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LETTER

Experience matters: prior exposure to plant toxins enhances diversity of gut microbes in herbivores

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Abstract

For decades, ecologists have hypothesised that exposure to plant secondary compounds (PSCs) modifies herbivore-associated microbial community composition. This notion has not been critically evaluated in wild mammalian herbivores on evolutionary timescales. We investigated responses of the microbial communities of two woodrat species (*Neotoma bryanti* and *N. lepida*). For each species, we compared experienced populations that independently converged to feed on the same toxic plant (creosote bush, *Larrea tridentata*) to naïve populations with no exposure to creosote toxins. The addition of dietary PSCs significantly altered gut microbial community structure, and the response was dependent on previous experience. Microbial diversity and relative abundances of several dominant phyla increased in experienced woodrats in response to PSCs; however, opposite effects were observed in naïve woodrats. These differential responses were convergent in experienced populations of both species. We hypothesise that adaptation of the foregut microbiota to creosote PSCs in experienced woodrats drives this differential response.

Keywords

Community structure, gut microbiota, mammalian herbivores, *Neotoma*, symbiotic microbes.

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INTRODUCTION

Mammalian herbivores are repeatedly faced with the challenge of consuming food sources that are low in nitrogen and high in indigestible material, such as fibre. To overcome these challenges, herbivores maintain communities of symbiotic microbes that increase the nutritive quality of foliage through the synthesis of essential amino acids or fermentation of fibre (Muegge *et al.* 2011). Herbivores maintain microbial communities that differ drastically from other mammals in terms of both composition and function, including an increased abundance of species with genes associated with the above processes (Ley *et al.* 2008; Muegge *et al.* 2011). However, plants also produce a wide array of plant secondary compounds (PSCs) that discourage or reduce consumption by altering the homeostasis of the herbivore (Dearing *et al.* 2005). In addition, PSCs, and not nutrient constraints, may be more important in limiting the ability of mammalian herbivores to specialise on certain plant species (Dearing *et al.* 2000).

Nearly four decades ago, Freeland and Janzen predicted that a mammalian herbivore's exposure to PSCs sculpted its gut microbial community (Freeland & Janzen 1974). The intense selective pressure of these potentially lethal and antibacterial compounds on herbivore hosts and their microbiota should favour a certain composition of microbes associated with detoxification and tolerance (Barboza *et al.* 2010). Work on herbivorous insects lends support to this hypothesis (Kelley & Dobler 2011). However, to date, only two studies have addressed this issue in mammalian herbivores (Jones & Megarrity 1986; Sundset *et al.* 2010). These studies focused on particular species of microbes or individual PSCs, and not alterations in total community structure with respect to complex mixtures of PSCs. The hypothesis that herbivore experience with PSCs influences mammalian gut microbial communities has not yet been critically evaluated using both experimental and comparative approaches.

To address this gap in our understanding of the effects of PSCs on gut microflora, we investigated the gut microbial communities from populations of two species of woodrats, Bryant's woodrat (*Neotoma bryanti*) and the desert woodrat (*N. lepida*). Both of these species have independently converged on the same PSC-rich diet of creosote bush, (*Larrea tridentata*). The dietary strategies and evolutionary histories of woodrats have been well documented, particularly for *N. bryanti* and *N. lepida* (Atsatt & Ingram 1983; Dial 1988; Patton *et al.* 2007). These two species diverged roughly 1.6 million years ago; their diets at the time of speciation are unknown (Patton *et al.* 2007). However, both species underwent a radical dietary shift 17 000 years ago when creosote naturally invaded their habitat (Hunter *et al.* 2001). Juniper (*Juniperus spp.*) had been the predominant shrub in the area for at least the past 40 000 years, but was extirpated by natural changes in climate (Van Devender & Spaulding 1979; Hunter *et al.* 2001). The leaves of creosote bush are covered in a phenolic-rich resin that can comprise 10–25% of the dry mass (Mabry *et al.* 1977). Creosote resin is a complex mixture of hundreds of chemical products, including phenolics, O-methylated flavones and flavonols, catechols, vinyl ketones and saponins (Mabry *et al.* 1977). The majority of this resin is composed of nordihydroguaiaretic acid, a phenolic compound that causes kidney cysts in lab rats (Goodman *et al.* 1970). Due to creosote's limited range, there are populations of woodrats that have never experienced creosote bush ('naïve populations'), as well as those that have up to 17 000 years of ecological experience with creosote ('experienced populations'). Experienced populations consume roughly 75% creosote bush in the wild (Karasov 1989), and are able to consume 25% more creosote resin in the laboratory compared with naïve populations (Mangione *et al.* 2000). Currently, naïve populations of *N. bryanti* feed primarily on cactus (*Opuntia occidentalis)* and sage *(Salvia spp.)*, which produce low-molecular weight PSCs such as oxalates or diterpenes, and thus have different plant chemistry profiles compared with creosote bush (Atsatt & Ingram 1983; Stintzing

& Carle 2005; Abreu *et al.* 2008). Naïve *N. lepida* persist in the ancestral habitat of juniper woodlands, which contain monoterpenes, phenolics and condensed tannins (Adams *et al.* 1981; Utsumi *et al.* 2009). Thus, populations of both species feed on diets with unique PSC profiles.

In addition, the gut morphology of woodrats makes them ideal herbivores in which to investigate interactions between PSCs and microbial communities. To deal with the high fibre content of plant material, woodrats, like many rodents, maintain a hindgut fermentation chamber, known as the caecum. However, woodrats also have highly segmented stomach morphology, the function of which is unknown (Carleton 1973). Woodrats possess a foregut chamber (termed 'pregastric stomach' in Kohl *et al.* 2011) that consists of non-secretory epithelium (Carleton 1973), and so should maintain a more neutral pH and facilitate more microbial growth compared with the acidic, gastric chamber. Interestingly, the microbes present in the faeces of woodrats more closely resemble the patterns observed in distantly related, foregut fermenting Artiodactyls rather than closely related rodents, suggesting that this foregut structure and its resident microbes could be important to host physiology (Kohl *et al.* 2011). Microbial detoxification would be most beneficial to a host if it were to occur proximally in the gastrointestinal tract to permit detoxification prior to absorption in the small intestine (Freeland & Janzen 1974). Thus, the foregut of woodrats could be critical to facilitating growth of microbes important for detoxification of PSCs.

We tested the hypothesis that the foregut of woodrats houses a microbial community. In addition, we investigated the determinants of microbial community structure when all individuals were placed on a novel, non-toxic diet. Finally, we examined whether evolutionary history or convergence on similar natural diets of creosote bush affected the response of the microbial community to the PSCs. Together, these studies represent the first comparative and experimental investigation into the interactions between PSCs and gut microbes in wild herbivores.

MATERIAL AND METHODS

Animal collection and maintenance

Details of trapping locations and dates for each population are described in Appendix S1 in Supporting Information. All animals used in our experiment were collected with Sherman live traps at locations given in Appendix S1. Woodrats were transported to the University of Utah Department of Biology Animal Facility and housed in individual cages (48 × 27 × 20 cm) under a 12:12-h light:dark cycle, with 28 °C ambient temperature and 20% humidity.

Diet treatments

Prior to experimentation, animals were maintained on a diet of high-fibre rabbit chow (Harland Teklad formula 2031). During experimentation, animals were fed the same chow except in a powdered form to prevent caching of food. Four individuals from each population served as control animals and were fed powdered rabbit chow in cages for 8 days. Four individuals from each population were fed the control diet for 3 days, followed by the same diet with increasing amounts of creosote resin (1 and 2% creosote resin for 2 and 3 days respectively). A diet of 2% creosote resin is the maximum concentration at which naïve individuals maintain body mass (Mangione *et al.* 2000). Moreover, it represents a tolerable diet for experienced individuals, as they consume a diet containing ~ 7.5% resin in the wild (Mabry *et al.* 1977; Karasov 1989). The 5-day experimental treatment is sufficient for observing changes in the microbial community given the retention time for woodrats (Karasov *et al.* 1986).

To prepare diet treatments containing creosote resin, creosote leaves were collected from trapping sites and frozen at −20 °C prior to resin extraction. We performed surface extractions from creosote leaves using techniques adapted from Mabry *et al.* (1977). Resin was extracted by soaking leaves in acetone (1:6, wet leaf mass:volume solvent) for 45 min. Solvent and resin were filtered (Whatman filter paper grade 1) to remove large particles and evaporated using a rotovap until the resin was highly viscous, at which point it was transferred to a vacuum pump for 48 h to remove any remaining acetone. Extracted resin was stored at −20 °C prior to use.

Creosote diet treatments were prepared by dissolving the appropriate amount of resin in a volume of acetone equal to 25% of the dry weight of ground rabbit chow to which it was added. Control diet (0%) was prepared by adding an identical ratio of acetone, without creosote resin. Acetone was evaporated from all diets in a fume hood and complete evaporation was confirmed gravimetrically. Acetone is a common disinfectant and sterilizing agent (Drews 1977).

Following diet treatments, animals were euthanised under CO_2 and immediately dissected. Contents of the foregut were removed and frozen at −80 °C until DNA isolation.

DNA isolation and sequencing

Foregut contents were thawed on ice and a small amount (~ 25 mg) was incubated with 180 μL enzymatic lysis buffer at 37 °C for 30 min to degrade the cell walls of gram-positive bacteria. The lysis buffer consisted of 20 mM Tris–Cl, pH 8.0, 2 mM sodium EDTA and 1.2% TritonX-100 dissolved in deionized water, with 20 mg mL^{-1} lysozyme added before use. DNA was extracted from faecal material using a DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA). Microbial 16S rRNA genes were amplified and sequenced using tag-encoded FLX amplicon pyrosequencing (TEFAP) using the universal primers 28F (5′-GAGTTTGATCNTGGCTCAG-3′) and 519R (5′ACCGCGGCTGCTGGCAC-3′). TEFAP was conducted by Research and Testing Laboratories (Lubbock, TX, USA), with methods described in detail in Sun *et al.* (2011).

Sequence analysis

Sequences were analysed using the QIIME software package (Caporaso *et al.* 2010). Sequences underwent standard quality control, and were removed from analysis if they lacked the primer sequence or had either quality scores < 25, read lengths < 200 bp or ambiguous base reads. Remaining sequences were assigned to specific samples using 8-bp barcodes assigned by Research and Testing Laboratories. Sequences were grouped into operational taxonomic units (OTUs) using UCLUST (Edgar 2010) with a minimum sequence identity of 99%. The use of 99% minimum sequence identity over 97% allows for better phylogenetic resolution. The most abundant sequences within each OTU were designated as a 'representative sequence', and then aligned against the Greengenes core set (DeSantis *et al.* 2006) using PyNAST (Caporaso *et al.* 2009) with default parameters set by QIIME. A PH

Lane mask supplied by QIIME was used to remove hypervariable regions from aligned sequences. FastTree (Price *et al.* 2009) was used to create a phylogenetic tree of representative sequences.

Diversity of the woodrat foregut, and determinants of diversity on a non-toxic diet

To investigate diversity within the woodrat foregut, OTUs were classified using the Ribosomal Database Project classifier with a the standard minimum support threshold of 80% (Wang *et al.* 2007). Sequences identified as chloroplasts were removed from analysis. Relative abundances of taxa were averaged across all individuals to give a representation of taxa residing in the woodrat foregut. In addition, we compared relative abundances of the dominant phyla from individuals fed the control diet using a two-way ANOVA with species and experience (naïve vs. experienced) as the main effects. Insignificant interactions were removed from the final analysis.

Effect of creosote resin on microbial community representation

We compared community memberships (presence or absence of lineages, and not their relative abundances) of treatment groups. We calculated unweighted UniFrac scores and conducted Principal Coordinates Analysis. To investigate how similar treatment groups were to one another, we combined individuals within treatments after normalising for the number of sequences per sample. Diversity shared between treatment groups (β diversity) was measured using the UniFrac metric, which utilises the fraction of branch length shared between two samples in the phylogenetic tree created from all representative sequences. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) hierarchical clustering of treatment groups was carried out using unweighted UniFrac distances.

Effect of creosote resin on microbial community structure

Several α diversity measurements were calculated for each sample. We calculated the Shannon Diversity Index, a biodiversity measure that incorporates both richness and evenness. We calculated evenness, or how similar in abundance the OTUs in a sample are, as well as Chao1, which estimates the asymptote on a species accumulation curve to estimate OTU richness. However, these diversity metrics weight all OTUs equally regardless of phylogenetic relationships. Therefore, we calculated a measurement of phylogenetic diversity (Faith 1992), which measures the cumulative branch lengths from randomly sampling OTUs from each sample. For each sample, we calculated the mean of 20 iterations for a sub-sampling of 1441 sequences and calculated the mean for each treatment.

To evaluate the role of previous experience with creosote on microbiota diversity, relative abundances of taxa, Shannon Index, evenness, Chao1 and Phylogenetic Diversity scores were compared using three-factor ANOVAs, using species, ecological experience and diet as main effects. If interaction terms were insignificant they were removed from analysis. Statistical analyses were conducted in JMP 9.0.

Data deposition

The bacterial 16S rRNA gene sequences from our effort were deposited in the GenBank Sequence Read Archive under accession number SRA048649.1.

RESULTS

Diversity of the woodrat foregut

We obtained a total of 144 863 high quality microbial 16S rRNA sequences (4527 ± 334 sequences per individual) across all samples from the woodrat foregut. These high quality reads were obtained after quality control removed roughly 0.4% of original sequence reads, and alignment and filtering for chloroplast removed ~ 20% (Appendix S2). Clustering of sequences with 99% sequence similarity identified 4825 OTUs. Within these OTUs, we identified seven microbial phyla (Table 1) The dominant genera present were *Lactobacillus* (73.1 ± 4.3%) and *Allobaculum* (2.5 ± 0.1%).

Determinants of diversity on a non-toxic diet

The two host species had foregut microbial communities with distinct characteristics when feeding on the non-toxic laboratory diet (Fig. 1). *N. lepida* hosted significantly fewer Firmicutes and more Bacteroidetes and Actinobacteria compared to *N. bryanti* (species effect: $P < 0.01$ for all phyla; Appendix S3).

Responses of the foregut microbiota to creosote resin

Community membership (the presence of lineages) of the foregut was determined by experience with creosote resin, as well as host species association. Populations of *N. lepida* share similar microbial community memberships (Fig. 2; Appendix S4). Interestingly, the microbiota of experienced *N. bryanti* was more similar to that of *N. lepida* (regardless of experience) than naïve *N. bryanti* (Fig. 2; Appendix S4). Diet treatment in the laboratory did not affect community membership, i.e., similar gut microbes were present in woodrats regardless of feeding on creosote resin (Fig. 2; Appendix S4).

Changes in community structure (relative abundances of lineages) due to addition of PSCs depended on previous ecological experience with creosote bush. We found significant diet by experience interactions for the relative abundances of the two dominant phyla residing in the woodrat foregut (Figs 1 and 3, Appendix S5). Experienced individuals had a lower relative abundance of Firmicutes and a greater relative abundance of Bacteroidetes when fed creosote resin, whereas naïve individuals showed an inverse pattern (Figs 1 and 3; Appendix S5). There was a trend for increased relative abundance of Actinobacteria when woodrats fed on creosote, particularly in experienced individuals (Appendix S5).

Similar significant interactions between diet treatment and ecological experience were observed across several of the biodiversity

Table 1 Dominant phyla residing in the woodrat foregut

Phylum	Relative abundance
Firmicutes	79.8 ± 3.7
Bacteroidetes	11.5 ± 2.2
Actinobacteria	6.5 ± 1.6
TM7	0.3 ± 0.1
Proteobacteria	0.3 ± 0.1
Fusobacteria	0.01 ± 0.006
Spirochaetes	0.01 ± 0.006
Other	1.57 ± 1.08

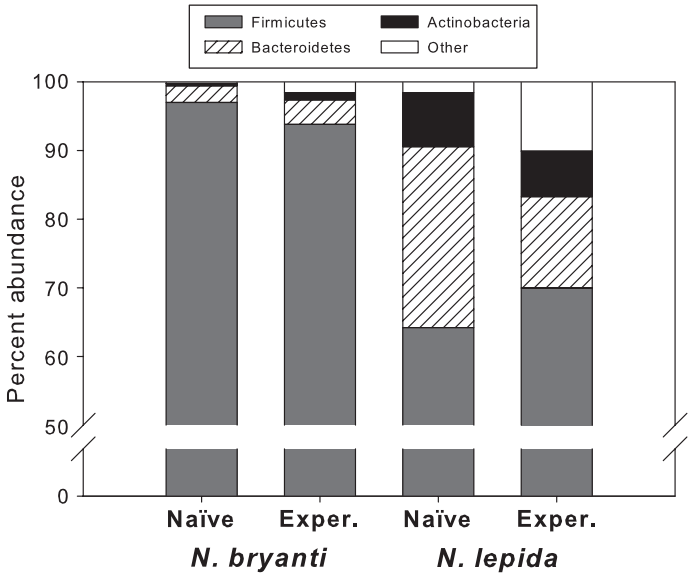

Figure 1 Relative abundances of microbial taxa from foreguts of four woodrat populations fed the control diet.

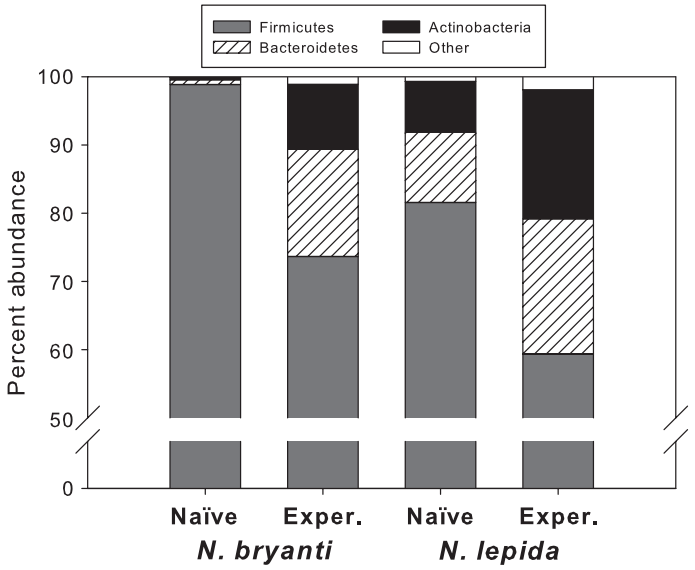

Figure 3 Relative abundances of microbial taxa from woodrat foreguts of four woodrat populations fed the creosote diet.

measurements, whereas the main effects of diet and ecological experience alone had no influence on any estimates of biodiversity (Table 2; Appendix S6). Microbial diversity was 20% greater in experienced individuals fed creosote resin compared with the control diet as measured by the Shannon Index, whereas naïve individuals exhibited ~ 5% lower diversity (Table 2; Fig. 4A). This effect is most likely driven by evenness of microbial species, which showed similar patterns (Table 2; Fig. 4B), whereas estimates of species richness (Chao1) did not differ (Table 2; Fig. 4C). Phylogenetic diversity also showed a significant diet by experience interaction (Table 2), such that phylogenetic diversity was higher in experienced individuals fed creosote resin compared with the control diet, whereas naïve individuals exhibited lower phylogenetic diversity (Fig. 4D). Thus, the overall response of individuals to dietary creosote resin seems to be largely influenced by previous ecological experience.

DISCUSSION

Plant secondary compounds represent a persistent challenge to mammalian herbivores, yet their interactions with the gut microbiota have been largely understudied in comparison with other dietary components, such as high fibre or low nitrogen. In this study, we inventoried the microbial community of two mammalian herbivores and investigated how evolutionary history and ecological exposure to PSCs influences the microbial community membership, as well as changes in community structure in response to dietary PSCs. This work represents the first experimental study on how the microbial communities of wild herbivores respond to dietary toxins. Below, we discuss how PSCs seem to have shaped the microbial communities of the woodrat foregut and how differential responses might have functional consequences for herbivores attempting to utilise new food sources.

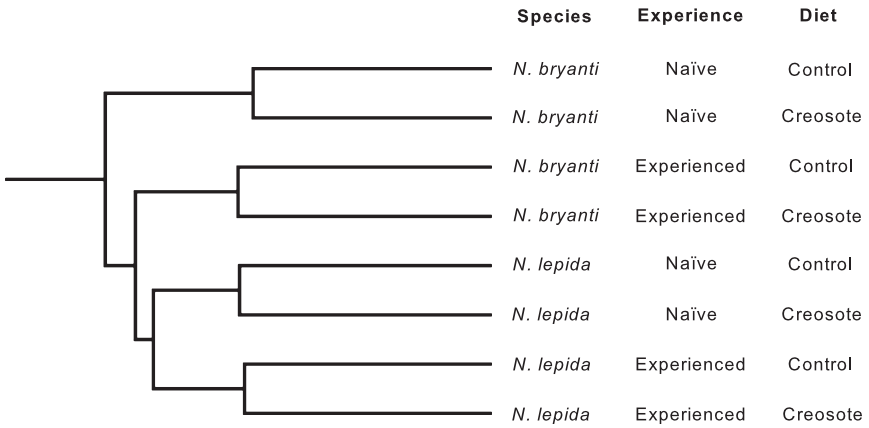

Figure 2 Results of UPGMA hierarchical clustering of treatment groups using unweighted UniFrac distances. Operational taxonomic unit abundances were normalised per individual and combined by treatment.

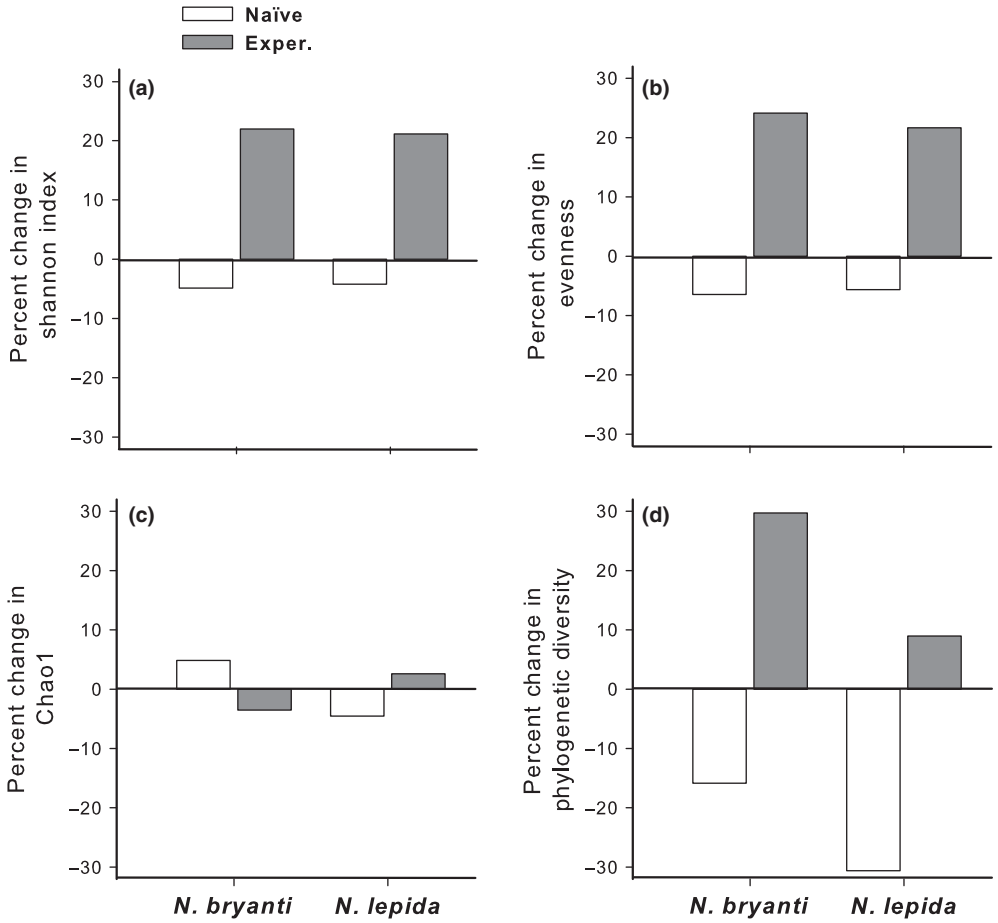

Figure 4 Per cent change in α diversity indices from control diet to feeding on a creosote diet. Data for each dietary treatment are presented in Table 2. Details of statistical analysis are presented in Appendix S6.

Table 2 Means ± 1 SEM and significant effects for measurements of α diversity indices from woodrats on control and creosote diets. Insignificant interactions were removed from analysis

	Control				Creosote				
	Neotoma bryanti		*Neotoma lepida*		*Neotoma bryanti*		*Neotoma lepida*		
	Naïve	Exper.	Naïve	Exper.	Naïve	Exper.	Naïve	Exper.	Effects†
Shannon index	5.53 ± 0.25	5.19 ± 0.60	6.40 ± 0.37	5.40 ± 0.52	5.26 ± 0.47	6.33 ± 0.35	6.13 ± 0.10	6.54 ± 0.03	S*; E × D*
Evenness	0.62 ± 0.03	0.58 ± 0.06	0.71 ± 0.05	0.60 ± 0.05	0.58 ± 0.05	0.72 ± 0.04	0.67 ± .01	0.73 ± 0.01	E × D**
Chao1	850.1 ± 93.3	803.9 ± 74.2	985.3 ± 125.1	864.2 ± 94.6	891.4 ± 62.5	775.5 ± 90.7	940.6 ± 2.9	886.7 ± 46.9	
Phylogenetic diversity	9.97 ± 1.37	11.51 ± 1.82	18.78 ± 1.42	13.95 ± 1.46	8.39 ± 1.37	14.93 ± 2.09	13.03 ± 1.17	15.20 ± 0.99	S**; S × E*; E × D**

†Indicates which treatment effects are significant (S = species, E = experience, D = diet). Interactions are denoted with '×'. Details of statistical analysis are presented in Appendix S6. Asterisks indicate the level of significance, determined by ANOVA.
*$P < 0.05$; **$P < 0.005$.

Diversity of the woodrat foregut

The microbial community of the woodrat foregut consists primarily of Firmicutes (~ 80%). This high representation of Firmicutes drastically differs from that of other foregut structures such as the bovine rumen, where Firmicutes contribute to ca. 30% of the microbial population (Callaway *et al.* 2010). As the functional repertoire of microbial genes can be predicted in part by microbial community structure (Muegge *et al.* 2011), and because of the differences between the microbiota of the woodrat foregut to those present in other foreguts, the function of the woodrat foregut is unlikely to be analogous to that of the bovine rumen. In addition, the smaller size of woodrat foregut coupled with probable shorter residence time of material in comparison to that of the caecum

(Kohl *et al.* 2011), suggests that the foregut is unlikely to play a key role in cellulolytic fermentation.

Rather, our results support the hypothesis that the woodrat foregut serves as a detoxification chamber. We found seven microbial phyla, many of which are known to play a role in toxin metabolism. The most predominant genus, *Lactobacillus*, degrades plant phenolics (Rodríguez *et al.* 2008) and dissociates tannin-protein complexes (Shimada *et al.* 2006). Moreover, this genus seems to be essential for the ability of the Japanese wood mouse (*Apodemus speciosus*) to feed on polyphenolic-rich acorns (Shimada *et al.* 2006). The phylum Actinobacteria is well known for its biotransformation abilities, such as oxidation of small cyclic hydrocarbons similar in structure to PSCs (Donova 2007). For example, Actinobacteria are thought to be important in the degradation of plant phenolics in the guts of termites (Le Roes-Hill *et al.* 2011). It is also possible that other less abundant microbes play a role in detoxification, or perhaps other functions related to detoxification processes, such as free-radical scavenging. In the future, we plan to conduct metagenomic sequencing to better understand the function of the woodrat foregut.

Determinants of diversity on a non-toxic diet

Our results show that species maintain unique assemblages of microbes, even when housed in the same animal room and after experiencing similar laboratory conditions. At this point, we cannot speculate how these communities change from natural to laboratory conditions. These distinct assemblages could be the products of unique host-microbial interactions among populations. For example, some hosts produce molecules (glycans) that enhance particular microbial species and generate unique communities (Hooper & Gordon 2001). Alternatively, as these animals were captured in the wild, we cannot exclude the possibility that the distinct assemblages are the legacy of unique founder microbial populations from each habitat.

Determinants of microbial community membership

Diet did not significantly alter microbial community membership, as shown by UPGMA hierarchical clustering. This suggests that the addition of creosote resin does not add or remove a significant number of microbial lineages from the woodrat foregut. Rather, experience with PSCs and evolutionary history determined foregut community membership. This is in contrast to herbivorous insects, where PSC class, and not evolutionary history, determine microbial diversity (Kelley & Dobler 2011). This difference may be due to the fact that mammals inherit their microbial communities from their mothers, whereas insects seem to acquire microbes from the environment (Kelley & Dobler 2011). Individuals of *N. lepida*, regardless of experience, shared similar microbial communities. However, experienced *N. bryanti* had microbial communities more similar to *N. lepida* (regardless of experience) rather than naïve *N. bryanti*. This pattern could also be the result of similar PSC profiles in the diets of the experienced woodrats and naïve *N. lepida*. Both juniper (the diet of naïve *N. lepida*) and creosote contain high concentrations of phenolics (Mabry *et al.* 1977; Utsumi *et al.* 2009). Thus, the microbial communities of *N. lepida* and experienced *N. bryanti* may be specialised for degrading phenolics. However, juniper also contains high concentrations of monoterpenes (Adams *et al.* 1981), and so it is puzzling why we find such similar communities between naïve and experienced *N. lepida*. However, experienced *N. lepida* retain the ability and detoxification machinery to feed on their ancestral diet of juniper (Magnanou *et al.* 2009), and may have also retained juniper-specific microbes while adding several microbial taxa to aid in the metabolism of creosote toxins.

In contrast, the diet of naïve *N. bryanti* contains low-molecular weight PSCs, such as oxalates or diterpenes (Stintzing & Carle 2005; Abreu *et al.* 2008). Therefore, the diet switch that occurred within *N. bryanti* represents a larger change in PSC profiles compared with *N. lepida*, and may have selected for the disparate communities observed. The microbial community of naïve *N. bryanti* may specialise in the degradation of the PSC classes it naturally encounters, such as oxalates. Indeed, microbes are important for oxalate degradation in another woodrat species, *N. albigula* (Shirley & Schmidt-Nielsen 1967). However, we cannot exclude the idea that the length time in captivity contributed to the unique microbial community observed in naïve *N. bryanti*.

Response to creosote resin

The response of the microbiota to creosote PSCs depended largely on previous experience with creosote. For example, experienced woodrats fed on creosote resin exhibit lower relative abundances of Firmicutes and higher relative abundances of Bacteroidetes compared with the control diet. In addition, the relative abundance of Actinobacteria was higher in experienced woodrats fed creosote, but showed less change in naïve woodrats. Likewise, biodiversity was higher in the foreguts of experienced woodrats fed creosote resin, but lower in naïve. As the functions of collective microbial genes can be predicted by microbial community structure (Muegge *et al.* 2011), we predict that this shift in microbial diversity is matched by a shift in microbiome function. Microbial biodiversity is often positively correlated with ecosystem function (Bell *et al.* 2009). It is possible that the ingestion of a novel toxin skews the relative abundance of microbial genes away from ideal representation, such as less representation of important fermentation or amino acid synthesis genes, which could then compromise the nutritional status of the animal. Thus, adaptation of the microbiota to specific PSC profiles may limit an herbivore's ability to shift diets quickly or utilise novel plant species. This specialisation within the microbiota, and impaired function due to novel PSCs may be a mechanism by which specialist herbivores are unable to utilise plants with novel PSCs as effectively as generalist herbivores (Sorensen *et al.* 2005).

The mechanism of these differential responses still remains unclear. They could be driven in part by host physiology, such that experienced hosts change the profile of their glycan or mucin diversity in response to creosote resin to select a certain microbial population. In addition, responses could have been driven by various mechanisms through which the microbiota of experienced woodrats have become adapted to an environment rich in PSCs, such as descent with modification within the microbiota, or more likely horizontal transfer of genes from transient microbes to the resident microbiota. Indeed, horizontal gene transfer within gut-associated bacteria occurs at a rate 25 × higher than other bacteria (Smillie *et al.* 2011), and is important for the acquisition of new metabolic capabilities to allow gut microbes to cope with plant compounds (Hehemann *et al.* 2010; Nelson *et al.* 2010). Through these mechanisms, representatives of Bacteroidetes and Actinobacteria residing

within experienced hosts may have accumulated genes important for the detoxification of creosote, allowing them to increase in relative abundance when the host is consuming creosote resin.

It is also noteworthy that the responses of experienced herbivores are convergent across species. Other studies have documented that nutritive factors cause convergence in the microbial communities of herbivores from disparate mammalian lineages (Ley *et al.* 2008; Muegge *et al.* 2011). Our study reveals that PSCs appear to have selective pressures similar to that of nutrients on the microbial communities of herbivores. Community convergence is exhibited by similar changes in biodiversity measures and relative abundances of dominant phyla in response to creosote PSCs in the experienced woodrat microbial communities. Metagenomic comparisons may elucidate whether the microbial communities of each woodrat species have convergently or uniquely adapted to PSCs at the microorganism or gene level.

Our results highlight the importance of considering ecological experience when investigating microbial responses to PSCs and perhaps other xenobiotics. Recently, there has been a large effort to use PSCs as modifiers of the rumen ecosystem in cattle and sheep due to the increasing restrictions on growth-promoting antibiotics in agriculture (Patra & Saxena 2009). In addition, the gut microbiota seems to play a large role in human obesity and modification of the gut microbiota through the use of PSCs is being investigated as a potential treatment (Rastmanesh 2011). However, our study shows differential responses within the same host species, and so such endeavours should consider host experience when conducting experiments or translating results to other systems.

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AUTHORSHIP

KDK conducted the experiment, participated in data interpretation and wrote the first draft of the manuscript. MDD participated in the data interpretation, contributed to revisions of the manuscript and oversaw the project.

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