by the Center for Environmental Research (UfZ), Leipzig, Germany.

SCFA measurements in blood and feces.*Metabolite extraction:*

Chemicals: Acetonitrile, formic acid and methanol were purchased from Sigma Aldrich (St Louis, MO, USA). D7-butyric acid was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). All short chain fatty acids standards (SCFAs) used for linear regression and quantitation were purchased from Sigma Aldrich (St Louis, MO, USA). All solvents for MS were of analytical grade purity. Experimental water (resistivity of 18.2 MΩ cm) was purified using a Milli-Q system (Millipore, Milford, MA, USA).

For SCFAs the method of Han et al. (2015) was modified. First, 100 mg feces were mixed with 500 µl ACN:Water (1:1, v/v) and homogenized using a TissueLyser II (30 Hz, 10 min; Retsch Qiagen). After short centrifugation (2 min, 14000 rpm) 100 µl of the supernatant were added to 500 µl ACN:Water:methanol (3:1:2, v/v/v) and the sample was vortexed for 5 min. After sonication (5 min) and centrifugation (14.000 rpm, 4°C, 5 min) 550 µl of the supernatant were transferred into a new tube and evaporated to dryness. Pellet was reconstituted in 100 µl 50% and 38 µl used for further derivatization. Next, 20 µl serum and 2 µl of standards were diluted with 18 µl and 38 µl 100% ACN, respectively. For derivatization, both specimen, serum and feces supernatant, were combined with 2 µl D7-butyric acid (2 mM) used as internal standard, 20 µl 3-nitrophenylhydrazine in 50% ACN (200 mM) and 20 µl N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride in 50% ACN with 6% pyridine (120 mM). Incubation of the mixture was done for 30 min at 40 °C in a thermomixer (Eppendorf, Hamburg, Germany).

Prior to measurement, the resulting derivative was diluted 1:50 in 10% ACN. Of each sample 10 µl were injected into the UltiMate 3000 HPLC system (ThermoFisher Scientific™, Waltham, MA, USA) coupled online to a QTRAP® 5500 mass spectrometer (Sciex, Framingham, USA). Chromatographic separation of SCFAs was performed on an Acquity UPLC BEH C18 column (1.7µm, 2.1 x 100 mm) with H2O + 0.01 % formic acid and ACN + 0.01% formic acid as mobile phases. Constant flow rate was set to 0.35 ml and linear LC gradient was as follows: 0-2 min at 15% B, 2-17 min 15-50% B, 17-18 min 100 % B, 18-18.1 min 100-15% B, 18.1 -21 min 15 % B. Mass spectrometric measurement was performed in negative ionization mode. For identification and quantitation, a scheduled multiple reaction monitoring (MRM) method was used, with specific transitions for every SCFA. Peak areas of all samples and standards for linear regression were determined in Analyst® Software (v. 1.6.2, AB Sciex) and areas for single SCFAs were exported. Normalization and statistics were performed with in-house written R scripts.

Note that fecal concentrations were higher compared to serum concentrations, and that the number of available samples dropped considerably due to methodological challenges in SCFA

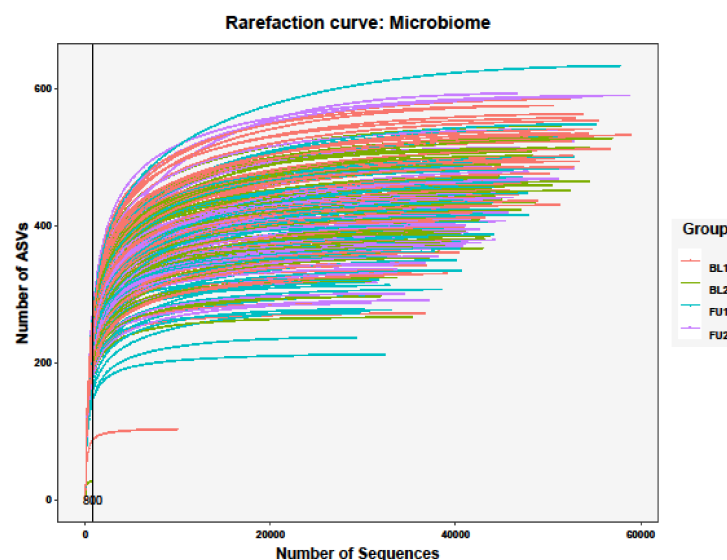
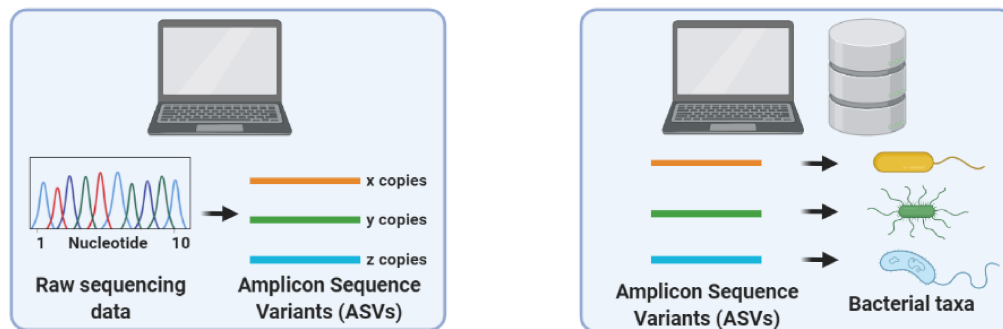
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measurements, including lack of remaining blood and feces material after the preceding analyses.

16S rRNA sequencing.

Sample preparation and sequencing was performed by GENEWIZ (GENEWIZ Germany GmbH, Leipzig) for sequencing. Briefly, following GENEWIZ standardized workflow. For each sample, paired-end reads were joined, low-quality reads were removed, reads were corrected, chimeras removed and Amplicon Sequence Variants (ASVs) were obtained. Taxonomy was annotated to the ASVs using the RDP database 95. The read counts per ASV with taxonomic annotation were normalized and relative abundances of each ASV and taxa were calculated using the R scripts Rhea. Visualization of all library-indexed genera was done as in 96 by inhouse written R-tools using ggplot2. Group statistics (4 groups: intervention, timepoint) consists of paired ANOVA Benjamini-Hochberg adjusted with pairwise post hoc Games-Howell.



SI Figure 1: 16S-rRNA sequencing to ASV extraction.

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Results – Microbial outcome measures after intervention.

SI Table 1: Significant shifts in gut-related variables of interest after prebiotic intervention, according to linear effect modelling.

	intervention*timepoint effects				ANOVA null model comparison
	n _{subj}	n _{obs}	b	t	p
Stool frequency	59	201	1.24*	2.05*	0.04
Bristol Stool Scale	57	196	-0.26	-0.88	0.38
Richness	58	200	-51.63*	-3.23	0.001
Evenness	58	200	-0.0085*	-5.12	<0.001
Linear mixed modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with p < 0.05): with the Formula: variable_of_interest ~ timepoint * intervention + timepoint + intervention + (1 + (timepoint+intervention) subject) + age + gender.					
Shannon Effective	58	200	-34.68*	-4.81	<0.001
Simpson Effective	58	200	-28.63*	-5.34	<0.001
Linear mixed modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with p < 0.05): with the Formula: variable_of_interest ~ timepoint * intervention + timepoint + intervention + (1 + (timepoint+intervention) subject) + age + gender + time_of_day.					

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SI-Table 2: Significant shifts in microbiota relative abundances on the genera level after prebiotic intervention, according to 16S-rRNA sequencing and linear mixed effects modelling.

increased abundance:	Interaction effect time (follow-up) * intervention (prebiotic)		ANOVA null model comparison	
	b	t	p	p _{adj}
Akkermansia	0.20	2.19	0.029	0.16
Anaerostipes	0.73	3.01	0.003	0.017
Bifidobacterium	9.82	10.42	< 0.001	< 0.001
Catenibacterium	0.11	2.19	0.029	0.16
Collinsella	2.66	4.96	< 0.001	< 0.001
Defluviitaleaceace UCG 011	0.02	2.12	0.034	0.19
Epulopiscium	0.01	1.96	0.049	0.27
Hafnia Obseumbacterium	0.03	0.02	0.047	0.26
Holdemanella	0.37	3.13	0.002	0.011
Lachnospiraceae FCS020 group	0.21	3.31	0.001	0.006
Lacticaseibacillus	0.10	2.05	< 0.001	0.002
Lactiplantibacillus	0.03	2.82	< 0.001	< 0.001
Lactobacillus¹	2.08	2.65	0.008	0.045
Libanicoccus	0.20	2.35	0.019	0.11
Ligilactobacillus	0.28	2.67	0.008	0.045
Limosilactobacillus	0.28	5.10	< 0.001	< 0.001
Neisseria	<0.01	2.01	0.043	0.24
Weissella	0.08	2.05	0.041	0.23
decreased abundance:				
Acetanaerobacterium	-0.01	-2.14	0.032	0.18
Actinomyces	-0.11	-2.38	0.018	0.10
Anaerofustis	-0.02	-2.22	0.027	0.15
Anaerotruncus	-0.02	-2.17	0.031	0.17
Bilophila	-0.10	-2.25	0.025	0.14
Blautia	-1.85	-2.14	0.033	0.18
Candidatus Saccharimonas	-0.01	-1.97	0.048	0.27
Catenibacillus	-0.01	-2.08	0.039	0.21

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Clostridium innocuum group	-0.05	-2.06	0.042	0.23
Corynebacterium	-0.01	-2.56	0.011	0.06
Desulfovibrio	-0.20	-3.41	0.001	0.006
Eggerthella	-0.33	-3.46	0.001	0.006
Erysipelatoclostridium	-0.19	-2.20	0.028	0.16
Eubacterium brachy group	-0.11	-3.18	0.002	0.011
Eubacterium eligens group	-0.21	-2.76	0.006	0.033
Eubacterium ventriosum group	-0.18	-2.00	0.046	0.26
Faecalitalea	-0.08	-2.12	0.029	0.16
Family XIII AD3011 group	-0.28	-2.50	0.013	0.07
Family XIII UCG 001	-0.08	-2.32	0.021	0.12
Gemella	-0.02	-2.26	0.025	0.14
Gordonibacter	-0.10	-2.49	0.013	0.07
Holdemania	-0.02	-2.34	0.019	0.11
Incertae Sedis	-0.35	-2.08	0.037	0.21
Lachnospira	-0.16	-1.99	0.047	0.26
Lachnospiraceae NK4A136 group	-0.45	-2.56	0.011	0.06
Levilactobacillus	-0.01	-2.23	0.026	0.15
Natronaerovirga	-0.01	-2.06	0.039	0.22
Roseburia	-1.10	-3.86	< 0.001	0.001
Rothia	-0.01	-1.97	0.049	0.27
Ruminococcus gauvreauii group	-0.69	-3.86	< 0.001	0.001
Ruminococcus torques group	-0.87	-2.63	0.009	0.05
Shuttleworthia	-0.08	-2.78	0.006	0.033
Subdoligranulum	-1.30	-2.82	0.005	0.028
Tyzzereella	-0.23	-2.37	0.019	0.10
UCG 003	-0.16	-3.42	< 0.001	0.004

Linear mixed effects modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with $p < 0.05$): with the Formula: $\text{bacterial_genus_of_interest} \sim \text{timepoint} * \text{intervention} + \text{timepoint} + \text{intervention} + (1 + (\text{intervention} + \text{timepoint}) | \text{subject})$. All models run on $n_{\text{obs}} = 204$ in $n_{\text{subj}} = 58$ and listed in alphabetical order of genera of interest. 1, statistics refer to models without random slopes due to non-convergence.

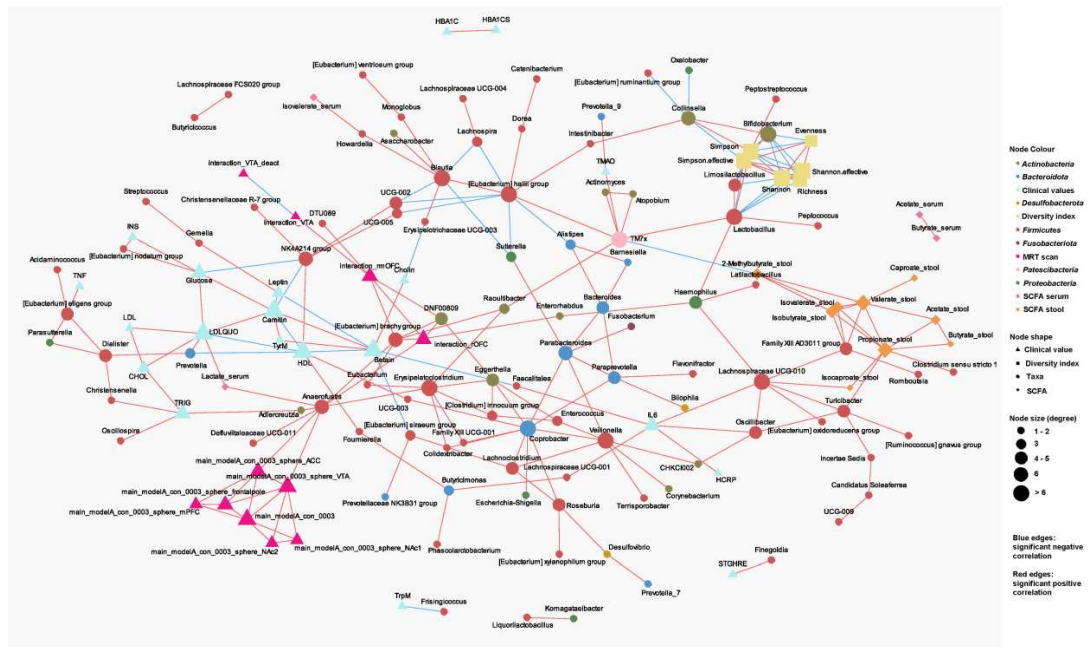
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Exploratory weighted network analysis (WGNCA)

Using weighted network analysis we clustered microbiota genera to modules. In detail, data from participants with complete measures from all four timepoints ($n_{\text{subj}} = 35$) entered these network analyses and 4 out of 13 taxa modules were significantly correlated to prebiotic intervention (M05 $r = 0.51, p < 0.001$; M06 $r = -0.23, p = 0.006$; M08 $r = -0.22, p = 0.007$; M09 $r = -0.20, p = 0.018$). However, none of those 4 clusters correlated with prebiotic-induced changes in brain activation during decision-making. Similarly, neither hubs nor clusters of microbiota abundance differences before compared to after prebiotic intervention, nor hubs nor clusters of the microbiota pattern after prebiotic per se, correlated significantly with brain activation.

Results – Network analysis.



SI Figure 2: Network analysis.

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KEGG analysis

We conducted functional capacity prediction of 16S-rRNA gene profiling data using the Tax4fun R-package and the Kyoto Encyclopedia of Genes and Genomes (KEGG) [2]. This resulted in 8800 KEGG functional orthologues.

SI Table 3: KEGG pathway relative abundance group posthoc pairwise PERMANOVA test (p-adjusted Benjamini Hochberg).

Pairs	F.Model	R2	p.value	p.adjusted	sign.
BL_placebo vs. BL_prebiotics	0.625	0.007	0.517	0.777	ns
BL_placebo vs. FU_placebo	0.105	0.001	0.987	0.987	ns
BL_placebo vs. FU_prebiotics	12.76	0.127	0.001	0.002	**
BL_prebiotics vs. FU_placebo	0.473	0.005	0.655	0.786	ns
BL_prebiotics vs. FU_prebiotics	11.459	0.115	0.001	0.002	**
FU_placebo vs. FU_prebiotics	14.433	0.141	0.001	0.002	**

SI Table 4: Significant shifts in functional pathway capacity after prebiotic intervention, according to KEGG analysis and linear effect modelling.

KEGG pathway	intervention*timepoint effects (prebiotics * follow-up)		ANOVA null model comparison	
	b	t	p	p _{adj}
increased post-prebiotic intervention:				
ABC transporters (ko02010)	0.55	2.77	0.01	0.029
Acarbose and validamycin biosynthesis (ko00525)	0.01	3.12	0.004	0.014
Alanine aspartate and glutamate metabolism (ko00250)	0.12	5.39	<0.001	<0.001
Aminoacyl tRNA biosynthesis (ko00970)	0.10	2.66	0.011	0.03

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Arginine biosynthesis (ko00220)	0.10	4.19	<0.001	0.001
Carbapenem biosynthesis (ko00332)	0.02	4.14	<0.001	0.001
Cyanoamino acid metabolism (ko00460)	0.03	2.43	0.019	0.045
Cysteine and methionine metabolism (ko00270)	0.25	5.39	<0.001	<0.001
D Glutamine and D glutamate metabolism (ko00471)	0.01	2.69	0.008	0.025
DNA replication (ko03030)	0.06	2.61	0.012	0.032
Ferroptosis (ko04216)	0.03	3.79	<0.001	0.002
Galactose metabolism (ko00052)	0.09	2.44	0.02	0.046
Glucosinolate biosynthesis (ko00966)	0.004	2.53	0.022	0.049
Glycine serine and threonine metabolism (ko00260)	0.09	5.63	<0.001	<0.001
Homologous recombination (ko03440)	0.11	3.85	<0.001	0.002
Isoquinoline alkaloid biosynthesis (ko00950)	0.05	5.36	<0.001	<0.001
Lysine biosynthesis (ko00300)	0.05	2.26	0.029	0.062
Mismatch repair (ko03430)	0.06	3.04	0.004	0.015
Nicotinate and nicotinamide metabolism (ko00760)	0.08	6.79	<0.001	<0.001
Nucleotide excision repair (ko03420)	0.08	3.89	<0.001	0.001
Phenylalanine tyrosine and tryptophan biosynthesis (ko00400)	0.10	2.97	0.006	0.019
Phenylpropanoid biosynthesis (ko00940)	0.03	2.66	0.011	0.029
Polyketide sugar unit biosynthesis (ko00523)	0.02	2.07	0.057	0.11
Primary bile acid biosynthesis (ko00120)	0.01	3.35	0.001	0.003
Proteasome (ko03050)	0.01	3.79	<0.001	0.002
Purine metabolism (ko00230)	0.19	4.33	<0.001	0.001
Pyrimidine metabolism (ko00240)	0.12	3.34	0.002	0.008
Quorum sensing (ko02024)	0.19	2.62	0.014	0.035
Ribosome (ko03010)	0.27	3.08	0.003	0.014
RNA degradation (ko03018)	0.04	2.81	0.007	0.022

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RNA polymerase (ko03020)	0.03	3.39	0.001	0.006
Secondary bile acid biosynthesis (ko00121)	0.01	3.09	0.002	0.008
Selenocompound metabolism (ko00450)	0.08	6.61	<0.001	<0.001
Starch and sucrose metabolism (ko00500)	0.18	2.98	0.005	0.017
Taurine and hypotaurine metabolism (ko00430)	0.03	7.04	<0.001	<0.001
Terpenoid backbone biosynthesis (ko00900)	0.04	2.71	0.009	0.027
Thiamine metabolism (ko00730)	0.05	3.21	0.002	0.007
Tropane piperidine and pyridine alkaloid biosynthesis (ko00960)	0.04	4.78	<0.001	<0.001
Valine leucine and isoleucine biosynthesis (ko00290)	0.04	2.86	0.008	0.026
Vitamin B6 metabolism (ko00750)	0.02	5.86	<0.001	<0.001
Zeatin biosynthesis (ko00908)	0.004	2.28	0.029	0.063
decreased post-prebiotic intervention:	b	t	p	p_{adj}
Amino sugar and nucleotide sugar metabolism (ko00520)	-0.07	-2.63	0.011	0.030
Arachidonic acid metabolism (ko00590)	-0.01	-2.90	0.005	0.017
Atrazine degradation (ko00791)	-0.01	-3.33	0.002	0.008
Basal transcription factors (ko03022)	-0.004	-3.41	0.001	0.005
beta Alanine metabolism (ko00410)	-0.04	-2.19	0.039	0.080
Biofilm formation - Pseudomonas aeruginosa (ko02025)	-0.09	-2.83	0.005	0.17
Biosynthesis of siderophore group nonribosomal peptides (ko01053)	-0.04	-2.47	0.019	0.045
Biosynthesis of terpenoids and steroids (ko01062)	0.00	-3.97	< 0.001	< 0.001
Biosynthesis of type II polyketide products (ko01057)	-0.004	-2.84	0.009	0.027
Biosynthesis of unsaturated fatty acids (ko01040)	-0.03	-2.70	0.010	0.028

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Biotin metabolism (ko00780)	-0.08	-3.52	0.001	0.004
Caprolactam degradation (ko00930)	-0.01	-2.53	0.014	0.034
Carotenoid biosynthesis (ko00906)	-0.004	-3.07	< 0.001	0.001
Cell cycle - Caulobacter (ko04112)	-0.06	-2.13	0.033	0.069
Citrate cycle - TCA cycle (ko00020)	-0.05	-4.54	< 0.001	< 0.001
Fatty acid biosynthesis (ko00061)	-0.05	-3.38	0.001	0.007
Flavonoid biosynthesis (ko00941)	0.00	-2.30	0.031	0.064
Fluorobenzoate degradation (ko00364)	-0.01	-2.16	0.041	0.082
Fructose and mannose metabolism (ko00051)	-0.16	-2.47	0.017	0.042
Glycerophospholipid metabolism (ko00564)	-0.02	-2.12	0.048	0.09
Glycolysis - Gluconeogenesis (ko00010)	-0.08	-2.59	0.010	0.28
Indole alkaloid biosynthesis (ko00901)	0.00	-3.09	0.004	0.014
Inositol phosphate metabolism (ko00562)	-0.02	-4.14	< 0.001	0.001
Methane metabolism (ko00680)	-0.06	-4.55	< 0.001	< 0.001
Nitrotoluene degradation (ko00633)	-0.02	-3.14	0.004	0.015
Non homologous end joining (ko03450)	-0.003	-4.30	< 0.001	0.001
Nonribosomal peptide structures (ko01054)	-0.01	-2.37	0.021	0.047
Oxidative phosphorylation (ko00190)	-0.10	-4.95	< 0.001	< 0.001
Phosphonate and phosphinate metabolism (ko00440)	-0.01	-2.28	0.027	0.059
Polycyclic aromatic hydrocarbon degradation (ko00624)	-0.003	-2.73	0.010	0.028
Porphyrin and chlorophyll metabolism (ko00860)	-0.16	-7.03	< 0.001	< 0.001
Pyruvate metabolism (ko00620)	-0.10	-4.58	< 0.001	< 0.001
RNA transport (ko03013)	-0.02	-6.00	< 0.001	< 0.001
Steroid degradation (ko00984)	-0.01	-4.06	< 0.001	0.001
Stilbenoid, diarylheptanoid and gingerol biosynthesis (ko00945)	0.00	-2.30	0.031	0.064
Styrene degradation (ko00643)	-0.01	-2.94	0.006	0.019
Sulfur metabolism (ko00920)	-0.08	-2.44	0.024	0.054

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Sulfur relay system (ko04122)	-0.04	-3.96	< 0.001	0.001
Tryptophan metabolism (ko00380)	-0.04	-2.15	0.043	0.085
Two component system (ko02020)	-1.01	-5.55	< 0.001	< 0.001
Xylene degradation (ko00622)	-0.02	-3.04	0.005	0.017

Linear mixed modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with $p < 0.05$ (uncorrected) and $p_{adj} < 0.05$ (FDR corrected, marked in bold)): with the Formula: `pathway_of_interest ~ timepoint * intervention + timepoint + intervention + (1 + (timepoint+intervention) | subject)`. All models on $n = 205$ observations in $n = 58$ individuals and listed in alphabetical order of genera of interest.

SI Figure 2: Heatmap of bivariate correlations between significant changes in reward-related brain activation and changes in microbial markers after prebiotics. A: brain activation, blood markers and microbiota genera. **B:** brain activation, blood markers and predicted microbial functional pathways. Color according to Spearman's r , red, positive correlations, blue, negative correlations. Written R values relate to corresponding p -values of $p < 0.05$. VTA, ventral tegmental are, OFC, orbitofrontal cortex, r, right, m, middle.

----- see supplementary files *SI_Figure2A/B.tiff*-----

References.

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- 2 Ogata H, Goto S, Fujibuchi W, *et al.* Computation with the KEGG pathway database. *BioSystems* 1998;**47**:119–28. doi:10.1016/S0303-2647(98)00017-3