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Supplemental Information

Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production

Edward C. Deehan, Chen Yang, Maria Elisa Perez-Muñoz, Nguyen K. Nguyen, Christopher C. Cheng, Lucila Triador, Zhengxiao Zhang, Jeffrey A. Bakal, and Jens Walter

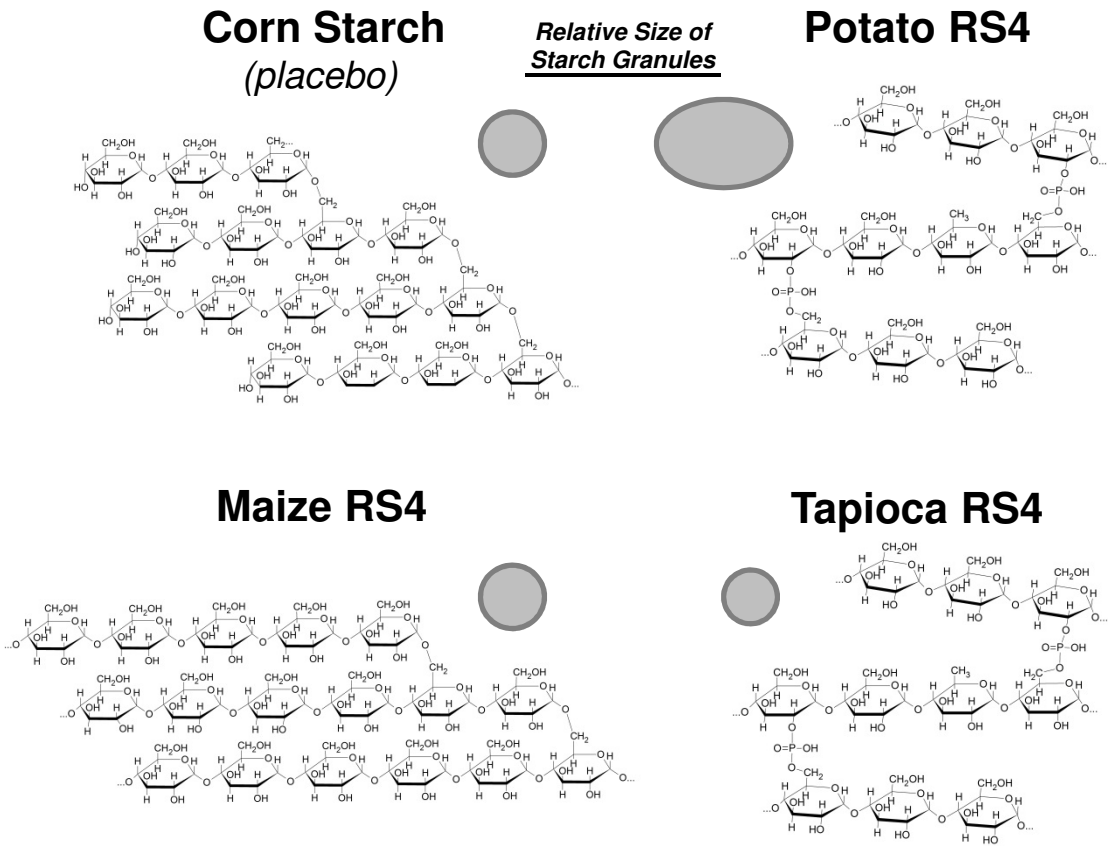

Figure S1: Schematic diagrams of the chemical structure and relative granule size of each supplement. Related to STAR Methods.

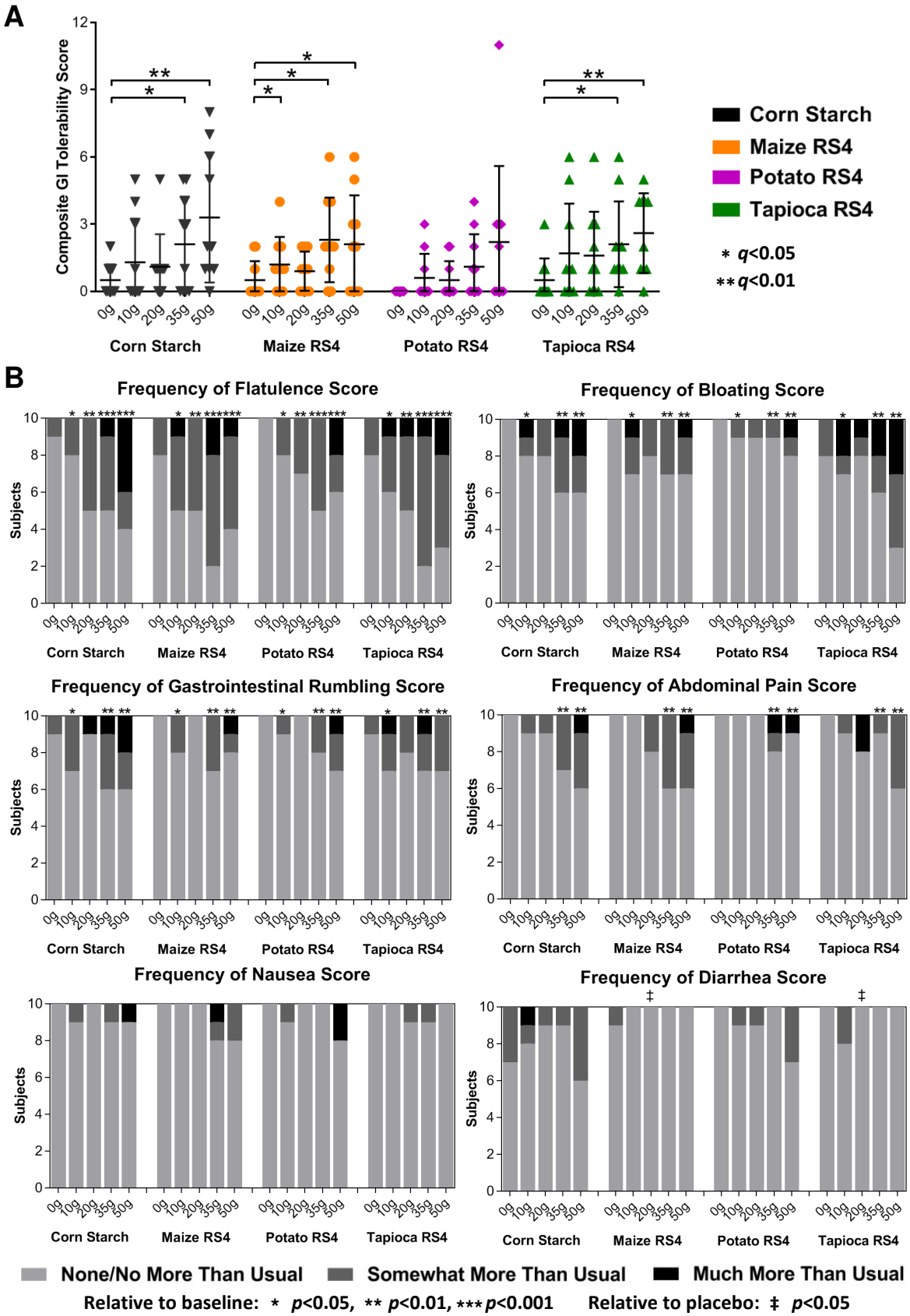

Figure S2: Composite GI tolerability scores and frequency of GI symptoms were affected by RS4 and placebo consumption, with high individual variability. Related to Figure 1.

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(A) Composite GI tolerability score data were analysed by generalized estimating equation models (with FDR correction) to assess differences within- and between-groups. All treatments, apart from Potato RS4, significantly increased composite scores relative to baseline, with no differences detected relative to placebo (Corn Starch). Symbols represent individual tolerance reports; lines represent mean ± SD. **(B)** Frequency of individual GI symptoms were analysed by cumulative link models relative to baseline (considering all 4 treatment groups) and to placebo (considering all 5 time-points).

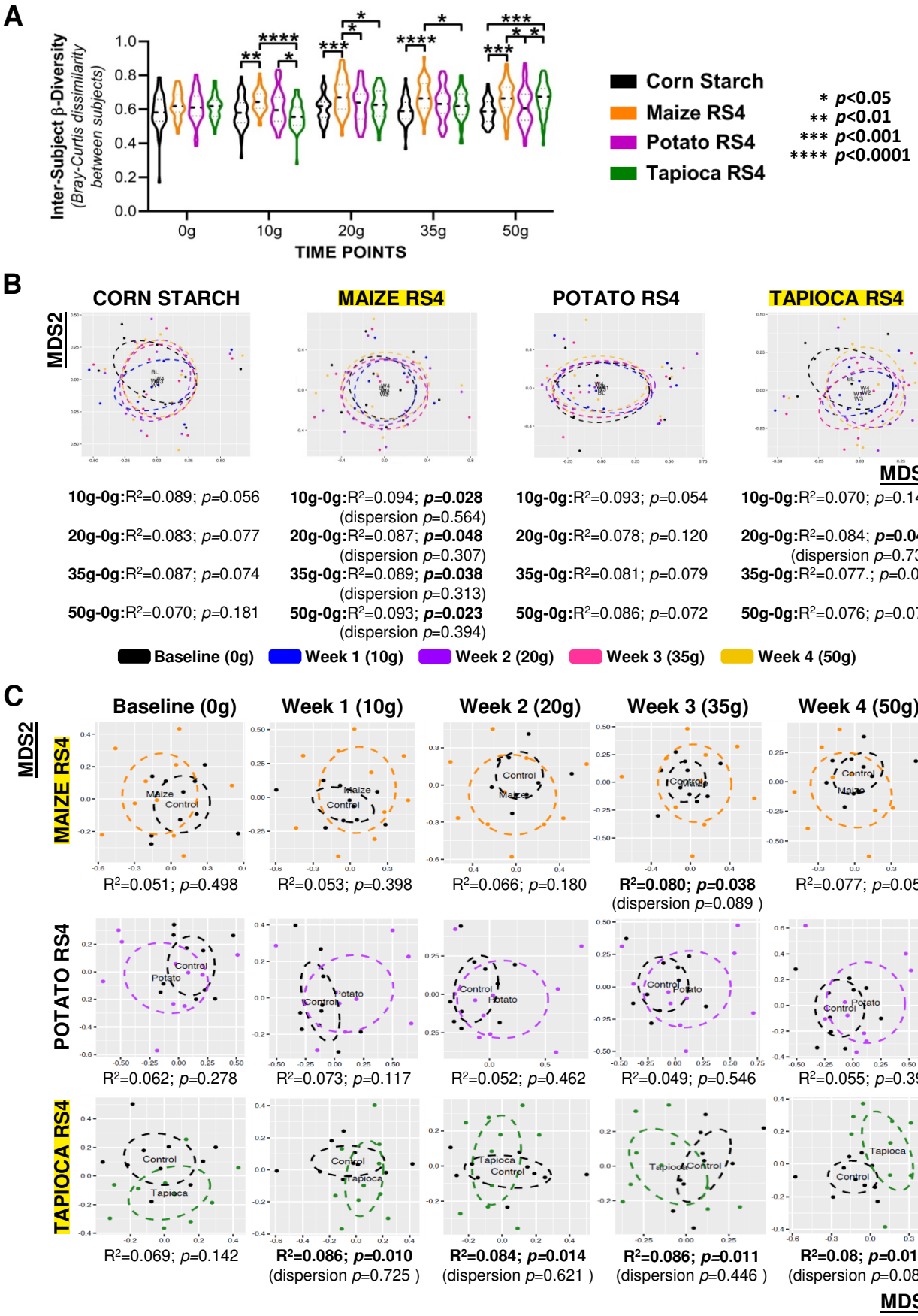

Figure S3: Effects of different RS4s and placebo on fecal bacterial β-diversity. Related to Figure 2. *Legend continued on next page.*

(A) Violin plots of Bray Curtis distances comparing between-treatment groups the fecal microbiomes of subjects at each dose/time-point (inter-subject β-diversity). Non-metric multidimensional scaling (NMDS) plots based on Bray Curtis distance metrics of RS4s and placebo at each dose (*i.e.* 0 g/d to 50 g/d) showing changes in the distance between subjects as DF dose increased for **(B)** each treatment relative to their baseline and **(C)** between RS4 treatments relative to placebo. Data analysed for **(A)** using 2-way rANOVA (with Holm-Šídák correction) and for **(B-C)** using PERMANOVA and PERMDISP.

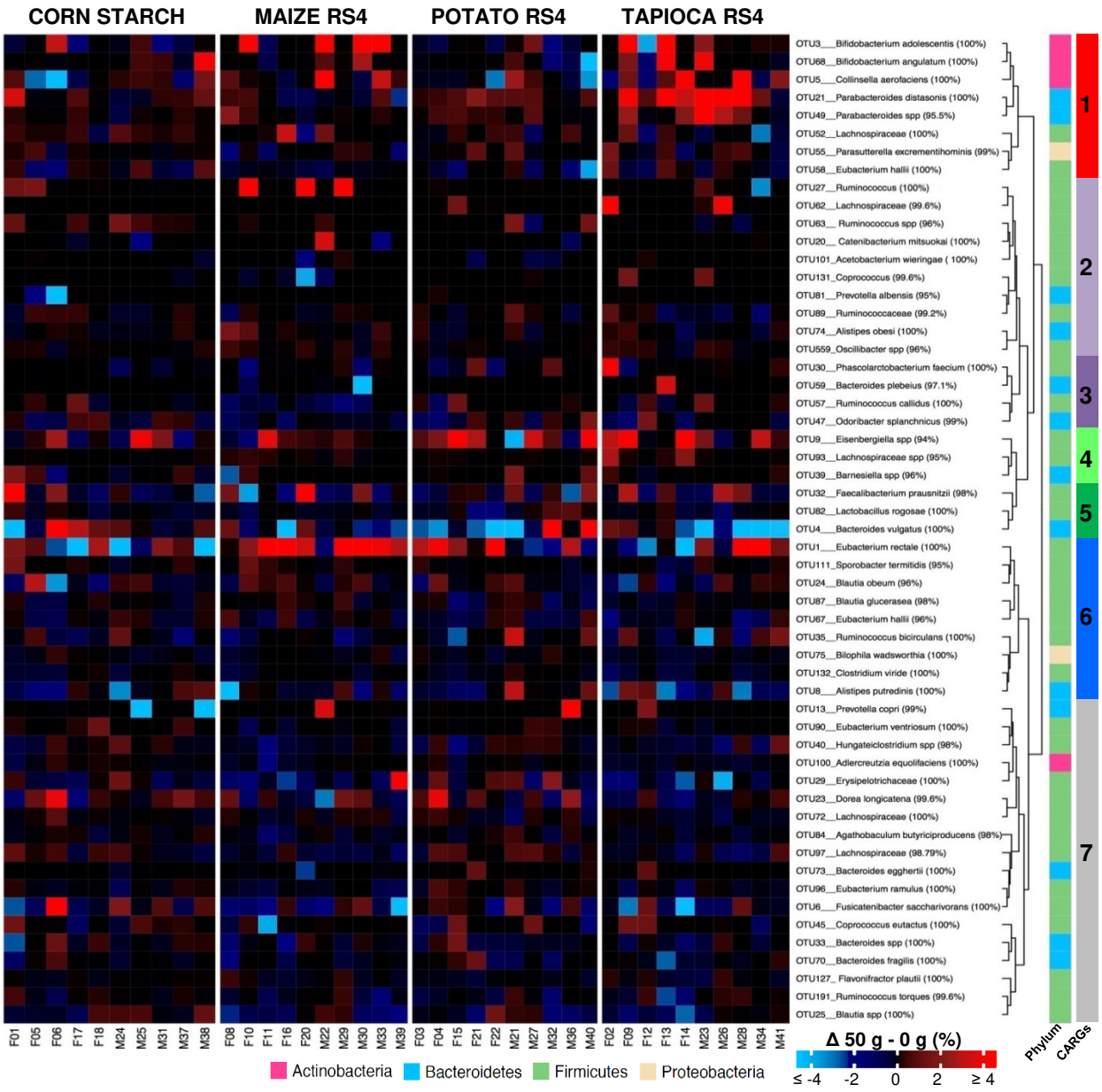

Figure S4: Identification of co-abundance response groups (CARGs). Related to Figure 3. Heatmap of shifts from baseline to week 4 (0 to 50 g/d) in relative abundance of 55 operational taxonomic units (OTUs) affected by RS4 treatment (dose/interaction effect unadjusted $p<0.20$, 2-way rANOVA). OTUs were clustered by Ward cluster algorithm based on Spearman's correlation distances that considered shifts across the 3 treatment arms (without placebo). Then OTUs were grouped on the Spearman's Hierarchical tree into 7 CARGs by PERMANOVA ($p\leq0.1$; CARGs 1-5) and visual inspection of the tree (CARGs 6-7).

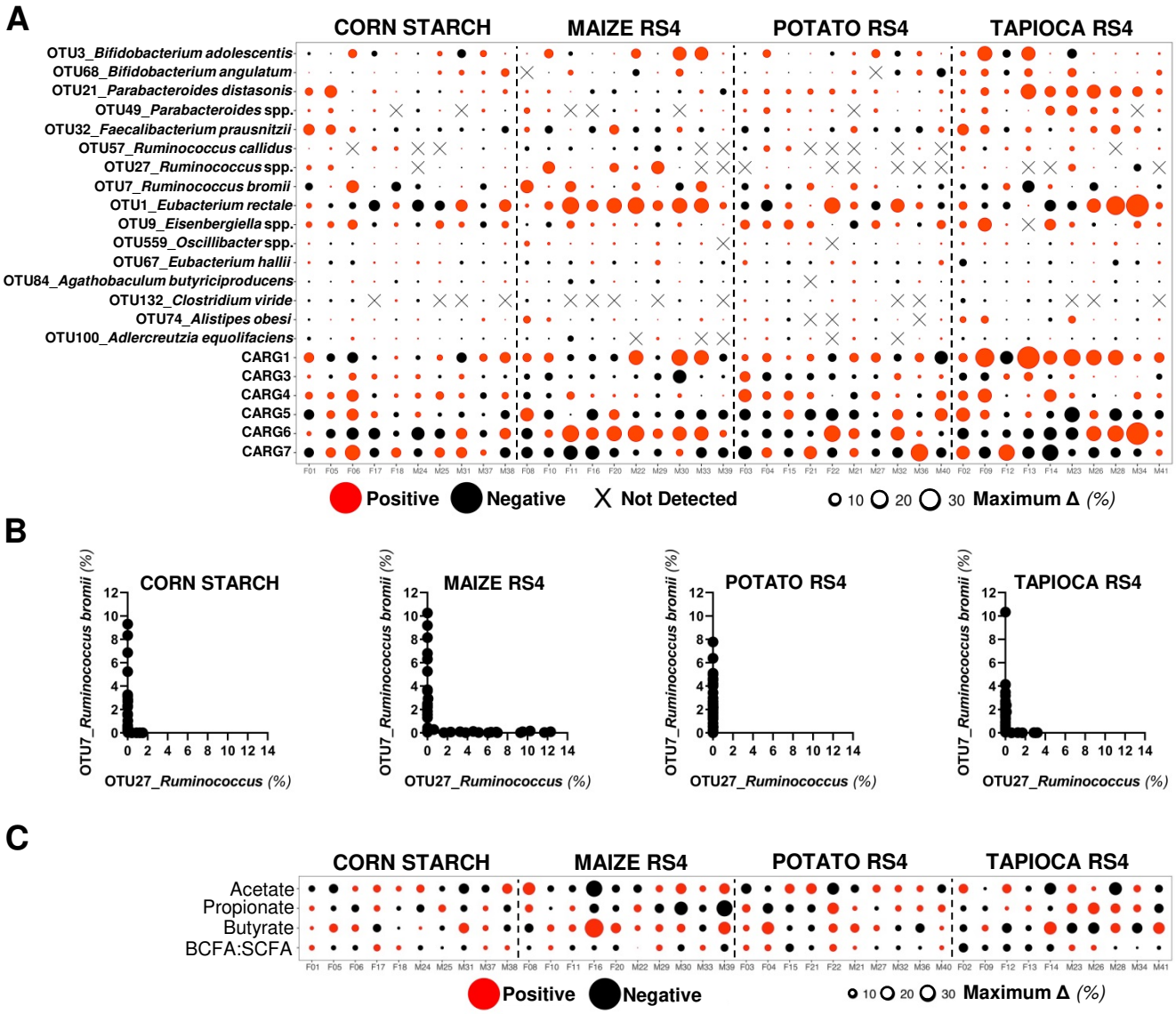

Figure S5: Individual differences in the magnitude of OTU, CARG, and SCFA response to RS4s and placebo. Related to Figures 3 and 4.

(A) Bubble plots showing maximum differences in relative abundance of OTUs and CARGs (percentage of total microbiota composition) detected during Maize and Tapioca RS4 treatment relative to baseline. **(B)** Scatter plots showing the association between the relative abundance of OTU7 (*Ruminococcus bromii*) and OTU27 (*Ruminococcus* spp.; also classified as *Anaeromassilibacillus* spp.) during the study (*i.e.* 0g to 50g), which suggests a potential co-exclusion relationship between these OTUs in response to Maize RS4. **(C)** Bubble plots showing maximum differences in relative proportion of acetate, propionate, butyrate (percentage of total SCFAs) and in the ratio of BCFA:SCFA during RS4 treatment relative to baseline. For (A,C), the red circles represent a mean positive change in relative abundance during the intervention; black circles represent a mean negative change. The circle size is proportional to the magnitude of the largest difference relative to baseline (dependent on direction of mean change). BCFA:SCFA, total branched short-chain to short-chain fatty acid ratio; CARG, co-abundance response group; OTU, operational taxonomic unit.

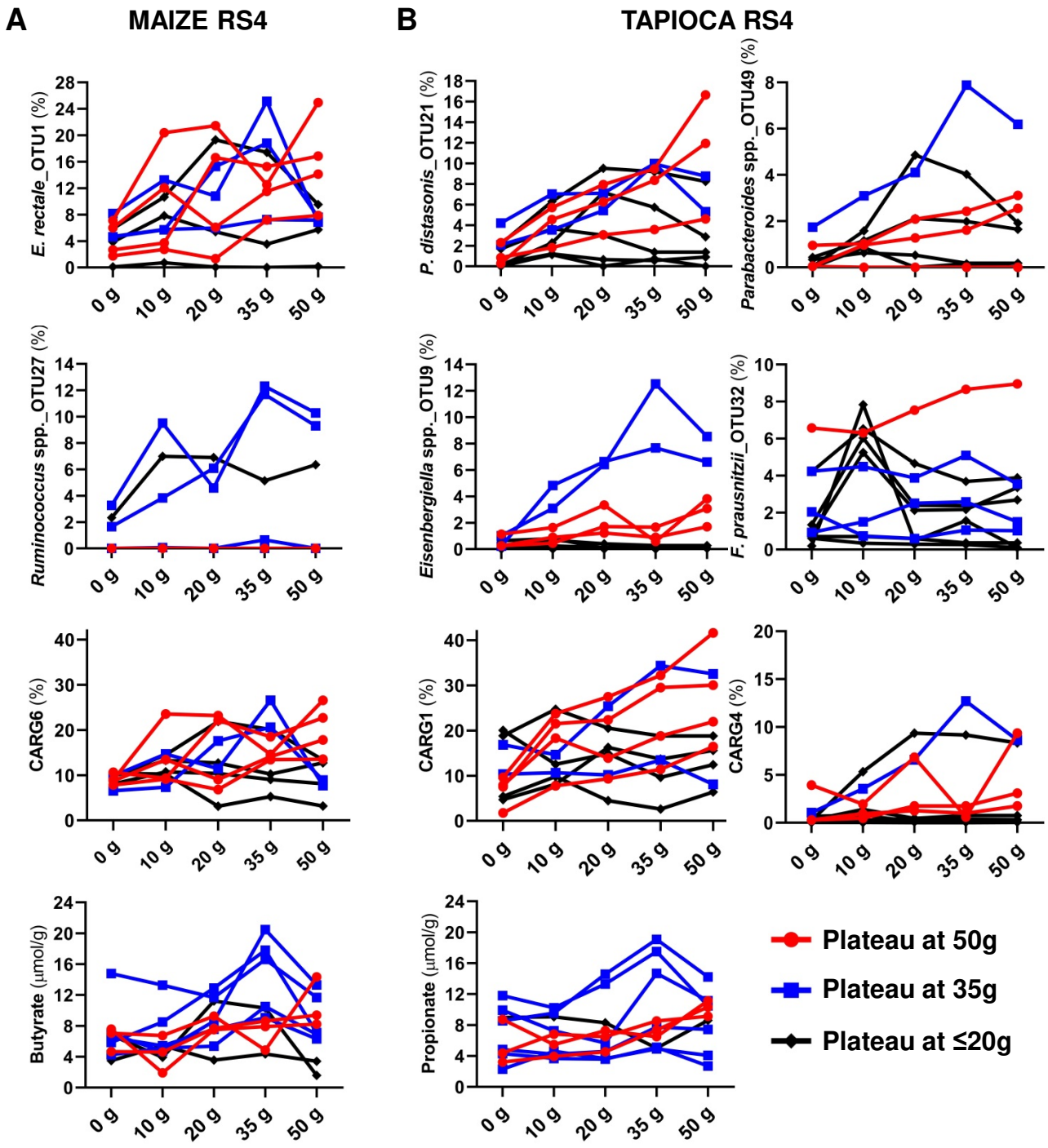

Figure S6: Individual dose-dependent effects of Maize and Tapioca RS4 on fecal bacterial composition and function. Related to Figure 5.

Individual dose-response curves of each operational taxonomic unit (OTU; mean change >0.75% relative abundance), co-abundance response group (CARG), and short-chain fatty acid that showed an increase (in either relative abundance or concentration) during **(A)** Maize RS4 or **(B)** Tapioca RS4 treatment. Red lines represent a maximum increase in relative abundance or concentration detected at 50 g/d, blue lines represent a maximum increase detected at 35 g/d, and black lines represent a maximum increase detected at a dose ≤20 g/d.

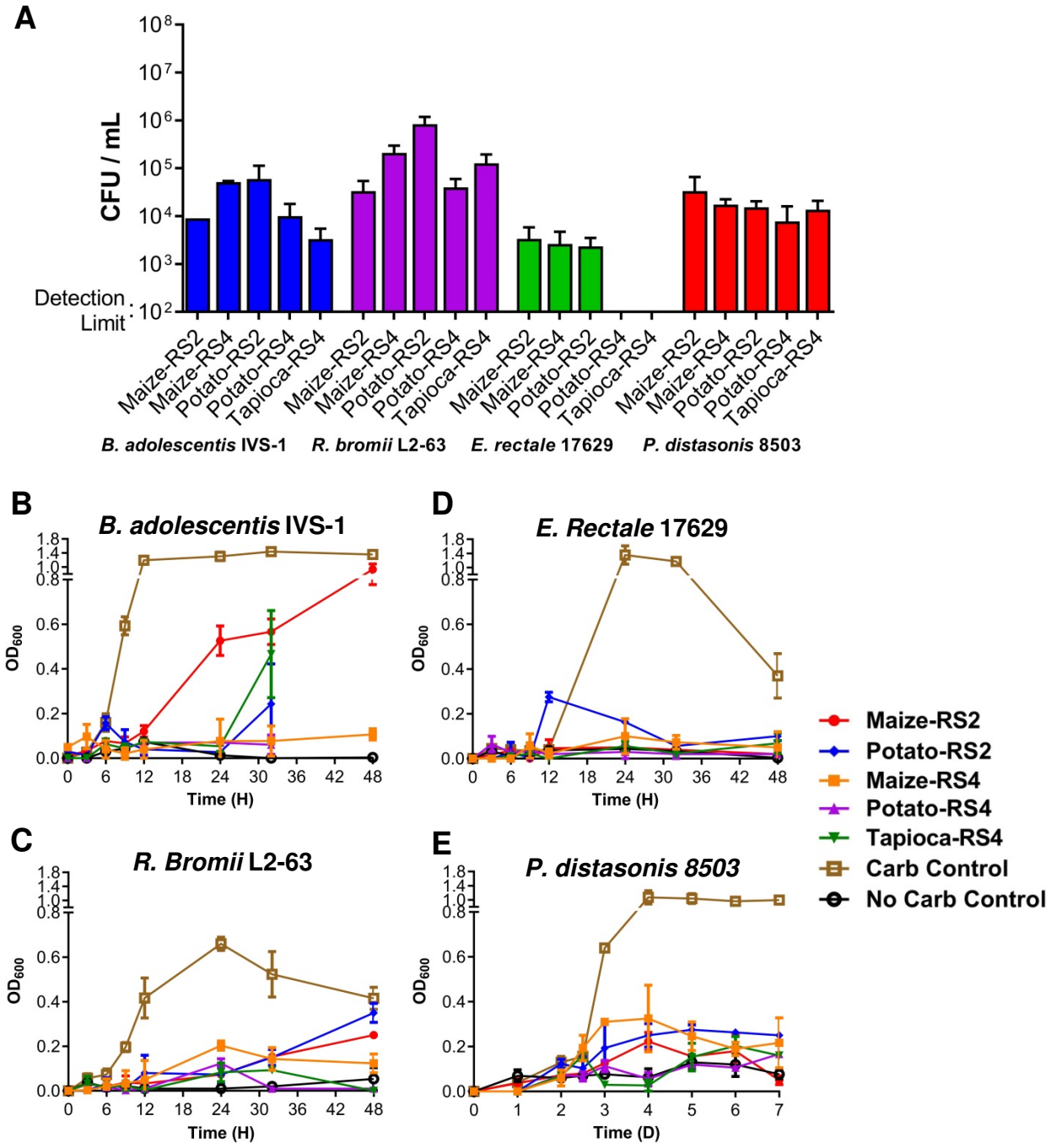

Figure S7: Assessing the adherence and utilization of human-gut-derived amylolytic bacteria on type-II and type-IV RSs. Related to Figure 7.

(A) Total CFUs (CFU/ml) of *Bifidobacterium adolescentis* IVS-1, *Ruminococcus bromii* L2-63, *Eubacterium rectale* DSM 17629, and *Parabacteroides distasonis* ATCC 8503 recovered from RS4s and RS2s after *in vitro* binding assay with the respective RS. Growth curves (OD_{600}) of **(B)** *B. adolescentis* IVS-1, **(C)** *R. bromii* L2-63, **(D)** *E. rectale* DSM 17629, and **(E)** *P. distasonis* ATCC 8503 in YCFA medium containing either 0.2% of the indicated RS, a carbohydrate mixture (positive control), or no carbohydrate (negative control).

Table S1. Supplement specifications. Related to STAR Methods.

Treatment Name	Product Name	Chemical Processing	Chemical Modifications	Total Fiber [a] *(dwb/as-is - %)*
Corn Starch *(placebo)*	AMIOCA™ TF	None; native high amylopectin corn starch	None	0.0/0.0
Maize RS4	VERSAFIBE™ 2470	Acid hydrolysis & annealing of native high-amylose maize starch	Removed digestible material & reorganized starch granule structure	65.0/58.2
Potato RS4	VERSAFIBE™ 1490	Phosphorylation of native potato starch with phosphorus oxychloride	Cross-linked the surface of the starch granule	90.0/78.7
Tapioca RS4	VERSAFIBE™ 3490	Phosphorylation of native tapioca starch with phosphorus oxychloride	Cross-linked the surface of the starch granule	96.0/85.3

[a] Total dietary fiber content was determined by AOAC 2009.01. as-is, adjusted for moisture content; dwb, dry weight basis.

Table S2. Subject characteristics at baseline. Related to Figure 1.

Characteristic	Treatments				Between Group
	Corn Starch	Maize RS4	Potato RS4	Tapioca RS4	*p* value [a]
Population (*n*)	10	10	10	10	
Gender (M/F)	5/5	5/5	5/5	5/5	
Age (y) [b]	27 ± 7.8	25 ± 8.3	31 ± 8.4	29 ± 7.7	0.44
Height (cm)	173.5 ± 10.1	170.5 ± 9.3	169.5 ± 9.0	173.2 ± 10.0	0.74
Weight (kg) [b]	72.3 ± 10.4	66.7 ± 12.7	71.6 ± 14.0	73.4 ± 18.2	0.71
BMI (kg/m^2) [b]	24.1 ± 3.7	22.8 ± 2.1	24.7 ± 2.8	24.3 ± 3.9	0.56
Perceived stress score	4.2 ± 3.1	4.1 ± 1.8	4.7 ± 3.2	5.4 ± 2.2	0.68
MET score [b]	3741 ± 2395	2794 ± 1568	2326 ± 2402	2507 ± 1837	0.29
Ethnicity					0.13
Caucasian	8 (80%)	3 (30%)	4 (40%)	5 (50%)	
Asian	1 (10%)	7 (70%)	5 (50%)	4 (40%)	
Other	1 (10%)	0 (0%)	1 (10%)	1 (10%)	
Education level					0.96
High school diploma	2 (20%)	4 (40%)	2 (20%)	3 (30%)	
Bachelor's degree	5 (50%)	3 (30%)	4 (40%)	3 (30%)	
Graduate degree	2 (20%)	1 (10%)	3 (30%)	3 (30%)	
Other	1 (10%)	2 (20%)	1 (10%)	1 (10%)	
Employment status					0.31
Student	7 (70%)	6 (60%)	5 (50%)	3 (30%)	
Employed	3 (30%)	3 (30%)	5 (50%)	7 (70%)	
Unemployed	0 (0%)	1 (10%)	0 (0%)	0 (0%)	
Household income					0.23
Less than $40,000	5 (50%)	3 (30%)	2 (20%)	3 (30%)	
$40,000 -- $69,000	0 (0%)	5 (50%)	3 (30%)	2 (20%)	
$70,000 -- $99,000	2 (2%)	2 (20%)	1 (10%)	2 (20%)	
$100,000 or more	3 (30%)	0 (0%)	4 (40%)	3 (30%)	

[a] Data were analyzed using either one-way ANOVA (continuous variable) or Fisher's exact test (count variable). No significant differences between the intervention arms at baseline ($p>0.05$). Data reported as mean ± SD or count (%). BMI, body mass index; MET, metabolic equivalent. [b] Square root transformed prior to statistical analysis with one-way ANOVA.

Table S3. Changes in anthropometric and lifestyle characteristics after the 4-week intervention. Related to Figure 1.

Δ Characteristic (W4 - BL)	Treatments				Treatment *p* value [a]	Time *p* value	Interaction *p* value
	Corn Starch	Maize RS4	Potato RS4	Tapioca RS4			
Weight (kg)	0.0 ± 1.1	0.9 ± 1.1	-0.3 ± 1.5	0.4 ± 1.1	0.753	0.195	0.174
BMI (kg/m^2)	0.0 ± 0.4	0.3 ± 0.4	-0.1 ± 0.5	0.1 ± 0.4	0.620	0.163	0.224
Perceived stress	1.4 ± 2.5	-0.2 ± 2.1	-0.3 ± 2.2	0.4 ± 1.6	0.624	0.337	0.269
MET score	105 ± 1162	-230 ± 1326	-852 ± 1268	-423 ± 594	0.168	0.057	0.303
Dietary Intake							
Energy (kcal)	-107 ± 569	348 ± 897	-103 ± 518	234 ± 391	0.652	0.350	0.258
Carbohydrate (g)	4.1 ± 57.5	18.7 ± 102.4	-14.0 ± 49.1	16.7 ± 68.2	0.914	0.582	0.730
Total sugar (g)	4.1 ± 41.3	-6.6 ± 57.2	-12.4 ± 47.8	16.7 ± 35.9	0.535	0.950	0.521
Total fiber [b] (g)	1.2 ± 6.8	-0.2 ± 6.9	-0.2 ± 9.0	-2.2 ± 9.1	0.521	0.856	0.939
Protein (g)	-13.5 ± 41.0	27.6 ± 49.9	-17.1 ± 26.2	20.1 ± 33.3	0.212	0.488	**0.025**
Total fat (g)	-2.0 ± 45.4	17.4 ± 46.9	3.0 ± 36.0	4.5 ± 28.0	0.382	0.369	0.733
SFA (g)	-1.5 ± 15.6	3.9 ± 16.9	2.1 ± 18.1	2.7 ± 12.0	0.196	0.476	0.886
USFA (g)	0.0 ± 27.6	12.8 ± 28.5	0.2 ± 16.5	0.9 ± 15.5	0.618	0.341	0.537
Cholesterol (g)	-53.0 ± 230.3	155.2 ± 319.4	-19.4 ± 202.3	55.2 ± 238.8	0.115	0.391	0.275

[a] Data were analyzed using two-way rANOVA (with Holm-Šídák correction). No significant changes within- or between-treatment groups after correction for multiple comparisons. Data reported as mean change from baseline ± SD. BMI, body mass index; MET, metabolic equivalent, SFA, saturated fatty acids; USFA, unsaturated fatty acids. [b] Total dietary fiber provided by the diet without the added fiber supplement.

Table S4. Effect of RS4 treatments and placebo on bowel movement habits. Related to Figure 1.

Treatments	Study Weeks					Treatment Effect *p* value [a]
	Baseline (0g)	Week 1 (10g)	Week 2 (20g)	Week 3 (35g)	Week 4 (50g)	
Frequency (stools/day)						
Corn Starch	1.5 ± 0.7	1.7 ± 0.7	1.9 ± 1.4	1.7 ± 0.6	1.6 ± 0.7	–
Maize RS4	1.1 ± 0.6	1.2 ± 0.6	1.4 ± 0.8	1.1 ± 0.7	1.3 ± 0.7	0.077
Potato RS4	1.6 ± 0.6	1.8 ± 0.6	1.3 ± 0.3	1.8 ± 1.1	**1.9 ± 0.7***	1.000
Tapioca RS4	1.2 ± 1.0	1.3 ± 0.9	1.6 ± 1.0	1.3 ± 0.9	1.6 ± 0.9	0.416
Bristol stool scale [b]						
Corn Starch	4.1 ± 1.0	4.1 ± 0.7	4.2 ± 1.1	3.4 ± 1.1	4.1 ± 1.0	–
Maize RS4	4.0 ± 0.8	4.0 ± 1.0	3.8 ± 0.8	3.7 ± 0.9	3.5 ± 0.8	0.520
Potato RS4	3.8 ± 1.1	3.8 ± 1.0	3.7 ± 0.9	3.9 ± 0.8	4.1 ± 1.1	0.690
Tapioca RS4	3.8 ± 1.1	4.1 ± 1.0	3.9 ± 0.6	4.2 ± 0.7	3.8 ± 0.9	0.930
Fecal hardness [c]						
Corn Starch	1.8 ± 0.6	1.6 ± 0.5	1.6 ± 0.6	2.0 ± 0.8	1.7 ± 0.6	–
Maize RS4	1.6 ± 0.5	1.8 ± 0.6	1.6 ± 0.4	1.9 ± 0.6	1.6 ± 0.6	0.679
Potato RS4	2.1 ± 0.5	1.8 ± 0.4	1.9 ± 0.6	**1.8 ± 0.4***	**1.6 ± 0.5***	0.574
Tapioca RS4	2.0 ± 0.7	1.7 ± 0.5	1.7 ± 0.4	1.6 ± 0.5	1.7 ± 0.5	0.965
Straining [d]						
Corn Starch	1.3 ± 0.4	1.6 ± 0.5	1.4 ± 0.4	1.8 ± 0.8	1.5 ± 0.3	–
Maize RS4	1.6 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	1.6 ± 0.4	1.8 ± 0.7	0.682
Potato RS4	1.7 ± 0.6	1.6 ± 0.4	1.6 ± 0.7	1.6 ± 0.5	1.4 ± 0.5	0.713
Tapioca RS4	1.8 ± 0.8	1.4 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	1.5 ± 0.5	0.920
Discomfort [d]						
Corn Starch	1.4 ± 0.4	1.7 ± 0.8	1.2 ± 0.4	1.7 ± 0.7	1.7 ± 0.6	–
Maize RS4	1.3 ± 0.5	1.4 ± 0.7	1.3 ± 0.4	1.3 ± 0.4	1.6 ± 0.6	0.419
Potato RS4	1.3 ± 0.5	1.3 ± 0.4	1.4 ± 0.5	1.2 ± 0.4	1.2 ± 0.5	0.183
Tapioca RS4	1.2 ± 0.5	1.2 ± 0.3	1.2 ± 0.5	1.1 ± 0.2	1.2 ± 0.5	**0.023**
Incomplete evacuation [d]						
Corn Starch	1.5 ± 0.5	1.7 ± 0.8	1.5 ± 0.6	1.6 ± 0.6	1.3 ± 0.4	–
Maize RS4	1.4 ± 0.7	1.3 ± 0.6	1.1 ± 0.1	1.2 ± 0.2	1.6 ± 0.9	0.330
Potato RS4	1.5 ± 0.6	1.3 ± 0.4	1.6 ± 0.6	1.5 ± 0.5	1.6 ± 0.5	0.880
Tapioca RS4	1.6 ± 0.7	1.2 ± 0.4	1.6 ± 0.8	1.3 ± 0.4	1.3 ± 0.5	0.400

[a] Data were analyzed by generalized estimating equation models to assess the effect of treatment and dose, with FDR corrected pair-wise comparisons within each treatment to assess change from baseline, *$p<0.05$. Data reported as mean ± SD. [b] Rated on a scale of 1 (hard) to 7 (liquid); [c] Rated on a scale of 1 (soft) to 4 (very hard); [d] Rated on a scale of 1 (none) to 4 (severe).