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Natural diets promote retention of the native gut microbiota in captive rodents

Rodolfo Martínez-Mota[1] · Kevin D. Kohl[2] · Teri J. Orr[1] · M. Denise Dearing[1]

Abstract

Wild animals entering captivity experience radical lifestyle changes resulting in microbiome alterations. However, little is known about the factors that drive microbial community shifts in captivity, and what actions could mitigate microbial changes. Using white-throated woodrats (*Neotoma albigula*), we tested whether offering natural diets in captivity facilitates retention of native microbial communities of captive animals. Wild-caught woodrats were brought to laboratory conditions. Woodrats received either a natural diet of *Opuntia* cactus or an artificial diet of commercial chow over three weeks. Microbial inventories from woodrat feces at the time of capture and in captivity were generated using Illumina 16S rRNA sequencing. We found that providing woodrats with wild-natural diets significantly mitigated alterations in their microbiota, promoting a 90% retention of native microbial communities across the experiment. In contrast, the artificial diet significantly impacted microbial structure to the extent that 38% of the natural microflora was lost. Core bacteria including *Bifidobacterium* and *Allobaculum* were lost, and abundances of microbes related to oxalate degradation decreased in individuals fed artificial but not natural diets. These results highlight the importance of supplementing captive diets with natural foods to maintain native microbiomes of animals kept in artificial conditions for scientific or conservation purposes.

Introduction

Numerous studies have demonstrated that entrance into captivity largely alters the gut microbiota of vertebrate hosts [1, 2]. Animals brought into captivity must cope with drastic changes imposed by numerous artificial conditions, such as space limitations, unnatural housing structures and bedding, standardized diets, as well as loss of conspecific social networks. These unnatural conditions can disrupt the diversity and composition of microbial communities of animals [3]. Research on amphibians, birds, and mammals indicate that microbial diversity is reduced in captive hosts across diverse taxonomic groups [2, 4–6]. However, this pattern may be species specific. A recent study on 41 mammal species yielded mixed results when comparing the gut microbiota between wild and captive counterparts [1]. Certain groups of mammals showed lowered microbial diversity when in captivity (e.g., equids, canids, and primates), while others (e.g., bovids) did not exhibit significant differences in diversity. These results call for more controlled studies to disentangle specific factors that impact the gut microbiome in captive animals.

The changes in gut microbial diversity associated with a transition to captivity have been shown to be driven by a number of different factors. For example, restricted exposure to natural microbial sources can decrease microbial diversity in confined mammalian hosts due to the inability to replenish the gut community [7, 8]. Moreover, artificial housing environments can alter host internal organ morphology (e.g., kidney mass, gut size), as well as hormonal and immunological stress responses in vertebrates [9, 10], which could lead to general disturbances in the gut microbiome [11, 12]. In particular, shifts in dietary regimes in captivity may drive rapid modifications of the gut microbial structure due to different availability of substrates [13].

✉ M. Denise Dearing
denise.dearing@utah.edu

1   School of Biological Sciences, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA

2   Department of Biological Sciences, University of Pittsburgh, 4249 Fifth Avenue, Pittsburgh, PA 15260, USA

Diet stands as one of the most important determinants of the gut microbiome in several species, including humans [14, 15] and rodents [16], and dietary shifts can have a significant impact on microbial abundance and function. In laboratory mice subjected to periodical dietary switching, the relative abundance of Bacteroidales and Clostridiales changed dramatically after just a single day on a new diet [16]. Furthermore, reductions in dietary fiber can cause extinction of certain gut microbes and alter host metabolism [17]. This phenomenon is exemplified by a study on the microbiota of captive folivorous primates (*Alouatta palliata*, *Pygathrix nemaeus*), in which individuals lost microbial diversity and showed lower relative abundances of metabolic pathways related to fiber degradation, due to a reduction in dietary fiber intake [6]. Overall, this information suggests that diets supplemented with natural foods may help to reduce significant changes in the gut microbiome of animals when brought into captivity. However, to our knowledge no studies have specifically examined the effect of diet vs other myriad of effects that captivity may have on the microflora of animals.

In this study, we examined whether consumption of natural diets by animals brought into captivity facilitates retention of their native gut microbiota. The white-throated woodrat (*Neotoma albigula*) is an excellent model system to explore gut microbial changes associated with lifestyle modifications imposed by captivity, because their natural diet can be replicated in captive settings. Although *N. albigula* is considered a generalist herbivore, certain populations in the southwest of the United States feed almost exclusively on cacti of the genus *Opuntia* [18, 19]. We took advantage of this dietary specialization and the feasibility of recreating the natural diet in captive conditions. From here on, we will refer to the diet consumed by woodrats in nature as 'wild', and to captive diets based on *Opuntia* cactus as 'natural' and on chow as 'artificial'.

We compared microbial diversity between animals fed natural and artificial diets, and compared changes in microbial diversity as these animals entered captivity. If changes in the microbiome are primarily driven by alterations in physiological function caused by captivity, such as hormonal or immunological changes, then woodrats fed either natural or artificial diets were predicted to exhibit similar changes in microbial diversity. Conversely, if microbiome changes are driven solely by diet, then we predicted that woodrats fed natural diets in captivity would retain the majority of their natural microbial communities. These two factors, diet and captivity, may interact such that the provisioning of the natural diet in captivity would facilitate the retention of a greater proportion of the natural diversity of the microbiome relative to a fully artificial diet.

Materials and methods

Animal collection

Twelve white-throated woodrats (*N. albigula*) hereon 'woodrats' were collected from Castle Valley, UT, USA (38.37° N, 109.22° W) in February 2017. Sherman live traps were baited with a mix of peanut butter and oats and placed near the entrance of woodrat middens (i.e., nests). We collected fecal samples from Sherman traps to obtain a baseline value representing each individual's wild-microbial community. Fecal samples collected in this manner from traps have been shown to be representative of gut microbial communities [20]. Fecal samples were stored on dry ice in the field, and stored in a $-80\,^{\circ}\mathrm{C}$ freezer upon return to the laboratory.

Captured animals were placed in individual shoebox rodent cages and transported to the School of Biological Sciences Animal Facility at the University of Utah. While in the field and during transport all individuals were provided *ad libitum* access to *Opuntia* spp. cactus pads, the main component of the natural diet for this population [18]. Following the methods of Kohl et al. [18], we further analyzed stable isotopes from hair of collected woodrats and confirmed that these individuals specialized on cacti (Fig. S1).

Captivity experiment and fecal collection

Upon entrance to captivity animals were split into two treatment groups. Six individuals ($n = 2$ males, $n = 4$ females) were fed an artificial diet (i.e., high-fiber rabbit chow, Teklad Global High Fiber Rabbit Diet-Envigo), and the other six ($n = 2$ males, $n = 4$ females) were fed their natural diet of *Opuntia* spp. cactus pads. Cacti were collected at the same site where woodrats were trapped. These food items were stored in a cold room ($4\,^{\circ}\mathrm{C}$) to keep them fresh over the course of the experiment. All individuals were fed either artificial or natural diets *ad libitum* for 21 days. Woodrats had *ad libitum* access to water. All animals were maintained under a 12/12 light/dark cycle at $27\,^{\circ}\mathrm{C}$ and 22% humidity.

Fecal samples from captive woodrats were collected from each individual 3, 7, 14, and 21 days after the animals entered captivity. Fecal samples were stored at $-80\,^{\circ}\mathrm{C}$ until DNA extractions were performed. We could not collect fecal samples from two females on the natural diet at day 21 because they gave birth. All methods were approved by the Institutional Animal Care and Use Committee—University of Utah (protocol 16-02011).

Microbial analysis

Total DNA was extracted from 58 woodrat fecal samples using QIAamp® PowerFecal® DNA kits. In addition, three blanks were included as extraction controls. The contribution of these controls to woodrat microbial communities was assessed by Source-Tracker analysis [21]. DNA also was extracted from six swabs of the surface of cactus pads and from three whole pellets of chow, to determine the contribution of microbes from food to the woodrat microbial communities. The extracted DNA was processed at the DNA Service Facility at the University of Illinois—Chicago. The V4 hypervariable region of the 16S rRNA gene was amplified using the 515F–806R primers as recommended by the Earth Microbiome Project [22]. Amplicons were sequenced using the Illumina MiniSeq platform.

Microbial sequences were analyzed in the Quantitative Insights into Microbial Ecology (QIIME) pipeline version 1.9.1 [23]. We implemented standard quality control settings and split sequences into libraries using default parameters in QIIME. Sequences were grouped into operational taxonomic units (OTUs) based on a 99% sequence identity using the command line *pick_open_reference_otus.py*. Singletons (OTUs represented by only a single read) and sequences identified as chloroplasts or mitochondria were removed. To control for the effects of variable sequencing depth, all samples were rarefied to 10,000 reads prior to downstream analyses. This depth is sufficient to uncover differences across groups [24]. This resulted in the exclusion of one sample, which did not achieve our rarefaction threshold, corresponding to an individual feeding on cactus on day 7. Therefore, subsequent analyses were based on a data set of 57 samples. Our finalized OTU table is uploaded as supplementary document (Table S1). From here on, to keep with our terminology for diet types, we will refer to fecal samples collected in the field as "wild", those collected from captive individuals fed chow as "artificial", and those collected from woodrats fed cactus as "natural" samples, respectively.

Alpha diversity analysis

We investigated four metrics of microbial alpha diversity: the number of observed OTUs, Faith's phylogenetic diversity, the Shannon index, and evenness. Each measure of alpha diversity was compared between diets and over time with generalized linear mixed models (GLMMs), using the *glmmADMB* package [25] in the R software version 3.4.3 [26]. The number of observed OTUs, the Shannon index, and the Faith's phylogenetic diversity index followed a normal distribution (Shapiro–Wilk test *p-value* > 0.05). Models with these diversity metrics were fitted with a

Gaussian distribution and identity function. Since values of the evenness index range between 0 and 1, the model for this metric was fitted with a beta distribution and a logit function. Individual woodrat ID was set as a random factor in all models. A likelihood ratio test was used to compare the fit of each full model against a null model that included only the intercept. Multiple comparisons with false-discovery rate (*FDR*) adjusted *p* values were run in R using the *multcomp* package [27].

Beta diversity analysis

We analyzed shifts in bacterial communities among wild, artificial, and natural samples using Principal Coordinate Analysis. Unweighted and weighted UniFrac distance matrices were used as measures of beta diversity [28]. These distance matrices consider microbial community membership (unweighted UniFrac, which only utilizes presence/absence of OTUs) and community structure (weighted UniFrac, which also incorporates the relative abundances of OTUs). To test the effects of diet and time on changes in community membership and structure, permutational multivariate analysis of variance (PERMANOVA) tests were run in R software, using the function *adonis* implemented in the *vegan* [29] and *qiimer* [30] packages. PERMANOVA tests were based on 999 permutations. Woodrat ID, diet, time and a diet × time-point interaction term were all included as factors in these analyses.

In addition, we compared relative differences in microbial communities between wild samples and samples collected in captivity at different time points. We used pairwise unweighted and weighted UniFrac distances between captive samples and the corresponding wild sample for the same individual. Here, larger distance measurements are indicative of less similar microbial communities. These distances were analyzed with GLMMs in R using the *glmmADMB* package and fitted with a beta distribution [25]. We included diet, time, and diet × time interaction terms as predictors, and included individual ID as a random effect. The significance of each model was tested with a likelihood ratio test as previously described.

We also determined the contribution of various bacterial taxa to dissimilarity in microbial communities between wild and artificial samples, and wild and natural samples over time. We performed similarity percentage (SIMPER) analyses using the *vegan* R package [29]. SIMPER analyses were conducted at the family taxonomic level using the Bray–Curtis dissimilarity matrix. We chose family for these analyses because at lower taxonomic levels there were several unclassified taxa. The overall dissimilarity (i.e., the sum of all individual-family contributions to differences between communities) was compared between diets and time using GLMMs as previously described.

Taxonomic analysis and changes in core microbiome

Changes in the relative abundances of microbial phyla between diets and over time were compared with GLMMs as previously described. Given that data were not normally distributed, relative abundances were first arcsine square root transformed. Furthermore, we characterized the woodrat core microbiome from wild samples to assess whether the abundance of core OTUs was perturbed by captivity. The core microbiome, defined as the OTUs that were present in 100% of wild samples, was estimated with the command line *compute_core_microbiome.py*. A heatmap was created in QIIME to show changes in core OTU abundance in both natural and artificial samples with respect to wild samples. Significant differences in core OTU abundances between wild and natural, and wild and artificial diets were tested by means of Mann–Whitney-U tests, with *FDR p* values adjusted for multiple comparisons. Core microbiome analyses were conducted in QIIME.

Source-Tracker analysis: tracking the origin of microbes

To explore whether a natural diet in captivity results in the maintenance of native microbiota as seen in the wild, we tracked the most likely origin of microbial communities observed in captivity using the SourceTracker 0.9.5 software implemented in QIIME [21]. In this analysis, samples from captive individuals fed artificial and natural diets were set as 'sinks', and samples from the wild, as well as microbial samples from food swabs were set as 'sources'. Proportions of the microbiome that originated from the wild were compared between diets and time with GLMMs as previously described.

Inferring changes in microbial function through PICRUSt

We implemented PICRUSt analysis to infer changes in microbial function associated with the different dietary treatments described above [31]. Although PICRUSt does not have the accuracy of shotgun metagenomics, it has been found to be a useful tool for predicting to a certain extent microbial function [32, 33]. We compared with a Kruskal–Wallis test, the relative abundance of sequences related to metabolism categories using KEGG pathways (level 2) across fecal community samples from the dietary treatments.

Results

Our sequencing effort resulted in 4,846,921 reads that were clustered into 58,732 OTUs. The mean number of reads per fecal sample was $83,568 \pm$ SD 29,653. We found that

blanks had a negligible contribution (i.e., 0.02%) to microbial communities of woodrats.

Alpha diversity

There were no differences in initial microbial diversity (i.e., wild condition) between the two treatment groups (*FDR p > 0.05* for the four alpha diversity metrics). Alpha diversity metrics were significantly affected by diet and its interaction with time spent in captivity (Table S2). Specifically, there were significant differences between the natural and artificial diets with respect to the number of observed OTUs (diet: $F_{1,35} = 16.3$, *FDR p < 0.001*). Although time in captivity did not significantly affect the number of observed OTUs (time: $F_{3,35} = 0.84$, *FDR p = 0.480*), there was a significant interaction between diet and time (diet × time: $F_{3,35} = 6.5$, *FDR p = 0.001*). Similarly, both diet and a diet × time interaction significantly influenced Faith's phylogenetic diversity (diet: $F_{1,35} = 10.7$, *FDR p = 0.002*; time: $F_{3,35} = 1.1$, *FDR p = 0.360*; diet × time: $F_{3,35} = 2.5$, *FDR p = 0.080*), and the Shannon index (diet: $F_{1,35} = 17.7$, *FDR p < 0.001*; time: $F_{3,35} = 0.98$, *FDR p = 0.410*; diet × time: $F_{3,35} = 3.7$, *FDR p = 0.020*). Only diet significantly affected the evenness index (diet: $F_{1,35} = 14.7$, *FDR p < 0.001*; time: $F_{3,35} = 0.76$, *FDR p = 0.520*; diet × time: $F_{3,35} = 1.9$, *FDR p = 0.130*). These trends were driven by differences observed on the third day in captivity, and not during other sampling periods of time (Fig. S2). On the third day of the experiment, woodrats fed the artificial diet exhibited significantly higher diversity for all metrics of diversity (30.1% higher number of observed OTUs, 16.2% higher phylogenetic diversity, 11.3% higher Shannon index, and 9.6% higher evenness) when compared with individuals fed the natural diet. There were no differences in alpha diversity between the two treatment groups at days 7, 14, or 21.

Beta diversity

Microbial communities clustered distinctly by diet and time spent in captivity for both beta diversity metrics (unweighted UniFrac and weighted UniFrac, Fig. 1a, b). Woodrats fed the artificial diet harbored communities that were distinct from those observed in the wild condition, and distinct from communities hosted by individuals fed the natural diet. In contrast, microbial communities from woodrats fed the natural diet shared the same multidimensional space with the communities found in their precaptivity wild sample (Fig. 1a, b). Only diet and woodrat ID had significant effects on microbial membership (PERMANOVA, unweighted UniFrac; Table 1). Changes in community structure were primarily driven by diet ($R^2 = 0.40$) and host ID ($R^2 = 0.16$), and to a lesser extent by time spent in
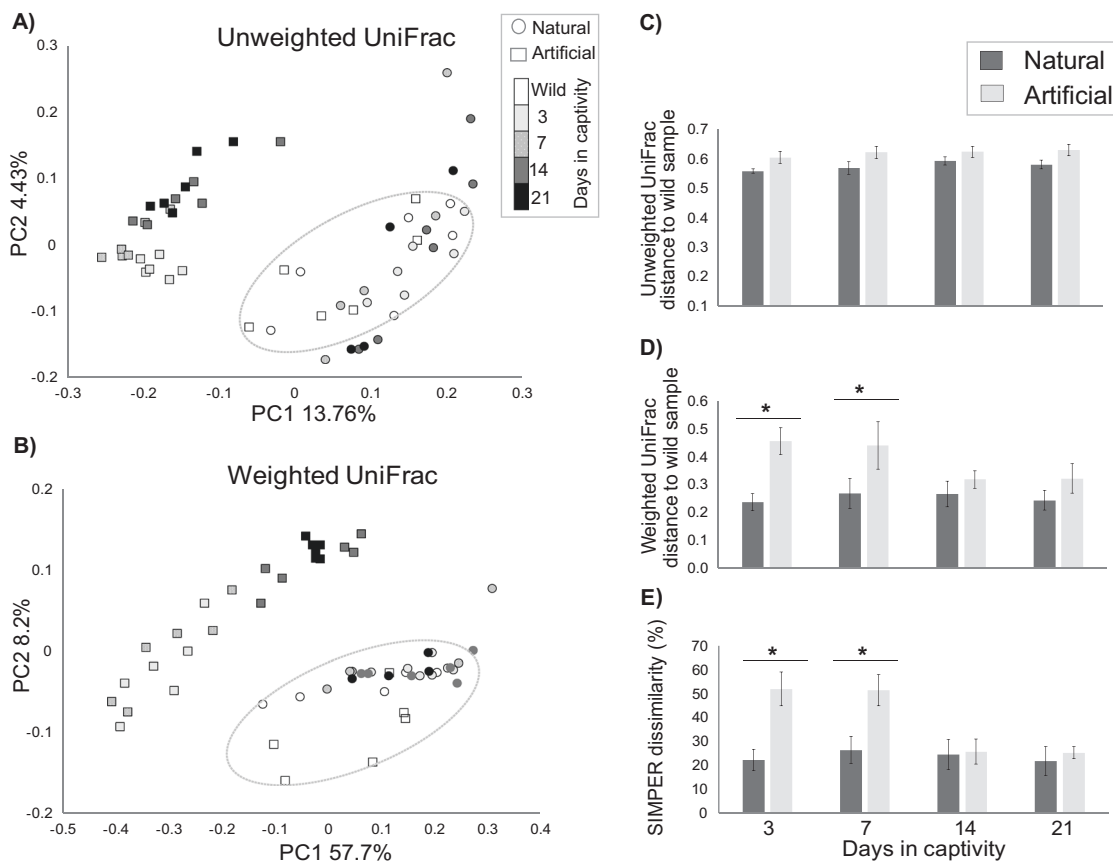
Fig. 1 Microbial community shifts associated with the intake of natural and artificial diets in captive white-throated woodrats (*Neotoma albigula*). **a**, **b** principal coordinate analysis (unweighted and weighted UniFrac) of microbial communities from individuals fed natural (circles) and artificial (squares) diets. Communities from wild samples are represented by open circles and squares, and ellipses denote the space encompassed by communities in the wild condition. Time spent in captivity is represented by gray scales. Comparisons of **c** unweighted and **d** weighted UniFrac distances, and **e** SIMPER average dissimilarity-to-wild-microbial communities between woodrats fed natural and artificial diets in captivity. Mean (±SE) values are shown. Pairwise significant differences are denoted with *$p < 0.001$ (*FDR*)

Table 1 Results of PERMANOVA tests examining the effects of diet, time spent in captivity, the interaction diet × time, and the individual identity on two beta diversity metrics (unweighted and weighted UniFrac distance matrices)

	Unweighted UniFrac			Weighted UniFrac		
	F	p	R^2	F	p	R^2
Diet	4.1	<0.001	0.17	20.6	<0.001	0.40
Time	1.2	0.054	0.05	5.3	<0.010	0.10
ID	1.8	<0.001	0.24	2.5	<0.001	0.16
Diet × time	1.1	0.190	0.04	4.3	<0.001	0.08

captivity ($R^2 = 0.10$) or to a marginal extent by the interaction term diet × time ($R^2 = 0.08$; PERMANOVA, weighted UniFrac; Table 1).

Microbial community distances were influenced differently by diet and time. Unweighted UniFrac distances between wild and captive samples were generally larger in woodrats fed the artificial diet than in individuals fed the natural diet during the entire experiment (diet: $F_{1,35} = 4.9$, FDR $p = 0.030$; time: $F_{3,35} = 1.4$, FDR $p = 0.260$; diet × time: $F_{3,35} = 0.5$, FDR $p = 0.690$; Fig. 1c). In contrast, there was an interaction between diet and time in weighted UniFrac scores. Here, differences between diets decreased towards the end of the experiment (diet: $F_{1,35} = 12.5$, FDR $p = 0.001$; time: $F_{3,35} = 0.2$, FDR $p = 0.930$; diet × time: $F_{3,35} = 3.6$, FDR $p = 0.020$). Pairwise comparisons indicated that weighted UniFrac distances from artificial to wild samples were >70% greater than distances from natural to wild samples during the first seven days spent in captivity (Fig. 1d). No significant differences were found at other time points.

A GLMM showed that the overall dissimilarity of microbial communities was affected by the interaction between diet and time (diet: $F_{1,35} = 12.2$, FDR $p = 0.001$; time: $F_{3,35} = 0.15$, FDR $p = 0.930$; diet × time: $F_{3,35} = 11.6$, FDR $p < 0.001$). The dissimilarity in communities between wild and artificial samples was significantly higher than the wild-natural dissimilarity during the first seven days in

Table 2 Results of GLMMs comparing the relative abundance (%) of microbial phyla between captive woodrats fed natural and artificial diets over time

Phylum	F	df	FDR p
Firmicutes			
Diet	59.4	1	<0.001
Time	0.1	3	0.980
Diet × time	16.8	3	<0.001
Bacteroidetes			
Diet	60.0	1	<0.001
Time	0.3	3	0.830
Diet × time	17.7	3	<0.001
Actinobacteria			
Diet	16.8	1	<0.001
Time	13.2	3	<0.001
Diet × time	6.7	3	<0.001

captivity; but after 14 days it returned to the levels of dissimilarity that were observed between wild and natural samples (Fig. 1e). SIMPER analysis indicated that five families were responsible for >80% of the dissimilarity (day 3: maximum dissimilarity) between bacterial communities of animals from the wild vs those on the artificial diet (Table S3). These were the candidate family S24-7, an unknown family in the order Clostridiales, and Ruminococcaceae, Lactobacillaceae, and Lachnospiraceae. These families contributed 29.4, 23.7, 11.7, 10.5, and 7.1%, respectively, to the dissimilarity. At the end of the experiment, the same families were responsible for 63% of the dissimilarity between microbial communities (Table S4).

Taxonomic analysis

At a broad taxonomic level, the relative abundances of three microbial phyla significantly changed according to the diet offered in captivity (Table 2). Pairwise comparisons indicate that individuals fed the artificial diet showed higher relative abundances of Firmicutes and lower relative abundances of Bacteroidetes during the first 14 days spent in captivity when compared with woodrats fed natural diets (FDR $p < 0.001$, Fig. 2a). There were no significant differences in these phyla between the two diet treatments at the end of the experiment. The relative abundance of Actinobacteria was significantly lower in woodrats fed the artificial diet across the whole feeding experiment compared with individuals fed natural diets (Fig. 2a).

At the family level, the relative abundances of specific bacterial phylotypes that were responsible for microbial community shifts also varied with diets offered in captivity (Fig. 2b, c). During the first seven days spent in captivity, woodrats fed the artificial diet exhibited lower abundances of the family S24-7 (2.5-fold decrease) when compared with wild samples (Fig. 2c); in contrast, the abundances of this family increased in animals fed natural diets (1.2-fold increase, Fig. 2b). Individuals fed the artificial diet exhibited increased abundances of microbes from the families Ruminococcaceae (2.7-fold increase), Lachnospiraceae (3.8-fold increase), and an unknown family within the Clostridiales (2.8-fold increase) when compared with wild samples (Fig. 2c), while individuals fed the natural diet experienced minimal changes in these families (Fig. 2b). Woodrats fed either artificial or natural diets had lower abundances of Lactobacillaceae compared with the wild condition (11.6- and 1.9-fold decrease, respectively).

Core microbiome changes

We identified core OTUs as those that were present across 100% of samples collected from 12 wild woodrats. We detected 36 wild core OTUs, the most abundant of which was *Lactobacillus* sp. (OTU592160), followed by an unclassified OTU from the family S24-7 (OTU1718), *L. reuteri* (OTU588197), and *Allobaculum* sp. (OTU1147). Mann–Whitney-U tests showed that diet treatments significantly affected abundances of core OTUs in captivity (Table 3). That is, the abundances of 22 core OTUs significantly differed between wild and artificial diets, in which 17 of these OTUs were reduced and five were increased in woodrats on the artificial diet (Fig. 3). In contrast, the abundance of only one core OTU (OTU1403) differed between the wild condition and individuals fed the natural diet (Fig. 3). Moreover, woodrats fed the artificial diet showed a drastic reduction in core OTUs of the genus *Lactobacillus*, and the genera *Bifidobacterium* and *Allobaculum* were lost in these individuals.

Tracking the origin of microbial communities

Source-Tracker analysis revealed that a large proportion of microbes in the feces of captive woodrats had a plausible origin from the natural microbiota (Fig. 4). Overall, 90% (± SD 3.0) of microbial communities from woodrats fed the natural diet had the wild condition as the microbial source. This proportion was maintained over the course of the captivity experiment. In contrast, only 60% (± SD 10.0) of microbial communities from individuals fed the artificial diet were sourced from the wild microbiome, and this proportion differed significantly from results in woodrats fed natural diets (diet: $F_{1,35} = 65.4$, FDR $p < 0.001$; time: $F_{3,35} = 2.0$, FDR $p = 0.120$; diet × time: $F_{3,35} = 1.6$, FDR $p = 0.200$). In these individuals, however, the proportion of microbes linked to a wild origin increased significantly at the end with respect to the beginning of the experiment (1.2-fold increase, FDR $p = 0.007$). The Source-Tracker
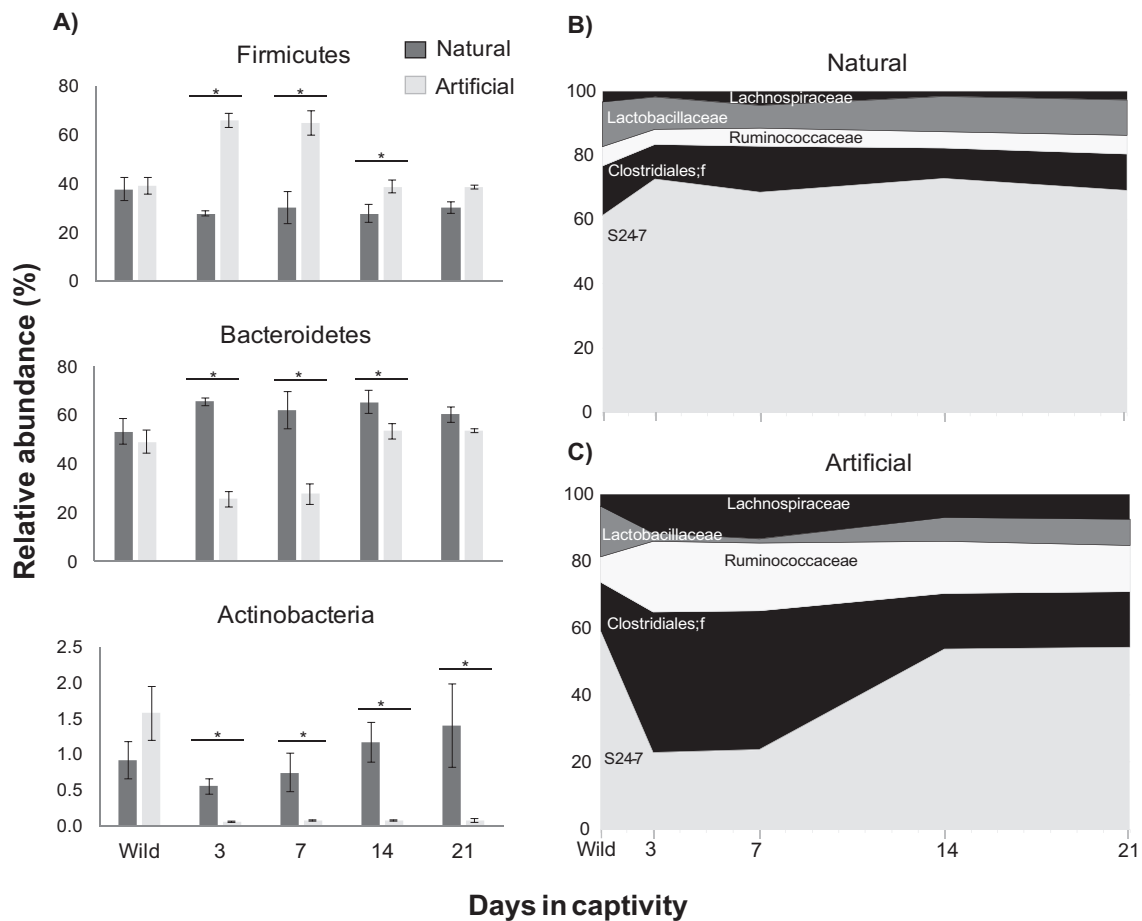
Fig. 2 Fluctuations in relative abundance of microbial phyla (**a**) and families that contributed the most to microbial community dissimilarities between wild and natural (**b**) and artificial diets (**c**)

analysis also showed that microbes located on the surface of food offered to captive woodrats were virtually absent in fecal microbial communities (Tables S5 and S6).

Changes in microbial function inferred by PICRUSt

The predictive profiles generated by PICRUSt indicated that the intake of captive diets modified microbial functions related to six metabolism categories (Fig. S3). Individuals fed the artificial diet had 7–12% higher relative abundance of sequences associated with amino acid metabolism (*FDR* $p < 0.001$), glycan biosynthesis (*FDR* $p < 0.05$), and biosynthesis of "other" secondary metabolites (*FDR* $p < 0.001$), and 5–10% lower relative abundance of sequences related to nucleotide metabolism (*FDR* $p < 0.001$), xenobiotic biodegradation (*FDR* $p < 0.05$), and metabolism of terpenoids and polyketides (*FDR* $p < 0.05$), compared with individuals collected from the wild and those fed natural diets in captivity, which did not show significant differences in these metabolism categories.

Discussion

Captivity is known to affect the gut microbiome, but it was unclear whether shifts in diet or other lifestyle changes associated with captivity, or both, were responsible for the observed changes in microbial communities. We addressed this knowledge gap using an experimental system where we could recreate natural diets under captive conditions and account for individual microbiomes before and after transition to captivity. In general, we found that dietary changes associated with captivity, i.e., administration of a commercial chow, had a large impact on the woodrat gut microbiome. Providing a natural diet to captive animals considerably mitigated alterations in the microbiota and aided in the retention of a large proportion of native microbial communities. Interestingly, the microbiota of animals fed the artificial diet gradually returned to be more similar to the wild state despite dietary changes, though a number of core taxa were still missing at the end of the study. We discuss these results in more detail below.

Table 3 Results of Mann–Whitney-U test comparing core OTU abundance between woodrats fed wild and artificial diets, and wild and natural diets

Taxonomic classification	Core OTU	Wild vs Artificial		Wild vs Natural	
		Test-statistic	FDR p	Test-statistic	FDR p
Family: S24-7	OTU1672	**72.0**	**<0.05**	69.5	0.35
Family: S24-7	OTU1403	108.0	0.28	**39.5**	**<0.05**
Family: S24-7	OTU674	**22.5**	**<0.001**	101.5	0.80
Family: S24-7	OTU475	129.0	0.68	115.5	0.95
Family: S24-7	OTU211	84.0	0.07	125.0	0.99
Family: S24-7	OTU1019	**3.5**	**<0.0001**	116.5	0.95
Family: S24-7	OTU1077336	**67.0**	**<0.05**	75.0	0.35
Family: S24-7	OTU183477	**59.0**	**<0.01**	123.0	0.95
Family: S24-7	OTU1225	**31.5**	**<0.001**	102.0	0.80
Family: S24-7	OTU701	**70.0**	**<0.05**	55.5	0.16
Genus: *Desulfovibrio*	OTU334	**68.5**	**<0.05**	90.5	0.73
Family: F16	OTU401717	**64.5**	**<0.05**	100.5	0.80
Family: F16	OTU1740	134.0	0.77	114.0	0.95
Genus: *Allobaculum*	OTU1147	**0.0**	**<0.0001**	114.0	0.95
Genus: *Allobaculum*	OTU901	**0.0**	**<0.0001**	104.0	0.81
Order: RF39	OTU4343981	**41.5**	**<0.01**	118.0	0.95
Order: Clostridiales	OTU239562	97.5	0.16	114.0	0.95
Order: Clostridiales	OTU4480176	83.0	0.06	123.0	0.95
Order: Clostridiales	OTU271602	122.0	0.53	104.5	0.81
Order: Clostridiales	OTU461487	135.5	0.79	123.0	0.95
Order: Clostridiales	OTU826541	101.0	0.2	98.0	0.78
Order: Clostridiales	OTU159007	85.5	0.07	96.0	0.78
Order: Clostridiales	OTU268410	**39.0**	**<0.01**	118.5	0.95
Order: Clostridiales	OTU288193	133.0	0.76	121.5	0.95
Order: Clostridiales	OTU825939	117.0	0.43	119.0	0.95
Genus: *Lactobacillus*	OTU316515	101.5	0.2	74.0	0.35
Family: Ruminococcaceae	OTU313216	93.5	0.12	95.0	0.78
Genus: *Oscillospira*	OTU443620	**68.5**	**<0.05**	120.0	0.95
Genus: *Lactobacillus*	OTU553352	**3.0**	**<0.0001**	97.5	0.78
Genus: *Lactobacillus*	OTU592160	**4.0**	**<0.0001**	84.0	0.62
Genus: *Lactobacillus*	OTU509452	**19.5**	**<0.0001**	88.0	0.71
Species: *L. reuteri*	OTU588197	**71.5**	**<0.05**	114.0	0.95
Genus: *Bifidobacterium*	OTU1108	**0.0**	**<0.0001**	74.0	0.35
Genus: *Bifidobacterium*	OTU295384	**0.0**	**<0.0001**	116.5	0.95
Family: S24-7	OTU530	**73.5**	**<0.05**	122.5	0.95
Family: S24-7	OTU1718	**77.0**	**<0.05**	98.0	0.78

Significant effects are in bold

The artificial diet was a strong selective agent that significantly impacted the diversity and structure of gut microbial communities in captive woodrats. The alfalfa-based chow used in these experiments contains high amounts of dietary fiber (39.4% NDF, 22.0% ADF) and is intended to be a comparable substitute for the wild diet of captive herbivorous small mammals [34]. This diet is also appropriate from a dietary standard to maintain woodrats specialized in consuming *Opuntia* cacti, which are high in fiber content (45.5% NDF, 9.4% ADF [22]). In animal models and humans, dietary fiber intake positively correlates with microbial diversity, the abundance of fiber-degrading bacteria, and enriches bacterial functional pathways associated with nondigestible carbohydrate metabolism [35, 36]. Consistent with these results, we found significant increases in alpha diversity metrics in woodrats fed the artificial diet three days after the start of dietary experiments. Furthermore, the artificial diet altered microbial communities by increasing the abundance of bacteria from the order Clostridiales and families Ruminoccocaceae
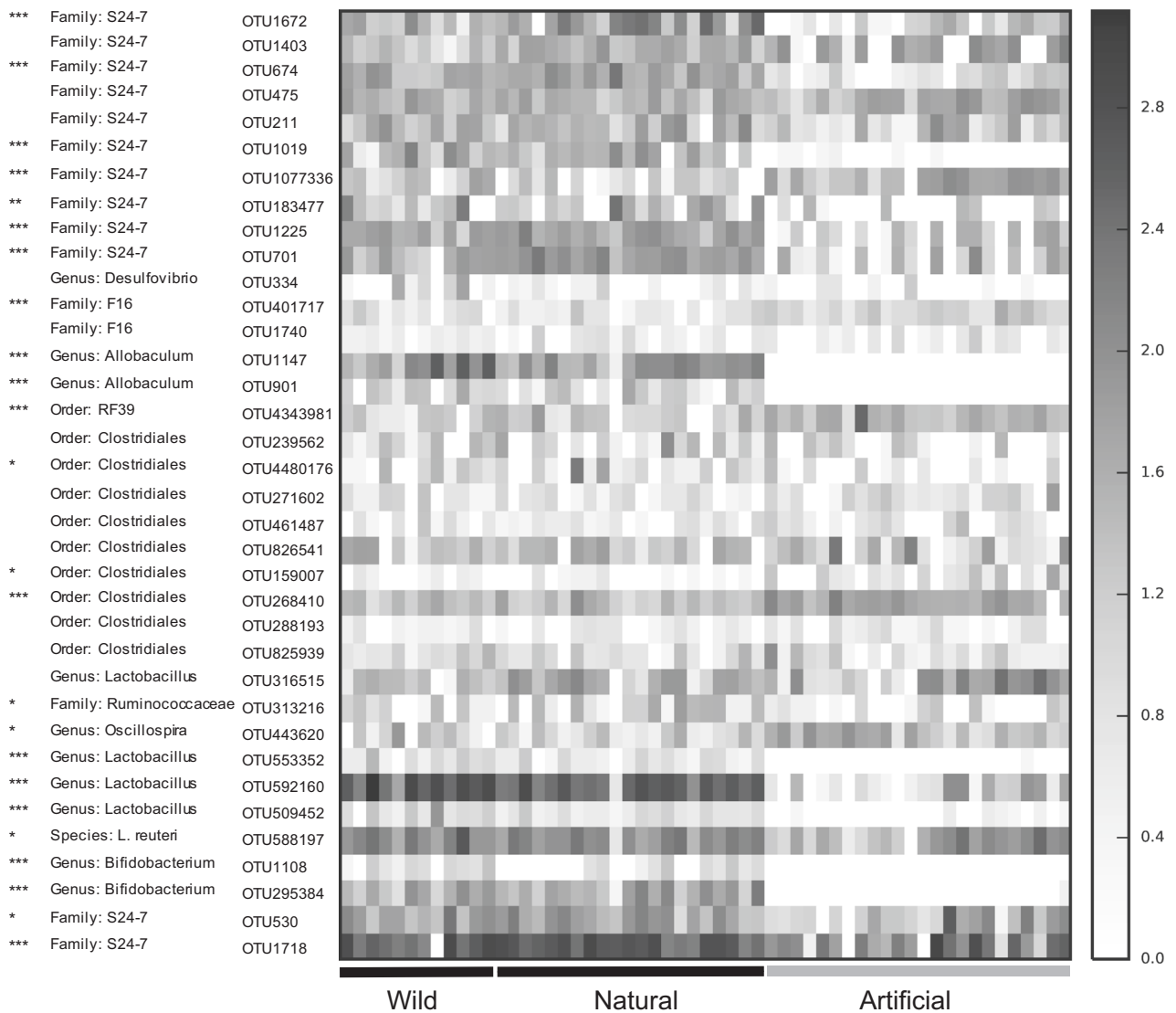
Fig. 3 Changes in log-abundance of core microbial OTUs associated with the intake of wild, natural, and artificial diets. Columns are arranged chronologically within each captive diet category, and each column represents an individual sample. Darker shades indicate higher abundances and lighter shades represent lower abundances. Core OTUs are shown to the lower taxonomic unit. *FDR*: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

and Lachnospiraceae, which are known to be involved in the metabolic conversion of nondigestible carbohydrates and production of butyric acid through their multiple carbohydrate active enzymes [37, 38]. These results suggest that the immediate exposure to a higher and different source of fiber not found in the natural diet, provided novel substrates that favored a temporary growth and expansion of normally rare microbial taxa. In addition, microbe–microbe competition could have occurred [39], and bacteria that used the artificial substrate might have suppressed the growth of microbes maintained by natural substrates.

The microbial community structure partially returned to the original wild state in woodrats fed the artificial diet. After the second week in captivity, the initially declining

abundances of several bacterial groups (unclassified family of Clostridiales and S24-7) in the artificial dietary treatment tended to recover to levels similar to the wild samples. These results suggest that some microbial taxa of the woodrats' gut microbiota exhibit resilience (i.e., rate of recovery after disturbance) when exposed to sudden dietary perturbations. Resilience is an attribute observed in several microbial communities [40] that could enable these microbial families to recover after a period of time on the artificial diet. Also, gut microbial communities may contain resident strains that contribute to long-term community stability [41]. These microbial taxa may have rapid, flexible responses allowing them to exploit new energy sources contained in the artificial diet, and through this, increase
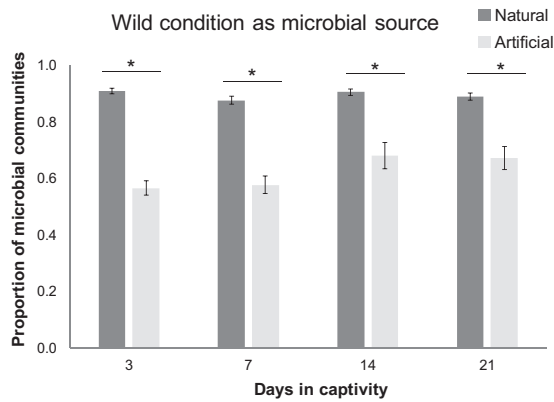
Fig. 4 Proportion of microbial communities from captive woodrats having the wild condition as most likely origin. Results based on Source-Tracker analysis. Mean proportions (± SE) over time are shown for individuals fed natural and artificial diets. *$p < 0.001$ (*FDR*)

their abundance after a period of time [42]. As a species, *N. albigula* can utilize numerous food items across its distribution, with varying nutritional and chemical composition [43]. Thus, this species has likely experienced extreme selection for the development of a microbiome with bacteria capable of using different substrates and responding rapidly to dietary changes in this host species.

We found that providing natural items (i.e., *Opuntia* cacti) ameliorated the effects of captivity on the gut microbiome and contributed to the retention of the native microbiota. Woodrats brought into captivity but fed the natural diet showed a far greater retention of wild core OTUs, and had microbial communities that were more similar to the wild condition, than animals on the artificial diet. These findings indicate that offering a diet of the naturally consumed items to captive woodrats was crucial for sustaining native microbial communities outside the woodrat habitat. The shift in microbiome structure not accounted for by diet, however, could be related to a loss of nutritional substrates associated with food items that are consumed only occasionally in the wild, and which we did not offer in our experiment. Alternatively, captive woodrats may have lost microbial inoculation from other sources, such as soil or vegetation used in nest construction, or they may have experienced physiological stress that prevented the full retention of their wild-microbial communities.

Woodrats fed the natural diet showed increases in the relative abundance of the candidate family S24-7 over the entire experiment (1.2-fold increase), and this microbe was identified as a core member of the woodrat wild microbiome. Genomic characterization from other studies have indicated that the family S24-7 is enriched with enzyme genes associated with oxalate degradation, such as formyl-CoA transferase and oxalyl-CoA decarboxylase [44]. Oxalate is the primary secondary metabolite present in high abundances in Cactaceae such as *Opuntia* cacti [45], and we

have previously shown that the microbiome plays an important role in the ability of woodrats to degrade ingested oxalate [46, 47]. Our results here suggest that the provisioning of captive animals with *Opuntia* cacti during the experiment maintained oxalate availability, supplying suitable substrates for bacteria that belong to the S24-7 family.

The artificial diet resulted in the extirpation of several members of the core microbiome, i.e., microbes shared among individuals that presumably play critical metabolic functions for the host [48, 49]. We found that core OTUs assigned to the genus *Bifidobacterium*, *Allobaculum*, and *Lactobacillus* were nearly undetectable during the entire study in woodrats fed the artificial diet. This loss indicates a strong relationship between specific OTUs and wild diets. These bacterial taxa are sensitive to drastic dietary changes, and their abundances also were observed to decrease in laboratory mice fed experimental high-fat diets [50]. Both *Bifidobacterium* and *Lactobacillus* are considered important key taxa for maintaining healthy gastrointestinal tracts, due to their active roles in biotransformation of carbohydrates and fermentation, and in protection against pathogens and colonic diseases [51, 52]. Similarly, bacteria related to oxalate degradation (family S24-7) were reduced. The clearance of core bacteria resulting from chronic dietary perturbations could lead to microbial extinctions over generations [17], to the extent that former bacterial communities cannot be restored even after returning animals to predisturbance conditions. This phenomenon was exemplified in a study with a dietary specialist of juniper (*Juniperus* sp.), the Stephen's woodrat (*N. stephensi*). These woodrats were fed an artificial diet treatment for six months followed by a natural juniper-based diet. Even after reexposure to the natural diet the gut microbiome did not fully recover [2]. Although it is unclear what the immediate functional consequence of this loss is for captive woodrats, dysbiosis resulting from disruption of bacterial homeostasis can increase the risk of metabolic and digestive diseases [53].

The artificial diet not only modified microbial composition but, putatively, also microbial function. Based on PICRUSt predictive profiling, the artificial diet increased microbial metabolism of amino acids and glycan biosynthesis, and decreased microbial metabolism linked to xenobiotics and secondary compounds (e.g., terpenoids and polyketides) degradation. These changes are most likely related to the nutritional and chemical properties of the ingested food. For example, the artificial diet is supplemented with 18 amino acids and rich in complex carbohydrates, but lacks natural plant secondary compounds commonly found in the woodrat wild diet. These results suggest that metabolic functions carried out by gut microbes are impacted by commercial diets offered in captivity. Given that PICRUSt is a predictive tool, we interpret these findings cautiously.

Our results have important implications for the welfare of animals kept in captivity and laboratory conditions. We demonstrated that providing natural diets, even for few days, considerably enhances the retention of the native gut microbiome of captive animals. We are aware that recreating wild diets in captivity may not be feasible for certain animals, however, supplementing diets with more natural food items may be an alternative to support microbial communities, as observed in this research, as well as in a study with sifakas (*Propithecus coquereli* [54]). Alternatively, captive programs should carefully adjust artificial diets to reduce the impact on the gut microbiome in other ways. For example, we have observed that woodrats feeding on high-fiber chow may retain up to 64% of native microbial communities ([55], present study). Nonetheless, loss of specific microbial functions (e.g., oxalate degradation) can occur in animals on artificial diets, which in turn might be detrimental for reintroduction programs aimed at releasing animals into the wild. Altogether, these results highlight the importance of offering natural food items that are part of the regular wild diet, or at least food with similar nutritional and chemical properties as those found in nature, to maintain a wild microbiome in wild-caught animals kept in captivity for scientific, recreational, or conservation purposes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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