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

3

4 Short Title: Cohort specific effects of diet supplementation

5

6

7

8 Chieh Jason Chou^{1*}, Chris Lauber¹, Anirikh Chakrabarti¹, Jay Siddharth¹, Anne

9 Chalut-Carpentier², Zoltan Pataky², Alain Golay^{2¶}, Scott Parkinson^{1¶}

10

11 ¹Nestlé Institute of Health Sciences S.A., Lausanne, Switzerland

12

13 ² Service of Therapeutic Education for Chronic Diseases, WHO Collaborating

14 Centre, University Hospitals of Geneva and University of Geneva, Geneva,

15 Switzerland

16

17

18

19

20

21 [¶] Senior authors

22

23 * Corresponding author. Nestlé Institute of Health Sciences, SA. 1015 Lausanne,

24 Switzerland. Email: christianlynn.lauber@rd.nestle.com.

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 β -
 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.

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

responsiveness of the microbiome in similar but distinctive cohorts may help to define the limits of fiber supplementation via changes in the microbiome.

Overweight diabetic (OD) and overweight non-diabetics (OND) represent two metabolic states that can be distinguished by the degree of insulin resistance. However, it is not known whether the two populations exhibit cohort specific effects of fiber supplementation on the bacterial communities and host metabolism. To answer this question, we conducted a fiber supplementation study in OD and OND subjects with a cereal bar containing 1.4 g β -glucan. We investigated how the bacterial communities responded to consumption of two cereal bars per day and determined whether there were unique features of the response in OND vs OD subjects. Specifically we predicted that fiber supplementation would increase abundances of Bacteroidetes and Verrucomicrobia while decreasing Firmicutes and Proteobacteria in both cohorts with concomitant improvement in host metabolic markers.

Results

Biological Parameters

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

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

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.

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 ⁻²)	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 ⁻¹)	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)
Total Cholesterol (mmol L ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	3.5 (0.61)	3.4 (0.68)	3.6 (0.65) ^a	2.8 (0.74)	2.7 (0.7)	2.7 (0.67) ^a
Glucose (mmol L ⁻¹)	5 (0.68)	5 (0.63)	5.1 (0.46) ^a	6.4 (2)	6.5 (2.2)	6.3 (1.5) ^a
Insulin (uIU ml ⁻¹)	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)
FFA (mmol L ⁻¹)	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 ⁻¹)	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)

139

140

Taxon abundance and KEGG functions pre- and post-supplementation

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

153

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

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

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

171

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

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

186

Fig 2. Effect size of cereal bar supplementation on biological (A), phyla (B), 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 β -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 β -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

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

As we had predicted, the abundance of Bacteroidetes responded positively to the fiber supplementation in the OD cohort while there was a simultaneous decrease in the abundance of Firmicutes, suggesting small additions of β -glucan in cereal bars can alter taxonomic abundances in gut bacterial communities. These observations are in line with studies highlighting the saccharolytic nature of the Bacteroidetes in the human gut [23]. Interestingly, we did not observe any significant change in Bacteroidetes or Firmicutes abundances in the OND participants. One possible explanation is that the specific strains of Bacteroidetes and Firmicutes in the OND group are somehow resistant to change with such a small infusion of fiber. As community 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 β -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 β -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

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

300

301 **Materials and Methods**

302 **Study population**

A total of 46 overweight subjects with Body Mass Index (BMI) between 20 and 30 kg/m² were recruited in the Service of Therapeutic education for Chronic Diseases of the University Hospitals of Geneva. After the inclusion and according to the results of the initial screening, patients were assigned to either group 1 – with type 2 diabetes mellitus (OD, n=21) or group 2 – without type 2 diabetes mellitus (OND, n=25). We defined type 2 diabetes mellitus as fasting plasma glucose > 7.0 mmol/l and/or HbA1c >7% and/or the presence of any glucose-lowering treatment. OND subjects were matched for age, gender, BMI and ethnic

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

Study design and intervention

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

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

Fecal Sample Collection, DNA extraction, PCR and sequencing

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

Sequence analysis

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

374

375 **Data analysis**

From the initial cohort we chose an age-matched sub set of patients to assess changes in host metabolic measurements, taxonomic abundance, and KEGG functions in response to the β -glucan cereal bar supplementation. Using Kruskal-Wallis tests implemented in Spotfire® (Göteborg, Sweden), we compared group means within (e.g. pre- vs post-supplementation in each cohort) and between cohorts (e.g. OND vs OD pre- supplementation and post-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 Es= effect size, m = mean, and σ =
 390 standard deviation, were compared using Kruskal-Wallis tests to determine of
 391 there were statistical differences in response.

392

393

394 Eq. 1

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

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.**

410

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.**

415

416 **S3 Table. Means, standard deviations, and effect sizes for taxa and Shannon**

417 **Diversity for the age-matched cohort.**

418

419 **S4 Table. Means, standard deviations, and effect sizes for the KEGG**

420 **functions predicted by PiCRUSt for the age-matched cohort.**

421

422

423

424 **References**

425

426 1. World Health Organization, 2003.

427 2. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al.

428 Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes.

429 Science. 2011; 334:105-8. doi: 10.1126/science.1208344. PMID:3368382.

- 430 3. Matijašić B, Obermajer T, Lipoglavšek L, Grabnar I, Avguštin G, Rogelj I.
431 Association of dietary type with fecal microbiota in vegetarians and
432 omnivores in Slovenia. *European Journal of Nutrition*. 2014; 53:1051-64.
433 doi: 10.1007/s00394-013-0607-6. PMID: 24173964
- 434 4. Kabeerdoss J, Shobana Devi R, Regina Mary R, Ramakrishna BS. Faecal
435 microbiota composition in vegetarians: comparison with omnivores in a
436 cohort of young women in southern India. *British Journal of Nutrition*.
437 2012; 108:953-7. doi:10.1017/S0007114511006362. PMID: 22182464
- 438 5. Glick-Bauer M, Yeh M-C. The Health Advantage of a Vegan Diet: Exploring
439 the Gut Microbiota Connection. *Nutrients*. 2014; 6:4822-38 doi:
440 10.3390/nu6114822. PMID: 25365383
- 441 6. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S,
442 et al. Impact of diet in shaping gut microbiota revealed by a comparative
443 study in children from Europe and rural Africa. *Proceedings of the*
444 *National Academy of Sciences of the United States of America*. 2010;
445 107:14691-6. doi: 10.1073/pnas.1005963107. PMID: 20679230.
- 446 7. Ley RE, Hamady M, Lozupone C, Turnbaugh P, Ramey RR, Bircher JS, et al.
447 Evolution of mammals and their gut microbes. *Science*. 2008. 320:1647-
448 51. doi: 10.1126/science.1155725. PMID:2649005.
- 449 8. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A
450 decrease of the butyrate-producing species *Roseburia hominis* and
451 *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative
452 colitis. *Gut*. 2014; 63:1275-83. doi: 10.1136/gutjnl-2013-304833. PMID:
453 24021287
- 454 9. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of

- 455 complex carbohydrates in the gut. Gut Microbes. 2012; 3:289-306. doi:
456 10.4161/gmic.19897. PMID: 22572875
- 457 10. Salyers AA, Vercellotti JR, West SE, Wilkins TD. Fermentation of mucin
458 and plant polysaccharides by strains of Bacteroides from the human
459 colon. Applied and Environmental Microbiology. 1977; 33:319-22. PMID:
460 848954
- 461 11. Sartor RB, Mazmanian SK. Intestinal Microbes in Inflammatory Bowel
462 Diseases. American Journal of Gastroenterology. Suppl. 2012; 1:15-21
463 doi:10.1038/ajgsup.2012.4. PMID: 19107650
- 464 12. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W,
465 Ren B, et al. The treatment-naïve microbiome in new-onset Crohn's
466 disease. Cell Host & Microbe. 2014; 15:382-92.
467 doi:10.1016/j.chom.2014.02.005. PMID: 4059512
- 468 13. Flint HJ, Duncan SH, Scott KP, Louis P. Interactions and competition
469 within the microbial community of the human colon: links between diet
470 and health. Environmental Microbiology. 2007; 9:1101-11. doi:
471 10.1111/j.1462-2920.2007.01281.x. PMID: 17472627
- 472 14. Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on
473 bacterial metabolism in the large intestine. Journal of Applied
474 Microbiology. 2007; 1197-208. doi: 10.1111/j.1365-2672.2007.03322.x.
475 PMID: 17448155
- 476 15. De Angelis M, Montemurno E, Vannini L, Cosola C, Cavallo N, Gozzi G, et al.
477 The role of whole-grain barley on human fecal microbiota and
478 metabolome. Applied and Environmental Microbiology. 2015; doi:
479 10.1128/aem.02507-15. PMID: 26386056

- 480 16. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PGB, Neyrinck AM, et al.
481 Insight into the prebiotic concept: lessons from an exploratory, double
482 blind intervention study with inulin-type fructans in obese women. Gut.
483 2013; 62:1112-21. doi: 10.1136/gutjnl-2012-303304. PMID:3711491.
- 484 17. Martinez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, et al. Gut
485 microbiome composition is linked to whole grain-induced immunological
486 improvements. The ISME Journal. 2013; 7:269-80.
487 doi:10.1038/ismej.2012.104. PMID: 23038174
- 488 18. Tap J, Furet J-P, Bensaada M, Philippe C, Roth H, Rabot S, et al. Gut
489 microbiota richness promotes its stability upon increased dietary fibre
490 intake in healthy adults. Environmental Microbiology. 2015; doi:
491 10.1111/1462-2920.13006. PMID: 26235304
- 492 19. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al.
493 Dominant and diet-responsive groups of bacteria within the human
494 colonic microbiota. The ISME Journal. 2011; 5:220-30.
495 doi:10.1038/ismej.2010.118. PMID: 20686513
- 496 20. Hooda S, Boler BMV, Serao MCR, Brulc JM, Staeger MA, Boileau TW, et al.
497 454 Pyrosequencing Reveals a Shift in Fecal Microbiota of Healthy Adult
498 Men Consuming Polydextrose or Soluble Corn Fiber. The Journal of
499 Nutrition. 2012; 142:1259-65. doi: 10.3945/jn.112.158766. PMID:
500 22649263
- 501 21. Benus RFJ, van der Werf TS, Welling GW, Judd PA, Taylor MA, Harmsen
502 HJM, et al. Association between Faecalibacterium prausnitzii and dietary
503 fibre in colonic fermentation in healthy human subjects. British Journal of
504 Nutrition. 2010; 104:693-700. doi:10.1017/S0007114510001030.

- 505 PMID: 20346190.
- 506 22. US Food and Drug Administration. TITLE 21—Food and drugs Chapter 1,
507 subchapter B –Food for human consumption. 2015.
508 [w.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.81](http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.81)
- 509 23. Martens EC, Chiang HC, Gordon JI. Mucosal Glycan Foraging Enhances
510 Fitness and Transmission of a Saccharolytic Human Gut Bacterial
511 Symbiont. *Cell host & microbe*. 2008; 4(5):447-57. doi:
512 [10.1016/j.chom.2008.09.007](https://doi.org/10.1016/j.chom.2008.09.007). PMID: 18996345
- 513 24. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M,
514 Chen YY, et al. High-Fat Diet Determines the Composition of the Murine
515 Gut Microbiome Independently of Obesity. *Gastroenterology*. 2009;
516 137:1716-24.e1-2. doi: [10.1053/j.gastro.2009.08.042](https://doi.org/10.1053/j.gastro.2009.08.042). PMID: 19706296
- 517 25. Shin N-R, Whon TW, Bae J-W. Proteobacteria: microbial signature of
518 dysbiosis in gut microbiota. *Trends in Biotechnology*. 2015; 33:496-503.
519 doi: doi.org/10.1016/j.tibtech.2015.06.011. PMID: 26210164
- 520 26. Carvalho Frederic A, Koren O, Goodrich Julia K, Johansson Malin EV,
521 Nalbantoglu I, Aitken Jesse D, et al. Transient Inability to Manage
522 Proteobacteria Promotes Chronic Gut Inflammation in TLR5-Deficient
523 Mice. *Cell Host & Microbe*. 2012; 12:139-52.
524 doi:[10.1016/j.chom.2012.07.004](https://doi.org/10.1016/j.chom.2012.07.004). PMID: 22863420
- 525
- 526 27. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al.
527 Cross-talk between *Akkermansia muciniphila* and intestinal epithelium
528 controls diet-induced obesity. *Proceedings of the National Academy of*
529 *Sciences*. 2013; 110:9066-71. doi: [10.1073/pnas.1219451110](https://doi.org/10.1073/pnas.1219451110). PMID:

530 23671105

531 28. Larsen N, Vogensen FK, van den Berg FWJ, Nielsen DS, Andreasen AS,
532 Pedersen BK, et al. Gut Microbiota in Human Adults with Type 2 Diabetes
533 Differs from Non-Diabetic Adults. PLoS ONE. 2010; 5:e9085. doi:
534 10.1371/journal.pone.0009085. PMID: 20140211

535 29. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An
536 obesity-associated gut microbiome with increased capacity for energy
537 harvest. Nature. 2006; 444:1027-131. doi:10.1038/nature05414. PMID:
538 17183312

539 30. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et
540 al. Ultra-high-throughput microbial community analysis on the Illumina
541 HiSeq and MiSeq platforms. The ISME Journal 2012; 6:1621-4.
542 doi:10.1038/ismej.2012.8. PMID: 22402401

543 31. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello
544 EK, et al. QIIME allows analysis of high-throughput community
545 sequencing data. Nat Meth. 2010;7:335-6. doi:10.1038/nmeth.f.303.
546 PMID: 20383131

547 32. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon GI, Knight R, et al.
548 Quality-filtering vastly improves diversity estimates from Illumina
549 amplicon sequencing. Nature Methods. 2013; 10:57-59.
550 doi:10.1038/nmeth.2276. PMID: 23202435

551 33. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et
552 al. Predictive functional profiling of microbial communities using 16S
553 rRNA marker gene sequences. Nature Biotechnology. 2013; 814-21.
554 doi:10.1038/nbt.2676. PMID: 23975157

555 34. Sullivan GM, Feinn R. Using Effect Size—or Why the P Value Is Not
556 Enough. Journal of Graduate Medical Education. 2012; 4(3):279-82.
557 doi: 10.4300/JGME-D-12-00156.1. PMID: 23997866

558

559

560

561

562

563

564

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

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 ⁻²)	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 ⁻¹)	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)
Total Cholesterol (mmol L ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	3.5 (0.61)	3.4 (0.68)	3.6 (0.65) ^a	2.8 (0.74)	2.7 (0.7)	2.7 (0.67) ^a
Glucose (mmol L ⁻¹)	5 (0.68)	5 (0.63)	5.1 (0.46) ^a	6.4 (2)	6.5 (2.2)	6.3 (1.5) ^a
Insulin (uIU ml ⁻¹)	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)
FFA (mmol L ⁻¹)	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 ⁻¹)	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)

606

607

608

609

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.
617

627

628

629

630

631

632

633

634
635
636
637
638
639
640
641
642
643
644
645
646
647
648

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.

649
650
651
652
653

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

	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 ²)	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 ⁻¹)	73 (11)	81 (19)
Total Cholesterol (mmol L ⁻¹)	5.0 (0.8)	4.7 (1.3)
Triglyceride (mmol L ⁻¹)	1.1 (0.4)	2.9 (4.2)
HDL cholesterol (mmol L ⁻¹)	1.1 (0.2)	0.9 (0.2)
LDL (mmol L ⁻¹)	3.4 (0.7)	2.8 (0.9)
Glucose (mmol L ⁻¹)	4.9 (0.6)	6.9 (1.9)
Insulin (uIU ml ⁻¹)	18 (11)	23 (19)
HOMA_IR	4.0 (2.8)	7.4 (6.2)
LBP (ug ml ⁻¹)	25 (8)	29 (7.6)
FFA (mmol L ⁻¹)	0.45 (0.21)	0.54 (0.21)

686

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

	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 ⁻²)	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 ⁻¹)	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)	0.1277	0.2319	0.0731	0.0223
Total Cholesterol (mmol L ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	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

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

	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)				

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

	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
KEGG Functions										
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