

# Heterogeneity of gut microbial responses in healthy household dogs transitioning from an extruded to a mildly cooked diet

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## ABSTRACT

**Background:** The gut microbiota (GM) is associated with canine health and can be impacted by diet. Dog owners in the U.S. have increasingly shown an interest in feeding their dogs a mildly cooked (MC) diet. However, its impact on canine GM and health remains largely unknown.

**Methods:** Healthy household dogs were tracked upon switching from various brands of extruded to MC diets for four weeks. A health assessment was completed and stool samples were collected by each owner before (day 0) and after the diet transition (day 28). Shotgun metagenomic sequencing was performed at both time points to characterize the GM.

**Results:** Dogs completed the study by either completing the health assessments ( $n = 31$ ) or providing stool samples at both time points ( $n = 28$ ). All owners reported either better or no change in overall health at the end of the study (61% and 39%, respectively), and none reported worse overall health. Defecation frequency was also reported to be lower (58%) or about the same (35%). Principal coordinate (PCo) analysis showed a significant shift ( $p = 0.004$ ) in the  $\beta$ -diversity of the GM upon diet transition (34.2% and 10.3% explained by the first two axes). The abundances of 70 species increased after the diet change (adjusted  $p < 0.05$ ), 67% and 24% of which belonged to the Lactobacillales and the Enterobacterales orders respectively.

The abundances of 28 species decreased (adjusted  $p < 0.05$ ), 46%, 18%, and 11% of which belonged to the Clostridiales, Bacillales, and Bacteroidales orders, respectively. Lower Lactobacillales and Enterobacterales, and higher Bacteroidales at baseline were associated with a greater shift along the PCo1 axis. Protein content of the baseline diet was correlated with the shift along the PCo1 axis ( $\rho = 0.67$ ,  $p = 0.006$ ).

**Conclusion:** Owners reported either improvement or no change in health in dogs transitioning from extruded kibble to MC diets for 4 weeks, but this report of health perception requires further exploration in a controlled trial. Diet change also led to a significant shift in the GM profile of healthy dogs. The magnitude of shift was associated with baseline GM and dietary protein, and warrants further examination of individualized responses and personalized nutrition in companion dogs.

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These results also support future investigation of the impact of a MC diet on health maintenance given its increasing popularity.

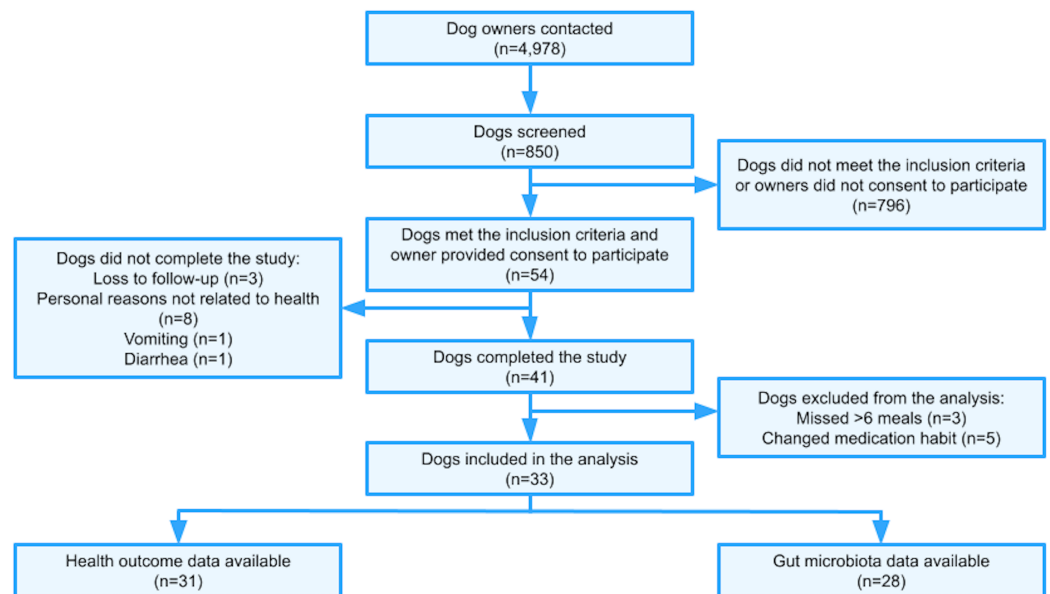
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## INTRODUCTION

For several millennia humans and dogs coevolved, sharing food and shelter (*Axelsson et al., 2013*). However, extruded dry food, commonly known as kibble, remains the most common form of feeding pet dogs in the United States. Extrusion cooking refers to a process where a mixture of ingredients is steam conditioned, compressed, and forced through the die of an extruder (*Tran, Hendriks & Van der Poel, 2008*). Mildly cooked (MC) diets, composed of ingredients that are cooked without extrusion, have been gaining in popularity in recent years among dog owners in the U.S. (*PRWeb, 2015; Algya et al., 2018*). Commercial MC canine diets are increasingly available, and must conform with the American Association Feed Control Officials (AAFCO) nutrient profiles or have been tested in feeding trials for the labeled life stages when complete and balanced. The nutritional profile of a MC diet may or may not differ significantly from dried and extruded pet food. Compared to dried and extruded food, MC diets have higher moisture content, higher digestibility of fats and protein, and dogs fed a MC diet for 28 days had lower levels of triglycerides (*Algya et al., 2018; Oba et al., 2020; Do et al., 2021*). Additionally, positive health perception was reported by dog owners after feeding their dogs a veterinarian-formulated MC diet (*Johnson et al., 2016*). However, the nutritional contents of MC diets and their effect on overall canine health remain understudied compared to other types of commercial dog foods.

Dietary changes are generally accompanied by alterations of the gut microbiota (GM) (*Wernimont et al., 2020*), which can have profound impact on canine health and disease (*Redfern, Suchodolski & Jergens, 2017; Pilla & Suchodolski, 2019*). Many studies have shown microbiome compositional changes upon switching to raw food diets, which are often higher in protein and fat and lower in carbohydrates (including fiber) than kibble diets (reviewed in (*Wernimont et al., 2020*)). These studies have shown that raw food diets lead to increased levels of certain Fusobacteria and Proteobacteria (*Sandri et al., 2017; Algya et al., 2018; Schmidt et al., 2018*). Similarly, MC diets have been associated with higher levels of certain Bacteroidetes, Fusobacteria and Proteobacteria, and lower levels of certain Actinobacteria and Firmicutes as compared to an extruded kibble diet (*Algya et al., 2018; Do et al., 2021*). However, these studies were conducted in only a small group of dogs under a laboratory setting. Thus, the extent that a MC diet influences the gut microbiomes of real world pet dogs with heterogeneous characteristics such as age, breeds, living conditions, and body size remains unexplored.

An investigation of the effect of dietary transition to a MC diet on GM and owner-reported subjective health metrics in healthy dogs was carried out to improve understanding of the effects of this increasingly popular diet type. Dogs switched from an



**Figure 1** Trial flowchart.

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extruded kibble to a MC diet for four weeks were assessed for changes relative to baseline in: (1) the gut microbiome by profiling the taxonomic and functional compositions of feces; and (2) perceived health outcomes using an owner-reported survey. Due to the variety of differences between diets, we hypothesized we would observe a shift in the diversity and abundance of GM upon diet change. Enrolled dogs were healthy at recruitment, however we aimed to determine if any additional positive impacts were reported by owners, including outcomes likely to change based on the literature for similar diets (e.g fecal output).

## MATERIALS AND METHODS

Dog owners gave electronic informed consent to the use of their dog's non-identifying data for scientific study prior to study enrollment. All dog owners willingly underwent diet transition regardless of study participation. As previously explained (*Tanprasertsuk et al., 2021*), since fecal samples were collected non-invasively by the dog owners, no institutional animal ethical review was required.

### Animals and study design

A total of 4,978 dog owners were contacted via email to gauge interest in study participation (Fig. 1). All owners were incoming customers of NomNomNow, Inc. (a direct-to-consumer pet food and health company in Nashville, TN, USA), who intended to switch their dog's diet to a MC diet regardless of study participation. A total of 850 dogs of various breeds were screened using an online questionnaire; 54 dogs met the inclusion criteria described below and were enrolled in the study.

Dogs were eligible to participate in the study if they met the following owner-assessed criteria at enrollment: aged 1–14 years, body condition score (BCS) of 4–6 on the 9-point

scale (*Laflamme, 1997*), currently feeding two meals per day exclusively of an extruded kibble diet with treats comprising  $\leq 10\%$  of overall caloric intake, absent of gastrointestinal (GI) issues (including chronic diarrhea/vomiting or diarrhea/vomiting within the previous 30 days), absent of any infections or major chronic diseases (pancreatitis, diabetes, kidney disease or failure, liver disease, heart disease, cancer, severe GI issues when young (<6 months old), and not pregnant or lactating). Only one dog per household was permitted to enroll. Dogs were not eligible to participate if they had consumed any prebiotics, probiotics, cultured foods, or antibiotics within the last three months, had a significant change in their diet within the last month, or had surgery within the previous three months. The criteria were similar to those previously described (*Tanprasertsuk et al., 2021*).

Participants were asked to provide the brand and formula of the baseline extruded kibble diet, and guaranteed analysis of nutrients was assembled when possible. Online health assessments were administered and stool samples were collected by owners one day before the diet transition (day 0) and again four weeks after the diet transition (day 28). On day 1, dogs began the transition from their previous extruded diet to a MC diet. An email reminder was sent out to all dog owners weekly to ensure adherence to the study diet and maintenance of baseline habits (supplements, medication, and exercise), as well as provide owners with the opportunity to report any observed adverse effects.

### **Dietary interventions**

Dog owners chose one to four commercially-available Nom Nom recipes as their study MC diet (Beef, Chicken, Pork, Turkey). Mildly cooking includes cooking techniques designed to reach internal temperatures for reduction of pathogens but without canning or extrusion, and the term is used to describe similar diets in a previous study (*Algya et al., 2018*). However, unlike the MC diet in the referenced paper, the protein sources were not ground and emulsified, but mixed into a homogenous blend with vitamin and mineral supplements. After cooking, the diets were then refrigerated or frozen and thawed prior to feeding. Dietary information for participants who completed the study is summarized in [Fig. S1](#). The ingredients and guaranteed nutrient analysis of each recipe are available in [Table 1](#). All recipes were formulated to meet AAFCO standards for all life stages. All dog owners were instructed to feed two MC meals daily from day 1 to the completion of the study on day 28. The meal portion for each dog was calculated using a proprietary custom-portioning algorithm which takes owner-reported ideal body weight and physical activity level into account. The amount of food not consumed was also reported at the end of the study to account for dietary adherence.

### **Health assessment**

An online health assessment was completed by each owner prior to diet transition (day 0). The assessment was composed of five broadly targeted questionnaires containing questions about signalment, overall wellness, diet and lifestyle, medical history, oral health, and behavior, and was previously used in a research study (*Jha et al., 2020*). Questions included single-choice checkbox, multiple-choice radio-button, dropdown, and fill-in-the-

**Table 1** Guaranteed analysis and ingredients of each mildly cooked recipe.

Recipe	Guaranteed analysis				
	Calories, kcal/kg FW	Crude protein, min %AF	Crude fat, min %AF	Crude fiber, max %AF	Moisture, max %AF
Beef	1,239	10	5	1	73
Chicken	1,255	8.5	6	1	77
Pork	1,246	8	5	2	75
Turkey	1,479	11	5	1	70
Recipe	Ingredients				
Beef	Ground beef, russet potatoes, eggs, carrots, peas, dicalcium phosphate, calcium carbonate, salt, fish oil, sunflower oil, vinegar, citric acid (preservative), taurine, choline bitartrate, zinc gluconate, ferrous sulfate, vitamin E supplement, copper gluconate, manganese gluconate, thiamine mononitrate (vitamin B1), selenium yeast, riboflavin (vitamin B2), vitamin B12 supplement, cholecalciferol (source of vitamin D3), potassium iodide				
Chicken	Diced chicken, sweet potatoes, yellow squash, spinach, sunflower oil, canola oil, dicalcium phosphate, calcium carbonate, fish oil, vinegar, citric acid, taurine, choline bitartrate, zinc gluconate, ferrous sulfate, vitamin E supplement, copper gluconate, manganese gluconate, thiamine mononitrate (vitamin B1), selenium yeast, riboflavin (vitamin B2), vitamin B12 supplement, cholecalciferol (source of vitamin D3), potassium iodide				
Pork	Ground pork, russet potatoes, green beans, yellow squash, kale, brown mushrooms, dicalcium phosphate, salt, fish oil, vinegar, citric acid, taurine, choline bitartrate, zinc gluconate, ferrous sulfate, vitamin E supplement, copper gluconate, manganese gluconate, thiamine mononitrate (vitamin B1), selenium yeast, riboflavin (vitamin B2), vitamin B12 supplement, cholecalciferol (source of vitamin D3), potassium iodide				
Turkey	Ground turkey, brown rice, eggs, carrots, spinach, dicalcium phosphate, calcium carbonate, salt, fish oil, vinegar, citric acid, taurine, choline bitartrate, zinc gluconate, ferrous sulfate, vitamin E supplement, copper gluconate, manganese gluconate, thiamine mononitrate (vitamin B1), selenium yeast, riboflavin (vitamin B2), vitamin B12 supplement, cholecalciferol (source of vitamin D3), potassium iodide				

**Note:**

FW: fresh weight, AF: as fed.

blank questions. A second set of online assessment questions was administered to assess change in health outcomes from baseline as perceived by the owner on day 28. The questions are written verbatim in [Table S1](#). A visual stool chart was used to assess the fecal quality ([Lewis & Heaton, 1997](#)), and a previously validated 9-point system was used to assess the BCS ([Laflamme, 1997](#)).

### Stool sample collection and DNA extraction

Stool samples from each dog were collected by owners with Nom Nom Plus Microbiome Testing Kits (NomNomNow, Inc., Nashville, TN, USA) at baseline (day 0) and four weeks after the diet transition (day 28). The kits were previously used in research studies ([Jha et al., 2020](#); [Tanprasertsuk et al., 2021](#)). All stool samples were processed and sequenced in a single batch at Diversigen, Inc. (New Brighton, MN, USA). DNA extraction and library construction protocols were performed as previously described ([Johnson et al., 2019](#); [Tanprasertsuk et al., 2021](#)), with the exception that DNA was extracted using the Zymogen Quick-DNA Fecal/Soil Microbe 96 Mag Bead kit (Zymo Research, Irvine, CA, USA) using Powerbead Pro (Qiagen, Redwood City, CA, USA) plates with 0.5 and 0.1 mm ceramic beads. Extraction controls included a no template control (water) and a characterized homogenized stool. All samples were quantified with Quant-iT Picogreen dsDNA Assay (Invitrogen, Carlsbad, CA, USA). Subsequently, DNA amplification and

library construction were performed with the Nextera XT DNA Library Preparation Kit (Illumina Inc, Foster City, CA, USA).

### **GM shotgun metagenomic sequencing and annotation**

Whole genome shotgun sequence reads used for this study are available on the NCBI Short Read Archive associated with the NCBI BioProject [PRJNA714112](#). Shotgun metagenomic sequencing was performed at Diversigen, Inc. (New Brighton, MN, USA) using their Boostershot shallow metagenomic sequencing service (2 million reads/sample), as previously described ([Johnson et al., 2019](#)). For quality control, single-end shotgun reads were trimmed and processed using Shi7 ([Al-Ghalith et al., 2018](#)). The sequences were then aligned to the NCBI RefSeq representative prokaryotic genome collection at 97% identity using BURST with default settings ([Al-Ghalith & Knights, 2017](#)). A filtering step was performed to remove taxa present in <5% of the samples and those belonging to unknown phyla. The resulting taxonomy table was also aggregated at higher taxonomy levels (for example, species, genus, family). After filtering, a total of 959 taxa were used for the analysis ([Fig. S2](#)). The average sequencing depth was  $2,481,073 \pm 909,748$ , and the depth per sample ranged from 476,329 to 4,819,202 reads. It has been shown that this particular method of low-depth metagenomic sequencing can reach species-level taxonomic assignment and allows for the assessment of functional profiles ([Hillmann et al., 2018](#)).

### **Functional annotation**

Sequenced reads were matched directly using alignment at 97% identity to the Kyoto Encyclopedia of Genes and Genomes Orthology groups (KEGG KOs) derived from the NCBI RefSeq representative prokaryotic genome collection. A filtering step was performed to remove KO terms present in <5% of the samples and those whose absolute count was  $\leq 1$ . After 401 KO terms were removed, 4,216 terms were used in subsequent analyses.

### **Statistical analysis**

Continuous variables are expressed as mean  $\pm$  SD, unless specified as mean  $\pm$  SEM. GM relative abundances are expressed as median (IQR). Categorical variables are presented as count (%). All analyses were performed using R Studio version 1.2.5033. Statistical significance level was set at  $\alpha = 0.05$ .

### **Analysis of health outcomes**

Wilcoxon signed rank test (continuous variables) and a Fisher's exact test (categorical variables) were performed to compare the observed values with the expected values under the null hypothesis (equal ratios of subjects in each category for categorical variables, and change equal to zero for continuous variables). Following the Fisher exact test, the Benjamini Hochberg method was performed to control the false discovery rate (FDR) due to multiple comparisons among categories of responses.



### ***Analysis of GM diversity, abundance, function***

Similar to the approach previously described ([Jha et al., 2020](#)), species richness and Shannon's diversity indices were computed by rarefying the samples to various depths starting from 10,000–470,000 sequences per sample by increasing the sequencing depth by 10,000 reads. One hundred iterations were performed at each depth and mean values were used as the estimate of these measures in each sample. Wilcoxon signed rank test was used to compare changes of alpha diversity metrics (evenness, diversity indices) from baseline to week 4.

The non-rarefied count data were log-transformed and principal coordinate analysis (PCoA) was performed using Bray–Curtis dissimilarity calculated with the vegan package at the species level ([Oksanen et al., 2014](#); [Tanprasertsuk et al., 2021](#)). Using the vegan package, permutational multivariate analysis of variance (PERMANOVA) was performed using Bray–Curtis dissimilarity with 10,000 randomizations by including timepoints to assess the differences in community composition ([Oksanen et al., 2014](#)). Partitioning around the medoids (PAM) Clustering was also performed to investigate the dissimilarity of bacterial profiles in samples collected at two different time points using the cluster package ([Maechler et al., 2015](#)). Fisher's exact test was performed to investigate the association between cluster membership and timepoint. Kruskal–Wallis test with Dunn's post hoc test, Wilcoxon signed rank test, and McNemar's Chi-squared test were performed to compare various measures among different groups as specified throughout the manuscript.

Differential abundances of bacterial taxa between timepoints were assessed at each taxonomic level using a negative binomial generalized linear model (GLM), using the differential expression analysis for sequence count data version 2 package (DESeq2) ([Love, Huber & Anders, 2014](#); [Jha et al., 2020](#)). Taxa with absolute  $\log_2$ (fold change (FC)) > 2 and FDR-adjusted  $p$ -values < 0.05 were considered significant.

To investigate the dissimilarity of bacterial profiles among subjects at baseline, we utilized the same statistical methods described above (PCoA using Bray–Curtis dissimilarity, PAM Clustering, negative binomial GLM with DESeq2). Additionally, random forest classifiers were constructed using repeated k-fold cross-validation and random search implemented in the R-package caret ([Khun, 2014](#)). As previously described ([Jha et al., 2020](#)), the data were randomly divided into training and validation sets containing 70% and 30% of the samples, respectively, and bacterial species were used as the predictors. The models were trained by optimizing the tuning parameters using 10-fold cross-validation repeated three times, and accuracy was used to select the optimal model. The performance of the classifiers was assessed by generating area under the receiver operating characteristic curves using the R-package ROCR ([Sing et al., 2005](#)).

Similarly, the non-rarefied count data of KO terms were log-transformed and PCoA was performed using the Bray–Curtis dissimilarity as described above. A negative binomial GLM using the DESeq2 was performed to identify differentially abundant KO terms between the two time points. The identified KO terms were used for KEGG pathway mapping against the PATHWAY database ([Kanehisa & Sato, 2020](#)).

### **Correlation analysis**

Correlation analysis was performed to investigate the relationship between: (1) the PCo1 score and the abundance of Enterobacterales and Lactobacillales at baseline; and (2) the PCo1 score and the macronutrient composition in the baseline extruded diet. Spearman's correlation coefficients are reported.

## **RESULTS**

### **Cohort description**

Of 54 dogs initially enrolled, 13 did not complete the study due to the following reasons: vomiting ( $n = 1$ ), diarrhea ( $n = 1$ ), loss to follow-up ( $n = 3$ ), and owners' personal reasons not related to dog health ( $n = 8$ ) (Fig. 1). No major adverse effect was reported. Eight cases (14.8%) of diarrhea and nine cases (16.7%) of vomiting were reported in those who enrolled, regardless of study completion. The rates of diarrhea and vomiting were 1 case per 27 dog-weeks and 1 case per 24 dog-weeks, respectively.

The remaining 41 participants completed the study by either filling out the online health assessment, collecting a stool sample, or both. Among those who completed the study, three dogs missed >6 meals of the study diet and five dogs changed their medication habit. These included four cases of antibiotic use (two cases of urinary tract infection, one case of diarrhea, one case of common cold) and one case of Prednisone use (to treat a physical injury not related to the study). After the exclusion of these eight dogs, data for 33 dogs were available for subsequent analyses ( $n = 5$  only completed the online health assessment,  $n = 2$  only provided fecal samples,  $n = 26$  provided both). In other words, of the 33 dogs that completed the study, we included 31 dogs for health data analysis and 28 for the microbiome data analysis.

### **Subject characteristics**

Characteristics of the 31 dogs with health assessment data and the 28 dogs with fecal samples are described in Table 2. The wide distribution of body weights reflect the diversity of breeds. Age, sex, weight, and breed information for each individual dog is available in Table S2. The baseline extruded kibble diets were diverse in terms of ingredients and nutrient profiles (data not shown). Reported fecal consistency scores were on average between 3 (sausage-shaped with cracks on surface) and 4 (sausage-shaped or snake like, smooth and soft).

### **Health outcomes**

Dog owners' perceptions of changes in health outcomes 28 days after the diet transition are listed in Table 3. Of 31 dog owners, 19 (61%) reported better overall health, 15 (48%) reported higher physical activity, 15 (48%) reported improved coat condition, and 19 (61%) reported higher appetite. Post-hoc pairwise comparisons showed the proportion of owners that reported no change in these outcomes was not significantly different from those that reported an improvement. None of the dog owners reported worsened overall health, coat condition, or lower physical activity level after transitioning from extruded kibble to MC diet. Furthermore, 18 (58%) reported lower defecation frequency. Fecal score



**Table 2** Baseline characteristics of 33 dogs whose owner provided survey data ( $n = 31$ ) or fecal samples ( $n = 28$ ).

Feature	Health assessment data ( $n = 31$ )	Fecal samples ( $n = 28$ )
Age, in years	4.8 ± 3.6	5.2 ± 3.7
Male	16 (52%)	14 (50%)
Spayed or neutered <sup>1</sup>	24 (83%)	24 (86%)
Body weight, in kg	10.2 ± 8.4	10.7 ± 8.7
Ideal body weight, in kg	10.0 ± 8.2	10.5 ± 8.5
Body condition score <sup>1</sup>		
4–5	20 (77%)	20 (74%)
6	6 (23%)	7 (26%)
Physical activity level <sup>1</sup>		
Very active	2 (7%)	2 (7%)
Active	8 (30%)	8 (30%)
Average active	8 (30%)	6 (22%)
Somewhat active	8 (30%)	10 (37%)
Not active	1 (4%)	1 (4%)
Overall health <sup>1</sup>		
Excellent	17 (63%)	17 (63%)
Good	9 (33%)	8 (30%)
Fair	1 (4%)	2 (7%)
Poor	0 (0%)	0 (0%)
Bad	0 (0%)	0 (0%)
Food motivation <sup>1</sup>		
Very motivated	12 (44%)	11 (41%)
Somewhat motivated	11 (41%)	12 (44%)
Average motivated	1 (4%)	1 (4%)
Mildly motivated	1 (4%)	1 (4%)
Not at all motivated	2 (7%)	2 (7%)
Fecal consistency score <sup>1</sup>	3.1 ± 1.3	3.0 ± 1.2
Defecation frequency <sup>1</sup>		
≥ 3 times/day	7 (26%)	8 (30%)
2 times/day	18 (67%)	17 (63%)
1 time/day	2 (7%)	2 (7%)
Flatulence frequency <sup>1</sup>		
Multiple times per day	1 (4%)	1 (4%)
Daily	5 (19%)	5 (19%)
Once in a while	11 (41%)	11 (41%)
Never	10 (37%)	10 (37%)

**Notes:**

<sup>1</sup> Spayed/neutered, body condition, physical activity level, overall health, food motivation, fecal score, defecation frequency, and flatulence frequency data were not available in all dogs. Spayed/neutered was not available in 2 dogs in the first column. Body condition score and fecal consistency score were not available in 4 dogs in the first column and 1 dog in the second column.

Data are expressed as mean ± SD or  $n$  (%).

**Table 3** Health outcomes 28 days after dietary intervention compared to baseline ( $n = 31$ ).

Outcome	$n$ (%) or mean $\pm$ SD	$P$ -value <sup>1</sup>
Overall health		<0.001
Better	19 (61%) <sup>a</sup>	
About the same	12 (39%) <sup>a</sup>	
Worse	0 (0%) <sup>b</sup>	
Physical activity		0.001
More active	15 (48%) <sup>a</sup>	
About the same	16 (52%) <sup>a</sup>	
Less active	0 (0%) <sup>b</sup>	
Fecal consistency score		
Post-intervention	2.5 $\pm$ 0.9	
Change from baseline <sup>2</sup>	-0.6 $\pm$ 1.6	0.091
Defecation frequency		0.020
Higher	2 (6%) <sup>a</sup>	
No effect	11 (35%) <sup>a,b</sup>	
Lower	18 (58%) <sup>b</sup>	
Flatulence frequency		0.016
Higher	2 (6%) <sup>a</sup>	
No effect	19 (61%) <sup>b</sup>	
Lower	10 (32%) <sup>a,b</sup>	
Coat condition		0.001
Better	15 (48%) <sup>a</sup>	
No effect	16 (52%) <sup>a</sup>	
Worse	0 (0%) <sup>b</sup>	
Appetite		0.005
Increased	19 (61%) <sup>a</sup>	
No effect	11 (35%) <sup>a</sup>	
Decreased	1 (3%) <sup>b</sup>	
Body weight change, in %	0.8 $\pm$ 9.0	0.536

**Notes:**

<sup>1</sup> Fisher exact test (categorical variables) and Wilcoxon signed-rank test (continuous variables) were performed to compare the observed data with the expected data under the null hypothesis (equal ratios of subjects in each category for categorical variables and change equal to zero for continuous variables). Those that do not share superscripts (a and b) are significantly different in pairwise comparisons with false discovery rate (FDR) adjustment.

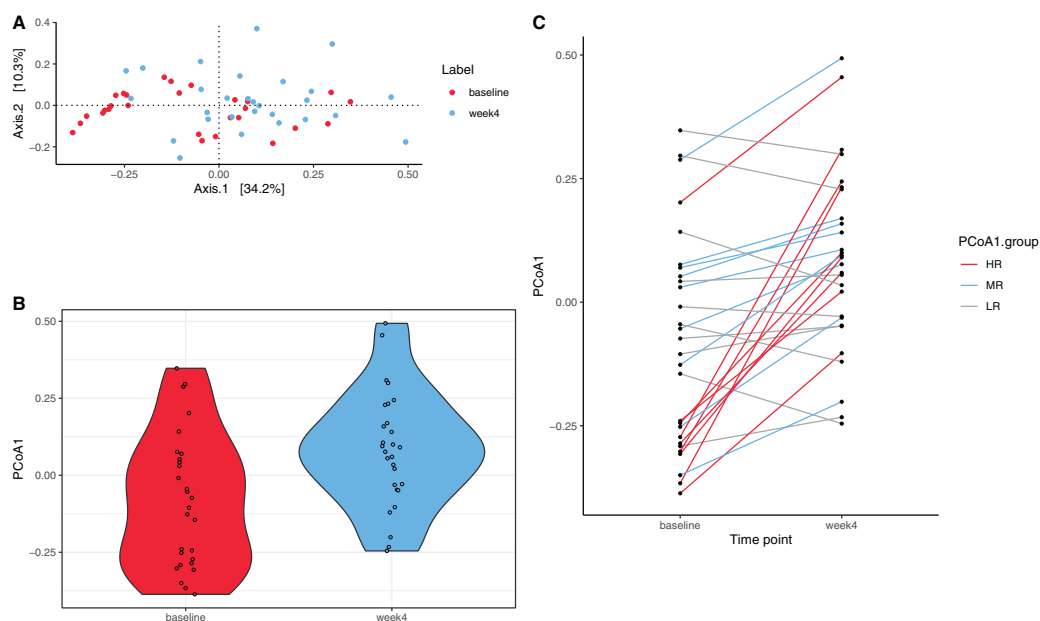
<sup>2</sup> Data not available for 4 subjects.

showed a small decrease from  $3.0 \pm 1.3$  to  $2.5 \pm 0.9$  after diet transition but it was not statistically significant ( $p = 0.091$ ). Body weight on average was maintained throughout the study period.

## Gut microbiome

### Diversity

To investigate the effect of diet transition on GM  $\alpha$ -diversity, we calculated two standard  $\alpha$ -diversity measures, species richness and Shannon's diversity index. A rarefaction curve generated for sequencing depth 10,000–470,000 reads demonstrated sufficient



**Figure 2** Species level principal coordinate analysis (PCoA). (A) PCo1 (Axis 1) and PCo2 (Axis 2) respectively explain 34.2% and 10.3% of species abundance variance (56 samples from 28 dogs). PERMANOVA using Bray–Curtis distance shows significant spatial separation between baseline (red) and week 4 (blue) ( $p = 0.004$ ). (B) A significant shift along the PCo1 axis from baseline (red) to week 4 (blue) is observed ( $p = 1.72E-04$ , Wilcoxon signed rank test). (C) Varying degrees of shift along the PCo1 axis are observable from baseline to week 4. Subjects were divided into the first tertile labeled high-responders (HR, maximal PCo1 score increase, red,  $n = 9$ ), those in the second tertile mid-responders (MR, blue,  $n = 9$ ), and those in the third tertile low-responders (LR, PCo1 score decrease or minimal PCo1 score increase, gray,  $n = 10$ ).

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sequencing depth to assess the alpha diversity (Figs. S3A and S3B). Using sequencing depth at 470,000 reads, these  $\alpha$ -diversity measures were not significantly different between baseline and four weeks after diet change ( $p > 0.05$ , Wilcoxon signed rank test).

PCoA was performed to investigate changes in  $\beta$ -diversity. The first two axes (PCo1 and PCo2), which respectively explained 34.2% and 10.3% of variation at the species level (Fig. 2A.) Eigenvalues for the first 25 PCo axes are displayed in Fig. S4. Spatial separation between the two timepoints along the first two PCo axes was demonstrated ( $p = 0.004$ , PERMANOVA Bray–Curtis dissimilarity matrices). A shift in PCo1 score, but not PCo2-5 scores, was also observed after 4 weeks of diet change ( $p = 1.72E-04$ , Wilcoxon signed rank test, Fig. 2B).

Using PAM Clustering analysis to investigate changes in  $\beta$ -diversity, the 56 samples (two samples from each of the 28 dogs) could be partitioned into two clusters (Fig. S5). The first cluster (C1) consisted primarily of baseline samples (15 samples collected at baseline and five samples collected at week 4), while the second cluster (C2) consisted of 13 samples collected at baseline and 23 samples collected at week 4. A significantly higher proportion of subjects had their baseline samples assigned to C1 and their week 4 samples assigned to C2 than the null hypothesis ( $p = 0.009$ , McNemar's Chi-squared test). This

result further confirms a significant shift in GM composition occurred due to dietary shift from extruded kibble to MC in these participants, although the presence of 13 baseline samples in C2 also indicates heterogeneous responses to diet change.

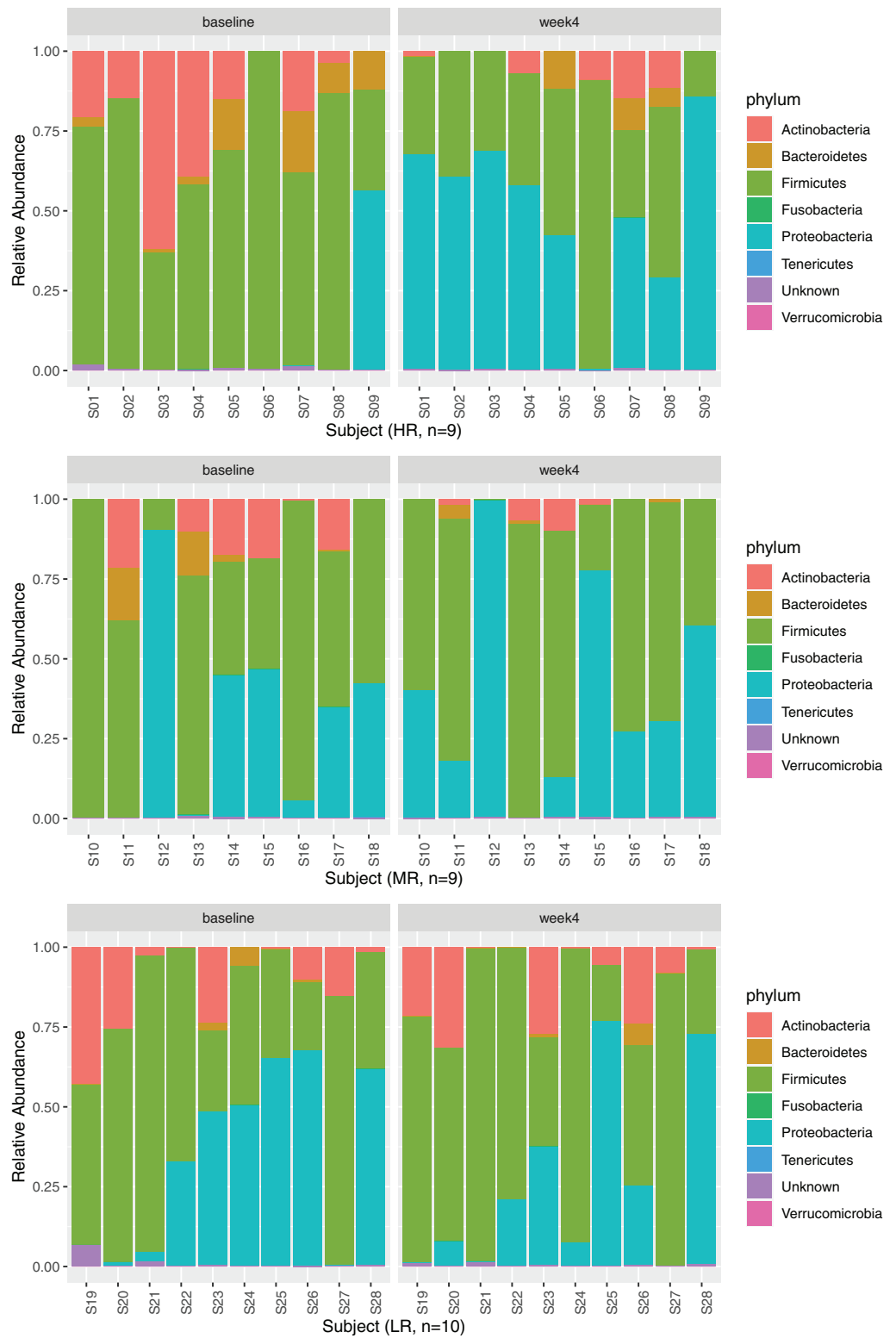
### **Relative abundances**

Four phyla, Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes, dominated the GM (Fig. 3, 28 subjects are displayed in three separate rows based on the magnitude of MB response as explained in section Identification of high-, mid- and low-responders). No significant changes in relative abundances were observed between the timepoints at phylum or class levels after diet change (adjusted  $p > 0.05$ , negative binomial GLM with DESeq2). The relative abundance of the order Micrococcales significantly increased after the diet change (Table S3). At the family level, the relative abundances of four families significantly increased at week 4 (Leuconostocaceae, Hafniaceae, Lactobacillaceae, and Morganellaceae), while four families significantly decreased after 4 weeks of diet change (Prevotellaceae, Sutterellaceae, Atopobiaceae and Microbacteriaceae).

The effect of diet change was more pronounced at the genus and species levels. Relative abundances of 16 genera increased and 12 genera decreased significantly after the diet change (Table S3). Of the genera that increased, nine (56%) belong to the Enterobacterales order (*Hafnia*, *Raoultella*, *Morganella*, *Proteus*, *Edwardsiella*, *Erwinia*, *Lelliottia*, *Yersinia*, *Leclercia*), and seven (44%) belong to the Lactobacillales order (*Leuconostoc*, *Tetragenococcus*, *Lactobacillus*, *Weissella*, *Carnobacterium*, *Pediococcus*, *Lactococcus*). Half of the 12 decreasing genera were in the Bacillales order (*Rummeliibacillus*, *Brevibacillus*, *Aneurinibacillus*, *Thermobacillus*, *Lysinbacillus*, *Bacillus*). The relative abundances of 70 bacterial species significantly increased after the diet change (Fig. 4, Table 4). Among these species, 47 (67%) belong to the Lactobacillales order (also known as lactic acid bacteria or LAB), and 17 (24%) belong to the Enterobacterales order. Meanwhile, the relative abundances of 28 species significantly decreased, 13 (46%) of which belong to the Clostridiales order, 5 (18%) of which belong to the Bacillales order, and 3 (11%) of which belong to the Bacteroidales order.

### **Gut microbiome functionality**

A total of 4,617 KO terms from all samples were identified, and after filtering 4,216 remained for subsequent analyses. PCoA was performed to investigate changes between time points. Fig. 5A shows the first two principal coordinate axes (which respectively explain 67.1% and 11.3% of the variation) and the eigenvalues for the first 25 axes are shown in Fig. S6. Spatial separation between the two timepoints along the first two PCoA axes was demonstrated ( $p = 0.007$ , PERMANOVA using the Bray–Curtis dissimilarity matrices). A significant shift in the PCo1 score after 4 weeks of diet change was observed ( $p = 0.013$ , Wilcoxon signed rank test, Fig. 5B). As listed in Table 5, a significant increase in the abundances of 14 KO terms was observed using a negative binomial GLM, while the abundances of 11 KO terms significantly decreased. Pathway mapping demonstrated differential abundances of pathways including the biosynthesis of secondary metabolites (ko01110: K02203, K13548, K16011), amino sugar and nucleotide sugar

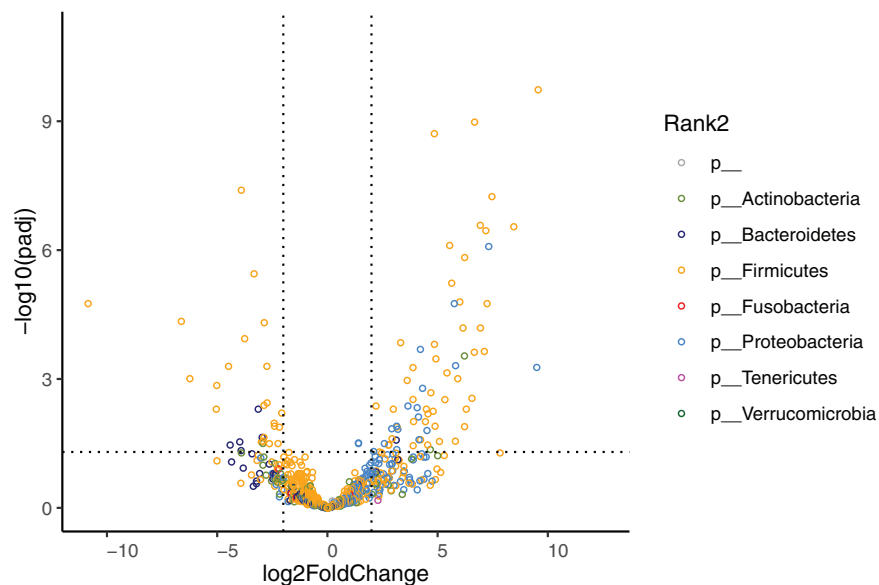


**Figure 3** Relative abundances at phylum level at baseline and week 4. The phyla Firmicutes (baseline: 59.18 (36.39–77.09)%, week 4: 49.59 (31.00–77.05)%), Proteobacteria (baseline: 2.12 (0.06–46.75)%, week 4: 33.67 (11.35–61.92)%), Actinobacteria (baseline: 12.59 (0.34–19.35)%, week 4: 1.67 (0.05–9.23)%), and

**Figure 3** (continued)

Bacteroidetes (baseline: 0.39 (0.03–3.79)%, week 4: 0.05 (0.00–0.88)%) dominated the GM at baseline and at week 4. Data are displayed separately for high- (HR,  $n = 9$ ), mid- (MR,  $n = 9$ ), and low-responders (LR,  $n = 10$ ) (see section Identification of high-, mid- and low-responders for the definition of HR, MR and LR).

Full-size  DOI: [10.7717/peerj.11648/fig-3](https://doi.org/10.7717/peerj.11648/fig-3)



**Figure 4** Volcano plot demonstrating fold-change (FC) in species abundance at week 4 compared to baseline. Vertical dashed lines represent log<sub>2</sub>FC at 1 and -1 (i.e. FC at 2 and -2), horizontal dashed line adjusted  $p = 0.05$ . Adjustments of  $p$  values were performed with false discovery rate. Points are colored by phylum.

Full-size  DOI: [10.7717/peerj.11648/fig-4](https://doi.org/10.7717/peerj.11648/fig-4)

metabolism (ko00520: K13019, K16011, K17716), O-antigen nucleotide sugar biosynthesis (ko00541: K13019, K16011, K17716), and microbial metabolism in diverse environments (ko01120: K00385, K02203, K16165).

## Differential gut microbiome responses to diet transition

### Identification of high-, mid- and low-responders

While a significant shift in PCo1 score ( $0.16 \pm 0.20$ ) was observed across all subjects over the four week period, there were interindividual differences in the magnitude of the shift along PCo1 (Fig. 2C). Given this wide range of responses, subjects were further divided into tertiles based on the magnitude of PCo1 score change to examine the differential response. Those with the highest magnitude of positive change were termed high-responders (HR,  $n = 9$ ), those in the second tertile mid-responders (MR,  $n = 9$ ), and those in the third tertile low-responders (LR,  $n = 10$ ). PCo1 score was significantly different among HR, MR, and LR at baseline ( $p = 0.017$ , Kruskal–Wallis test), but not at week 4 ( $p = 0.082$ , Kruskal–Wallis test). PCo1 score at baseline in HR was significantly lower than that of MR (FDR-adjusted  $p = 0.035$ , Dunn’s post hoc test) and LR (FDR-adjusted  $p = 0.025$ , Dunn’s post hoc test).



Table 4 Species with differential abundances between baseline and week 4 ( $n = 28$ ,  $\log_2[\text{fold change}] \geq 2$  and FDR-adjusted  $p < 0.05$ ).

Phylum	Class	Order	Family	Genus	Species	Relative abundance (%)		Week 4 vs Baseline	
						Baseline Median (IQR)	Week 4	Log <sub>2</sub> FC mean $\pm$ SE	Adjusted P -value <sup>1</sup>
<b>Increased at week 4 (70 species)</b>									
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	algidus	0.00E+00 (0.00E+00 to 1.64E-04)	3.54E-03 (1.48E-04 to 1.50E-01)	12.53 $\pm$ 1.11	1.12E-26
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	gelidum	5.01E-05 (0.00E+00 to 2.71E-04)	8.20E-03 (3.38E-04 to 1.43E+00)	11.90 $\pm$ 1.17	9.78E-22
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	sakei	2.62E-05 (0.00E+00 to 3.44E-04)	3.00E-03 (5.95E-05 to 7.40E-02)	11.61 $\pm$ 1.16	3.24E-21
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	fuchuensis	0.00E+00 (0.00E+00 to 5.26E-05)	1.12E-04 (0.00E+00 to 4.20E-03)	9.56 $\pm$ 1.34	1.85E-10
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Morganellaceae	Proteus	no data	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.06E-05)	9.50 $\pm$ 2.25	5.37E-04
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	carnosum	0.00E+00 (0.00E+00 to 1.80E-05)	3.22E-05 (0.00E+00 to 2.10E-02)	8.46 $\pm$ 1.43	2.85E-07
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	curvatus	0.00E+00 (0.00E+00 to 2.06E-04)	8.89E-04 (5.95E-05 to 1.83E-02)	7.46 $\pm$ 1.21	5.66E-08
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Morganellaceae	Proteus	mirabilis	0.00E+00 (0.00E+00 to 5.66E-05)	7.21E-05 (0.00E+00 to 1.79E-03)	7.32 $\pm$ 1.29	8.23E-07
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	siliginis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.18E-03)	7.24 $\pm$ 1.43	1.76E-05
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	no data	0.00E+00 (0.00E+00 to 1.11E-04)	1.52E-03 (0.00E+00 to 3.58E-02)	7.19 $\pm$ 1.23	3.52E-07
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	kimchii	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 2.26E-03)	7.12 $\pm$ 1.60	2.28E-04
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	sanfranciscensis	0.00E+00 (0.00E+00 to 9.13E-05)	1.94E-04 (0.00E+00 to 6.55E-03)	6.94 $\pm$ 1.46	6.48E-05
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	pseudomesenteroides	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 7.94E-04)	6.94 $\pm$ 1.17	2.64E-07
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	citreum	2.35E-05 (0.00E+00 to 2.98E-04)	7.62E-04 (2.16E-04 to 2.33E-02)	6.68 $\pm$ 0.97	1.04E-09
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	fallax	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 7.02E-04)	6.67 $\pm$ 1.50	2.38E-04
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Tetragenococcus	halophilus	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 8.40E-05)	6.56 $\pm$ 1.74	2.81E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	saniviri	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 7.28E-05)	6.30 $\pm$ 1.77	5.03E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	plantarum	4.21E-05 (0.00E+00 to 2.57E-04)	1.37E-04 (0.00E+00 to 1.90E-03)	6.23 $\pm$ 1.12	1.48E-06

(Continued)

Table 4 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance (%)		Week 4 vs Baseline	
						Median (IQR)	Baseline	Week 4	Log <sub>2</sub> FC mean ± SE
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	parlamentarius	0.00E+00 (0.00E+00 to 4.38E-05)	0.00E+00 (0.00E+00 to 1.61E-05 (0.00E+00 to 1.75E-04)	6.22 ± 1.91	1.28E-02
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	no data	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 2.25E-04)	6.22 ± 1.42	2.90E-04
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	hellenica	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 6.72E-04)	6.15 ± 1.29	6.48E-05
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	cibaria	0.00E+00 (0.00E+00 to 6.30E-05)	0.00E+00 (0.00E+00 to 2.25E-04 (0.00E+00 to 2.10E-03)	6.01 ± 1.18	1.61E-05
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	coryniformis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 3.87E-04)	5.91 ± 1.46	9.81E-04
Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Morganella	morganii	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 6.20E-05)	5.81 ± 1.37	4.88E-04
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	capillatus	0.00E+00 (0.00E+00 to 6.65E-05)	0.00E+00 (0.00E+00 to 1.29E-05 (0.00E+00 to 2.49E-03)	5.81 ± 1.95	2.80E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Kosakonia	sp S29	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 2.83E-05 (0.00E+00 to 1.35E-04)	5.76 ± 1.14	1.76E-05
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	messengeroides	8.43E-05 (0.00E+00 to 2.37E-04)	3.02E-04 (0.00E+00 to 1.46E-02)	5.64 ± 1.07	5.87E-06
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	brevis	2.63E-05 (0.00E+00 to 1.26E-04)	9.84E-05 (0.00E+00 to 7.25E-04)	5.55 ± 0.97	7.77E-07
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	pentostus	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.39E-04)	5.43 ± 1.31	7.19E-04
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	jogaejeotgali	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.78E-04)	5.33 ± 1.43	3.02E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus	inopinatus	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 9.05E-05)	5.08 ± 1.71	2.85E-02
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	confusa	0.00E+00 (0.00E+00 to 1.12E-04)	6.25E-05 (0.00E+00 to 1.90E-04)	4.92 ± 1.13	3.39E-04
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	oligofermentans	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 4.01E-04)	4.89 ± 1.50	1.25E-02
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	no data	0.00E+00 (0.00E+00 to 1.11E-04)	5.12E-05 (0.00E+00 to 6.32E-04)	4.86 ± 1.07	1.56E-04
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	no data	1.61E-03 (4.32E-04 to 4.28E-03)	1.57E-02 (7.43E-04 to 7.92E-02)	4.85 ± 0.72	1.94E-09
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	paraplantarum	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 2.48E-04)	4.80 ± 1.36	5.63E-03
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	paramesenteroides	0.00E+00 (0.00E+00 to 5.53E-05)	0.00E+00 (0.00E+00 to 2.43E-04)	4.70 ± 1.22	2.08E-03

Table 4 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance (%)		Week 4 vs Baseline	
						Median (IQR)	Baseline	Week 4	Log <sub>2</sub> FC mean ± SE
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	animalis	0.00E+00 (0.00E+00 to 1.59E-04)	0.00E+00 (0.00E+00 to 6.60E-06)	4.67 ± 1.68	4.39E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	hyovaginalis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.18E-04)	4.57 ± 1.31	6.47E-03
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	korensis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 4.02E-04)	4.55 ± 1.59	3.43E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	alvei	0.00E+00 (0.00E+00 to 7.61E-05)	9.66E-05 (0.00E+00 to 2.41E-03)	4.52 ± 1.42	1.58E-02
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	lactis	0.00E+00 (0.00E+00 to 2.35E-05)	7.59E-05 (0.00E+00 to 8.90E-04)	4.49 ± 1.26	5.03E-03
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	garlicum	0.00E+00 (0.00E+00 to 0.00E+00)	7.89E-05 (0.00E+00 to 1.36E-03)	4.45 ± 1.33	9.80E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Morganeliaceae	Proteus	sp HMSC10D02	0.00E+00 (0.00E+00 to 6.23E-05)	0.00E+00 (0.00E+00 to 1.45E-04)	4.32 ± 1.10	1.65E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	parafarraginis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 4.81E-05)	4.23 ± 1.40	2.59E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Raoultella	ornithinolytica	1.59E-05 (0.00E+00 to 3.26E-04)	1.35E-04 (0.00E+00 to 1.01E-03)	4.22 ± 0.94	2.04E-04
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	sp HMSC25G12	0.00E+00 (0.00E+00 to 9.44E-06)	0.00E+00 (0.00E+00 to 1.63E-04)	4.16 ± 1.38	2.59E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Edwardsiella	tarda	0.00E+00 (0.00E+00 to 4.38E-05)	7.19E-05 (0.00E+00 to 6.58E-04)	4.13 ± 1.20	7.62E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Cronobacter	dublinensis	0.00E+00 (0.00E+00 to 1.00E-04)	2.29E-05 (0.00E+00 to 2.66E-04)	4.07 ± 1.13	4.65E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp kppr-6	0.00E+00 (0.00E+00 to 7.30E-05)	3.30E-05 (0.00E+00 to 8.30E-05)	3.91 ± 1.16	9.79E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	kefranofaciens	8.20E-05 (0.00E+00 to 2.80E-04)	1.43E-04 (2.66E-05 to 1.52E-03)	3.89 ± 1.37	3.57E-02
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	maltromaticum	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.46E-04)	3.89 ± 0.92	5.42E-04
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	divergens	0.00E+00 (0.00E+00 to 1.58E-04)	1.63E-04 (0.00E+00 to 2.02E-03)	3.88 ± 1.04	2.99E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	cf. planticola B43	0.00E+00 (0.00E+00 to 3.96E-05)	2.68E-05 (0.00E+00 to 4.17E-05)	3.66 ± 1.01	4.25E-03
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp CL-2	3.68E-05 (0.00E+00 to 3.50E-04)	6.46E-05 (0.00E+00 to 6.24E-04)	3.62 ± 0.90	1.08E-03
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	piscium	1.70E-04 (2.10E-05 to 3.65E-04)	3.60E-04 (8.80E-05 to 4.30E-03)	3.32 ± 0.73	1.43E-04

(Continued)

Table 4 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance (%)		Week 4 vs Baseline FC mean ± SE
						Median (IQR)	Week 4	
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Raouliella	planticola	0.00E+00 (0.00E+00 to 7.77E-05)	6.66E-05 (0.00E+00 to 5.63E-04)	3.20 ± 1.00 1.48E-02
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	fermentum	4.90E-05 (0.00E+00 to 2.86E-04)	1.73E-04 (0.00E+00 to 5.50E-04)	3.16 ± 0.97 1.31E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Lelliottia	jeotgali	0.00E+00 (0.00E+00 to 3.33E-05)	3.11E-05 (0.00E+00 to 7.24E-05)	3.15 ± 1.13 4.39E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter	cloacae complex Hoffmann cluster III	0.00E+00 (0.00E+00 to 2.78E-04)	1.62E-04 (0.00E+00 to 8.01E-04)	3.14 ± 0.96 1.25E-02
Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	Parabacteroides	merdae	1.54E-04 (0.00E+00 to 1.50E-03)	2.75E-05 (0.00E+00 to 1.05E-03)	3.12 ± 1.04 2.64E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	lactis	3.81E-03 (9.03E-04 to 2.35E-02)	7.09E-03 (2.76E-03 to 3.68E-02)	2.99 ± 0.84 5.03E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC067C01	1.45E-04 (0.00E+00 to 1.67E-03)	5.45E-04 (9.33E-05 to 1.77E-02)	2.93 ± 0.97 2.45E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella	no data	1.44E-03 (0.00E+00 to 9.01E-03)	7.64E-03 (9.62E-04 to 2.58E-02)	2.92 ± 0.91 1.43E-02
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus	acidilactici	2.62E-05 (0.00E+00 to 1.36E-04)	5.89E-05 (0.00E+00 to 4.94E-04)	2.79 ± 0.97 3.39E-02
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Terrisporobacter	glycolicus	9.90E-05 (0.00E+00 to 1.49E-03)	2.07E-04 (0.00E+00 to 3.80E-03)	2.77 ± 0.97 3.47E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter	asburiae	0.00E+00 (0.00E+00 to 5.62E-04)	2.42E-04 (3.63E-05 to 1.35E-03)	2.58 ± 0.89 3.18E-02
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	parabuchneri	8.76E-05 (0.00E+00 to 2.69E-04)	6.09E-05 (0.00E+00 to 2.71E-04)	2.36 ± 0.87 4.99E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzzeria	nexilis	3.93E-02 (1.98E-02 to 5.81E-02)	2.04E-02 (4.61E-03 to 6.78E-02)	2.21 ± 0.61 4.25E-03
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Pseudeshcherichia	vulneris	0.00E+00 (0.00E+00 to 8.89E-05)	1.10E-04 (3.63E-05 to 2.01E-04)	2.10 ± 0.77 4.86E-02
<b>Decreased at week 4 (28 species)</b>								
Firmicutes	Bacilli	Bacillales	Planococcaceae	Rummeliibacillus	stabekisii	0.00E+00 (0.00E+00 to 8.97E-05)	0.00E+00 (0.00E+00 to 0.00E+00)	-10.85 ± 2.15 1.76E-05
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	ginsengihumi	0.00E+00 (0.00E+00 to 1.49E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	-6.63 ± 1.37 4.55E-05
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	borstelensis	0.00E+00 (0.00E+00 to 9.69E-05)	0.00E+00 (0.00E+00 to 0.00E+00)	-6.24 ± 1.54 9.81E-04
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Aneurimbacillus	no data	0.00E+00 (0.00E+00 to 1.84E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	-5.04 ± 1.42 5.03E-03

Table 4 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance (%)		Week 4 vs Baseline	
						Median (IQR)	Week 4	Log <sub>2</sub> FC	Adjusted P-value <sup>1</sup>
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	canintestini	4.60E-05 (0.00E+00 to 3.30E-03)	1.06E-04 (0.00E+00 to 4.47E-04)	-5.02 ± 1.41E-03	1.27
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	hirae	2.01E-04 (2.70E-05 to 5.32E-03)	1.40E-03 (0.00E+00 to 8.16E-03)	-4.48 ± 5.07E-04	1.06
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri	5.71E-05 (0.00E+00 to 1.20E-01)	2.94E-05 (0.00E+00 to 3.01E-04)	-4.41 ± 3.44E-02	1.54
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	stercora	0.00E+00 (0.00E+00 to 6.00E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	-3.97 ± 2.90E-02	1.35
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	buccalis	0.00E+00 (0.00E+00 to 2.28E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	-3.91 ± 4.63E-02	1.42
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	wexterae	4.99E-02 (1.54E-02 to 1.35E-01)	2.52E-02 (5.28E-03 to 3.90E-02)	-3.90 ± 4.02E-08	0.62
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	sp Marseille-P2398	1.90E-03 (7.51E-04 to 4.41E-03)	6.13E-04 (0.00E+00 to 1.33E-03)	-3.75 ± 1.15E-04	0.81
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	no data	2.12E-01 (9.30E-02 to 3.45E-01)	6.61E-02 (8.53E-03 to 2.10E-01)	-3.33 ± 3.55E-06	0.62
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	thetaitoamicron	1.96E-04 (3.66E-05 to 9.82E-04)	0.00E+00 (0.00E+00 to 1.73E-04)	-3.14 ± 5.03E-03	0.88
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	baratii	1.05E-04 (0.00E+00 to 5.42E-04)	3.42E-05 (0.00E+00 to 4.11E-04)	-2.99 ± 2.90E-02	1.01
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	coprophilus	3.78E-04 (4.00E-05 to 2.33E-03)	2.95E-05 (0.00E+00 to 1.87E-04)	-2.96 ± 2.28E-02	0.97
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	longum	1.30E-04 (0.00E+00 to 1.04E-03)	7.44E-05 (0.00E+00 to 5.36E-04)	-2.93 ± 3.15E-02	1.00
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus	russellii	1.60E-04 (0.00E+00 to 5.47E-04)	0.00E+00 (0.00E+00 to 4.79E-05)	-2.92 ± 2.80E-02	0.98
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Traorella	massiliensis	8.35E-05 (0.00E+00 to 2.42E-04)	5.39E-05 (0.00E+00 to 1.39E-04)	-2.88 ± 2.28E-02	0.94
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp L2-50	4.28E-05 (0.00E+00 to 4.24E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	-2.88 ± 4.10E-03	0.79
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	obeum	2.29E-02 (9.54E-03 to 5.48E-02)	7.10E-03 (1.23E-03 to 2.10E-02)	-2.86 ± 4.88E-05	0.59
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	no data	no data	1.04E-03 (1.82E-04 to 4.44E-03)	1.81E-04 (0.00E+00 to 7.61E-04)	-2.75 ± 5.07E-04	0.65
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	hominis	2.63E-04 (2.39E-05 to 1.91E-03)	9.08E-05 (0.00E+00 to 3.07E-04)	-2.74 ± 3.60E-03	0.74
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Faecalitalea	sp Marseille-P3755	1.04E-04 (0.00E+00 to 2.68E-04)	0.00E+00 (0.00E+00 to 5.27E-05)	-2.63 ± 3.20E-02	0.91

(Continued)

Table 4 (continued)

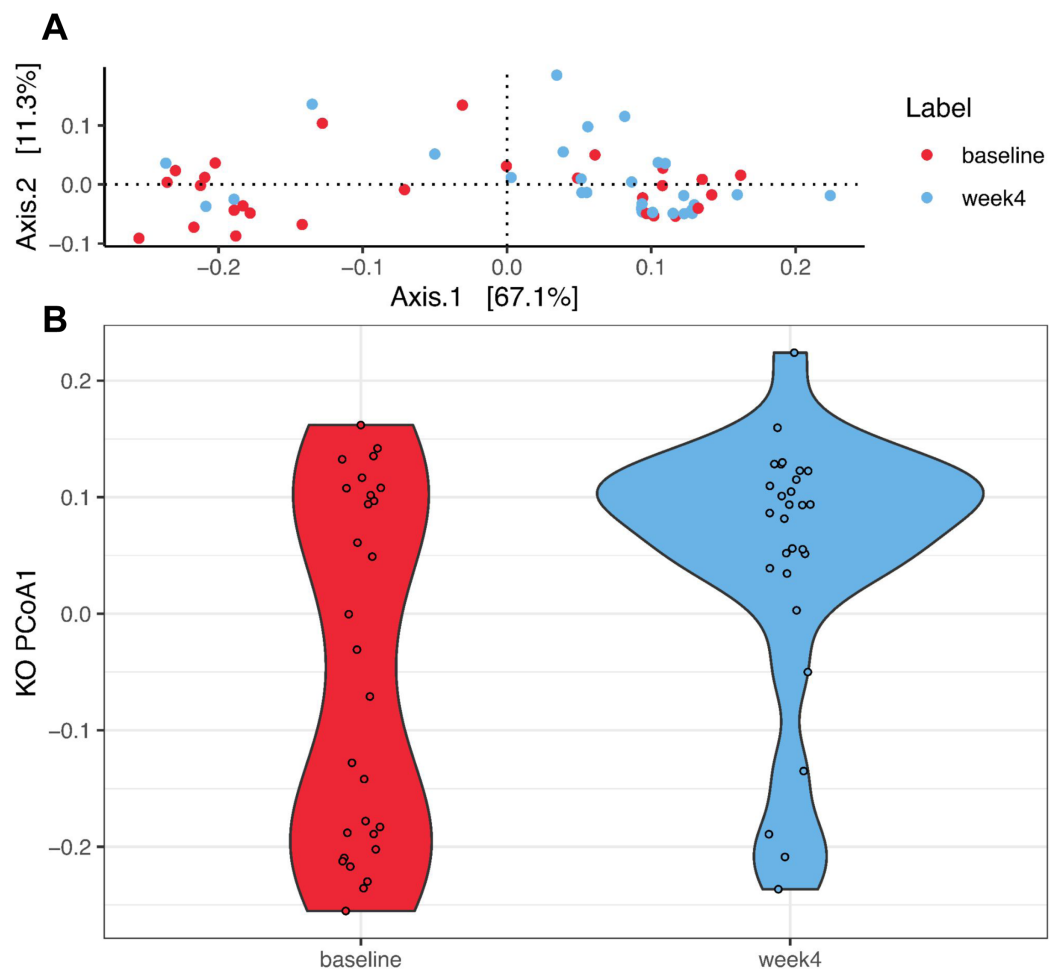
Phylum	Class	Order	Family	Genus	Species	Relative abundance (%) Median (IQR)		Week 4 vs Baseline	
						Baseline	Week 4	Log <sub>2</sub> FC mean ± SE	Adjusted P-value <sup>1</sup>
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	no data	bacterium 28-4	5.43E-05 (2.65E-05 to 1.67E-04)	0.00E+00 (0.00E+00 to 4.85E-05)	-2.42 ± 0.73	1.07E-02
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	no data	4.89E-04 (1.37E-04 to 1.13E-03)	5.78E-05 (0.00E+00 to 2.96E-04)	-2.40 ± 0.73	1.25E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Faecalicatena	contorta	7.58E-04 (7.63E-05 to 1.82E-03)	9.69E-05 (0.00E+00 to 4.14E-04)	-2.24 ± 0.77	3.18E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	no data	Bacterium 1 4 56FAA	2.36E-02 (8.91E-03 to 7.52E-02)	7.29E-03 (1.38E-03 to 1.72E-02)	-2.17 ± 0.67	1.32E-02
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	prausnitzii	9.43E-03 (3.19E-03 to 2.18E-02)	1.46E-03 (1.30E-04 to 8.07E-03)	-2.07 ± 0.59	6.19E-03

**Notes:**

<sup>1</sup> P values were adjusted with false discovery rate for multiple comparisons

FC, fold change; SE, standard error.





**Figure 5** Principal coordinate analysis (PCoA) of the Kyoto Encyclopedia of Genes and Genomes Orthology (KO) terms. (A) PCo1 (Axis 1) and PCo2 (Axis 2) respectively explain 67.1% and 11.3% of abundance variance (56 samples from 28 dogs). PERMANOVA using Bray–Curtis distance shows significant spatial separation between baseline (red) and week 4 (blue) ( $p = 0.007$ ). (B) A significant shift along the PCo1 axis from baseline (red) to week 4 (blue) is observed ( $p = 0.013$ , Wilcoxon signed rank test). Full-size [DOI: 10.7717/peerj.11648/fig-5](https://doi.org/10.7717/peerj.11648/fig-5)

To better understand the observed differences in the GM composition among the differential response groups (HR, MR, and LR), the abundances at the species level of the 28 samples collected at baseline were further analyzed. PCoA was performed and the first two axes (PCo1 and PCo2) explained 37.4% and 10.0% of the variation. Spatial separation among HR, MR, and LR along the first two PCo axes was demonstrated ( $p = 0.022$ , PERMANOVA Bray–Curtis dissimilarity matrices) (Fig. S7). PAM Clustering analysis revealed two clusters. GM clusters using Bray–Curtis dissimilarity matrices and a MDS ordination assigned 8 HR, 2 MR and 2 LR samples to one cluster (C1), while 94% of samples assigned to the other cluster (C2) contained samples from MR or LR ( $p = 0.004$ , Fisher’s exact test). In other words, a significantly higher proportion of HR samples were assigned to C1 and a significantly higher proportion of MR and LR samples were assigned to C2. We also ran a random forest classification model on the samples collected at

**Table 5** Kyoto Encyclopedia of Genes and Genomes Orthology (KO) terms with differential abundances between baseline and week 4 ( $n = 28$ , |fold change|  $\geq 2$  and adjusted  $p < 0.05$ ).

KO term	Description	Week 4 vs. Baseline	
		Log 2 FC mean $\pm$ SE	Adjusted $P$ -value <sup>1</sup>
<b>Increased abundance at week 4</b>			
K16323	purine nucleoside transport protein	7.2 $\pm$ 1.24	1.80E-05
K13548	2-deoxy-scylo-inosamine dehydrogenase [EC:1.1.1.329]	6.29 $\pm$ 1.39	0.005
K11628	mycocerosic acid synthase [EC:2.3.1.111]	5.99 $\pm$ 1.58	0.034
K20707	lysine racemase [EC:5.1.1.5]	5.91 $\pm$ 1.62	0.046
K16959	L-cystine transport system permease protein	4.95 $\pm$ 1.15	0.009
K00638	chloramphenicol O-acetyltransferase type B [EC:2.3.1.28]	4.45 $\pm$ 1.14	0.025
K16846	(2R)-sulfolactate sulfo-lyase subunit beta [EC:4.4.1.24]	3.97 $\pm$ 0.97	0.016
K03457	nucleobase:cation symporter-1, NCS1 family	3.32 $\pm$ 0.72	0.004
K12442	phthiocerol/phenolphthiocerol synthesis type-I polyketide synthase C [EC:2.3.1.292]	3.13 $\pm$ 0.85	0.045
K12953	cation-transporting P-type ATPase F [EC:7.2.2.-]	3.07 $\pm$ 0.79	0.025
K19279	aminoglycoside 9-adenylyltransferase [EC:2.7.7.-]	2.66 $\pm$ 0.63	0.009
K18104	ATP-binding cassette, subfamily B, bacterial AbcA/BmrA [EC:7.6.2.2]	2.65 $\pm$ 0.68	0.025
K19422	glycosyltransferase EpsD [EC:2.4.-.-]	2.32 $\pm$ 0.59	0.025
K15125	filamentous hemagglutinin	1.91 $\pm$ 0.53	0.048
<b>Decreased abundance at week 4</b>			
K13925	plasmin and fibronectin-binding protein A	-8.43 $\pm$ 1.90	0.006
K02203	phosphoserine / homoserine phosphotransferase [EC:3.1.3.3 2.7.1.39]	-5.08 $\pm$ 1.03	0.001
K13532	two-component system, sporulation sensor kinase D [EC:2.7.13.3]	-4.17 $\pm$ 1.15	0.047
K09759	nondiscriminating aspartyl-tRNA synthetase [EC:6.1.1.23]	-4.06 $\pm$ 0.78	0.000
K00351	Na <sup>+</sup> -transporting NADH:ubiquinone oxidoreductase subunit F [EC:7.2.1.1]	-3.88 $\pm$ 0.95	0.016
K14136	decaprenyl-phosphate phosphoribosyltransferase [EC:2.4.2.45]	-3.33 $\pm$ 0.87	0.030
K16165	fumarylpyruvate hydrolase [EC:3.7.1.20]	-2.97 $\pm$ 0.79	0.036
K16011	mannose-1-phosphate guanylyltransferase / mannose-6-phosphate isomerase [EC:2.7.7.13 5.3.1.8]	-2.65 $\pm$ 0.63	0.011
K13019	UDP-GlcNAc3NAcA epimerase [EC:5.1.3.23]	-2.01 $\pm$ 0.54	0.040
K17716	UDP-glucose 4-epimerase [EC:5.1.3.2]	-1.72 $\pm$ 0.46	0.034
K00385	anaerobic sulfite reductase subunit C	-1.64 $\pm$ 0.38	0.008

**Notes:**<sup>1</sup>  $P$  values were adjusted with false discovery rate for multiple comparisons.

baseline (70% of the samples used as a training data set and 30% used as a validation data set), which was able to differentiate between the two classes (HR vs. MR/LR) with 78% accuracy. Classification error for HR and MR/LR were 33% and 17% respectively; AUC = 0.94. These results collectively demonstrate that the magnitude of GM response to diet change along the PCo1 axis was dependent on the initial gut microbiota.

***Gut microbiome relative abundance at baseline in high- and low-responders***

The relative abundances at the phylum level are displayed for HR, MR, and LR (Fig. 3). Comparisons of species abundances demonstrate a distinctive pattern between HR and LR at baseline (Table 6, Fig. 6A). Species belonging to the order Bacteroidales were

**Table 6** Bacterial species with differential abundances (fold change  $\geq 2$  and FDR-adjusted  $p < 0.05$ ) between high-responders (HR,  $n = 9$ ) and low-responders (LR,  $n = 10$ ) at baseline.

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		Baseline HR vs LR	
						HR ( $n = 9$ )	LR ( $n = 10$ )	Log <sub>2</sub> FC	Adjusted P value <sup>1</sup> SE
<b>Higher in HR (40 species)</b>									
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	sp 4 3 47FAA	3.88E-04 (5.24E-05 to 1.19E-02)	0.00E+00 (0.00E+00 to 2.96E-05)	10.02 ± 1.89	6.31E-06
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	josui	8.20E-04 (5.24E-05 to 7.18E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	7.37 ± 1.84	1.40E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	bivia	5.01E-04 (0.00E+00 to 3.85E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	7.29 ± 2.01	3.97E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri	2.09E-01 (1.01E-04 to 2.07E+00)	5.71E-05 (6.58E-06 to 1.21E-02)	7.06 ± 1.92	3.48E-03
Firmicutes	Negativicutes	Selenomonadales	Selenomonadaceae	Megamonas	sp Cal98-2	3.92E-01 (1.17E-02 to 5.17E-01)	8.40E-04 (0.00E+00 to 4.30E-03)	7.01 ± 1.67	9.19E-04
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	sp 3 1 33FAA	9.99E-04 (4.56E-05 to 1.46E-03)	0.00E+00 (0.00E+00 to 2.10E-05)	6.52 ± 1.49	4.47E-04
Firmicutes	Negativicutes	Selenomonadales	Selenomonadaceae	Megamonas	no data	3.38E+00 (1.43E-01 to 4.20E+00)	7.07E-03 (0.00E+00 to 4.27E-02)	6.38 ± 1.82	5.45E-03
Firmicutes	Negativicutes	Selenomonadales	Selenomonadaceae	Megamonas	funiformis	5.96E-01 (3.11E-02 to 7.66E-01)	1.25E-03 (0.00E+00 to 7.41E-03)	6.25 ± 1.71	3.53E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	buccalis	2.28E-04 (0.00E+00 to 7.57E-03)	0.00E+00 (0.00E+00 to 3.66E-05)	6.14 ± 1.53	1.40E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	sp 9 1 42FAA	1.82E-04 (6.11E-05 to 2.11E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	5.68 ± 1.81	1.45E-02
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	vulgatus	6.41E-03 (2.69E-03 to 5.92E-02)	1.68E-04 (5.80E-05 to 2.84E-03)	5.63 ± 1.56	4.19E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	dorei	1.22E-03 (1.37E-04 to 1.38E-02)	7.04E-05 (0.00E+00 to 3.40E-04)	5.60 ± 1.62	5.82E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	no data	2.01E-03 (2.28E-04 to 7.25E-02)	0.00E+00 (0.00E+00 to 1.08E-04)	5.35 ± 1.73	1.57E-02
Firmicutes	Negativicutes	Selenomonadales	Selenomonadaceae	Megamonas	hypermegale	4.10E-03 (1.14E-04 to 8.73E-03)	0.00E+00 (0.00E+00 to 7.09E-05)	5.32 ± 1.48	4.22E-03
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	panachumi	4.48E-05 (0.00E+00 to 7.34E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.16 ± 2.02	4.77E-02
Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	carbonis	1.82E-04 (0.00E+00 to 1.47E-03)	0.00E+00 (0.00E+00 to 2.96E-05)	5.10 ± 1.75	2.34E-02
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	thermoruber	0.00E+00 (0.00E+00 to 1.14E-03)	0.00E+00 (0.00E+00 to 2.10E-05)	5.10 ± 1.82	2.88E-02
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	bryantii	4.56E-05 (0.00E+00 to 9.71E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.00 ± 1.98	4.99E-02

(Continued)

Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		LR (n = 10)	Baseline HR vs LR	
						HR (n = 9)	LR (n = 10)		Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Mediterranea	massiliensis	6.29E-04 (5.04E-04 to 1.95E-03)	0.00E+00 (0.00E+00 to 3.52E-05)	4.98 ± 1.50	9.25E-03	
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	borstelensis	1.11E-04 (0.00E+00 to 4.04E-03)	0.00E+00 (0.00E+00 to 7.20E-05)	4.96 ± 1.74	2.66E-02	
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	ginsengihumi	1.11E-04 (0.00E+00 to 4.84E-03)	0.00E+00 (0.00E+00 to 7.47E-05)	4.84 ± 1.67	2.47E-02	
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	stercora	8.42E-04 (9.12E-05 to 6.78E-03)	0.00E+00 (0.00E+00 to 3.52E-05)	4.78 ± 1.52	1.42E-02	
Firmicutes	Negativicutes	Selenomonadales	Selenomonadaceae	Megamonas	rupellensis	1.22E-01 (7.40E-03 to 1.67E-01)	2.47E-04 (0.00E+00 to 1.11E-03)	4.61 ± 1.72	3.58E-02	
Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	Tannerella	sp 6 1 58FAA CT1	9.12E-05 (0.00E+00 to 5.49E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	4.58 ± 1.75	3.98E-02	
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Paraprevotella	clara	1.66E-04 (4.21E-05 to 7.94E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	4.57 ± 1.45	1.42E-02	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	sp Marseille-P3166	1.47E-03 (3.03E-04 to 3.30E-03)	0.00E+00 (0.00E+00 to 2.45E-05)	4.42 ± 1.45	1.74E-02	
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	wexlerae	1.05E-01 (2.46E-02 to 3.83E+00)	1.98E-02 (1.25E-02 to 3.64E-02)	4.35 ± 0.96	2.41E-04	
Bacteroidetes	Bacteroidia	Bacteroidales	Odoribacteraceae	Odoribacter	laneus	8.07E-04 (0.00E+00 to 2.87E-03)	1.40E-05 (0.00E+00 to 4.98E-05)	4.31 ± 1.43	1.87E-02	
Proteobacteria	Betaproteobacteria	Burkholderiales	Sutterellaceae	Sutterella	no data	1.14E-03 (0.00E+00 to 1.26E-03)	4.21E-05 (9.86E-06 to 1.87E-04)	4.27 ± 1.57	3.52E-02	
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	multisaccharivorax	1.26E-04 (0.00E+00 to 2.91E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	4.18 ± 1.56	3.58E-02	
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	minor	1.27E-02 (1.85E-03 to 3.57E-02)	5.68E-04 (7.98E-05 to 1.16E-03)	3.94 ± 1.27	1.52E-02	
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Solobacterium	moorei	2.33E-03 (5.01E-04 to 1.45E-02)	0.00E+00 (0.00E+00 to 5.80E-05)	3.85 ± 1.51	4.79E-02	
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp AT4	8.65E-02 (6.45E-03 to 1.48E-01)	4.29E-03 (1.64E-03 to 8.18E-03)	3.78 ± 1.02	3.29E-03	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	coprocola	2.32E-02 (7.57E-03 to 1.06E-01)	5.86E-04 (1.76E-04 to 2.05E-03)	3.64 ± 1.36	3.58E-02	
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	no data	6.00E-01 (1.76E-01 to 8.44E+00)	1.27E-01 (4.23E-02 to 2.47E-01)	3.63 ± 0.88	9.84E-04	
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	obeum	5.40E-02 (1.96E-02 to 5.43E-01)	1.08E-02 (3.61E-03 to 2.24E-02)	3.30 ± 0.97	7.18E-03	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	mediterraneensis	1.14E-03 (3.03E-04 to 2.57E-03)	5.38E-05 (9.01E-06 to 1.01E-04)	2.96 ± 1.08	3.41E-02	

Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Baseline HR vs LR	
						HR (n = 9)	LR (n = 10)	Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Marvinbryantia	formatexigens	3.58E-03 (8.07E-04 to 3.87E-03)	2.23E-04 (5.25E-05 to 4.42E-04)	2.78 ± 0.98	2.68E-02
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	barnesiiae	7.33E-04 (2.28E-04 to 1.31E-03)	2.80E-05 (0.00E+00 to 7.93E-05)	2.75 ± 1.02	3.58E-02
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	no data	no data	3.05E-03 (7.17E-04 to 1.23E-02)	4.25E-04 (7.09E-05 to 1.13E-03)	2.43 ± 0.86	2.75E-02
<b>Lower in HR (97 species)</b>									
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	alvei	0.00E+00 (0.00E+00 to 0.00E+00)	1.32E-05 (0.00E+00 to 3.62E-04)	-2.649 ± 2.97	9.00E-17
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	braakii	0.00E+00 (0.00E+00 to 0.00E+00)	2.13E-03 (0.00E+00 to 8.85E-03)	-2.622 ± 2.42	7.87E-25
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Yersiniaceae	Serratia	marcescens	0.00E+00 (0.00E+00 to 0.00E+00)	1.51E-04 (6.58E-06 to 4.23E-03)	-25.50 ± 2.22	9.94E-28
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	sputorum	0.00E+00 (0.00E+00 to 0.00E+00)	2.80E-05 (0.00E+00 to 1.38E-03)	-2.456 ± 2.97	1.96E-14
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	sp MGH99	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 9.63E-04)	-23.88 ± 2.97	1.05E-13
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	no data	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.97E-05)	-23.75 ± 2.97	1.24E-13
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	portucalensis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.13E-04)	-23.27 ± 2.97	3.96E-13
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	cloacae complex sp ECNIH7	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	-22.05 ± 2.97	8.72E-12
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Edwardiella	piscicida	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 1.97E-05)	-21.90 ± 2.97	1.14E-11
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Cloaca complex sp TREC1	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 4.54E-04)	-21.17 ± 2.97	6.51E-11
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	canintestini	0.00E+00 (0.00E+00 to 0.00E+00)	9.29E-03 (1.63E-05 to 2.42E-01)	-9.45 ± 2.53	3.12E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	hormaechei	0.00E+00 (0.00E+00 to 0.00E+00)	2.04E-04 (0.00E+00 to 3.08E-03)	-9.31 ± 2.14	5.01E-04
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	dispar	0.00E+00 (0.00E+00 to 0.00E+00)	8.72E-04 (2.15E-04 to 6.38E-03)	-9.06 ± 1.73	8.87E-06
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	thailandicus	0.00E+00 (0.00E+00 to 0.00E+00)	2.71E-04 (5.50E-05 to 2.74E-03)	-8.92 ± 1.75	1.61E-05
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp FDAARGOS 163	0.00E+00 (0.00E+00 to 0.00E+00)	1.32E-05 (0.00E+00 to 7.07E-04)	-8.85 ± 2.20	1.40E-03

(Continued)

Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		LR (n = 10)	Baseline HR vs LR	
						HR (n = 9)	LR (n = 10)		Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
Firmicutes	Bacilli	Bacillales	Planococcaceae	Rummelibacillus	stabekisii	0.00E+00 (0.00E+00 to 5.24E-05)	0.00E+00 (0.00E+00 to 4.72E-04)	0.00E+00 (0.00E+00 to 4.72E-04)	-8.73 ± 2.93	2.00E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	asburiae	0.00E+00 (0.00E+00 to 0.00E+00)	4.31E-04 (0.00E+00 to 1.06E-03)	0.00E+00 (0.00E+00 to 1.06E-03)	-8.61 ± 2.13	1.40E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp 4E1 DIV0656	0.00E+00 (0.00E+00 to 0.00E+00)	1.80E-05 (0.00E+00 to 1.27E-04)	0.00E+00 (0.00E+00 to 1.27E-04)	-8.48 ± 2.54	8.51E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	sakai	0.00E+00 (0.00E+00 to 6.11E-05)	9.38E-05 (0.00E+00 to 3.41E-03)	0.00E+00 (0.00E+00 to 3.41E-03)	-8.45 ± 2.03	9.81E-04
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	cloacae complex Hoffmann cluster III	0.00E+00 (0.00E+00 to 0.00E+00)	1.15E-04 (9.01E-06 to 1.22E-03)	0.00E+00 (0.00E+00 to 1.22E-03)	-8.30 ± 1.93	6.02E-04
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Cronobacter	sakazakii	0.00E+00 (0.00E+00 to 0.00E+00)	1.01E-03 (3.61E-05 to 3.11E-03)	0.00E+00 (0.00E+00 to 3.11E-03)	-8.03 ± 2.17	3.34E-03
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	indicus	0.00E+00 (0.00E+00 to 0.00E+00)	5.33E-05 (0.00E+00 to 8.20E-04)	0.00E+00 (0.00E+00 to 8.20E-04)	-7.91 ± 2.27	5.56E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	sp ODB01	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	-7.82 ± 2.97	3.96E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC058D07	0.00E+00 (0.00E+00 to 0.00E+00)	6.00E-05 (0.00E+00 to 1.67E-03)	0.00E+00 (0.00E+00 to 1.67E-03)	-7.80 ± 2.18	4.39E-03
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	epidermidis	0.00E+00 (0.00E+00 to 0.00E+00)	4.53E-04 (6.70E-05 to 2.18E-03)	0.00E+00 (0.00E+00 to 2.18E-03)	-7.76 ± 1.94	1.40E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	faecalis	0.00E+00 (0.00E+00 to 2.24E-04)	7.52E-03 (8.40E-05 to 3.24E-01)	0.00E+00 (0.00E+00 to 3.24E-01)	-7.57 ± 2.15	5.26E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	quasipneumoniae	0.00E+00 (0.00E+00 to 0.00E+00)	1.05E-04 (3.75E-05 to 9.22E-04)	0.00E+00 (0.00E+00 to 9.22E-04)	-7.43 ± 1.99	3.12E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	sp KTE151	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 3.29E-04)	0.00E+00 (0.00E+00 to 3.29E-04)	-7.27 ± 2.70	3.58E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	freundii complex s CFNH3	0.00E+00 (0.00E+00 to 0.00E+00)	3.29E-05 (0.00E+00 to 6.20E-04)	0.00E+00 (0.00E+00 to 6.20E-04)	-7.25 ± 2.48	2.32E-02
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp CL-2	0.00E+00 (0.00E+00 to 1.01E-04)	2.67E-05 (0.00E+00 to 2.68E-04)	0.00E+00 (0.00E+00 to 2.68E-04)	-7.16 ± 2.25	1.32E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	sp S-77	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 4.98E-04)	0.00E+00 (0.00E+00 to 4.98E-04)	-7.14 ± 2.67	3.58E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erwiniaceae	Pantoea	sesami	0.00E+00 (0.00E+00 to 0.00E+00)	8.43E-05 (0.00E+00 to 1.47E-03)	0.00E+00 (0.00E+00 to 1.47E-03)	-7.05 ± 2.64	3.58E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Francomibacter	helveticus	0.00E+00 (0.00E+00 to 0.00E+00)	2.50E-04 (9.01E-06 to 4.04E-04)	0.00E+00 (0.00E+00 to 4.04E-04)	-6.90 ± 1.99	5.78E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	freundii complex sp CFNH2	0.00E+00 (0.00E+00 to 0.00E+00)	2.99E-04 (6.31E-05 to 5.02E-04)	0.00E+00 (0.00E+00 to 5.02E-04)	-6.88 ± 1.94	4.73E-03



Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Baseline HR vs LR	
						HR (n = 9)	LR (n = 10)	Log <sub>2</sub> FC	Adjusted P value <sup>1</sup> SE
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	werkmanii	0.00E+00 (0.00E+00 to 0.00E+00)	5.38E-05 (9.01E-06 to 3.96E-04)	-6.70 ± 1.82	3.43E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	cloacae complex Hoffmann cluster IV	0.00E+00 (0.00E+00 to 0.00E+00)	3.91E-04 (4.80E-05 to 8.27E-04)	-6.70 ± 1.83	3.56E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	curvatus	1.14E-04 (0.00E+00 to 1.79E-04)	1.64E-04 (0.00E+00 to 3.71E-03)	-6.69 ± 1.71	1.80E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	michiganensis	0.00E+00 (0.00E+00 to 0.00E+00)	6.77E-04 (1.45E-04 to 2.32E-03)	-6.60 ± 1.65	1.40E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pluralibacter	gergoviae	0.00E+00 (0.00E+00 to 0.00E+00)	6.58E-05 (8.17E-06 to 5.18E-04)	-6.56 ± 1.75	3.12E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Shigella	no data	0.00E+00 (0.00E+00 to 0.00E+00)	1.67E-01 (1.76E-02 to 1.01E+00)	-6.46 ± 2.31	2.88E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erwiniaceae	Erwinia	telegrylli	0.00E+00 (0.00E+00 to 0.00E+00)	9.93E-05 (8.17E-06 to 6.65E-04)	-6.45 ± 1.71	3.12E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE96	0.00E+00 (0.00E+00 to 0.00E+00)	4.75E-04 (9.01E-06 to 3.41E-03)	-6.43 ± 2.21	2.43E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE11	0.00E+00 (0.00E+00 to 0.00E+00)	1.40E-05 (0.00E+00 to 2.35E-03)	-6.38 ± 2.38	3.58E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Shigella	sonnei	0.00E+00 (0.00E+00 to 0.00E+00)	9.59E-02 (9.56E-03 to 2.82E-01)	-6.38 ± 2.21	2.52E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp FDAARGOS 375	0.00E+00 (0.00E+00 to 0.00E+00)	6.27E-05 (0.00E+00 to 4.75E-04)	-6.31 ± 2.28	3.09E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Cronobacter	malonicus	0.00E+00 (0.00E+00 to 0.00E+00)	6.41E-05 (1.05E-05 to 1.06E-03)	-6.27 ± 1.99	1.42E-02
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter	sp ATCC35328	0.00E+00 (0.00E+00 to 0.00E+00)	9.52E-03 (1.18E-03 to 1.99E-02)	-6.16 ± 2.04	1.89E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	oxytoca	0.00E+00 (0.00E+00 to 0.00E+00)	7.20E-04 (2.11E-05 to 3.25E-03)	-6.16 ± 1.88	1.04E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE159	0.00E+00 (0.00E+00 to 0.00E+00)	1.01E-02 (2.82E-04 to 3.19E-02)	-6.16 ± 2.14	2.54E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	no data	0.00E+00 (0.00E+00 to 0.00E+00)	6.56E-03 (2.91E-04 to 3.21E-02)	-6.15 ± 2.21	3.05E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	massiliensis	0.00E+00 (0.00E+00 to 9.71E-05)	1.97E-04 (3.94E-05 to 8.56E-04)	-6.06 ± 1.79	7.55E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	no data	no data	2.28E-04 (9.71E-05 to 4.19E-04)	8.92E+00 (5.93E-01 to 3.02E+01)	-6.05 ± 1.81	8.53E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	hirae	8.42E-05 (0.00E+00 to 2.62E-04)	3.64E-04 (4.34E-05 to 4.62E-03)	-5.98 ± 1.67	4.37E-03

(Continued)

Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		Baseline HR vs LR	
						HR (n = 9)	LR (n = 10)	Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	equinus	4.55E-04 (1.94E-04 to 1.50E-03)	1.76E-03 (1.06E-04 to 8.90E-01)	-5.94 ± 1.93	1.61E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	raffinosis	0.00E+00 (0.00E+00 to 0.00E+00)	8.85E-05 (0.00E+00 to 6.18E-04)	-5.90 ± 2.19	3.58E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE52	0.00E+00 (0.00E+00 to 0.00E+00)	2.20E-03 (1.54E-04 to 1.79E-02)	-5.90 ± 1.97	1.94E-02
Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium	freudenreichii	1.11E-04 (0.00E+00 to 2.02E-04)	6.80E-05 (1.05E-05 to 2.17E-03)	-5.89 ± 1.84	1.29E-02
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	sp VT-16-81	0.00E+00 (0.00E+00 to 0.00E+00)	3.73E-05 (0.00E+00 to 1.08E-04)	-5.87 ± 2.04	2.54E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Shigella	boydii	0.00E+00 (0.00E+00 to 0.00E+00)	8.74E-02 (7.11E-03 to 2.18E-01)	-5.87 ± 2.18	3.58E-02
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	gallinarum	0.00E+00 (0.00E+00 to 0.00E+00)	3.77E-05 (0.00E+00 to 7.99E-04)	-5.85 ± 2.17	3.58E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	luteitensis	8.42E-05 (0.00E+00 to 4.62E-03)	1.49E-04 (6.23E-05 to 3.44E+00)	-5.85 ± 2.30	4.89E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC34G12	0.00E+00 (0.00E+00 to 0.00E+00)	7.47E-05 (0.00E+00 to 2.38E-04)	-5.83 ± 2.08	2.88E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	no data	3.68E-02 (8.00E-03 to 5.84E-01)	1.91E-01 (1.39E-02 to 2.36E+01)	-5.81 ± 1.55	3.12E-03
Fusobacteria	Fusobacteria	Fusobacteriales	Leptotrichiaceae	Streptobacillus	moniliformis	0.00E+00 (0.00E+00 to 0.00E+00)	3.73E-03 (6.84E-04 to 1.09E-02)	-5.78 ± 1.92	1.91E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp 1 1 43	0.00E+00 (0.00E+00 to 0.00E+00)	8.41E-03 (8.32E-04 to 9.78E-03)	-5.68 ± 1.83	1.52E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	infantarius	8.55E-04 (0.00E+00 to 1.39E-03)	6.33E-04 (1.34E-04 to 8.77E-01)	-5.65 ± 2.00	2.75E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	canis	0.00E+00 (0.00E+00 to 0.00E+00)	3.32E-04 (9.01E-06 to 1.50E-03)	-5.59 ± 2.16	4.44E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC035C10	0.00E+00 (0.00E+00 to 9.12E-05)	5.28E-04 (4.21E-05 to 9.66E-04)	-5.59 ± 1.77	1.42E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	villorum	0.00E+00 (0.00E+00 to 8.42E-05)	2.35E-05 (0.00E+00 to 1.47E-04)	-5.56 ± 2.06	3.58E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp 255 E5PC	0.00E+00 (0.00E+00 to 0.00E+00)	1.52E-04 (9.01E-06 to 4.48E-03)	-5.54 ± 1.92	2.47E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Lelliottia	amnigena	0.00E+00 (0.00E+00 to 0.00E+00)	9.27E-05 (1.89E-05 to 3.69E-04)	-5.53 ± 1.84	1.91E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus	0.00E+00 (0.00E+00 to 8.42E-05)	7.61E-04 (1.17E-05 to 3.15E-03)	-5.46 ± 1.77	1.58E-02

Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Baseline HR vs LR	
						HR (n = 9)	LR (n = 10)	Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erwiniaceae	Erwinia	gerundensis	0.00E+00 (0.00E+00 to 0.00E+00)	9.82E-05 (0.00E+00 to 1.26E-04)	-5.45 ± 1.82	1.92E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	no data	0.00E+00 (0.00E+00 to 0.00E+00)	3.09E-03 (3.87E-04 to 1.20E-02)	-5.39 ± 2.00	3.58E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC072F02	0.00E+00 (0.00E+00 to 5.05E-04)	5.25E-04 (8.41E-05 to 6.68E-03)	-5.29 ± 1.68	1.42E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	no data	bacterium ENNIH1	0.00E+00 (0.00E+00 to 0.00E+00)	5.49E-05 (0.00E+00 to 1.18E-04)	-5.28 ± 1.85	2.60E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Raoultella	planticola	0.00E+00 (0.00E+00 to 0.00E+00)	1.89E-05 (0.00E+00 to 1.16E-04)	-5.17 ± 2.03	4.79E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	gallinarum	0.00E+00 (0.00E+00 to 5.55E-05)	2.51E-03 (1.32E-03 to 1.13E-02)	-5.12 ± 1.57	1.04E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Yersiniaceae	Yersinia	no data	0.00E+00 (0.00E+00 to 0.00E+00)	1.41E-04 (9.01E-06 to 2.87E-04)	-5.08 ± 1.86	3.41E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Raoultella	ornithinolytica	0.00E+00 (0.00E+00 to 0.00E+00)	9.13E-05 (9.44E-06 to 7.79E-04)	-5.06 ± 1.90	3.62E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	no data	6.11E-05 (0.00E+00 to 2.10E-04)	1.21E+00 (5.58E-01 to 7.20E+00)	-5.02 ± 1.99	4.99E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	sp MGH 55	0.00E+00 (0.00E+00 to 0.00E+00)	5.88E-05 (9.01E-06 to 2.40E-04)	-5.00 ± 1.77	2.75E-02
Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	no data	5.55E-05 (0.00E+00 to 3.14E-04)	2.03E-04 (0.00E+00 to 7.70E-04)	-4.89 ± 1.50	1.04E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	thermophilus	1.82E-04 (6.11E-05 to 6.81E-04)	1.08E-02 (5.86E-04 to 2.09E-02)	-4.67 ± 1.35	6.07E-03
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	macedonicus	1.01E-04 (0.00E+00 to 1.48E-02)	2.99E-03 (4.25E-04 to 6.15E-02)	-4.62 ± 1.70	3.52E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	pasteurianus	8.73E-04 (5.69E-04 to 3.33E-03)	6.32E-03 (4.42E-04 to 4.98E-02)	-4.60 ± 1.17	1.80E-03
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	suus	3.88E-04 (3.19E-04 to 9.27E-04)	8.34E-04 (2.27E-04 to 3.00E-03)	-4.10 ± 1.03	1.40E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	durans	1.26E-04 (0.00E+00 to 3.19E-04)	7.94E-04 (4.20E-05 to 2.29E-03)	-4.04 ± 1.53	3.96E-02
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	no data	5.92E-04 (2.53E-04 to 1.92E-03)	3.83E-03 (1.86E-03 to 1.66E-02)	-4.00 ± 1.07	3.12E-03
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	lactis	1.26E-03 (8.88E-04 to 3.54E-03)	2.40E-02 (3.15E-03 to 2.71E-02)	-3.96 ± 0.95	9.52E-04
Firmicutes	Clostridia	Clostridiales	Clostridiales	Emergencia	timonensis	3.05E-04 (1.37E-04 to 7.34E-04)	4.46E-04 (2.25E-04 to 1.29E-03)	-3.84 ± 1.16	9.41E-03

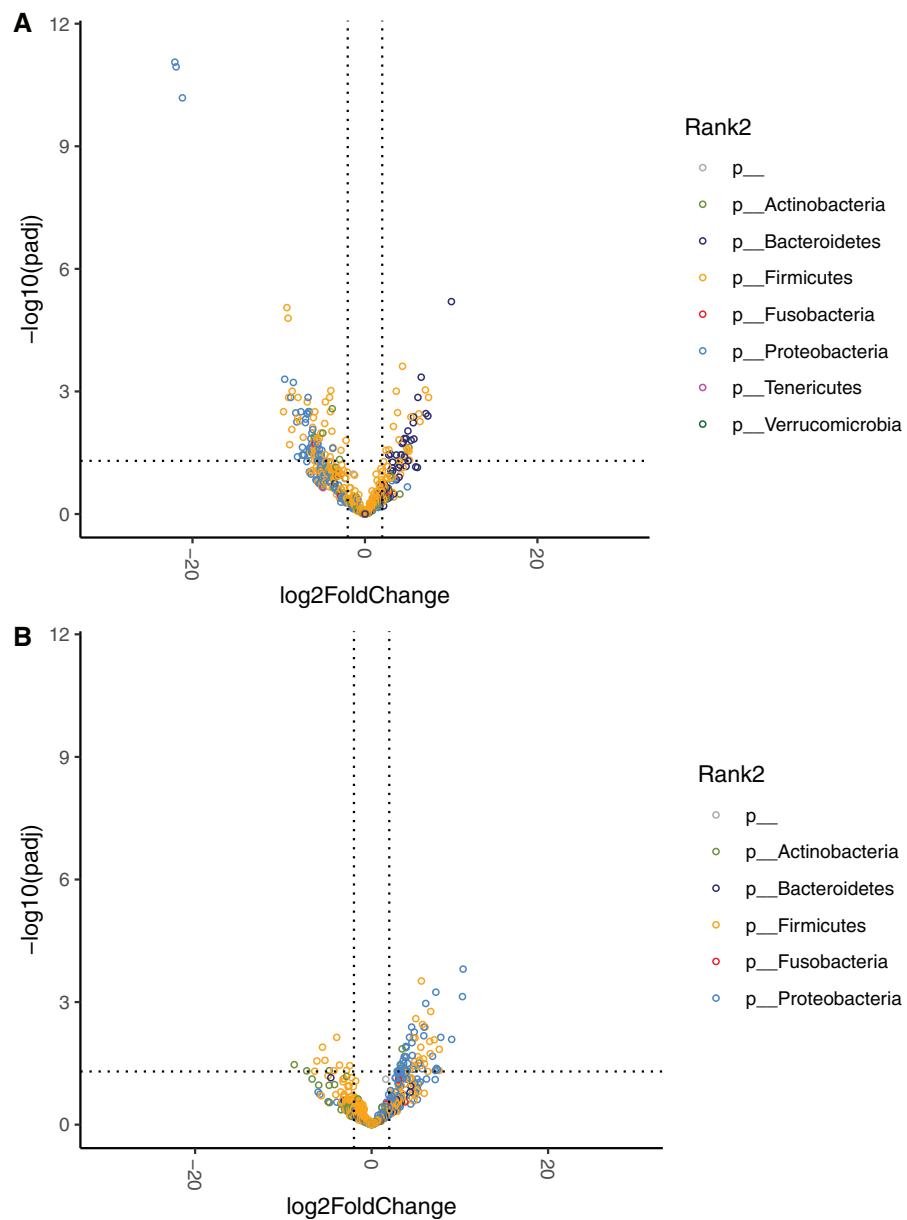
(Continued)

Table 6 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Baseline HR vs LR	
						Median (IQR)	LR (n = 10)	Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	breve	3.05E-03 (1.65E-03 to 3.41E-03)	1.63E-03 (6.32E-04 to 4.35E-03)	-3.79 ± 0.99	2.64E-03
Firmicutes	Bacilli	no data	no data	no data	no data	8.66E-04 (1.34E-04 to 4.72E-03)	4.48E-03 (1.65E-03 to 3.28E-02)	-3.73 ± 1.28	2.34E-02
Proteobacteria	Gammaproteobacteria	no data	no data	no data	no data	5.24E-05 (4.21E-05 to 1.14E-04)	1.39E-02 (3.37E-03 to 3.15E-02)	-3.73 ± 1.29	2.47E-02
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	no data	5.55E-05 (0.00E+00 to 2.91E-04)	9.61E-05 (3.85E-05 to 3.39E-04)	-2.94 ± 1.14	4.61E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	no data	bacterium 3 1 46FAA	1.14E-02 (2.69E-03 to 2.41E-02)	1.05E-02 (1.24E-03 to 6.32E-02)	-2.78 ± 0.99	2.75E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	parasanguinis	7.76E-04 (6.72E-04 to 1.21E-03)	8.33E-04 (3.19E-04 to 1.59E-03)	-2.19 ± 0.71	1.58E-02

**Notes:**<sup>1</sup> P values were adjusted with false discovery rate for multiple comparisons.

FC, fold change; SE, standard error.



**Figure 6** Volcano plots demonstrating fold-change (FC) of species abundance between high-responders (HR,  $n = 9$ ) and low-responders (LR,  $n = 10$ ) at (A) baseline and (B) week 4. FC is calculated from HR/LR. Vertical dashed lines show  $\log_2FC$  at 1 and  $-1$  (i.e. FC at 2 and  $-2$ ). Horizontal dashed line shows adjusted  $p = 0.05$ . Adjustment of  $p$  values is performed with false discovery rate. Points are colored by phylum. [Full-size !\[\]\(b345a1c4255362eec3746050dd71ccac\_img.jpg\) DOI: 10.7717/peerj.11648/fig-6](https://doi.org/10.7717/peerj.11648/fig-6)

observed to have higher, and never lower, abundances in HR as compared to LR (Fig. S8A). On the contrary, species belonging to the Enterobacterales or the Lactobacillales order were observed to have lower, and never higher, abundances in HR as compared to LR, with a single exception of *Streptococcus minor* (Lactobacillales order).

#### *Bacteroidales and Clostridiales dominated the gut of high-responders*

Among 40 bacterial species whose abundance were higher in HR, 20 (50%) belonged to the Bacteroidales order (Table 6, Fig. S8A). They included 9 *Bacteroides* species and 7 *Prevotella* species. Seven species (18%) of the Clostridiales order and 5 *Megamonas* species (13%) were also observed to dominate the gut of the HR at baseline.

#### *Enterobacterales and Lactobacillales dominated the gut of low-responders*

On the other hand, 97 species had lower abundances in the guts of HR as compared to LR dogs (Table 6, Fig. S8A). Forty seven of these species (48%) were in the Enterobacterales order. Species in the Lactobacillales order were also underrepresented in the guts of HR and accounted for 35% of the 97 species. It is worth noting that the abundances of many Enterobacterales and Lactobacillales species increased as a result of diet change when all subjects were included in the analysis (Table 4).

Given the enrichment of Enterobacterales and Lactobacillales in LR as compared to HR, correlative analysis was performed in all dogs. The read counts and the relative abundances of total Enterobacterales were negatively correlated with the magnitude of PCo1 change (read count: Spearman's  $\rho = -0.74$ ,  $p = 6.66E-06$ , relative abundance: Spearman's  $\rho = -0.69$ ,  $p = 6.77E-05$ ). Significant correlation was not observed with total Lactobacillales.

#### **Changes in the gut microbiome abundance from baseline to week 4 in high- and low-responders**

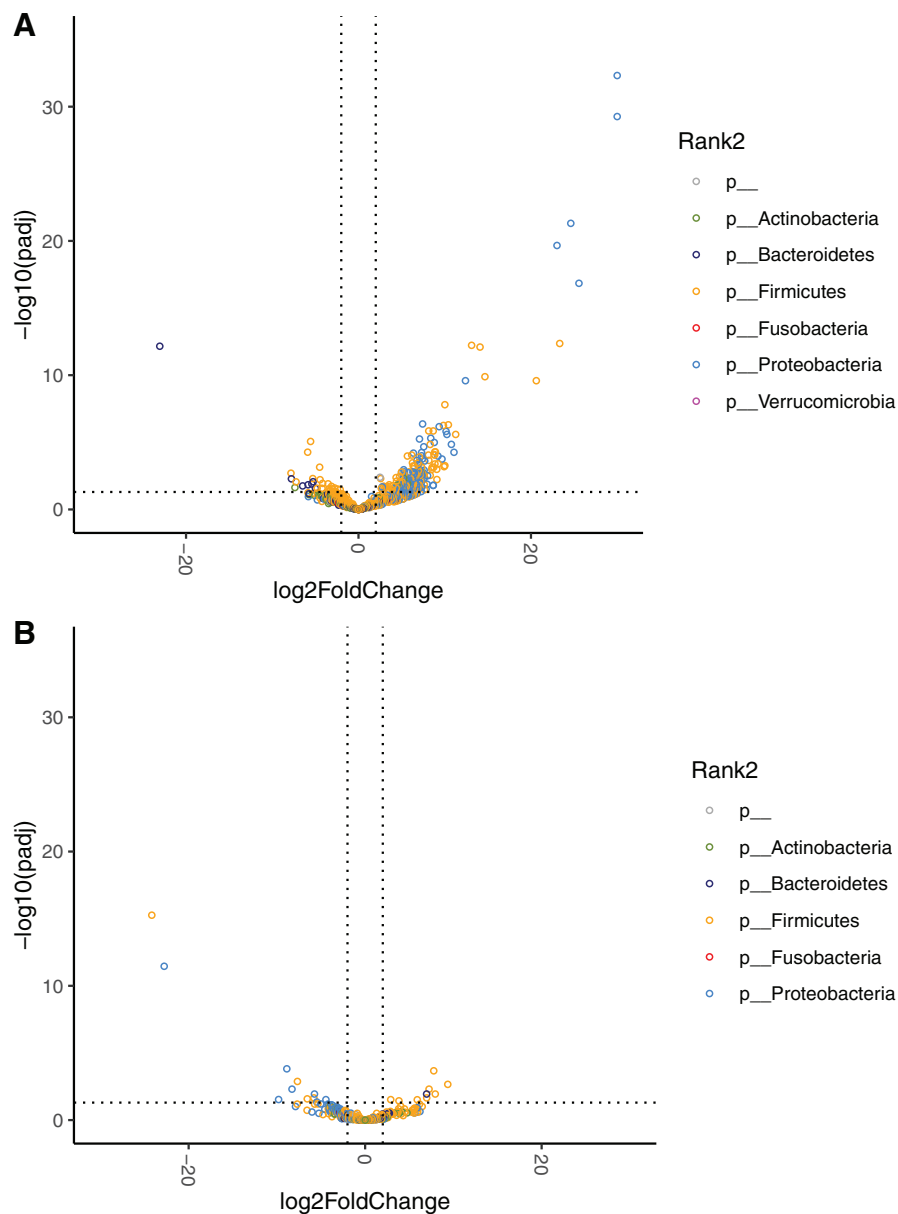
Not only was the GM of HR and LR different at baseline, HR and LR also responded differently to the diet transition (Table S4, Figs. 7A and 7B).

#### *Enterobacterales and Lactobacillales increased predominantly in high-responders*

Over the 4 week period in HR the abundances of 97 species of Enterobacterales increased and none decreased (Table S4 and Fig. 7A). Whereas in LR no increase in abundance of any Enterobacterales species was observed, but rather seven Enterobacterales species were reduced (Fig. 7B). Similarly, the abundances of 77 species in the Lactobacillales order significantly increased and none decreased at week 4 in HR, whereas only five species of Lactobacillales increased and one decreased in LR.

#### *Clostridiales and Bacteroidales had distinctive responses in high- vs. low-responders*

A distinctive pattern of change in the Clostridiales and Bacteroidales orders was also noted between HR and LR. In HR, the abundances of 17 species of Clostridiales were reduced whereas there were increased abundances of five species (three of which were members of the genus *Clostridium*) (Table S4 and Fig. 7A). In LR the abundances of only three species of Clostridiales increased and one species decreased (Fig. 7B). Additionally, the abundance of any Bacteroidales did not significantly change in LR, while seven species of Bacteroidales (six of which were in the genus *Prevotella*) were reduced in HR.



**Figure 7** Volcano plots demonstrating fold-change (FC) of species abundance between week 4 and baseline in (A) high-responders ( $n = 9$ ) and (B) low-responders ( $n = 10$ ). Vertical dashed lines show  $\log_2FC$  at 1 and  $-1$  (i.e. FC at 2 and  $-2$ ). Horizontal dashed line shows adjusted  $p = 0.05$ . Adjustment of  $p$  values is performed with false discovery rate. Points are colored by phylum.

Full-size DOI: [10.7717/peerj.11648/fig-7](https://doi.org/10.7717/peerj.11648/fig-7)

### ***Gut microbiome relative abundance at week 4 in high- and low-responders***

Consistent with a distinctive GM profile at baseline and differential changes between HR and LR, the microbial profiles of HR and LR at week 4 also differed (Table 7, Fig. 6B). A summary of overall GM profile and changes in the abundance of Bacteroidales, Clostridiales, Lactobacillales, and Proteobacteria is also provided in Table 8.



Table 7 Bacterial species with differential abundances (fold change  $\geq 2$  and  $p < 0.05$ ) between high-responders (HR,  $n = 9$ ) and low-responders (LR,  $n = 10$ ) at week 4.

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Week 4 HR vs LR
						Median (IQR)	Log <sub>2</sub> FC	
						HR ( $n = 9$ )	LR ( $n = 10$ )	Adjusted P -value <sup>1</sup> mean $\pm$ SE
<b>Higher in HR (68 species)</b>								
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	alvei	1.88E-03 (1.47E-03 to 1.06E+00)	4.81E-05 (0.00E+00 to 6.65E-05)	10.37 $\pm$ 2.02 1.56E-04
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	no data	3.57E-04 (1.66E-04 to 1.54E-01)	0.00E+00 (0.00E+00 to 3.58E-05)	10.30 $\pm$ 2.26 7.33E-04
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Morganellaceae	Morganella	sp HMSC11D09	0.00E+00 (0.00E+00 to 4.86E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	9.07 $\pm$ 2.50 8.16E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	sp HMSC23F03	3.96E-03 (2.77E-05 to 2.24E-02)	0.00E+00 (0.00E+00 to 1.06E-04)	7.84 $\pm$ 2.12 7.29E-03
Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria	monocytogenes	3.24E-05 (0.00E+00 to 2.65E-04)	0.00E+00 (0.00E+00 to 5.61E-05)	7.67 $\pm$ 2.27 1.43E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Solobacterium	moorei	0.00E+00 (0.00E+00 to 1.95E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	7.64 $\pm$ 2.77 4.71E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	sp MGH 7	0.00E+00 (0.00E+00 to 5.95E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	7.46 $\pm$ 2.68 4.51E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	sp AA405	0.00E+00 (0.00E+00 to 1.33E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	7.36 $\pm$ 2.59 4.18E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Cronobacter	dublinensis	1.94E-04 (1.47E-04 to 9.28E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	7.28 $\pm$ 1.56 5.73E-04
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	sp Ep R1	0.00E+00 (0.00E+00 to 6.63E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	7.22 $\pm$ 2.61 4.71E-02
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	sp VT-16-81	1.19E-04 (0.00E+00 to 3.01E-03)	0.00E+00 (0.00E+00 to 4.34E-05)	7.11 $\pm$ 1.97 8.34E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Edwardsiella	hoshimae	2.08E-05 (0.00E+00 to 5.27E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	6.91 $\pm$ 2.13 2.12E-02
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp CL-2	3.97E-04 (5.54E-05 to 1.66E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	6.70 $\pm$ 1.56 1.70E-03
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	terrium	6.77E-05 (0.00E+00 to 7.83E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	6.65 $\pm$ 2.01 1.83E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC072H05	2.65E-04 (0.00E+00 to 7.83E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	6.59 $\pm$ 1.84 9.15E-03
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	josui	0.00E+00 (0.00E+00 to 1.81E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	6.37 $\pm$ 2.37 4.97E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	no data	1.06E-02 (7.68E-03 to 1.80E-01)	1.36E-03 (3.94E-04 to 6.31E-03)	6.14 $\pm$ 1.39 1.08E-03
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	maltaromaticum	1.08E-03 (2.66E-05 to 3.65E-03)	1.31E-04 (5.01E-05 to 4.23E-04)	6.06 $\pm$ 1.54 4.09E-03

Table 7 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>	Week 4 HR vs LR
						HR (n = 9)	LR (n = 10)			
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	avium	1.77E-04 (0.00E+00 to 2.88E-02)	0.00E+00 (0.00E+00 to 4.11E-04)	5.99 ± 2.00	3.24E-02	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE11	2.54E-03 (1.06E-03 to 6.28E-03)	0.00E+00 (0.00E+00 to 6.79E-05)	5.94 ± 1.50	4.09E-03	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erwiniaceae	Pantoea	ananatis	4.52E-04 (2.28E-04 to 6.10E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.91 ± 1.56	6.66E-03	
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp 7 2 43FAA	1.62E-04 (0.00E+00 to 7.34E-04)	0.00E+00 (0.00E+00 to 3.58E-05)	5.88 ± 1.87	2.46E-02	
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	minor	3.48E-03 (5.75E-04 to 6.97E-03)	9.53E-04 (1.21E-04 to 3.04E-03)	5.77 ± 1.43	3.54E-03	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	sp MS 92-3	3.97E-05 (0.00E+00 to 2.21E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.72 ± 2.03	4.28E-02	
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	no data	6.10E-03 (5.07E-03 to 1.04E-02)	1.36E-03 (3.72E-04 to 3.53E-03)	5.64 ± 1.16	3.04E-04	
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Terrisporobacter	glycolicus	1.22E-03 (0.00E+00 to 3.90E-03)	0.00E+00 (0.00E+00 to 4.39E-05)	5.57 ± 1.90	3.59E-02	
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelatoclostridium	ramosum	4.70E-03 (1.30E-04 to 2.69E-02)	8.23E-05 (1.32E-05 to 1.54E-03)	5.57 ± 1.62	1.27E-02	
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sartagoforme	9.73E-05 (0.00E+00 to 9.12E-04)	0.00E+00 (0.00E+00 to 2.42E-05)	5.44 ± 1.75	2.66E-02	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Raoultella	no data	2.66E-05 (0.00E+00 to 3.72E-04)	0.00E+00 (0.00E+00 to 5.18E-05)	5.35 ± 1.77	3.10E-02	
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	celatum	2.41E-04 (1.45E-04 to 4.40E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.22 ± 1.63	2.16E-02	
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	parapatrificum	7.33E-03 (1.55E-03 to 3.41E-02)	2.06E-03 (5.15E-04 to 9.22E-03)	5.01 ± 1.20	2.54E-03	
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	sp 3 3 56FAA	3.47E-03 (1.95E-04 to 4.56E-02)	1.39E-03 (2.32E-04 to 4.99E-03)	4.92 ± 1.34	7.51E-03	
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	mesenteroides	2.21E-03 (2.70E-04 to 1.31E-01)	2.38E-04 (4.92E-05 to 1.15E-02)	4.92 ± 1.75	4.28E-02	
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	no data	1.54E-02 (1.10E-03 to 4.62E-02)	5.15E-04 (1.98E-05 to 7.03E-03)	4.91 ± 1.60	2.85E-02	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	quasipneumoniae	3.91E-04 (3.45E-04 to 4.74E-03)	9.33E-05 (0.00E+00 to 2.56E-04)	4.83 ± 1.65	3.59E-02	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erwiniaceae	Erwinia	gerundensis	1.77E-04 (1.11E-04 to 2.71E-04)	0.00E+00 (0.00E+00 to 4.76E-05)	4.83 ± 1.25	5.40E-03	
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Turicibacter	sanguinis	8.27E-04 (5.54E-05 to 1.52E-03)	2.39E-05 (0.00E+00 to 2.95E-04)	4.76 ± 1.63	3.59E-02	

(Continued)

Table 7 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
						HR (n = 9)	LR (n = 10)		
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	no data	no data	3.08E-01 (1.35E-02 to 2.33E+00)	1.07E-01 (1.37E-02 to 1.31E-01)	4.73 ± 1.28	7.46E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Raoultella	ornithinolytica	1.86E-04 (4.89E-05 to 3.96E-03)	3.45E-05 (0.00E+00 to 1.38E-04)	4.68 ± 1.67	4.40E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	no data	9.48E-03 (8.22E-03 to 7.28E-02)	1.61E-03 (2.52E-04 to 8.19E-03)	4.61 ± 1.41	2.02E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE172	1.67E-02 (9.42E-03 to 1.64E-01)	4.98E-03 (8.28E-04 to 1.45E-02)	4.57 ± 1.29	9.85E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp 1 1 43	1.57E-02 (5.90E-03 to 2.83E-02)	1.07E-03 (3.16E-04 to 5.30E-03)	4.54 ± 1.15	4.09E-03
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Turicibacter	no data	1.03E-03 (3.09E-04 to 2.31E-03)	2.11E-04 (0.00E+00 to 1.64E-03)	4.32 ± 1.54	4.35E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Shigella	no data	9.74E-01 (2.15E-01 to 1.97E+00)	4.37E-02 (1.42E-02 to 1.51E-01)	4.29 ± 1.15	7.29E-03
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	pneumoniae	2.68E-03 (9.12E-04 to 1.63E-02)	5.40E-04 (8.12E-05 to 1.14E-03)	4.09 ± 1.36	3.19E-02
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	indicus	1.14E-03 (1.33E-04 to 1.81E-03)	0.00E+00 (0.00E+00 to 1.26E-04)	3.99 ± 1.48	4.97E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	sp KTE96	1.83E-03 (4.83E-04 to 3.30E-03)	9.68E-05 (0.00E+00 to 7.69E-04)	3.98 ± 1.45	4.76E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	freundii complex sp CFNIH2	2.13E-04 (1.66E-04 to 7.21E-04)	4.00E-05 (0.00E+00 to 9.02E-05)	3.94 ± 1.45	4.81E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	cloacae	1.49E-03 (3.42E-04 to 1.95E-03)	1.08E-04 (0.00E+00 to 3.00E-04)	3.92 ± 1.23	2.26E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Shigella	flexneri	3.42E-01 (2.39E-01 to 7.99E-01)	4.10E-02 (7.24E-03 to 1.49E-01)	3.90 ± 1.14	1.30E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Siccibacter	turicensis	1.96E-04 (1.33E-04 to 2.08E-04)	4.84E-05 (0.00E+00 to 1.21E-04)	3.89 ± 1.12	1.21E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	no data	bacterium strain FGI 57	3.05E-03 (9.31E-04 to 8.49E-03)	7.14E-04 (1.58E-04 to 1.31E-03)	3.86 ± 1.20	2.15E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	freundii	5.34E-03 (9.58E-04 to 2.11E-02)	6.44E-04 (2.16E-04 to 1.19E-03)	3.83 ± 1.42	4.97E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	sp 8 2 54BFAA	5.63E-03 (5.86E-04 to 2.25E-02)	2.28E-03 (2.22E-04 to 5.50E-03)	3.72 ± 1.15	2.15E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Yokenella	regensburgi	5.32E-04 (3.33E-04 to 9.28E-04)	9.74E-05 (4.14E-05 to 2.38E-04)	3.71 ± 1.16	2.16E-02
Proteobacteria	Gammaproteobacteria	no data	no data	no data	no data	2.66E-02 (6.47E-03 to 1.03E-01)	2.62E-03 (7.30E-04 to 9.95E-03)	3.70 ± 1.29	4.11E-02

Table 7 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)		Log <sub>2</sub> FC mean ± SE	Adjusted P -value <sup>1</sup>
						HR (n = 9)	LR (n = 10)		
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Citrobacter	werkmanii	3.72E-04 (5.32E-05 to 2.93E-03)	5.87E-05 (3.61E-05 to 2.19E-04)	3.63 ± 1.20	3.19E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Shigella	sonnei	2.11E-01 (1.90E-01 to 4.21E-01)	2.15E-02 (5.35E-03 to 1.19E-01)	3.58 ± 1.15	2.66E-02
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	striatum	5.55E-04 (4.42E-04 to 6.65E-04)	1.20E-04 (1.73E-05 to 2.52E-04)	3.46 ± 1.02	1.40E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	no data	no data	2.40E+01 (1.57E+01 to 3.93E+01)	3.26E+00 (3.15E-01 to 1.05E+01)	3.34 ± 1.17	4.11E-02
Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	opacus	6.19E-04 (4.44E-04 to 7.18E-04)	7.17E-05 (0.00E+00 to 3.01E-04)	3.32 ± 1.13	3.53E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	sp D7	3.57E-03 (4.86E-04 to 1.51E-02)	2.00E-03 (4.98E-04 to 5.34E-03)	3.29 ± 1.15	4.11E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Escherichia	albertii	3.53E-01 (1.71E-01 to 5.09E-01)	6.62E-02 (1.44E-02 to 1.43E-01)	3.29 ± 1.15	4.16E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Citrobacter	sp MGH110	1.66E-04 (1.45E-04 to 6.09E-04)	6.33E-05 (0.00E+00 to 1.34E-04)	3.26 ± 1.15	4.28E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Kluyvera	cryocrescens	1.04E-03 (7.06E-04 to 1.46E-03)	1.46E-04 (8.05E-06 to 5.91E-04)	3.25 ± 1.09	3.39E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter	no data	5.65E-03 (4.07E-03 to 8.89E-03)	7.39E-04 (1.65E-04 to 2.59E-03)	3.09 ± 1.13	4.81E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Kosakonia	cowanii	4.42E-04 (3.17E-04 to 4.79E-04)	5.90E-05 (0.00E+00 to 3.21E-04)	2.99 ± 1.10	4.90E-02
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Shigella	boydii	2.11E-01 (7.81E-02 to 3.71E-01)	2.20E-02 (6.29E-03 to 7.84E-02)	2.90 ± 1.07	4.90E-02
<b>Lower in HR (12 species)</b>									
Actinobacteria	Coriobacteriia	Eggerthellales	Eggerthellaceae	Eggerthella	no data	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 9.63E-05)	-8.77 ± 2.94	3.39E-02
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	gallinarum	0.00E+00 (0.00E+00 to 1.47E-04)	0.00E+00 (0.00E+00 to 1.97E-02)	-7.36 ± 2.70	4.81E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	caccae	0.00E+00 (0.00E+00 to 0.00E+00)	2.42E-05 (0.00E+00 to 4.28E-03)	-6.45 ± 2.38	4.90E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	no data	2.08E-05 (0.00E+00 to 1.33E-04)	1.23E-04 (8.06E-06 to 4.72E-02)	-6.20 ± 2.01	2.76E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	canintestini	7.98E-05 (0.00E+00 to 2.92E-04)	3.40E-04 (3.54E-05 to 1.04E-02)	-5.57 ± 1.62	1.27E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Catenibacterium	mitsuokai	8.32E-05 (0.00E+00 to 7.59E-04)	2.97E-02 (1.35E-03 to 5.00E-01)	-5.29 ± 1.71	2.66E-02

(Continued)

Table 7 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Week 4 HR vs LR	
						Median (IQR)	LR (n = 10)		
						HR (n = 9)	LR (n = 10)	Log <sub>2</sub> FC mean ± SE	Adjusted P-value <sup>1</sup>
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	no data	0.00E+00 (0.00E+00 to 2.22E-04)	3.54E-04 (3.63E-05 to 1.07E-02)	-4.80 ± 1.75	4.76E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	pasteurianus	4.89E-04 (1.38E-04 to 3.65E-03)	6.72E-03 (1.91E-03 to 2.25E-01)	-4.26 ± 1.55	4.76E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	sp 3 2 56FAA	1.55E-03 (2.92E-04 to 1.01E-02)	2.29E-02 (5.94E-03 to 4.40E-02)	-3.96 ± 1.07	7.29E-03
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	no data	bacterium CHKCI004	0.00E+00 (0.00E+00 to 3.97E-05)	1.54E-04 (5.61E-05 to 5.19E-04)	-3.63 ± 1.23	3.48E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	no data	bacterium 3 1 46FAA	5.06E-04 (1.35E-04 to 3.67E-03)	5.23E-03 (2.61E-03 to 3.45E-02)	-2.85 ± 1.03	4.71E-02
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	sp SS2/1	2.93E-04 (6.49E-05 to 6.85E-04)	1.51E-03 (7.01E-04 to 4.71E-03)	-2.47 ± 0.85	3.59E-02

**Notes:**<sup>1</sup> P values were adjusted with false discovery rate for multiple comparisons.

FC, fold change; SE, standard error.

**Table 8** Summary of the differential abundances of Bacteroidales, Clostridiales, Lactobacillales, and Enterobacterales at baseline and week 4 in all dogs ( $n = 28$ ), high- ( $n = 9$ ), and low-responders ( $n = 10$ ).

Bacteria (Order)	Subjects	Baseline	Change in the abundance	Week 4
<b>Bacteroidales</b> 96 species compared	All	–	↑ 1 species (1.0%) ↓ 5 species (5.2%)	–
	HR	HR>LR: 20 species (20.8%)	↓ 7 species (7.3%)	HR>LR: 0 species (0%)
	LR	HR<LR: 0 species (0%)	↑ 1 species (1.0%)	HR<LR: 0 species (0%)
<b>Clostridiales</b> 205 species compared	All	–	↑ 3 species (1.5%) ↓ 13 species (6.3%)	–
	HR	HR>LR: 7 species (3.4%) HR<LR: 3 species (1.5%)	↑ 5 species (2.4%) ↓ 17 species (8.3%)	HR>LR: 9 species (4.4%) HR<LR: 6 species (2.9%)
	LR		↑ 3 species (1.5%) ↓ 1 species (0.5%)	
<b>Lactobacillales</b> 205 species compared	All	–	↑ 47 species (22.9%) ↓ 2 species (1.0%)	–
	HR	HR<LR: 34 species (16.6%)	↑ 77 species (37.6%)	HR>LR: 5 species (2.4%)
	LR	HR>LR: 1 species (0.5%)	↑ 5 species (2.4%) ↓ 1 species (0.5%)	HR<LR: 2 species (1.0%)
<b>Enterobacterales</b> 205 species compared	All	–	↑ 17 species (8.3%)	–
	HR	HR<LR: 47 species (22.9%)	↑ 97 species (47.3%)	HR<LR: 0 species (0%)
	LR	HR>LR: 0 species (0%)	↓ 7 species (3.4%)	HR>LR: 38 species (18.5%)

**Notes:**

Data expressed as numbers of species and percentages of all species being compared in each order.

*Enterobacterales and Lactobacillales dominated the gut of high-responders*

Among 68 bacterial species whose abundances were higher in HR, 38 (56%) were Enterobacterales (Table 7, Fig. S8B). No Enterobacterales species was observed to be higher in LR at week 4. This was the opposite of baseline GM where 47 species of Enterobacterales were higher in LR (Table 6). For Lactobacillales, five species were enriched in the gut of HR while two species were enriched in the gut of LR at week 4.

*Clostridiales in the gut of high- and low-responders*

Nine (13%) out of 68 species with higher abundances among HR were in the Clostridiales order, while six (50%) out of 12 species with higher abundances among LR were in the Clostridiales order (Table 7, Fig. S8B).

*Bacteroidales was no longer different in high- vs low-responders*

The 20 species in the Bacteroidales order observed to have higher abundances in HR at baseline were no longer different between HR and LR at week 4 (Table 7, Fig. S8B).

***Protein content of the baseline diet associated with the magnitude of PCo1 change***

In addition to baseline GM, the magnitude of PCo1 change was also associated with baseline diet composition. Data on the baseline extruded kibble diets were assembled; however, guaranteed nutrient analysis could only be determined for 15 dogs whose specific formula was provided (Table S5). Protein content of the baseline diet statistically differed

among HR, MR, and LR, when expressed as either % dry matter ( $p = 0.040$ ) or gram per 1,000 kcal diet ( $p = 0.027$ , Kruskal–Wallis test). In addition, % protein dry matter was also negatively correlated with PCo1 score at baseline (Spearman's  $\rho = -0.54$ ,  $p = 0.039$ ) (Fig. S9A), and positively correlated with PCo1 score change over the 4 week (Spearman's  $\rho = 0.67$ ,  $p = 0.006$ ) (Fig. S9B). A similar finding was seen when protein content was expressed as g/1000 kcal (Spearman's  $\rho = -0.59$ ,  $p = 0.024$  for correlation with PCo1 score at baseline; Spearman's  $\rho = 0.78$ ,  $p = 9.9E-04$  for correlation with PCo1 score change) (Figs. S9C, S9D). No association with PCo1 score was observed for fat, fiber, or calorie density. Changes in PCo1 score were also not associated with any of the four different recipes of the intervention diet.

### **Subject characteristics and $\alpha$ -diversity in high-, mid- and low-responders**

Other subject characteristics among the HR, MR, and LR are summarized in Table S6A. Age, sex, body weight, BCS and other subject characteristics at baseline were not significantly different among the groups. Likewise, health outcomes reported at week 4 were also not associated with the magnitude of PCo1 score change (Table S6B). Additionally, no measure of  $\alpha$ -diversity was different among HR, MR, and LR at baseline or at week 4 at a sequencing coverage of 470,000 reads (Table S7).

### **Gut microbiome and change in overall health**

To investigate the association between changes in the microbial profile and overall health, the GM of dogs with reported “better” ( $n = 19$ ) and “no change” ( $n = 12$ ) in overall health were compared at the species level at week 4 (Tables 3, 9, Fig. 8). Dogs whose owners reported improved health had higher abundances of 35 species, 24 (69%) of which are in the Lactobacillales order and two of which are in the Enterobacterales order. Five (14%) species of the Erysipelotrichaceae family were also higher in dogs reported as having better overall health. Two species, both *Bacteroides*, were lower in those reported as having better overall health.

Since the overall health status at baseline was comparable between these two groups of dogs, as a control the same analysis was performed on the sample collected at baseline. Only 8 species were identified to be differentially present between the two groups (Table S8). None of the identified species were a Lactobacillales, an Enterobacterales, or an Erysipelotrichaceae.

The magnitude of change in PCo1 score was not significantly different between dogs reported as having improved versus no change in overall health. Species evenness, richness, and Shannon's diversity index were not different between these two groups at baseline at the sequencing coverage of 470,000 reads. At week 4, however, the species evenness in those reporting improved overall health was significantly higher than those reporting no change in overall health ( $0.59 \pm 0.10$  vs  $0.52 \pm 0.11$ ,  $p = 0.023$ , Kruskal–Wallis test). Likewise, Shannon's diversity index at week 4 was higher in those reported to have improved overall health ( $2.39 \pm 0.39$  vs  $2.10 \pm 0.44$ ,  $p = 0.023$ , Kruskal–Wallis test).

**Table 9** Bacterial species whose abundance at week 4 was significantly different ( $|\text{fold change}| \geq 2$  and FDR-adjusted  $p < 0.05$ ) between those reporting “better” ( $n = 16$ ) and “about the same” overall health ( $n = 10$ ) after diet change.

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Better vs About the same health Week 4
						Better overall health ( $n = 16$ ) Median (IQR)	About the same overall health ( $n = 10$ )	
<b>Higher in “better overall health” (35 species)</b>								
Firmicutes	Bacilli	Bacillales	Bacillaceae	Lysinibacillus	sphaericus	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	25.39 ± 3.04 3.41E-14
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	animalis	0.00E+00 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	10.52 ± 3.04 1.54E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	avium	1.85E-04 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 3.11E-05)	8.40 ± 2.03 3.09E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp 3C7 DIV0644	6.49E-05 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	8.39 ± 1.94 2.71E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	plantarum	3.25E-04 (8.20E-05 to 1.99E-03)	1.18E-04 (0.00E+00 to 1.64E-03)	8.35 ± 1.68 1.84E-04
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp 3G6 DIV0642	8.56E-04 (0.00E+00 to 0.00E+00)	0.00E+00 (0.00E+00 to 0.00E+00)	7.89 ± 1.84 2.71E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMS072H05	1.33E-05 (0.00E+00 to 2.88E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	7.87 ± 2.36 1.88E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp FDAARGOS 163	1.35E-04 (0.00E+00 to 3.44E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	7.30 ± 1.95 6.49E-03
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	no data	8.09E-05 (0.00E+00 to 3.75E-04)	0.00E+00 (0.00E+00 to 3.29E-05)	7.05 ± 1.83 5.68E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp 5B3 DIV0040	4.23E-04 (0.00E+00 to 5.17E-03)	0.00E+00 (0.00E+00 to 2.42E-05)	6.66 ± 1.64 3.09E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp CR-Ec1	7.63E-05 (0.00E+00 to 1.59E-03)	0.00E+00 (0.00E+00 to 0.00E+00)	6.39 ± 1.85 1.54E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp kprr-6	5.33E-05 (0.00E+00 to 4.47E-03)	0.00E+00 (0.00E+00 to 2.42E-05)	6.17 ± 1.63 6.25E-03

(Continued)



Table 9 (continued)

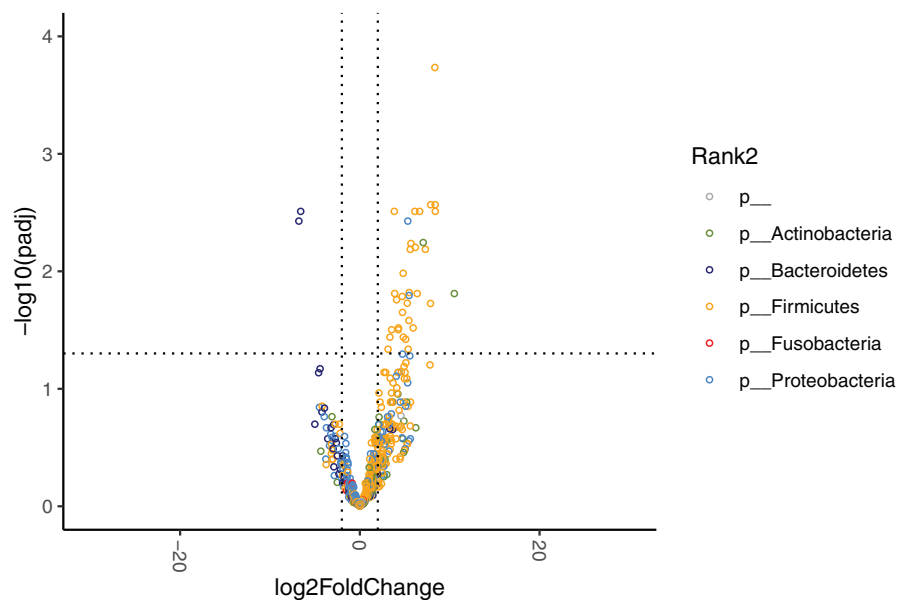
Phylum	Class	Order	Family	Genus	Species	Relative abundance, in % Median (IQR)	Better vs About the same health		Adjusted P-value <sup>1</sup>
							About the same overall health (n = 10)	Log <sub>2</sub> FC mean ± SE	
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp FDAARGOS 375	9.02E-04 (2.08E-05 to 1.91E-02)	4.83E-05 (0.00E+00 to 2.76E-04)	6.12 ± 1.50	3.09E-03
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	orisratti	1.02E-04 (0.00E+00 to 7.83E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.94 ± 1.88	3.03E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	casseliflavus	4.22E-03 (3.18E-04 to 5.93E-02)	5.53E-05 (6.27E-06 to 4.45E-04)	5.68 ± 1.49	5.79E-03
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	brevis	2.64E-04 (0.00E+00 to 8.82E-04)	5.09E-05 (3.45E-05 to 5.27E-04)	5.61 ± 1.49	6.49E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	pseudoavium	7.17E-05 (0.00E+00 to 3.14E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.49 ± 1.57	1.51E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia	alvei	8.40E-04 (5.64E-05 to 1.16E-01)	3.45E-05 (0.00E+00 to 4.21E-04)	5.48 ± 1.60	1.60E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Catenibacterium	mitsuokai	4.69E-03 (3.99E-05 to 7.00E-01)	7.95E-04 (1.71E-04 to 2.00E-02)	5.46 ± 1.69	2.62E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp 6D12 DIV0197	2.71E-04 (0.00E+00 to 9.29E-03)	0.00E+00 (0.00E+00 to 6.22E-05)	5.38 ± 1.81	4.60E-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Raoultella	ornithinolytica	1.96E-04 (0.00E+00 to 3.51E-03)	5.90E-05 (8.27E-06 to 1.22E-04)	5.34 ± 1.34	3.73E-03
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	sp HMSC05C03	1.11E-04 (0.00E+00 to 8.99E-04)	0.00E+00 (0.00E+00 to 0.00E+00)	5.28 ± 1.58	1.87E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	canis	1.46E-04 (0.00E+00 to 1.36E-03)	3.45E-05 (0.00E+00 to 1.93E-04)	5.11 ± 1.67	3.78E-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	no data	1.82E-04 (0.00E+00 to 2.27E-02)	4.25E-05 (5.19E-06 to 3.35E-03)	4.83 ± 1.57	3.63E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	gallinarum	1.84E-03 (2.06E-04 to 8.66E-03)	8.58E-05 (0.00E+00 to 1.66E-03)	4.82 ± 1.34	1.04E-02

Table 9 (continued)

Phylum	Class	Order	Family	Genus	Species	Relative abundance, in %		Better vs About the same health Week 4	
						Better overall health (n = 16) Median (IQR)	About the same overall health (n = 10)		
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	dispar	7.80E-05 (0.00E+00 to 1.00E-03)	0.00E+00 (0.00E+00 to 6.09E-05)	4.75 ± 1.45	2.23E-02
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	malodoratus	1.66E-04 (3.32E-05 to 5.32E-04)	0.00E+00 (0.00E+00 to 3.67E-05)	4.72 ± 1.38	1.64E-02
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	malromaticum	2.01E-04 (9.79E-05 to 3.96E-03)	2.93E-05 (0.00E+00 to 9.28E-04)	4.30 ± 1.36	3.03E-02
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	fermentum	3.96E-04 (0.00E+00 to 1.01E-03)	6.09E-05 (0.00E+00 to 2.59E-04)	4.29 ± 1.37	3.14E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	garvieae	4.12E-04 (1.69E-04 to 1.80E-03)	1.88E-04 (0.00E+00 to 4.24E-04)	4.09 ± 1.21	1.74E-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	lactis	2.18E-02 (2.76E-03 to 1.32E-01)	6.68E-03 (3.89E-03 to 2.35E-02)	3.88 ± 1.12	1.54E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelatoclostridium	innocuum	6.12E-04 (2.26E-04 to 3.63E-03)	1.46E-04 (3.54E-05 to 2.50E-04)	3.83 ± 0.94	3.09E-03
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	sp 3 3 56FAA	4.90E-03 (8.45E-04 to 1.62E-02)	8.80E-04 (1.98E-05 to 3.02E-03)	3.54 ± 1.13	3.14E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	no data	no data	2.01E-01 (1.77E-02 to 1.16E+00)	2.66E-02 (4.94E-03 to 2.53E-01)	3.35 ± 1.09	3.63E-02
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	sp 8 2 54BFAA	6.89E-03 (2.25E-03 to 1.77E-02)	5.31E-04 (5.01E-05 to 4.51E-03)	3.15 ± 1.06	4.60E-02
<b>Lower in "better overall health" (2 species)</b>									
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	stercoris	7.21E-05 (0.00E+00 to 1.05E-03)	1.43E-03 (0.00E+00 to 1.59E-01)	-6.79 ± 1.71	3.73E-03
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	coprocola	1.50E-05 (0.00E+00 to 5.17E-04)	2.17E-04 (2.38E-05 to 5.16E-02)	-6.58 ± 1.60	3.09E-03

**Notes:**

<sup>1</sup> P values were adjusted with false discovery rate for multiple comparisons.  
FC, fold change; SE, standard error; NS, not significant.



**Figure 8** Volcano plot demonstrating fold-change (FC) of species abundance between week 4 and baseline in those reporting better overall health ( $n = 16$ ) compared to those reporting about the same overall health ( $n = 10$ ). Vertical dashed lines show  $\log_2\text{FC}$  at 1 and  $-1$  (i.e. FC at 2 and  $-2$ ). Horizontal dashed line shows adjusted  $p = 0.05$ . Adjustment of  $p$  values is performed with false discovery rate. Points are colored by phylum. [Full-size !\[\]\(ba1b80118482ccef74a5d718ca4d7242\_img.jpg\) DOI: 10.7717/peerj.11648/fig-8](https://doi.org/10.7717/peerj.11648/fig-8)

## DISCUSSION

We describe GM composition and owner-reported health perception of dogs switching from various brands of kibble to commercial MC diets. To our knowledge, this is the first MC diet study performed in household dogs, including male and female dogs of various breeds across a broad age range. As a result of this diverse and free-living study population these findings may be more generalizable to the dog population than other studies that focus on a single breed or a single diet. Two previously published studies using a similar diet comparison were conducted, however they consisted of eight and 12 Beagles in a laboratory setting (Algya et al., 2018; Do et al., 2021). Dogs in the present study maintained their body weight throughout the study period, therefore changes in health and GM were independent of weight change. Finally, the characterization of gut bacteria in this study was performed with metagenomic sequencing, a powerful technique enabling enhanced taxonomic resolution and accuracy, as well as increased detection of diversity and functional genes, a technique that has not yet been widely used in canine research (Ranjan et al., 2016; Salas-Mani et al., 2018; Xu et al., 2019; Alessandri et al., 2019; Connelly et al., 2019; Do et al., 2021).

All dogs were perceived by their owners as having either better or no change in overall health after diet transition. It is possible that this observation could be subject to bias and may not directly correlate to more objective health outcomes, but as argued by Yeates & Main (2009), owner-reported health assessment is a separate and independent outcome that is especially important in this type of research study. A chance of bias may also derive from the willingness of participants to report the health impact associated with the diet

transition, and the observation that a significant portion of participants (24%) did not complete the study. However the association between a MC diet and positive health perception has been previously reported using a survey administered to a group of dog owners after a veterinarian formulated MC diets for the dogs (*Johnson et al., 2016*). Dogs accepted MC diets well and remained healthy in two additional feeding trials (*Algya et al., 2018; Do et al., 2021*).

Incidents of vomiting and diarrhea were recorded over the four weeks after diet transition. Currently there is not much data on the prevalence and frequency of these symptoms in household dogs, regardless of diet transition. A 2005 survey collecting responses from 772 dog owners in England found that the rate of spontaneous vomiting and diarrhea was one case per 10.6 dog-weeks and one case per 13.4 dog-weeks, respectively (*Hubbard et al., 2007*). A diet change was suggested to be a cause of diarrhea in some dogs. These are slightly higher than the rates in this present study. Likewise, 27% of dogs experienced diarrhea within seven weeks after boiled minced beef replaced 75% of the calories in an extruded diet (one case per 25.6 dog-weeks) (*Herstad et al., 2017, 2018*). Other diet transition studies using a raw or MC diet did not report cases of diarrhea or vomiting (*Sandri et al., 2017; Algya et al., 2018; Do et al., 2021*). Therefore, the rates of vomiting and diarrhea appear to be consistent with data available from other household dog populations.

Fifty eight percent of owners reported lower defecation frequency upon diet transition in this study, although this proportion was not significantly different from those reporting no change. Fecal output has been reported to be significantly lower in dogs fed MC diets compared to extruded diets, despite dogs maintaining the same body weight on different diets (*Oba et al., 2020; Do et al., 2021*). The extremely high nutrient and energy digestibility of MC diets is suggested to be the main driver of the reduction in fecal output (*Do et al., 2021*). There was also a non-significant trend towards firmer stool when dogs switched to the test diets in our study. In studies using veterinarian-assessed data or an objective analysis of fecal moisture, fecal consistency was reported to be firmer (*Sandri et al., 2017; Bermingham et al., 2017*) or comparable (*Algya et al., 2018; Schmidt et al., 2018; Do et al., 2021*) among healthy dogs fed MC or raw diets compared to those fed a traditional extruded diet.

The intervention diet in this study consisted of many nutrients and dietary components that may have an influence on both health and GM composition, including water (*Cameron et al., 2011; Deng et al., 2014; Alexander, Colyer & Morris, 2014*), prebiotic fibers (*Middelbos et al., 2010; Panasevich et al., 2015; Alexander et al., 2018; Eisenhauer et al., 2019*), omega-3 fatty acids (*Bauer, 2011*), carotenoids, and polyphenols (*Kolchin et al., 1991; Kim et al., 2000; Chew et al., 2000; Massimino et al., 2003; Hall et al., 2006; Carrera-Quintanar et al., 2018; Lyu et al., 2018*), although high-quality extruded diets may include these nutrients and compounds as well. Nutrient and energy digestibility has also been observed to be different between extruded kibble and MC diets (*Algya et al., 2018*), and it may facilitate differential impacts on health and GM. Due to the study design, it is impossible to pinpoint which component(s) of the MC diets contributed to the change in

health and GM profile. Similarly, the four MC diet recipes were different in nutrient and in ingredient composition, adding an additional confounding factor.

Changes in  $\alpha$ -diversity in response to dietary interventions have varied between studies, likely because they are dependent on the diets being compared. Similar to *Do et al. (2021)*, we did not observe any change in the GM  $\alpha$ -diversity between kibble and MC diets. A cross-sectional study performed in household dogs also reported no differences in  $\alpha$ -diversity between those fed raw and extruded diets (*Schmidt et al., 2018*). However these findings contrast with previous reports that demonstrate reduced  $\alpha$ -diversity upon transitioning to a MC diet in 8 or 11 dogs (*Herstad et al., 2017; Algysa et al., 2018*), or increased  $\alpha$ -diversity upon transitioning to a raw food diet in eight dogs (*Sandri et al., 2017*).  $\alpha$ -Diversity was also higher in six household dogs fed raw diets as compared to five household dogs fed commercial extruded diets (*Kim et al., 2017*). Herstad et al. argued that decreased  $\alpha$ -diversity with a cooked beef diet may be due to higher fat content inducing bile production (*Herstad et al., 2017*), which has been shown to be antimicrobial (*Yokota et al., 2012; Herstad et al., 2017*). As the fat and protein content in the baseline extruded diets were not statistically different from the intervention diet in this present study, it is possible that the lack of macronutrient composition difference is responsible for the lack of a change in  $\alpha$ -diversity. The variety of baseline diets and the utilization of four different MC recipes may also mask the effect of diet transition on  $\alpha$ -diversity. Often, the  $\alpha$ -diversity is observed to be lower in diseased populations (*Wernimont et al., 2020*). In this study the Shannon's diversity index in a subgroup of dogs whose overall health reported to be improved at week four was significantly higher than those that remained unchanged. However, the use of  $\alpha$ -diversity and its clinical importance have been recently challenged (*Moya & Ferrer, 2016*).

Consistent with previous shotgun metagenomic sequencing studies in household dogs (*Alessandri et al., 2019; Jha et al., 2020*), four phyla, Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes, dominated the GM. A variety of taxonomic differences in the microbiome were observed after dietary transition, including increased abundances of many LAB. The abundances of the Lactobacillaceae and Leuconostocaceae families and the *Lactobacillus* genus were similarly reported to be higher in dogs fed a raw diet compared to a kibble diet (*Birmingham et al., 2017; Schmidt et al., 2018*). On the contrary, *Do et al. (2021)* reported lower levels of *Lactobacillus* in dogs fed MC diets compared to an extruded kibble, but the kibble contained an *L. acidophilus* fermentation product and high chicory content which were postulated to contribute to the growth of LAB. In many mammals LAB have been suggested to be allochthonous, meaning they are ingested through their natural occurrence on food rather than being true inhabitants of the distal part of the host GI tract (*Walter, 2008*). Owing to their demonstrated ability to improve GI health (*Grześkowiak et al., 2015; Jensen & Bjørnvad, 2019*), selective LAB are often included in probiotic food or supplements. We also observed a significant association between increased abundances of Lactobacillales and dogs reported as having better health after four weeks of the intervention diet.

An increase of Enterobacterales was also observed with diet transition. Similar increases were previously observed in healthy dogs fed either a MC or a raw diet (*Sandri et al., 2017*;

*Algya et al., 2018; Schmidt et al., 2018*). While there are pathogens within this order, many species of Enterobacterales, such as *Salmonella* and *E. coli*, are also members of the healthy human and canine GM, and only opportunistically cause disease in humans, for instance, when an immune system is compromised (*Moon et al., 2018*). Frequently, only specific strains are pathogenic. It is unknown whether some species of Enterobacterales are also associated with increased disease risk in dogs, particularly since some known human pathogens (e.g. *Escherichia/Shigella* spp. (*Sandri et al., 2017*),  $\beta$ -lactamase producing *E. coli* (*Baede et al., 2015*), *Salmonella* (*Reimschuessel et al., 2017*), and *Campylobacter* spp. (*Bojanić et al., 2017*)) are present in the GI tract of healthy dogs. Additionally, Enterobacterales are diverse and have a considerably wide range of abundances in the present study. A wide range of Proteobacteria, whose members predominantly include Enterobacterales, have been previously reported in other cohorts of healthy dogs using metagenomic sequencing (IQR: 3–52% in (*Jha et al., 2020*)) or 16S rRNA sequencing (range: 0–22% in (*Hand et al., 2013*), range: 0–17% in (*Garcia-Mazcorro et al., 2012*)). Therefore the impact of an increased abundance of Enterobacterales on canine health remains to be determined.

Switching to the MC diet also resulted in reduced abundances of some taxa, including species in the order Clostridiales and certain genera, including *Blautia*, *Prevotella*, *Peptostreptococcus*, and *Faecalibacterium*. This result shares some similarities with previous reports with a transition from an extruded kibble to a raw diet (*Sandri et al., 2017; Bermingham et al., 2017; Algya et al., 2018; Schmidt et al., 2018*). A MC diet also decreased *Prevotella* in dogs switching from an extruded kibble diet (*Do et al., 2021*), but not *Clostridium*, *Blautia*, or *Faecalibacterium*. Many of these bacteria are present in a healthy human GI tract, but their relevance is unknown in dogs. *F. prausnitzii*, which was lower after the intervention diet, was also shown to be lower in dogs fed minced beef than those fed an extruded diet (*Herstad et al., 2017*). This bacterium is thought to be beneficial because of its ability to produce butyrate, a short-chain fatty acid that serves as an energy source for colonocytes (*Louis & Flint, 2009; Donohoe et al., 2011*). However, its reduced abundance in MC diets may be due to other butyrate-producing bacteria becoming more abundant (e.g. Firmicutes (*Macfarlane & Macfarlane, 2003*)), as it has been shown with increased levels of *Roseburia* and butyrate in dogs fed minced beef (*Herstad et al., 2017*), but not in the previous trial (*Do et al., 2021*), or in the present study. This is further supported by the observation that the KO terms related to short-chain fatty acid metabolism remained unchanged upon diet transition in this study.

Our results indicate a differential microbiomic response to dietary transition based on the magnitude of change in  $\beta$ -diversity (i.e. HR, MR and LR). While similar analytical approaches have been adopted in human research (*Salonen et al., 2014; Mills et al., 2019*), to our knowledge this is the first study to report distinct varied microbial responses in dogs in response to diet change. Using assorted multivariate analyses, the GM at baseline was demonstrated to be different among HR, MR and LR. Specifically, HRs had lower abundances of Lactobacillales and Enterobacterales, and higher abundances of Bacteroidales than the LRs, and they responded to the MC diet differently and more



robustly. We hypothesize that the niche occupied by Bacteroidales at baseline becomes more favorable for Enterobacterales and LAB growth upon diet transition. This may explain why the lower abundance of Enterobacterales at baseline was associated with greater PCo1 score change. Moreover, the protein content of the baseline diet was positively correlated with the magnitude of PCo1 change, although we acknowledge the small sample size ( $n = 15$ ) as well as a previous analysis of 750 commercial dry pet foods demonstrating 1.6% difference between the guaranteed and actual analysis of crude protein (Hill *et al.*, 2009). Given that the protein content of the baseline and the intervention diets were statistically comparable, and that Enterobacterales are able to thrive on proteins, carbohydrates, or lipids (Moon *et al.*, 2018), the observed correlation may be explained by the change in the ingredients despite an unmodified macronutrient profile. For example, replacing chicken with whole cricket meal led to a small change in the GM profile in dogs (Jarett *et al.*, 2019), and replacing cellulose and brewer's rice with potato fiber up to 6% also led to an increase of *Faecalibacterium* (Panasevich *et al.*, 2015). Such associative findings between baseline characteristics and magnitude of responses to the intervention can serve as a basis for future studies that aim to construct predictive models, and ultimately, dietary plans tailored to each individual based on existing microbiome composition and function for optimized health outcomes (Zmora *et al.*, 2018).

Changes in GM function characterized by KEGG were detected with a multivariate analysis (PCoA), but remained less defined with univariate analysis (negative binomial GLM with DESeq2) in this study. The univariate method is inferior in terms of its ability to capture small changes cumulatively. GM functional redundancy under different dietary environments has been demonstrated (Moya & Ferrer, 2016), and changes in GM profile in healthy dogs may not reflect a drastic change in the functionality as compared to those with improved or worsened health status after an intervention (Guard *et al.*, 2015). Thus the lack of any drastic change in functionality is likely at least partially reflective of dogs being healthy at both baseline and week 4. Availability of data from other platforms such as transcriptional profiling or targeted metabolomics may complement metagenomic sequencing in capturing finer changes in GM functionality.

In conclusion, we have demonstrated significant changes in the GM profile of healthy dogs transitioning from a variety of kibble diets to MC diets. The magnitude of shift is associated with baseline GM profile and protein content of the baseline diet. The test diets were well accepted throughout the study, and the majority of dog owners report an improvement or no change in specific health outcomes. Despite its novelty, there are some limitations to the study. A control group where dogs remained on the baseline diet for four weeks was not included, thus any direct causal relationship cannot be established between diet transition and outcomes. However, observed changes in the GM were likely to be caused by diet change as minimal changes were expected if baseline diets were maintained for four weeks. While the four different MC diet recipes may have dampened the signal, most dogs were on a mixed-recipe diet and a wide variety of baseline diets may have provided an opportunity to investigate the wide range of diets and

responses among subjects. Finally, while no clinical measures were available, the health data do reflect the owner's perceptions of health.

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Jirayu Tanprasertsuk, Justin Shmalberg, LeeAnn M. Perry, Ryan W. Honaker are employees of and/or hold stocks or stock options in NomNomNow, Inc. Aashish R. Jha, Heather Maughan, Devon E. Tate consult for NomNomNow, Inc.

### Author Contributions

- Jirayu Tanprasertsuk conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Justin Shmalberg conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Heather Maughan analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Devon E. Tate analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- LeeAnn M. Perry analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Aashish R. Jha analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Ryan W. Honaker conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.



## DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The metagenomic sequences are available at NCBI: BioProject [PRJNA714112](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA714112).

The raw sequence data is also available at FigShare:

[https://figshare.com/projects/Gut\\_microbiome\\_and\\_heterogeneity\\_of\\_responses\\_in\\_healthy\\_household\\_dogs\\_transitioning\\_from\\_an\\_extruded\\_to\\_a\\_mildly\\_cooked\\_diet/100469](https://figshare.com/projects/Gut_microbiome_and_heterogeneity_of_responses_in_healthy_household_dogs_transitioning_from_an_extruded_to_a_mildly_cooked_diet/100469).

## Data Availability

The following information was supplied regarding data availability:

The R codes, phylogenetic sequencing data (read counts), alpha-diversity, and health assessment data are available as [Supplemental Files](#).

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.11648#supplemental-information>.

## REFERENCES

- Al-Ghalith GA, Hillmann B, Ang K, Shields-Cutler R, Knights D. 2018. SHI7 is a self-learning pipeline for multipurpose short-read DNA quality control. *mSystems* 3(3):e00202-17 DOI 10.1128/mSystems.00202-17.
- Al-Ghalith G, Knights D. 2017. BURST enables optimal exhaustive DNA alignment for big data. GitHub. Available at <https://github.com/knights-lab/burst>.
- Alessandri G, Milani C, Mancabelli L, Mangifesta M, Lugli GA, Viappiani A, Duranti S, Turroni F, Ossiprandi MC, Van Sinderen D, Ventura M. 2019. Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features. *Environmental Microbiology* 21(4):1331–1343 DOI 10.1111/1462-2920.14540.
- Alexander JE, Colyer A, Morris PJ. 2014. The effect of reducing dietary energy density via the addition of water to a dry diet, on body weight, energy intake and physical activity in adult neutered cats. *Journal of nutritional science* 3:e21 DOI 10.1017/jns.2014.22.
- Alexander C, Cross T-WL, Devendran S, Neumer F, Theis S, Ridlon JM, Suchodolski JS, De Godoy MRC, Swanson KS. 2018. Effects of prebiotic inulin-type fructans on blood metabolite and hormone concentrations and faecal microbiota and metabolites in overweight dogs. *The British Journal of Nutrition* 120(6):1–10 DOI 10.1017/S0007114518001952.
- Algya KM, Cross T-WL, Leuck KN, Kastner ME, Baba T, Lye L, De Godoy MRC, Swanson KS. 2018. Apparent total-tract macronutrient digestibility, serum chemistry, urinalysis, and fecal characteristics, metabolites and microbiota of adult dogs fed extruded, mildly cooked, and raw diets<sup>1</sup>. *Journal of Animal Science* 96(9):3670–3683 DOI 10.1093/jas/sky235.
- Axelsson E, Ratnakumar A, Arendt M-L, Maqbool K, Webster MT, Perloski M, Liberg O, Arnemo JM, Hedhammar A, Lindblad-Toh K. 2013. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495(7441):360–364 DOI 10.1038/nature11837.
- Baede VO, Wagenaar JA, Broens EM, Duim B, Dohmen W, Nijse R, Timmerman AJ, Hordijk J. 2015. Longitudinal study of extended-spectrum- $\beta$ -lactamase- and AmpC-producing Enterobacteriaceae in household dogs. *Antimicrobial agents and chemotherapy* 59(6):3117–3124 DOI 10.1128/AAC.04576-14.

- Bauer JE. 2011.** Therapeutic use of fish oils in companion animals. *Journal of the American Veterinary Medical Association* **239(11)**:1441–1451 DOI [10.2460/javma.239.11.1441](https://doi.org/10.2460/javma.239.11.1441).
- Bermingham EN, Maclean P, Thomas DG, Cave NJ, Young W. 2017.** Key bacterial families (Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae) are related to the digestion of protein and energy in dogs. *PeerJ* **5**:e3019 DOI [10.7717/peerj.3019](https://doi.org/10.7717/peerj.3019).
- Bojanić K, Midwinter AC, Marshall JC, Rogers LE, Biggs PJ, Acke E. 2017.** Isolation of *Campylobacter* spp. from client-owned dogs and cats, and retail raw meat pet food in the Manawatu, New Zealand. *Zoonoses and Public Health* **64(6)**:438–449 DOI [10.1111/zph.12323](https://doi.org/10.1111/zph.12323).
- Cameron KM, Morris PJ, Hackett RM, Speakman JR. 2011.** The effects of increasing water content to reduce the energy density of the diet on body mass changes following caloric restriction in domestic cats. *Journal of Animal Physiology and Animal Nutrition* **95(3)**:399–408 DOI [10.1111/j.1439-0396.2010.01107.x](https://doi.org/10.1111/j.1439-0396.2010.01107.x).
- Carrera-Quintanar L, López Roa RI, Quintero-Fabián S, Sánchez-Sánchez MA, Vizmanos B, Ortuño-Sahagún D. 2018.** Phytochemicals that influence gut microbiota as prophylactics and for the treatment of obesity and inflammatory diseases. *Mediators of Inflammation* **2018**:9734845 DOI [10.1155/2018/9734845](https://doi.org/10.1155/2018/9734845).
- Chew BP, Park JS, Wong TS, Kim HW, Weng BB, Byrne KM, Hayek MG, Reinhart GA. 2000.** Dietary  $\beta$ -carotene stimulates cell-mediated and humoral immune response in dogs. *The Journal of Nutrition* **130(8)**:1910–1913 DOI [10.1093/jn/130.8.1910](https://doi.org/10.1093/jn/130.8.1910).
- Connelly S, Fanelli B, Hasan NA, Colwell RR, Kaleko M. 2019.** Low dose oral beta-lactamase protects the gut microbiome from oral beta-lactam-mediated damage in dogs. *AIMS Public Health* **6(4)**:477–487 DOI [10.3934/publichealth.2019.4.477](https://doi.org/10.3934/publichealth.2019.4.477).
- Deng P, Iwazaki E, Suchy SA, Pallotto MR, Swanson KS. 2014.** Effects of feeding frequency and dietary water content on voluntary physical activity in healthy adult cats. *Journal of Animal Science* **92(3)**:1271–1277 DOI [10.2527/jas.2013-7235](https://doi.org/10.2527/jas.2013-7235).
- Do S, Phungviwatnikul T, De Godoy MRC, Swanson KS. 2021.** Nutrient digestibility and fecal characteristics, microbiota, and metabolites in dogs fed human-grade foods. *Journal of Animal Science* **99(2)**:114 DOI [10.1093/jas/skab028](https://doi.org/10.1093/jas/skab028).
- Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, Bultman SJ. 2011.** The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism* **13(5)**:517–526 DOI [10.1016/j.cmet.2011.02.018](https://doi.org/10.1016/j.cmet.2011.02.018).
- Eisenhauer L, Vahjen W, Dadi T, Kohn B, Zentek J. 2019.** Effects of brewer's spent grain and carrot pomace on digestibility, fecal microbiota, and fecal and urinary metabolites in dogs fed low or high protein diets. *Journal of Animal Science* **97(10)**:4124–4133 DOI [10.1093/jas/skz264](https://doi.org/10.1093/jas/skz264).
- Garcia-Mazcorro JF, Dowd SE, Poulsen J, Steiner JM, Suchodolski JS. 2012.** Abundance and short-term temporal variability of fecal microbiota in healthy dogs. *MicrobiologyOpen* **1(3)**:340–347 DOI [10.1002/mbo3.36](https://doi.org/10.1002/mbo3.36).
- Grześkowiak Ł, Endo A, Beasley S, Salminen S. 2015.** Microbiota and probiotics in canine and feline welfare. *Anaerobe* **34**:14–23 DOI [10.1016/j.anaerobe.2015.04.002](https://doi.org/10.1016/j.anaerobe.2015.04.002).
- Guard BC, Barr JW, Reddivari L, Klemashevich C, Jayaraman A, Steiner JM, Vanamala J, Suchodolski JS. 2015.** Characterization of microbial dysbiosis and metabolomic changes in dogs with acute diarrhea. *PLOS ONE* **10(5)**:e0127259 DOI [10.1371/journal.pone.0127259](https://doi.org/10.1371/journal.pone.0127259).
- Hall JA, Picton RA, Finneran PS, Bird KE, Skinner MM, Jewell DE, Zicker S. 2006.** Dietary antioxidants and behavioral enrichment enhance neutrophil phagocytosis in geriatric Beagles. *Veterinary Immunology and Immunopathology* **113(1–2)**:224–233 DOI [10.1016/j.vetimm.2006.03.019](https://doi.org/10.1016/j.vetimm.2006.03.019).

- Hand D, Wallis C, Colyer A, Penn CW. 2013.** Pyrosequencing the canine faecal microbiota: breadth and depth of biodiversity. *PLOS ONE* **8(1)**:e53115 DOI [10.1371/journal.pone.0053115](https://doi.org/10.1371/journal.pone.0053115).
- Herstad KMV, Gajardo K, Bakke AM, Moe L, Ludvigsen J, Rudi K, Rud I, Sekelja M, Skancke E. 2017.** A diet change from dry food to beef induces reversible changes on the faecal microbiota in healthy, adult client-owned dogs. *BMC Veterinary Research* **13(1)**:147 DOI [10.1186/s12917-017-1073-9](https://doi.org/10.1186/s12917-017-1073-9).
- Herstad KMV, Rønning HT, Bakke AM, Moe L, Skancke E. 2018.** Changes in the faecal bile acid profile in dogs fed dry food vs high content of beef: a pilot study. *Acta Veterinaria Scandinavica* **60(1)**:29 DOI [10.1186/s13028-018-0383-7](https://doi.org/10.1186/s13028-018-0383-7).
- Hill RC, Choate CJ, Scott KC, Molenberghs G. 2009.** Comparison of the guaranteed analysis with the measured nutrient composition of commercial pet foods. *Journal of the American Veterinary Medical Association* **234(3)**:347–351 DOI [10.2460/javma.234.3.347](https://doi.org/10.2460/javma.234.3.347).
- Hillmann B, Al-Ghalith GA, Shields-Cutler RR, Zhu Q, Gohl DM, Beckman KB, Knight R, Knights D. 2018.** Evaluating the information content of shallow shotgun metagenomics. *mSystems* **3(6)**:e00069-18 DOI [10.1128/mSystems.00069-18](https://doi.org/10.1128/mSystems.00069-18).
- Hubbard K, Skelly BJ, McKelvie J, Wood JLN. 2007.** Risk of vomiting and diarrhoea in dogs. *The Veterinary Record* **161(22)**:755–757 DOI [10.1136/vr.161.22.755](https://doi.org/10.1136/vr.161.22.755).
- Jarett JK, Carlson A, Rossoni Serao M, Strickland J, Serfilippi L, Ganz HH. 2019.** Diets with and without edible cricket support a similar level of diversity in the gut microbiome of dogs. *PeerJ* **7(10)**:e7661 DOI [10.7717/peerj.7661](https://doi.org/10.7717/peerj.7661).
- Jensen AP, Bjørnvad CR. 2019.** Clinical effect of probiotics in prevention or treatment of gastrointestinal disease in dogs: a systematic review. *Journal of Veterinary Internal Medicine/ American College of Veterinary Internal Medicine* **33(5)**:1849–1864 DOI [10.1111/jvim.15554](https://doi.org/10.1111/jvim.15554).
- Jha AR, Shmalberg J, Tanprasertsuk J, Perry L, Massey D, Honaker RW. 2020.** Characterization of gut microbiomes of household pets in the United States using a direct-to-consumer approach. *PLOS ONE* **15(2)**:e0227289 DOI [10.1371/journal.pone.0227289](https://doi.org/10.1371/journal.pone.0227289).
- Johnson LN, Linder DE, Heinze CR, Kehs RL, Freeman LM. 2016.** Evaluation of owner experiences and adherence to home-cooked diet recipes for dogs. *The Journal of Small Animal Practice* **57(1)**:23–27 DOI [10.1111/jsap.12412](https://doi.org/10.1111/jsap.12412).
- Johnson AJ, Vangay P, Al-Ghalith GA, Hillmann BM, Ward TL, Shields-Cutler RR, Kim AD, Shmagel AK, Syed AN, Walter J, Menon R, Koecher K, Knights D, Personalized Microbiome Class Students. 2019.** Daily sampling reveals personalized diet-microbiome associations in humans. *Cell Host & Microbe* **25(6)**:789–802.e5 DOI [10.1016/j.chom.2019.05.005](https://doi.org/10.1016/j.chom.2019.05.005).
- Kanehisa M, Sato Y. 2020.** KEGG Mapper for inferring cellular functions from protein sequences. *Protein Science: A Publication of the Protein Society* **29(1)**:28–35 DOI [10.1002/pro.3711](https://doi.org/10.1002/pro.3711).
- Khun M. 2014.** *Caret: classification and regression training*. R package version 6.0-35. Available at <https://cran.r-project.org/package=caret>.
- Kim J, An J-U, Kim W, Lee S, Cho S. 2017.** Differences in the gut microbiota of dogs (*Canis lupus familiaris*) fed a natural diet or a commercial feed revealed by the Illumina MiSeq platform. *Gut Pathogens* **9(1)**:68 DOI [10.1186/s13099-017-0218-5](https://doi.org/10.1186/s13099-017-0218-5).
- Kim HW, Chew BP, Wong TS, Park JS, Weng BB, Byrne KM, Hayek MG, Reinhart GA. 2000.** Dietary lutein stimulates immune response in the canine. *Veterinary immunology and immunopathology* **74(3–4)**:315–327 DOI [10.1016/S0165-2427\(00\)00180-X](https://doi.org/10.1016/S0165-2427(00)00180-X).
- Kolchin IN, Maksiutina NP, Balanda PP, Luik AI, Bulakh VN, Moibenko AA. 1991.** The cardioprotective action of quercetin in experimental occlusion and reperfusion of the coronary artery in dogs. *Farmakologija i Toksikologija* **54**:20–23.

- Laflamme D.** 1997. Development and validation of a body condition score system for dogs. *Canine Practice* 22:10–15.
- Lewis SJ, Heaton KW.** 1997. Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterology* 32(9):920–924 DOI 10.3109/00365529709011203.
- Louis P, Flint HJ.** 2009. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters* 294(1):1–8 DOI 10.1111/j.1574-6968.2009.01514.x.
- Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biology* 15(12):550 DOI 10.1186/s13059-014-0550-8.
- Lyu Y, Wu L, Wang F, Shen X, Lin D.** 2018. Carotenoid supplementation and retinoic acid in immunoglobulin A regulation of the gut microbiota dysbiosis. *Experimental Biology and Medicine* 243(7):613–620 DOI 10.1177/1535370218763760.
- Macfarlane S, Macfarlane GT.** 2003. Regulation of short-chain fatty acid production. *The Proceedings of the Nutrition Society* 62(1):67–72 DOI 10.1079/PNS2002207.
- Maechler M, Rousseeuw P, Struyt A, Hubert M.** 2015. *Cluster: cluster analysis basics and extensions*. R package version 2.0.1. Available at <https://cran.r-project.org/package=cluster>.
- Massimino S, Kearns RJ, Loos KM, Burr J, Park JS, Chew B, Adams S, Hayek MG.** 2003. Effects of age and dietary  $\beta$ -carotene on immunological variables in dogs. *Journal of Veterinary Internal Medicine/American College of Veterinary Internal Medicine* 17(6):835–842 DOI 10.1111/j.1939-1676.2003.tb02523.x.
- Middelbos IS, Vester Boler BM, Qu A, White BA, Swanson KS, Fahey GC Jr.** 2010. Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. *PLOS ONE* 5(3):e9768 DOI 10.1371/journal.pone.0009768.
- Mills S, Lane JA, Smith GJ, Grimaldi KA, Ross RP, Stanton C.** 2019. Precision nutrition and the microbiome part II: potential opportunities and pathways to commercialisation. *Nutrients* 11(7):1468 DOI 10.3390/nu11071468.
- Moon CD, Young W, Maclean PH, Cookson AL, Bermingham EN.** 2018. Metagenomic insights into the roles of *Proteobacteria* in the gastrointestinal microbiomes of healthy dogs and cats. *MicrobiologyOpen* 7(5):e00677 DOI 10.1002/mbo3.677.
- Moya A, Ferrer M.** 2016. Functional redundancy-induced stability of gut microbiota subjected to disturbance. *Trends in Microbiology* 24(5):402–413 DOI 10.1016/j.tim.2016.02.002.
- Oba PM, Utterback PL, Parsons CM, Swanson KS.** 2020. True nutrient and amino acid digestibility of dog foods made with human-grade ingredients using the precision-fed cecectomized rooster assay. *Translational Animal Science* 4(1):442–451 DOI 10.1093/tas/txz175.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H.** 2014. *Vegan: community ecology package*. R package version 2.2-0. Available at <https://cran.r-project.org/package=vegan>.
- Panasevich MR, Kerr KR, Dilger RN, Fahey GC Jr, Guérin-Deremaux L, Lynch GL, Wils D, Suchodolski JS, Steer JM, Dowd SE, Swanson KS.** 2015. Modulation of the faecal microbiome of healthy adult dogs by inclusion of potato fibre in the diet. *The British Journal of Nutrition* 113(1):125–133 DOI 10.1017/S0007114514003274.
- Pilla R, Suchodolski JS.** 2019. The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. *Frontiers in Veterinary Science* 6:498 DOI 10.3389/fvets.2019.00498.
- PRWeb.** 2015. Allprovide study shows thirty-three percent of U.S. pet owners are interested in the fresh, natural pet food diet. Available at <https://www.prweb.com/releases/2015/06/prweb12787651.htm> (accessed 16 September 2020).

- Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. 2016. Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochemical and Biophysical Research Communications* 469(4):967–977 DOI 10.1016/j.bbrc.2015.12.083.
- Redfern A, Suchodolski J, Jergens A. 2017. Role of the gastrointestinal microbiota in small animal health and disease. *Veterinary Record* 181(14):370 DOI 10.1136/vr.103826.
- Reimschuessel R, Grabenstein M, Guag J, Nemser SM, Song K, Qiu J, Clothier KA, Byrne BA, Marks SL, Cadmus K, Pabilonia K, Sanchez S, Rajeev S, Ensley S, Frana TS, Jergens AE, Chappell KH, Thakur S, Byrum B, Cui J, Zhang Y, Erdman MM, Rankin SC, Daly R, Das S, Ruesch L, Lawhon SD, Zhang S, Baszler T, Diaz-Campos D, Hartmann F, Okwumabua O. 2017. Multilaboratory survey to evaluate Salmonella prevalence in diarrheic and nondiarrheic dogs and cats in the United States between 2012 and 2014. *Journal of Clinical Microbiology* 55(5):1350–1368 DOI 10.1128/JCM.02137-16.
- Salas-Mani A, Jeusette I, Castillo I, Manuelian CL, Lionnet C, Iraculis N, Sanchez N, Fernández S, Vilaseca L, Torre C. 2018. Fecal microbiota composition changes after a BW loss diet in Beagle dogs. *Journal of Animal Science* 96(8):3102–3111 DOI 10.1093/jas/sky193.
- Salonen A, Lahti L, Salojärvi J, Holtrop G, Korpela K, Duncan SH, Date P, Farquharson F, Johnstone AM, Lobley GE, Louis P, Flint HJ, De Vos WM. 2014. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *The ISME Journal* 8(11):2218–2230 DOI 10.1038/ismej.2014.63.
- Sandri M, Dal Monego S, Conte G, Sgorlon S, Stefanon B. 2017. Raw meat based diet influences faecal microbiome and end products of fermentation in healthy dogs. *BMC Veterinary Research* 13(1):65 DOI 10.1186/s12917-017-0981-z.
- Schmidt M, Unterer S, Suchodolski JS, Honneffer JB, Guard BC, Lidbury JA, Steiner JM, Fritz J, Kölle P. 2018. The fecal microbiome and metabolome differs between dogs fed Bones and Raw Food (BARF) diets and dogs fed commercial diets. *PLOS ONE* 13(8):e0201279 DOI 10.1371/journal.pone.0201279.
- Sing T, Sander O, Beerenwinkel N, Lengauer T. 2005. ROCr: visualizing classifier performance in R. *Bioinformatics* 21(20):3940–3941 DOI 10.1093/bioinformatics/bti623.
- Tanprasertsuk J, Jha AR, Shmalberg J, Jones RB, Perry LM, Maughan H, Honaker RW. 2021. The microbiota of healthy dogs demonstrates individualized responses to synbiotic supplementation in a randomized controlled trial. *Animal Microbiome* 3(1):36 DOI 10.1186/s42523-021-00098-0.
- Tran QD, Hendriks WH, Van der Poel AFB. 2008. Effects of extrusion processing on nutrients in dry pet food. *Journal of the Science of Food and Agriculture* 88(9):1487–1493 DOI 10.1002/jsfa.3247.
- Walter J. 2008. Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Applied and Environmental Microbiology* 74(16):4985–4996 DOI 10.1128/AEM.00753-08.
- Wernimont SM, Radosevich J, Jackson MI, Ephraim E, Badri DV, MacLeay JM, Jewell DE, Suchodolski JS. 2020. The effects of nutrition on the gastrointestinal microbiome of cats and dogs: impact on health and disease. *Frontiers in Microbiology* 11:1266 DOI 10.3389/fmicb.2020.01266.
- Xu H, Zhao F, Hou Q, Huang W, Liu Y, Zhang H, Sun Z. 2019. Metagenomic analysis revealed beneficial effects of probiotics in improving the composition and function of the gut microbiota in dogs with diarrhoea. *Food & Function* 10(5):2618–2629 DOI 10.1039/C9FO00087A.
- Yeates J, Main D. 2009. Assessment of companion animal quality of life in veterinary practice and research. *The Journal of Small Animal Practice* 50(6):274–281 DOI 10.1111/j.1748-5827.2009.00755.x.

- Yokota A, Fukiya S, Islam KBMS, Ooka T, Ogura Y, Hayashi T, Hagio M, Ishizuka S. 2012.** Is bile acid a determinant of the gut microbiota on a high-fat diet? *Gut Microbes* 3(5):455–459 DOI [10.4161/gmic.21216](https://doi.org/10.4161/gmic.21216).
- Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB-Z, Federici S, Cohen Y, Linevsky R, Rothschild D, Moor AE, Ben-Moshe S, Harmelin A, Itzkovitz S, Maharshak N, Shibolet O, Shapiro H, Pevsner-Fischer M, Sharon I, Halpern Z, Segal E, Elinav E. 2018.** Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell* 174(6):1388–1405.e21 DOI [10.1016/j.cell.2018.08.041](https://doi.org/10.1016/j.cell.2018.08.041).