Rodents consuming the same toxic diet harbor a unique taxonomic and functional core microbiome

Tess E. Stapleton (tstapleton06@gmail.com)
University of Utah
LeAnn M. Lindsey
University of Utah
Hari Sundar
University of Utah
M. Denise Dearing
University of Utah

Research Article

Keywords: Metagenomics, Herbivore, Plant-Herbivore Interactions, Wild

Posted Date: October 11th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3419959/v1

Additional Declarations: No competing interests reported.

Abstract

Gut microbiota are intrinsic to an herbivorous lifestyle, but very little is known about how plant secondary compounds (PSCs), which are often toxic, influence these symbiotic partners. Using 16S rRNA gene and shotgun metagenomic sequencing, we investigated the possibility of a unique taxonomic and functional core microbiome in populations of two species of woodrats (*Neotoma lepida* and *bryanti*) that have independently converged to feed on the same toxic diet (creosote bush; *Larrea tridentata*). In each gut region (foregut, cecum, and hindgut) sampled, we found a distinct taxonomic core set of microbes in the populations feeding on creosote that were not designated as core members in noncreosote-feeding populations. The core members in creosote feeders were significantly enriched and occurred more frequently than predicted by chance, suggesting that they may benefit the host. Some of the functions identified within the functional core include the metabolism of aromatic rings and thus may be involved in the degradation of PSCs. Overall, our results characterize the functional profiles of the gut microbiota in a wild herbivore and identify key taxa that may aid herbivores in subsisting on their toxic diet.

Introduction

The microbial communities of the mammalian gut are well known for their roles in defending against pathogens, training the immune system, and synthesizing nutrients (Moran, Ochman, & Hammer, 2019). These microbial communities can be highly variable among hosts due to differences in genetics, physiological status, and diet (Turnbaugh et al., 2007; Weinstein et al., 2021). Despite this variation, it is hypothesized that within a species, population, or dietary strategy, a subset of gut microbes or microbial functions persists, known as the “core microbiome”. These core microbes are proposed to be key ecological and functional members of the community, and recent studies have revealed numerous insights into the microbial ecology found in an assortment of host species in a variety of environments (Ainsworth et al., 2015; Jorge, Dheilly, & Poulin, 2020; Mendes, Kuramae, Navarrete, Van Veen, & Tsai, 2014). For example, among domestic ruminants, a distinctive core microbiome occurs in the rumens of 32 host species (Henderson et al., 2015). Microbes belonging to the rumen core may represent taxa that are fundamental to this dietary strategy. However, microbial community structure does not always link with function, as some functions are restricted to certain taxa, while others are more widespread (Shade & Handelsman, 2012). Thus, a host population may not share a similar taxonomic core of microbes but may host microbes with shared functional capability or a functional core microbiome. While many studies have investigated the presence of a shared taxonomic core, few have extended the work to examine the functionality of the core.

In herbivores consuming a similar diet, core microbes may be involved in critical functions. Herbivores rely completely on their gut microbiota for fermentation of indigestible, complex plant carbohydrates, such as fiber and cellulose, into simple sugars usable by the host (Karasov & Martínez del Rio, 2008). In addition, to deter herbivores, plants produce plant secondary compounds (PSCs), defensive toxins that can cause a wide range of negative physiological effects on the consumer (Freeland & Janzen, 1974). It has long been hypothesized that herbivores house gut microbiota to aid in the degradation of PSCs

(Freeland & Janzen, 1974), and recently, studies have provided evidence for this microbial function in a multiplicity of herbivorous hosts (Berasategui et al., 2017; Blyton et al., 2019; Hammer & Bowers, 2015; Itoh, Tago, Hayatsu, & Kikuchi, 2018; Kohl, Stengel, & Dearing, 2016; Kohl, Weiss, Cox, Dale, & Dearing, 2014). Plant secondary compounds also significantly influence the taxonomic structure of gut microbial communities (Kohl & Dearing, 2012; Stapleton, Kohl, & Dearing, 2022). Such a complex diet may select for a taxonomic core microbiome of organisms capable of fiber breakdown or interacting with PSCs. Alternatively, a lack of a discernable taxonomic core microbiome may mean that the ability to breakdown PSCs is more spread among various taxa, i.e., a functional core. While the presence of a core has been investigated across domestic, ruminant herbivores (Henderson et al., 2015), there has been little investigation across different gut regions within a host, across different hosts consuming the same diet, or hosts in a natural setting.

The herbivore gut is highly specialized for ingestion of its complex diet, including adaptations for housing these communities of symbiotic microbes. Fiber-degrading microbes reside in specialized, nongastric stomach chambers in foregut-fermenting animals such as ruminants (Karasov & Martínez del Rio, 2008) and in a more distal fermentation chamber, the cecum, for hindgut-fermenting animals such as equids, rabbits, and rodents (Karasov & Martínez del Rio, 2008). Some rodents, in addition to the cecum, have a sacculated foregut chamber proximal to the gastric stomach (Carleton, 1973). This chamber does not extensively ferment fiber (Kohl, Miller, Marvin, Mackie, & Dearing, 2014) and houses microbes capable of metabolizing PSCs (Kohl, Miller, et al., 2014). Little is known about the extent to which other species of herbivore host PSC-degrading microbes and whether these microbes are similar across different host populations consuming the same diet (Dearing & Weinstein, 2022).

To advance our understanding of how PSCs affect the gut microbiota of mammalian herbivores, we investigated the presence of a unique core microbiome in woodrat populations (*Neotoma* spp.) that have converged to feed on the same toxic diet, creosote bush (*Larrea tridentata*). These herbivorous rodents consume a variety of diets across their range (Kohl & Dearing, 2016; Weinstein et al., 2021). Some populations of both the desert woodrat (*Neotoma lepida*) and Bryant's woodrat (*Neotoma bryanti*), species that diverged approximately 1.6 mya (Patton, Huckaby, Álvarez-Castañeda, & Ticul, 2014), independently converged to feed on creosote bush (Malenke, Skopec, & Dearing, 2014). Creosote bush leaves are coated in a phenolic-rich resin composed of hundreds of PSCs, such as phenolics, flavones, and saponins (Schmutz, Mabry, Hunziker, & Difeo, 1978). Many of these compounds, such as nordihydroguaiaretic acid (NDGA), are toxic to mammals (Mangione, Dearing, & Karasov, 2004; Ríos, Mangione, & Gianello, 2008) and antimicrobial (Guzmán-Beltrán, Rubio-Badillo, Juárez, Hernández-Sánchez, & Torres, 2016; Kyselka et al., 2017). These PSCs not only strongly affect the diversity of the gut microbial community but may also select for microbes that use these compounds as substrates (Stapleton, Kohl, et al., 2022). Additionally, previous studies have revealed that the gut microbiota plays a critical role in facilitating the ingestion of creosote bush in *N. lepida* (Kohl, Weiss, et al., 2014). Therefore, the PSCs in creosote bush may have selected for the same taxonomic or functional core microbiome in populations of *N. lepida* and *bryanti* that feed on creosote. To investigate the presence of a creosote-related core microbiome, we surveyed the microbial communities in the foregut, cecum, and hindgut of

populations of *N. lepida* and *bryanti* that consume creosote ("creosote feeders") and compared them to microbial communities from populations outside the natural range of creosote bush ("noncreosote feeders") to identify core microbes specific to a host diet rather than host species. We expected core microbes related to creosote feeding in the foregut, as this structure has been documented to house microbes capable of degrading PSCs (Kohl & Dearing, 2016; Miller, Oakeson, Dale, & Dearing, 2016). Furthermore, we predicted that this chamber would harbor more gut microbiota capable of breaking down PSCs compared to the cecum, which should house primarily fiber-degrading bacteria. Finally, because the most abundant PSC produced by creosote bush (NDGA) is a phenolic, we anticipated that we would see microbial functions associated with the metabolism of xenobiotics and aromatic compounds in the functional core.

Experimental Procedures

Sample Collection

To examine the core microbiota of woodrats consuming a creosote diet, we collected 3–10 individuals from 20 populations across two woodrat species in 2017–2018 (Table S1). Populations spanned the southwestern United States (California, Utah, and Nevada). We live-trapped animals using Sherman traps baited with oats; previous work has shown that this trapping method does not significantly affect the microbiome of woodrats (Kohl, Luong, & Dearing, 2015). For all individuals, a fecal sample was collected at the time of capture. Fecal samples were used as a representation of the hindgut microbial community because the microbiota in feces often resemble the hindgut of mammalian hosts (Dill-Mcfarland, Weimer, Pauli, Peery, & Suen, 2016; Dougal et al., 2012; Kohl, Miller, et al., 2014). Foregut and cecum contents were sampled from a subset of populations. These animals were dispatched after capture and immediately dissected (Table S1). Feces, foregut content, and cecum content were stored in liquid nitrogen in the field and then held at − 80°C until DNA extraction.

DNA Extraction and Amplicon Sequencing

We isolated DNA from woodrat feces, foregut content, and cecum content using QIAamp PowerFecal DNA kits (Qiagen) following the manufacturer's protocols. Two negative controls were sequenced for each extraction kit. All DNA amplification, library preparation, and sequencing were conducted at the DNA Service Facility at the University of Illinois-Chicago (Naqib et al., 2018). To determine the gut microbial community of our sampled woodrats, we amplified the V4 hypervariable region of the 16S rRNA gene locus using the 515F and 806R primers (Caporaso et al., 2018). To determine dietary content, we amplified the P6 loop of the chloroplast *trnL* (UAA) intron using the *g* and *h* primers (Taberlet et al., 2007). All amplicon sequencing was conducted on an Illumina MiniSeq platform (2 x 150 bp paired-end reads).

16S rRNA Gene Sequence Processing

All 16S rRNA gene sequences were processed in QIIME2 version 2021.8 (Bolyen et al., 2019). Primers were removed using Cutadapt (Martin, 2011), and the resulting sequences were filtered for quality control

in QIIME2. Sequences were grouped into amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016), resulting in 5,508 unique ASVs that were assigned to taxonomy using the Silva database release 138.1 (Quast et al., 2013). We removed identified chimeras, sequences that appeared in fewer than four samples, sequences that appeared less than ten times total across all samples, and sequences that were identified as chloroplast or mitochondria. After filtering, the samples contained 6,884,401 total reads with an average of 27,985 reads per sample. To control for differential sequencing depth, samples were rarefied to a sequencing depth of 4,499 reads per sample (the lowest coverage in any one sample that contained sufficient reads). Rarefaction resulted in the removal of four samples. The identification of core microbes was performed using unrarefied data, as rarefaction can skew the estimation of core microbes (Neu, Allen, & Roy, 2021; Ramakodi, 2021). All other analyses were performed on rarefied data.

trnL Sequence Processing

To determine the diets of animals, we used *trnL* plant metabarcoding. Plant sequences were processed using QIIME2 version 2019.4 as previously described (Stapleton, Weinstein, Greenhalgh, & Dearing, 2022). Sequences with taxonomy that did not resolve to at least the family level were considered unclassified (< 4% of sequences). After examining the content of our negative controls and based on previous work (Stapleton, Weinstein, et al., 2022), families that represented less than 1% of each population's total relative abundance were removed. Populations were considered 'creosote feeders' if creosote reads were present in diet samples after this filtering step.

Identification of Core Members

To determine the creosote-feeding core microbiome, we used a two-step process. First, we identified the core ASVs across all woodrat samples as those that were present in ≥ 50% of *N. lepida* and *N. bryanti* samples (Lloyd-Price et al., 2017; Risely, 2020; Turnbaugh et al., 2007). This threshold was chosen based on precedence from other studies that suggest that using a more stringent threshold generates diversity scores that correlate poorly with unfiltered data and reduces the ability to compare core microbiomes across studies. Additionally, we sampled across many geographically distinct populations, which is known to significantly influence the structure of the microbiome (Ainsworth et al., 2015; Gomez-Arango et al., 2017; Graystock, Rehan, & McFrederick, 2017; Hammer, Janzen, Hallwachs, Jaffe, & Fierer, 2017; John Wallace et al., 2019; Risely, 2020; Sullam et al., 2015; Weinstein et al., 2021). Then, we removed all core woodrat microbes that were not unique to creosote-fed woodrats to yield the creosote core. Using this two-step process, we defined core ASVs for the cecum, foregut, and hindgut of *N. lepida* and *N. bryanti* in creosote-feeding populations and in noncreosote-feeding populations. We considered ASVs part of the 'creosote-feeding core microbiome' for each sampled gut region if they met the ≥ 50% threshold for the general core in both *N. lepida* and *N. bryanti* samples and were not considered core in noncreosote-feeding populations. Microbes meeting these thresholds are designated as 'core' or 'creosote-feeding core' hereafter. In addition, because we had more hindgut samples than foregut and cecum samples, we evaluated the effect of sample size on estimates of core microbes by restricting the hindgut dataset to only samples that had a matching foregut and cecum sample and comparing the results from this subset to the results from all hindgut samples.

Statistical Analysis

Alpha diversity of the microbiome was measured using Shannon's index and observed ASVs, and these values were compared across groups using Kruskal–Wallis tests implemented in R. We measured beta diversity using Bray–Curtis distances (community structure) and Jaccard distances (community membership). Differences in beta diversity were compared using permutational multivariate analysis of variance (PERMANOVAs) implemented using the vegan package in R. To determine whether core ASVs were under host selection, we fit the prokaryotic neutral model to all ASVs found in each gut region following methods described in Burns et al. 2015. Using this model, all ASVs were classified as either overrepresented, neutral, or underrepresented. In addition, we applied the neutral model to each population to determine whether there was greater selection on creosote populations than on noncreosote populations. Finally, we estimated differentially abundant ASVs using DESeq2 (Love, Huber, & Anders, 2014). We used the package ashr to shrink log2fold changes generated by DESeq2, changes in relative abundance were considered significant if the FDR corrected p value was < 0.01 and the log fold change was ≥ 1.5.

Metagenomic Analysis

We performed metagenomic sequencing on 45 fecal samples from 9 populations (n = 3 per species, per population; Table S1). We extracted DNA as previously described, and library preparation, amplification, and final sequencing were completed at the DNA Service Facility at the University of Illinois-Chicago. Library prep was performed using the Swift 2S Turbo DNA Library Kit with enzymatic fragmentation (catalog 44024 Swift Biosciences Ann Arbor, MI) followed by PCR performed according to the manufacturer's protocol. Final libraries were size-selected, pooled, and sequenced on an Illumina NovaSeq 6000 with 2x150 bp sequencing and a 1% phiX spike-in. Metagenomic sequencing resulted in a total of 452,316,506 reads with an average of 10,051,478 reads per sample (S.D. 1,719,434)

Previous research has shown that read-based and assembly based methods can produce different results (Tamames, Cobo-Simón, & Puente-Sánchez, 2019); therefore, we characterized the functional profile of the gut microbiota using both unassembled and assembled reads. For the unassembled reads, we used MEGAN6 to conduct an analysis of gene function on forward reads (Huson et al., 2016). Adaptors were trimmed from all sequences using FastP (Chen, Zhou, Chen, & Gu, 2018). We removed host reads from sequences by mapping the reads to all of the following host genomes: *Peromyscus leucopus, P. maniculatus, P. nasutus*, and *Neotoma lepida*. We ran DIAMOND (v. 2.0.9) (Buchfink, Reuter, & Drost, 2021) to blast the remaining reads against the UniRef100 database (Suzek, Wang, Huang, McGarvey, & Wu, 2015) with an e-value cutoff of 0.001. These host-filtered, annotated forward reads were uploaded into MEGAN6, community edition and classified to KEGG Orthologs (KOs) using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa, Sato, & Kawashima, 2022). Genes were classified as belonging to the creosote core microbiome as previously described for the taxonomic core. We used DESeq2 to investigate genes that were differentially abundant between creosote and noncreosote feeders as previously described.

In addition, to identify bacteria associated with creosote feeding, we assembled metagenomic sequences into metagenome assembled genomes (MAGs). We used Metaspades (SPAdes v. 3.15.3) on a large memory node to coassemble all 45 samples, resulting in 16,336,611 total contigs. Then, we used MetaBat2 to bin contigs with default parameters, resulting in 649 total bins. We measured MAG completeness and contamination using checkM (v. 1.1.3) and dereplicated the MAGs to 99% ANI using dRep (Olm, Brown, Brooks, & Banfield, 2017). All unique MAGs identified as > 50% complete and with < 10% contamination were kept for further analysis, resulting in 271 total MAGs identified across all samples. We classified MAGs as part of the 'core creosote-feeding microbiome' using the methods described above for ASVs (i.e., creosote-feeding core). We assigned taxonomy to the MAGs using the Genome Taxonomy Database (GTDB) and GTDB-Tk (Chaumeil, Mussig, Hugenholtz, & Parks, 2022). The functional capability of core MAGs was determined by identifying KOs in each sample. First, coding regions were identified using Prodigal and then extracted using GffRead (Pertea & Pertea, 2020). To reduce gene redundancy, we clustered the resulting genes at 95% sequence similarity using CD-HIT (Fu, Niu, Zhu, Wu, & Li, 2012). Nonredundant genes were kept and blasted against the UniRef100 database using DIAMOND for functional annotation. We then generated gene abundance profiles by mapping reads from each sample to nonredundant genes using BWA (Li & Durbin, 2009). We investigated the presence of differentially abundant genes in creosote and noncreosote feeders using DESeq2, which has been validated for use with metagenomes (Jonsson, Österlund, Nerman, & Kristiansson, 2016).

Results

Community composition across and within gut regions

For all woodrat populations, gut microbial diversity varied across the foregut, cecum, and hindgut. Alpha-diversity metrics were significantly different across the three gut communities (Kruskal–Wallis, Shannon index $H(2) = 84$, $p < 0.001$; observed ASVs $H(2) = 68$, $p < 0.001$; Fig. S1). The cecum proved highly diverse, with the highest alpha diversity for both the Shannon index and observed ASVs, while the foregut was the least diverse (Table 1). The microbial community of the three gut regions significantly differed in both community membership (PERMANOVA, Jaccard, pseudo-F = 4.6, $R^2 = 0.04$, $p < 0.001$) and community structure (PERMANOVA, Bray–Curtis, pseudo-F = 7.7, $R^2 = 0.06$ $p < 0.001$). The variable with the most explanatory power was the most abundant plant family in the host diet rather than the gut region (PERMANOVA, Jaccard, diet $R^2 = 0.14$, $p < 0.001$; Bray–Curtis, diet $R^2 = 0.20$, $p < 0.001$). Within each gut region, the microbial communities of creosote-fed populations significantly differed from populations that did not feed on creosote (PERMANOVA, Jaccard and Bray–Curtis, $p < 0.001$ for all gut regions; Fig. 1), with levels of dietary creosote explaining 7% of the variation (PERMANOVA, Jaccard and Bray–Curtis, $R^2 < 0.07$, $p < 0.001$ for all gut regions; Table S2).

Table 1
Average alpha-diversity metrics (Shannon's Index and Observed ASVs) found in each gut region.

	Avg. Shannon's Index	Standard Error	Avg. Observed ASVs	Standard Error
Foregut	4.3	± 0.87	307	± 142
Cecum	5.4	± 0.34	499	± 82
Hindgut	5.0	± 0.33	358	± 93

Creosote-feeding Core Microbiome Membership

The cecum, foregut, and hindgut predominantly harbor distinct creosote-feeding core microbiomes. With respect to the core microbiome unique to creosote feeders, hereafter 'core', 25 ASVs were identified as being core in more than one gut region, while two ASVs, both in the family *Lactobacillaceae*, were core microbes across all three regions (Fig. 2A). The majority of core ASVs shared between the two gut regions belonged to *Muribaculaceae* (Table S3). The cecum harbored the largest overall core microbiome of the gut regions. Of the 2,973 total identified ASVs in the cecum, 164 were classified as core ASVs (Fig. 2C) represented by 18 microbial families. The families containing the most core ASVs in the cecum were *Lachnospiraceae* (64 ASVs), *Oscillospiraceae* (26 ASVs), and *Muribaculaceae* (29 ASVs).

The foregut had the smallest core of the three gut regions (Fig. 2B). The foregut harbored a total of 2,207 identified ASVs, 36 of which were identified as core ASVs. Foregut core ASVs were represented by 9 families, with *Muribaculaceae* (12 ASVs), *Lachnospiraceae* (6 ASVs), *Desulfovibrionaceae* (5 ASVs), and *Eggerthellaceae* (3 ASVs) containing the most. Finally, hindgut samples contained a total of 3,781 identified ASVs, with 40 ASVs classified as core, belonging to 10 families (Fig. 2D). *Muribaculaceae* and *Oscillospiraceae* also contained the most core hindgut ASVs, followed by the family *Lachnospiraceae* (15, 6, and 5 ASVs, respectively).

The sample size of the hindgut dataset affected the size of the core microbiota. When we restricted our hindgut samples to match the animals included in the cecum and foregut samples, the number of microbes identified as core increased. For the smaller hindgut dataset, we observed 2,627 ASVs, 66 of which were classified as core. Core microbes were represented by 13 different families, with the majority of ASVs belonging to *Muribaculaceae, Lachnospiraceae*, and *Oscillospiraceae* (25, 12, and 9 ASVs, respectively). None of the identified core members belonged to *Butyricoccaceae*, despite that family being present for core members in the full hindgut core dataset. Notably, although the core microbiota increased compared to the larger hindgut dataset, the cecum still harbored a larger core microbiome.

Core ASVs did not comprise the majority of the microbiome but were more abundant than other ASVs. The relative abundance of core ASVs was significantly higher than that of noncore ASVs within each gut region (Kruskal–Wallis, $p < 0.001$, cecum, foregut, and hindgut). Within each gut region, the average relative abundance of any one core ASV never exceeded 1.5%. In the cecum, the most abundant core ASV belonged to the family *Lachnospiraceae* and had a total relative abundance across all creosote-feeding

samples of 0.07%. Collectively, cecum core microbes comprised a total relative abundance of 19.1%, while core foregut ASVs comprised 15.7% of the total. The most abundant core ASV in the foregut had the highest relative abundance of any core ASV across the gut regions, with a total relative abundance of 6.1% (family *Lactobacillaceae*). The most abundant core ASV in the hindgut also belonged to the family *Lactobacillaceae* and had a total relative abundance in all creosote-feeding samples of 1.1%. The cumulative relative abundance of all core ASVs in the hindgut was 10.4% of the microbiome. Although no core ASVs comprised the majority of the microbiome in any gut region, no single ASV outside the core had a relative abundance higher than 7.6% (foregut, noncore ASV).

Taxonomic Differences in Creosote Feeding Populations

Core ASVs were significantly enriched in the cecum, foregut, and hindgut of creosote-fed populations compared to noncreosote-fed populations. Using DESeq2, within the cecum, we found 111 ASVs belonging to 13 microbial families significantly enriched in creosote-fed animals compared to noncreosote-fed animals; 36 of these ASVs were core (Fig. 3A). Enriched core ASVs were represented by 9 microbial families (Table S4). Amplicon sequence variants with the largest log changes compared to noncreosote feeders belonged to the families *Muribaculaceae* and *Lachnospiraceae* (Table S4). Forty-eight ASVs were significantly enriched in the foregut of creosote-fed animals, 16 of which were core (DESeq2; Fig. 3A). Significantly enriched core ASVs in the foregut belonged to the families *Eggerthellaceae*, *Lactobacillaceae*, *Lachnospiraceae*, and *Pasteurellaceae* (Table S5). The hindgut of creosote feeders contained the highest number of significantly enriched microbes compared to noncreosote feeders, with 151 enriched ASVs, 18 of which were classified as core (DESeq2; Fig. 3A). These enriched core microbes were represented by 7 microbial families; the largest log changes were observed in ASVs belonging to *Muribaculaceae* and *Lactobacillaceae* (Table S6).

Neutral Selection of ASVs

To determine whether core ASVs were under selection, we assessed the fit of the prokaryotic neutral model to occupancy and abundance distributions of all ASVs in each gut region. Deviations from this model (i.e., reduced model fit) are indicative of nonneutral processes, such as selection from the host or host diet (Burns et al., 2015). Model fit was greatest for all ASVs in the hindgut (R^2 = 0.46); however, when we restricted the dataset to only include hindgut samples from hosts that had matching cecum and foregut samples, the model fit decreased (R^2 = 0.27) and was more similar to the other gut regions (cecum R^2 = 0.30; foregut R^2 = 0.26). Additionally, there was no significant difference in prokaryotic neutral model fit on all ASVs between creosote and noncreosote populations (Student's t test, t(13) = -0.31, p = 0.78), nor was there a relationship between the amount of creosote in each population's diet and model fit (linear regression, R^2 = 0.24, p = 0.12). Among the ASVs identified as under selection, many were core microbes from each of the different gut regions. Fewer core microbes were selected against or underrepresented than under selection (Table 2). Across all gut regions, there was underrepresentation of core ASVs belonging to *Lactobacillaceae, Muribaculaceae, Clostridia_UCG-014, Pasteurellaceae,*

Lachnospiraceae, and *Oscillospiraceae* and overrepresentation of core ASVs belonging to many families found in the core (Fig. 3B, 3C).

Table 2
Proportion of core microbes identified as being selected for or selected against by the prokaryotic neutral model.

	core selected for	core selected against
Foregut	30.50%	8.30%
Cecum	39.02%	5.49%
Hindgut	47.50%	15.00%

Characterizing the Functional Core Microbiome

To characterize the potential functional core microbiome of creosote-fed woodrats, we compared the gene content of the gut microbial communities of creosote- and noncreosote-fed woodrats using KOs. Using the unassembled reads, we investigated the abundance of high-level functional pathways and found that most functions were unclassified (46%), with the next most abundant pathways being functions associated with metabolism (Fig. 4A; Table S7). We also investigated whether there existed a functional core in creosote feeders using KOs associated with the degradation of xenobiotics at the protein level. Of the 208 KOs within the database, 4 were identified as part of the creosote feeding functional core. The core KOs unique to creosote feeders coded for the enzymes enoyl-CoA hydratase (K01692), 4-hydroxybenzoate decarboxylase (K01612), benzoyl-CoA reductase subunit B (K04113), and 2-pyrone-4,6-dicarboxylate lactonase (K10221). The three most abundant KOs in creosote feeders were associated with the biosynthesis or metabolism of pyrimidines and purines (Fig. 4B). The next most abundant KO coded for 4-carboxymuconolactone decarboxylase, an enzyme involved in the degradation of benzoate (Fig. 4B). More than half of the KOs (106) were shared across the functional core of creosote and noncreosote feeders, with no hierarchical clustering of functional profiles by diet type (Fig. 4A, B; Table S8). Principal component analysis of the KOs at the xenobiotic degradation protein level did not show separation of the functional profiles of woodrat gut microbial communities by diet (Fig. 5A). In addition, no xenobiotic degradation KOs were identified as significantly enriched in creosote feeders compared to noncreosote feeders (DESeq2).

Coassembly of the short-read data resulted in 271 total MAGs. These MAGs primarily belonged to the families *Muribaculaceae* (124), *Lachnospiraceae* (48), and *Ruminococcaceae* (15; Table S9, S10). Only one MAG belonged to the creosote-feeding core microbiome. This MAG in the family *Treponemataceae* has an estimated 447 KOs, some of which, such as multidrug resistance transporters, could enable microbial survival and growth in a high toxin environment (Table S11). No MAGs were significantly enriched in creosote feeders compared to noncreosote feeders (DESeq2). In addition, there was large overlap of the identified functional profiles of MAGs within creosote and noncreosote feeders (Fig. 5B). However, 367 microbial genes were significantly more abundant in creosote feeders (Table S12). When

blasted against the UniRef100 database, we found that these differentially abundant genes with an existing KO classification were associated with a wide range of functions, with the majority coding for enzymes or cellular transporters, specifically ABC transporters (Table S12). None were identified as being involved in xenobiotic degradation.

Discussion

Diet heavily influences the mammalian gut microbiome (David et al., 2014; Knight, 2015; Ley et al., 2008; Youngblut et al., 2019). For mammalian herbivores in particular, diet should exert strong selection on the gut microbial community for microbes that provide ecologically relevant functions across hosts or core microbiota. Little investigation has been done on whether diet exerts this selection for shared microbial taxa and/or functions. Here, we addressed this gap in our knowledge by investigating the taxonomic and functional core microbiome in wild woodrats consuming the same toxic diet. We found core microbes unique to woodrats that consume creosote and unique core communities across different gut regions that may aid the host in subsisting on an herbivorous diet. We also identified a functional core unique to creosote-fed woodrats consisting of several KOs that may be involved in the degradation of PSCs.

Based on taxonomy, creosote-fed woodrats harbored distinct gut microbial communities and core microbes in the foregut, cecum, and hindgut. This is consistent with previous studies that also found that microbial communities differ between the midgut and hindgut in various herbivorous hosts (Jones et al., 2018; Kohl et al., 2017; Kohl, Miller, et al., 2014; Pardesi et al., 2022). These gut regions carry out different functions within the host, and these physiological differences present unique environments for microbes that likely shape the gut microbial community. The cecum, for instance, is a large fermentation chamber that houses high densities of microbes that ferment dietary fiber (Karasov & Martínez del Rio, 2008; Kohl, Miller, et al., 2014). Indeed, we found that the cecum had the highest diversity of microbes and the largest creosote-feeding core microbiome. In addition, most core microbes belonged to the families *Lachnospiraceae* and *Oscillospiraceae*, families known for fiber fermentation in the rumen (Anderson & Fernando, 2021) and previously identified as part of the ruminant core microbiome (Henderson et al., 2015). The large cecum core microbiome of woodrats resembling that of ruminant herbivores may indicate that these taxa are widespread across and functionally important for fiber degradation in many mammalian herbivores. Future work could investigate this pattern across even more diverse hosts that consume diets high in fiber, such as reptiles and birds, to determine whether the same microbial taxa have been selected for, as previously hypothesized (Colston & Jackson, 2016).

In contrast to the cecum, the foregut is a small, sacculated region thought to house microbes capable of using PSCs as substrates (Kohl & Dearing, 2016; Miller et al., 2016). The foregut had the largest number of core members belonging to the family *Eggerthellaceae*. Notably, several microbes that belong to *Eggerthellaceae* are capable of degrading PSCs (Beltrán, Romo-Vaquero, Espín, Tomás-Barberán, & Selma, 2018; Bess et al., 2020; Bode et al., 2013; Haiser et al., 2013; Koppel, Bisanz, Pandelia, Turnbaugh, & Balskus, 2018). These microbes may metabolize PSCs within the foregut prior to absorption in the small intestine. More core members were identified as *Eggerthellaceae* in the foregut than in the cecum;

however, this family was ubiquitously identified as part of the core microbiome across gut regions. Other core families in the foregut were either families commonly found in the woodrat gut microbiome (*Lactobacillaceae, Muribaculaceae, Desulfovibrionaceae*) or known fiber degraders (*Lachnospiraceae, Oscillospiraceae).* The retention time of food in the foregut is not long enough for extensive fermentation (Kohl, Miller, et al., 2014), but as it precedes the cecum in the gastrointestinal tract, it is possible that some fermentation of simple sugars and volatile fatty acids begins in this region (Kohl, Miller, et al., 2014).

The foregut microbiome was less diverse than the cecum, with the core microbiome consisting of ~ 16% as many ASVs as that of the cecum. This lower diversity may stem from higher concentrations of PSCs in the undigested diet, which can reduce microbial growth (Compean & Ynalvez, 2014), as well as a low pH (~ 4.5), which may make the foregut inhospitable to some gut microbiota (Kamada, Chen, Inohara, & Núñez, 2013). Alternatively, the cecum may harbor a large and taxonomically diverse core microbiome due to the diversity of fibers, waxes, and pectins found in a plant-based diet. Creosote contains 2–3 times more fiber than resin by dry mass, and different plant species produce various kinds of fibers that may require different microbes to breakdown (Meyer & Karasov, 1989). The smaller, more taxonomically varied core in the foregut may indicate that the ability to degrade PSCs is not unique to a few microbial taxa but is possibly a conserved function across microbial lineages.

The hindgut also harbored a smaller core than the cecum, i.e., approximately 24% of that found in the cecum core. Many of these core microbes belong to families also identified as core microbes in the cecum, such as *Muribaculaceae, Oscillospiraceae*, and *Lachnospiraceae*. It is possible that the hindgut core retained fewer members than the cecum because there is little selection from creosote resin once digesta has reached the colon, as most of the resin components would be absorbed in the small intestine or fermented by cecal microbes. In addition, we used fecal samples to represent the hindgut, and although feces are often used as a proxy for the hindgut, these microbial communities can be similar or discordant, depending on the host and sampling methods (Reyman, van Houten, Arp, Sanders, & Bogaert, 2019; Tang et al., 2020; Yan et al., 2019). Some of this variation may stem from the fact that fecal microbial communities may shift after defecation or become contaminated by the environment. In some mammalian species, using feces as a proxy for the hindgut obscures ecological and phylogenetic signals (Ingala et al., 2018). Although a previous study found that greater than 80% of microbes from woodrat feces collected aseptically were retained in feces collected from a trap (Kohl et al., 2015), even this loss of native microbes may impact the number of shared core microbes found using fecal samples.

Sample size affected the number of core members identified. We collected far more fecal samples than cecum and foregut samples because fecal sample collection is noninvasive. This difference in sample size altered estimates of the core microbiome. When hindgut samples were reduced to the same sample sizes as the other regions, the number of core microbiota increased, although the hindgut microbial community still harbored a smaller core than the cecum. In addition, different families were identified as belonging to the core in the smaller dataset than in the full dataset. This has important implications for core microbiome studies, as the number of samples used could artificially inflate or shrink the number of

microbes identified as core and even miss taxa of ecological importance. Differential sequencing depth has a similar effect where analyses at different depths identify different core microbiota within different taxonomic groups (Ramakodi, 2021). While sample size can be difficult to keep consistent in studies of the microbial communities of wild organisms, we caution researchers to take this into consideration when designing core microbiome studies or comparing across studies.

Core microbes were more abundant than other ASVs, enriched in creosote-fed woodrats, and occurred more frequently than would be predicted by chance. While the core microbes did not make up a majority of the gut microbiome, they were more abundant than other ASVs and did represent a cumulative relative abundance of >15% in the foregut and the cecum. This finding is consistent with other studies that classified their core microbiome at the ASV level (Ainsworth et al., 2015a; Simonin et al., 2020). In addition, several core microbes were enriched in creosote feeders and identified as overrepresented by neutral models. These overrepresented core members were taxonomically widespread, indicating that these taxa may be beneficial and are potentially selected for within a host (Burns et al., 2015). Indeed, several of these enriched or selected core microbes belonged to families that can provide useful functions to an herbivore, such as fiber fermentation (*Ruminococcaceae*) or degradation of PSCs (*Eggerthellaceae*). Taken together, these results suggest that the selected core members are keystone taxa (Banerjee, Schlaeppi, & van der Heijden, 2018). Our samples were collected from a large number of geographically distant populations and two different species, factors that significantly influence the community composition of the gut microbial community (Weinstein et al., 2021). Despite samples coming from two species and populations occurring over wide geographic distances (as far as ~ 700 km between creosote feeders), we identified a core microbiome only found in animals consuming creosote, further signifying the possible importance of these cecum and foregut microbes to their host. Experimental manipulation or sequencing of the genome of potential keystone taxa may further elucidate the roles these microbes play in the host gut microbiome.

Some KOs identified as belonging to the functional creosote-feeding core microbiome were related to the metabolism of PSCs. Three of the identified core KOs (4-hydroxybenzoate decarboxylase, benzoyl-CoA reductase subunit B, and 2-pyrone-4,6-dicarboxylate lactonase) coded for enzymes that play important roles in the metabolism of lignans and aromatic rings (Boll & Fuchs, 1995; Breinig, Schiltz, & Fuchs, 2000; Hobbs et al., 2012). Creosote resin is composed of many phenolics, primarily NDGA (Schmutz et al., 1978), a lignan with aromatic rings. Therefore, these proteins in the core may play a role in degrading creosote PSCs in the wood rat gut. This finding is consistent with previous work and warrants further investigation into the particular microbial taxa and pathways that metabolize creosote resin. We also identified several KOs coding for ABC transporters that were significantly more abundant in the gut microbial communities of creosote feeders than in those of noncreosote feeders. Microorganisms use ABC transporters to efflux toxins, including PSCs, to protect the cell from detrimental effects (Fleißner, Sopalla, & Weltring, 2002; Zwiers, Stergiopoulos, Gielkens, Goodall, & De Waard, 2003). Thus, microbes in the creosote-feeding gut microbial community may be using transporters to efflux creosote toxins out of the cell. Indeed, the only core MAG had several KOs related to multidrug resistance, which are usually transporter genes.

When characterizing the functional profile of the gut microbiome of woodrats, nearly half of the metagenomic data were not assigned to a functional pathway, severely limiting our ability to detect a functional core. It is possible that there exists a larger functional core microbiome in creosote-fed woodrats than we were able to observe due to these pathways being largely uncharacterized. One of the major advantages of metagenomic sequencing is that, in addition to taxonomic information, it provides information on the genomic content of microbes found in a region of interest. Indeed, the main goal of most studies utilizing metagenomic sequencing, including ours, is to characterize the metabolic capacity of microbial communities. Metagenomics is often touted as providing more in-depth and accurate results than 16S rRNA gene sequencing, and this has been demonstrated in well-studied systems, such as the human microbiome, where microbial functions have been established (Durazzi et al., 2021; Hillmann et al., 2018; Jovel et al., 2016; Laudadio et al., 2018). However, for poorly studied hosts living in natural environments, much of the genetic information recovered cannot be classified with currently available databases, as was the case in this study and many others (Gibson et al., 2019; Huang et al., 2018; Riiser et al., 2019; Stewart et al., 2018; Youngblut et al., 2020). This highlights the need for the sequencing and incorporation of more diverse, wild systems in order to access the wealth of novel, microbial functions that currently remain unknown.

In both unassembled reads and assembled MAGs, we identified a creosote-feeding functional core microbiome; however, a majority of the xenobiotic degradation KOs were shared between creosote- and noncreosote-fed woodrats. This result could indicate that enzymes capable of xenobiotic degradation are more pervasive across the woodrat gut microbiota than predicted. These enzymes could be conserved across gut microbiomes, as they often perform other essential services, such as nutrient synthesis. In addition, while not all woodrats consume creosote bush, all woodrats subsist on natural diets that contain PSCs (Kohl, & Dearing, 2016). Therefore, although they are consuming different plant species, these animals may be exposed to similar suites of PSCs. The woodrats in the noncreosote populations feed on plants from the families *Krameriaceae, Fagaceae, Polygonaceae, Ephedraceae*, and *Salicaceae*, all of which can produce phenolics, the predominant class of PSCs in creosote resin (Atsatt & Ingram, 1983; Ayer, Browne, & Kasitu, 1990; Ibragic & Sofić, 2015; Jiménez-Estrada et al., 2013; Julkunen-Tiitto, 1989; Ochmian, Oszmiański, & Skupień, 2009; Schofield, Hagerman, & Harold, 1998). Eating a diet that contains any phenolics may select for similar microbial functions, regardless of phenolic structure or abundance. Further evidence for this notion is that, using our neutral models, we did not see increased selection of the microbiota of creosote feeders compared to that of noncreosote feeders. This result may indicate that all woodrat diets exert selective pressure on gut microbiota for microbes that utilize the particular resources ingested by the host (Weinstein et al., 2021). Additionally, the functional pathways involved in the degradation of PSCs are not well understood. It is possible that many of the same microbial proteins or protein families are involved in metabolizing diverse arrays of PSCs, making them more universally prevalent in hosts consuming toxic diets (Koppel, Rekdal, & Balskus, 2017). Much work has been done to investigate the effect of diet on the structure, composition, and functional profile of the microbiome in domestic herbivores (Cholewińska, Czyż, Nowakowski, & Wyrostek, 2020; Cholewińska, Górniak, & Wojnarowski, 2021; Gruninger, Ribeiro, Cameron, & McAllister, 2019). Comparatively little work

has been done on the interplay of PSCs and gut microbial communities, although nearly all plants defend themselves with a wide array of these toxins (Dearing, Foley, & McLean, 2005; Wink, 2008). Future work could focus on how these toxic compounds affect the microbiome and which microbial enzymes and functional pathways are involved in this process.

In conclusion, this work presents a detailed characterization of the unique taxonomic and functional core microbiome of herbivorous mammals fed the same toxic diet. Our work demonstrates that there are core microbes and microbial functions found only in populations of woodrats consuming this toxic diet that are not present in other woodrat populations of the same species that consume different diets. In addition, our work advances our knowledge of which microbes and microbial functions may be involved in the degradation of these naturally occurring PSCs present in all herbivore diets. Most of the metagenomic data could not be classified, suggesting that much remains unknown about the functional profile of the gut microbiome of wild herbivores and highlighting the need for further studies. Investigating functional pathways and microbes capable of breaking down these PSCs may improve our understanding of the importance of the gut microbiota to an herbivorous host.

Declarations

Ethics approval and consent to participate: Animal use and procedures were approved by the University of Utah IACUC (16-02011). Animal samples were collected under permits from California (SC-8123), Utah (1COLL5194-1, 2), and Nevada (333663) issued to MDD and California Department of Fish and Wildlife permit SC-2105 issued to Jim Patton.

Consent for publication: Not applicable.

Availability of Data and Material: Raw sequence data associated with this project are accessible through the NCBI Sequence Read Archive under BioProject PRJNA875083. All code associated with this work can be found at: https://github.com/tess-stapleton/neotoma-creosote-core

Competing interests: The authors declare that they have no competing interests.

Funding: We thank the Scientific Computing and Imaging Institute at the University of Utah for computing resources. The computing resources on Frontera were provided through an allocation by the Texas Advanced Computing Center PHY20033. We also thank Mission Creek Preserve and Ronald W. Casper's Wilderness Park for housing in the field and collection of samples. This project was funded by NSF DEB-1342615 and NSF IOS 1656497 to MDD and NSF grants 1808652 and 2008772 to HS and was also supported through the NSF IR/D program to MDD. Animal use was approved by the University of Utah Institutional Animal Care and Use Committee (16-02011) and conducted under permits from California (SC-8123), Utah (1COLL5194-1, 2), and Nevada (333663).

Author's Contributions: TS conceived the study, collected all field samples, extracted and prepped all samples for sequencing, analyzed all data, and wrote the manuscript, LL analyzed the metagenomic

data, HS provided funding, and MDD conceived the study, wrote the manuscript, and provided funding.

Acknowledgements: We thank KayLene Yamada, Lane Mulvey, Madeline Nelson, Drs. Dylan Klure, Sara Weinstein, Teri Orr, Jim Patton, Rodolfo Martínez-Mota, and Cecilia Noecker for their assistance in the field, collection of samples, and assistance with data analysis. We also thank Drs. Margaret Doolin, Phyllis Coley, Kevin Kohl, William Brazelton, June Round, and Colin Dale for their comments on the manuscript.

References

1. Ainsworth, T. D., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., … Leggat, W. (2015). The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *The ISME Journal*, *9*, 2261–2274. doi: 10.1038/ismej.2015.39
2. Anderson, C. L., & Fernando, S. C. (2021). Insights into rumen microbial biosynthesis-related gene cluster diversity through genome-resolved metagenomics. *Communications Biology 2021 4:1*, *4*(1), 1–12. doi: 10.1038/s42003-021-02331-7
3. Atsatt, P. R., & Ingram, T. (1983). Adaptation to oak and other fibrous, phenolic-rich foliage by a small mammal, Neotoma fuscipes. *Oecologia*, *60*(1), 135–142. doi: 10.1007/BF00379333
4. Ayer, W., Browne, L., & Kasitu, G. (1990). Metabolites of *Eriogonum umbellatum*. *Planta Medica*, *56*(03), 336–336. doi: 10.1055/s-2006-960977
5. Banerjee, S., Schlaeppi, K., & van der Heijden, M. G. A. (2018). Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology 2018 16:9*, *16*(9), 567–576. doi: 10.1038/s41579-018-0024-1
6. Beltrán, D., Romo-Vaquero, M., Espín, J. C., Tomás-Barberán, F. A., & Selma, M. V. (2018). Ellagibacter isourolithinifaciens gen. Nov., sp. Nov., a new member of the family Eggerthellaceae, isolated from human gut. *International Journal of Systematic and Evolutionary Microbiology*, *68*(5), 1707–1712. doi: 10.1099/IJSEM.0.002735/CITE/REFWORKS
7. Berasategui, A., Salem, H., Paetz, C., Santoro, M., Gershenzon, J., Kaltenpoth, M., & Schmidt, A. (2017). Gut microbiota of the pine weevil degrades conifer diterpenes and increases insect fitness. *Molecular Ecology*, *26*(15), 4099–4110. doi: 10.1111/MEC.14186
8. Bess, E. N., Bisanz, J. E., Yarza, F., Bustion, A., Rich, B. E., Li, X., … Turnbaugh, P. J. (2020, January 1). Genetic basis for the cooperative bioactivation of plant lignans by Eggerthella lenta and other human gut bacteria. *Nature Microbiology*, Vol. 5, pp. 56–66. Nature Research. doi: 10.1038/s41564-019-0596-1
9. Blyton, M. D. J., Soo, R. M., Whisson, D., Marsh, K. J., Pascoe, J., Le Pla, M., … Moore, B. D. (2019). Fecal inoculations alter the gastrointestinal microbiome and allow dietary expansion in a wild specialist herbivore, the koala. *Animal Microbiome 2019 1:1*, *1*(1), 1–18. doi: 10.1186/S42523-019-0008-0

10. Bode, L. M., Bunzel, D., Huch, M., Cho, G. S., Ruhland, D., Bunzel, M., … Kulling, S. E. (2013). In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. *The American Journal of Clinical Nutrition*, *97*(2), 295–309. doi: 10.3945/AJCN.112.049379
11. Boll, M., & Fuchs, G. (1995). Benzoyl-coenzyme A reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism ATP dependence of the reaction, purification and some properties of the enzyme from Thauera aromaticu strain K172. *Eur. J. Biochem*, *234*, 921–933.
12. Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. doi: 10.1038/s41587-019-0209-9
13. Breinig, S., Schiltz, E., & Fuchs, G. (2000). Genes Involved in Anaerobic Metabolism of Phenol in the Bacterium Thauera aromatica. *Journal of Bacteriology*, *182*(20), 5849. doi: 10.1128/JB.182.20.5849-5863.2000
14. Brucker, R., Kolodkin-Gal, I., Andersen, S. B., Kohl, K. D., & Dearing, M. D. (2016). *The Woodrat Gut Microbiota as an Experimental System for Understanding Microbial Metabolism of Dietary Toxins*. doi: 10.3389/fmicb.2016.01165
15. Buchfink, B., Reuter, K., & Drost, H. G. (2021). Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods 2021 18:4*, *18*(4), 366–368. doi: 10.1038/s41592-021-01101-x
16. Burns, A. R., Stephens, W. Z., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., & Bohannan, B. J. M. (2015). Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *The ISME Journal 2016 10:3*, *10*(3), 655–664. doi: 10.1038/ismej.2015.142
17. Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. doi: 10.1038/nmeth.3869
18. Caporaso, J. G., Ackermann, G., Apprill, A., Bauer, M., Berg-Lyons, D., Betley, J., … Weber, L. (2018). Earth microbiome project: EMP 16S Illumina Amplicon Protocol. *Protocols.Io*, 1–7. doi: 10.17504/protocols.io.nuudeww
19. Carleton, M. D. (1973). A survey of gross stomach morphology in New World Cricetinae (Rodentia, Muroidea), with comments on Functional Interpretations. *Miscellaneous Publications Museum of Zoology, University of Michigan*, (146), 1–43.
20. Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2022). GTDB-Tk v2: memory friendly classification with the Genome Taxonomy Database. *BioRxiv*, 2022.07.11.499641. doi: 10.1101/2022.07.11.499641
21. Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultrafast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. doi: 10.1093/BIOINFORMATICS/BTY560
22. Cholewińska, P., Czyż, K., Nowakowski, P., & Wyrostek, A. (2020). *Animal Health Research Reviews The microbiome of the digestive system of ruminants-a review*. doi: 10.1017/S1466252319000069

23. Cholewińska, P., Górniak, W., & Wojnarowski, K. (2021). Impact of selected environmental factors on microbiome of the digestive tract of ruminants. *BMC Veterinary Research*, *17*(1), 1–10. doi: 10.1186/S12917-021-02742-Y/FIGURES/1
24. Colston, T. J., & Jackson, C. R. (2016). Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. *Molecular Ecology*, *25*(16), 3776–3800. doi: 10.1111/MEC.13730
25. Compean, K. L., & Ynalvez, R. A. (2014). Antimicrobial activity of plant secondary metabolites: A review. *Research Journal of Medicinal Plant*, *8*(5), 204–213. doi: 10.3923/RJMP.2014.204.213
26. David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. doi: 10.1038/nature12820
27. Dearing, M. D., Foley, W. J., & McLean, S. (2005). The Influence of Plant Secondary Metabolites on the Nutritional Ecology of Herbivorous Terrestrial Vertebrates. *Http://Dx.Doi.Org/10.1146/Annurev.Ecolsys.36.102003.152617*, *36*, 169–189. doi: 10.1146/ANNUREV.ECOLSYS.36.102003.152617
28. Dearing, M. D., & Weinstein, S. B. (2022). Metabolic Enabling and Detoxification by Mammalian Gut Microbes. doi: 10.1146/ANNUREV-MICRO-111121-085333
29. Dill-Mcfarland, K. A., Weimer, P. J., Pauli, J. N., Peery, M. Z., & Suen, G. (2016). Diet specialization selects for an unusual and simplified gut microbiota in two- and three-toed sloths. *Environmental Microbiology*, *18*(5), 1391–1402. doi: 10.1111/1462-2920.13022
30. Dougal, K., Harris, P. A., Edwards, A., Pachebat, J. A., Blackmore, T. M., Worgan, H. J., & Newbold, C. J. (2012). A comparison of the microbiome and the metabolome of different regions of the equine hindgut. *FEMS Microbiology Ecology*, *82*(3), 642–652. doi: 10.1111/J.1574-6941.2012.01441.X
31. Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., & De Cesare, A. (2021). Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Scientific Reports 2021 11:1*, *11*(1), 1–10. doi: 10.1038/s41598-021-82726-y
32. Fleißner, A., Sopalla, C., & Weltring, K. M. (2002). An ATP-binding cassette multidrug-resistance transporter is necessary for tolerance of Gibberella pulicaris to phytoalexins and virulence on potato tubers. *Molecular Plant–Microbe Interactions*, *15*(2), 102–108. doi: 10.1094/mpmi.2002.15.2.102
33. Freeland, W. J., & Janzen, D. H. (1974). Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. *The American Naturalist*, *108*(961), 269–289. doi: 10.1086/282907
34. Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics*, *28*(23), 3150–3152. doi: 10.1093/BIOINFORMATICS/BTS565
35. Gibson, K. M., Nguyen, B. N., Neumann, L. M., Miller, M., Buss, P., Daniels, S., … Pukazhenthi, B. (2019). Gut microbiome differences between wild and captive black rhinoceros – implications for rhino health. *Scientific Reports 2019 9:1*, *9*(1), 1–11. doi: 10.1038/s41598-019-43875-3
36. Gomez-Arango, L. F., Barrett, H. L., McIntyre, H. D., Callaway, L. K., Morrison, M., & Nitert, M. D. (2017). Contributions of the maternal oral and gut microbiome to placental microbial colonization in

overweight and obese pregnant women. *Scientific Reports 2017 7:1*, *7*(1), 1–10. doi: 10.1038/s41598-017-03066-4

37. Graystock, P., Rehan, S. M., & McFrederick, Q. S. (2017). Hunting for healthy microbiomes: determining the core microbiomes of Ceratina, Megalopta, and Apis bees and how they associate with microbes in bee collected pollen. *Conservation Genetics*, *18*(3), 701–711. doi: 10.1007/s10592-017-0937-7
38. Gruninger, R. J., Ribeiro, G. O., Cameron, A., & McAllister, T. A. (2019). Invited review: Application of meta-omics to understand the dynamic nature of the rumen microbiome and how it responds to diet in ruminants. *Animal*, *13*(9), 1843–1854. doi: 10.1017/S1751731119000752
39. Guzmán-Beltrán, S., Rubio-Badillo, M. Á., Juárez, E., Hernández-Sánchez, F., & Torres, M. (2016). Nordihydroguaiaretic acid (NDGA) and α-mangostin inhibit the growth of Mycobacterium tuberculosis by inducing autophagy. *International Immunopharmacology*, *31*, 149–157. doi: 10.1016/j.intimp.2015.12.027
40. Haiser, H. J., Gootenberg, D. B., Chatman, K., Sirasani, G., Balskus, E. P., & Turnbaugh, P. J. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium Eggerthella lenta. *Science*, *341*(6143), 295–298. doi: 10.1126/SCIENCE.1235872/SUPPL_FILE/1235872.HAISER.SM.PDF
41. Hammer, T. J., & Bowers, M. D. (2015). Gut microbes may facilitate insect herbivory of chemically defended plants. *Oecologia 2015 179:1*, *179*(1), 1–14. doi: 10.1007/S00442-015-3327-1
42. Hammer, T. J., Janzen, D. H., Hallwachs, W., Jaffe, S. P., & Fierer, N. (2017). Caterpillars lack a resident gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(36), 9641–9646. doi: 10.1073/PNAS.1707186114/SUPPL_FILE/PNAS.1707186114.SAPP.PDF
43. Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Janssen, P. H., … Zunino, P. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, *5*, 14567. doi: 10.1038/srep14567
44. Hillmann, B., Al-Ghalith, G. A., Shields-Cutler, R. R., Zhu, Q., Gohl, D. M., Beckman, K. B., … Knights, D. (2018). Evaluating the Information Content of Shallow Shotgun Metagenomics. *MSystems*, *3*(6). doi: 10.1128/msystems.00069-18
45. Hobbs, M. E., Malashkevich, V., Williams, H. J., Xu, C., Sauder, J. M., Burley, S. K., … Raushel, F. M. (2012). Structure and Catalytic Mechanism of LigI: Insight into the Amidohydrolase Enzymes of cog3618 and Lignin Degradation. *Biochemistry*, *51*(16), 3497. doi: 10.1021/BI300307B
46. Huang, P., Zhang, Y., Xiao, K., Jiang, F., Wang, H., Tang, D., … Zeng, J. (2018). The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids 06 Biological Sciences 0605 Microbiology. *Microbiome*, *6*(1), 1DUMY. doi: 10.1186/S40168-018-0590-5/FIGURES/5
47. Huson, D. H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., … Tappu, R. (2016). MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. *PLOS Computational Biology*, *12*(6), e1004957. doi: 10.1371/JOURNAL.PCBI.1004957

48. Ibragic, S., & Sofić, E. (2015). Chemical composition of various ephedra species. *Bosnian Journal of Basic Medical Sciences*, *15*(3), 21–27. doi: 10.17305/bjbms.2015.539

49. Ingala, M. R., Simmons, N. B., Wultsch, C., Krampis, K., Speer, K. A., & Perkins, S. L. (2018). Comparing microbiome sampling methods in a wild mammal: Fecal and intestinal samples record different signals of host ecology, evolution. *Frontiers in Microbiology*, *9*(MAY), 803. doi: 10.3389/FMICB.2018.00803/BIBTEX

50. Itoh, H., Tago, K., Hayatsu, M., & Kikuchi, Y. (2018). Detoxifying symbiosis: microbe-mediated detoxification of phytotoxins and pesticides in insects. *Natural Product Reports*, *35*(5), 434–454. doi: 10.1039/C7NP00051K

51. Jiménez-Estrada, M., Velázquez-Contreras, C., Garibay-Escobar, A., Sierras-Canchola, D., Lapizco-Vázquez, R., Ortiz-Sandoval, C., … Robles-Zepeda, R. E. (2013). In vitro antioxidant and antiproliferative activities of plants of the ethnopharmacopeia from northwest of Mexico. *BMC Complementary and Alternative Medicine*, *13*(1), 1–8. doi: 10.1186/1472-6882-13-12

52. John Wallace, R., Sasson, G., Garnsworthy, P. C., Tapio, I., Gregson, E., Bani, P., … Mizrahi, I. (2019). A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. *Science Advances*, *5*(7), 8391–8394. doi: 10.1126/SCIADV.AAV8391/SUPPL_FILE/AAV8391_SM.PDF

53. Jones, J., DiBattista, J. D., Stat, M., Bunce, M., Boyce, M. C., Fairclough, D. V., … Huggett, M. J. (2018). The microbiome of the gastrointestinal tract of a range-shifting marine herbivorous fish. *Frontiers in Microbiology*, *9*(AUG), 2000. doi: 10.3389/FMICB.2018.02000/BIBTEX

54. Jonsson, V., Österlund, T., Nerman, O., & Kristiansson, E. (2016). Statistical evaluation of methods for identification of differentially abundant genes in comparative metagenomics. *BMC Genomics*, *17*(1), 1–14. doi: 10.1186/S12864-016-2386-Y/FIGURES/5

55. Jorge, F., Dheilly, N. M., & Poulin, R. (2020). Persistence of a Core Microbiome Through the Ontogeny of a Multi-Host Parasite. *Frontiers in Microbiology*, *11*, 954. doi: 10.3389/FMICB.2020.00954/BIBTEX

56. Jovel, J., Patterson, J., Wang, W., Hotte, N., O'Keefe, S., Mitchel, T., … Wong, G. K. S. (2016). Characterization of the gut microbiome using 16S or shotgun metagenomics. *Frontiers in Microbiology*, *7*(APR), 459. doi: 10.3389/FMICB.2016.00459/BIBTEX

57. Julkunen-Tiitto, R. (1989). Phenolic constituents of Salix: A chemotaxonomic survey of further finnish species. *Phytochemistry*, *28*(8), 2115–2125. doi: 10.1016/S0031-9422(00)97930-5

58. Kamada, N., Chen, G. Y., Inohara, N., & Núñez, G. (2013). Control of pathogens and pathobionts by the gut microbiota. *Nature Immunology 2013 14:7*, *14*(7), 685–690. doi: 10.1038/ni.2608

59. Kanehisa, M., Sato, Y., & Kawashima, M. (2022). KEGG mapping tools for uncovering hidden features in biological data. *Protein Science*, *31*(1), 47–53. doi: 10.1002/PRO.4172

60. Karasov, W. H., & Martínez del Rio, C. (2008). *Physiological ecology: how animals process energy, nutrients, and toxins* (Vol. 45). Princeton University Press. doi: 10.5860/choice.45-4381

61. Knight, R. (2015). Dietary effects on human gut microbiome diversity. *The British Journal of Nutrition*, Vol. 113, pp. S1–S5. doi: 10.1017/S0007114514004127

62. Kohl, K. D., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J. C., … Bordenstein, S. R. (2017). Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. *Molecular Ecology*, *26*(4), 1175–1189. doi: 10.1111/MEC.13921
63. Kohl, K. D., & Dearing, M. D. (2012). Experience matters: Prior exposure to plant toxins enhances diversity of gut microbes in herbivores. *Ecology Letters*, *15*(9), 1008–1015. doi: 10.1111/j.1461-0248.2012.01822.x
64. Kohl, K. D., & Dearing, M. D. (2016). The woodrat gut microbiota as an experimental system for understanding microbial metabolism of dietary toxins. *Frontiers in Microbiology*, Vol. 7, p. 1165. Frontiers Media S.A. doi: 10.3389/fmicb.2016.01165
65. Kohl, K. D., Luong, K., & Dearing, M. D. (2015). Validating the use of trap-collected feces for studying the gut microbiota of a small mammal (Neotoma lepida). *Journal of Mammalogy*, *96*(1), 90–93. doi: 10.1093/jmammal/gyu008
66. Kohl, K. D., Miller, A. W., Marvin, J. E., Mackie, R., & Dearing, M. D. (2014). Herbivorous rodents (Neotoma spp.) harbor abundant and active foregut microbiota. *Environmental Microbiology*, *16*(9), 2869–2878. doi: 10.1111/1462-2920.12376
67. Kohl, K. D., Stengel, A., & Dearing, M. D. (2016). Inoculation of tannin-degrading bacteria into novel hosts increases performance on tannin-rich diets. *Environmental Microbiology*, *18*(6), 1720–1729. doi: 10.1111/1462-2920.12841
68. Kohl, K. D., Weiss, R. B., Cox, J., Dale, C., & Dearing, M. D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. *Ecology Letters*, *17*(10), 1238–1246. doi: 10.1111/ele.12329
69. Koppel, N., Bisanz, J. E., Pandelia, M. E., Turnbaugh, P. J., & Balskus, E. P. (2018). Discovery and characterization of a prevalent human gut bacterial enzyme sufficient for the inactivation of a family of plant toxins. *ELife*, *7*. doi: 10.7554/eLife.33953
70. Koppel, N., Rekdal, V. M., & Balskus, E. P. (2017). Chemical transformation of xenobiotics by the human gut microbiota. *Science*, *356*(6344), 1246–1257. doi: 10.1126/SCIENCE.AAG2770/ASSET/7F3BFA3D-4441-425F-A5E7-A8C2BB8E8AEF/ASSETS/GRAPHIC/356_AAG2770_F4.JPEG
71. Kyselka, J., Rabiej, D., Dragoun, M., Kreps, F., Burčová, Z., Němečková, I., … Filip, V. (2017). Antioxidant and antimicrobial activity of linseed lignans and phenolic acids. *European Food Research and Technology 2017 243:9*, *243*(9), 1633–1644. doi: 10.1007/S00217-017-2871-9
72. Laudadio, I., Fulci, V., Palone, F., Stronati, L., Cucchiara, S., & Carissimi, C. (2018). Quantitative Assessment of Shotgun Metagenomics and 16S rDNA Amplicon Sequencing in the Study of Human Gut Microbiome. *OMICS A Journal of Integrative Biology*, *22*(4), 248–254. doi: 10.1089/omi.2018.0013
73. Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., … Gordon, J. I. (2008). Beltrán, Romo-VaquerBeltrán, Romo-Vaquer. *Science*, *320*(5883), 1647–1651. doi:

10.1126/SCIENCE.1155725/SUPPL_FILE/LEY.SOM.PDF

74. Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics (Oxford, England)*, *25*(14), 1754–1760. doi: 10.1093/BIOINFORMATICS/BTP324
75. Lloyd-Price, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A. B., … Huttenhower, C. (2017). Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature 2017 550:7674*, *550*(7674), 61–66. doi: 10.1038/nature23889
76. Love, M. I., 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
77. Malenke, J. R., Skopec, M. M., & Dearing, M. D. (2014). Evidence for functional convergence in genes upregulated by herbivores ingesting plant secondary compounds. *BMC Ecology*, *14*(1), 1–16. doi: 10.1186/1472-6785-14-23/TABLES/6
78. Mangione, A. M., Dearing, M. D., & Karasov, W. H. (2004). Creosote bush (Larrea tridentata) resin increases water demands and reduces energy availability in desert woodrats (Neotoma lepida). *Journal of Chemical Ecology*, *30*(7), 1409–1429. doi: 10.1023/B:JOEC.0000037748.19927.a1
79. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. doi: 10.14806/ej.17.1.200
80. Mendes, L. W., Kuramae, E. E., Navarrete, A. A., Van Veen, J. A., & Tsai, S. M. (2014). Taxonomical and functional microbial community selection in soybean rhizosphere. *The ISME Journal*, *8*, 1577–1587. doi: 10.1038/ismej.2014.17
81. Meyer, M. W., & Karasov, W. H. (1989). Antiherbivore chemistry of Larrea tridentata: effects on woodrat (*Neotoma lepida*) feeding and nutrition. *Ecology*, *70*(4), 953–961. doi: 10.2307/1941362
82. Miller, A. W., Oakeson, K. F., Dale, C., & Dearing, M. D. (2016). Microbial Community Transplant Results in Increased and Long-Term Oxalate Degradation. *Microbial Ecology*, *72*(2), 470–478. doi: 10.1007/s00248-016-0800-2
83. Moran, N. A., Ochman, H., & Hammer, T. J. (2019). Evolutionary and ecological consequences of gut microbial communities. *Annual Review of Ecology, Evolution, and Systematics*, *50*(1), 451–475. doi: 10.1146/ANNUREV-ECOLSYS-110617-062453
84. Naqib, A., Poggi, S., Wang, W., Hyde, M., Kunstman, K., & Green, S. J. (2018). Making and sequencing heavily multiplexed, high-throughput 16S ribosomal RNA gene amplicon libraries using a flexible, two-stage PCR protocol. *Methods in Molecular Biology*, *1783*, 149–169. doi: 10.1007/978-1-4939-7834-2_7/FIGURES/4
85. Neu, A. T., Allen, E. E., & Roy, K. (2021). Defining and quantifying the core microbiome: Challenges and prospects. *Proceedings of the National Academy of Sciences*, *118*(51), e2104429118. doi: 10.1073/PNAS.2104429118/-/DCSUPPLEMENTAL
86. Ochmian, I., Oszmiański, J., & Skupień, K. (2009). Chemical composition, phenolics, and firmness of small black fruits. *Journal of Applied Botany and Food Quality*, *83*(1), 64–69. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20103040866

87. Olm, M. R., Brown, C. T., Brooks, B., & Banfield, J. F. (2017). dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through dereplication. *The ISME Journal 2017 11:12*, *11*(12), 2864–2868. doi: 10.1038/ismej.2017.126

88. Pardesi, B., Roberton, A. M., Lee, K. C., Angert, E. R., Rosendale, D. I., Boycheva, S., … Clements, K. D. (2022). Distinct microbiota composition and fermentation products indicate functional compartmentalization in the hindgut of a marine herbivorous fish. *Molecular Ecology*, *00*, 1–16. doi: 10.1111/MEC.16394

89. Patton, A., Huckaby, J. L., Álvarez-Castañeda, D. G., & Ticul, S. (2014). *The Evolutionary History and a Systematic Revision of Woodrats of the Neotoma lepida Group*. Berkley, California: University of California Press.

90. Pertea, M., & Pertea, G. (2020). GFF Utilities: GffRead and GffCompare. *F1000Research*, *9*, 304. doi: 10.12688/F1000RESEARCH.23297.1

91. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1). doi: 10.1093/nar/gks1219

92. Ramakodi, M. P. (2021). Effect of Amplicon Sequencing Depth in Environmental Microbiome Research. *Current Microbiology*, *78*(3), 1026–1033. doi: 10.1007/S00284-021-02345-8

93. Reyman, M., van Houten, M. A., Arp, K., Sanders, E. A. M., & Bogaert, D. (2019). Rectal swabs are a reliable proxy for fecal samples in infant gut microbiota research based on 16S-rRNA sequencing. *Scientific Reports 2019 9:1*, *9*(1), 1–8. doi: 10.1038/s41598-019-52549-z

94. Riiser, E. S., Haverkamp, T. H. A., Varadharajan, S., Borgan, Ø., Jakobsen, K. S., Jentoft, S., & Star, B. (2019). Switching on the light: using metagenomic shotgun sequencing to characterize the intestinal microbiome of Atlantic cod. *Environmental Microbiology*, *21*(7), 2576–2594. doi: 10.1111/1462-2920.14652

95. Ríos, J. M., Mangione, A. M., & Gianello, J. C. (2008). Effects of natural phenolic compounds from a desert dominant shrub Larrea divaricata Cav. on toxicity and survival in mice. *Revista Chilena de Historia Natural*, *81*(2), 292–302. doi: 10.4067/S0716-078X2008000200011

96. Risely, A. (2020, July 1). Applying the core microbiome to understand host–microbe systems. *Journal of Animal Ecology*, Vol. 89, pp. 1549–1558. John Wiley & Sons, Ltd. doi: 10.1111/1365-2656.13229

97. Risely, A., Gillingham, M. A. F., Béchet, A., Brändel, S., Heni, A. C., Heurich, M., … Sommer, S. (2021). Phylogeny- and Abundance-Based Metrics Allow for the Consistent Comparison of Core Gut Microbiome Diversity Indices Across Host Species. *Frontiers in Microbiology*, *12*. doi: 10.3389/FMICB.2021.659918/FULL

98. Schmutz, E. M., Mabry, T. J., Hunziker, J. H., & Difeo, D. R. (1978). Creosote Bush. Biology and Chemistry of Larrea in New World Deserts. *Journal of Range Management*, *31*(5), 399. doi: 10.2307/3897371

99. Schofield, J. A., Hagerman, A. E., & Harold, A. (1998). Loss of Tannins and Other Phenolics from Willow Leaf Litter. *Journal of Chemical Ecology 1998 24:8*, *24*(8), 1409–1421. doi: 10.1023/A:1021287018787
100. Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology*, *14*(1), 4–12. doi: 10.1111/J.1462-2920.2011.02585.X
101. Simonin, M., Dasilva, C., Terzi, V., Ngonkeu, E. L. M., Diouf, Di., Kane, A., … Moulin, L. (2020). Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidence for a core microbiome across eight African and European soils. *FEMS Microbiology Ecology*, *96*(6). doi: 10.1093/FEMSEC/FIAA067
102. Stapleton, T. E., Kohl, K. D., & Denise Dearing, M. (2022). Plant secondary compound- and antibiotic-induced community disturbances improve the establishment of foreign gut microbiota. *FEMS Microbiology Ecology*, *98*(1), 1–13. doi: 10.1093/FEMSEC/FIAC005
103. Stapleton, T. E., Weinstein, S. B., Greenhalgh, R., & Dearing, M. D. (2022). Successes and limitations of quantitative diet metabarcoding in a small, herbivorous mammal. *Molecular Ecology Resources*. doi: 10.1111/1755-0998.13643
104. Stewart, R. D., Auffret, M. D., Warr, A., Wiser, A. H., Press, M. O., Langford, K. W., … Watson, M. (2018). Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nature Communications 2018 9:1*, *9*(1), 1–11. doi: 10.1038/s41467-018-03317-6
105. Sullam, K. E., Rubin, B. E. R., Dalton, C. M., Kilham, S. S., Flecker, A. S., & Russell, J. A. (2015). Divergence across diet, time and populations rules out parallel evolution in the gut microbiomes of Trinidadian guppies. *The ISME Journal 2015 9:7*, *9*(7), 1508–1522. doi: 10.1038/ismej.2014.231
106. Suzek, B. E., Wang, Y., Huang, H., McGarvey, P. B., & Wu, C. H. (2015). UniRef clusters: a comprehensive and scalable alternative for improving sequence similarity searches. *Bioinformatics*, *31*(6), 926–932. doi: 10.1093/BIOINFORMATICS/BTU739
107. Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., … Willerslev, E. (2007). Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research*, *35*(3), e14. doi: 10.1093/NAR/GKL938
108. Tamames, J., Cobo-Simón, M., & Puente-Sánchez, F. (2019). Assessing the performance of different approaches for functional and taxonomic annotation of metagenomes. *BMC Genomics*, *20*(1), 1–16. doi: 10.1186/S12864-019-6289-6/FIGURES/10
109. Tang, Q., Jin, G., Wang, G., Liu, T., Liu, X., Wang, B., & Cao, H. (2020). Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. *Frontiers in Cellular and Infection Microbiology*, *10*, 151. doi: 10.3389/FCIMB.2020.00151/BIBTEX
110. Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature*, *449*(7164), 804. doi: 10.1038/NATURE06244
111. Weinstein, S. B., Martinez-Mota, R., Stapleton, T. E., Klure, D. M., Greenhalgh, R., Orr, T. J., … Denise Dearing, M. (2021). Microbiome stability and structure is governed by host phylogeny over diet and

geography in woodrats (Neotoma spp.). *Proceedings of the National Academy of Sciences of the United States of America*, *118*(47). doi: 10.1073/pnas.2108787118

112. Wink, M. (2008). Plant secondary metabolism: Diversity, function and its evolution. *Natural Product Communications*, Vol. 3, pp. 1205–1216. doi: 10.1177/1934578x0800300801
113. Yan, W., Sun, C., Zheng, J., Wen, C., Ji, C., Zhang, D., … Yang, N. (2019). Efficacy of Fecal Sampling as a Gut Proxy in the Study of Chicken Gut Microbiota. *Frontiers in Microbiology*, *10*, 2126. doi: 10.3389/FMICB.2019.02126/BIBTEX
114. Youngblut, N. D., de la Cuesta-Zuluaga, J., Reischer, G. H., Dauser, S., Schuster, N., Walzer, C., … Ley, R. E. (2020). Large-Scale Metagenome Assembly Reveals Novel Animal-Associated Microbial Genomes, Biosynthesis-related Gene Clusters, and Other Genetic Diversity. *MSystems*, *5*(6). doi: 10.1128/MSYSTEMS.01045-20
115. Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., … Farnleitner, A. H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications 2019 10:1*, *10*(1), 1–15. doi: 10.1038/s41467-019-10191-3
116. Zwiers, L. H., Stergiopoulos, I., Gielkens, M. M. C., Goodall, S. D., & De Waard, M. A. (2003). ABC transporters of the wheat pathogen Mycosphaerella graminicola function as protectants against biotic and xenobiotic toxic compounds. *Molecular Genetics and Genomics*, *269*(4), 499–507. doi: 10.1007/s00438-003-0855-x

Figures

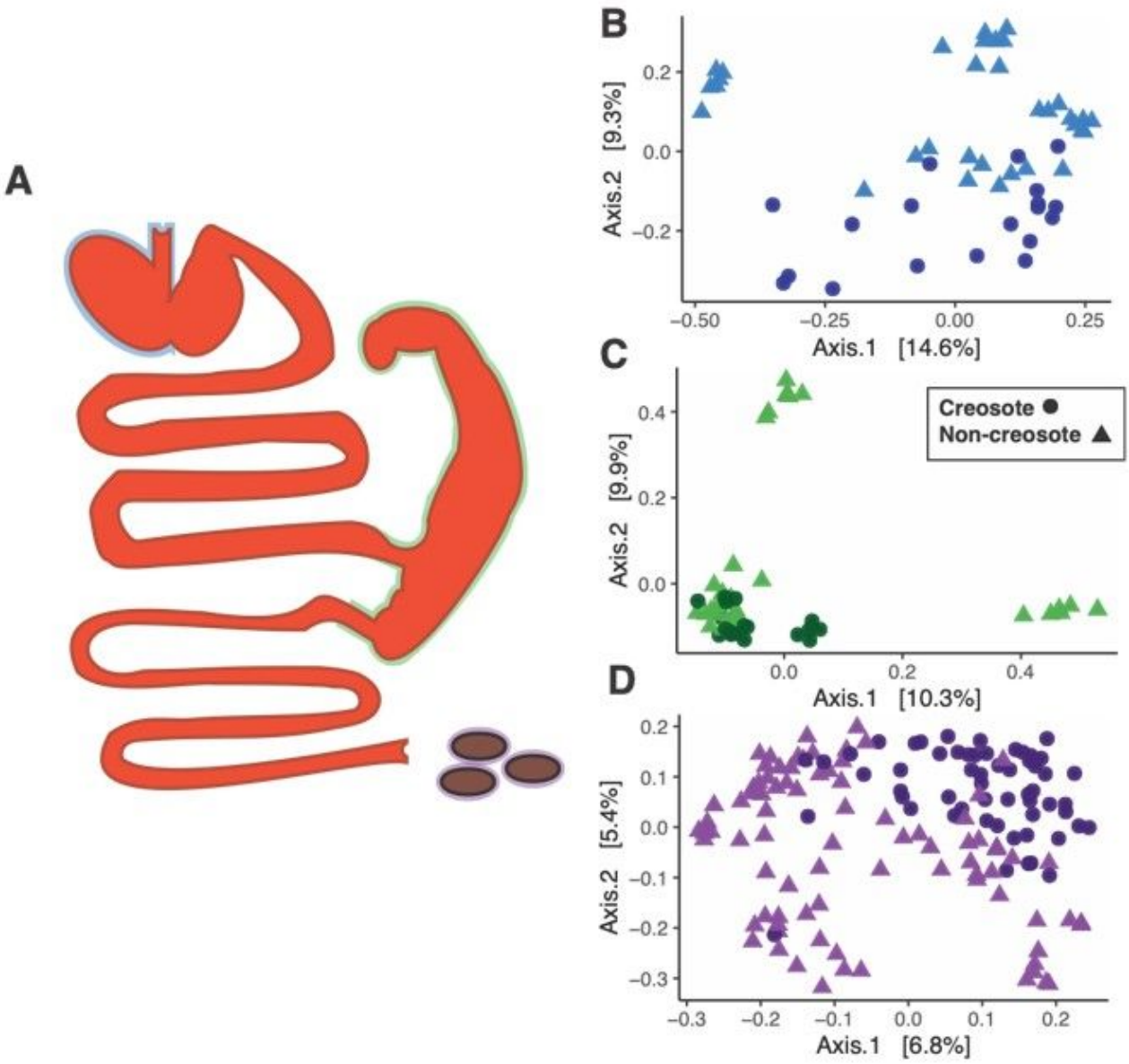

Figure 1

Gut microbial community structure and membership between creosote and non-creosote feeding woodrat populations. (A) Diagram of the gastrointestinal tract of woodrats, highlighted segments represent gut regions we sampled: the foregut (blue), cecum (green), and hindgut (purple). Principle coordinate analysis of Bray-Curtis distances between creosote and non-creosote feeders for the foregut (B), cecum (C), and hindgut (D); there were significant differences between creosote and non-creosote feeders across all gut regions (statistics reported in the text).

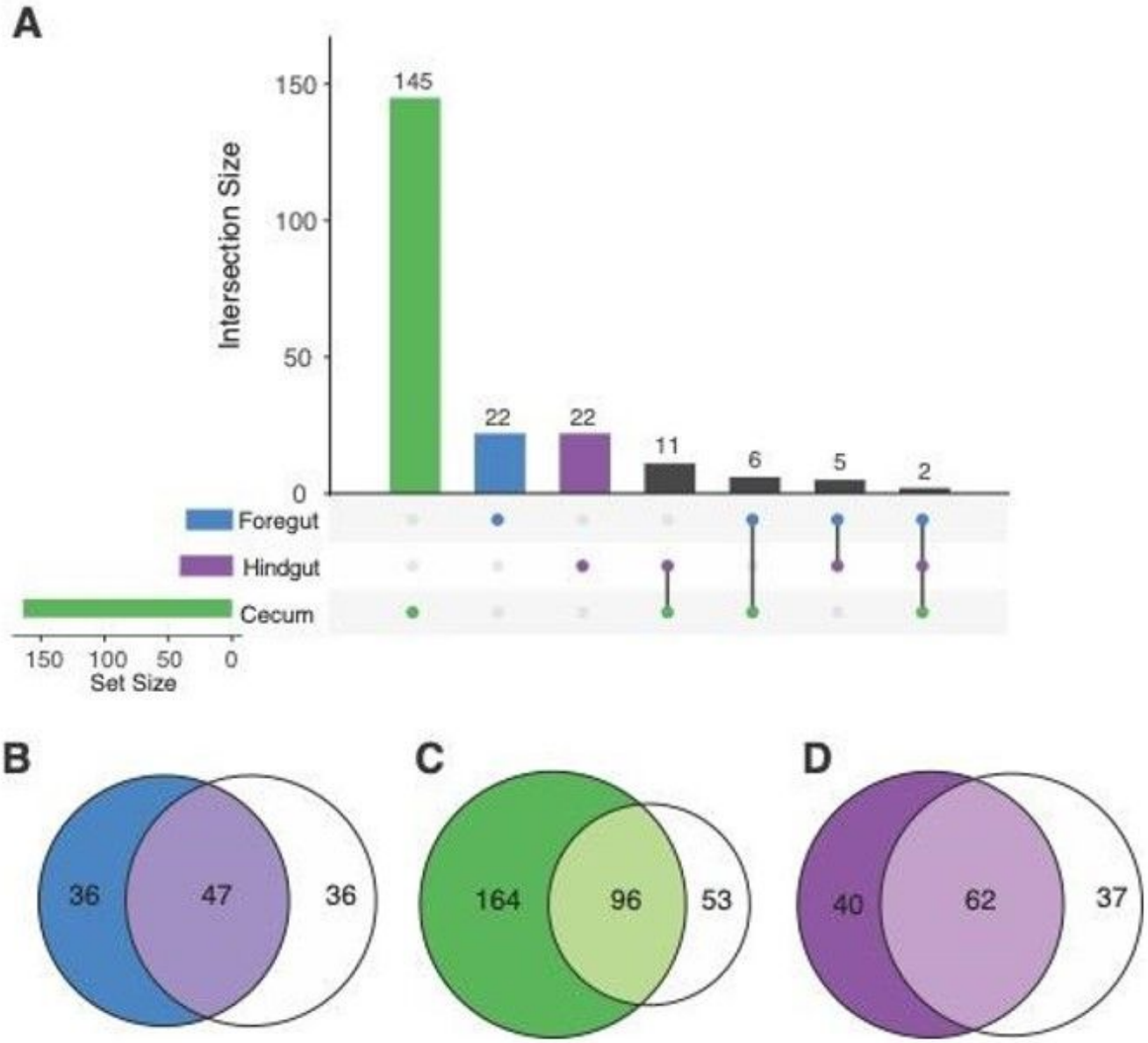

Figure 2

Gut regions harbored distinct core microbiotas. (A) UpSet diagrams of core microbes shared between all gut regions. Few core microbes were shared across gut regions. Euler diagrams of core microbes that were unique to creosote feeders (left), unique to non-creosote feeders (right), and shared by all populations (center) in the foregut (B), cecum (C), and hindgut (D).

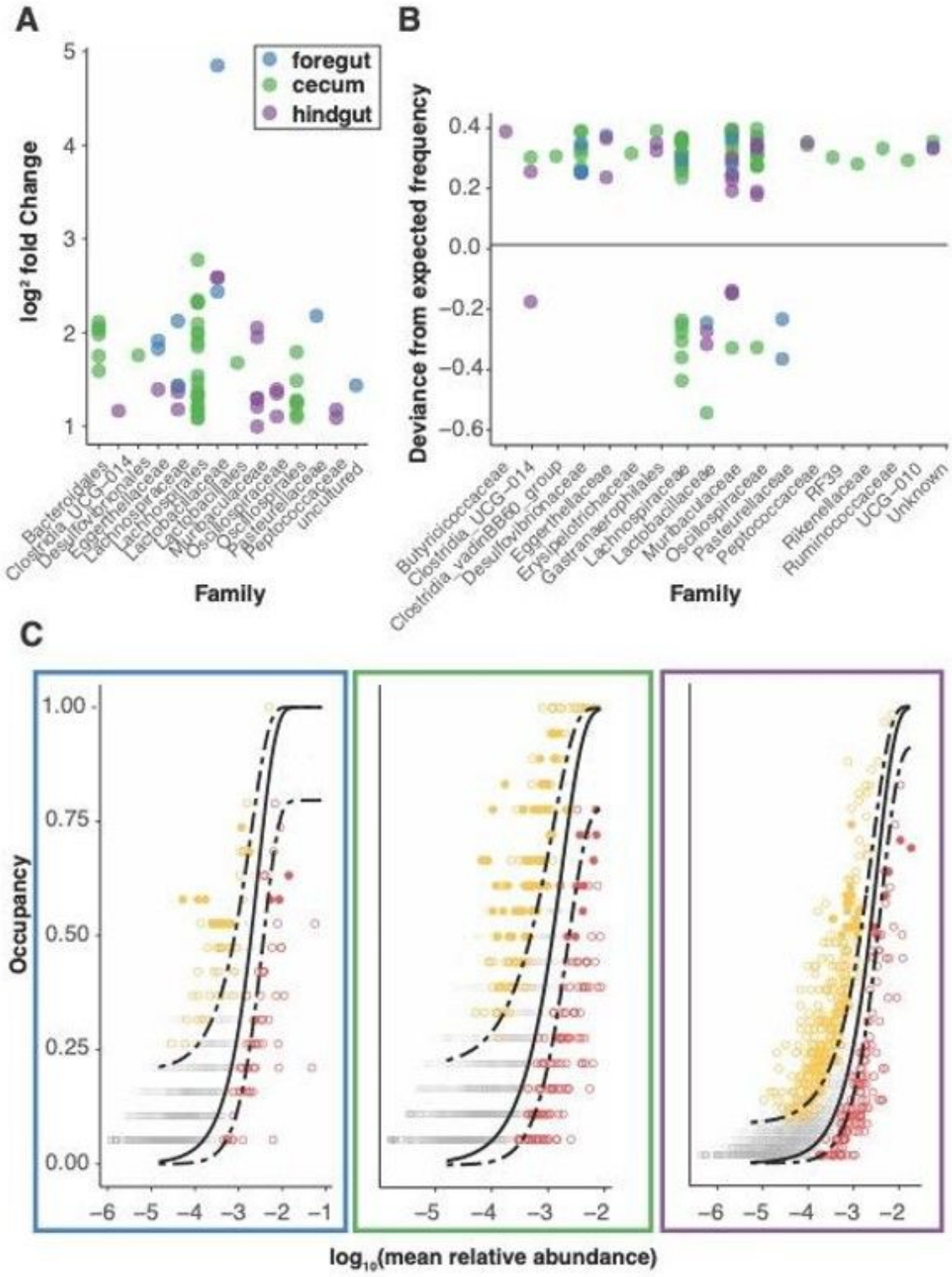

Figure 3

Core microbes enriched and selected for in the foregut, cecum, and hindgut of creosote feeders. (A) Log2 fold enrichment of core microbes in creosote feeders compared to non-creosote feeders. (B) Deviance of core ASVs from neutral model fit. Overrepresented ASVs appear above the line and underrepresented ASVs beneath the line. Points are colored by gut region. (C) Fit of the prokaryotic neutral model to all ASVs in the foregut (blue outline), cecum (green outline), and hindgut (purple outline). Closed circles

represent members of the core microbiome, open circles represent non-core ASVs. ASVs are classified as overrepresented (yellow), underrepresented (red), or neutral (gray). The solid line represents the predicted frequency of occurrence and the dashed line is 95% confidence intervals.

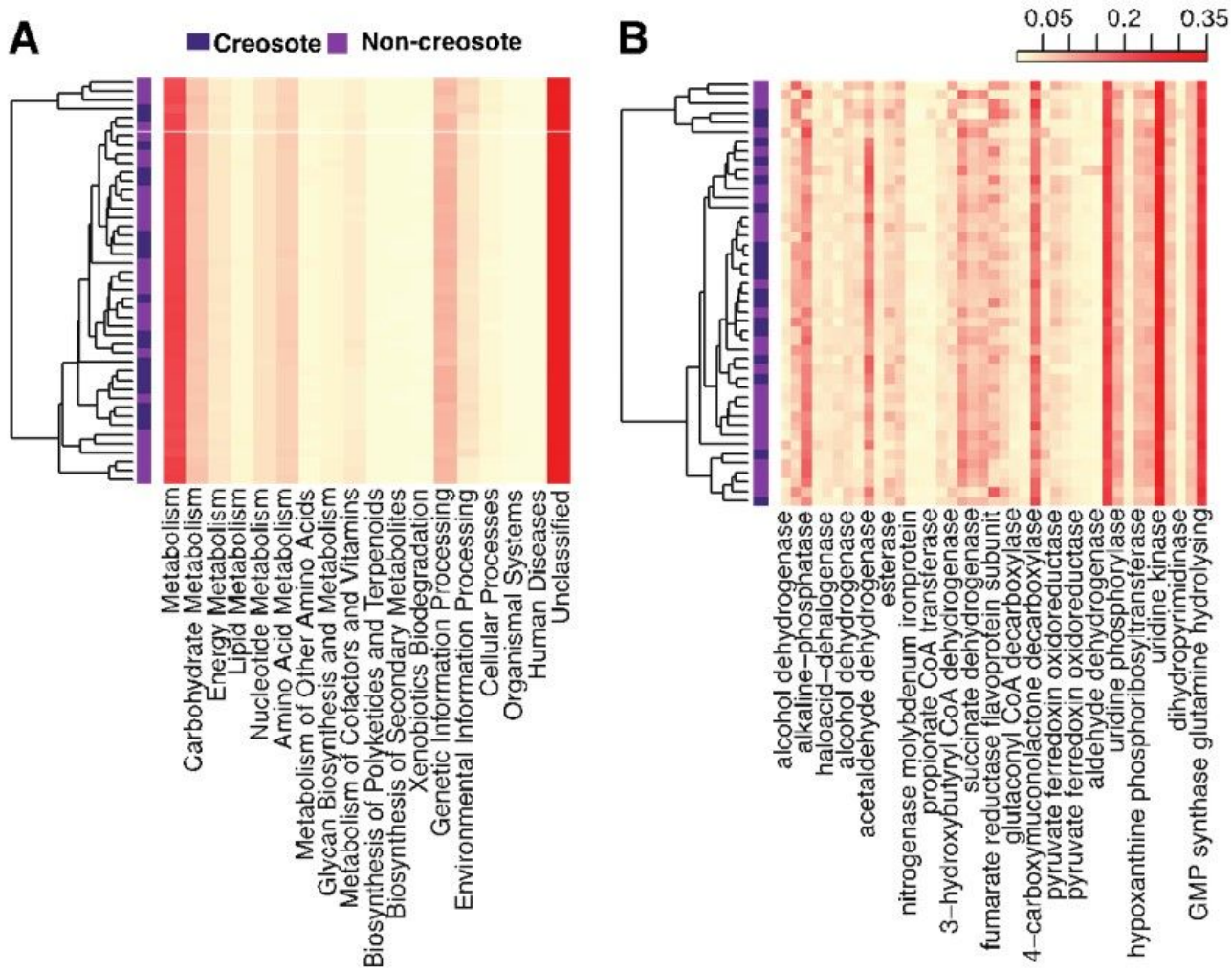

Figure 4

There was no difference in the functional profiles of gut microbial communities in creosote and non-creosote feeding woodrats using unassembled reads. Heatmaps of relative abundances of the most abundant KEGG pathways (A) and the most abundant KEGG-assigned xenobiotic degradation proteins (B) in both creosote and non-creosote feeding animals. The dendrogram left of each heatmap represents hierarchical clustering of creosote (dark purple) and non-creosote (light purple) individuals based on Bray-Curtis dissimilarity of KEGG pathway or xenobiotic degradation protein abundance counts. See Tables S11 and S12 for full relative abundance information.

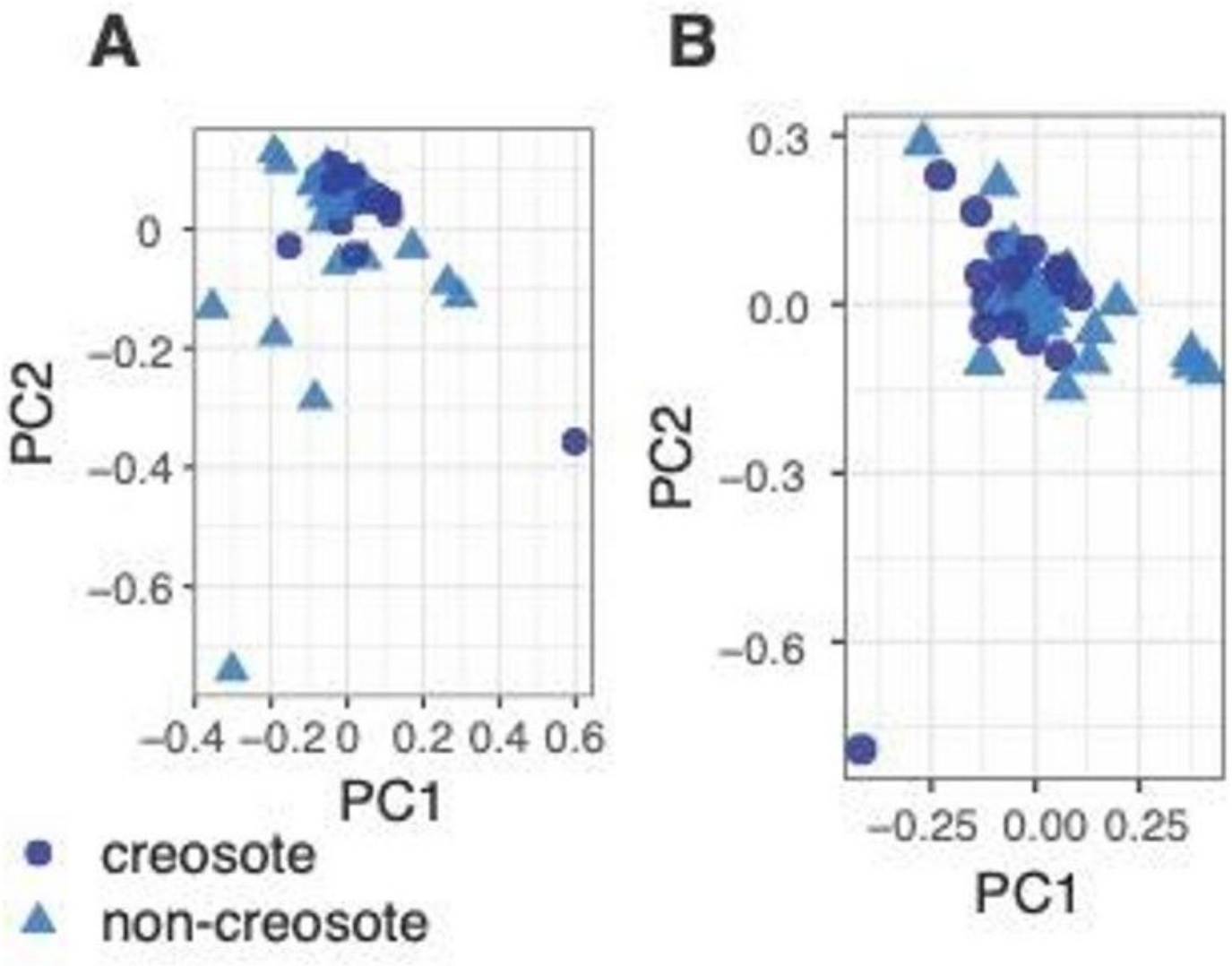

Figure 5

There was large overlap of gut microbiome functional profiles between creosote and non-creosote feeders. Principal component analysis of Bray-Curtis distances generated from KO counts at the protein level for (A) unassembled reads and (B) assembled MAGs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalMaterialCorePaper.xlsx