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The Effect of Dietary Oxalate on the Gut Microbiota of the Mammalian Herbivore *Neotoma albigula*

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Running head: Gut Microbiota Response to Dietary Oxalate

Abstract

Diet is one of the primary drivers that sculpts the form and function of the mammalian gut microbiota. However, the enormous taxonomic and metabolic diversity held within the gut microbiota makes it difficult to isolate specific diet-microbe interactions. The objective of the current study was to elucidate interactions between the gut microbiota of the mammalian herbivore, *Neotoma albigula,* and dietary oxalate, a plant secondary compound (PSC) degraded exclusively by the gut microbiota. We quantified oxalate degradation in *N. albigula* fed increasing amounts of oxalate over time and tracked the response of the fecal microbiota using high-throughput sequencing. The amount of oxalate degraded *in vivo* was linearly correlated with oxalate consumed. The addition of dietary oxalate was found to impact microbial species diversity by increasing the representation of certain taxa, some of which are known to be capable of degrading oxalate (e.g., *Oxalobacter* spp.). Furthermore, the relative abundance of 117 OTUs exhibited a significant correlation with oxalate consumption. The results of this study indicate that dietary oxalate induces complex interactions within the gut microbiota that includes an increase in relative abundance of a community of bacteria that may contribute either directly or indirectly to oxalate degradation in mammalian herbivores.

Key words: oxalate-degrading bacteria; gut microbiota; plant secondary compounds; oxalate; biotransformation

Introduction

Mammals live in a complex and largely symbiotic relationship with a gut microbiota. This microbiota harbors 150 times more genes than the host and exhibits complex interactions with the host's diet (1-3). In mammalian herbivores, diverse intestinal bacteria ferment a diet high in recalcitrant cellulose and in turn synthesize nutrients from the diet in a form more amenable to absorption by the host (4). Furthermore, mammalian herbivores harbor greater microbial diversity in their gut than either omnivores or carnivores (1). Despite the progress of research into the interactions between the mammalian gut microbiota and diet, the isolation of specific diet-microbe interactions in such a complex system has proven to be difficult (5,6).

In addition to fermentation, microbes play an important role in the biotransformation of dietary toxins in mammalian herbivores (4,7-10). For some toxins, such as oxalate or 3,4-dihydroxy pyridine (DHP), a single species of bacteria is capable of biotransforming the toxin, and this function can be transferred to other mammals through microbial transplants (7,8,11, 12). For other toxins, such as creosote resin, whole microbial community transplantation into other mammals can increase tolerance (10).

Oxalate, a widely produced and ingested plant secondary compound (PSC), serves as an excellent model to study diet-microbe interactions (13). It is the simplest organic acid and is toxic to mammals (14-16). Oxalate can bind to free calcium ions in the blood and aggregate in the kidneys to form kidney stones (17). In fact, oxalate is a constituent in 80% of kidney stones in humans (17).

Oxalate is not metabolized by mammalian enzymes, but rather is biotransformed into formate and CO_2 by gut microbes (7,18-21). While some oxalate-degrading bacteria such as *Oxalobacter formigenes* biotransform oxalate for use as a carbon and energy source, the growth of other oxalate-degrading bacteria such as *Lactobacillus acidophilus* is inhibited by the presence of oxalate, even though these bacteria will biotransform the compound when present (7,22). Additionally, the by-products of microbial oxalate degradation, formate and CO_2, may be used by a number of bacteria in the process of acetogenesis or methanogenesis, potentially benefitting other gut bacteria not directly involved in the oxalate degradation function (23). While there is no direct evidence for either acetogenesis or methanogenesis, several known acetogenic taxa are prevalent in the *N. albigula* gut, such as *Clostridium, Streptococcus,* and *Ruminococcus* (24-26). These attributes make for a unique system to isolate the interactions between dietary toxins and gut microbiota, along with their contribution to the overall metabolism of the host.

The wild mammalian herbivore *Neotoma albigula* (white-throated woodrat) is an ideal species to study the effects of dietary oxalate. Some populations of *N. albigula* consume a diet composed of nearly 100% *Opuntia spp.* cactus, which contains a high oxalate content (1.5% dry weight; 26). *Neotoma albigula* can degrade >90% of dietary oxalate when fed artificial diets of up to 9% oxalate (dry weight). This high level of oxalate degradation has been hypothesized to be the result of microbial metabolism (27,28). Furthermore, *N. albigula* harbors a diversity of known and potential oxalate-degrading bacteria distributed across the

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gastrointestinal tract, including *Oxalobacter, Lactobacillus, Clostridium,* and *Enterococcus* among others (26). Thus, *N. albigula* regularly consumes high amounts of oxalate and harbors a diversity of bacteria that exhibit complex interactions with oxalate, making it an ideal species to elucidate oxalate-microbiota interactions.

The purpose of the current study was to identify the ecological and functional interactions between dietary oxalate and the gut microbiota of *N. albigula*. This study has two primary objectives. The first is to quantify the effect of increasing oxalate consumption on oxalate degradation *in vivo.* The second is to determine if the gut microbiota of *N. albigula* exhibits a community-level response to oxalate consumption. Given the previously identified differential responses of oxalate-degrading bacteria to the presence of oxalate, we predicted that oxalate would stimulate the growth of some microbial taxa, inhibit the growth of others, while having a neutral effect on the remaining community. Our data supports the hypothesis that a specialized microbial network of bacteria is responsible for oxalate degradation in *N. albigula*.

Materials and Methods

Location, collection, and diet of animals

Six individuals of the white-throated woodrat, *N. albigula,* were collected with Sherman live traps from Castle Valley, Utah (38.63ºN, 109.41ºW), in October, 2012. Woodrats were immediately transported to the University of Utah Department of Biology Animal Facility and housed in individual cages (48 x 27 x 20 cm) under a 12:12-hr light:dark cycle, at 28°C and 20% humidity. Animals

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were maintained in captivity and fed high-fiber rabbit chow with 0.2% oxalate (Harlan Teklad formula 2031, Denver, CO, USA) for six months prior to experimentation. All methods were approved by the IACUC under protocol #12-12010.

To examine the interactions between dietary oxalate and gut microbes, animals were placed in a diet trial where oxalate was gradually increased over time (Table 1). The five-day time periods for the 0.05% oxalate diet were chosen to ensure that any effect of oxalate on the microbiota was removed while the three days for each of the oxalate diet periods were chosen based on Belenguer et al. (2013) in which three days on oxalate was long enough to elicit a microbial response. Metabolic cages were used to separate urine and feces and allow for the quantification of food and water intake, which were given *ad libitum.* In metabolic cages, *N. albigula* had access to direct coprophagy (consuming feces from the anus), but not indirect coprophagy (cache feces to consume later). To minimize the oxalate concentration of the rabbit chow without reducing food intake, a 3:1 ratio of powdered purified rat chow (Harlan, Denver, CO, USA) to powdered rabbit chow (Harlan) was used in the study. This diet contained an oxalate concentration of 0.05%, which will be referred to as “no oxalate” from here on (Table S1). Oxalate diets were prepared by mixing sodium oxalate (Fisher Scientific, Pittsburgh, PA, USA) into the powdered chow on a dry weight basis. At the end of the diet trial, all animals were returned to the no oxalate diet to ensure that any effect on the microbiota was the result of oxalate and not some other factor. Urine and feces were collected daily in sterile 50ml falcon

tubes for oxalate assays and microbial inventories. Additionally we collected data on body mass, food and water intake, along with fecal and urinary output, daily. Using the food intake and fecal output data, we estimated dry matter digestibility (DMD) as 1 – (dry fecal output ÷ food consumed). These data were evaluated with repeated measures ANOVA.

Oxalate Assays

Oxalate in the urine was quantified by following a modified protocol described by Ingale *et al.* (29). Urine samples were collected daily from each animal for the assays and pooled together for each treatment period. Urine samples were acidified with 3M HNO_3 to a pH of <3 to solubilize any oxalate crystals. Acidified urine was centrifuged to remove precipitates and the supernatant reserved. The pH of the supernatant was brought up to 7 with NaOH. Approximately 0.1g of $CaCl_2$ was added and mixed to precipitate oxalate. Samples were then centrifuged and decanted. A volume of distilled water matching the total urinary volume was added to calcium oxalate precipitate. Samples were then titrated as described below.

For fecal oxalate assays, feces for each animal were collected daily, dried at 45ºC overnight, and pooled by animal at the end of each treatment period. Oxalate assays were conducted following a modified protocol from Justice (28). Approximately 0.4g of dried feces were ground and added to 5ml 6N H_2SO_4 for 15 minutes to solubilize oxalate. After 15 minutes, 25ml of distilled water was added and the entire solution was filtered through Grade 4 Whatman filter paper.

The filtrate was brought up to a pH of 7 with NaOH and 0.1g $CaCl_2$ was added to precipitate oxalate. The samples were centrifuged and decanted. After centrifugation, a volume of distilled water equal to that recovered after filtration was added and the samples were titrated.

The urine and fecal extracts containing calcium oxalate were titrated in 5ml aliquots with 0.01M $KMnO_4$ in triplicate. Aliquots were first acidified with 1ml 6N H_2SO_4 and heated to 70-90ºC. The $KMnO_4$ was then added until a pink color persisted for 30sec and the volume of $KMnO_4$ was recorded. These volumes were then compared to a standard curve. Standard curves were made by adding 0mM, 5mM, 10mM, 15mM, or 20mM of sodium oxalate to the urine or feces of wood rats consuming 0.05% oxalate. After extraction and titration, the volume of $KMnO_4$ required to titrate samples with no oxalate added was subtracted from all samples to account for endogenous oxalate production. With these methods, we are able to recover 102.69 ± 12.94% of the oxalate from urine and 97.47 ± 6.78% of the oxalate from feces. Both titration curves were linear with r^2 values >0.9.

To estimate how much dietary oxalate was being degraded, we quantified the difference between oxalate consumed and total oxalate excreted. This estimate is conservative given that some endogenously produced oxalate is excreted in the urine and feces that is not accounted for with this method. However, our estimates of total oxalate excretion on the no oxalate diet indicate that the endogenous contribution is typically small (<10% of oxalate consumed on a 0.5% oxalate diet). Furthermore, given that endogenous oxalate production is determined by the consumption of certain dietary precursors, it should not

change under the diet regime used in this study and is unlikely to impact the conclusions drawn (15).

Microbial Inventories

We collected fresh feces for microbial inventories on the last day of each diet treatment, which were frozen at -80ºC until DNA extraction. DNA was extracted from 180-220g of feces using the QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD, USA). DNA extractions were also performed on oxalate, food, and reagents of the extraction kit to identify potential sources of contamination. Microbial inventories from a total of 36 fecal samples were generated by amplifying the V4 region of the 16S rRNA gene with the primers 515F and 806R (31). Primers contained a 12 base barcode sequence, which allowed for multiplexing of samples within a single-lane sequencing run on an Illumina MiSeq with paired end sequencing of 150 base pairs each, as previously described (31).

Sequences were analyzed using QIIME (32). Standard quality control was conducted and sequences were demultiplexed using default parameters in QIIME. A *de novo* picking strategy was used to classify operational taxonomic units (OTUs) with UCLUST (33) with a minimum sequence identity of 97%. This strategy resulted in an OTU table and phylogenetic tree, which were used in downstream analyses. Sequences identified as chloroplasts, mitochondria, or that had fewer than 10 representations across the dataset were removed. Additionally, samples of microbial communities with fewer than 3000 sequence reads total were removed from further data analysis. For comparative analyses,

197 samples were rarified to an equal sampling depth of 27378, which was the
198 highest number that included all samples remaining after quality control.

199 We calculated the α-diversity metrics species richness (Margalef's
200 richness index), evenness (equitability), and Shannon-Index. Community
201 membership and structure were determined using unweighted and weighted
202 UniFrac analyses respectively to compare microbial community similarity across
203 individuals and diet treatments. Unweighted UniFrac analysis compares
204 community membership, whereas the weighted analysis also takes into
205 consideration relative abundance (34). Comparisons were made with an analysis
206 of similarity (ANOSIM) after 999 permutations. Additionally, a repeated
207 measures Pearson correlation analysis between the relative abundance of an
208 OTU and oxalate consumption was conducted for all samples and OTUs. The
209 open source software QIIME was used for diversity, ANOSIM, and correlation
210 metrics with a False Discovery Rate (FDR) correction for the Pearson correlation.
211 Significance was set at a P-value <0.05 for all analyses.

212 **Results**

213 *Oxalate Degradation*

214 Body mass, food intake, DMD, water intake, and urine output, did not differ
215 significantly among treatments (Table 2). Oxalate intake increased significantly
216 with increasing dietary oxalate concentration (P<0.001), and the amount of
217 oxalate degraded correlated significantly with oxalate consumption (Figure 1).
218 When excretion of endogenous oxalate is taken into consideration (i.e., by

subtracting the amount excreted on the no oxalate diet), oxalate degradation exceeded 90% of that consumed regardless of concentration in the diet. Furthermore, 94-99% of the excreted dietary oxalate was found in the feces, indicating that little oxalate was absorbed into the blood.

Response of Gut Microbiota

High-throughput sequencing yielded a total of 2,208,347 high-quality sequences of 150 overlapping base pairs. The dataset from one animal was removed because two of the microbial inventories contained <3000 sequence reads. Furthermore, a total of 38,723 OTUs were removed from dataset, having fewer than 10 sequence reads total. The remaining inventories contained an average of 69,010 ± 5,353 sequences per sample. Rarefaction analysis concluded that the diversity at 27,378 is a good estimate of the true diversity (Figure S1). With a cut-off of 27.378, and additional 15 OTUs were removed from the dataset. When DNA was extracted from oxalate or food, and used as template for PCR using universal 16S rRNA primers, no amplification products were detected following gel electrophoresis. Similarly, the DNA extraction reagents used in the study yielded no PCR amplification of 16S rRNA, indicating that there was no detectable contamination. Across all fecal samples, sequences were assigned to 6232 OTUs. Of these OTUs, 97.6% were assigned to 14 bacterial phyla with 25.3% assigned to 88 genera. The fecal microbiota showed a composition typical of woodrats and other mammals that was dominated by *Bacteroidetes,* particularly the family S24-7 that comprised between 49.8-67.2% of the microbiota (26). There were no significant differences in community

membership or community structure across treatments, based on ANOSIM analysis (P=0.496 and 0.691 respectively; Figure 2). Species richness and Shannon Index increased significantly with dietary oxalate concentration; however, evenness did not differ significantly (Figure 3). Species richness correlated significantly with oxalate consumption (Figure 4). However, a repeated measures ANOVA followed by a post-hoc Tukey's analysis revealed that only the species richness on a 3% oxalate diet was significantly different than on the no oxalate diet (Figure 3A). This shift in α-diversity prompted us to further investigate the microbial involvement in oxalate biotransformation.

Of the 6232 identified OTUs, a total of 116 OTUs exhibited a significant positive correlation (P<0.05 after a False Discovery Rate correction) with oxalate consumption while one OTU exhibited a negative correlation (Table 3). Those OTUs exhibiting a positive correlation included known oxalate-degrading bacteria: *Oxalobacter,* another *Oxalobacteraceae sp., Clostridiales,* and *Lachnospiraceae* among others. The taxonomic clade with the greatest number of OTUs that exhibited a positive correlation was the S24-7 family.

A subset of identified OTUs were shared across all animals and treatments. A total of 103 OTUs were present in all six animals on the no oxalate diet and 282 OTUs were shared on the 3% oxalate diet, including all of those present in all animals on the no oxalate diet (Table S2).

Discussion

The current study sought to address two important gaps in gut microbiota research. First, there is a need to understand the factors that contribute to changes in the form and function of the mammalian gut microbiota, both to aid in the development of personalized therapies and to advance ecological theories (5,6). However, studying these factors is confounded by the complexity inherent within the gut microbiota with its immense and variable diversity, and considerable microbe-microbe and microbe-host interactions (2,6,35). Second, there is a need to understand how oxalate affects the mammalian gut microbiota as a whole. Previous research has focused on the role of individual taxa in oxalate degradation (7,20,36,37,38). However, several oxalate-degrading taxa have now been identified from the mammalian gut and other taxa may be affected by oxalate in obscure ways (20,26,39,40). To address the gaps, we combined controlled laboratory diet trials, physiological assays, and microbial ecology to examine the taxonomic and functional response of the whole gut microbiota in a mammalian herbivore, *N. albigula,* which naturally consumes high amounts of oxalate in its diet, a simple compound that is metabolized exclusively by the gut microbiota (18,26).

The microbiota of *N. albigula* is exceptional in its capacity to degrade oxalate. The animals exhibited no adverse effects associated with oxalate intake (Table 1) and the microbiota was capable of degrading >90% of dietary oxalate regardless of the amount of oxalate consumed, showing a strong microbial response to oxalate consumption. Studies conducted on other mammals indicate that the level of dietary oxalate degradation in *N. albigula* is unique (27,28). The

Norway rat, *Rattus norvegicus,* becomes hyperoxaluric on a 1.5% oxalate diet (42, unpublished data), whereas another study demonstrated that *N. albigula* can tolerate 9% oxalate with no detrimental effect (27). One potential morphological characteristic that may facilitate oxalate degradation in *N. albigula* is the presence of a foregut that houses a microbiota with a high potential for oxalate degradation (10,26). In metabolic cages, *N. albigula* have access to direct coprophagy (consuming feces from the anus), which may help to inoculate the foregut with oxalate-degrading bacteria. Given the results of the current and previous studies, the gut microbiota of *N. albigula* appears to have a considerable capacity for oxalate degradation, indicative of a rapid microbial response to oxalate consumption.

Our work shows that dietary oxalate affects both the microbial community diversity as a whole in *N. albigula* as well as the relative abundance of specific OTUs. The correlation between oxalate consumption and species richness (Figure 3A) suggests that OTUs that were present below detectable limits on a no oxalate diet increased in relative abundance with higher oxalate consumption to detectable levels. Such a correlation between the consumption of (natural) dietary toxins and gut microbiota diversity has been observed in other woodrat studies and is likely indicative of a dynamic, community-wide adaptation to dietary change (43). Although there was a strong individual signature to the gut microbiota in the current study, some OTUs were both broadly distributed among animals in general and exhibited a significant correlation with oxalate consumption (Tables 3,S2). The subset of microbes that increased with oxalate

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consumption may represent a core community of microbes essential for the function of oxalate degradation, or an “oxalate microbiome”.

A core gut microbiota has previously been associated with diverse mammalian host phenotypes (44-46). In the current study, we have identified a core set of bacteria that are commonly distributed across individuals and are responsive to oxalate, suggesting that this microbial network may be important in reducing oxalate absorption in *N. albigula.* Some of the bacteria in this group, such as *Oxalobacter,* may engage in oxalate degradation directly. Others such as *Oscillospora* and *Clostridiales* may benefit indirectly from oxalate degradation possibly via acetogenesis and facilitate the continued presence of those bacteria that degrade oxalate.

Strategies to utilize known oxalate-degrading bacteria as probiotic therapies to reduce urinary oxalate excretion in humans and rat models typically only result in an ephemeral reduction of urinary oxalate and a transient colonization by the probiotic bacteria (11,37,42,47,48). This is in contrast to mammals that are natural hosts to oxalate-degrading bacteria, such as the animals in the current study, that maintain those populations and their associated functions across generations and respond to increasing dietary oxalate even after long periods of time without oxalate in the diet (38,49,50). The transient colonization of the oxalate-degrading bacteria following probiotic treatment suggests that these transplanted bacteria are unable to integrate successfully into a foreign community, implying that there are underlying mechanisms of support for these bacteria in their natural hosts.

The S24-7 family appears to play a critical role both in the oxalate microbiome specifically and in the gut microbiota of *N. albigula* generally. This family comprised 43% of the OTUs that exhibited a significant correlation with oxalate consumption and consistently makes up >50% of the entire gut microbiota in *N. albigula* (10, this study). This family is commonly found in rats, mice, goats, and humans, and has also been correlated with a high fat diet, immunoglobin A, tapeworms, etc. Thus, the S24-7 family may generally be sensitive to dietary shifts (51-55). Given the widespread distribution of this family and correlation with a number of dietary components, S24-7 represents a significant gap in our understanding of the gut microbiota form and function.

Oxalate is a simple molecular compound with characteristics that make it amenable to elucidating specific diet-microbiota interactions within the mammalian gut. In the current study, we were able to predict identities for a sub-community of microbes that exhibits a strong, rapid response to oxalate ingestion. Our results suggest that a distinct oxalate metabolizing microbiome exists that increases in abundance when oxalate is consumed. Furthermore, we have shown that the methods utilized here are effective at identifying sub-communities within the mammalian gut microbiota that engage in a particular function of interest that may be useful to manipulate in a therapeutic context.

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References

1. **Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R.** 2008. Evolution of mammals and their gut microbes. Science **320**(5883):1647-1651.

2. **Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, MetaHIT Consortium, Bork P, Ehrlich SD, Wang J.** 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature **464**(7285):59-65.

3. **Muegge BD, Kuczynski J, Knights D, Clemente JC, Gonzalez A, Fontana L, Henrissat B, Knight R, Gordon JI.** 2011 Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science **332**(6032):970-974.

4. **Karasov WH, Carey HV.** 2009. Metabolic teamwork between gut microbes and host. Micro. **4**(7):323-328.

5. **Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R.** 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. **6**(5):1007-1017.

6. **Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R.** (2012) Diversity, stability and resilience of the human gut microbiota. Nature **489**(7415):220-230.

7. **Allison MJ, Dawson KA, Mayberry WR, Foss JG.** 1985. *Oxalobacter formigenes* gen. nov., sp. nov.: Oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch. Microbiol. **141**(1):1-7.

8. **Jones R, Megarrity, R.** 1986. Successful transfer of DHP-degrading bacteria from hawaiian goats to australian ruminants to overcome the toxicity of *Leucaena*. Aust. Vet. J. **63**(8):259-262.

9. **Sundset MA, Barboza PS, Green TK, Folkow LP, Blix AS, Mathiesen SD.** 2010. Microbial degradation of usnic acid in the reindeer rumen. Naturwissenschaften **97**(3):273-278.

10. **Kohl KD, Weiss RB, Cox J, Dale C, Dearing MD.** 2014. Gut microbes of mammalian herbivores facilitate intake of plant toxins. Ecol. Lett. **17**(10):1238-1246.

11. **Hatch M, Gjymishka A, Salido EC, Allison MJ, Freel RW.** 2011. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with O*xalobacter*. Am. J. Physiol. Gastrointest. Liver Physiol. **300**(3):G461-9.

12. **Wallace JR.** 2008. Gut microbiology-broad genetic diversity, yet specific metabolic niches. Animal. **2**:661-668.

13. **Franceschi VR, Nakata PA.** 2005. Calcium oxalate in plants: Formation and function. Ann. Rev. Plant Bio. **56**:41-71.

14. **James LF, Butcher JE.** 1972. *Halogeton* poisoning of sheep: Effect of high level oxalate intake. J. Anim. Sci. **35**(6):1233-1238.

15. **Conyers RA, Bais R, Rofe AM.** 1990. The relation of clinical catastrophes, endogenous oxalate production, and urolithiasis. Clin. Chem. **36**(10):1717-1730.

16. **Massey LK, Roman-Smith H, Sutton RA.** 1993. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. J. Am. Diet. Assoc. **93**(8):901-906.

17. **Moe OW.** 2006. Kidney stones: pathophysiology and medical management. The Lancet **367**(9507):333-344.

18. **Hodgkinson A.** 1977. Oxalic acid in biology and medicine. New York: Academic Press.

418 19. **Allison MJ, Cook HM, Milne DB, Gallagher S, Clayman RV.** 1986. Oxalate
419 degradation by gastrointestinal bacteria from humans. J. Nutr. **116**(3):455-460.
420 20. **Hokama S, Honma Y, Toma C, Ogawa Y.** 2000. Oxalate-Degrading
421 *Enterococcus faecalis*. Microbiol. Immunol. **44**(4):235-240.
422 21. **Ren Z, Pan C, Jiang L, Wu C, Liu Y, Zhong Z, Ran L, Ren F, Chen X,**
423 **Wang Y, Zhu Y, Huang K.** 2011. Oxalate-degrading capacities of lactic acid
424 bacteria in canine feces. Vet. Microbiol. **152**(3):368-373.
425 22. **Campieri C, Campieri M, Bertuzzi V, Swennen E, Matteuzzi D, Stefoni S,**
426 **Pirovano F, Centi C, Ulisse S, Famularo G, De Simone C.** 2001. Reduction of
427 oxaluria after an oral course of lactic acid bacteria at high concentration. Kidney
428 Int. **60**(3):1097-1105.
429 23. **Drake HL** 2012. Acetogenesis. Springer Science and Business Media: New
430 York, NY.
431 24. **Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal**
432 **J, Garcia P, Cai J, Hippe H, Farrow JAE.** 1994. The phylogeny of the genus
433 *Clostridium*: proposal of five new genera and eleven new species combinations.
434 Int. J. Syst. Bacter. **44**(4): 812-826
435 25. **Leclerc M, Bernalier A, Donadille G, Lelait M.** 1997. H_2/CO_2 metabolism in
436 acetogenic bacteria isolated from the human colon. Anaerobe. **3**(5): 307-315.
437 26. **Miller AW, Kohl KD, Dearing MD.** 2014. The gastrointestinal tract of the
438 white-throated woodrat (*Neotoma albigula*) harbors distinct consortia of oxalate-
439 degrading bacteria. Appl. Environ. Microbiol. **80**(5):1595-1601.

27. **Shirley EK, Schmidt-Nielsen K.** 1967. Oxalate metabolism in the pack rat, sand rat, hamster, and white rat. J. Nutr. **91**:496-502.

28. **Justice KE.** 1985. Oxalate digestibility in *Neotoma albigula* and *Neotoma mexicana*. Oecologia **67**(2):231-234.

29. **Ingale KG, Thakurdesai PA, Vyawahare NS.** 2012. Effect of *Hygrophila spinosa* in ethylene glycol induced nephrolithiasis in rats. Indian J. of Pharmacol. **44**(5):639.

30. **Caporaso JG, Lauber CL, Walters, WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R.** 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA **108**(1):4516-4522.

31. **Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens S, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R.** 2012. Ultra-high-throughput microbial community analysis on the illumina HiSeq and MiSeq platforms. ISME J. **6**(8):1621-1624.

32. **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R.** 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Meth **7**(5):335-336.

33. **Edgar RC.** 2010. Search and clustering orders of magnitude faster than BLAST. Bioinform. **26**(19):2460-2461.

463 34. **Lozupone CA, Hamady H, Knight R.** 2006. UniFrac – an online tool for
464 comparing microbial community diversity in a phylogenetic context. BMC
465 Bioinform. **7**(1):371.
466 35. **Costello EK, Stagaman K, Dethlefsen L, Bohannan BJ, Relman DA.**
467 2012. The application of ecological theory toward an understanding of the human
468 microbiome. Science **336**(6086):1255-1262.
469 36. **Daniel SL, Hartman PA, Allison MJ.** 1987. Intestinal colonization of
470 laboratory rats with *Oxalobacter formigenes*. Appl. Environ. Microbiol.
471 **53**(12):2767-2770.
472 37. **Hoppe B, Beck B, Gatter N, Von Unruh G, Tischer A, Hesse A, Laube N,**
473 **Kaul P, Sidhu H.** 2006. *Oxalobacter formigenes*: A potential tool for the
474 treatment of primary hyperoxaluria type 1. Kidney Int. **70**(7):1305-1311.
475 38. **Belenguer A, Ben Bati M, Hervás G, Toral PG, Yáñez-Ruiz DR, Frutos P.**
476 2013. Impact of oxalic acid on rumen function and bacterial community in sheep.
477 Ani. **7**(6): 940-947.
478 39. **Murphy C, Murphy S, O'Brien F, O'Donoghue M, Boileau T, Sunvold G,**
479 **Reinhart G, Kiely B, Shanahan F, O'Mahony L.** 2009. Metabolic activity of
480 probiotics-Oxalate degradation. Vet. Microbio. **136**(1-2):100-107.
481 40. **Turroni S, Bendazzoli C, Dipalo SCF, Candela M, Vitali B, Gotti R, Brigidi**
482 **P.** 2010. Oxalate-degrading activity in *Bifidobacterium animalis* subsp. *lactis:*
483 Impact of acidic conditions on the transcriptional levels of the oxalyl-CoA
484 decarboxylase and formyl-CoA transferase genes. Appl. Environ. Microbio.
485 **7**(16):5609-5620.

41. **Allison MJ, Cook HM.** 1981. Oxalate degradation by microbes of the large bowel of herbivores: The effect of dietary oxalate. Science **8**(212):675-676.

42. **Lieske JC, Tremaine WJ, De Simone C, O'Conner HM, Li X, Bergstralh E, Goldfarb DS.** 2010. Diet, but not oral probiotics, effectively reduces urinary oxalate excretion and calcium oxalate supersaturation. Kidney Int. **78**(11):1178-1185.

43. **Kohl KD, Dearing MD.** 2012. Experience matters: Prior exposure to plant toxins enhances diversity of gut microbiome in herbivores. Eco. Lett. **15**(9):1008-1015.

44. **Sekelja M, Berget I, Naes T, Rudi K.** 2011. Unveiling an abundant core microbiota in the human adult colon by a phylogroup-independent searching approach. ISME J. **5**(3):519-531.

45. **Pédron T, Mulet C, Dauga C, Frangeul L, Chervaux C, Grompone G, Sansonetti PJ.** 2012. A crypt-specific core microbiota resides in the mouse colon. M. Bio. **3**(3):e00116-12.

46. **Elli M, Colombo O, Tagliabue A.** 2010. A common core gut microbiota between obese individuals and their lean relatives? Evaluation of the predisposition to obesity on the basis of the fecal microflora profile. Med. Hypothes. **75**(4): 350-352.

47. **Sidhu H, Allison MJ, Chow JM, Clark A, Peck AB.** 2001. Rapid reversal of hyperoxaluria in a rat model after probiotic administration of *Oxalobacter formigenes*. J. Urol. **166**(4):1487-1491.

48. **Knight J, Deora R, Assimos DG, Holmes RP.** 2013. The genetic composition of *Oxalobacter formigenes* and its relationship to colonization and calcium oxalate stone disease. Urolithiasis **41**(3):187-196.

49. **Allison MJ, Littledike E, James L.** 1977. Changes in ruminal oxalate degradation rates associated with adaptation to oxalate ingestion. J. Anim. Sci. **45**(5):1173-1179.

50. Palgi N, Ronen Z, Pinshow B. 2008. Oxalate balance in fat sand rats feeding on high and low calcium diets. J. Comp. Phys. B **178:**617-622.

51. **Serino M, Luche E, Gres S, Baylac A, Bergé M, Cenac C, Waget A, Klopp P, Iacovoni J, Klopp C, Mariette J, Bouchez O, Lluch J, Ouarné F, Monsan P, Valet P, Roques C, Amar J, Bouloumié A, Théodorou V, Burcelin R.** 2012. Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut **61**(4):543-553.

52. **Kato T, Fukuda S, Fujiwara A, Suda W, Hattori M, Kikuchi J, Ohno H.** 2014. Mutiple omics uncovers host-gut microbial mutualism during prebiotic fructooligosaccharide supplementation. DNA Res. **21**(5):469-480.

53. **Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S, Dougherty J, Moulton L, Glawe A, Wang Y, Leone V, Antonopoulous DA, Smith D, Chang EB, Ciancio MJ.** 2014. Excercise prevents weight gain and alters the gut microbiota in a mouse model of high-fat diet induced obesity. PLOS One **9**(3): e92193.

54. **Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL,**

531 **Flavell RA.** 2014. Immunoglobulin A coating identifies colitogenic bacteria in
532 inflammatory bowel disease. Cell **158**(5):1000-1010.
533 55. **Kreisinger J, Bastien G, Hauffe HC, Marchesi J, Perkins SE.** 2015.
534 Interactions between multiple helminths and the gut microbiota in wild rodents.
535 Phil. Trans. R. Soc. B. **370**(1675):20140295.
536

Figure legends

Figure 1. The amount of oxalate consumed correlated with the amount of oxalate degraded (estimated by the differential between oxalate consumed and the total oxalate excreted in the urine and feces). Data was analyzed with a repeated measures Pearson correlation ($r = 0.99845$, $P < 0.001$). Oxalate consumed also increased significantly with increasing oxalate consumption as determined by a repeated measures ANOVA with a posthoc, Holm's corrected Tukey's analysis (statistical groups shown by bolded letters).

Figure 2. The relative abundance of the major phyla present within the gut at different dietary oxalate concentrations over time. The "other" category contains several phyla with minor contributions to the microbiota. Columns are ordered relative to the time series of the experiment. Neither community membership nor structure changed with oxalate treatment ($P = 0.496$ and 0.691 respectively).

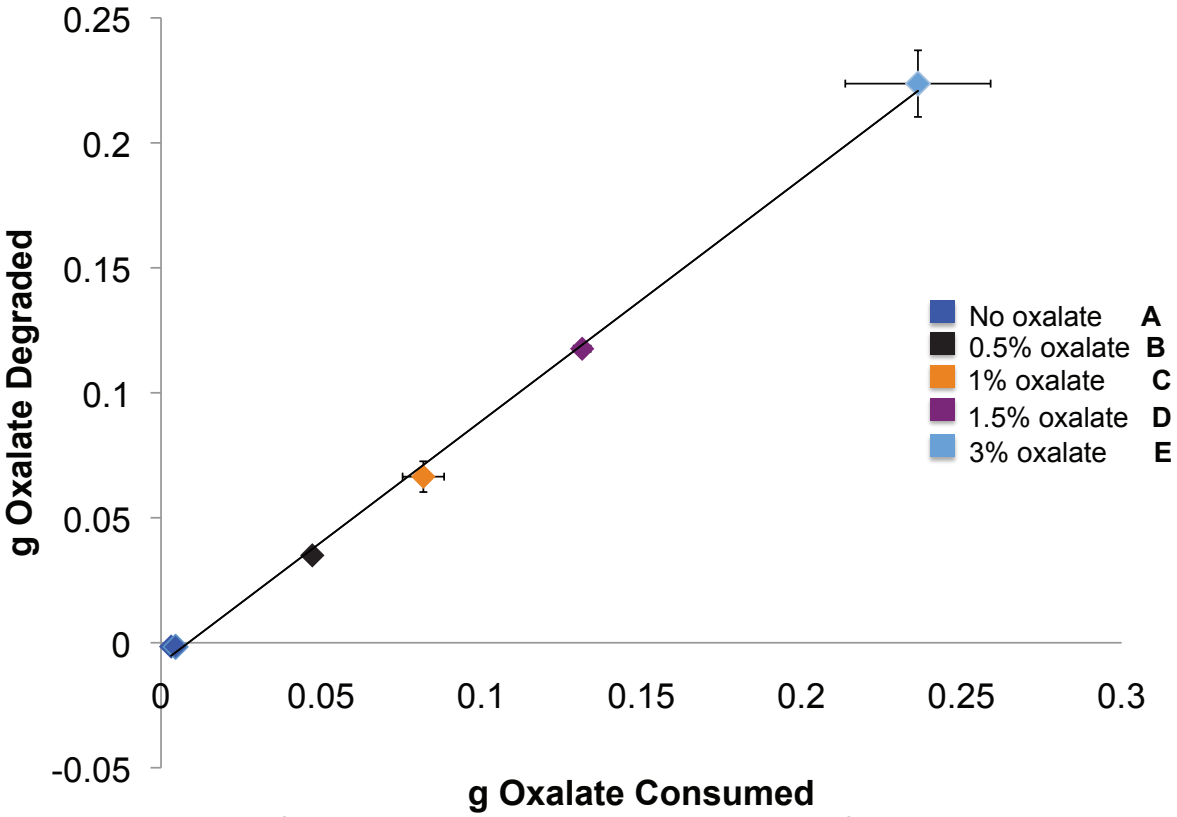
Figure 3. Alpha-diversity metrics between different dietary oxalate treatments. Species richness was determined with Margalef's richness index; species evenness was determined by their equitability; the Shannon Index is a combination of species richness and evenness. The statistical analyses were repeated measures ANOVA followed by a Holm's-corrected paired t-test. Similar letters indicate statistically similar treatment groups. Order of columns reflects the time series of the experiment.

3A) Species richness: Repeated measures ANOVA $F(5,30)=2.67$, $P=0.044$.

3B) Species evenness: Repeated measures ANOVA $F(5,30)= 1.1126$, $P=0.38$.

559 3C) The Shannon Index: Repeated measures ANOVA $F(5,30)=2.9928$, $P=0.031$.

560 Figure 4. Species richness was correlated with oxalate consumption (repeated
561 measures Pearson correlation with $P=0.001$ and $r=0.529$). Symbols represent
562 mean oxalate consumption and species richness for each treatment.



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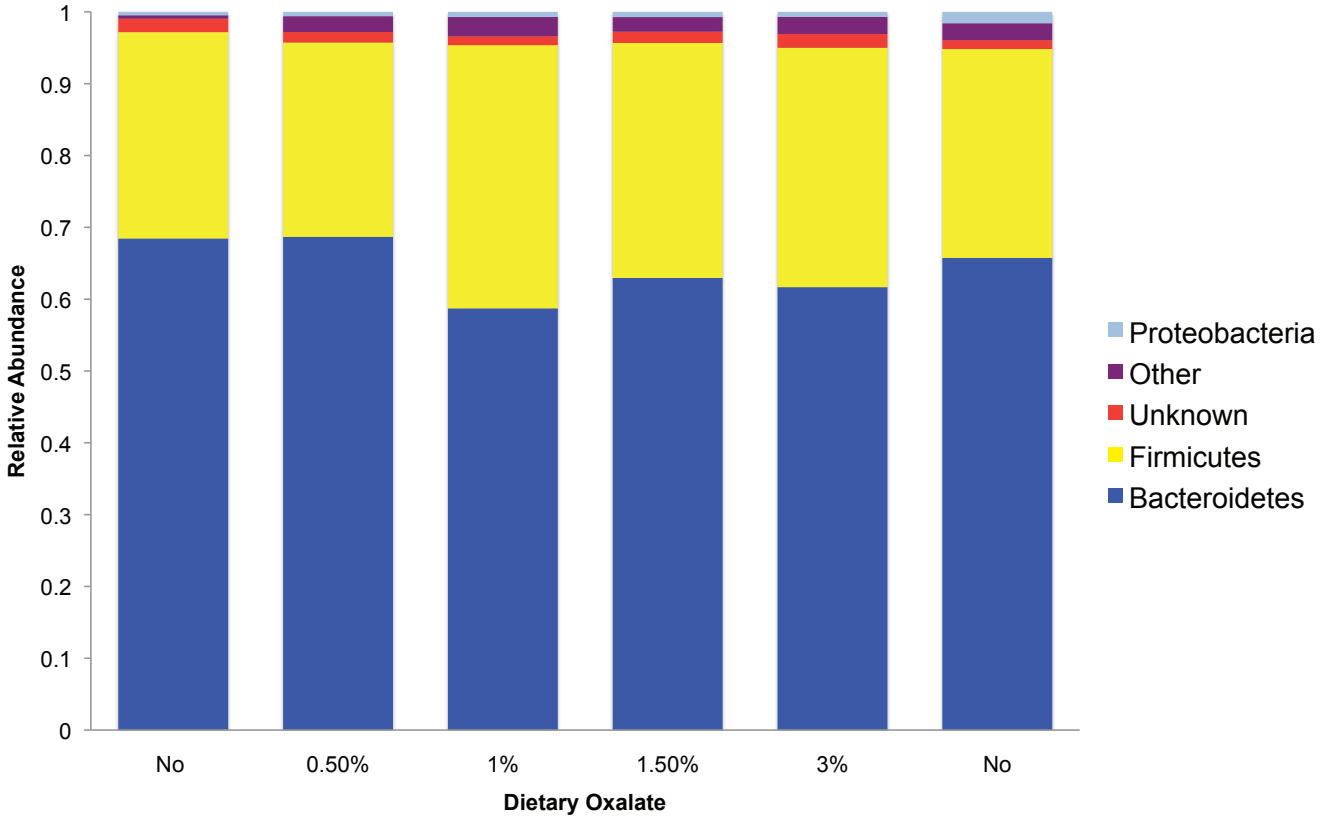

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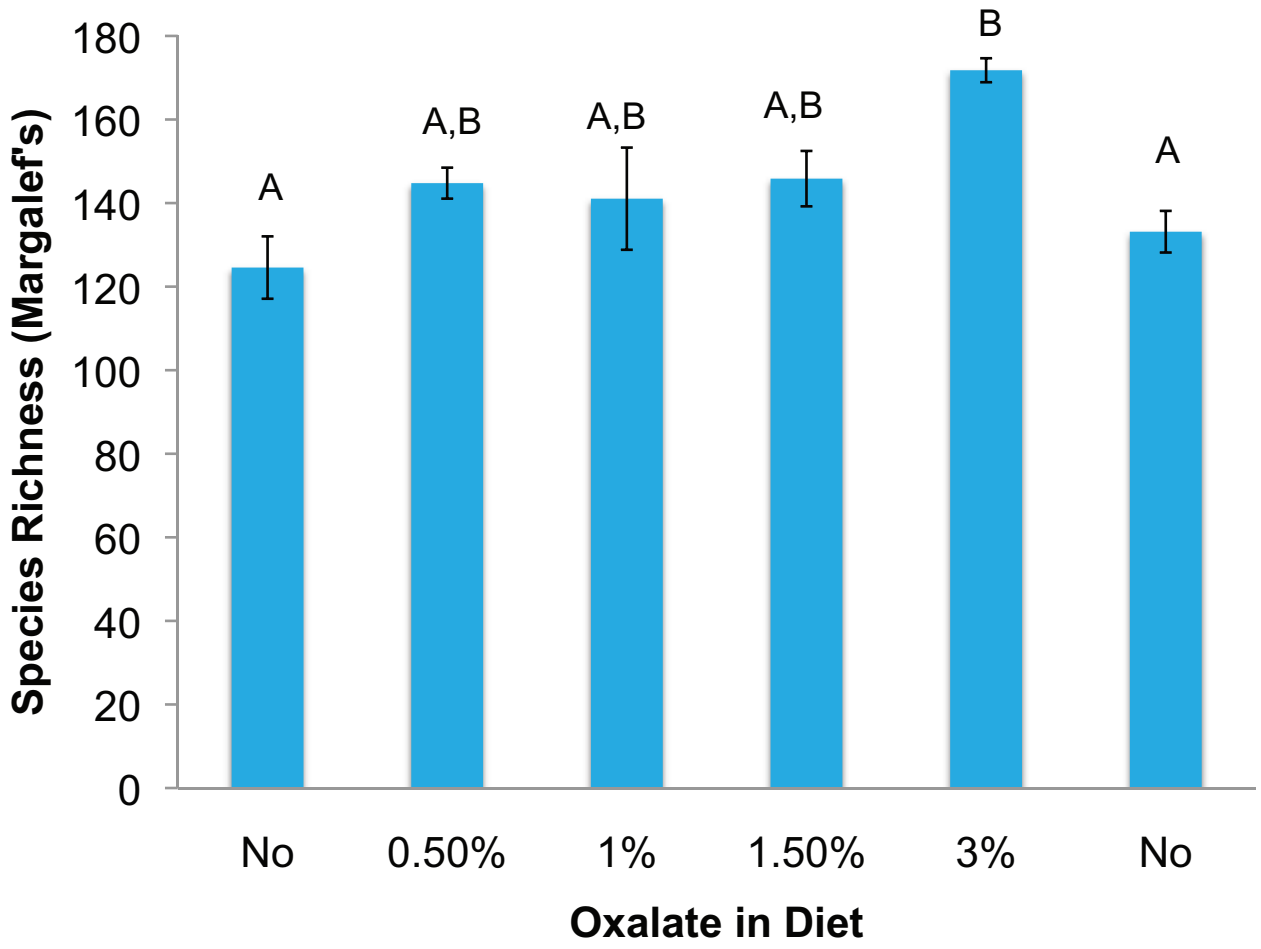

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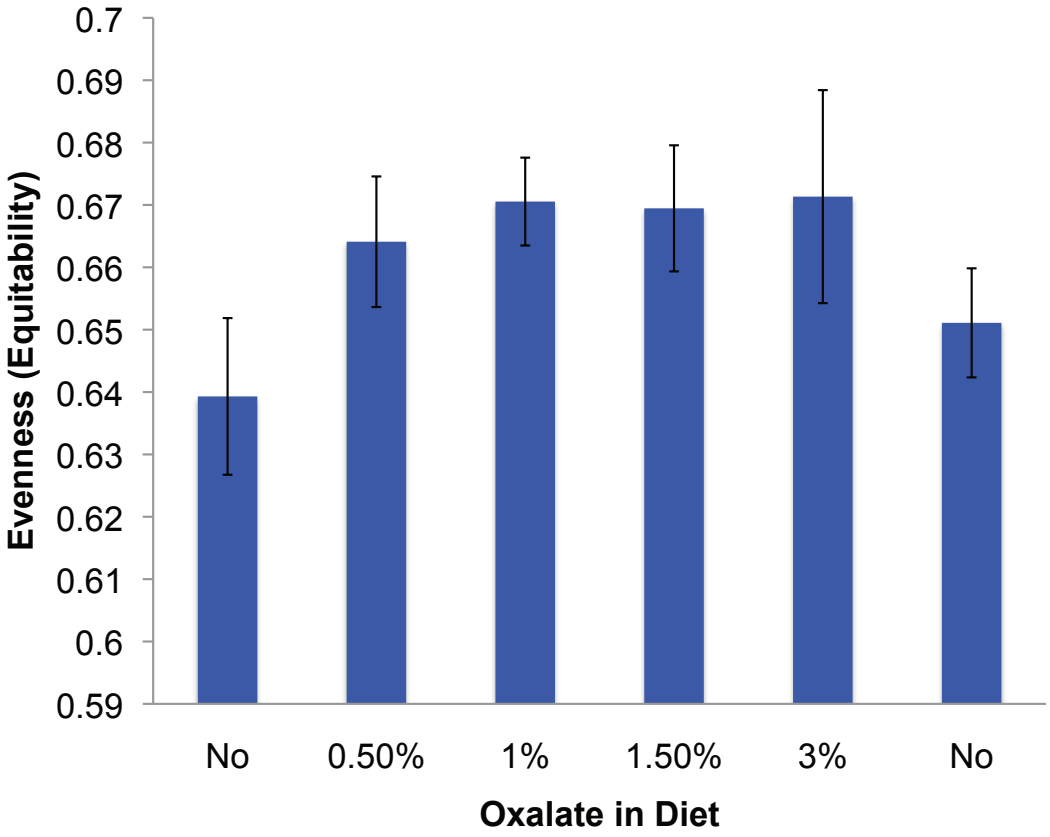

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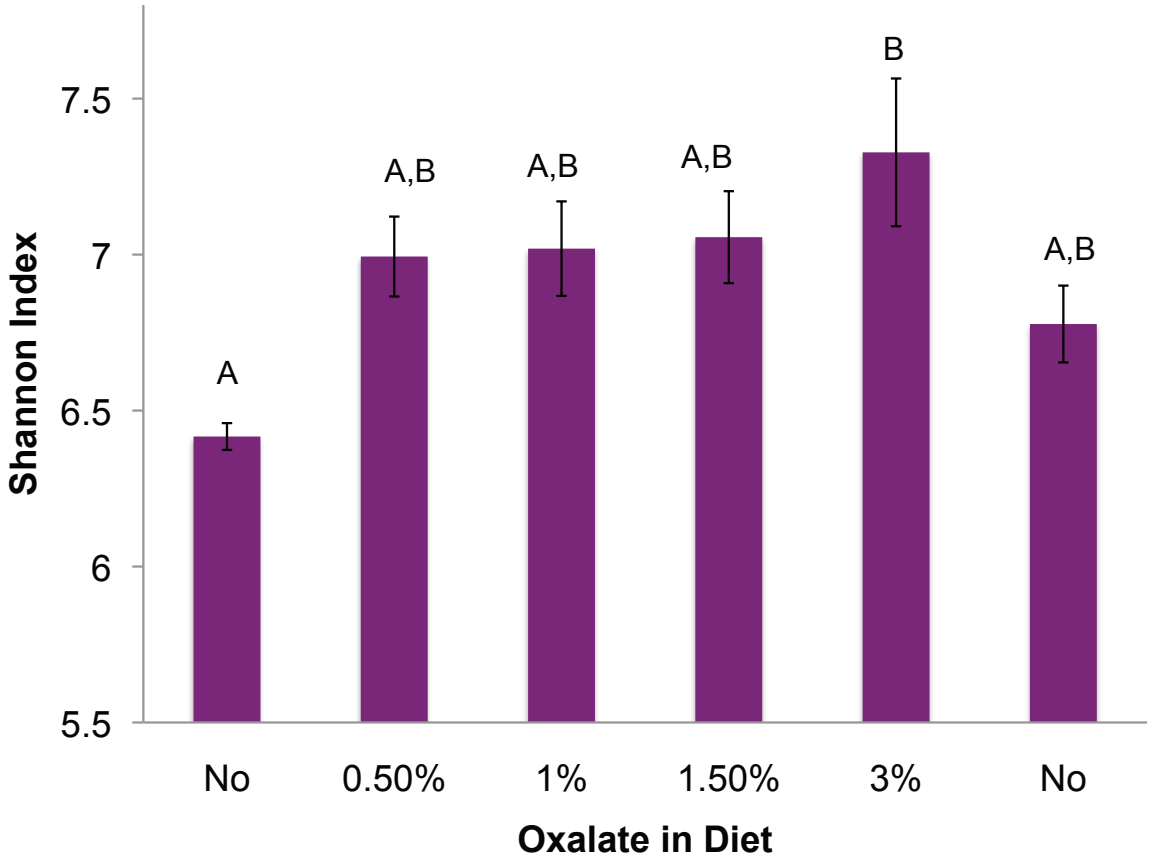

3C) The Shannon Index: Repeated measures ANOVA $F(5,30)=2.9928$, $P=0.031$.

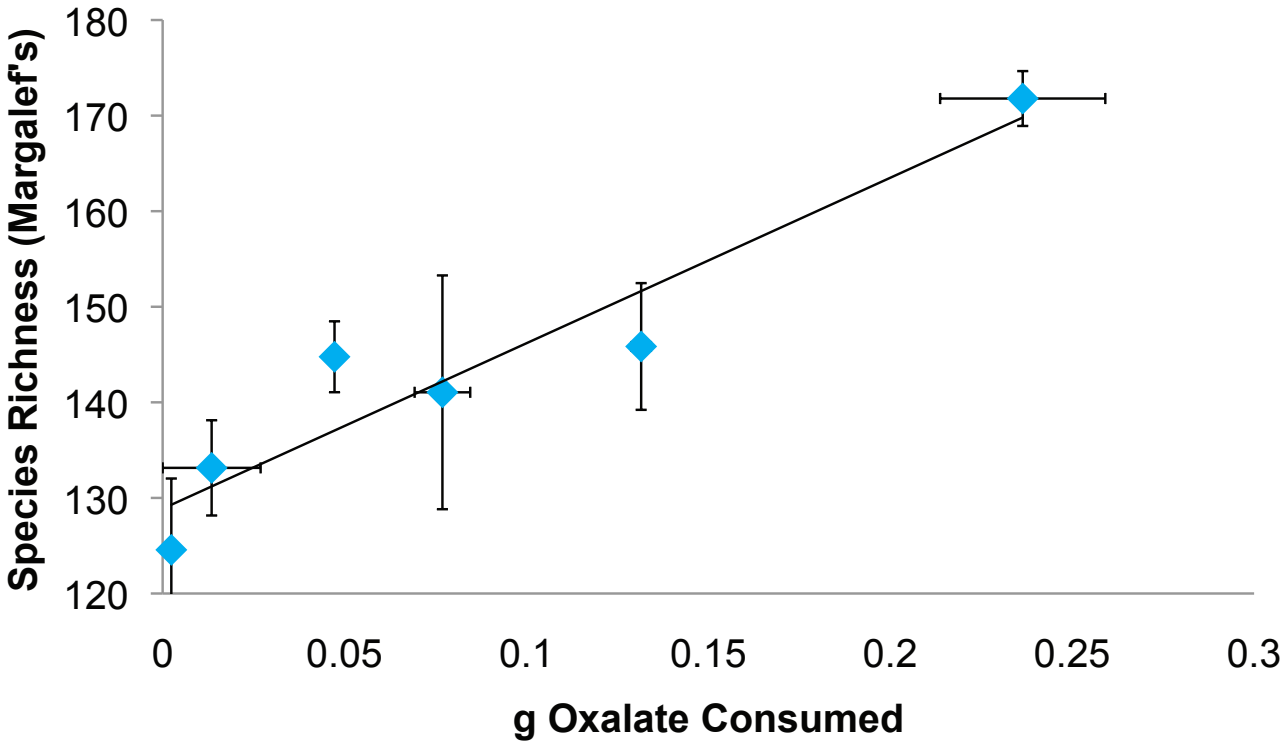

Figure 4. Species richness was correlated with oxalate consumption (repeated measures Pearson correlation with P=0.001 and r=0.529). Symbols represent mean oxalate consumption and species richness for each treatment.

Diet	Duration
No oxalate	5 days
0.5% oxalate	3 days
1% oxalate	3 days
1.5% oxalate	3 days
3% oxalate	3 days
No oxalate	5 days

Table 1. The design of the diet trial. Oxalate percentage was determined by mass.

Metric	Mean (g) +/- SE	F-value	P-value
Body Mass	171.08 +/- 12.83	0.06	1.00
Food Intake	18.71 +/- 0.06	1.66	0.18
Fecal Output	1.907 +/- 0.088	3.16	0.27
DMD	0.758 +/- 0.012	0.88	0.49
Water Intake	12.71 +/- 2.25	0.74	0.57
Urine Output	5.88 +/- 0.96	0.39	0.82

Table 2. Metrics associated with woodrats that were not significantly affected by oxalate. Means were compared over the course of the experiment with a repeated measures ANOVA (df = 5,30). Shown are the global mean for each metric.

Lowest Assigned Taxonomy	Taxonomic Level	# of OTUs	Relative Abundance 0% Oxalate	Relative Abundance 3% Oxalate	r	P
Oscillospira	genus	4	$1*10^{-4}$	$5.8*10^{-3}$	0.54 ± 0.037	0.009-0.037
Oxalobacter	genus	1	$6.39*10^{-5}$	$5.24*10^{-4}$	0.54	0.031
Clostridiales	order	16	9.13*10-6	$1.03*10^{-3}$	0.52 ± 0.015	0.009-0.04
Ruminococcus	genus	5	0	$3.29*10^{-4}$	0.52 ± 0.015	0.031-0.038
Allobaculum	genus	3	0	$3.29*10^{-4}$	0.51 ± 0.017	0.031-0.034
S24-7	family	50	$1.01*10^{-3}$	$1.27*10^{-2}$	0.51 ± 0.007	0.009-0.041
Lactobacillus	genus	1	0	$2.44*10^{-5}$	0.51	0.031
Oxalobacteraceae	family	1	$5.48*10^{-5}$	$6.09*10^{-4}$	0.51	0.032
RF39	order	1	0	$1.22*10^{-5}$	0.51	0.031
Bifidobacterium	genus	3	$9.13*10^{-6}$	$1.83*10^{-3}$	0.50 ± 0.022	0.031-0.04
Unassigned	N/A	19	$4.57*10^{-5}$	$8.52*10^{-4}$	0.49 ± 0.007	0.031-0.044
Ruminococcaceae	family	3	2.74*10-5	$3.17*10^{-4}$	0.48 ± 0.02	0.031-0.044
Lachnospiraceae	family	3	$3.65*10^{-5}$	$2.19*10^{-4}$	0.48 ± 0.017	0.031-0.043
Rikenellaceae	family	4	$9.13*10^{-6}$	$1.87*10^{-3}$	0.48 ± 0.004	0.034-0.037
Coprococcus	genus	1	0	2.44*10-5	0.48	0.034
Proteus	genus	1	0	2.44*10-5	0.46	0.04
Salinibacterium	genus	1	$2.74*10^{-5}$	0	-0.46	0.039

Table 3. Microbial OTUs (out of 6232) whose relative abundances were positively correlated with oxalate intake (Pearson Correlation regression analysis, with a False Discovery Rate (FDR) correction for multiple comparisons). For taxa with multiple OTUs that were correlated with oxalate consumption, the average r-

values and range of P-values are given and the relative abundance refers to the group of OTUs as a whole.

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