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Microbial transcriptional responses to host diet maintain gut microbiome homeostasis in the American cockroach

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Running title: Cockroach hindgut microbial response to host diet

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Abstract

Diet is considered a key determinant of gut microbiome composition and function. However, studies in the American cockroach have revealed surprising stability in hindgut microbiome taxonomic composition following shifts in host diet. To discover microbial activities underlying this stability, we analyzed microbial community transcriptomes from hindguts of cockroaches fed diverse diets. We used a taxon-centric approach in which we clustered genomes based on taxonomic relatedness and functional similarity and examined the transcriptional profiles of each cluster independently. In total, we analyzed a set of 18 such "genome clusters", including key taxa within *Bacteroidota*, *Bacillota*, *Desulfobacterota*, and *Euryarcheaeota* phyla. We found that microbial transcriptional responses to diet varied across diets and microbial functional profiles, with the strongest transcriptional shifts seen in taxa predicted to be primarily focused on degradation of complex dietary polysaccharides. These groups upregulated genes associated with utilization of diet-sourced polysaccharides in response to bran and dog food diets, while they upregulated genes for degradation of potentially host-derived polysaccharides in response to tuna, butter, and starvation diets. In contrast, chemolithotrophic taxa, such as *Desulfobacterota* and *Methanimicrococcus*, exhibited stable transcriptional profiles, suggesting that compensatory changes in the metabolism of other microbial community members are sufficient to support their activities across major dietary shifts. These results provide new insight into microbial activities supporting gut microbiome stability in the face of variable diets in omnivores.

Importance

The gut microbiome of omnivores must adjust to constantly changing dietary nutrient compositions. This study describes gut microbial transcriptional responses to dietary shifts that

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support gut microbiome homeostasis in omnivorous cockroaches. Our results provide insight into the complex web of metabolic interactions mediating gut microbiome composition and stability. We found a key role for polysaccharide-degrading microbes (including *Bacteroides* and *Dysgonomonas*) in supporting the metabolic activity of other gut microbes through utilization of diverse dietary polysaccharides or, in the absence of dietary polysaccharides, host-origin polysaccharides. Further, the results show remarkable stability in gene expression across diets for many gut microbes including secondary degraders of simple sugars, sulfate-reducing bacteria, and methanogens, showing that the metabolism of these microbes is more dependent on the metabolic products of primary degraders rather than dietary nutrients. This research provides new insights into the complexity of microbe-microbe and host-microbe interactions underlying gut microbiome homeostasis and dysbiosis.

Introduction

The gut microbiome shares an intimate relationship with its host, impacting digestion, nutrition, immunity, and more (1). Given the influence of microbes on their host, gut microbiome dysbiosis has been implicated in a wide range of diseases (2-7). In turn, the host can modify the gut microbiome through a variety of pathways (8-12). Of these, diet has long been considered one of the most direct drivers of gut microbiome composition (13-17). The fiber content of diets is thought to play a particularly important role in shaping gut microbiome composition, with many (18) studies reporting differences in the taxonomic and functional community profile of mice (19-21), humans (22, 23), and other mammals (13, 24-26) fed Western diets vs. high-fiber diets.

Despite the importance of diet in shaping the gut microbiome (23, 27, 28), short-term dietary interventions in humans often fail to elicit shifts in gut microbiome composition greater

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than pre-existing, individual-to-individual differences (27, 29, 30). Multiple long-term studies of individual humans have shown stability over time (23, 27, 28, 31). Even dietary interventions that have been shown to induce short-term responses in the gut microbiome have shown longer-term reversion towards the native composition before or after cessation of the intervention (32-34). Together, these results emphasize the importance of understanding microbe-microbe or host-microbe interactions that may assist in the maintenance of gut microbiome homeostasis.

We are exploring gut microbiome homeostasis in response to host dietary shifts using an omnivorous cockroach (*Periplaneta americana*) as a model system. The American cockroach hindgut microbiome is highly diverse and includes many taxa from clades found in mammalian and human guts (35-38). This gut microbiome also shows broad taxonomic and functional similarities to mammalian omnivores, including abundant *Bacteroidota* and *Bacillota* with taxonomic and functional profiles similar to their sister clades found in mammalian guts (39). However, the ability of *Periplaneta americana* to survive for long periods of time on diverse and nutrient-poor diets allows us to design studies using more extreme dietary shifts than allowable in mammalian studies. Studies have found that, like humans, the cockroach gut microbiome exhibits stability across highly divergent diets (35, 37, 40, 41). Therefore, we hypothesize that a combination of host-microbe and microbe-microbe interactions support gut microbiome homeostasis.

Here, we examined hindgut microbial community activity in cockroaches fed a control diet of dog food as well as high-fiber, high-protein, high-fat, and starvation diets. A previous study showed that these diets did not induce substantial, global changes in gut microbiome composition (35). Using metagenomic and metatranscriptomic data, we identified taxonomic and phylogenetic clusters of related microbes showing evidence of similar functional roles. We then

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generated pangenomes for each cluster and analyzed their transcriptional responses to diet independently. These results provide new insight into microbial activities underlying observed compositional stability in the cockroach hindgut microbiome.

Results

We sequenced shotgun community DNA and shotgun community mRNA reads from 45 individual insects fed one of five distinct dietary conditions: bran, dog food (laboratory maintenance diet), tuna, butter, and starvation. We obtained 25 metagenomes (5 from each diet) and 20 metatranscriptomes (4 from each diet). Following quality-filtering, removal of host DNA/RNA reads, and removal of remaining rRNA reads, we obtained 1,021,038,314 metagenome (870,360-533,070,442 per sample) and 78,022,090 metatranscriptome reads (891,624-5,551,790 per sample). After read translation, 35.14% of metagenome and 62.44% of metatranscriptome reads were mapped by homology search to protein sequences in RefSeq or our single cell genomes from the cockroach gut (39).

Taxonomic profiles and overall community composition

Overall, the metagenomes and metatranscriptomes were dominated by *Bacteroidota*, *Bacillota* (formerly *Firmicutes*), and *Desulfobacterota*. Alpha diversity (Shannon index) did not vary significantly across the diets for the metagenomes ($p = 0.28$, $p_{adj} \geq 0.39$) nor metatranscriptomes ($p = 0.04$, $p_{adj} > 0.08$) (Figure S1). However, we did observe large variability in metagenome alpha diversity (Shannon index) within the butter diet (Figure S1). PERMANOVA analysis of Bray-Curtis dissimilarities based on taxonomic profiles showed that while diet exerted little effect over metagenome community composition ($p = 0.20$), it was a significant driver of metatranscriptome composition ($p = 0.01$) (Figure 1A). Bran-fed cockroaches had lower dissimilarity compared to other diets for each of the datasets, while

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butter-fed cockroaches had the highest dissimilarity (Figure 1A). The high variability seen in butter-fed cockroaches was likely associated with "blooms" in the abundance of individual taxa among a subset of samples in both metagenome and metatranscriptome datasets (Figure 1).

Changes in microbial abundance and activity levels across diets

To evaluate how key taxa within the cockroach gut community respond to host dietary shifts, we employed a taxon-centric strategy for metatranscriptomic analyses. Pangenomes were constructed for eight genome clusters in *Bacteroidota*, seven in *Bacillota*, two in *Desulfobacterota*, and one in *Euryarchaeota*. *Bacteroidota* genome clusters represent roughly genus-level clusters of *Bacteroides*, *Parabacteroides*, *Dysgonomonas*, *Alistipes*, and *Odoribacter*, as well as unclassified *Paludibacteraceae*, *Azobacteroidaceae*, and *Bacteroidales*. *Bacillota* genome clusters often could not be built at genus level (42) but included three family-level clusters (*Clostridiaceae, Lactobacillaceae*, and *Entercoccaceae*) where co-occurrence data suggested a single population, while *Oscillospiraceae* and *Lachnospiraceae* were each split into two sub-populations that showed distinct co-occurrence and transcriptional profiles (Figure S2-S3); Other sub-populations identified within *Oscillospiraceae* and *Lachnospiraceae* did not have enough sequence coverage in the datasets to be analyzed further. For *Desulfobacterota*, clusters include *Frigididesulfovibrio* and *Desulfosarcina*. *Methanimicrococcus* was the only methanogen with transcriptional abundance high enough to generate a genome cluster. See Supplemental Table 1 for a full list of genome references used to build genome clusters.

Many individual genome clusters showed little change in average metagenome relative abundance between diet groups (Figure 2). However, *Dysgonomonas*, *Lactobacillaceae*, and *Enterococcaceae*, exhibited higher average metagenome relative abundance in the butter diet, much of which is attributable to "blooms" of different taxa in a subset of samples (Figures 1B,

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2). In contrast, the fraction of metatranscriptome reads assigned to individual genome clusters was more variable. *Bacteroides* had increases in relative abundance of transcripts for the bran diet while *Lachnospiraceae*_2 and *Clostridiaceae* had more transcription in the dog food diet. *Odoribacter*, unclassified *Bacteroidales*, and *Desulfosarcina* had more transcripts in the butter diet, while *Desulfosarcina* was very transcriptionally active in the tuna diet. Some groups, such as *Parabacteroides* and *Frigididesulfovibrio*, showed little to no change in abundance in the metagenome and metatranscriptome between diets (Figure 2).

High-level trends in gene expression of key metabolic pathways

Figure 3 shows expression levels of genes assigned to key functional categories in different diets for individual taxonomic clusters. These functional profiles underscore established patterns of gene expression for these groups, with carbohydrate-active enzymes (CAZymes) expressed by nearly all groups within *Bacteroidota* and *Bacillota* phyla, with exception of the *Bacteroidales* taxonomic cluster (Figure 3). *Bacteroides*, *Dysgonomonas*, and *Parabacteroides* showed some of the highest transcription of CAZymes (Figure 3) and are hypothesized to be primary degraders of polysaccharides (39). Beyond the primary degraders, other taxa transcribed CAZymes ($\geq$ 0.1% average relative abundance) and the difference in transcription varied by diet (Figure S4). Many of these taxa had more transcription of CAZymes in the bran and dog food diets, but the CAZyme type and specificity varied among taxa (Figure S4). Even in diets without abundant carbohydrates, there were CAZymes transcribed at high levels. For many taxa, particularly–*Bacteroidota*—CAZymes targeting eukaryotic components (GH109, GT41, CBM51) and mannose (GH92, GH38) were transcribed more during host starvation (Figure S4).

Not all *Bacteroidota* appeared to specialize in fiber degradation. *Odoribacter* and a group of unclassified *Bacteroidales* both transcribed genes related to carbon fixation at high levels

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(Figure 3) but showed differences in the specific carbon fixation genes utilized. *Odoribacter* primarily transcribed genes involved in converting acetyl-CoA and CO_2 to pyruvate (K14534 and K03737) (Figure S5A). Due to higher transcription of both genes, this implicates a role in the dicarboxylate-hydroxybutyrate cycle of carbon fixation. *Bacteroidales* primarily transcribed genes for the conversion of acetyl-CoA and CO_2 to phosphoenol-pyruvate (K03737, K01006) but also highly transcribed genes to convert succinyl-CoA and CO_2 to 2-oxoglutarate (K00174, K00175, and K00177) (Figure S5B). These are metabolic steps in both the dicarboxylate-hydroxybutyrate cycle and the reductive citrate cycle. However, *Bacteroidales* was not transcribing several genes in the dicarboxylate-hydroxybutyrate pathway that *Odoribacter* transcribed, suggesting that *Bacteroidales* utilizes these genes in the reductive citrate cycle (Figure S5).

While *Bacteroidota* and *Bacillota* groups transcribed CAZymes at high levels, they differed in nutrient acquisition strategies; *Bacteroidota* typically transcribed TonB/Sus type transporter systems and *Bacillota* primarily transcribed ABC-type transporters (Figure 3). In *Bacillota*, we observed diet- and taxon-driven differences in ABC transporter transcription (Figure 3). For example, *Lactobacillaceae* transcribed ABC transporters to a greater extent in the butter diet and less so in the tuna diet (Figure 3). For this group, only a single ATP-binding cassette (K20344, a subfamily C bacteriocin exporter), was upregulated in the tuna diet while the butter diet induced upregulation of diverse ABC transporters (Figure S6). Finally, *Lactobacillaceae* responded to host starvation by upregulating ABC transporters for nucleosides, ribose/D-Xylose, lincosamide, and streptogramin A (Figure S6).

Chemolithotrophic groups, including sulfate reducers and methanogens, were also highly transcriptionally active (Figure 3). Although transcription of sulfate reduction genes was largely

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not significantly changing in response to host diet, *Desulfosarcina* and *Frigididesulfovibrio* showed trends in transcription of sulfur metabolism genes. For the butter diet, *Desulfosarcina* had reduced transcription of some sulfur metabolism genes compared to the other diets (Figure S7). Conversely, *Frigididesulfovibrio* increased transcription of some sulfur metabolism genes in cockroaches fed butter, particularly genes involved in the conversion of PAPS to APS (K01082 and K06881), an anaerobic sulfite reductase (K16951), and an anaerobic dimethyl sulfoxide reductase (K07306) (Figure S7B). Methane metabolism genes for *Methanimicrococcus* did not change gene expression significantly in response to host diet shift. Although not significant, some methane metabolism genes were transcribed more in the bran and tuna diets (Figure S8). Additionally, there were transcriptional differences that suggest changes in preferred substrates between methanol and methylated amines depending on host diet (Figure S8).

Nearly all taxa increased transcription of genes associated with stress responses in the butter diet, evident by increased transcription of chaperones and folding catalysts (Figure 3). Specifically, DnaJK chaperones, heat-shock proteins, peroxiredoxin, and thioredoxin were all differentially expressed in the butter diet relative to all other diets (Figure S9). Taxa that showed significant differential expression of these stress transcripts included *Bacteroides*, *Dysgonomonas*, *Parabacteroides*, *Alistipes*, *Paludibacteraceae*, *Lactobacillaceae*, and *Lachnospiraceae*_1. The most consistent trend of increased transcription in these stress response genes was for DnaK (K04043) and chaperonin GroEL (K04077) in the butter diet (Figure S9). Despite a strong stress response seen in many taxonomic clusters, there was no decrease in the relative abundance of these groups in the metagenomes (Figure 2). However, some taxa exhibited increased transcriptional activity in the butter diet (as measured by relative abundance

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in the metatranscriptome), suggesting that they were more active under this condition and/or were less impacted by this stress than other taxa (Figure 2).

Some taxa also exhibited high transcription of stress-related genes in diets other than butter. For example, *Dysgonomonas* transcribed thioredoxin (K03671) and NADH-dependent peroxiredoxin subunit C (K24119) to a greater extent in starvation (Figure S9). *Lachnospiraceae*_1 exhibited greater transcription of NADH-dependent peroxiredoxin subunit C (K24119) in the tuna diet than in any of the other diets (Figure S9). *Clostridiaceae* and *Lachnospiraceae*_2 transcribed chaperoin GroEL (K04077) and DnaK (K04043) at higher levels than other taxa in all diets, not only the butter diet.

Significant gene expression changes in response to host diet shift

For each genome cluster, we analyzed genes that displayed significantly altered expression across diets using DESeq2 (43). Across all taxa and all pairwise comparisons of diets, a total of 1215 genes were identified as significantly changing with respect to host diet (Figure 4). However, there were striking differences in the number of significantly differentially expressed genes across taxa. Those previously identified as potential primary degraders (39) showed stronger transcriptional responses to diet than other organisms. Additionally, taxa varied in which diets elucidated the strongest responses, with some organisms responding most strongly to bran, while others responded most to tuna or butter diets (Figure 4).

Significant gene expression for Bacteroidota taxa

Members of the *Bacteroidota* phylum had the greatest number of significantly changing genes, with *Bacteroides* (520 significant genes) and *Dysgonomonas* (198 significant genes) having far more than other taxa (Figure S10). *Bacteroides* had the most changes in gene expression when comparing bran to tuna, butter, and starvation (Figure 4). In contrast to

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Bacteroides, *Dysgonomonas*, and *Parabacteroides*, other *Bacteroidota* taxa (*Alistipes*, *Azobacteroidaceae*, *Odoribacter*, *Paludibacteraceae*, and *Bacteroidales*) show substantially fewer differences in gene expression across diets despite having high read counts (Figure S10, Figure 4).

Bacteroides showed a particularly strong response in gene expression between the bran and other diets (Figure 4). When comparing gene expression between the tuna and bran diet, *Bacteroides* differentially expressed 127 genes, 16 of which were upregulated in the bran diet (Figure 4). Bran-responsive genes included four CAZyme genes: two that degrade diverse substrates (GH3, GH32) and two that degrade xylan (CE1, GH10) (Table S2). There were four transporters upregulated in the bran diet, including a putative fructose porter, SusD starch binding proteins, and SusD homologs. Other bran upregulated genes included a fructokinase, glycan binding proteins, TolC family proteins, and RagB/SusD family nutrient uptake proteins. The 20 CAZymes upregulated in the tuna (vs. bran) diet included CAZymes specific for pectin (CE8, PL1_2, PL1, GH28, GH105), chitin or peptidoglycan (CBM50), rhamnogalacturonan (PL11_1, GH106), and diverse substrates (GH43_4, GH43_10, GH2, CE12). Other genes upregulated in cockroaches fed tuna (vs. bran) included FAD-dependent oxidoreductase, NADH-dependent peroxiredoxin subunit C, fimbrillin family protein, and Mfa1 family fimbria major subunit (Table S2). When comparing bran and butter gene expression patterns, *Bacteroides* differentially expressed 119 genes, 40 of which were upregulated in the bran diet (Figure 4). The same four CAZymes upregulated in bran (vs. tuna) were also upregulated in response to butter (vs. bran), with the addition of three CAZymes that degrade diverse substrates (CE20, GH43_1, and GH67). Other genes upregulated in the butter diet (vs. bran) included chaperonin GroEL (*groL*), a putative lipoprotein, a possible iron receptor (RagA), the chloroplast envelope TIC

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translocase complex, and NADH-dependent peroxiredoxin subunit C. During host starvation, *Bacteroides* differentially expressed 122 genes compared to the bran diet. CAZymes that were upregulated in the bran diet (vs. starvation) were similar to those seen for other diets, including GH32 (diverse substrates), CE1 (xylan), GH10 (xylan), GH67 (alpha-glucuronidase), and GH43_24 (Exo-β-1,3-galactanase). Some upregulated genes in starvation (vs. bran) included FAD-dependent oxidoreductase, possible iron receptor (RagA), and NADH-dependent peroxiredoxin subunit C. There were no CAZyme genes that were significantly upregulated during host starvation (vs. bran). However, CAZymes that showed increased, if not significantly so, expression included those potentially involved in breakdown of host mucins, including GH109 and mannosidases (Figure S4) (44). In addition, a single gene, xylulokinase was upregulated in starvation when compared to the butter diet.

Dysgonomonas and *Parabacteroides* showed fewer significantly differentially expressed genes, but overall exhibited similar responses (Figure 4). Like *Bacteroides*, *Dysgonomonas* upregulated CAZymes targeting xylan, pectin, and diverse substrates in the bran diet (Table S3). *Dysgonomonas* also upregulated SusC and SusD transporters, L-arabinose isomerase, xylose isomerase, and L-ribulokinase in the bran diet (vs. butter). During starvation (vs. bran), *Dysgonomonas* upregulated NADH-dependent peroxiredoxin subunit F, DNA-binding ferritin-like protein (oxidative damage protectant), and a fatty acid repression mutant protein (predicted oxidoreductase). *Dysgonomonas* responded to the tuna diet (vs. bran) with pectin CAZymes (GH28, PL1_2, CE8). *Parabacteroides* responded to the bran diet with upregulation of CAZymes that target diverse substrates (Table S4). Other genes that were upregulated by *Parabacteroides* in the bran diet included SusC and SusD transporters (Table S4). Unlike *Bacteroides* and *Dysgonomonas, Parabacteroides* did not upregulate pectin CAZymes in

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response to the tuna diet (Table S4). In response to the tuna diet, *Parabacteroides* instead upregulated SusC and SusD transporters and other related starch binding outer membrane protein, SusD/RagB family. The butter diet triggered upregulation of GroEL (*groL*) and DnaK genes (Table S4). In addition, *Parabacteroides* only upregulated three genes during host starvation (vs. bran): NADH-dependent peroxiredoxin subunit F, cytochrome bd ubiquinol oxidase subunit I, and cytochrome bd ubiquinol oxidase subunit II (Table S4).

Alistipes had far fewer differentially expressed genes as compared to other members of *Bacteroidota*, largely changing gene expression in response to the butter diet (Figure 4). Many of the genes that were upregulated in the butter diet were stress response genes, including chaperonins and heat-shock related proteins. Six genes were upregulated in the dog food diet (vs. butter). Functions of these genes included outer membrane protein OmpA, Mfa1 family fimbria major subunit, and thioredoxin-dependent peroxiredoxin. Two other taxa of *Bacteroidota*, *Azobacteroidaceae* and *Paludibacteraceae*, differentially expressed genes in the bran to butter comparison (Figure 4). *Azobacteroidaceae* upregulated a putative arabinose porter and putative outer membrane porin in the bran diet (vs. butter) (Table S5). Some of the other genes upregulated in the bran diet included c-type cytochrome, IS982 family transposase, and L-arabinose isomerase. As was observed in many organisms, DnaK genes were upregulated in the butter diet compared to bran (Table S5). In addition, *Azobacteroidaceae* upregulated a hypothetical protein, T9SS type A sorting domain-containing protein, and an arabinose isomerase in the dog food diet (vs. butter). *Paludibacteraceae* differentially expressed 15 genes in the comparison of bran and butter diets (Figure 4), and 10 of these genes were upregulated in the bran diet (vs. butter). Some bran upregulated genes included elongation factor Tu, 50S ribosomal protein L11 (rplK), and 30S ribosomal protein S11 (rpsK) (Table S6). There was also

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upregulation of xylulokinase, RagB/SusD family nutrient uptake outer membrane protein, and ribulokinase in the bran diet (vs. butter). Butter upregulated genes included chloroplast envelope TIC translocase (TIC) complex, molecular chaperone protein (Hsp90 homologue), and HSP20 family protein, presented in highest to lowest transcription (Table S6). *Paludibacteraceae* upregulated a single gene for a glucoronate isomerase in the dog food diet (vs. butter). *Odoribacter* upregulated fewer genes, and only in the bran vs. butter and tuna vs. butter comparisons (Figure 4). Of these 19 upregulated genes, four were upregulated in the butter diet (vs. tuna), all of which were transporters; these transporters included an outer membrane protein transport protein, multidrug efflux pump, Triclosan resistance efflux pump, and TolC family protein. All genes that *Odoribacter* upregulated in the tuna and bran diet (vs. butter) included H^+-translocating ATPases (Table S7). *Bacteroidales* only had a single gene, a two-sector V-type ATPase or ATP synthase, that was upregulated in the butter diet (vs. tuna).

Significant gene expression for Bacillota taxa

Among the *Bacillota*, *Oscillospriaceae*_2 also showed a similar pattern of gene expression changes when comparing bran to tuna, butter, and starvation, suggesting a strong response to diets with a high polysaccharide content (Figure 4). However, the types of genes upregulated by *Oscillospiraceae*_2 in response to these diets were different from those observed in *Bacteroides*. When comparing tuna to bran, *Oscillospiraceae*_2 differentially expressed 26 genes, of which 15 genes were upregulated in the bran diet. None of these bran upregulated genes were CAZymes but instead included ABC transporters for arabinose, arabinose isomerase genes, and electron transporters involved in sulfur metabolism with anaerobic dimethyl sulfoxide reductase (Pyrogallol hydroxytransferase) (Table S8). Genes that were upregulated in the tuna

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316 diet included ferrous iron transporter B, amidohydrolase, and aminobenzoyl-glutamate utilization
317 protein B. The latter two genes are involved in lysine biosynthesis.

318 *Lactobacillaceae* (*Bacillota*) had 154 genes that were significantly changing with diet.
319 Interestingly, many of these differences in gene expression were identified in comparisons
320 between tuna and all other diets, suggesting a strong response to a high-protein diet (Figure 3).
321 *Lactobacillaceae* upregulated four CAZymes consistently in all diets compared to tuna,
322 including CBM50 (chitin or peptidoglycan), CE9 (N-acetylglucosamine-6-phosphate
323 deacetylase), GH1 (beta-glucosidases and beta-galactosidases), and GT2 (diverse substrates).
324 Only seven genes were upregulated in the tuna diet (vs. starvation), including
325 maltose/maltotriose/maltodextrin (up to 7 glucose units) transporters, NADP-dependent
326 phosphogluconate dehydrogenase, septation ring formation regulator, 3-phenylpropionate/trans-
327 cinnamate dioxygenase ferredoxin reductase component, Lipoprotein-anchoring transpeptidase,
328 and 6-phospho-beta-glucosidase. Many of the same genes were upregulated in the bran, butter,
329 and starvation diet when compared to tuna. Genes that were consistently upregulated in diets
330 other than tuna included formate C-acetyltransferase, phosphoenolpyruvate-protein
331 phosphotransferase (PTS system enzyme I), pyruvate kinase, and putative sigma-54 modulation
332 protein (Table S9).

333 *Lachnospiraceae*_1 (*Bacillota*) also had a strong response to the tuna diet, but only when
334 compared to the bran diet (Figure 4). There were 34 differentially expressed genes in this
335 comparison, 12 of which were upregulated in the bran diet. Bran upregulated genes included a
336 putative multiple sugar transport system, flagellin, and glycine/betaine/sarcosine/D-proline
337 family reductase selenoprotein B. Tuna upregulated genes (vs. bran) included those for
338 peptide/nickel transport system substrate-binding protein, IS21-like element helper ATPase and

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IS21 family transposase (Table S10). *Lachnospiraceae*_1 only upregulated a single CAZyme, which has N-acetylglucosamine-6-phophate deacetylase activity (CE9), for the tuna diet (vs. bran).

Significant gene expression of chemolithotrophs

The two sulfate reducing taxa, *Frigididesulfovibrio* and *Desulfosarcina*, did not respond strongly to host diet. *Desulfosarcina* did not differentially express any genes across the diets. *Frigididesulfovibrio* differentially expressed only 17 genes when comparing bran to butter (Figure 4), 15 of which were upregulated in the bran diet (vs. butter). Four of these bran-upregulated genes were transporters including a putative porin, a dicarboxylate (succinate, fumarate, malate) transporter, Na^+-dependent C4-dicarboxylate (fumarate, succinate) uptake transporter, and a 2,3-diketo-L-gulonate (2,3-DKG) transporter (Table S11). Other bran-upregulated genes included several related to choline metabolism, including five BCCT family transporters and choline TMA-lyase-activating enzyme. The two genes that were upregulated in the butter diet (vs. bran) were for a multidrug efflux pump subunit AcrA (membrane-fusion protein) and opacity protein LomR and related surface antigens. In the tuna diet (vs. butter), several genes related to histidine degradation were upregulated, including putative histidine porter and histidine ammonia-lyase. Further, *Fridgididesulfovibrio* upregulated two subunits of the 50S ribosome in both bran and tuna diets compared to butter.

Methanimicrococcus differentially expressed very few genes, with most gene expression changes identified in the bran vs. dog food comparison (Figure 4). Many of these differentially expressed genes were hypothetical proteins. *Methanimicrococcus* also showed a response between bran and butter diet with four differentially expressed genes. Three of these genes were

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upregulated in the butter diet (vs. bran) including methanol:5-hydroxybenzimidazolylcobamide Co-methyltransferase, ethanolamine permease, and peroxiredoxin (Table S12).

Discussion

The aim of this study was to utilize metagenomic and metatranscriptomic techniques to better understand how microbial transcriptional responses support the strong stability in taxonomic composition of the cockroach gut microbiome following dietary shifts. Although we observed somewhat higher variability in taxonomic composition across samples in the metagenome dataset (Figure 1B), the metagenome results support the key observation of the previous study that diet was not a strong driver of gut microbiome community composition (35). In contrast, metatranscriptome composition did exhibit changes in microbial abundance and transcript abundance in responses to dietary shifts. As a result, we focused primarily on analysis of transcriptional responses to diet that may support this stability.

We used a taxon-centric, pangenome approach to generate a composite transcriptome for clusters of closely related genomes. This approach was implemented to take maximal advantage of available reference genomes—including highly fragmented, host-relevant, single-cell sequenced genomes (39)—while avoiding having to select a single reference genome that may not perfectly match the unclassified dominant organisms in the sample. A similar approach has previously been used for analysis of transcriptional patterns among marine microorganisms (45-47). The set of organisms selected for our analysis included *Bacteroides*, *Dysgonomonas*, *Parabacteroides*, *Alistipes*, unclassified *Azobacteroidaceae*, *Paludibacteraceae*, *Bacteroidales*, *Clostridiaceae*, *Lactobacillaceae*, *Enterococcaceae*, *Lachnospiraceae*, *Oscillospiraceae*; *Frigididesulfovibrio*; *Desulfosarcina*; and *Methanimicrococcus*. These organisms were selected because their high relative abundance in the metatranscriptome. They represent a variety of

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putative trophic groups (48), including primary polysaccharide degraders, secondary degraders of polysaccharolytic byproducts, and chemolithotrophs including acetogens, sulfate reducers, and methanogens.

Through this work we have expanded what is known about a wide range of microorganisms' transcriptional response to host dietary shift. In general, changes in the relative abundance of individual taxa in the metatranscriptome, which is assumed to represent their overall transcriptional activity, broadly reflected hypothesized substrate specializations. Taxa that have previously been hypothesized to act as primary degraders, including *Bacteroides*, *Dysgonomonas*, and *Parabacteroides*, showed higher transcriptional activity in cockroaches fed bran and dog food diets (Figure 2) (49-52). Conversely, other members of *Bacteroidota* had higher transcriptional activity in the butter and/or tuna diets (Figure 2). Members of *Bacillota* varied in which diets they were most active in, with some more active in cockroaches fed butter, tuna, or dog food. Both *Desulfosarcina* and *Frigididesulfovibrio* exhibited reduced activity in the bran diet, whereas *Methanimicrococcus* were more active in the bran fed cockroaches (Figure 2).

Microbial responses to high-polysaccharide diets (bran and dog food)

The microbial community of bran-fed cockroaches had the most stable taxonomic community structure in both metagenome and metatranscriptome datasets (Figure 1A). *Bacteroides* had the highest transcriptional activity in the bran diet followed by *Dysgonomonas* and *Parabacteroides* (Figure 2). All three of these taxa transcribed a wide range of genes responsible for the degradation of complex carbohydrates, including genes containing inulin- and xylan-targeting CAZyme domains that are upregulated in cockroaches eating bran or dog food. This activity supports findings from *Bacteroides* spp. from other environments that release large amounts of polysaccharide degradation products extracellularly, providing the potential for these

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primary degraders to create an "everybody wins" scenario in terms of cross-feeding relationships (53, 54). Previous studies have implicated *Bacteroides* in cross-feeding of fermentation products with secondary degraders, sulfate reducers, and methanogens (54-58). Given the potential for cross-feeding relationships, gains in growth and carbohydrate degradation among the primary degraders may simultaneously promote growth of a wide range of other organisms, thereby minimizing compositional changes in gut microbiome composition, as observed here. Further studies are needed to assess specific cross-feeding interactions that occur between these primary degraders and other microbiome members in the cockroach hindgut.

Some *Bacillota* members also showed strong transcriptional responses to bran. Rather than breaking down primary carbohydrates, their transcriptional patterns suggest that these organisms are making use of by-products from primary polysaccharide degradation. *Bacillota* using simple sugars released from primary degraders has been shown in *Roseburia* (*Lachnospiraceae*) (59), while aldouronate (hemicellulose depolymerization product) is commonly metabolized by Gram-positive members of *Clostridiaceae* and *Oscillospiraceae* (60). In our data (Figure 3), *Bacillota* members such as *Lactobacillaceae*, *Lachnospiraceae*_1, *Oscillospiraceae*_1, and *Oscillospiraceae*_2 responded to a bran diet by upregulating ABC transporters that target mono- and short chain oligosaccharides such as maltose, oligosaccharide (glucuronate-linked to a xylo-oligosaccharide), ribose, and arabinose, suggesting that they are primarily acting as secondary degraders. Other *Bacillota* show evidence of amino acid utilization in the bran diet. *Lachnospiraceae*_1 and *Oscillospiraceae*_2 upregulated glycine betaine/sarcosine reductase and transporters associated with Stickland metabolism. Related organism *Closridium sporogenes* has been shown to reduce amino acids using the sugar fermentation by-product H_2 (61-63).

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Methanimicrococcus transcribed various genes related to methyl-reducing methanogenesis at higher levels in the bran diet (Figure S6), although many of these genes were not significantly upregulated. Previous studies have shown that *Methanimicrococcus* can utilize diverse methyl-containing organic compounds such as methylamines and methanol (39, 63, 64). Degradation of plant-sourced phosphatidylcholine by *Bacteroidota* and *Desulfobacterota* has been shown to release methylamines (65, 66), and methanol is associated with degradation of pectin (65, 66). In rumen isolates, consumption of these byproducts increased energy yield and fiber degradation of other fiber degraders in the gut (67, 68). Although not significantly differentially expressed, genes for the coenzyme 420 hydrogenanse (Frh) are transcribed more in the bran diet (Figure S8), suggesting that the activity of methanogens is closely tied to the activity of fiber degraders in the cockroach. *M. blatticola* lacks many enzymes of the methyl-branch of the Wood-Ljungdahl pathway. Therefore, any electrons transferred to Frh would be used for anabolism (69). This suggests a crucial role for methanogens in maintaining the low partial pressure of H_2 and utilizing fermentation byproducts that may impact fermentation activity in the gut. It also demonstrates how cross-feeding relationships may act to stabilize gut taxonomic composition.

In many cases, organisms that respond strongly to bran also respond to dog food, likely because it has the second highest carbohydrate content of the tested diets. Taxa that responded strongly to the dog food diet included *Dysgonomonas*, *Bacteroides*, *Lactobacillaceae*, and *Parabacteroides*, listed in highest to lowest number of dog food upregulated genes. Other organisms responding to dog food appear to be interacting with the amino acid content of this food including *Paludibacteraceae* and *Azobacteroidaceae*.

Microbial responses to a high-protein (tuna) diet

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In general, the tuna diet elicited a strong response to protein components by *Bacillota,* and *Frigididesulfovibrio*. Genes involved in amino acid degradation were upregulated in the tuna diet by *Lactobacillaceae, Lachnospiraceae*_1, *Oscillospiraceae*_1, *Oscillospiraceae*_2, and *Frigididesulfovibrio*. Proteolysis in the human gut is also largely attributed to *Bacillota* members, including activity on aromatic amino acids (70-72). *Lactobacillaceae* upregulated several genes involved in aromatic amino acid metabolism. *Frigididesulfovibrio* also responded to the tuna diet with upregulation of several genes for histidine metabolism. Although their primary metabolism remains unchanged by host diet, this may reflect an ability by this sulfate reducer to take advantage of additional electron donor substrates if present.

In contrast to the tuna diet response by *Bacillota* and *Frigididesulfovibrio*, *Bacteroides* and *Dysgonomonas* responded with transcriptional patterns suggesting utilization of polysaccharides in tuna. We did not expect polysaccharide degraders to engage extensively with this diet. However, there was strong upregulation of genes associated with pectin degradation by various primary and secondary degraders. We hypothesize this CAZyme activity is related to utilization of galacturonan, which—in addition to being a key component of pectin–is known to be present in tuna and tuna by-products (73). In addition, most canned tuna products contain vegetable broth as a flavor enhancer, which may also contribute to the pectin activity that we observed. Even in a diet very low in polysaccharides, such as tuna, these primary degraders identify and scavenge relevant substrates for their specialized metabolisms.

Microbial responses to a high-fat (butter) diet

Microbial responses to the butter diet represent one of the major surprises of this work. There was wide-spread transcriptional upregulation of stress response proteins including RND efflux pumps, oxidative stress, and heat shock proteins (Figure 3, Figure S7). Across many

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organisms, this stress response was associated with upregulation of *groEL* and *dnaK* (Figure 3, Figure S7). These two genes are known to allow bacteria to survive better in adverse conditions and are involved in adhesion and invasion processes (74, 75). GroEL is the bacterial counterpart of mammalian heat shock protein 60 and plays an essential role in bacterial survival during chemical, heat, or oxidative stress. DnaK is a chaperone that is part of the heat shock protein family and involved in stress response (76). Previous studies have shown that a high-fat diet induces oxidative stress in the host, which may be the reason we see a wide-spread upregulation of stress genes in the microbiome (77). A similar stress response to changes in redox state that has been noted in gut epithelial cells in mice on high-fat diets (78, 79). The butter diet in the cockroach also appears to induce lower gut microbiome stability (Figure 1). Instability has not been explicitly connected to high-fat diets, but studies in mice eating high-fat diets have indicated blooms of organisms (19, 20, 80). A study of human diet and microbiome composition also indicated greater variability in community profiles of individuals eating diets high in fats (81). Together, these results support findings that high fat diets may have profound impacts on the gut microbiome.

In addition to the observed stress response, *Bacteroides* and *Dysgonomonas* respond to the butter diet by transcribing CAZyme domains associated with degradation of GlcNAc and mannose in host tissue (Figure S2). These transcripts are also upregulated in response to starvation, which may reflect degradation of host mucins from the peritrophic membrane; this starvation response is discussed in more detail below. In addition, *Lactobacillaceae* upregulate genes with CAZyme domains that target diverse substrates. Despite primary polysaccharide degraders attempting to scavenge appropriate substrates here, much of their transcriptional activity is reduced, with remaining activity redirected towards survival in suboptimal conditions.

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Despite this reduced transcriptional activity in diets low in polysaccharides, primary degraders may avoid dropping in relative abundance in the metagenome because other taxa are experiencing similar stresses (82, 83).

In contrast, *Enterococcaceae* and *Lactobacillaceae* exhibited blooms in a subset of cockroaches eating butter (Figure 1), and both taxa had increased relative abundance in the transcriptome (Figure 2). *Enterococcaceae* and *Lactobacillaceae* spp. have been found in association with high-fat diets in mice (84-86). Although *Enterococcaceae* did not significantly upregulate any genes across the diets, *Enterococcus faecalis* is known for efficient glycerol metabolism (87, 88). This ability may provide *Enterococcaceae* an advantage in cockroaches eating butter. Furthermore, *Enterococcus* species are known to produce reactive oxygen species that damage host epithelial cells (89, 90). Oxygenation in the gut produces a colonization benefit for *Lactobacillus* in humans (91), as they are equipped to handle oxidative stress (92). *Enterococcus* activities may cause the observed stress response from microbes throughout the hindgut community, allowing *Lactobacillaceae* to take advantage of changing environmental conditions in the gut (89, 90).

Microbial responses to host starvation

Bacteroides, *Parabacteroides*, and *Dysgonomonas* responded to host starvation (and to a lesser extent butter) by upregulating GH109, a CAZyme that targets GlcNAc (93). *Bacteroides* and *Dysgonomonas* also transcribe gene clusters with mannose-targeting CAZyme domains (GH92, GH76). Although the hindgut of insects is surrounded by a chitinous ectoderm and lacks a mammalian-like mucus layer, the midgut of insects produces a peritrophic membrane that is continually extruded into the hindgut and may serve as a source of polysaccharides and glycans (94). From work in the Colorado potato beetle, glycosylation of the peritrophic membrane

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includes mannose and GlcNAc, especially the structures $GlcNAc_2Man_3$, $GlcNAc_2Man_3Fuc$ and $GlcNAc_2Man_5$ (44). *Dysgonomonas* also highly expresses gene clusters with mannose-specific CAZyme domains in low-carbohydrate diets and indicates lower expression of these genes in carbohydrate-rich diets (Table S3). This transcriptional adjustment likely allows them to use host-sourced polysaccharides in a diet short on complex carbohydrates. Studies in mammals have widely identified host mucins as an important substrate for gut fiber degraders in the absence of microbially accessible polysaccharides (95). These results suggests that the peritrophic membrane in cockroaches may serve a similar role in the ecology of the cockroach gut microbiome.

Microbial transcriptional stability across diets

While we focus on transcripts whose expression changed significantly across the diets, it is worth noting the overall stability of gene expression in many of these microbes. While primary degraders such as *Bacteroides*, *Dysgonomonas*, and *Parabacteroides* showed large number of significantly changing genes, many key taxa had few or even no transcripts that were found to be significantly different across diets or were responsive to only one diet (Figure 4). While the ability to detect significantly changing transcripts is somewhat dependent on transcriptome coverage, multiple groups with few or no diet-responsive transcripts, such as *Desulfosarcina* and *Frigididesulfovibrio*, did so despite transcriptome coverage similar to or greater than groups with larger numbers of significantly changing transcripts. These results suggest that they are not directly dependent on dietary components to support their metabolism and are supported by other host or microbial activities. Given the extensive shifts in metabolic activities observed in key primary degraders, and the likely position of less diet-responsive microbes at the 'bottom' of the microbial food web, this points towards a scenario in which the actions of these primary

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545 degraders are sufficient to compensate for dietary changes and in turn support the metabolism of
546 microbes further down the food chain. This ability is likely not infinite, which could in turn
547 explain why polysaccharide-rich diets are associated with the greatest overall compositional and
548 transcriptional stability. Together, these findings support a 'keystone taxa' role for fiber
549 degraders in structuring gut microbiome metabolism.

550 *Conclusions*

551 The primary goal of this work was to gain insight into microbial transcriptional activities that
552 may explain the remarkable compositional stability of the cockroach gut microbiome following
553 substantial dietary shifts. A key finding of this work suggests the activity of polysaccharide
554 degraders—including *Bacteroides* and *Dysgonomonas*—is highly responsive to diet and plays a
555 key role in supporting gut microbiome homeostasis. These organisms are highly versatile,
556 scavenging polysaccharides from even low-fiber foods such as tuna, and using components of
557 the peritrophic matrix under starvation conditions and on low-carbohydrate butter diets. Higher
558 fiber diets result in lower sample-to-sample variability in gut microbiome composition and
559 transcriptional activity. Secondary degraders like *Bacillota* appear to adapt to available polymer
560 degradation products—such as oligosaccharides and oligopeptides—depending on the level of
561 fiber and protein in the host's diet. In contrast, a high-fat diet is associated with upregulation of
562 stress proteins across many gut microbes. This stress was associated with reduced gut
563 microbiome stability, with individual samples showing large-scale blooms of different taxa. This
564 finding aligns well with literature concluding that the 'Western diet' rich in fats and simple
565 sugars can lead to gut microbiome dysbiosis in many organisms (18, 24). Overall, these results
566 suggest that the omnivorous cockroach is a promising model for disentangling microbial
567 responses to diet. In addition, it suggests that taxon-centric analysis of metatranscriptomes is

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568 highly promising for understanding the complex web of microbe-microbe and host-microbe
569 interactions underlying gut microbiome homeostasis and dysbiosis.
570
571 **Materials and Methods**
572 *Diet manipulation and sample collection*
573 Laboratory colonies of *Periplaneta americana* are maintained in mixed age, mixed sex
574 colonies in aquarium tanks at room temperature on a diet of dog food *ad libitum*. Each tank is
575 provided with corn cob bedding, cardboard tubes for nesting, and a cellulose sponge saturated
576 with water. Each dietary treatment group was housed in a single plastic tank that contained
577 pebbles for bedding, a large weigh boat for shelter, and food and water in weigh boats. Food,
578 water, and weigh boats were changed as needed, and any ootheca or deceased cockroaches were
579 removed daily. Treatments included dog food (Kroger Nutritionally Complete Bite Size Adult
580 Dog Food; composed of 21% protein, 8% fat, and 6% fiber), bran (Bob's Red Mill Organic High
581 Fiber Oat Bran Hot Cereal), butter (Kroger Unsalted Butter Sticks), tuna (StarKist Selects Low
582 Sodium Chunk Light Tuna in Water), and starvation (Table 1).
583 After 14 days on the assigned diet, all cockroaches were sacrificed and their hindgut
584 microbiota preserved for DNA or RNA extraction. Individual cockroaches were removed from
585 tanks, weighed, and placed on ice in sterile culture plates. Once sufficiently torpid, cockroaches
586 were dissected, and the entire gut was removed. Any visible debris, including fat bodies or
587 exoskeleton, was removed with forceps. The hindgut was then separated from the rest of the gut
588 using a scalpel and submerged in either 100 µL of 1XTE buffer or RNALater (Ambion, Austin,
589 TX, USA). Hindgut samples placed in 1XTE buffer were immediately stored at -80°C. Hindgut

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samples placed in RNALater were crushed with a sterile glass stirring rod. The suspended gut lumen was then removed and stored at -80°C.

DNA and RNA extraction, sequencing library preparation

Microbial DNA and RNA was extracted from hindgut samples as outlined previously in (39). Briefly, DNA was extracted using a modified version of the EZNA Bacteria Kit (Omega Biotek, Norcross, GA). Microbial RNA was extracted using a modified version of the HP Total RNA Kit (Omega Biotek, Norcross, GA). Detailed protocols for DNA and RNA extractions can be found here: dx.doi.org/10.17504/protocols.io.jz5cp86, https://dx.doi.org/10.17504/protocols.io.jz6cp9e. Sequencing libraries were prepared using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB) following product guidelines with an RNA fragmentation time of 10 min. The prepared library was submitted to the Georgia Genomics and Bioinformatics Core facility for normalization, pooling, and sequencing (Illumina HiSeq; Illumina, Inc., San Diego, CA).

Sheared DNA samples were end-repaired, dA-tailed, and adaptor ligated as instructed with the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs Inc). Adaptor-ligated fragments were size-selected with a magnetic bead protocol (Glenn et al., 2019) derived from (Rohland and Reich, 2012). Size-selected and adapter-ligated DNA was enriched via PCR using the NEBNext Ultra II DNA Library Prep Kit for Illumina with the NEBNext Multiplex Oligos for Illumina as dual index primers (New England BioLabs Inc.). A final metagenome pool was sent for sequencing at Novogene Corporation Inc. (Sacramento, CA) following their specifications.

Upon receiving sequences, FastQC and MultiQC were used to check for low-quality regions. Adapter sequences were trimmed using BBDuk from BBTools. Reads were then filtered

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to remove rRNA sequences using SortMeRNA (96). Sequences that aligned to the *Periplaneta americana* (97), *Blattabacterium* genome or plasmid (98) were removed using BBSplit. Paired-end reads were merged using BBMerge and reads that did not successfully merge were rescued by pseudo-merging using a custom laboratory script. For this, the forward read was joined with the reverse complement of the reverse read using a string 10 Ns as an internal spacer to denote a gap of unknown length.

Construction of reference genome clusters for key taxonomic clusters

An obstacle to RNA-seq analysis of gut microbiota is the relative lack of available reference genomes. To surmount this problem, we utilized a pangenome mapping approach based loosely on that employed in (45). This approach takes the available reference genomes associated with taxonomic clusters of interest to construct a genome cluster for analysis of group-level transcriptional activity.

We identified 18 genome clusters for analysis of the metatranscriptome data (see Table S1 for a full list of reference genomes used for constructing each cluster). Genome sequences for inclusion in each genome cluster were identified using a variety of metrics, including taxonomic annotation, phylogenetic information from 16S rRNA gene and genome trees, abundance in metagenome and metatranscriptome based on RefSeq homology searches as described below, and co-correlation analyses.

Pangenomes of orthologous gene clusters for each taxon were built with Anvi'o (99). We used *anvi-pan-genome* with DIAMOND (100) to calculate genome similarity within the taxon and bin individual genes into gene clusters using the MCL algorithm (101, 102). We used *anvi-pan-genome* with DIAMOND (100) to calculate genome similarity within the taxon and bin individual genes into gene clusters using the MCL algorithm (103, 104). Functional annotations

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for gene clusters were found using DIAMOND (100) to match functions and pathways in KEGG (105), COG (103, 104, 106), and CAZy (107, 108). Additional annotations from a DIAMOND search of each taxon's reference genomes to the Transporter Classification Database (TCDB) were added to gene clusters (109).

Metatranscriptome and metagenome reads were translated and mapped to protein sequences in RefSeq Prokaryotic Genomes (accessed 11/2021) (110) and to our dataset of 96 single-cell genomes from cockroach gut microbes (39, 111) using DIAMOND (100). The top-scoring hit (based on bit score) and all equal hits were kept. Custom scripts were used to map reads to genome clusters and build a per-sample gene cluster hit count table.

Analysis of genome cluster abundance and transcriptional activity

To calculate genome cluster abundance, the number of metatranscriptome and metagenome read hits to each taxon was divided by the number of reads in that sample with hits to the combined RefSeq and SAG reference database.

Weighted Bray-Curtis dissimilarities were calculated in R on whole-community taxonomic tables using *vegdist()* from the vegan package (112). Differences in dissimilarity values between diets were tested for significance in R with the Kruskal-Wallace method followed by Dunn's test with Bonferroni correction (*dunnTest()* from FSA package and *kruskal.test()* from stats package, respectively). Beta-dispersion in dissimilarity values were tested using the betadisper() function from the vegan package (112). Permutational multivariate analysis of variance (PERMANOVA) was carried out on dissimilarities using *adonis2()* from the vegan R package with host diet as the factor of variation.

For all genome clusters, DESeq2 (43, 113) was used for pairwise comparisons between diet treatments to find significantly differentially expressed gene clusters between diets. Relative

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transcriptional abundances of gene clusters found to be significantly differentially expressed between any pairwise diet comparison were visualized with heatmaps to determine their diet specificity.

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Data Availability:

Data associated with this study are available from NCBI SRA under BioProject accession number PRJNA994902. Scripts and workflow for the metatranscriptomic analyses can be found here: https://github.com/adepoy1/cockroach_metatranscriptome_manuscript.

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Figure Legends and tables

Table 1. Nutrient information for 100g serving of diet treatment. All cockroaches were raised on our laboratory control diet of dog food, composed of 21% protein, 8% fat, and 6% fiber. Carbohydrate contents of dog food are estimated based on levels of other macronutrients, ash, and moisture.

Diet Treatment	Calories	Protein (g)	Carbohydrate (g)	Fat (g)	Fiber (g)
Bran	375	17.5	67.5	5	17.5
Butter	714	0	0	79	0
Tuna	107	27	0	1	0
Starvation	N/A	N/A	N/A	N/A	N/A
Dog Food	350-399	21	45	8	6

Figure 1. Taxonomy and beta diversity metrics for the metagenome and metatranscriptome datasets (A)Within diet and all-diet comparison of weighted Bray-Curtis dissimilarities were calculated using taxonomic community profiles from the metagenome and metatranscriptome datasets. Tests of significance among the diets were done with a Kruskal test and Dunn's test.

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Beta dispersion was tested using the betadisper() function in vegan. (B) Relative abundance of families in metagenome samples. (C) Relative abundance of families in metatranscriptome samples. Each column represents a hindgut sample from a single cockroach. Families with a maximum relative abundance of at least 5% in either the metatranscriptome or metagenome datasets are shown.

Figure 2. Average relative abundance of genome clusters in metatranscriptome and metagenome datasets across diet treatments plotted using ggradar package for R.

Figure 3. Within-genome cluster transcriptional abundance of a manually curated list of KEGG pathways (binned at third hierarchy), RND efflux pumps (gene clusters classified as belonging to TCDB families 2.A.6 or 8.A.50), TonB-dependent transporters (gene clusters classified as belonging to TCDB families 1.B.14 or 2.C.1), and total summed CAZymes. Relative abundance was averaged within diet treatment.

Figure 4. Number of differentially expressed genes identified by DESeq2. Each number within each square is the number of differentially expressed genes when those two diets were compared.

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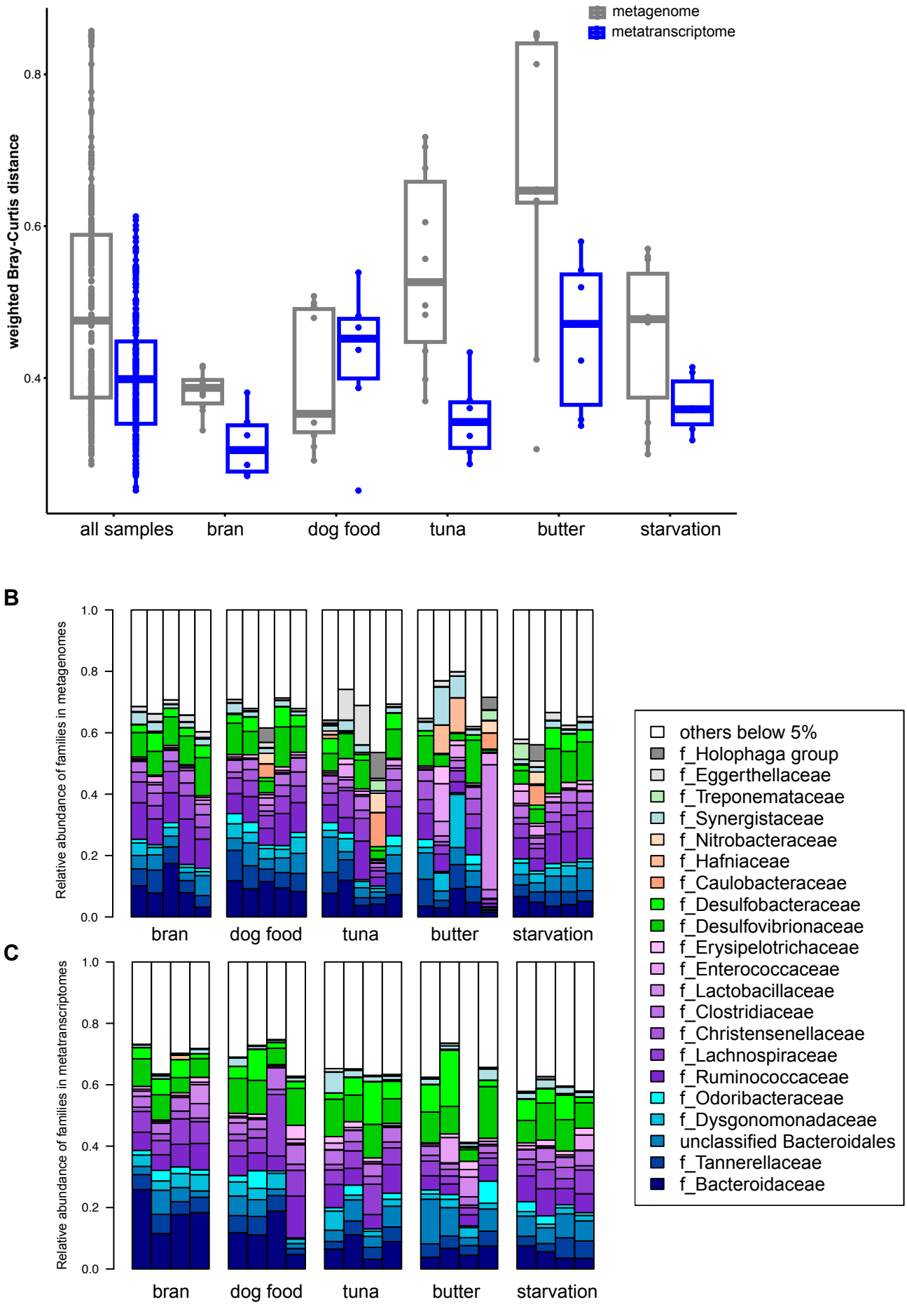

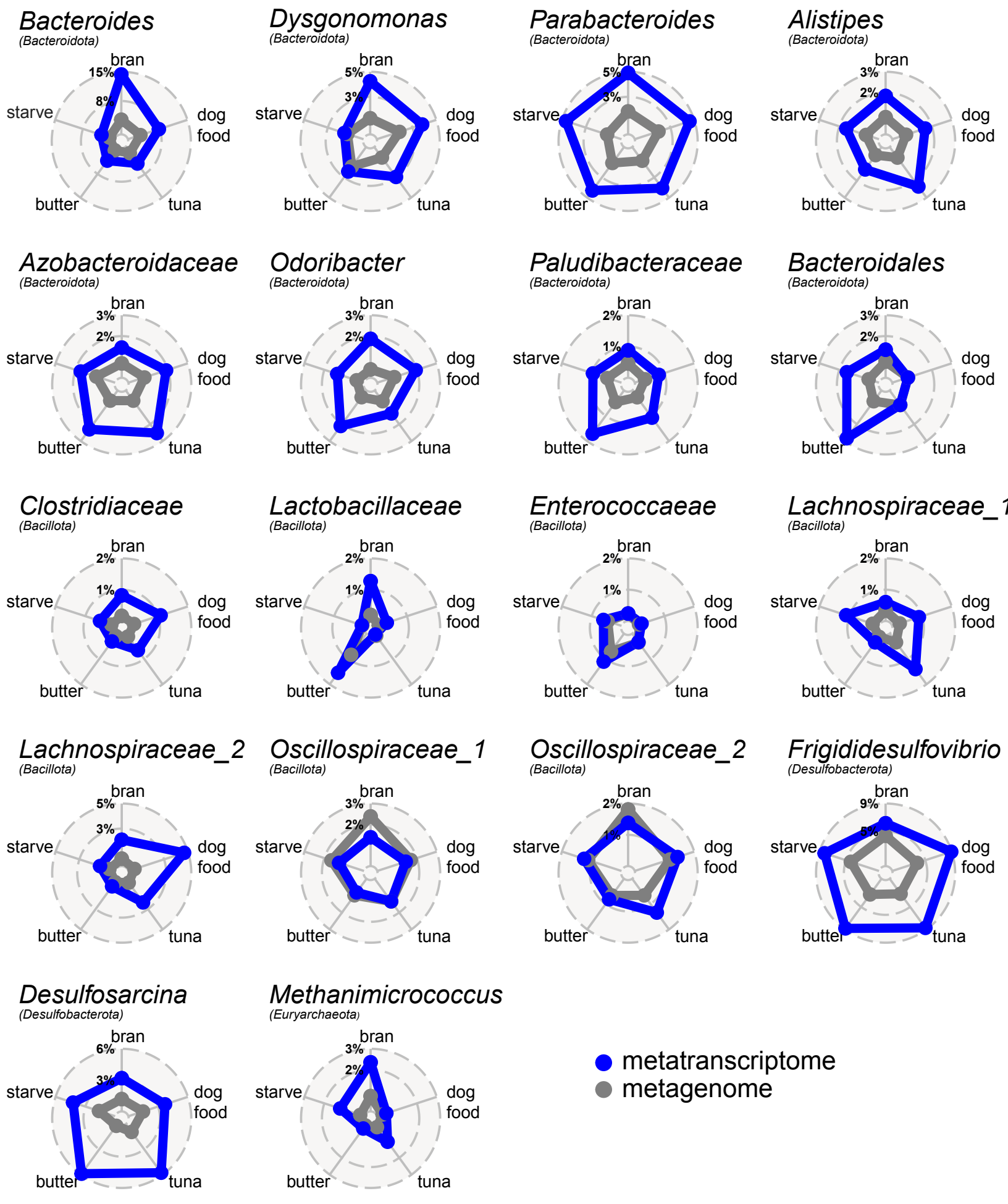
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Parabacteroides
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Alistipes
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Azobacteroidaceae
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Odoribacter
(Bacteroidota)
Paludibacteraceae
(Bacteroidota)
Bacteroidales
(Bacteroidota)
Clostridiaceae
(Bacillota)
Lactobacillaceae
(Bacillota)
Enterococcaeae
(Bacillota)
Lachnospiraceae_1
(Bacillota)
Lachnospiraceae_2
(Bacillota)
Oscillospiraceae_1
(Bacillota)
Oscillospiraceae_2
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Frigididesulfovibrio
(Desulfobacterota)
Desulfosarcina
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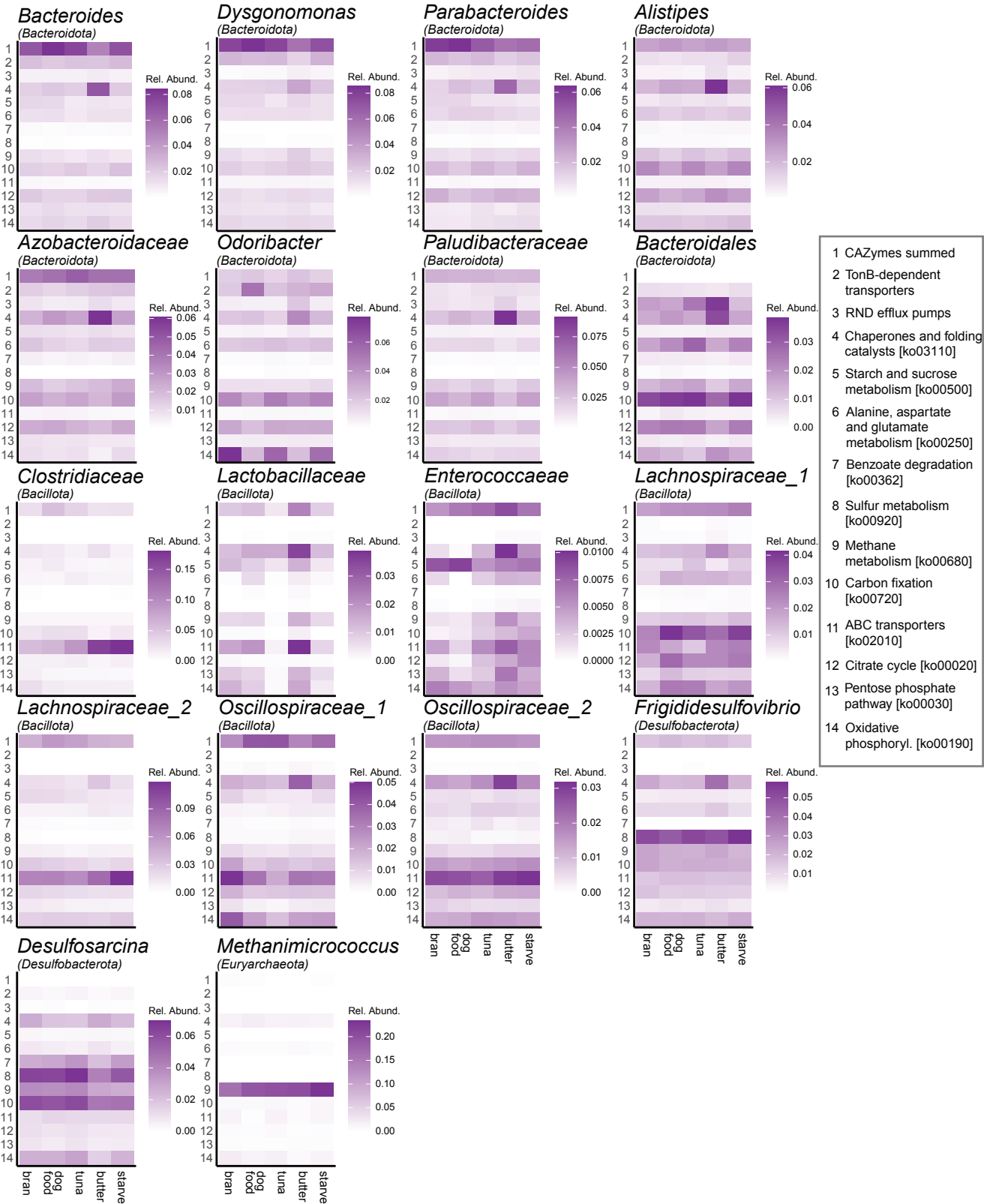
Methanimicrococcus
(Euryarchaeota)
bran
dog food
tuna
butter
starve
metatranscriptome
metagenome


Bacteroides
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Dysgonomonas
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Parabacteroides
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Alistipes
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Odoribacter
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(Bacteroidota)
Clostridiaceae
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Lactobacillaceae
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Enterococcaceae
(Bacillota)
Lachnospiraceae_1
(Bacillota)
Lachnospiraceae_2
(Bacillota)
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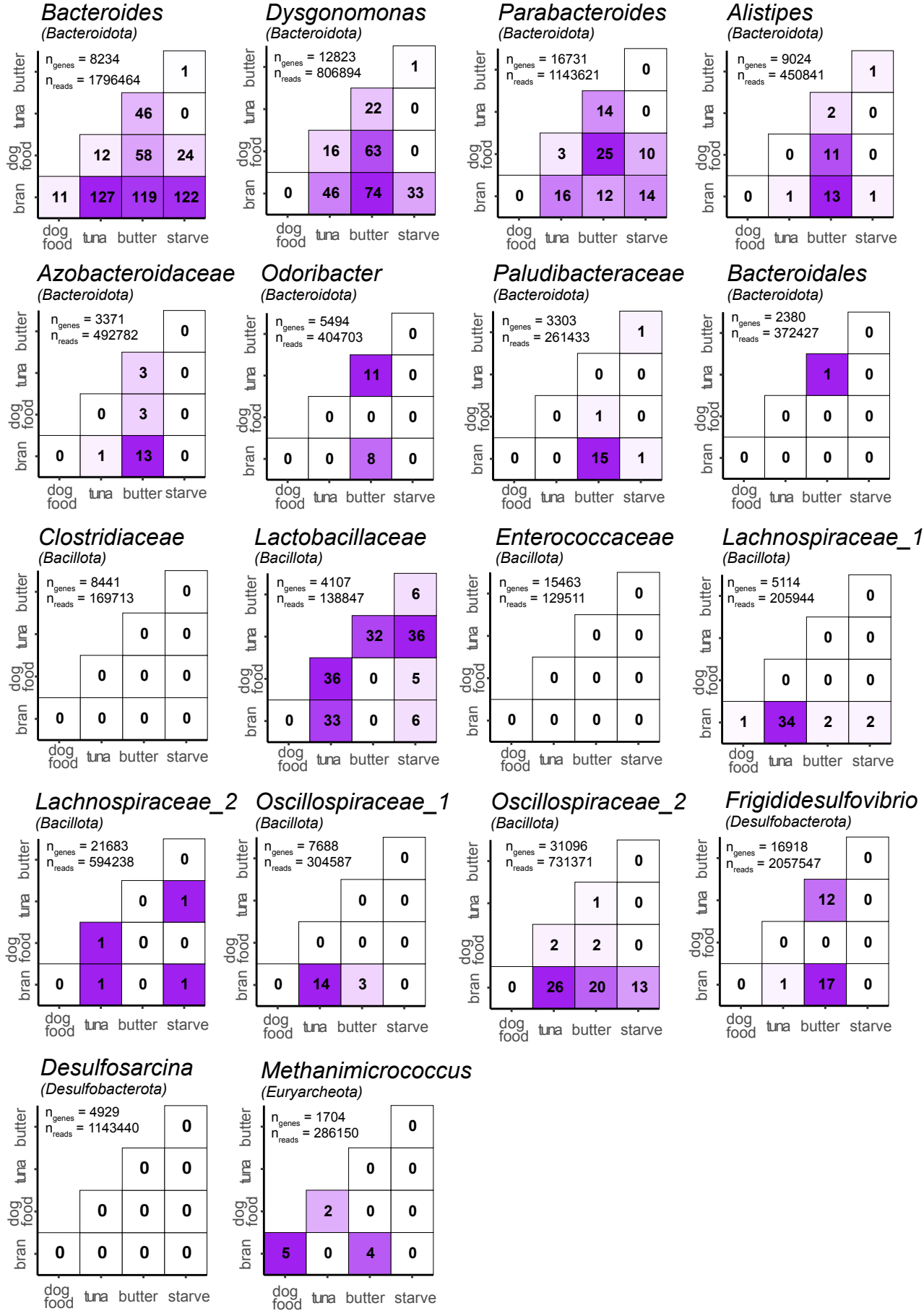
Frigididesulfovibrio
(Desulfobacterota)
Desulfosarcina
(Desulfobacterota)
Methanimicrococcus
(Euryarchaeota)
Rel. Abund.
bran
dog food
tuna
butter
starve
1 CAZymes summed
2 TonB-dependent transporters
3 RND efflux pumps
4 Chaperones and folding catalysts [ko03110]
5 Starch and sucrose metabolism [ko00500]
6 Alanine, aspartate and glutamate metabolism [ko00250]
7 Benzoate degradation [ko00362]
8 Sulfur metabolism [ko00920]
9 Methane metabolism [ko00680]
10 Carbon fixation [ko00720]
11 ABC transporters [ko02010]
12 Citrate cycle [ko00020]
13 Pentose phosphate pathway [ko00030]
14 Oxidative phosphoryl. [ko00190]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16 63 0
0 46 74 33
Parabacteroides
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0 16 12 14
Alistipes
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dog food tuna butter starve