

1 Title: Cohort Specific Effects Fiber Supplementation in Overweight Patients With  
2 or Without Type 2 Diabetes Mellitus

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4 Short Title: Cohort specific effects of diet supplementation

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25

## 26 **Abstract**

27           The importance of gut microbes to metabolic health is becoming more  
28 evident and nutrition-based therapies to alter the composition of bacterial  
29 communities to manage metabolic disease are an attractive avenue to ameliorate  
30 some effects of Western diets. While the composition of gut microbial  
31 communities can vary significantly across disease states, it is not well known if  
32 these communities have common responses to nutritional interventions. To  
33 better understand fiber-bacterial community interactions, we collected  
34 biological parameters and fecal samples of overweight non-diabetic (OND) and  
35 diabetic (OD) individuals before and after daily supplementation of 2.8 g  $\beta$ -  
36 glucan on their habitual diet for 30 days. Fecal bacterial communities in an age-  
37 matched cohort were measured by sequencing partial 16S rRNA genes and  
38 imputed metagenomic content. Unexpectedly, we observed disconnected  
39 responses of biological measurements and the bacterial community. Based on  
40 average effect size, biological measurements were greater in the OND group  
41 while effects on the bacterial community were greatest on the OD cohort, and we  
42 suspect these observations are due to the significantly lower alpha diversity in  
43 the OD cohort. Our data indicate that responses to fiber supplementation are  
44 cohort specific and this should be considered when manipulating the  
45 microbiome via fiber supplementation.

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47

## 48 **Introduction**

49

50           As a result of a globalization of the economy, obesity and diabetes are  
51 becoming major issues not just for the developed but the developing world. The  
52 global adoption of a Western lifestyle includes dietary modification to diets low  
53 in fiber and high energy density. Deaths accounted for by non-communicable  
54 diseases have now overtaken deaths due to communicable diseases virtually  
55 world wide [1]. This statistic highlights the dilemma for providing high quality  
56 fiber rich foods and avoiding over nutrition for both the developed and  
57 developing world.

58           The gut bacterial community serves as one conduit by which the  
59 consequences of nutrition and dietary choices integrate into human health, and  
60 therefore must be considered in the context of any nutritional intervention.  
61 Recent work shows abundances of dominant taxa in the distal gut vary widely  
62 across individuals and habitual food choices [2-6]. As Firmicutes and  
63 Bacteroidetes are by far the most prevalent taxa in the human gastro-intestinal  
64 tract [7], their presence and response to diet can have a significant impact on  
65 human health. For instance, members of the *Fecalbacterium* (a Firmicute)  
66 produce short-chain fatty acids such as butyrate [8] that serve as an energy  
67 source for colonic epithelia. *Bacteroides* and *Ruminococcaceae* (both  
68 Bacteroidetes) degrade polysaccharides found in foods containing fiber [9, 10].  
69 Conversely, abundant taxa such as the Proteobacteria are typically associated  
70 with dysbiosis observed in persons with inflammatory bowel disorders [11, 12]  
71 or in the guts of those consuming a high fat or typical Western style diet [2].  
72 Despite the inter- and intra-individual variability in the abundance of bacterial  
73 taxa, these and other studies demonstrate the importance of the bacterial  
74 communities to integrate diet and host metabolism. Dietary supplements aiming

75 to leverage the benefits of microbial function therefore may become practical  
76 solutions to Western lifestyle associated diseases if their efficacy and the  
77 molecular mechanisms underlying the bacteria-diet interactions could be  
78 demonstrated.

79         Fiber supplementation in the diet is one approach proposed to minimize  
80 the effects of Western life styles by potentially altering the composition and  
81 metabolism of the gut bacterial community [13, 14]. However it is not widely  
82 known if fiber has the same effect on microbiomes in subjects of related but  
83 distinct disease states. For instance, there are numerous studies that document  
84 fiber induced alterations in the microbiomes of monotonic cohorts [15-19].  
85 Hooda et al. [20] demonstrated the significant effect of corn derived fibers on  
86 abundances of genera of belonging to Firmicutes, Bacteroidetes and  
87 Verrucomicrobia in generally healthy subjects, while Benus et al. [21] further  
88 demonstrated that pea fiber and fructo-oligosaccharides supplementation was  
89 associated with increased abundances of butyrate producing bacteria in healthy  
90 subjects. Though these studies have given us great insight into the effect of fiber  
91 on the microbiome, each used unique diet amendments in cohorts that consisted  
92 almost exclusively of healthy, overweight, obese, or subjects with diagnosed  
93 metabolic disease. Given that bacterial community composition and diversity  
94 vary significantly across disease states, it is reasonable to assume that effects in  
95 one cohort may not be applicable to others. This lack of knowledge may be  
96 important when identifying patients whose microbiomes may be more or less  
97 responsive to fiber supplementation and thus resistant to improvement of host  
98 metabolic markers potentially mediated by gut bacteria. Thus quantifying the

99 responsiveness of the microbiome in similar but distinctive cohorts may help to  
100 define the limits of fiber supplementation via changes in the microbiome.  
101 Overweight diabetic (OD) and overweight non-diabetics (OND) represent  
102 two metabolic states that can be distinguished by the degree of insulin  
103 resistance. However, it is not known whether the two populations exhibit cohort  
104 specific effects of fiber supplementation on the bacterial communities and host  
105 metabolism. To answer this question, we conducted a fiber supplementation  
106 study in OD and OND subjects with a cereal bar containing 1.4 g  $\beta$ -glucan. We  
107 investigated how the bacterial communities responded to consumption of two  
108 cereal bars per day and determined whether there were unique features of the  
109 response in OND vs OD subjects. Specifically we predicted that fiber  
110 supplementation would increase abundances of Bacteroidetes and  
111 Verrucomicrobia while decreasing Firmicutes and Proteobacteria in both  
112 cohorts with concomitant improvement in host metabolic markers.

113

## 114 **Results**

### 115 **Biological Parameters**

116 We recruited 46 overweight and obese individuals with and without  
117 diabetes to assess the effect of a  $\beta$ -glucan containing cereal bar supplementation  
118 on biological parameters and the distal gut bacterial community. The subject  
119 characteristics are summarized in S1 Table. Subjects went through a diet  
120 normalization phase followed by consumption of 2 cereal bars per day for 30  
121 days to deliver 2.8g  $\beta$ -glucan fiber to the habitual diet. Twenty-six individuals  
122 were excluded for compliance issues, lack of sequencing success, or fell outside

123 the age-range, leaving 10 individuals in each group for analysis. Non-parametric  
 124 testing indicated no significant difference in any biological measurement  
 125 between the cohorts at the pre-supplementation time point. However, LDL and  
 126 triglyceride concentrations were statistically different between the OND and OD  
 127 post supplementation (Table 1, S2 Table), though these differences were not  
 128 significant improvements from their pre-supplementation values. The remaining  
 129 biological parameters were unaffected by consumption of the cereal bar. Lastly,  
 130 Kruskal-Wallis tests showed there were no differences within each cohort  
 131 between pre- and post-supplementation time points (Table1).

132  
 133 **Table 1. Biological parameters of age-matched cohorts at enrollment, pre-**  
 134 **supplementation, and post-supplementation.** The mean and standard  
 135 deviation (in parentheses) are shown. Except for \*Age and \*Height, the rest of  
 136 parameters were included in biological effect size calculation. Superscript “a”  
 137 indicates significant difference between cohorts post-supplementation.

138

	OND Mean			OD Mean		
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation
*Age	48 (3.5)	48 (3.5)	48 (3.5)	47 (4.3)	47 (4.3)	47 (4.3)
*Height (cm)	167 (10.3)	167 (10.3)	167 (10.3)	171 (10)	171 (10)	171 (10)
Weight (kg)	95 (14.7)	95 (14.1)	94 (14.4)	98 (17)	96 (17)	96 (16)
BMI (kg m <sup>-2</sup> )	34 (5.2)	34 (5.2)	34 (5.3)	34 (4.66)	33 (4.5)	33 (4.4)
Waist Circumference (cm)	104 (11.4)	105 (11.3)	106 (11.3)	113 (10)	113 (10)	113 (12)
Hip Circumference (cm)	117 (8.1)	117 (8.3)	116 (8.2)	116 (12)	114 (11)	115 (12)
Diastolic BP (mmHg)	95 (16)	87 (5.1)	89 (13)	87 (9.86)	85 (9.63)	88 (14)
Systolic BP (mmHg)	131 (15)	124 (13)	131 (21)	130 (17)	129 (22)	131 (17)
Cardiac Frequency (beats min <sup>-1</sup> )	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)
Total Cholesterol (mmol L <sup>-1</sup> )	5 (0.74)	4.9 (0.87)	5.2 (0.69)	4.9 (1.3)	4.6 (0.82)	4.7 (0.49)
Triglyceride (mmol L <sup>-1</sup> )	1.1 (0.42)	1.2 (0.51)	1.2 (0.43)	4.1 (5.8)	3.6 (4.3)	2.9 (2.1)
HDL cholesterol (mmol L <sup>-1</sup> )	1 (0.23)	1 (0.3)	1.1 (0.25)	0.9 (0.24)	0.8 (0.24)	0.8 (0.16)
LDL (mmol L <sup>-1</sup> )	3.5 (0.61)	3.4 (0.68)	3.6 (0.65) <sup>a</sup>	2.8 (0.74)	2.7 (0.7)	2.7 (0.67) <sup>a</sup>
Glucose (mmol L <sup>-1</sup> )	5 (0.68)	5 (0.63)	5.1 (0.46) <sup>a</sup>	6.4 (2)	6.5 (2.2)	6.3 (1.5) <sup>a</sup>
Insulin (uIU ml <sup>-1</sup> )	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)
FFA (mmol L <sup>-1</sup> )	0.5 (0.13)	0.5 (0.2)	0.6 (0.23)	0.6 (0.25)	0.5 (0.19)	0.5 (0.19)
HOMA_IR	3.9 (1.8)	4.4 (2.1)	4.3 (1.6)	6.2 (5.8)	5.1 (3.7)	5.5 (5)
LBP (ug ml <sup>-1</sup> )	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)

140

141 **Taxon abundance and KEGG functions pre- and post-**  
142 **supplementation**

143 Bacterial communities in each cohort were dominated by Firmicutes and  
144 Bacteroidetes, followed by the Actinobacteria, Proteobacteria and  
145 Verrucomicrobia (Fig 1A-1D, S3 Table). Abundances at the pre-supplementation  
146 time point were, however, not statistically different between OND and OD groups  
147 (Fig 1A-1D). Likewise the abundances of KEGG functions from predicted  
148 metagenomes were not different between the cohorts pre-supplementation (S4  
149 Table). However, we did observe a significant difference in alpha diversity  
150 between OND and OD groups at enrollment and before the intervention (Fig 1E,  
151 S3 Table). The OND cohorts had significantly greater community richness  
152 compared to the OD group (Fig 1E, S3 Table).

153

154 **Fig 1. Proportional abundances of dominant taxa and Shannon diversity at**  
155 **enrollment (EN), pre-supplementation (Pre) and post-supplementation**  
156 **(Post).** Panel A, Firmicutes; B, Bacteroidetes; C, Proteobacteria; D,  
157 Verrucomicrobia. Filled bars represent the OND cohort, open bars represent the  
158 OD group. Error bars depict the standard deviation for the mean of 10 subjects.  
159 p-values for significant differences are shown.

160 Post-supplementation, we observed a decline in Firmicutes (Fig 1A) with  
161 simultaneous increase in Bacteroidetes (Fig 1B) abundances in the OD cohort.  
162 However, only the Firmicutes were statistically different between the cohorts  
163 even though the Bacteroidetes increased by 12% over the pre-supplementation  
164 abundance in the OD group (S3 Table). The remaining taxon abundances were

165 not significantly impacted by the supplementation but we did observe that the  
166 Proteobacteria (Fig 1C) consistently decreased, while the Verrucomicrobia (Fig  
167 1D) consistently increased in abundance after supplementation. The abundance  
168 of individual KEGG functions were not significantly altered within or between  
169 cohorts post-supplementation (S3 Table). Alpha diversity (Shannon Index)  
170 remained significantly higher in the OND cohort at the last time point (Fig 1E).

171

## 172 **Changes in effect size pre- and post-supplementation**

173 We next examined the response to the  $\beta$ -glucan cereal bar  
174 supplementation by comparing average effect size across the biological  
175 parameters, phyla, and predicted metagenomic content within each cohort (Fig  
176 2, S2- S4 Tables). Firstly, effect size was not significantly different after  
177 supplementation in the OND cohort when compared to pre-supplementation  
178 abundances ( $p > 0.3$  in all cases, Fig 2A-2C). Mean effect size was largest for the  
179 phyla (mean 0.26, range 0.04-0.62, Fig 2B), followed by the biological (mean  
180 0.20, range 0.04-0.42, Fig 2A) and predicted metagenome content (mean 0.11,  
181 range 0.00-0.37, Fig 2C). In contrast,  $\beta$ -glucan supplementation had a significant  
182 effect in the OD cohort for all data categories (Fig 2A-C). Effects for the phyla and  
183 predicted metagenome content were significantly greater after supplementation  
184 while ( $p < 0.03$ , Fig 2B) while physiology effect size was significantly greater pre-  
185 supplementation for the OD ( $p = 0.04$ , Fig 2A, S3 Table).

186

187 **Fig 2. Effect size of cereal bar supplementation on biological (A), phyla (B),**  
188 **and predicted metagenome (C).** Box and whisker plots showing the mean, the

189 minimum and maximum values for each data set. Kruskal-Wallis comparisons  
190 with p-values are shown. EN-Pre = effect size for enrollment and pre-  
191 supplementation time points; Pre-Post = effect size for pre-supplementation and  
192 post-supplementation time points. Data can be found in S2-S4 Tables.

193 Finally, we compared the average effect size between cohorts using the  
194 Kruskal-Wallis test to determine which cohort was more responsive to the cereal  
195 bar supplementation. Comparing the two treated groups illustrated significant  
196 differences in average effect size on the biological parameters, phyla, and  
197 predicted metagenomic content ( $p < 0.02$ , Fig 2A-C). Average effect size on  
198 biological parameters was higher in the OND compared to OD (0.19 and 0.09,  
199 respectively, Fig 2A, S2 Table), whereas average effects on the phyla and  
200 predicted metagenomic content were both higher in the OD cohorts (Fig 2B and  
201 2C). Differences in effect sizes were most apparent for the predicted  
202 metagenomic content as there was a 4 fold greater effect in the OD versus the  
203 OND group (0.49 vs 0.11, respectively,  $p = 4.3 \times 10^{-9}$ , Fig 2C).

204

## 205 Discussion

206 We tested a cereal bar supplementation containing  $\beta$ -glucan for 30 days  
207 in OD and OND subjects with the goal of quantifying the effect in two similar  
208 disease cohorts. We chose cereal bar with the goal of increasing fiber  
209 consumption and minimizing side effects of sudden increase of fiber intake.  
210 However, we found that consumption of 2.8 g  $\beta$ -glucan per day did not  
211 significantly affect blood glucose in either cohort nor did we observe  
212 improvement in any of the other individual biological parameters. The

213 ineffectiveness of the cereal bar supplementation could result from several  
214 possibilities. For instance, the amount of fiber delivered in this study was  
215 relatively small even though it is near the FDA recommended amount for  
216 cholesterol reduction (CFR 21 101.81, [22]). Martinez et al., [17] has showed  
217 improvement in blood glucose with 60 g of additional oat fiber over four weeks  
218 in healthy individuals while DeAngelis et al. [15] showed a similar result with  
219 pasta containing  $\beta$ -glucan fiber in health individuals. In addition our subjects  
220 were asked not to alter their normal dietary habit and the habitual fiber  
221 consumption was not controlled, possibly confounding the effect of our fiber  
222 supplementation. We must also consider the possibility that our observations  
223 are related to the medications taken by the OD subjects as we asked the subjects  
224 not to change their drug treatment during the study. Regardless of the reasons  
225 behind our observations, we found no significant effect of 2.8 g per day  $\beta$ -glucan  
226 supplementation on biological parameters in our cohorts.

227         As we had predicted, the abundance of Bacteroidetes responded  
228 positively to the fiber supplementation in the OD cohort while there was a  
229 simultaneous decrease in the abundance of Firmicutes, suggesting small  
230 additions of  $\beta$ -glucan in cereal bars can alter taxonomic abundances in gut  
231 bacterial communities. These observations are in line with studies highlighting  
232 the saccharolytic nature of the Bacteroidetes in the human gut [23].  
233 Interestingly, we did not observe any significant change in Bacteroidetes or  
234 Firmicutes abundances in the OND participants. One possible explanation is that  
235 the specific strains of Bacteroidetes and Firmicutes in the OND group are  
236 somehow resistant to change with such a small infusion of fiber. As community  
237 analysis using 16S rRNA genes does not allow for strain level resolution, an

238 alternative approach may be necessary in order to identify specific members of  
239 the microbial community that are resistant or susceptible to cereal bar  
240 supplementations. However, it is also possible that abundances of Bacteroidetes  
241 and Firmicutes in the OND cohort are at levels where small additions of fiber can  
242 not overcome the inherent functional state of the bacterial communities  
243 requiring more intense interventions to shift the abundance of these taxa. As  
244 these two taxa represented more than 93% of the sequences in this study, the  
245 possibility exists that consuming fiber rich cereal bars may be insufficient to  
246 garner consistent and significant changes of dominant taxa across all patient  
247 cohorts.

248         Changes in abundance of Proteobacteria and Verrucomicrobia matched  
249 our *a priori* predictions in both cohorts. The decline of Proteobacteria is  
250 concomitant with studies indicating these bacteria are negatively associated with  
251 diets high in fiber and positively associated with high fat diets [24, 25]. The  
252 reduced abundance of the Proteobacteria has been linked to declines in  
253 inflammatory markers and general host inflammation [26]. The reduction of  
254 these organisms in human gut microbial communities is proposed to be  
255 beneficial. Conversely, increases in Verrucomicrobia are associated with healthy  
256 gut microbial communities [27] and diets high in fiber. The changes observed in  
257 both cohorts suggest that small supplements of fiber can alter abundances of  
258 these taxa. Though abundances of Proteobacteria and Verrucomicrobia did not  
259 change significantly, our data nevertheless indicates these taxa are responsive to  
260 fiber intake across similar disease cohorts possibly reflecting a general life  
261 history strategy of these organisms.

262           The alternative hypothesis that 2.8g  $\beta$ -glucan per day would have a  
263 significant impact on average effect size in both cohorts was not supported.  
264 Instead we observed significantly larger effects on the phyla and predicted  
265 metagenomic content of the OD cohort compared to the OND individuals and  
266 postulate that the lower species richness in the OD group is responsible for these  
267 observations. Greater mean effect sizes in the OD group indicate these gut  
268 communities were more susceptible to our cereal bar supplementation than  
269 were the OND group and coincide with recent report documenting richness as  
270 factor in determining microbial responses to fiber supplementations [18]. Our  
271 data build upon this observation and further suggest significantly changing  
272 microbiomes with  $\beta$ -glucan containing cereal bars may be limited to disease  
273 cohorts harboring microbial communities that are relatively species poor as  
274 these ecosystems have lost the functional flexibility needed to cope with a  
275 changing environment. Unfortunately our experimental design did not allow us  
276 to identify robust causal relationships between microbial communities and low  
277 intensity fiber supplementation (e.g. we used a single dosing level with small  
278 cohort size). Nonetheless, our data suggest that small amount of fiber  
279 supplementation delivered by the cereal bars have the potential to  
280 disproportionally affect low diversity microbial communities and thus may be  
281 useful in studies addressing ecological questions *in situ*, such as community  
282 stability, without altering host physiology.

283           Given the significant effect on the OD bacterial communities, we would  
284 have expected there to be a parallel effect on the biological parameters of these  
285 participants as gut microbes and host phenotypes are clearly linked [7, 28, 29].  
286 However, even though effect sizes were small in both cohorts, the physiology of

287 the OND cohort was more affected by the cereal bar supplementation. The  
288 intrinsic metabolic state of the host could explain this observation. In our  
289 subjects it may be possible that insulin resistance, pancreatic beta cell functions  
290 and even drug treatment dominate the biological set point of the diabetics and  
291 shifts in fiber consumption or bacterial communities had limited influence on the  
292 metabolic outcomes. Unlike the diabetics, these same intrinsic factors in non-  
293 diabetics may not be of equal intensity leaving a window for relatively small  
294 dietary supplementations to have some degree of influence on the host. Low  
295 dose fiber supplementation in healthy individuals reported by Martinez et al.  
296 [17] would support this observation. Altogether, the results of this study suggest  
297 bacterial community diversity in different cohorts may play a role in the  
298 response of microbes to dietary supplementation aimed to manage host  
299 metabolism.

300

## 301 **Materials and Methods**

### 302 **Study population**

303 A total of 46 overweight subjects with Body Mass Index (BMI) between 20  
304 and 30 kg/m<sup>2</sup> were recruited in the Service of Therapeutic education for Chronic  
305 Diseases of the University Hospitals of Geneva. After the inclusion and according  
306 to the results of the initial screening, patients were assigned to either group 1 –  
307 with type 2 diabetes mellitus (OD, n=21) or group 2 – without type 2 diabetes  
308 mellitus (OND, n=25). We defined type 2 diabetes mellitus as fasting plasma  
309 glucose > 7.0 mmol/l and/or HbA1c >7% and/or the presence of any glucose-  
310 lowering treatment. OND subjects were matched for age, gender, BMI and ethnic

311 background of the OD subjects. Exclusion criteria were based on use of drugs  
312 altering intestinal permeability (nonsteroidal anti-inflammatory drugs,  
313 corticoids) or intestinal digestion and absorption (Orlistat, Colestipol,  
314 anticoagulants,  $\alpha$ -glucosidase inhibitors) and antibiotics administered in the 4  
315 weeks preceding inclusion; previous abdominal surgery, gastro-intestinal  
316 diseases interfering with intestinal absorption, cancer, bulimia, pregnancy;  
317 parenteral nutrition or other ongoing dietary intervention, and diarrhea (>2  
318 stools/day) within 7 days before enrolment. From this initial population, 26  
319 were excluded for compliance with the supplementation protocol, they failed to  
320 contribute samples or the sequencing effort was insufficient at any visit. The  
321 resulting subset had a mean age of 42 yrs and 51 yrs for OND and OD,  
322 respectively. Ten individuals for each cohort with overlapping age range were  
323 selected for microbial analysis. Patient data is summarized in S1 Table.

324

## 325 **Study design and intervention**

326 A case controlled, single center prospective clinical trial design was used.  
327 The study consisted of 4 visits and 3 periods. The 3 periods were i. recruiting  
328 period (10 to 30 days), ii. Diet-normalization period (14 days), and iii.  
329 intervention period (30 days). After their recruitment, subjects received  
330 instructions by a nutritionist for dietary normalization. After the dietary  
331 normalization period, subjects received instructions for taking cereal bars per  
332 day (one between breakfast and lunch and the other one between lunch and  
333 dinner), rich in viscous soluble fiber  $\beta$ -glucan. Each cereal bar contained 65 Kcal.  
334 The total carbohydrate is 10.3 g that includes 3.8 g of sugars and 2.5 g of

335 fructose. The total protein is 1.9 g per bar. There is 1.8 g of total fat of which  
336 saturated fat is 0.7 g, monounsaturated fat is 0.7 g and polyunsaturated fat is 0.3  
337 g. Total dietary fiber is 4.4 g of which 1.4 g is  $\beta$ -glucan. Sodium content is 33 mg  
338 in each bar. The cereal bar was well tolerated by all patients without clinically  
339 significant side effects. Subjects submitted blood and fecal samples at the  
340 enrollment, pre- and post- cereal bar supplementation to monitor host  
341 physiology and bacterial community composition. The study protocol  
342 (06.42NRC) was approved by the Geneva ethical committee. Participants were  
343 informed about the aims of the study and gave their written consent.

344

## 345 **Fecal Sample Collection, DNA extraction, PCR and** 346 **sequencing**

347 Stools were collected in sterile plastic 50 mL containers and frozen at -  
348 80°C until processing. Frozen samples were partially thawed and from which  
349 0.25 g of fecal matter was placed in the lysis tube and extracted according to the  
350 manufacturers instructions. DNA was frozen at -20°C until its use in PCR  
351 reactions to generate barcoded amplicons for sequencing on the MiSeq platform  
352 [30]. Briefly, individual samples were amplified in triplicate, pooled then the  
353 PCR products were quantified using PicoGreen dsDNS reagent. Equal amounts of  
354 amplicon from each sample were then combined and sequenced on the MiSeq  
355 platform. Sequencing was performed at the Nestlé Institute of Health Sciences  
356 Functional Genomics Core facility.

357

## 358 **Sequence analysis**

359           Sequence data were quality filtered and demultiplexed in Qiime 1.8 [31]  
360    using the default settings for the `split_libraries_fastq.py` command followed by  
361    closed reference OTU picking at 97% sequence similarity against the `gg_13_5`  
362    release ([http://greengenes.secondgenome.com/downloads/database/13\\_5](http://greengenes.secondgenome.com/downloads/database/13_5))  
363    with the `parallel_pick_otus_uclust_ref.py` command. We additionally filtered out  
364    Cyanobacteria to avoid chloroplast sequences and filtered out low abundance  
365    OTUs according to Bokulich et al. 2013 [32] using the  
366    `filter_otus_from_otu_table.py` command. Samples were then rarefied to 50000  
367    sequences per sample (`single_rarefaction.py`) from which the relative abundance  
368    of taxa (classified to the phylum level using `summarize_taxa.py`) was calculated  
369    and used for downstream analysis. An estimate of bacterial richness was  
370    performed using the Shannon index (`alpha_diversity.py`) on the rarefied data.  
371    Predicted metagenomic content was also calculated using PiCRUST [33] and  
372    summarized to KEGG level 2 for statistical evaluation. All bacterial community  
373    data was expressed as the proportional abundance in each sample.

374

## 375    **Data analysis**

376           From the initial cohort we chose an age-matched sub set of patients to  
377    assess changes in host metabolic measurements, taxonomic abundance, and  
378    KEGG functions in response to the  $\beta$ -glucan cereal bar supplementation. Using  
379    Kruskal-Wallis tests implemented in Spotfire® (Göteborg, Sweden), we  
380    compared group means within (e.g. pre- vs post-supplementation in each cohort)  
381    and between cohorts (e.g. OND vs OD pre- supplementation and post-  
382    supplementation) with  $\alpha = 0.05$  for all individual measurements (e.g. blood

383 glucose, taxon abundance, and individual KEGG functions, etc.) to identify  
384 changes in these measurements. Bonferroni multiple comparison correction was  
385 applied to the p-values for the taxonomic abundances and KEGG functions. We  
386 next evaluated the magnitude of response of a data category (biological, phyla,  
387 and predicted metagenome; S2-S4 Tables) by testing average effect size between  
388 cohorts before and after cereal bar supplementation [34]. The absolute values of  
389 the effect size, calculated by Eq. 1 where  $E_s$  = effect size,  $m$  = mean, and  $\sigma$  =  
390 standard deviation, were compared using Kruskal-Wallis tests to determine of  
391 there were statistical differences in response.

392

393

394 Eq. 1

$$E_s = \frac{(m_1 - m_2)}{\sqrt{\frac{\sigma_1^2 + \sigma_2^2}{2}}}$$

395

396

397

398

## 399 **Acknowledgements**

400

401 We would like to thank Bernard Berger of the Nestlé Research Center Lausanne  
402 for his assistance, Patrick Descombes and Deborah Moine of the NIHS Functional  
403 Genomics core for their technical help with sequencing the 16S rRNA amplicons.

404

405

## 406 **Supporting Information**

407

408 **S1 Table. Mean and standard deviation of biological parameters of 46**  
409 **subjects at recruitment.**

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411 **S2 Table. Means, standard deviations, and effect sizes for biological**  
412 **parameters for the age-matched cohort. \*Age and \*height are not included in**  
413 **the mean effect size calculation. Superscript “a” indicates significant difference**  
414 **between cohorts post-supplementation.**

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416 **S3 Table. Means, standard deviations, and effect sizes for taxa and Shannon**  
417 **Diversity for the age-matched cohort.**

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419 **S4 Table. Means, standard deviations, and effect sizes for the KEGG**  
420 **functions predicted by PiCRUST for the age-matched cohort.**

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Table 1. Biological parameters of age-matched cohorts at enrollment, pre-supplementation, and post-supplementation. The mean and standard deviation (in parentheses) are shown. Except for \*Age and \*Height, the rest of parameters were included in biological effect size calculation. Superscript “a” indicates significant difference between cohorts post-supplementation.

	OND Mean			OD Mean		
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation
*Age	48 (3.5)	48 (3.5)	48 (3.5)	47 (4.3)	47 (4.3)	47 (4.3)
*Height (cm)	167 (10.3)	167 (10.3)	167 (10.3)	171 (10)	171 (10)	171 (10)
Weight (kg)	95 (14.7)	95 (14.1)	94 (14.4)	98 (17)	96 (17)	96 (16)
BMI (kg m <sup>-2</sup> )	34 (5.2)	34 (5.2)	34 (5.3)	34 (4.66)	33 (4.5)	33 (4.4)
Waist Circumference (cm)	104 (11.4)	105 (11.3)	106 (11.3)	113 (10)	113 (10)	113 (12)
Hip Circumference (cm)	117 (8.1)	117 (8.3)	116 (8.2)	116 (12)	114 (11)	115 (12)
Diastolic BP (mmHg)	95 (16)	87 (5.1)	89 (13)	87 (9.86)	85 (9.63)	88 (14)
Systolic BP (mmHg)	131 (15)	124 (13)	131 (21)	130 (17)	129 (22)	131 (17)
Cardiac Frequency (beats min <sup>-1</sup> )	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)
Total Cholesterol (mmol L <sup>-1</sup> )	5 (0.74)	4.9 (0.87)	5.2 (0.69)	4.9 (1.3)	4.6 (0.82)	4.7 (0.49)
Triglyceride (mmol L <sup>-1</sup> )	1.1 (0.42)	1.2 (0.51)	1.2 (0.43)	4.1 (5.8)	3.6 (4.3)	2.9 (2.1)
HDL cholesterol (mmol L <sup>-1</sup> )	1 (0.23)	1 (0.3)	1.1 (0.25)	0.9 (0.24)	0.8 (0.24)	0.8 (0.16)
LDL (mmol L <sup>-1</sup> )	3.5 (0.61)	3.4 (0.68)	3.6 (0.65) <sup>a</sup>	2.8 (0.74)	2.7 (0.7)	2.7 (0.67) <sup>a</sup>
Glucose (mmol L <sup>-1</sup> )	5 (0.68)	5 (0.63)	5.1 (0.46) <sup>a</sup>	6.4 (2)	6.5 (2.2)	6.3 (1.5) <sup>a</sup>
Insulin (uIU ml <sup>-1</sup> )	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)
FFA (mmol L <sup>-1</sup> )	0.5 (0.13)	0.5 (0.2)	0.6 (0.23)	0.6 (0.25)	0.5 (0.19)	0.5 (0.19)
HOMA_IR	3.9 (1.8)	4.4 (2.1)	4.3 (1.6)	6.2 (5.8)	5.1 (3.7)	5.5 (5)
LBP (ug ml <sup>-1</sup> )	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)

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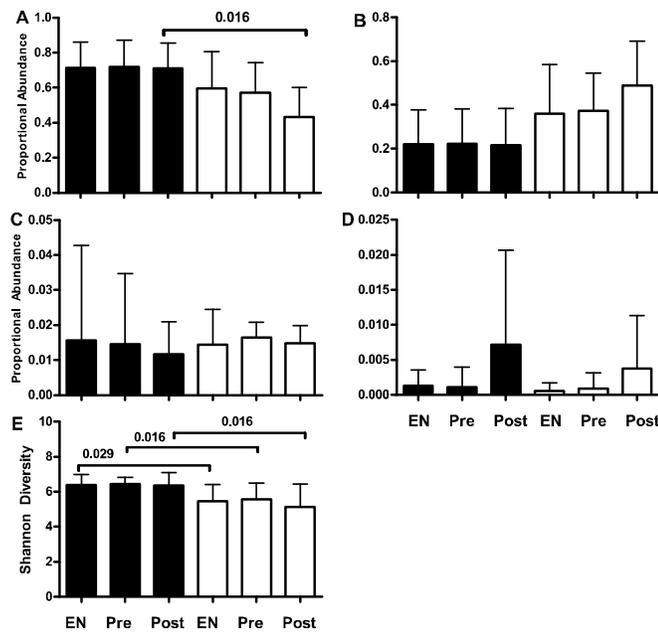
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610 **Fig 1. Proportional abundances of dominant taxa and Shannon diversity at**  
611 **enrollment (EN), pre-supplementation (Pre) and post-supplementation**  
612 **(Post).** Bar graphs for proportional abundances of dominant taxa are indicated  
613 as (A) Firmicutes, (B) Bacteroidetes, (C) Proteobacteria, and (D)  
614 Verrucomicrobia. Filled bars represent the OND cohort, open bars represent the  
615 OD group. Error bars depict the standard deviation for the mean of 10 subjects.  
616 p-values for significant differences are shown.  
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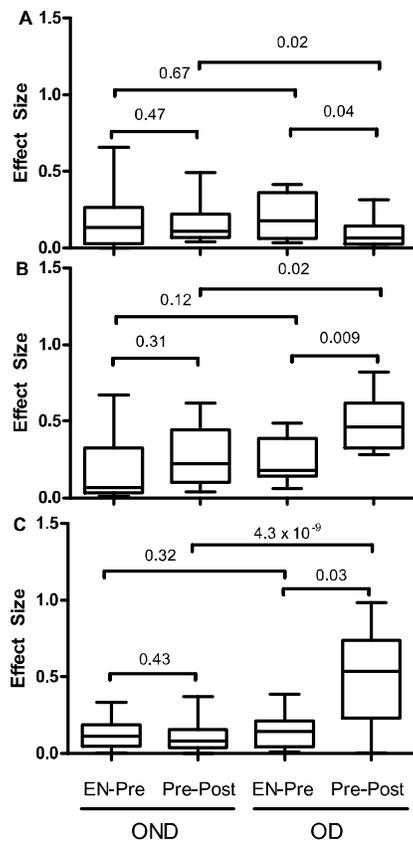
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**Fig 2. Effect size of cereal bar supplementation on measured parameters.**  
Box and whisker plots showing the mean, the minimum and maximum values for (A) biological, (B) phyla, and (C) predicted metagenome. Kruskal-Wallis comparisons with p-values are shown. EN-Pre = effect size for enrollment and pre-supplementation time points; Pre-Post = effect size for pre-supplementation and post-supplementation time points. Data can be found in S2-S4 Tables.



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658 S1 Table. Mean and standard deviation of biological parameters of 46 subjects at  
659 recruitment.

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	OND	OD
Subjects	25	21
Height (cm)	166 (8.7)	169 (9.5)
Age	43 (7.2)	50 (5.9)
Weight (kg)	91 (14)	95 (16)
BMI (kg m <sup>2</sup> )	33 (4.8)	33 (4.3)
Waist Circumference (cm)	102 (11)	112 (11)
Hip Circumference (cm)	116 (9.1)	113 (9.5)
Diastolic BP (mmHg)	89 (13)	87 (11)
Systolic BP (mmHg)	124 (15)	130 (16)
Cardiac Frequency (beats min <sup>-1</sup> )	73 (11)	81 (19)
Total Cholesterol (mmol L <sup>-1</sup> )	5.0 (0.8)	4.7 (1.3)
Triglyceride (mmol L <sup>-1</sup> )	1.1 (0.4)	2.9 (4.2)
HDL cholesterol (mmol L <sup>-1</sup> )	1.1 (0.2)	0.9 (0.2)
LDL (mmol L <sup>-1</sup> )	3.4 (0.7)	2.8 (0.9)
Glucose (mmol L <sup>-1</sup> )	4.9 (0.6)	6.9 (1.9)
Insulin (uIU ml <sup>-1</sup> )	1.8 (11)	23 (19)
HOMA_IR	4.0 (2.8)	7.4 (6.2)
LBP (ug ml <sup>-1</sup> )	25 (8)	29 (7.6)
FFA (mmol L <sup>-1</sup> )	0.45 (0.21)	0.54 (0.21)

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687 S2 Table. Means, standard deviations, and effect sizes for biological parameters for the age-matched cohort. \*Age and \*height are not  
 688 included in the mean effect size calculation. Superscript “a” indicates significant difference between cohorts post-supplementation.  
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	OND Mean			OD Mean			OND Effect Size		OD Effect Size	
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation	Enrolment vs Pre-supplementation	Pre-supplementation vs Post-supplementation	Enrolment vs Pre-supplementation	Pre-supplementation vs Post-supplementation
Biological Parameters										
*Age	48 (3.5)	48 (3.5)	48 (3.5)	47 (4.3)	47 (4.3)	47 (4.3)	0.0000	0.0000	0.0000	0.0000
*Height (cm)	167 (10.3)	167 (10.3)	167 (10.3)	171 (10)	171 (10)	171 (10)	0.0000	0.0000	0.0000	0.0000
Weight (kg)	95 (14.7)	95 (14.1)	94 (14.4)	98 (17)	96 (17)	96 (16)	0.0007	0.0653	0.0873	0.0356
BMI (kg m <sup>-2</sup> )	34 (5.2)	34 (5.2)	34 (5.3)	34 (4.66)	33 (4.5)	33 (4.4)	0.0046	0.0610	0.1135	0.0427
Waist Circumference (cm)	104 (11.4)	105 (11.3)	106 (11.3)	113 (10)	113 (10)	113 (12)	0.0746	0.0664	0.0529	0.0091
Hip Circumference (cm)	117 (8.1)	117 (8.3)	116 (8.2)	116 (12)	114 (11)	115 (12)	0.0000	0.1698	0.1457	0.1008
Diastolic BP (mmHg)	95 (16)	87 (5.1)	89 (13)	87 (9.86)	85 (9.63)	88 (14)	0.6578	0.1860	0.2156	0.3138
Systolic BP (mmHg)	131 (15)	124 (13)	131 (21)	130 (17)	129 (22)	131 (17)	0.4325	0.3575	0.0502	0.1059
Cardiac Frequency (beats min <sup>-1</sup> )	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)	0.1277	0.2319	0.0731	0.0223
Total Cholesterol (mmol L <sup>-1</sup> )	5 (0.74)	4.9 (0.87)	5.2 (0.69)	4.9 (1.3)	4.6 (0.82)	4.7 (0.49)	0.1384	0.3816	0.3066	0.1585
Triglyceride (mmol L <sup>-1</sup> )	1.1 (0.42)	1.2 (0.51)	1.2 (0.43)	4.1 (5.8)	3.6 (4.3)	2.9 (2.1)	0.0864	0.0352	0.1046	0.1999
HDL cholesterol (mmol L <sup>-1</sup> )	1 (0.23)	1 (0.3)	1.1 (0.25)	0.9 (0.24)	0.8 (0.24)	0.8 (0.16)	0.0224	0.1082	0.0955	0.0025
LDL (mmol L <sup>-1</sup> )	3.5 (0.61)	3.4 (0.68)	3.6 (0.65)	2.8 (0.74)	2.7 (0.7)	2.7 (0.67)	0.2127	0.4162	0.0680	0.0038
Glucose (mmol L <sup>-1</sup> )	5 (0.68)	5 (0.63)	5.1 (0.46)	6.4 (2)	6.5 (2.2)	6.3 (1.5)	0.0381	0.2262	0.0402	0.0813
Insulin (uIU ml <sup>-1</sup> )	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)	0.3149	0.0406	0.1913	0.0487
FFA (mmol L <sup>-1</sup> )	0.5 (0.13)	0.5 (0.2)	0.6 (0.23)	0.6 (0.25)	0.5 (0.19)	0.5 (0.19)	0.1759	0.3637	0.4917	0.0354
HOMA_IR	3.9 (1.8)	4.4 (2.1)	4.3 (1.6)	6.2 (5.8)	5.1 (3.7)	5.5 (5)	0.2842	0.0476	0.2236	0.0908
LBP (ug ml <sup>-1</sup> )	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)	0.1829	0.3826	0.4042	0.1908
Biological Mean Effect Size							0.1721	0.1962	0.1665	0.0901

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701 S3 Table. Means, standard deviations, and effect sizes for taxa and Shannon Diversity for the age-matched cohort.  
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	OND Mean			OD Mean			OND Effect Size		OD Effect Size	
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation	Enrolment vs Pre-supplementation	Pre-supplementation vs Post-supplementation	Enrolment vs Pre-supplementation	Pre-supplementation vs Post-supplementation
Taxa										
Euryarchaeota	0.003 (0.004)	0.001 (0.002)	0.003 (0.006)	0 (0)	0 (0)	0 (0)	0.3417	0.2984	0.1805	0.2959
Actinobacteria	0.043 (0.049)	0.039 (0.053)	0.048 (0.074)	0.028 (0.04)	0.036 (0.064)	0.059 (0.096)	0.0670	0.1410	0.1557	0.2819
Bacteroidetes	0.221 (0.157)	0.222 (0.16)	0.216 (0.169)	0.361 (0.225)	0.373 (0.172)	0.49 (0.202)	0.0100	0.0378	0.0606	0.6194
Firmicutes	0.714 (0.145)	0.718 (0.154)	0.71 (0.145)	0.596 (0.209)	0.572 (0.171)	0.432 (0.17)	0.0275	0.0573	0.1265	0.8221
Fusobacteria	0 (0)	0 (0)	0 (0)	0 (0.001)	0.002 (0.006)	0 (0)	0.6708	0.5855	0.3444	0.4316
Lentisphaerae	0 (0.001)	0 (0)	0 (0.001)	0 (0)	0 (0)	0 (0)	0.3087	0.2211	0.4884	0.6174
Proteobacteria	0.016 (0.027)	0.015 (0.02)	0.012 (0.009)	0.014 (0.01)	0.016 (0.004)	0.015 (0.005)	0.0405	0.1907	0.2602	0.3567
Tenericutes	0.002 (0.003)	0.003 (0.003)	0.004 (0.007)	0 (0)	0 (0)	0.001 (0.003)	0.0697	0.2273	0.4346	0.4626
Verrucomicrobia	0.00126 (0.002)	0.001118 (0.003)	0.00716 (0.014)	0.001 (0.001)	0.001 (0.002)	0.004 (0.008)	0.0549	0.6187	0.1758	0.5085
Phyla Mean Effect Size							0.1768	0.2642	0.2474	0.4885
Richness										
Shannon Index	6.4 (0.60)	6.4 (0.35)	6.4 (0.74)	5.5 (0.9)	5.6 (0.9)	5.1 (1.3)				

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719 S4 Table. Means, standard deviations, and effect sizes for the KEGG functions predicted by PiCRUST for the age-matched cohort.

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KEGG Functions	OND Mean			OD Mean			OND Effect Size		OD Effect Size	
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation	Enrollment vs Pre-supplementation	Pre-supplementation vs Post-supplementation	Enrollment vs Pre-supplementation	Pre-supplementation vs Post-supplementation
Amino Acid Metabolism	0.098 (0.002)	0.098 (0.0021)	0.098 (0.0013)	0.097 (0.0024)	0.097 (0.0026)	0.098 (0.0031)	0.0379	0.0803	0.0835	0.0915
Biosynthesis of Other Secondary Metab	0.009 (0.0003)	0.009 (0.0004)	0.009 (0.0003)	0.01 (0.0007)	0.01 (0.0007)	0.01 (0.0005)	0.1333	0.1794	0.3736	0.2317
Cancers	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.0435	0.0717	0.1256	0.2135
Carbohydrate Metabolism	0.109 (0.0043)	0.11 (0.004)	0.109 (0.0034)	0.11 (0.0043)	0.11 (0.0044)	0.108 (0.0075)	0.2028	0.2562	0.0830	0.2429
Cardiovascular Diseases	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1964	0.3691	0.3830	0.0027
Cell Growth and Death	0.005 (0.0002)	0.005 (0.0002)	0.005 (0.0002)	0.005 (0.0003)	0.005 (0.0003)	0.005 (0.0005)	0.1611	0.0653	0.1491	0.4353
Cell Motility	0.019 (0.003)	0.019 (0.0036)	0.019 (0.0027)	0.018 (0.0091)	0.02 (0.01)	0.016 (0.0057)	0.0145	0.0127	0.1990	0.5426
Cellular Processes and Signaling	0.041 (0.0008)	0.041 (0.001)	0.04 (0.0009)	0.042 (0.0012)	0.042 (0.0011)	0.042 (0.0018)	0.0197	0.0942	0.0184	0.1155
Circulatory System	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1634	0.3326	0.3871	0.0676
Digestive System	0 (0.0002)	0 (0.0002)	0 (0.0002)	0 (0.0003)	0.001 (0.0002)	0.001 (0.0005)	0.1368	0.0681	0.0717	0.5709
Endocrine System	0.003 (0.0003)	0.003 (0.0003)	0.003 (0.0003)	0.003 (0.0005)	0.003 (0.0004)	0.003 (0.0003)	0.1777	0.0156	0.0244	0.7313
Energy Metabolism	0.058 (0.0019)	0.058 (0.0019)	0.058 (0.0018)	0.06 (0.002)	0.059 (0.0016)	0.06 (0.0025)	0.0926	0.0766	0.3325	0.5827
Environmental Adaptation	0.002 (0.0001)	0.002 (0.0001)	0.002 (0.0001)	0.002 (0.0002)	0.002 (0.0002)	0.002 (0.0002)	0.0488	0.1554	0.2496	0.8562
Enzyme Families	0.022 (0.0006)	0.022 (0.0004)	0.022 (0.0005)	0.022 (0.0007)	0.022 (0.0007)	0.023 (0.0016)	0.2361	0.1106	0.1057	0.3593
Excretory System	0 (0.0001)	0 (0.0001)	0 (0.0001)	0 (0.0001)	0 (0.0001)	0 (0.0001)	0.1169	0.0384	0.0349	0.1777
Folding, Sorting and Degradation	0.024 (0.0008)	0.024 (0.0009)	0.024 (0.0008)	0.025 (0.0012)	0.025 (0.0011)	0.025 (0.002)	0.0702	0.0680	0.0853	0.5338
Genetic Information Processing	0.027 (0.0006)	0.026 (0.0005)	0.027 (0.0008)	0.026 (0.001)	0.025 (0.0009)	0.026 (0.0011)	0.2298	0.2076	0.0766	0.2411
Glycan Biosynthesis and Metabolism	0.02 (0.0028)	0.02 (0.0031)	0.02 (0.0028)	0.022 (0.005)	0.022 (0.0031)	0.026 (0.005)	0.0181	0.0321	0.0454	0.9108
Immune System	0.001 (0)	0.001 (0)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.0652	0.3149	0.1823	0.0025
Immune System Diseases	0 (0.0001)	0 (0)	0 (0.0001)	0 (0.0001)	0 (0)	0.001 (0.0001)	0.0743	0.0465	0.3842	0.7881
Infectious Diseases	0.003 (0.0001)	0.003 (0.0001)	0.003 (0.0001)	0.003 (0.0001)	0.003 (0.0001)	0.004 (0.0003)	0.0883	0.0247	0.0149	0.8136
Lipid Metabolism	0.029 (0.0013)	0.029 (0.0012)	0.029 (0.0012)	0.029 (0.0011)	0.028 (0.0011)	0.028 (0.0018)	0.1894	0.0815	0.1611	0.3154
Membrane Transport	0.126 (0.0103)	0.126 (0.0094)	0.127 (0.0097)	0.122 (0.0138)	0.122 (0.0106)	0.112 (0.0171)	0.0040	0.0993	0.0414	0.6641
Metabolic Diseases	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.0476	0.2004	0.1516	0.5964
Metabolism	0.023 (0.0003)	0.023 (0.0004)	0.024 (0.0004)	0.024 (0.0005)	0.024 (0.0006)	0.024 (0.001)	0.1877	0.1874	0.1942	0.2318
Metabolism of Cofactors and Vitamins	0.043 (0.0018)	0.042 (0.0018)	0.043 (0.0015)	0.044 (0.0018)	0.043 (0.0014)	0.045 (0.0024)	0.1382	0.0934	0.1551	0.8163
Metabolism of Other Amino Acids	0.014 (0.0005)	0.014 (0.0006)	0.014 (0.0004)	0.014 (0.0008)	0.015 (0.0007)	0.015 (0.0007)	0.1045	0.0167	0.3831	0.7379
Metabolism of Terpenoids and Polyketi	0.016 (0.0006)	0.016 (0.0005)	0.016 (0.0005)	0.016 (0.0012)	0.016 (0.0012)	0.017 (0.0015)	0.0044	0.0067	0.1608	0.6339
Nervous System	0.001 (0)	0.001 (0)	0.001 (0)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.0034	0.0734	0.1025	0.7113
Neurodegenerative Diseases	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.001 (0.0001)	0.2698	0.1135	0.3445	0.3910
Nucleotide Metabolism	0.041 (0.0016)	0.041 (0.0014)	0.041 (0.0013)	0.041 (0.0027)	0.041 (0.0026)	0.043 (0.0046)	0.1118	0.0006	0.0847	0.5354
Poorly Characterized	0.048 (0.0003)	0.048 (0.0006)	0.048 (0.0004)	0.048 (0.001)	0.048 (0.0009)	0.049 (0.0009)	0.1267	0.2076	0.1446	0.7982
Replication and Repair	0.091 (0.003)	0.091 (0.0024)	0.091 (0.0026)	0.09 (0.005)	0.09 (0.0049)	0.093 (0.0084)	0.0558	0.1214	0.0152	0.3754
Signal Transduction	0.014 (0.0008)	0.014 (0.0009)	0.014 (0.0007)	0.014 (0.0025)	0.015 (0.0028)	0.013 (0.0026)	0.0939	0.1203	0.2118	0.5708
Signaling Molecules and Interaction	0.002 (0.0001)	0.002 (0.0001)	0.002 (0.0001)	0.002 (0.0003)	0.002 (0.0003)	0.002 (0.0003)	0.2380	0.0203	0.0269	0.9032
Transcription	0.03 (0.001)	0.03 (0.0012)	0.03 (0.0009)	0.03 (0.0017)	0.029 (0.0009)	0.028 (0.0016)	0.0056	0.0337	0.2626	0.9466
Translation	0.059 (0.002)	0.059 (0.0017)	0.059 (0.0017)	0.057 (0.003)	0.057 (0.0031)	0.059 (0.0058)	0.2156	0.0461	0.0095	0.3133
Transport and Catabolism	0.002 (0.0004)	0.002 (0.0005)	0.002 (0.0005)	0.003 (0.0008)	0.003 (0.0005)	0.003 (0.0006)	0.1532	0.0483	0.0104	0.9835
Xenobiotics Biodegradation and Metab	0.016 (0.0009)	0.016 (0.0009)	0.016 (0.0009)	0.015 (0.0018)	0.015 (0.002)	0.015 (0.001)	0.3328	0.1151	0.1751	0.1909
Predicted Metagenome Mean Effect Size							0.1182	0.1078	0.1550	0.4930

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