Effects of dietary shift and altered helminth infection on the gut microbiota of two sympatric rodents in urban environments

都市化にともなう食性と寄生虫相の改変が同所的に生息する齧歯類の腸内細菌叢に及ぼす影響

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Summary

Urban areas represent the most extreme form of anthropogenic ecosystem modification and have a profound effect on the wildlife that inhabit them. One area of recent but rapidly increasing interest is how urbanization affects the gut microbial communities of wild animals. The gut microbiota is pivotal for proper development, nutritional uptake, and immune system function and therefore essential to maintaining overall health. Plasticity in what constitutes a healthy gut microbial community may aid in the successful adaption to rapidly changing environments. Although studies have shown distinct changes in the gut microbiome of urban animals as compared to conspecifics in less disturbed habitats, few studies have investigated the underlying causes. Furthermore, differences in among species life histories such as dietary niche should impact how the gut microbiota is altered within urban environments due to host species-specific requirements in functionality, yet no study has investigated changes in multiple species experiencing the same degree of urbanization. The focus of this thesis is to understand how the gut microbiota of two sympatric species of rodents, the large Japanese field mouse (*Apodemus speciosus*) and the grey red-backed vole (*Myodes rufocanus*), is altered within urban ecosystems and what factors may be affecting those changes. In chapter 2, I characterize differences in the gut microbial community along the gastrointestinal tract (i.e. small intestine, cecum, colon, and rectum) of each species as well as between species differences in individuals from a natural environment (i.e. national forest). I found distinct differences between the small intestine and the cecum, colon, and rectum in both species as well as host species specific gut microbial communities in all gut regions. In chapter 3, I examined differences in dietary niche between urban and natural populations using stable isotope analysis and how it partially explains the altered gut microbiota of urban populations. I found that both rodent species experienced a dietary niche expansion, but that the expansion was much larger for the omnivorous *A. speciosus*. Furthermore, both species have undergone a shift towards consuming different dietary items in concordance with their life histories within the urban environment that may be related to novel anthropogenic food resources. These dietary changes were associated with specific changes in the gut microbial community structure; however, the relationship was not always

clear. In chapter 4, I analyzed differences in the intestinal helminth communities between ecosystems and if their interactions with the gut microbial community is altered by urbanization, thereby affecting the gut microbiota. I found that urbanization had a negative impact on several species of helminths in both rodent species. Furthermore, I found that helminth prevalence and abundances were associated with changes in alpha diversity and community structure of the gut microbiota, but there was little consistency among gut region or ecosystem type suggesting a complex host-microbe-helminth-environmental interaction. Together, these results suggest that alteration of the gut microbiome in urban areas is species specific but determining the source of alteration is complex. By investigating multiple factors that may underlie these changes, it is possible to identify potential causes of dysbiosis as well as tools that could be used in management protocols to help urban wildlife maintain a proper gut microbiota, thereby improving the health of animals.

Chapter 1

General introduction

Urbanization is occurring at a rapid rate on a global scale with an ever increasing amount of land being converted into cities (Güneralp et al., 2020; Seto et al., 2012). This extreme form of ecosystem modification has an enormous impact on the wildlife that reside within them (Bradley & Altizer, 2006; Shochat et al., 2006). For example, forest fragmentation reduces available habitat and overall animal diversity (McKinney, 2008), artificial feeding allows for higher population densities (Contesse et al., 2004; Pagani-Núñez et al., 2019), while chemical, light, and noise pollution affect immune system function (Isaksson, 2015). It is imperative that we understand how this impacts wildlife health and what allows some species to successfully adapt while others fail not only for conservation but also public health; the high degree of human-wildlife interactions within urban areas may increase the transmission risk of zoonotic diseases such as Echinococcus, hantavirus, and Lyme disease (Bradley & Altizer, 2006; Mackenstedt et al., 2014). Unhealthy animals within urban cities may be more susceptible to such diseases, thereby increasing the chance of transmission to humans.

Recently, there has been a surge of interest in understanding how the gut microbiome of wildlife is affected by urbanization (Littleford-Colquhoun et al., 2019; Phillips et al., 2018; Sugden et al., 2020; Teyssier et al., 2018, 2020). A healthy gut microbiota in vertebrates is essential to maintaining proper health as it plays a pivotal role in digestion and nutritional uptake, development, and immune system function (Fraune & Bosch, 2010; Hooper et al., 2002; Schluter et al., 2020). Plasticity in what constitutes a healthy gut microbiota may help facilitate adaption to a rapidly changing environment as symbiotic microbes may aid in acquisition of nutrition from novel human derived food resources (Bäckhed et al., 2004; David et al., 2014), the breakdown of toxic chemicals (Kohl et al., 2014), or provide protection against the negative impacts of human activities. However, urbanization may disrupt the gut microbiome through diet simplification and low quality food or acute and chronic stress, thereby inducing a condition

known as dysbiosis (Amato et al., 2013; Maureen Murray et al., 2015). A dysbiotic gut is associated with an increase in diseases such as obesity, inflammatory bowel disease, and a general decrease in immune system function (Chin et al., 2000; Gao et al., 2018; Logan et al., 2016). Therefore, it is essential that we understand how the gut microbiota of wildlife is affected by urbanization so that new methods of wildlife management can be developed that prioritizes gut health and improves the well-being of animals in human modified environments.

Previous studies exploring the gut microbiota of urban animals have focused on a single animal species (e.g. house sparrows or coyotes) with each investigation conducted in different cities spread across three different continents, thereby limiting our ability to compare species-specific responses as the urban environments are likely be quite different (Littleford-Colquhoun et al., 2019; Phillips et al., 2018; Sugden et al., 2020; Teyssier et al., 2018, 2020). Just as each species of animal responds differently to urbanization, impacts on the gut microbial community structure are unlikely to be similar among all host species (Kark et al., 2007; Lowry et al., 2013). Gut microbes have been co-evolving with their host for millions of years leading to host species-specific microbial communities, and the transplantation from one animal species to another significantly reduces their ability to digest food or survive until adulthood (Brooks et al., 2016; Kohl et al., 2018; Ley et al., 2008). Consequently, what constitutes a healthy gut microbiota is not universal, highlighting the need to understand how the gut microbial communities of different species respond to the same urban environments and how it affects their ability to successfully adapt.

Dietary niche is one of the largest factors affecting the gut microbiota composition and urbanization is known to induce dietary changes in animals due to artificial feeding such as trash and feeder boxes (Pagani-Núñez et al., 2019). A large shift in diet in response to novel food items may negatively impact the microbial community within the gut and therefore host health (David et al., 2014). The gut microbiota of omnivores may experience less of an impact in response to the consumption of anthropogenic food resources as their characteristically wider dietary niche may allow their gut microbes to aid in digestion without undergoing a large shift in community composition. On the other hand, more specialized species such as herbivores may suffer if their gut microbiota deviates drastically due to the consumption of food items for which they are not typically suited.

Microbes are not the only organisms residing within he gastrointestinal tract, as intestinal helminths are ubiquitous throughout nature (Leung & Poulin., 2008). Although intestinal helminths are generally considered detrimental to their host, low abundances usually don't induce clinical signs of disease and the host tolerates them (Bilbo et al., 2011; Kutzer & Armitage, 2016). Furthermore, helminths are often host species specific and have been co-evolving with both the host and their symbiotic microbes. Similar to the microbiota, helminths are able to immunomodulate the host to avoid detection and destruction (Gause & Maizels, 2016; Midha et al., 2017). By altering the host immune system function such as the up-regulation of Th cytokines, it ultimately affects the gut microbial community structure and may help maintain gut homeostasis (Fricke et al., 2015; Kreisinger et al., 2015). Helminths have even been used as a therapeutic tool to remedy dysbiosis in captive macaques and cure them of chronic dysentery when standard antibiotic treatment did not work (Broadhurst et al., 2012). However, intestinal helminth communities of wildlife are often altered within urban areas (Anders et al., 2019; Delgado-V. & French, 2012; Werner & Nunn, 2020). Therefore, urbanization may indirectly affect the gut microbiota of animals through the alteration of their helminth communities. On the contrary, helminths that persist within the urban ecosystem may help animals maintain a healthy gut microbiota as long as they don't induce negative impacts on the host.

In this thesis, I investigated how the gut microbiota was differentially impacted by urbanization in two sympatric species of rodents, the omnivorous large Japanese field mouse (*Apodemus speciosus*) and the more herbivorous grey-red backed vole (*Myodes rufocanus*), and what factors may underly any observed changes. First, I compared the gut microbiota among four distinct gut regions (i.e. small intestine, cecum, colon, and rectum) within each species captured in a natural minimally disturbed ecosystem as well as characterized among species differences to determine how their gut microbiota differ. Second, I analyzed differences in dietary niche between natural and urban populations and if a dietary shift in response to anthropogenic food resources explains an altered gut microbiota in urban individuals. Specifically, I investigated if between species differences in dietary habit and utilization of different resources within the urban areas induced species specific changes in the gut microbiota. Third, I quantified differences in the intestinal helminth communities among natural and urban populations of both species, and if their interactions with the gut microbial community is altered

by urbanization, thereby impacting microbial community structure. Finally, I discuss how urbanization differentially impacts the gut microbiome of both host species integrating all findings. This thesis is the first study evaluating the effects of urbanization on the gut microbiota of multiple animal species and potential underlying causes.

Chapter 2

Comparing the gut microbiome along the gastrointestinal tract of three sympatric species of wild rodents

Abstract

Host-microbe interactions within the gastrointestinal tract (GIT) play a pivotal role in shaping host physiology, ecology, and life history. However, these interactions vary across gut regions due to changes in the physical environment or host immune system activity, thereby altering the microbial community. Each animal species may harbor their own unique microbial community due to host species-specific life history or physiological traits. While gut microbiota in wild animals has received much attention in the last decade, most studies comparing closely related species only utilized fecal or colon samples. In this study, we compare the gut microbial community from the small intestine, cecum, colon and rectum within three sympatric species of wild rodents (i.e. *Apodemus speciosus*, *A. argenteus*, and *Myodes rufocanus*) as well as compared each gut region among host species to determine the effect of both on the gut microbiota. We found that the small intestine harbored a unique microbiome as compared to the lower GIT in all three host species, with the probiotic *Lactobacillus* in particular having higher abundance in the small intestine of all three host species. There were clear interspecific differences in the microbiome within all gut regions, although some similarity in alpha diversity and community structure within the small intestine was found. Finally, when both phylogeny and species abundance were considered, the microbiome could be distinguished between the cecum, colon, and rectum indicating that rectum or fecal samples may not always be appropriate for studying the gut microbiome in these animal species.

Introduction

The vertebrate gastrointestinal tract (GIT) is a complex ecosystem occupied by a diverse community of microbes that impact many aspects of the host's biology such as behavior (Heijtz et al., 2011), digestion (Hooper et al., 2002), and immune system function through interactions with the host (Amato, 2013; Gerardo & Parker, 2014; Sekirov & Finlay, 2009). Therefore, understanding host-microbe interactions will help us to better understand the ecology and evolution of wildlife (Hird, 2017). However, interactions are not uni-directional, as the host helps shape the microbial community by actively destroying species that are pathogenic while allowing those that are beneficial to remain or tolerating those that cause no harm (Bevins & Salzman, 2011). Due to species specific physiological and dietary needs, this selective process leads to unique microbial community profiles even among sympatric species exposed to the same environmental microbes (Moeller et al., 2017) and often mirroring their evolution (Brucker & Bordenstein, 2012; Ingala et al., 2018; Kohl et al., 2018). Although this phylosymbiosis has already been demonstrated in several groups of taxon, only two studies have done so using multiple gut regions from the same individuals in lizards (Kohl et al., 2017) and mice (Kohl et al., 2018), with the later using laboratory reared animals.

The digestive tract is a complex environment that changes drastically in physical structure, immune system activity, oxygen concentration, and pH going from the oral cavity to the anus due to differing physiological functions as required by the host (Donaldson et al., 2016). This creates physical and physiological barriers that microbes must cross before establishing themselves. Therefore, unique microbial communities reside within each gut region, with those in the upper and lower digestive tract being distinctly different from each other as has been demonstrated in several groups of animals such as rodents (Gu et al., 2013; Kohl et al., 2018; T. A. Suzuki & Nachman, 2016), pigs (Kelly et al., 2017), chickens (Yan et al., 2019), and lizards (Kohl et al., 2017). Although we are only just beginning to understand the biogeography of the gut microbiota along the digestive tract of vertebrates, this has called into question the wide spread use of fecal samples to answer all manner of questions regarding the gut microbiome (Gu et al., 2013; Ingala et al., 2018; Yan et al., 2019). Fecal samples are easy to collect non-invasively and may provide a representation of the gut microbial flora in the lower GIT,

particularly species membership. However, it may not accurately reflect species abundances especially of the upper GIT (Yan et al., 2019).

In this study, we investigated the gut microbial communities of three sympatric species of wild caught small rodents, two field mice (*Apodemus speciosus* and *A. argenteus*) and one vole (*Myodes rufocanus*), to investigate differences in the microbiome among four gut regions (i.e. small intestine, cecum, colon, and the rectum) within each host species, as well as among species differences within the same gut regions. We hypothesized that within species, each gut region would harbor a unique microbiome, particularly between the small intestine and the lower GIT (i.e. cecum, colon, and rectum) because of differences in host physiological function (Donaldson et al., 2016; T. A. Suzuki & Nachman, 2016). Due to differences in life and evolutionary histories of each host species we predicted that all gut regions would show significant among species differences, with the largest between *M. rufocanus* and both species of field mice (i.e. phylosymbiosis (Kohl et al., 2017, 2018). Lastly, as our rectum samples was fecal matter taken directly from the GIT rather than after defecation, we wanted to see if the microbiome was an accurate representation of any specific gut region or if it provides an overall picture of the whole gut. We expected it to be most similar to the microbiome of the colon. By answering these questions, we hope to better understand the gut microbiome of wild animals so that future studies can target specific aspects of their ecology and life history that may help shape it.

Methods

Host species and field sampling

Three sympatric species of wild caught small rodents, two field mice (*Apodemus speciosus* and *A. argenteus*), and the grey red-backed vole (*Myodes rufocanus*), were captured in October 2019. *A. speciosus* and *A. argenteus* are common throughout the Japanese archipelago (H. Suzuki et al., 2004). Although they maintain overlap in their ecological niches, *A. speciosus* is entirely ground dwelling while *A. argenteus* is often arboreal, especially when the population density of *A. speciosus* is high (Sakamoto et al., 2012). *M. rufocanus* on the other hand, is widely distributed across Eurasia from Fennoscandia to Japan where it is only found within Hokkaido and associated small islands (Kaneko et al., 1998). All species are omnivorous, but the diet of *A.*

speciosus and *A. argenteus* largely consists of nuts, seeds, and insects while that of *M. rufocanus* is dominated by herbaceous plants and bamboo (Saitoh et al., 2007; Sato et al., 2018).

Field sampling was conducted at four field sites (Shirakkeyama, Chitoseyama, Harushinai, and Mukoyama) within the Kamikawa Chubu National Forest in central Hokkaido, Japan (Table S1) using Sherman traps baited with Oatmeal. Traps were set 10m apart in a four by10 grid pattern, with two trap grids at each site except for Shirakkeyama which contained three. Furthermore, due to constraints of the terrain at Mukoyama, the trap grids utilized were two by 20. Traps were checked within one hour after sunrise for two or three consecutive days at each site. Any trap containing an animal was replaced with a fresh trap and all living individuals were transported to the department of parasitology at Asahikawa Medical University, Asahikawa, Japan for processing. Experimental design and handling of animals was approved and carried out in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the National University Corporation Hokkaido University (reference number 15-0121). This study was carried out in compliance with the ARRIVE guidelines.

Gut content sampling

After euthanization by cervical dislocation, body weight, sex, and morphometric measurements were recorded. The entire digestive tract was removed and segmented into three parts corresponding to the small intestine, cecum, and large intestine. Using a small steel spatula (2mm width), feces was collected directly from the rectum to avoid environmental contamination, and gut content was collected from the ileum within the small intestine, the central part of the cecum, and the ascending colon. All samples were collected in sterile 2 ml vials and placed in a -80℃ freezer within one hour after collection. All tools were flame sterilized and all surfaces sterilized with 10% bleach followed by 70% ethanol before each use.

DNA extraction, PCR amplification, and NGS

All gut content and fecal samples were placed on dry ice and transported by car to the Laboratory of Parasitology in the Faculty of Veterinary Medicine at Hokkaido University,

Sapporo, Japan (two-and-a-half-hour journey), and immediately placed into a -80℃ freezer upon arrival. DNA extraction was performed using the QIAamp fast DNA Stool Mini Kit (Qiagen) after bead beating with four 3mm beads and 1mg of 0.1mm beads per sample following Hayakawa et al (2018). A negative control was included with each batch of 24 samples to determine potential contaminants introduced during processing. The V3-V4 region of the 16S rRNA gene was amplified by PCR using universal primers 341F-805R (Klindworth et al., 2013) in a solution recommend by Illumina with a slight modification and consisting of 0.5µl of each primer at a concentration of 10µM, 12.5µl of 2x Kapa HiFi HotStart Ready Mix (KAPA Biosystems), 9 µl of molecular water, and 2.5µl of extracted DNA from gut content and fecal samples. The following thermocycler conditions were used: initial denaturation at 95℃ for 3 min, then 25 cycles of denaturation at 95℃, annealing at 55℃, and extension at 72℃ for 30 sec each, followed by a final extension at 72℃ for 5 min. An additional negative control was included in each PCR. Both DNA extraction and PCR were performed under sterile conditions within a biosafety cabinet in which all equipment and surfaces were cleaned with 70% ethanol and sterilized for 20 minutes by UV light before use. Following the manufactures instructions, library preparation was performed using Nextera XT DNA Index Kit v2 set A, B, C, or D, and sequenced on an Illumina MiSeq 300bp paired-end platform using a v3 Reagent Kit. Raw sequence reads were submitted to the DNA database of Japan (DDBJ) with the accession number DRA011343.

Analyses

After demultiplexing and merging of forward and reverse paired-end reads in Qiime2 version 2020.2 (Bolyen et al., 2019), the standard DADA2 denoising pipeline (Callahan et al., 2016) was used for quality filtering and removal of chimeric sequences to produce a feature table of Amplicon Sequence Variants (ASVs). Using the Decontam package (Davis et al., 2018) in R version 4.0.2 (Core R Team, 2020), likely contaminants introduced during sample processing were determined by the frequency method with a threshold of 0.1 and all potential contaminants were checked manually in reference to our control samples and subsequently removed in Qiime2 using sequence identifiers. Taxonomic classification (Bokulich et al., 2018) of the decontaminated sequences were assigned using SILVA classifier (release 132). ASVs identified

as Archaea, Eukaryota, mitochondria, and Chloroplastida were removed as well as bacterial sequences not assigned to phylum level. A rooted phylogenetic tree was then generated using the FastTree method in Qiime2 (Price et al., 2010).

All samples were rarefied to a sampling depth of 10,000 reads for diversity analysis based on alpha rarefaction analysis, leading to the exclusion of three samples (two small intestine and one rectum from *M. rufocanus*) due to low sequence read counts. Microbial diversity was quantified using four α-diversity measurements (Shannon diversity, Faith's phylogenetic diversity (PD), Pielou's evenness, and number of ASVs) and four β-diversity metrics (Jaccard dissimilarity, Bray-Curtis dissimilarity, unweighted unifrac, and weighted unifrac) in Qiime2. To determine significant differences in α-diversity among the different gut regions within each species as well as the same gut region among species, a generalized linear mixed effects model with gaussian distribution was utilized. The response variable was log transformed alpha diversity with host species (or gut region), sex, and age (i.e. adult / sub-adult) as fixed effects and field site as the random effect as performed in R using the nlme package version 3.1-150 (Pinheiro et al., 2020). To determine the effect of host species or gut region on community structure of the gut microbiome, we first visualized the data using principle coordinate analysis (PCoA) plots based on the four distance matrices using the R package phyloseq (McMurdie & Holmes, 2013). We then analyzed the effect of field site, sex, and age (adult / sub-adult) on β-diversity, by using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and the by = "margin" option to account for all variables using the adonis2 function in the vegan package in R (Oksanen et al., 2007). We first included all gut regions (whole gut) for each species or all three species for among species comparison of each gut region before running a series of pairwise comparisons.

To determine differences in relative abundances of microbial genera, we ran linear discriminant analysis effect size (LEfSe) using the Huttenhower lab Galaxy pipeline (Segata et al., 2011). This was first done at the whole gut level for each species in which gut region (i.e. small intestine, cecum, colon, and rectum) was the class and host ID the subject to analyze within species variation. We then analyzed among species variation of each gut region including all three host species in which the class was host species. Pairwise analysis was then conducted

between each gut region within each host species (e.g. small intestine vs. colon in *A. speciosus*), as well each gut region between each species (e.g. cecum in *A. speciosus* vs. *M. rufocanus*).

Results

Host and gut content sampling

A total of 94 individuals were captured consisting of 42 *A. speciosus*, 9 *A. argenteus*, and 43 *M. rufocanus* (Table S1) with a total of 280 gut content and fecal matter (from the rectum) samples collected for microbiome analysis. Of those, 94 were from the small intestine (42 *A. speciosus*, 9 *A. argenteus*, 43 *M. rufocanus*), 50 from the cecum (27 *A. speciosus*, 9 *A. argenteus*, 14, *M. rufocanus*), 86 from the colon (41 *A. speciosus*, 7 *A. argenteus*, 38, *M. rufocanus*), and 50 from the rectum (25 *A. speciosus*, 9 *A. argenteus*, 16 *M. rufocanus*). Based on 16S rRNA metagenomics using Illumina Miseq, a total of 12,286,171 paired-end reads were obtained after quality filtering and chimeric removal. There was an average of 43,879 reads per sample, although it varied among species and gut region (Table S2). The lowest average number of reads were from the cecum (35048 ± 2310 SEM), small intestine (34063 ± 5044 SEM), and rectum (30623 ± 2636 SEM) for *A. speciosus, A. argenteus*, and *M. rufocanus* respectively while the highest were from the small intestine (51095 ± 1571 SEM), rectum (56242 ± 2960 SEM), and cecum (45232 ± 4857 SEM) respectively (Table S2).

Gut Microbiota Alpha diversity

Alpha diversity of the gut microbiota in the small intestine was significantly lower than the rectum, colon, and cecum in all three host species based on Shannon diversity, Faith's PD, evenness, and number of ASVs as expected (all $p < 0.01$; Fig. 1, S1, Table S3 to S6). There was no difference between the cecum, colon, or rectum within any species for any alpha diversity metric (all $p > 0.05$; Fig. 1, S1, Table S3 to S6). Sex significantly affected alpha diversity within *A. speciosus* and *A. argenteus* for all four metrics (all $p < 0.02$; Table S3 to S6), but not *M. rufocanus* (all $p > 0.05$; Table S3 to S6) while age had no effect (all $p > 0.05$; Table S3 to S6).

Among species, *M. rufocanus* had significantly higher alpha diversity in all four gut regions as compared to both *A. speciosus* and *A. argenteus* based on all four diversity measurements (all $p < 0.01$; Fig. 1, S1, Table S7 to S10) except for Faith's PD of the small intestine ($p > 0.05$; Fig. 1, Table S8). There were fewer significant differences in alpha diversity between *A. speciosus* and *A. argenteus* as expected with the colon exhibiting differences based on Faith's PD and evenness, as well as in the small intestine and cecum for Shannon diversity and evenness (all $p < 0.05$; Fig. 1, S1, Table S7 to 10). There were no significant differences of alpha diversity within the rectum between *Apodemus spp.*, nor was there an effect of age or sex on any alpha diversity measurement in any among species analysis (Fig. 1, S1, Table S7 to 10).

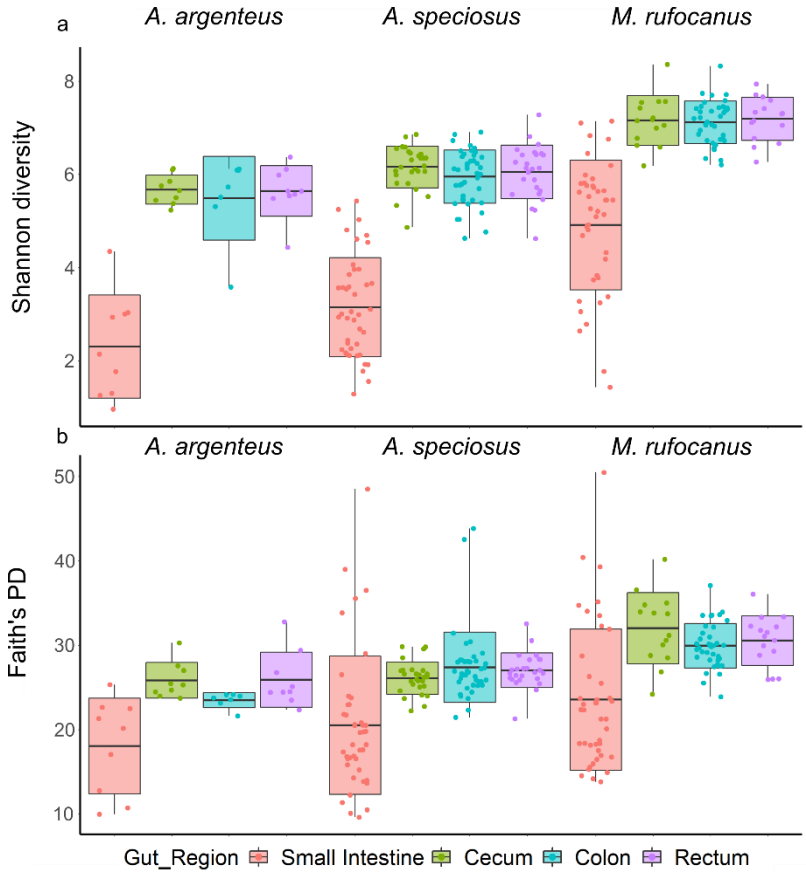


Fig. 1 Alpha diversity within each gut region of each species based on a) Shannon diversity and b) Faith's PD.

Gut microbiota community composition

When the whole gut was included for within species analysis of beta diversity, we found that gut region had a highly significant effect in all three host species regardless of dissimilarity matrix (all $p < 0.01$; Table S11 to S13). Age and field site also significantly impacted beta diversity for *A. speciosus* and *M. rufocanus* (all $p < 0.01$; Table S11, S13) while sex was significant for all except weighted unifrac in all three species ($p = 0.055$ to 0.266; Table S11 to S13) as well as unweighted unifrac in *M. rufocanus* ($F = 1.6$, $p = 0.071$; Table S13). The pairwise PERMANOVAs provided a more detailed picture of the community structure of microbes along the digestive tract in this study as gut region had a highly significant effect when the small intestine was compared to the cecum, colon, and rectum in all three species regardless of diversity matrix (all $p < 0.01$; Table S14 to S16). However, gut region was not always distinguishable among the cecum, colon, and rectum. Specifically, in pairwise comparisons between the three gut regions in *A. speciosus*, gut region had a significant effect based on Bray-Curtis and weighted unifrac (all $p < 0.05$), but not Jaccard or unweighted unifrac (all $p > 0.05$; Table S14). No significant effect was found among the same regions in *A. argenteus* (all $p > 0.05$; Table S15) while in *M. rufocanus*, gut region significantly impacted beta diversity when the colon and cecum ($F = 2.26$, $p = 0.041$), and the colon and rectum ($F = 2.35$, $p = 0.41$) were compared based on weighted unifrac alone (Table S16). Our PCoA plots showed similar results as samples from the small intestine clustered separate from the others, and a large degree of overlap occurred in the clustering of the cecum, colon, and rectum, but not entirely (Fig. 2, S2). Capture site played a significant role in most comparisons while age and sex were important for *A. speciosus* and *M. rufocanus* (Table S14, S16).

Host species had a significant effect on beta diversity for the small intestine, cecum, colon, and rectum for Jaccard and Bray-Curtis as well as unweighted and weighted unifrac distance matrices when all three species were included (all $p < 0.05$; Table S17 to S20). Capture site also had a significant impact (all $p < 0.05$; Table S17 to S20) except for weighted unifrac ($p = 0.156$ to 0.464; Table S17 to S20) while sex and age had no effect (all $p > 0.05$; Table S17 to S20). The PCoA plots confirmed these findings as there was clustering according to host species within each gut region (Fig. 3, S3). For the small intestine, however, there was a large overlap for weighted unifrac as well as a small sub-clustering for both *A. speciosus* and *M. rufocanus* that

could not be explained by site, age, or sex (Fig 3). Pairwise analysis found host species had a significant effect when the small intestine, cecum, colon, and rectum were compared between *M. rufocanus* and both species of *Apodemus* (all $p < 0.05$; Table S18, S20). When compared between *A. speciosus* and *A. argenteus*, host species was significant for Jaccard, Bray-Curtis, and unweighted unifrac for all gut regions (all $p < 0.01$), but only the cecum based on weighted unifrac ($F = 3.77$, $p = 0.003$; Table S19). Capture site was significant for most comparisons except for those based on weighted unifrac distances, while sex and age were rarely significant in any of the pairwise analysis (Table S18 to S20).

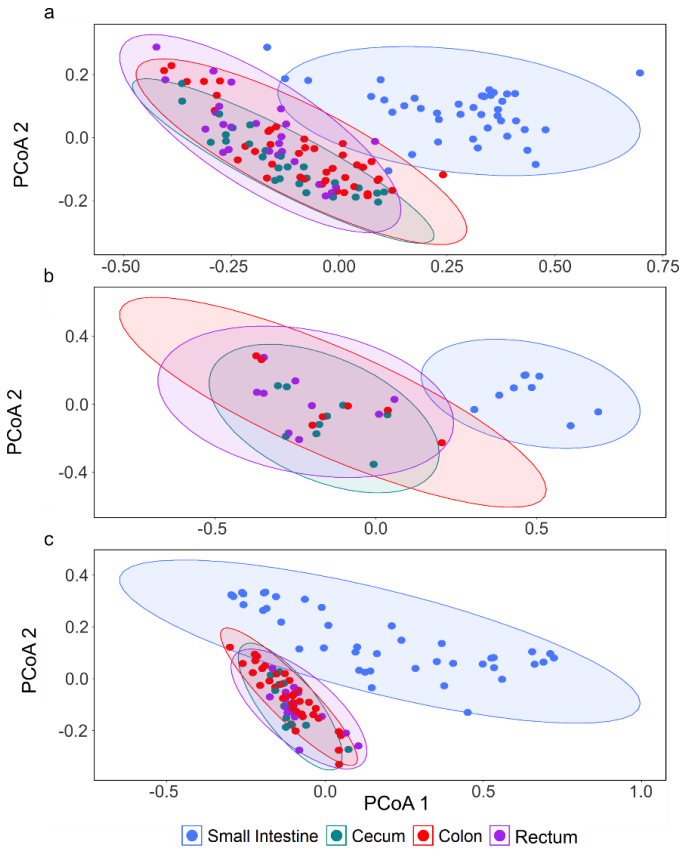


Fig. 2 Among gut region variation of the gut microbiome within each species based on weighted unifrac where a) is *A. speciosus*, b) is *A. argenteus*, and c) is *M. rufocanus*. Ellipses indicate 95% confidence interval.

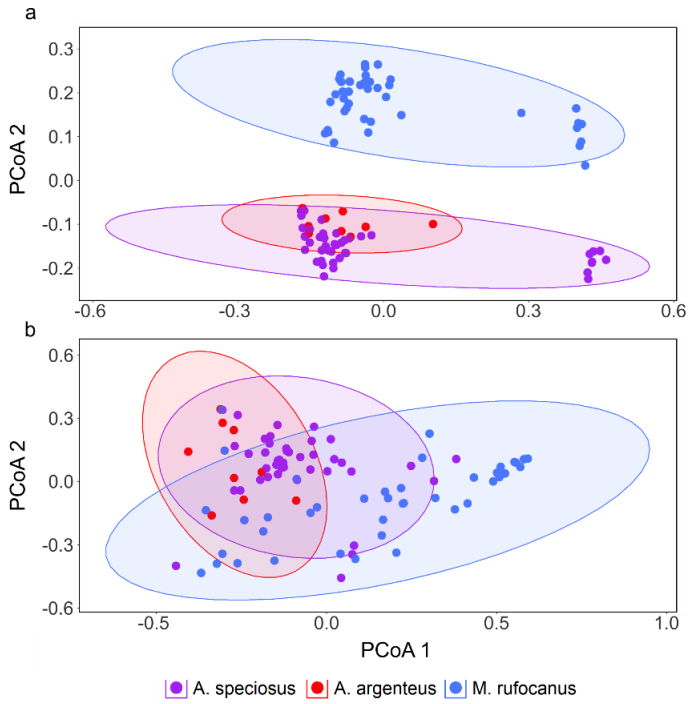


Fig. 3 Among species variation of the gut microbiome within the small intestine based on a) unweighted unifrac and b) weighted unifrac. Ellipses indicate 95% confidence interval.

Gut microbiota taxonomic composition

Firmicutes, Bacteroidetes, and Epsilonbacteraeota were the most abundant phylum in the cecum, colon, and rectum, of *A. speciosus* and *A. argenteus* accounting for 85 to 95 percent of all microbes, while Firmicutes, Bacteroides, and Spirochaetes were dominant in *M. rufocanus*, although relative abundances of each varied depending on host species and gut region (Fig. 4). In the small intestine of *M. rufocanus* the four most abundant phyla were Bacteroides (40.8%), Proteobacteria (20.1%), Firmicutes (18.5%), and Tenericutes (14.8%; Fig. 4). Firmicutes (55.7%), Tenericutes (15.5%), Bacteroides (13.4%), and Proteobacteria (12.4%) made up the largest portion of the microbiome in the small intestine of *A. speciosus* and the most dominant in *A. argenteus* were Tenericutes (26.4%), Proteobacteria (24.2%), Firmicutes (19.9%), and Spirochaetes (15.4%; Fig. 4). Notably, Tenericutes and Proteobacteria had higher relative abundance in the small intestine in all three host species as compared to the cecum, colon, or rectum (Fig. 4).

By comparing relative abundances of microbial genera along the GIT in each host species at the whole gut level using LEfSe analysis, we found Ruminococcaceae NK4A21 group was significantly more abundant in the rectum of *A. speciosus* and Treponema 2 was more abundant in the cecum of *M. rufocanus* (Fig. S4). Four genera in the small intestine, 13 in the cecum, five in the colon, and 17 in the rectum of *A. argenteus* were found to be significantly more abundant, suggesting highly differential microbiome communities along the length of the GIT (Fig. S4, Table S21).

Pairwise analysis suggested large differences in the community structure between the small intestine and the lower GIT in all three species (Table S5, S7, S9). An average of 21, eight, and 13 genera were found to be significantly more abundant in the small intestine of *A. speciosus*, *A. argenteus*, and *M. rufocanus* respectively when compared to the colon, cecum, or rectum where an average of 50, 36, and 46 genera were significantly more abundant in the three species respectively as compared to the small intestine (Table S22, Fig. S5, S7, S9). Some of the same bacterial genera such as *Lactobacillus* and *Veilonella* were found to be more abundant within the small intestine in all three host species regardless of which gut region it was compared to (Fig. S5, S7, S9). Others were host genus specific in this trend such as *Leptotrichia* in *Apodemus spp.* while many were host species specific such as *Helicobacter* in *M. rufocanus* (Fig. S5, S9). Similarly, some genera were found to have higher relative abundances throughout the lower GIT across the three host species such as *Oscilibacter* and *Ruminiclostridum*. However, most genera either exhibited host species specific higher abundance such as *Blautia* in *A. speciosus*, *Muribaculum* in *A. argenteus*, or *Hairflintia* in *M. rufocanus* or no clear trend was found (Fig. S5, S7, S9). Furthermore, when the colon and cecum were compared there was relatively little variation in abundances as only four, zero, and zero in the cecum and four, one, and six genera in the colon were found to be significantly more abundant in *A. speciosus, A. argenteus*, and *M. rufocanus* respectively, suggesting a high degree of similarity (Fig. S6, S8, S10, Table S22). Microbial genera relative abundances in the rectum were largely similar to both the colon and cecum, but more genera were found to have higher abundance as compared to the colon with seven and 12 as opposed to the cecum with five and three in *A. speciosus* and *M. rufocanus* respectively (Table S22, Fig. S6, S8, S10). Additionally, few taxa were found to be more abundant in the cecum with five, two, and one, or the colon with five, zero, and zero, genera as compared to the rectum in *A. speciosus, A. argenteus,* and *M. rufocanus* respectively

(Table S22, Fig. S6, S8, S10). Notably, there was little consistency in terms of which bacterial genera were found to be more abundant in each gut region (Fig. S6, S8, S10). However, *Oscillibacter* was found in higher abundances in the cecum of both *A. speciosus*, and *M. rufocanus* as compared to the colon and rectum (Fig. S6, S10), but not in *A. argenteus* (Fig. S8). Furthermore, *Ruminococcus* 1 and *Pygmaiobacter* in the rectum of *M. rufocanus* (Fig. S10), *Ruminococcaceae* NK4A214 in the rectum of *A. speciosus* (Fig. S6), and *Rodentibacter* in the rectum of *A. argenteus* (Fig. S8) were found to be more abundant as compared to the colon or cecum.

We found that variation in genera abundances were far greater among species within each gut region when non-pairwise (i.e. all three species included) LEfSe was used than we found among the different gut regions within each species when the whole gut was included (i.e. small intestine, cecum, colon, and rectum). A total of 17, eight, and 10, in the small intestine, 14, nine, and 15 in the cecum, 20, eight, and 17 in the colon, and 15, six, and 16 genera in the rectum were significantly more abundant in *A. speciosus*, *A. argenteus*, and *M. rufocanus* respectively (Table S23, Fig. S11). Pairwise analysis found the largest variation in abundances were between *M. rufocanus* and both species of *Apodemus* as predicted with an average of 29 and 20 significantly more abundant genera within the different gut regions as compared to *A. speciosus* with an average of 26 and *A. argenteus* with 25 on average respectively (Table S23, Fig. S12 to S15). Far fewer genera were found to be more abundant in either *A. speciosus* or *A. argenteus* when compared to each other with an average of nice and 10 respectively (Table S23, Fig. S12 to S15). Notably, many of the same genera such as *Ruminococcaceae* NK4A214 and *Ruminicolostridium* in *M. rufocanus*, *Lachnospiraceae* UCG_006 in both *A. speciosus* and *A. argenteus* as compared to *M. rufocanus*, *Streptococcus* in *A. speciosus* except in the cecum compared to *A. argenteus*, and *Prevotellaceae* UCG_003 in *A. argenteus* as compared to *M. rufocanus* were found to be more abundant regardless of whether it was the cecum, colon, or rectum being compared (Fig. S12 to S15).

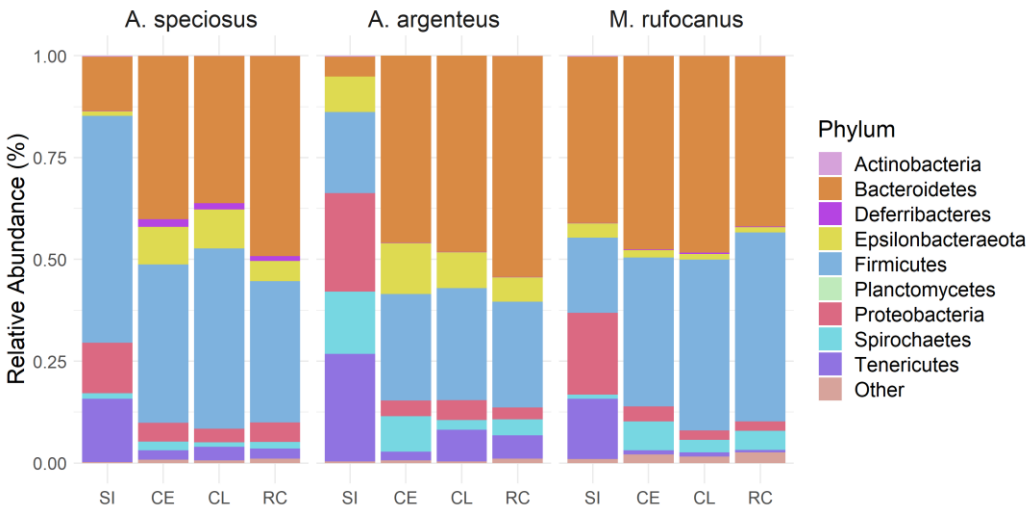


Fig. 4 Relative abundances of the nine most abundant microbial phyla within each gut region of each host species. SI is the small intestine, CE is the cecum, Cl is the colon, and RC is the rectum.

Discussion

Within species variation

We found a similar trend in gut microbiome diversity along the digestive track in our three host species as alpha diversity was lowest in the small intestine, but nearly identical in the cecum, colon, and rectum (Fig. 1, Table S3 to S6). Our PCoA plots demonstrated a similar pattern with a high degree of overlap in community structure within the lower GIT but was distinct within the small intestine (Fig. 2, Table S14 to S16). This is a common among hind gut fermenting animals such as rodents (Gu et al., 2013; Kohl et al., 2018; T. A. Suzuki & Nachman, 2016) and reptiles (Colston et al., 2015; Kohl et al., 2017). In mammals, the transit time of gut content through the small intestine is 10 times faster than through the cecum or colon (DeSesso & Williams, 2008). This has led some to postulate that only those bacterial species that are able to adhere to the mucosal wall can become established while the rest pass into the lower GIT (Donaldson et al., 2016), ultimately limiting the number of microbes and subsequently the

number of species that reside in the region permanently. Furthermore, the host immune system also has the highest activity level within the small intestine as compared to the cecum or colon through the secretion of antimicrobial peptides by Paneth cells in the epithelial wall (Bevins & Salzman, 2011). This not only helps defend against pathogens, but also shapes the microbial community by restricting which microbes can successfully colonize the mucosa, many of which are beneficial for the host. For example, in all three species we found significantly higher abundance of the probiotic genus *Lactobacillus* within the small intestine (Fig. S5, S7, S9), not an uncommon finding (Gu et al., 2013; T. A. Suzuki & Nachman, 2016).This microbial genus is known to upregulate the immune system of the host helping to protect against pathogens (Christensen et al., 2002; Sekirov & Finlay, 2009).

Despite PCoA and alpha diversity suggesting highly similar microbial community structure among the cecum, colon, and rectum, within each species, PERMANOVA analysis suggests far more variability (Table S14 to S16, Fig. 1, S2). Specifically, both Jaccard and unweighted unifrac found no difference among these three regions in *A. speciosus*, but Bray-Curtis and weighted unifrac did, while in *M. rufocanus*, there was a difference in the microbial community structure between the cecum and colon, as well as the colon and rectum according to weighted unifrac (Table S14, S15). Interestingly, this suggests that although species membership may be similar throughout the lower GIT, their abundances are not.

While no differences in relative abundance were found for the majority of microbial genera among the different gut regions of the lower GIT based on pairwise LEfSe analysis, there were several exceptions. For example, there was higher abundance of *Ruminococcous* 1 in the rectum of *M. rufocanus*, *Ruminococcaceae* NK4A214 in the rectum of *A. speciosus*, and *Oscillibacter* in the cecum of both *A. speciosus* and *M. rufocanus*. This partially supports the results of PERMANOVA (Fig. S6, S8, S10) suggesting that any difference in community structure is driven by a few highly abundant taxa within each gut region. The high degree of similarity in abundances within the cecum and colon is of no surprise as there is similarity in physiological functionality. However, they are not identical as the cecum's main role is fermentation and energy absorption while reabsorption of water occurs in the colon before defecation causing the physical environments to differ (Bowcutt et al., 2014; Donaldson et al., 2016) possibly explaining the observed differences. Alternatively, micro-geographic differences

in the microbiome can occur within the same gut region affecting our results as we did not sample the entirety of each (Huse et al., 2014). But our sampling technique which took gut content from a large portion of each gut region, as opposed to a biopsy, as well as our large sample size should control for this. Therefore, we believe that when only interested in presence or absence of microbes within the lower GIT, sampling from the cecum, colon, or rectum is appropriate. Furthermore, because the rectum samples were fecal matter collected before defecation, thereby controlling for environmental microbial contaminants, feces may be an appropriate tool for non-invasive sampling when studying the microbial communities of the lower GIT in these host species. However, caution should be taken when bacterial abundances are of concern or when interested in the microbial community of the small intestine.

Among species variation

Not only was the gut microbiome distinct among the more distantly related vole and the two field mice in all four gut regions, we also found distinct differences between *A. speciosus* and *A. argenteus*. The cecum, and to a lesser extent the colon, is especially important for the breakdown of plant polysaccharides in hind gut fermenting herbivorous species such as *M. rufocanus* (Rechkemmer et al., 1988). In fact, we found that alpha diversity of the cecum, colon, and rectum was highest within *M. rufocanus* (Fig. 1, S1, Table S7 to 10) as is typically seen in herbivores as compared to either omnivores or carnivores (Ley et al., 2008). Furthermore, many of the more abundant genera in the lower gut region such as *Ruminococcaceae* NK4A214 and *Ruminiclostridium* in *M. rufocanus* (Fig. S13 to S15), Lachnospiraceae UCG_006 from both *A. speciosus* and *A. argenteus* (Fig. S13 to S15), *Streptococcus* in *A. speciosus* (Fig. S13 to S15), and *Prevotellaceae* UCG_003 in *A. argenteus* (Fig. S13 to S15) are known fermenters of various food materials (Biddle et al., 2013; Fosses et al., 2017; Shah et al., 2015; Whiley & Hardie, 2015). Therefore, the primary driver of microbial composition within the lower GIT in the three host species in our study is likely related to dietary niche. However, *A. speciosus* and *A. argenteus* share a large degree of overlap in dietary preferences (Sato et al., 2018). Therefore, the observed differences could be due to exposure to different microbes in their micro-habitat specific environments as *A. argenteus* is more arboreal (Sakamoto et al., 2012).

Interestingly, there was no difference in alpha diversity within the small intestine in any of the three species based on Faith's PD (Fig. 1, Table S8), nor between *A. speciosus* and *A. argenteus* according to the number of ASVs (Fig. S1, Table S10). Furthermore, there was a large degree of overlap in the microbial community based on weighted unifrac PCoA alone (Fig. 3, S3) suggesting that when both phylogenetic relatedness and abundances are considered, there is more similarity in the gut microbiota within the small intestine than the lower GIT. Kohl et al. (2018) postulated that the lower GIT could be subject to stronger host-microbe co-evolutionary forces because it is critically important for energy acquisition by the host as aided through microbial fermentation. However, the mammalian immune system function is highly conserved even among distantly related species such as lab mice and humans (Mestas & Hughes, 2004; Waterston et al., 2002) and the small intestine is the most immunologically active area within the body (Bowcutt et al., 2014). Therefore, perhaps the environment is far too harsh or restrictive, and too similar among rodent species to promote a high degree of host-microbe co-evolution. Therefore, differential exposure to environmental microbes due to geographic location or species-specific life history may play a larger role in determining the microbiota species membership within the small intestine, but not necessarily abundances. In addition, the small sub cluster in the unweighted unifrac PCoA for *A. speciosus* (Fig. 3a) could be caused by an altered immune state due to an infection as four of the eight individuals had an enlarged spleen. However, no obvious symptoms of disease were found within the subclustered individuals of *M. rufocanus*. Additionally, these results were not supported by PERMANOVA between *M. rufocanus* and *A. speciosus* nor *A. argenteus* but was between *Apodemus spp.* for weighted unifrac (Table S18 to 20). Further research must be conducted as few comparative immunology studies of immune specific responses among different species have been conducted (Haley, 2003).

Despite some similarities in the microbial community structure of the small intestine in our three host species, we found multiple genera to be significantly more abundant within each. In particular, we found that the probiotic genus *Lactobacillus* was most abundant in *A. argenteus* and least abundant in *A. speciosus*. However, this was likely caused by a single individual of *A. argenteus* within our small sample size with 75% of sequences identified as *Lactobacillus* while only 4% on average were in the remaining eight individuals. Furthermore, while the majority of *Lactobacillus* sequences were of an unidentified species, we also found host specific species

such as *L. gasseri* in 26% of *A. speciosus* and *L. rodentium* in 72% of *M. rufocanus*. Perhaps this is due to host selection as these sympatric species of rodent would likely have been exposed to many of the same microbes due to an overlap in life history and dietary niche.

Conclusion

By sampling the gut microbiome in multiple locations across the GIT, we were able to show that the microbial community within the small intestine is distinct from the lower GIT in three sympatric species of wild rodents, similar to previous studies (Gu et al., 2013; Kohl et al., 2018; T. A. Suzuki & Nachman, 2016). Within the lower GIT, there was no difference in alpha diversity among the cecum, colon, nor rectum, and rarely did gut region affect beta diversity except when both phylogenetic relationships and abundance were considered (i.e. weighted unifrac analysis). Furthermore, few genera were found to differ in relative abundance, not as many as when comparing the upper and lower GIT. Therefore, feces may be appropriate for studying the gut microbiota of the lower GIT except when abundances of specific microbes are of interest. We did not test for phylosymbiosis directly, but we found that each species harbored a unique microbiome within each gut region, especially when comparing the distantly related *M. rufocanus* to both *A. speciosus* and *A. argenteus*. The main driver for these differences is likely due to dietary niche as many of the genera that differed in relative abundance are known to aid in digestion of plant polysaccharides within the lower GIT. In this study, the small intestine microbiome may be more similar among species than the lower GIT based on weighted unifrac PCoA and Faiths PD, perhaps suggesting evolutionarily conserved functionality of the immune system. However, this was not confirmed by PERMANOVA, nor was immune system function measured directly. Future research identifying specific differences in diet and immune response associated with species specific gut microbiome profiles must be conducted.

Appendices

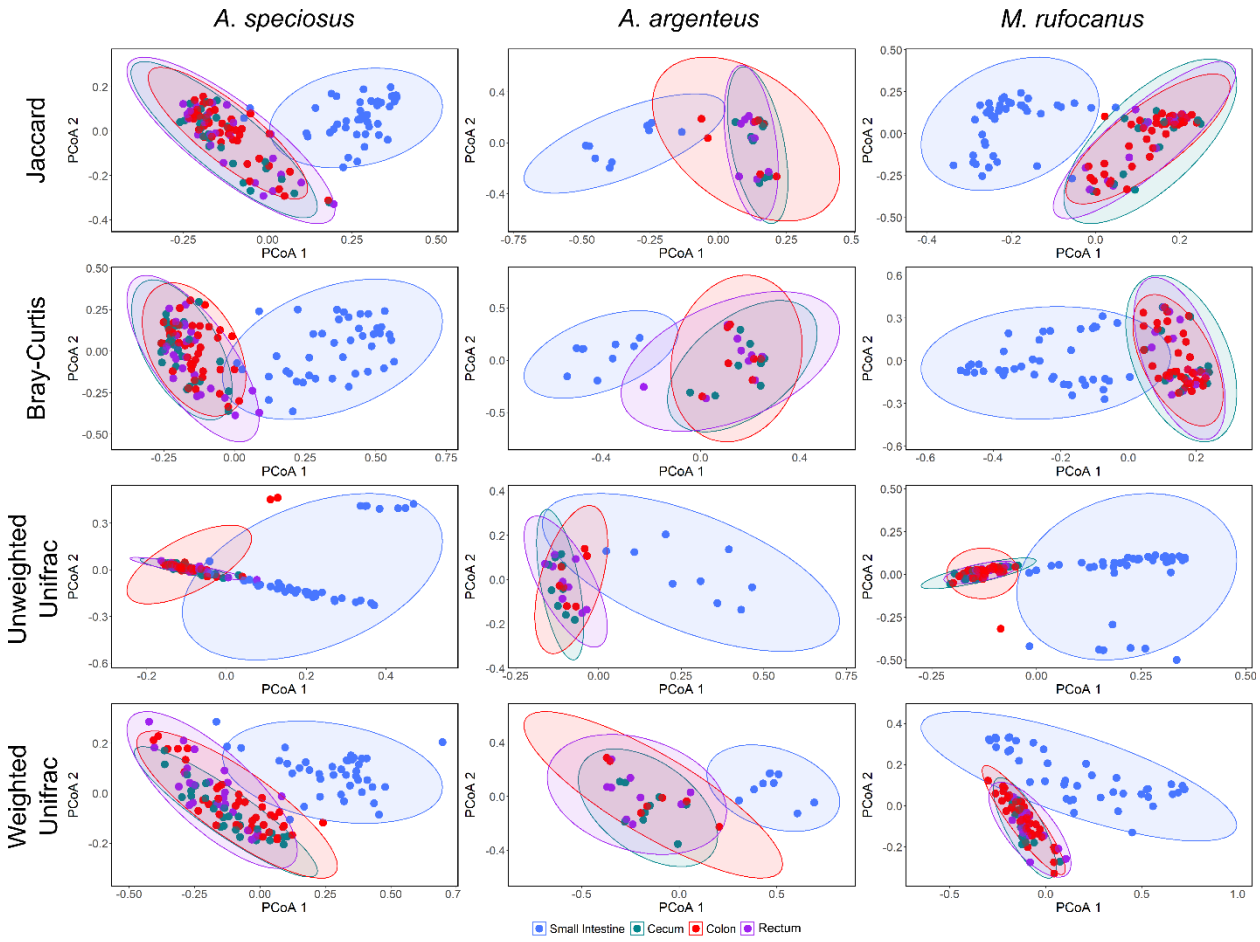


Fig. S2 Variation in the gut microbiome among gut regions within each species where column is host species and row is dissimilarity matrix. Blue is the small intestine, green is cecum, red is colon, and purple is rectum. Ellipses indicate 95% confidence interval.

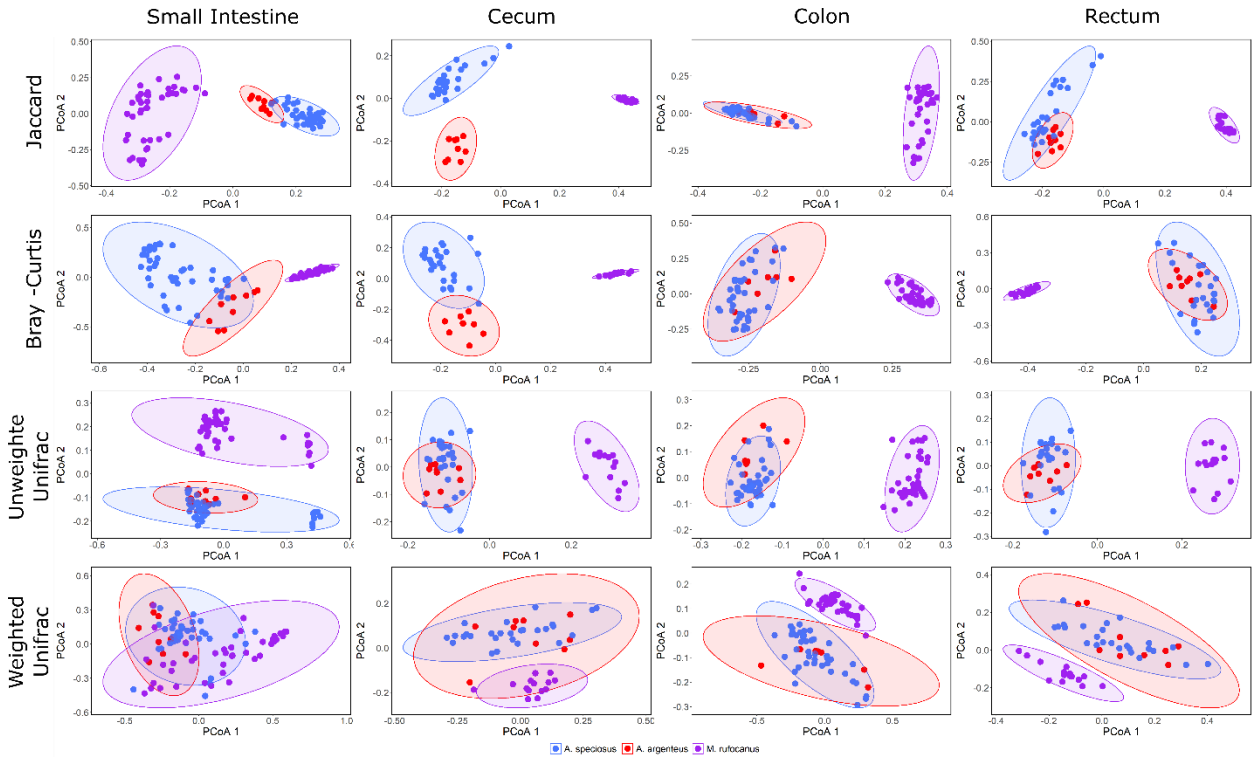


Fig. S3 Variation in the gut microbiome among species within each gut region where column is gut region and row is dissimilarity matrix. Blue is *A. speciosus*, red is *A. argenteus*, and purple is *M. rufocanus*. Ellipses indicate 95% confidence interval.

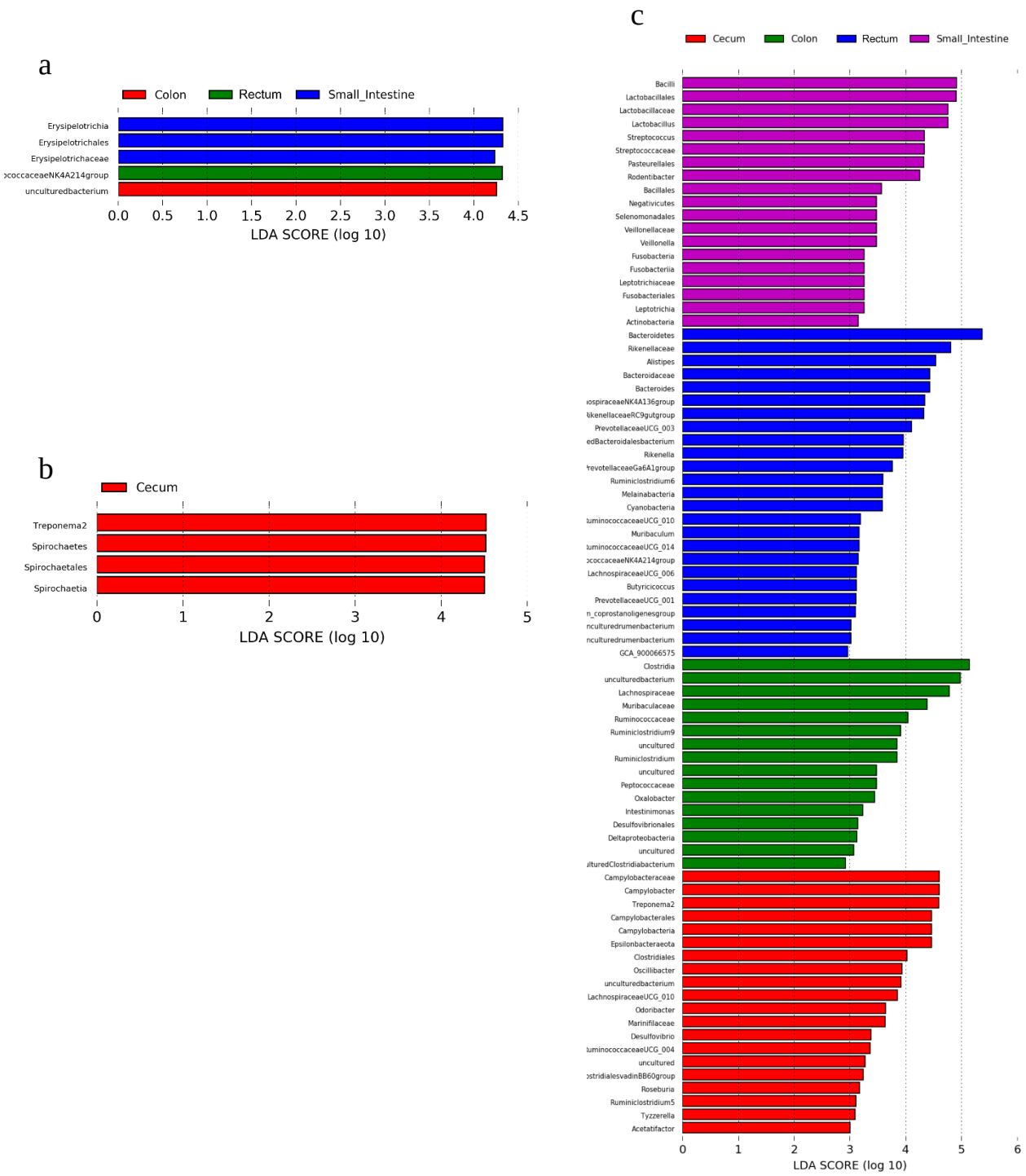


Fig. S4 LEfSe results showing more abundant genera within each gut region when all gut regions (whole gut) were included for a) *A. speciosus*, b) *M. rufocanus* and c) *A. argenteus*.

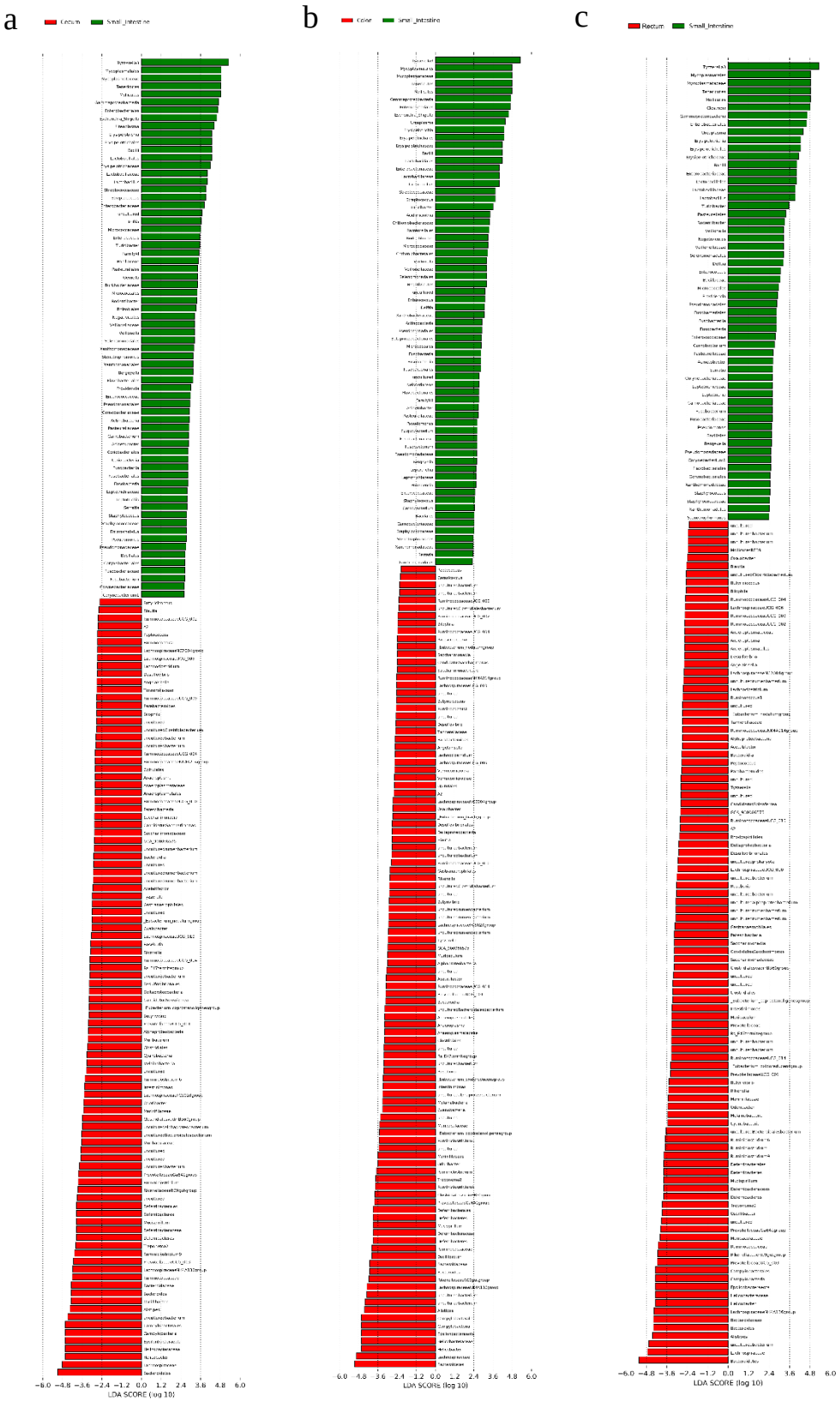


Fig. S5 More abundant taxon in the small intestine as compared to the a) cecum, b) colon, and c) rectum in *A. speciosus* based on pairwise LEfSe analysis.

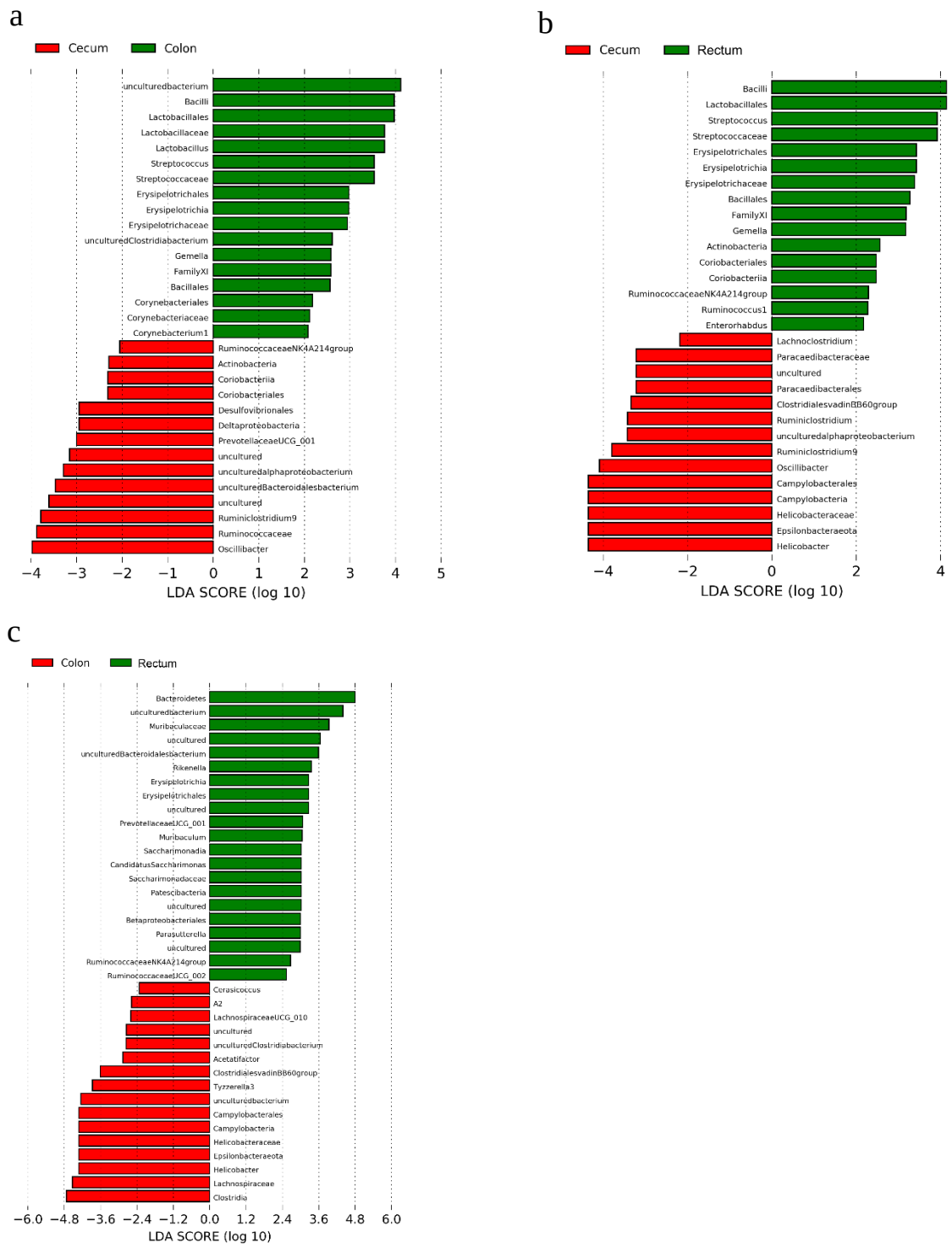


Fig. S6 More abundant taxon in the different regions of the lower GIT in *A. speciosus* based on pairwise LEfSe analysis between a) the cecum and colon, b) the cecum and rectum, and c) the colon and rectum.

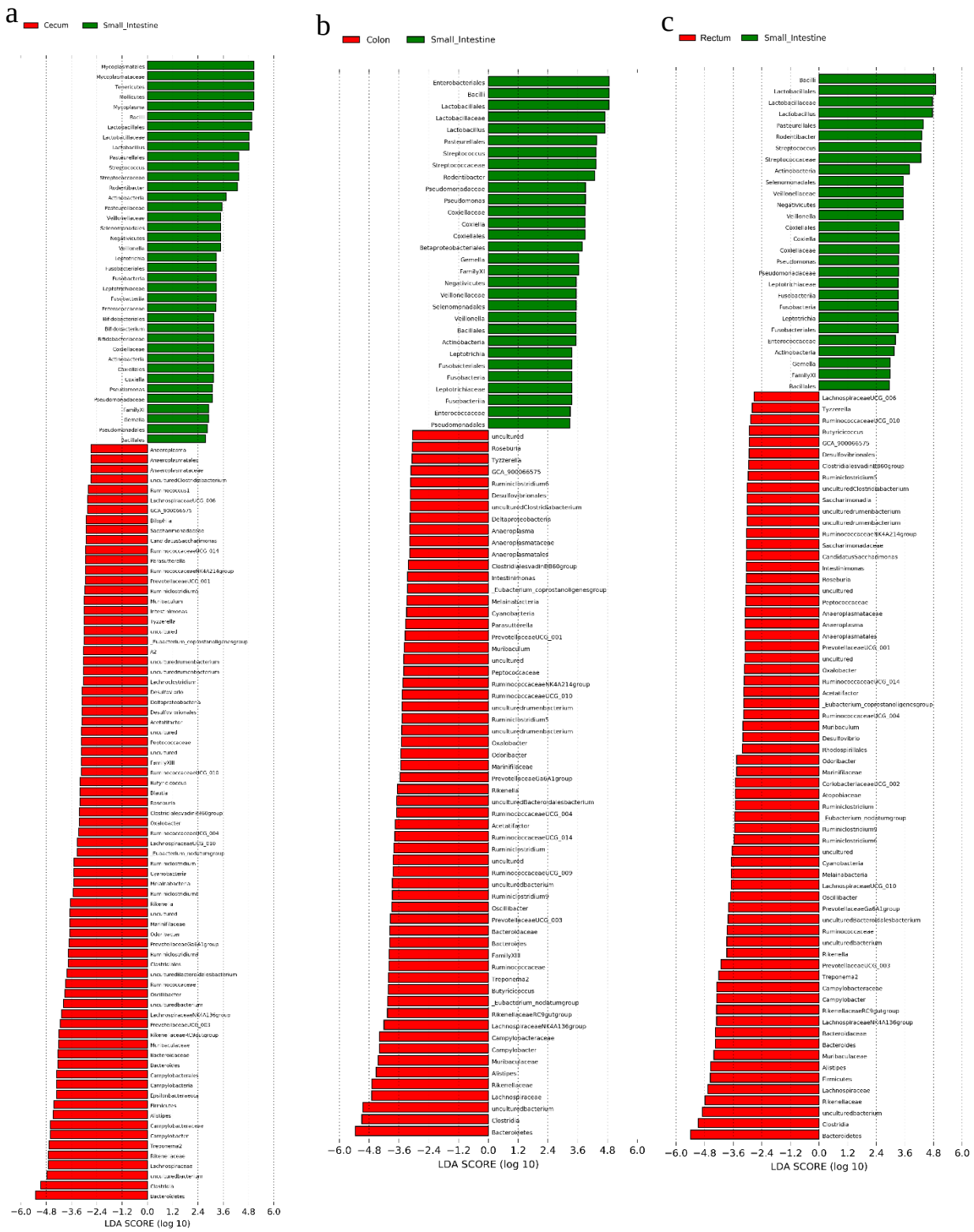


Fig. S7 More abundant taxon in the small intestine as compared to the a) cecum, b) colon, and c) rectum in *A. argenteus* based on pairwise LEfSe analysis.

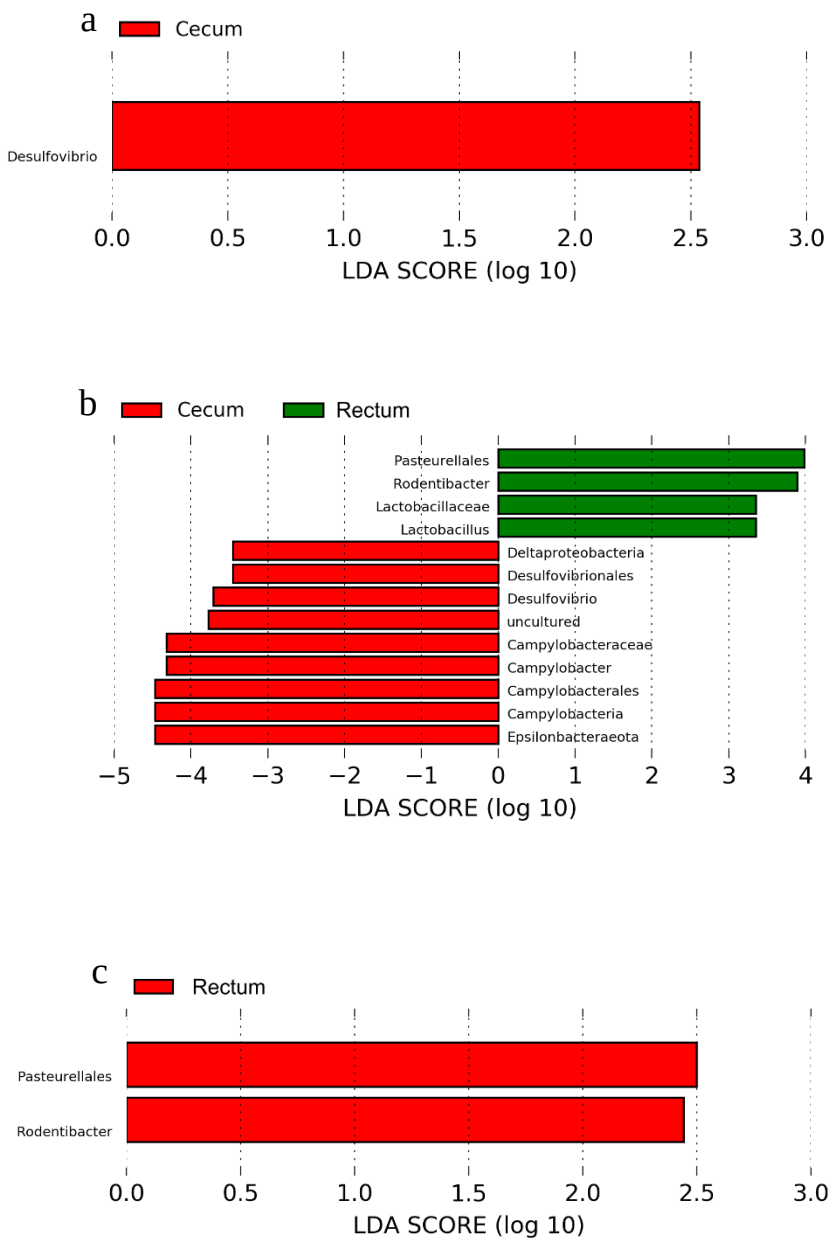


Fig. S8 More abundant taxon in the different regions of the lower GIT in *A. argenteus* based on pairwise LEfSe analysis between a) the cecum and colon, b) the cecum and rectum, and c) the colon and rectum.

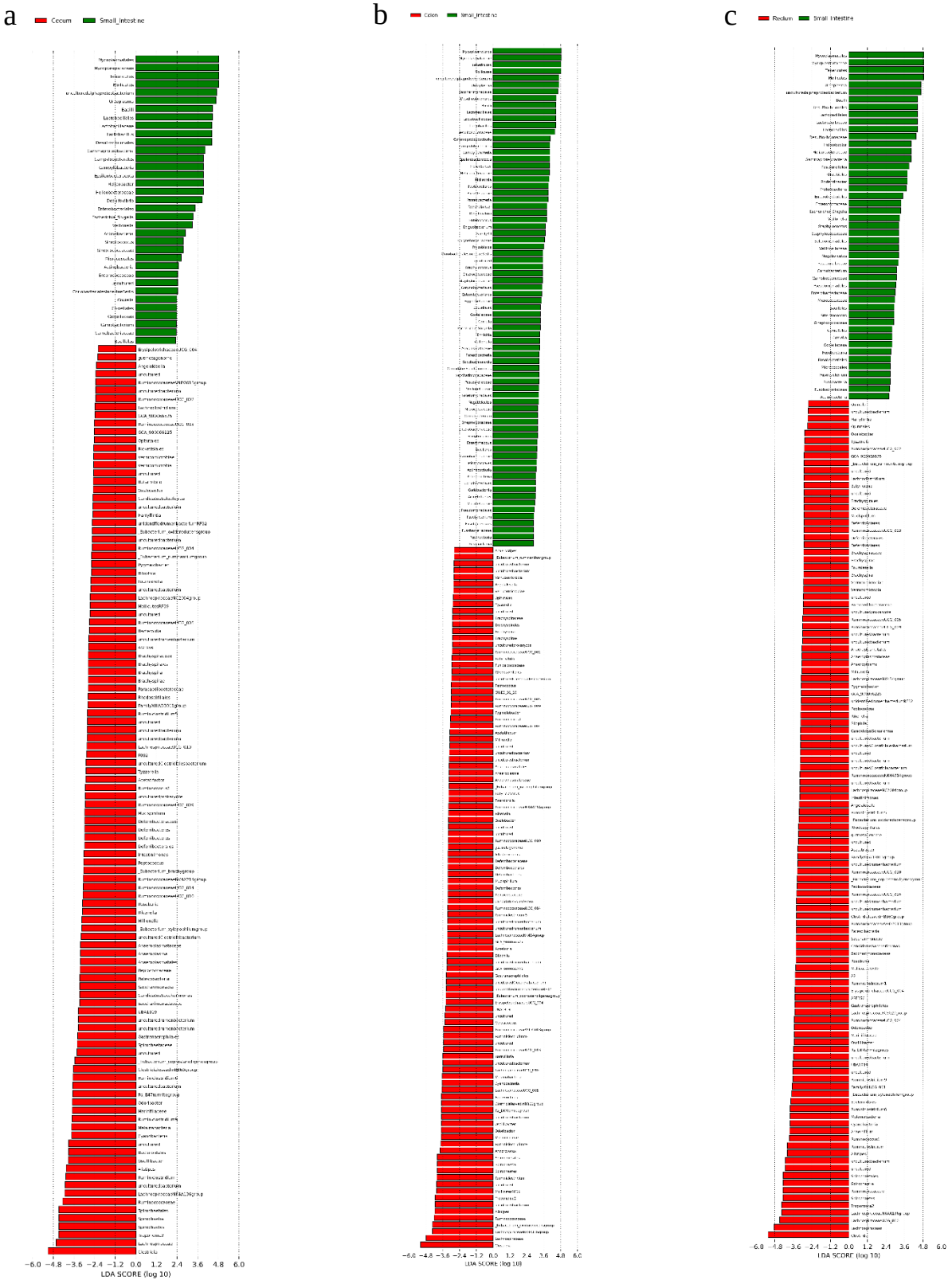


Fig. S9 More abundant taxon in the small intestine as compared to the a) cecum, b) colon, and c) rectum in *M. rufocanus* based on pairwise LEfSe analysis.

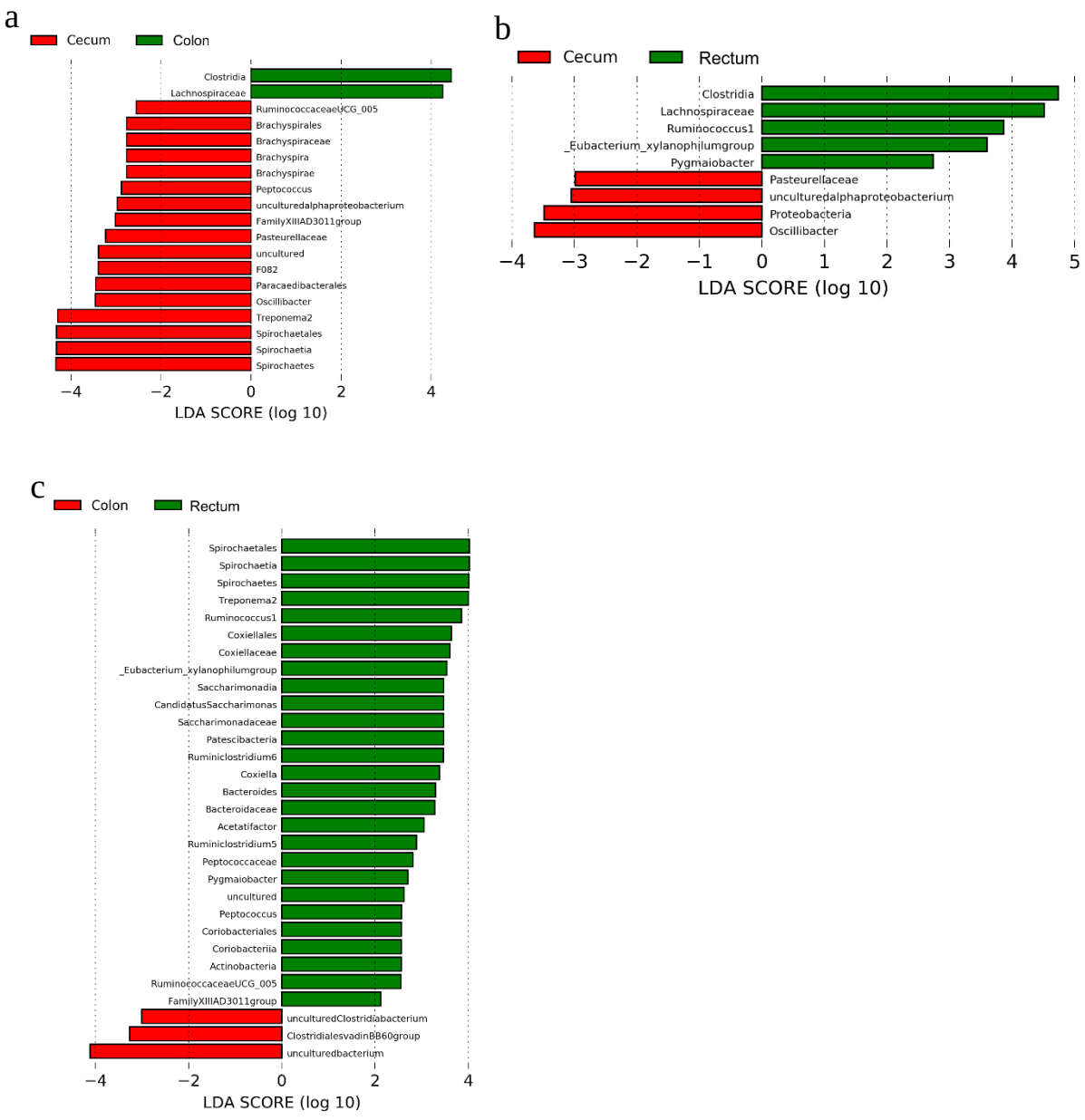


Fig. S10 More abundant taxon in the different regions of the lower GIT in *A. argenteus* based on pairwise LEfSe analysis between a) the cecum and colon, b) the cecum and rectum, and c) the colon and rectum.

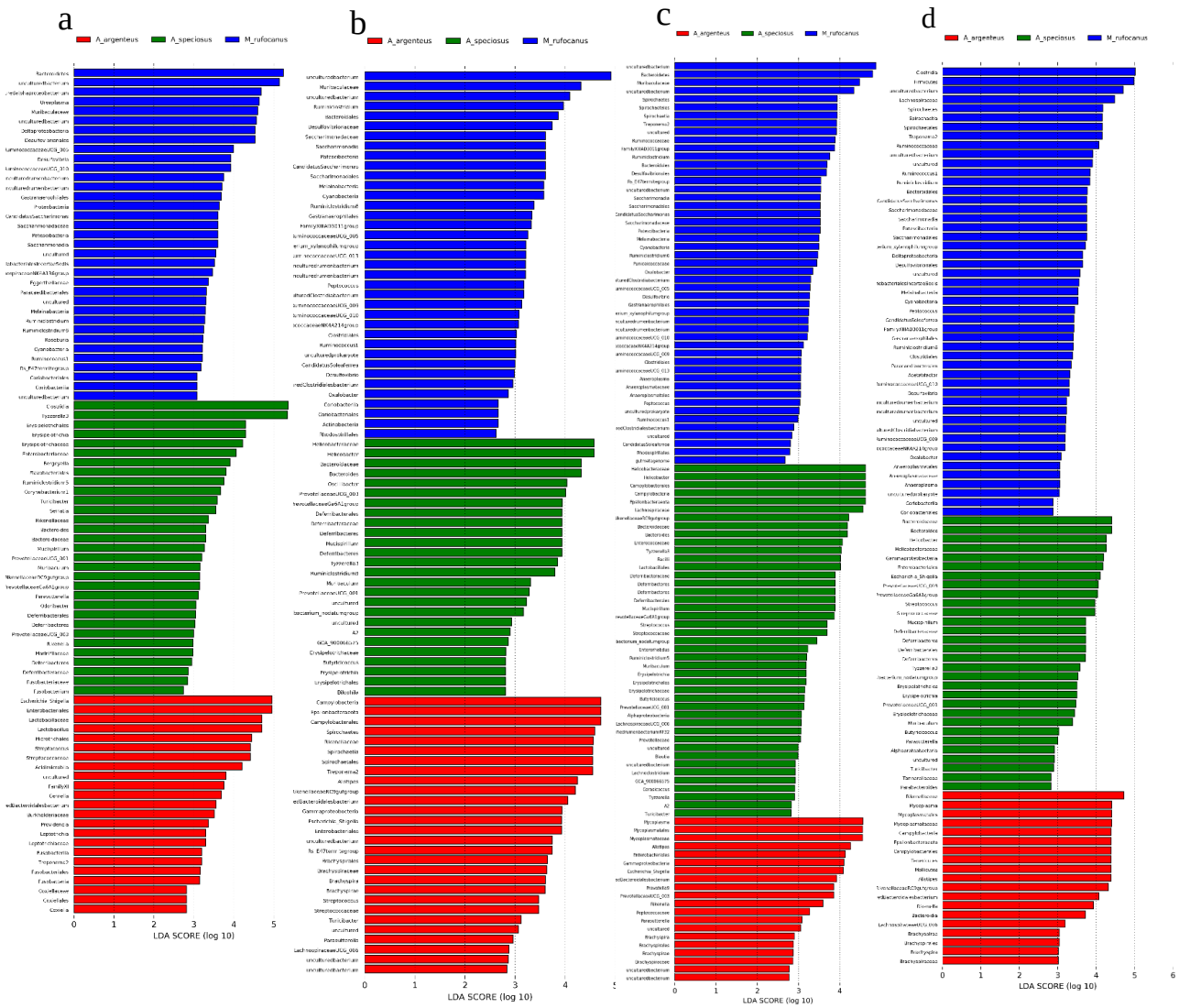


Fig. S11 More abundant taxon within each gut section of each host species when all three hosts were included in LEfSe analysis. a) is the small intestine, b) is the cecum, c) the colon, and d) the rectum.

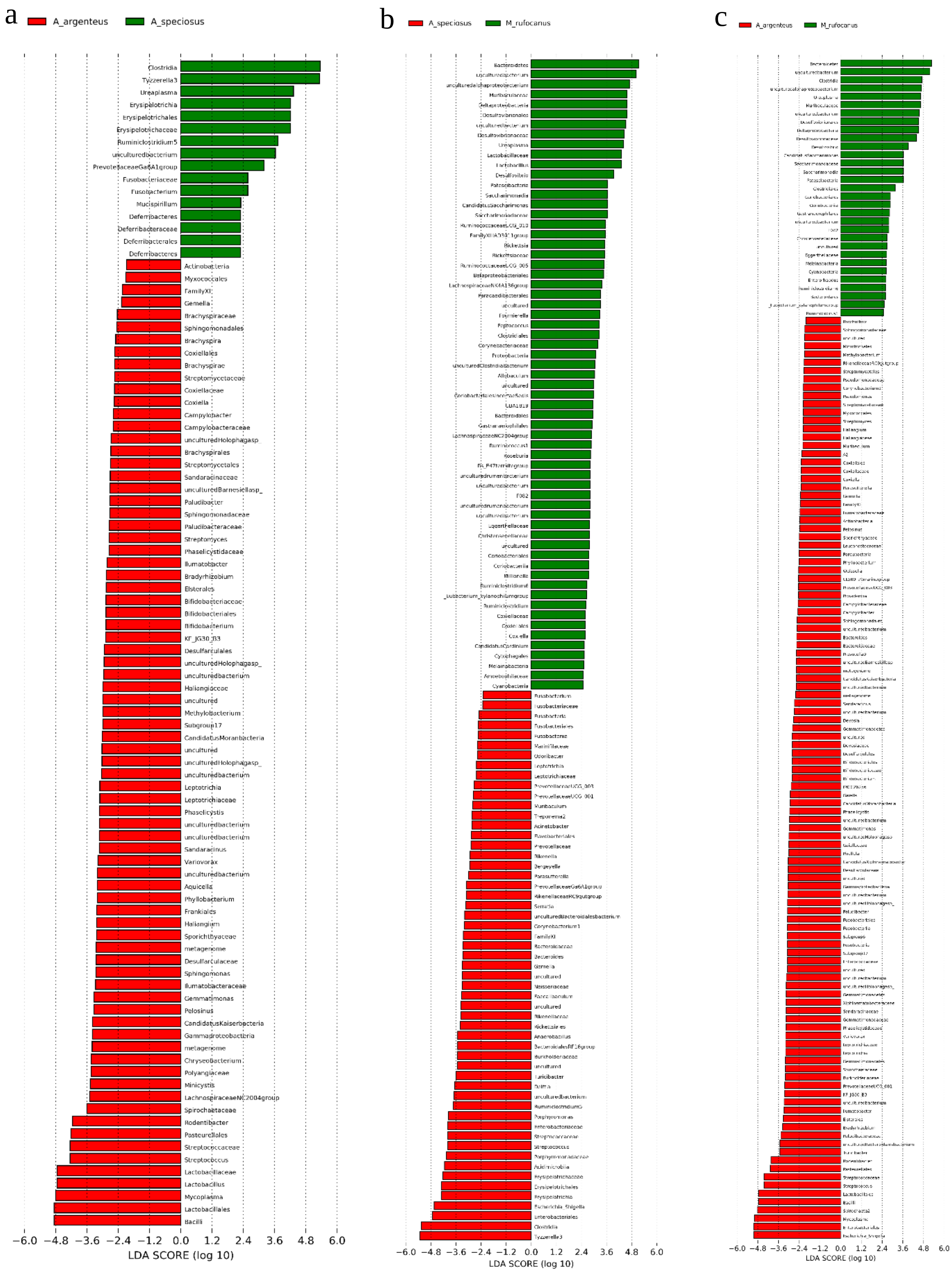


Fig. S12 More abundant taxon in the small intestine of each host species based on pairwise LEfSe analysis between a) *A. speciosus* and *A. argenteus*, b) *A. speciosus* and *M. rufocanus*, and c) *A. argenteus* and *M. rufocanus*.

Fig. S13 More abundant taxon in the cecum of each host species based on pairwise LEfSe analysis between a) *A. speciosus* and *A. argenteus*, b) *A. speciosus* and *M. rufocanus*, and c) *A. argenteus* and *M. rufocanus*.

Fig. S14 More abundant taxon in the colon of each host species based on pairwise LEfSe analysis between a) *A. speciosus* and *A. argenteus*, b) *A. speciosus* and *M. rufocanus*, and c) *A. argenteus* and *M. rufocanus*.

Fig. S15 More abundant taxon in the rectum of each host species based on pairwise LEfSe analysis between a) *A. speciosus* and *A. argenteus*, b) *A. speciosus* and *M. rufocanus*, and c) *A. argenteus* and *M. rufocanus*.

Site	Latitude	Longitude	*A. speciosus*	*A. argenteus*	*M. rufocanus*
Chitoseyama	N: 43° 56' 30.64"	E: 142° 24' 10.09"	6	0	16
Harushinai	N: 43° 42' 19.82"	E: 142° 12' 08.28"	5	0	20
Mukoyama	N: 43° 34' 07.41"	E: 142° 21' 34.57"	11	5	3
Shirakkeyama	N: 43° 56' 14.15"	E: 142° 13' 21.56"	20	4	4
Total			42	9	43

Table S1 Coordinates of each field site and the number of individuals of each species captured at each.

Species	All	Small Intestine	Cecum	Colon	Rectum
A. speciosus	45406 ± 1004	51095 ± 1571	35048 ± 2310	48865 ± 1301	46763 ± 2174
A. argenteus	46650 ± 2600	34063 ± 5044	55879 ± 3700	38635 ± 3980	56242 ± 2960
M. rufocanus	39956 ± 1543	41959 ± 3099	45232 ± 4857	39674 ± 1649	30623 ± 2636

Table S2 Average number of sequences per sample for each gut region in each species plus or minus standard error of the mean.

		Small Intestine			Cecum			Colon			Rectum		
Species	**Gut Region**	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
A. speciosus	Small intestine	–	–	–	-0.729	0.05	**< *.001***	-0.688	0.044	**< *.001***	-0.714	0.051	**< *.001***
	Cecum	0.729	0.05	**< .001**	–	–	–	0.042	0.05	0.409	0.015	0.056	0.786
	Colon	0.688	0.044	**< .001**	-0.042	0.05	0.409	–	–	–	-0.026	0.051	0.608
	Rectum	0.714	0.051	**< .001**	-0.015	0.056	0.786	0.026	0.051	0.608	–	–	–
	Sex	0.133	0.036	**< .001**	0.133	0.036	**< .001**	0.133	0.036	**< .001**	0.133	0.036	**< .001**
	Age	0.065	0.043	0.136	0.065	0.043	0.136	0.065	0.043	0.136	0.065	0.043	0.136
A. argenteus	Small intestine	–	–	–	-1.006	0.11	**< .001**	-0.956	0.118	**< .001**	-0.998	0.11	**< .001**
	Cecum	1.006	0.11	**< .001**	–	–	–	0.05	0.118	0.675	0.008	0.11	0.941
	Colon	0.956	0.118	**< .001**	-0.05	0.118	0.675	–	–	–	-0.042	0.118	0.726
	Rectum	0.998	0.11	**< .001**	-0.008	0.11	0.941	0.042	0.118	0.726	–	–	–
	Sex	-0.302	0.081	**< .001**	-0.302	0.081	**< .001**	-0.302	0.081	**< .001**	-0.302	0.081	**< .001**
	Age	–	–	–	–	–	–	–	–	–	–	–	–
M. rufocanus	Small intestine	–	–	–	-0.004	0.083	0.963	-0.423	0.05	**< .001**	-0.448	0.068	**< .001**
	Cecum	0.451	0.07	**< .001**	-0.028	0.07	0.69	0.028	0.07	0.69	0.004	0.083	0.963
	Colon	0.423	0.05	**< .001**	–	–	–	–	–	–	-0.024	0.068	0.722
	Rectum	0.448	0.068	**< .001**	-0.451	0.07	**< .001**	0.024	0.068	0.722	–	–	–
	Sex	0.03	0.045	0.508	0.03	0.045	0.508	0.03	0.045	0.508	0.03	0.045	0.508
	Age	-0.044	0.053	0.405	-0.044	0.053	0.405	-0.044	0.053	0.405	-0.044	0.053	0.405

Table S3 Linear mixed effects model results for Shannon diversity comparing gut region within each species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

Species	Gut Region	Small Intestine			Cecum			Colon			Rectum		
		b	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
A. speciosus	Small intestine	–	–	–	-0.289	0.052	**< .001**	-0.344	0.046	**< .001**	-0.327	0.054	**< .001**
	Cecum	0.289	0.052	**< .001**	–	–	–	-0.056	0.053	0.294	-0.039	0.058	0.507
	Colon	0.344	0.046	**< .001**	0.056	0.053	0.294	–	–	–	0.017	0.054	0.755
	Rectum	0.327	0.054	**< .001**	0.039	0.058	0.507	-0.017	0.054	0.755	–	–	–
	Sex	0.106	0.038	**0.006**	0.106	0.038	**0.006**	0.106	0.038	**0.006**	0.106	0.038	**0.006**
	Age	0.013	0.047	0.784	0.013	0.047	0.784	0.013	0.047	0.784	0.013	0.047	0.784
A. argenteus	Small intestine	–	–	–	-0.406	0.083	**< .001**	-0.31	0.088	**0.001**	-0.405	0.083	**< .001**
	Cecum	0.406	0.083	**< .001**	–	–	–	0.096	0.088	0.287	0.0004	0.083	0.996
	Colon	0.31	0.088	**0.002**	-0.096	0.088	0.287	–	–	–	-0.096	0.088	0.289
	Rectum	0.405	0.083	**< .001**	-0.0004	0.083	0.996	0.096	0.088	0.289	–	–	–
	Sex	-0.176	0.061	**0.007**	-0.176	0.061	**0.007**	-0.176	0.061	**0.007**	-0.176	0.061	**0.007**
	Age	–	–	–	–	–	–	–	–	–	–	–	–
M. rufocanus	Small intestine	–	–	–	-0.389	0.066	**< .001**	-0.293	0.047	**< .001**	-0.331	0.064	**< .001**
	Cecum	0.389	0.066	**< .001**	–	–	–	0.096	0.067	0.155	0.058	0.078	0.459
	Colon	0.293	0.047	**< .001**	-0.096	0.067	0.155	–	–	–	-0.038	0.065	0.559
	Rectum	0.331	0.064	**< .001**	-0.058	0.078	0.459	0.038	0.065	0.559	–	–	–
	Sex	0.021	0.043	0.628	0.021	0.043	0.628	0.021	0.043	0.628	0.021	0.043	0.628
	Age	-0.07	0.051	0.17	-0.07	0.051	0.17	-0.07	0.051	0.17	-0.07	0.051	0.17

Table S4 Linear mixed effects model results for Faith's phylogenetic diversity comparing gut region within each species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

Species	Gut Region	Small Intestine			Cecum			Colon			Rectum		
		b	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
A. speciosus	Small intestine	–	–	–	-0.512	0.037	**< .001**	-0.461	0.033	**< .001**	-0.487	0.038	**< .001**
	Cecum	0.512	0.037	**< .001**	–	–	–	0.051	0.038	0.178	0.025	0.042	0.556
	Colon	0.461	0.033	**< .001**	-0.051	0.038	0.178	–	–	–	-0.026	0.039	0.498
	Rectum	0.487	0.038	**< .001**	-0.025	0.042	0.556	0.026	0.039	0.498	–	–	–
	Sex	0.07	0.027	**0.011**	0.07	0.027	**0.011**	0.07	0.027	**0.011**	0.07	0.027	**0.011**
	Age	0.043	0.032	0.191	0.043	0.032	0.191	0.043	0.032	0.191	0.043	0.032	0.191
A. argenteus	Small intestine	–	–	–	-0.698	0.078	**< .001**	-0.694	0.083	**< .001**	-0.7	0.078	**< .001**
	Cecum	0.698	0.078	**< .001**	–	–	–	0.005	0.083	0.954	-0.001	0.078	0.983
	Colon	0.694	0.083	**< .001**	-0.005	0.083	0.954	–	–	–	-0.007	0.083	0.938
	Rectum	0.7	0.078	**< .001**	0.002	0.078	0.983	0.007	0.083	0.938	–	–	–
	Sex	-0.199	0.057	**0.001**	-0.199	0.057	**0.002**	-0.199	0.057	**0.002**	-0.199	0.057	**0.002**
	Age	–	–	–	–	–	–	–	–	–	–	–	–
M. rufocanus	Small intestine	–	–	–	-0.259	0.051	**< .001**	-0.252	0.036	**< .001**	-0.273	0.049	**< .001**
	Cecum	0.259	0.051	**< .001**	–	–	–	0.007	0.051	0.895	-0.014	0.06	0.814
	Colon	0.252	0.036	**< .001**	-0.007	0.051	0.895	–	–	–	-0.021	0.049	0.675
	Rectum	0.273	0.049	**< .001**	0.014	0.06	0.814	0.021	0.049	0.675	–	–	–
	Sex	0.038	0.032	0.24	0.038	0.032	0.24	0.038	0.032	0.24	0.038	0.032	0.24
	Age	-0.024	0.038	0.538	-0.024	0.038	0.536	-0.024	0.038	0.536	-0.024	0.038	0.536

Table S5 Linear mixed effects model results for Pielou's evenness comparing gut region within each species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

Species	Gut Region	Small Intestine			Cecum			Colon			Rectum		
		b	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
A. speciosus	Small intestine	–	–	–	-1.056	0.081	**< .001**	-1.107	0.071	**< .001**	-1.108	0.083	**< .001**
	Cecum	1.056	0.081	**< .001**	–	–	–	-0.051	0.081	0.531	-0.051	0.09	0.572
	Colon	1.107	0.071	**< .001**	0.051	0.081	0.531	–	–	–	-0.0002	0.083	0.998
	Rectum	1.108	0.083	**< .001**	0.051	0.09	0.572	0.0002	0.083	0.998	–	–	–
	Sex	0.3	0.059	**< .001**	0.3	0.059	**< .001**	0.3	0.059	**< .001**	0.3	0.059	**< .001**
	Age	0.120	0.07	0.087	0.12	0.07	0.087	0.12	0.07	0.087	0.12	0.07	0.087
A. argenteus	Small intestine	–	–	–	-1.408	0.174	**< .001**	-1.169	0.187	**< .001**	-1.354	0.174	**< .001**
	Cecum	1.408	0.174	**< .001**	–	–	–	0.239	0.187	0.211	0.054	0.174	0.761
	Colon	1.169	0.187	**< .001**	-0.239	0.187	0.211	–	–	–	-0.185	0.187	0.33
	Rectum	1.354	0.174	**< .001**	-0.054	0.174	0.761	0.185	0.187	0.33	–	–	–
	Sex	-0.432	0.128	**0.002**	-0.432	0.128	**0.002**	-0.432	0.128	**0.002**	-0.432	0.128	**0.002**
	Age	–	–	–	–	–	–	–	–	–	–	–	–
M. rufocanus	Small intestine	–	–	–	-1.028	0.129	**< .001**	-0.905	0.092	**< .001**	-0.92	0.124	**< .001**
	Cecum	1.028	0.129	**< .001**	–	–	–	0.123	0.129	0.343	0.108	0.151	0.478
	Colon	0.905	0.092	**< .001**	-0.123	0.129	0.343	–	–	–	-0.015	0.125	0.904
	Rectum	0.920	0.124	**< .001**	-0.108	0.151	0.478	0.015	0.125	0.904	–	–	–
	Sex	-0.043	0.082	0.6	-0.043	0.082	0.6	-0.043	0.082	0.6	-0.043	0.082	0.6
	Age	-0.11	0.097	0.261	-0.11	0.097	0.261	-0.11	0.097	0.261	-0.11	0.097	0.261

Table S6 Linear mixed effects model results for ASV abundance comparing gut region within each species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

Gut Region	Species	*A. speciosus*			*A. argenteus*			*M. rufocanus*		
		b	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
Small Intestine	*A. speciosus*	–	–	–	0.372	0.138	**0.009**	-0.476	0.092	**< .001**
	A. argenteus	-0.373	0.138	**0.009**	–	–	–	-0.849	0.153	**< .001**
	M. rufocanus	0.476	0.092	**< .001**	0.849	0.153	**< .001**	–	–	–
	Sex	0.07	0.077	0.368	0.07	0.077	0.368	0.070	0.077	0.368
	Age	-0.048	0.092	0.604	-0.048	0.092	0.604	-0.048	0.092	0.604
Cecum	*A. speciosus*	–	–	–	0.079	0.029	**0.009**	-0.150	0.030	**< .001**
	A. argenteus	-0.079	0.029	**0.009**	–	–	–	-0.228	0.039	**< .001**
	M. rufocanus	0.15	0.03	**< .001**	0.228	0.039	**< .001**	–	–	–
	Sex	0.014	0.021	0.507	0.013	0.021	0.507	0.014	0.021	0.507
	Age	-0.002	0.03	0.955	-0.002	0.03	0.955	-0.002	0.030	0.955
Colon	*A. speciosus*	–	–	–	0.084	0.04	**0.039**	-0.174	0.025	**< .001**
	A. argenteus	-0.084	0.04	**0.039**	–	–	–	-0.259	0.045	**< .001**
	M. rufocanus	0.174	0.025	**< .001**	0.259	0.045	**< .001**	–	–	–
	Sex	-0.001	0.021	0.981	-0.001	0.021	0.981	0.000	0.021	0.981
	Age	0.015	0.024	0.554	0.014	0.024	0.554	0.014	0.024	0.554
Rectum	*A. speciosus*	–	–	–	0.064	0.036	0.079	-0.165	0.035	**< .001**
	A. argenteus	-0.064	0.036	0.079	–	–	–	-0.229	0.047	**< .001**
	M. rufocanus	0.165	0.035	**< .001**	0.229	0.047	**< .001**	–	–	–
	Sex	0.004	0.026	0.877	0.004	0.026	0.877	0.004	0.026	0.877
	Age	0.019	0.037	0.618	0.019	0.037	0.618	0.019	0.037	0.618

Table S7 Linear mixed effects model results for Shannon diversity comparing each gut region among species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

		A. speciosus			*A. argenteus*			*M. rufocanus*		
Gut Region	**Species**	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
Small Intestine	*A. speciosus*	–	–	–	0.088	0.124	0.478	-0.057	0.095	0.55
	A. argenteus	-0.088	0.124	0.478	–	–	–	-0.146	0.154	0.346
	M. rufocanus	0.057	0.095	0.55	0.146	0.154	0.346	–	–	–
	Sex	0.062	0.067	0.361	0.062	0.067	0.361	0.062	0.067	0.361
	Age	-0.072	0.083	0.383	-0.072	0.083	0.383	-0.072	0.083	0.383
Cecum	*A. speciosus*	–	–	–	0.014	0.037	0.712	-0.228	0.038	**< .001**
	A. argenteus	-0.014	0.037	0.712	–	–	–	-0.242	0.05	**< .001**
	M. rufocanus	0.228	0.038	**< .001**	0.242	0.05	**< .001**	–	–	–
	Sex	0.003	0.027	0.906	0.003	0.027	0.906	0.003	0.027	0.906
	Age	-0.054	0.039	0.176	-0.054	0.039	0.176	-0.054	0.039	0.176
Colon	*A. speciosus*	–	–	–	0.147	0.046	**0.002**	-0.096	0.029	**0.001**
	A. argenteus	-0.147	0.046	**0.002**	–	–	–	-0.242	0.051	**< .001**
	M. rufocanus	0.096	0.029	**0.001**	0.242	0.051	**< .001**	–	–	–
	Sex	0.011	0.024	0.643	0.011	0.024	0.643	0.011	0.024	0.643
	Age	-0.008	0.028	0.763	-0.008	0.028	0.763	-0.008	0.028	0.763
Rectum	*A. speciosus*	–	–	–	0.053	0.036	0.152	-0.143	0.035	**<.001**
	A. argenteus	-0.053	0.036	0.152	–	–	–	-0.196	0.046	**< .001**
	M. rufocanus	0.143	0.035	**< .001**	0.196	0.046	**< .001**	–	–	–
	Sex	-0.022	0.027	0.418	-0.022	0.027	0.418	-0.022	0.027	0.418
	Age	-0.044	0.036	0.234	-0.044	0.036	0.234	-0.0438	0.036	0.234

Table S8 Linear mixed effects model results for Faith's phylogenetic diversity comparing each gut region among species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

		A. speciosus			*A. argenteus*			*M. rufocanus*		
Gut Region	**Species**	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
Small Intestine	*A. speciosus*	–	–	–	0.273	0.1	**0.008**	-0.341	0.066	**< .001**
	A. argenteus	-0.273	0.1	**0.008**	–	–	–	-0.614	0.11	**< .001**
	M. rufocanus	0.341	0.066	**< .001**	0.614	0.11	**< .001**	–	–	–
	Sex	0.055	0.056	0.327	0.055	0.056	0.327	0.055	0.056	0.327
	Age	-0.018	0.066	0.785	-0.018	0.066	0.785	-0.018	0.066	0.785
Cecum	*A. speciosus*	–	–	–	0.078	0.019	**< .001**	-0.054	0.02	**0.009**
	A. argenteus	-0.078	0.019	**< .001**	–	–	–	-0.132	0.026	**< .001**
	M. rufocanus	0.054	0.02	**0.009**	0.132	0.026	**< .001**	–	–	–
	Sex	0.003	0.013	0.83	0.003	0.013	0.83	0.003	0.013	0.83
	Age	0.012	0.02	0.563	0.012	0.02	0.563	0.012	0.02	0.563
Colon	*A. speciosus*	–	–	–	0.028	0.029	0.339	-0.109	0.019	**< .001**
	A. argenteus	-0.028	0.029	0.339	–	–	–	-0.137	0.033	**< .001**
	M. rufocanus	0.109	0.019	**< .001**	0.137	0.033	**< .001**	–	–	–
	Sex	-0.004	0.015	0.803	-0.004	0.015	0.803	-0.004	0.015	0.803
	Age	0.015	0.018	0.412	0.015	0.018	0.412	0.015	0.018	0.412
Rectum	*A. speciosus*	–	–	–	0.046	0.025	0.073	-0.099	0.025	**< .001**
	A. argenteus	-0.046	0.025	0.073	–	–	–	-0.145	0.033	**< .001**
	M. rufocanus	0.1	0.025	**< .001**	0.145	0.033	**< .001**	–	–	–
	Sex	-0.003	0.018	0.858	-0.003	0.018	0.858	-0.003	0.018	0.858
	Age	0.02	0.026	0.459	0.02	0.026	0.459	0.02	0.026	0.459

Table S9 Linear mixed effects model results for Pielou's evenness comparing each gut region among species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance.

		A. speciosus			*A. argenteus*			*M. rufocanus*		
Gut Region	**Species**	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
Small Intestine	*A. speciosus*	–	–	–	0.399	0.229	0.0851	-0.634	0.154	**< .001**
	A. argenteus	-0.399	0.229	0.085	–	–	–	-1.033	0.255	**< .001**
	M. rufocanus	0.634	0.154	**< .001**	1.033	0.255	**< .001**	–	–	–
	Sex	0.069	0.128	0.589	0.069	0.128	0.589	0.069	0.128	0.589
	Age	-0.131	0.152	0.391	-0.131	0.152	0.391	-0.131	0.152	0.391
Cecum	*A. speciosus*	–	–	–	0.008	0.089	0.93	-0.529	0.091	**< .001**
	A. argenteus	-0.008	0.089	0.93	–	–	–	-0.537	0.117	**< .001**
	M. rufocanus	0.529	0.091	**< .001**	0.537	0.117	**< .001**	–	–	–
	Sex	0.062	0.065	0.345	0.062	0.065	0.345	0.062	0.065	0.345
	Age	-0.039	0.091	0.668	-0.039	0.091	0.668	-0.039	0.091	0.668
Colon	*A. speciosus*	–	–	–	0.297	0.084	**< .001**	-0.369	0.051	**< .001**
	A. argenteus	-0.297	0.084	**< .001**	–	–	–	-0.667	0.092	**< .001**
	M. rufocanus	0.369	0.051	**< .001**	0.667	0.092	**< .001**	–	–	–
	Sex	0.019	0.044	0.66	0.019	0.043	0.66	0.019	0.044	0.66
	Age	-0.002	0.051	0.962	-0.002	0.051	0.962	-0.002	0.051	0.962
Rectum	*A. speciosus*	–	–	–	0.1	0.074	0.188	-0.377	0.072	**< .001**
	A. argenteus	-0.1	0.074	0.188	–	–	–	-0.477	0.094	**< .001**
	M. rufocanus	0.377	0.072	**< .001**	0.477	0.094	**< .001**	–	–	–
	Sex	0.037	0.055	0.499	0.037	0.055	0.499	0.037	0.055	0.499
	Age	0.002	0.074	0.977	0.002	0.074	0.977	0.002	0.074	0.977

Table S10 Linear mixed effects model results for ASV abundance comparing each gut region among species, values rounded to the thousandth place. Row as compared to column. Bolded text indicates significance

Species	Dissimilarity Index	Variable	Sum of Squares	R2	F	*p*
A. speciosus	Jaccard	Gut Region	5.021	0.10766	5.7211	**0.001**
		Site	3.309	0.07096	3.7708	**0.001**
		Sex	0.799	0.01713	2.7304	**0.001**
		Age	0.647	0.01388	2.2126	**0.002**
	Bray-Curtis	Gut Region	8.511	0.17782	10.3656	**0.001**
		Site	3.272	0.06837	3.9852	**0.001**
		Sex	0.793	0.01658	2.8988	**0.002**
		Age	0.8	0.01672	2.9242	**0.002**
	Unweighted unifrac	Gut Region	3.1658	0.20183	12.2717	**0.001**
		Site	1.167	0.0744	4.5235	**0.001**
		Sex	0.2774	0.01769	3.2259	**0.004**
		Age	0.2402	0.01531	2.7934	**0.003**
	Weighted unifrac	Gut Region	5.6009	0.342	24.7671	**0.001**
		Site	0.7453	0.04551	3.2956	**0.002**
		Sex	0.1828	0.01116	2.4248	0.055
		Age	0.3501	0.02138	4.644	**0.002**

Table S11 PERMANOVA results comparing all gut regions (whole gut) within *A. speciosus*. Bolded text indicates significance.

Species	Dissimilarity Index	Variable	Sum of Squares	R2	F	*p*
A. argenteus	Jaccard	Gut Region	1.737	0.15734	2.0728	**0.001**
		Site	0.8825	0.07994	3.1595	**0.001**
		Sex	0.5989	0.05425	2.1442	**0.002**
	Bray-Curtis	Gut Region	2.3742	0.20174	2.8253	**0.001**
		Site	0.8956	0.0761	3.1972	**0.001**
		Sex	0.6556	0.0557	2.3403	**0.006**
	Unweighted unifrac	Gut Region	1.0521	0.29497	4.6851	**0.001**
		Site	0.2206	0.06186	2.9473	**0.006**
		Sex	0.1981	0.05554	2.6464	**0.014**
	Weighted unifrac	Gut Region	3.1108	0.47008	10.0174	**0.001**
		Site	0.4098	0.06193	3.9593	**0.014**
		Sex	0.1986	0.03001	1.9183	0.115

Table S12 PERMANOVA results comparing all gut regions (whole gut) within *A. argenteus*. Bolded text indicates significance.

Species	Dissimilarity Index	Variable	Sum of Squares	R2	F	*p*
M. rufocanus	Jaccard	Gut Region	3.5	0.08417	3.5317	**0.001**
		Site	4.086	0.09826	4.1229	**0.001**
		Sex	0.621	0.01493	1.8801	**0.003**
		Age	0.674	0.0162	2.0394	**0.001**
	Bray-Curtis	Gut Region	4.629	0.11182	5.0003	**0.001**
		Site	4.697	0.11345	5.0732	**0.001**
		Sex	0.646	0.01561	2.0943	**0.001**
		Age	0.874	0.02112	2.8334	**0.001**
	Unweighted unifrac	Gut Region	3.2729	0.2294	11.5249	**0.001**
		Site	1.2335	0.08646	4.3436	**0.001**
		Sex	0.1512	0.0106	1.5972	0.071
		Age	0.2382	0.0167	2.5167	**0.007**
	Weighted unifrac	Gut Region	3.5355	0.25814	12.9768	**0.001**
		Site	0.6179	0.04511	2.2678	**0.026**
		Sex	0.1099	0.00802	1.2096	0.266
		Age	0.4423	0.03229	4.8702	**0.006**

Table S13: PERMANOVA results comparing all gut regions (whole gut) within *M. rufocanus*. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine - Colon				Small Intestine - Cecum				Small Intestine - Rectum			
		Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*
Jaccard	Gut Region	3.2263	0.10765	10.2769	**0.001**	2.7766	0.11057	8.7412	**0.001**	2.4068	0.09892	7.4833	**0.001**
	Site	1.881	0.06276	1.9972	**0.001**	1.741	0.06933	1.827	**0.001**	1.7266	0.07096	1.7894	**0.001**
	Sex	0.5181	0.01729	1.6504	**0.009**	0.528	0.02102	1.6622	**0.01**	0.5192	0.02134	1.6143	**0.008**
	Age	0.4845	0.01617	1.5434	**0.026**	0.3723	0.01482	1.172	0.139	0.3804	0.01564	1.1828	0.114
Bray-Curtis	Gut Region	5.2863	0.17607	18.4936	**0.001**	4.9493	0.20059	17.7443	**0.001**	4.1016	0.17185	14.3382	**0.001**
	Site	2.0776	0.0692	2.4227	**0.001**	1.6124	0.06535	1.9269	**0.001**	1.7401	0.07291	2.0277	**0.003**
	Sex	0.4588	0.01528	1.6052	0.068	0.4413	0.01789	1.5823	0.072	0.4688	0.01964	1.6388	0.058
	Age	0.4773	0.0159	1.6698	**0.05**	0.3769	0.01528	1.3513	0.139	0.3925	0.01645	1.372	0.125
Unweighted unifrac	Gut Region	2.1938	0.20393	22.9577	**0.001**	1.7389	0.19544	17.7268	**0.001**	1.5892	0.18596	16.0504	**0.001**
	Site	0.8773	0.08155	3.0601	**0.001**	0.7339	0.08248	2.4938	**0.002**	0.6843	0.08007	2.3037	**0.001**
	Sex	0.2519	0.02341	2.6359	**0.014**	0.2304	0.02589	2.3483	**0.023**	0.2257	0.02641	2.2796	**0.024**
	Age	0.1724	0.01602	1.8041	**0.07**	0.1124	0.01264	1.1462	0.293	0.1057	0.01237	1.0678	0.357
Weighted unifrac	Gut Region	3.2353	0.30236	37.7693	**0.001**	3.2278	0.35068	38.4697	**0.001**	3.455	0.36075	39.0967	**0.001**
	Site	0.6223	0.05816	2.4216	**0.007**	0.4872	0.05293	1.9354	**0.033**	0.5295	0.05528	1.9972	**0.038**
	Sex	0.1497	0.01399	1.748	0.123	0.1982	0.02154	2.3627	0.066	0.2001	0.02089	2.2638	0.059
	Age	0.1825	0.01706	2.131	0.077	0.089	0.00967	1.061	0.327	0.0905	0.00945	1.0242	0.373
Dissimilarity Index	**Variable**	**Cecum - Rectum**				**Colon - Cecum**				**Colon - Rectum**			
		Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*
Jaccard	Gut Region	0.1699	0.01125	0.6435	0.983	0.2749	0.01376	1.0176	0.413	0.3165	0.01615	1.1588	0.129
	Site	2.0954	0.13866	2.6447	**0.001**	2.2219	0.11127	2.7421	**0.001**	2.1915	0.11184	2.6743	**0.001**
	Sex	0.5117	0.03386	1.9373	**0.001**	0.5082	0.02545	1.8815	**0.001**	0.4807	0.02453	1.7599	**0.003**
	Age	0.4507	0.02982	1.7063	**0.005**	0.4883	0.02445	1.808	**0.001**	0.4903	0.02502	1.7948	**0.002**
Bray-Curtis	Gut Region	0.4834	0.03205	1.879	**0.005**	0.5686	0.02823	2.144	**0.001**	0.4786	0.02377	1.7421	**0.009**
	Site	1.8524	0.1228	2.4001	**0.001**	2.1865	0.10855	2.7482	**0.001**	2.2107	0.10981	2.6826	**0.001**
	Sex	0.5169	0.03427	2.0093	**0.005**	0.5016	0.0249	1.8913	**0.005**	0.5203	0.02584	1.8939	**0.006**
	Age	0.655	0.04342	2.546	**0.002**	0.7085	0.03517	2.6713	**0.001**	0.7159	0.03556	2.6062	**0.001**
Unweighted unifrac	Gut Region	0.0546	0.01486	0.8597	0.68	0.0672	0.01415	1.0655	0.357	0.0881	0.01776	1.2976	0.102
	Site	0.4848	0.13187	2.5424	**0.001**	0.5333	0.11224	2.8184	**0.001**	0.5635	0.1136	2.7671	**0.001**
	Sex	0.1265	0.03441	1.9904	**0.007**	0.1377	0.02898	2.1833	**0.003**	0.1387	0.02796	2.043	**0.002**
	Age	0.15	0.0408	2.3599	**0.003**	0.1656	0.03485	2.6254	**0.002**	0.165	0.03326	2.4305	**0.002**
Weighted unifrac	Gut Region	0.1996	0.05713	3.6447	**0.011**	0.1469	0.03054	2.4485	**0.04**	0.2602	0.05145	4.1045	**0.006**
	Site	0.3712	0.10626	2.2596	**0.018**	0.3991	0.08295	2.217	**0.013**	0.465	0.09195	2.4452	**0.005**
	Sex	0.1282	0.03671	2.342	**0.047**	0.1318	0.02739	2.196	0.059	0.1691	0.03344	2.6678	**0.026**
	Age	0.3302	0.09452	6.0301	**0.001**	0.4732	0.09834	7.8853	**0.001**	0.4229	0.08362	6.6707	**0.001**

Table S14 Pairwise PERMANOVA results comparing the different gut regions (whole gut) within *A. speciosus*. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine - Colon				Small Intestine - Cecum				Small Intestine - Rectum			
		Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*
Jaccard	Gut Region	0.9049	0.15698	2.7296	**0.001**	1.1474	0.17836	3.6504	**0.001**	1.0643	0.16777	3.4144	**0.001**
	Site	0.4479	0.07769	1.351	0.072	0.4819	0.07491	1.5331	**0.049**	0.4847	0.0764	1.5548	**0.039**
	Sex	0.4336	0.07522	1.3079	0.103	0.4032	0.06269	1.283	0.121	0.4309	0.06793	1.3824	0.078
Bray-Curtis	Gut Region	1.277	0.21026	3.9726	**0.001**	1.5953	0.24005	5.283	**0.001**	1.3492	0.20287	4.3108	**0.001**
	Site	0.5194	0.08552	1.6157	0.058	0.4569	0.06875	1.513	0.087	0.4976	0.07482	1.5897	**0.07**
	Sex	0.4196	0.06909	1.3053	0.169	0.3658	0.05505	1.2115	0.237	0.4219	0.06344	1.348	0.156
Unweighted unifrac	Gut Region	0.56323	0.28141	5.838	**0.001**	0.75253	0.32092	8.177	**0.001**	0.74469	0.32155	8.4404	**0.001**
	Site	0.09295	0.04644	0.9635	0.385	0.12238	0.05219	1.3298	0.208	0.14274	0.06163	1.6178	0.104
	Sex	0.18757	0.09372	1.9442	**0.049**	0.18161	0.07745	1.9734	0.063	0.19331	0.08347	2.191	**0.046**
Weighted unifrac	Gut Region	1.5613	0.41869	10.6232	**0.001**	1.9941	0.50749	16.7913	**0.001**	2.225	0.52283	18.3957	**0.001**
	Site	0.2119	0.05683	1.442	0.191	0.1108	0.0282	0.9331	0.379	0.1655	0.0389	1.3686	0.225
	Sex	0.1921	0.05153	1.3073	0.237	0.1618	0.04118	1.3626	0.226	0.1718	0.04038	1.4207	0.208
Dissimilarity Index	**Variable**	**Cecum - Rectum**				**Colon - Cecum**				**Colon - Rectum**			
		Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*
Jaccard	Gut Region	0.0922	0.02049	0.3875	0.986	0.1474	0.03588	0.5968	0.894	0.1114	0.02683	0.4397	0.984
	Site	0.6567	0.14596	2.7608	**0.001**	0.5944	0.14466	2.4061	**0.001**	0.603	0.14518	2.3791	**0.001**
	Sex	0.4203	0.09341	1.7668	**0.01**	0.4028	0.09802	1.6304	**0.022**	0.3977	0.09574	1.5689	**0.04**
Bray-Curtis	Gut Region	0.1461	0.0308	0.5764	0.878	0.1406	0.03317	0.5432	0.912	0.131	0.0293	0.4851	0.947
	Site	0.6613	0.13944	2.6097	**0.004**	0.5645	0.13314	2.1808	**0.008**	0.6637	0.14842	2.4572	**0.001**
	Sex	0.3875	0.0817	1.5289	0.094	0.4285	0.10107	1.6555	0.061	0.4357	0.09744	1.6131	0.059
Unweighted unifrac	Gut Region	0.02864	0.02698	0.5118	0.936	0.0444	0.046	0.7767	0.709	0.02653	0.02806	0.4579	0.973
	Site	0.15127	0.14254	2.7037	**0.003**	0.13992	0.14495	2.4473	**0.003**	0.1376	0.14552	2.3749	**0.001**
	Sex	0.09809	0.09243	1.7533	0.035	0.09491	0.09833	1.6602	0.055	0.08618	0.09114	1.4875	0.11
Weighted unifrac	Gut Region	0.1019	0.07118	1.5253	0.172	0.08783	0.05638	1.0206	0.387	0.10634	0.06438	1.3562	0.213
	Site	0.32197	0.22491	4.8194	**0.001**	0.31455	0.2019	3.655	**0.007**	0.4726	0.28612	6.0277	**0.002**
	Sex	0.07241	0.05058	1.0839	0.364	0.12286	0.07886	1.4276	0.199	0.13194	0.07988	1.6828	0.141

Table S15 Pairwise PERMANOVA results comparing the different gut regions (whole gut) within *A. argenteus*. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine - Colon				Small Intestine - Cecum				Small Intestine - Rectum			
		Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*
Jaccard	Gut Region	2.3792	0.0768	6.8205	**0.001**	1.4535	0.06693	4.0903	**0.001**	1.3684	0.0616	3.8015	**0.001**
	Site	2.563	0.08274	2.4492	**0.001**	2.3521	0.10832	2.2064	**0.001**	2.3327	0.10501	2.1601	**0.001**
	Sex	0.4127	0.01332	1.1831	0.13	0.3657	0.01684	1.0291	0.347	0.3844	0.0173	1.0679	0.257
	Age	0.5075	0.01638	1.4548	**0.018**	0.4868	0.02242	1.3699	**0.027**	0.4903	0.02207	1.3619	**0.025**
Bray-Curtis	Gut Region	3.2344	0.10469	9.7927	**0.001**	1.9177	0.08953	5.7594	**0.001**	1.9786	0.08974	5.8709	**0.001**
	Site	2.7804	0.09	2.8061	**0.001**	2.4535	0.11455	2.4562	**0.001**	2.4501	0.11112	2.4233	**0.001**
	Sex	0.4024	0.01302	1.2182	0.143	0.3547	0.01656	1.0653	0.33	0.3737	0.01695	1.109	0.279
	Age	0.6967	0.02255	2.1095	**0.001**	0.7103	0.03316	2.1331	**0.002**	0.7319	0.03319	2.1715	**0.002**
Unweighted unifrac	Gut Region	2.4149	0.22128	23.4817	**0.001**	1.4157	0.17379	12.0178	**0.001**	1.3607	0.16447	11.2724	**0.001**
	Site	0.8109	0.0743	2.6283	**0.001**	0.803	0.09858	2.2722	**0.003**	0.7393	0.08936	2.0414	**0.002**
	Sex	0.1067	0.00978	1.038	0.318	0.0911	0.01119	0.7738	0.648	0.0882	0.01067	0.731	0.732
	Age	0.1761	0.01614	1.7125	0.062	0.1817	0.02231	1.5426	0.094	0.1701	0.02056	1.4092	0.144
Weighted unifrac	Gut Region	2.5543	0.21908	22.953	**0.001**	1.5439	0.16104	10.7244	**0.001**	1.5094	0.15706	10.6542	**0.001**
	Site	0.656	0.05626	1.9649	0.055	0.5964	0.06221	1.381	0.177	0.6114	0.06362	1.4385	0.159
	Sex	0.0712	0.00611	0.6398	0.596	0.0655	0.00683	0.4551	0.787	0.0704	0.00732	0.4969	0.756
	Age	0.3652	0.03132	3.2819	**0.029**	0.4708	0.04911	3.2702	**0.024**	0.4775	0.04968	3.3701	**0.023**
Dissimilarity Index	**Variable**	**Cecum - Rectum**				**Colon - Cecum**				**Colon - Rectum**			
		Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*	Sum of Squares	R2	F	*p*
Jaccard	Gut Region	0.1941	0.01999	0.707	0.916	0.2469	0.01409	0.8036	0.928	0.2572	0.01419	0.8193	0.912
	Site	2.4425	0.2515	2.9648	**0.001**	2.5426	0.14508	2.7583	**0.001**	2.5503	0.14069	2.7078	**0.001**
	Sex	0.531	0.05467	1.9335	**0.004**	0.4544	0.02593	1.4788	**0.015**	0.4456	0.02458	1.4194	**0.019**
	Age	0.5027	0.05176	1.8307	**0.002**	0.4545	0.02594	1.4793	**0.011**	0.4321	0.02384	1.3765	**0.026**
Bray-Curtis	Gut Region	0.1664	0.01804	0.7026	0.873	0.2305	0.01362	0.8015	0.856	0.267	0.01527	0.9144	0.643
	Site	2.7607	0.29933	3.885	**0.001**	2.8832	0.17041	3.3419	**0.001**	2.9234	0.16723	3.3377	**0.001**
	Sex	0.5524	0.0599	2.3324	**0.002**	0.4099	0.02423	1.4253	**0.031**	0.4159	0.02379	1.4244	**0.04**
	Age	0.5322	0.0577	2.2468	**0.003**	0.4547	0.02688	1.5813	**0.022**	0.4452	0.02547	1.525	**0.022**
Unweighted unifrac	Gut Region	0.06482	0.02881	1.119	0.292	0.0879	0.02213	1.3233	0.096	0.0666	0.01614	0.9697	0.476
	Site	0.68059	0.30254	3.9162	**0.001**	0.6834	0.17202	3.4292	**0.001**	0.6886	0.16698	3.3437	**0.001**
	Sex	0.11133	0.04949	1.9219	**0.008**	0.0872	0.02196	1.3132	0.099	0.091	0.02206	1.3254	0.078
	Age	0.11839	0.05263	2.0437	**0.003**	0.125	0.03148	1.8825	**0.009**	0.12	0.02909	1.7478	**0.013**
Weighted unifrac	Gut Region	0.04721	0.04381	1.7634	0.089	0.08447	0.03909	2.2624	**0.041**	0.0878	0.03967	2.3492	**0.041**
	Site	0.26678	0.24756	3.3216	**0.001**	0.23425	0.10841	2.0913	**0.004**	0.22869	0.10334	2.0397	**0.014**
	Sex	0.08093	0.0751	3.023	**0.004**	0.05941	0.02749	1.5911	0.112	0.07802	0.03526	2.0876	0.063
	Age	0.09371	0.08696	3.5004	**0.003**	0.10243	0.0474	2.7433	**0.015**	0.09925	0.04485	2.6557	**0.019**

Table S16 Pairwise PERMANOVA results comparing the different gut regions (whole gut) within *M. rufocanus*. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine				Cecum			
		Sum of squares	R2	F	*p*	Sum of squares	R2	F	*p*
Jaccard	Species	6.148	0.1553	8.3689	**0.001**	4.2995	0.2308	7.2481	**0.001**
	Site	1.81	0.04572	1.6426	**0.001**	1.2947	0.0695	1.4551	**0.013**
	Sex	0.367	0.00927	0.9988	0.353	0.2894	0.01553	0.9756	0.391
	Age	0.41	0.01035	1.1158	0.199	0.2879	0.01546	0.9708	0.354
Bray-Curtis	Species	8.045	0.20842	11.9986	**0.001**	4.9895	0.26674	8.7541	**0.001**
	Site	1.582	0.04098	1.5728	**0.012**	1.1855	0.06338	1.3867	**0.028**
	Sex	0.335	0.00869	1.0001	0.43	0.2238	0.01196	0.7852	0.796
	Age	0.477	0.01235	1.4216	0.093	0.3375	0.01804	1.1842	0.197
Unweighted unifrac	Species	2.7611	0.17991	10.4283	**0.001**	1.8215	0.34797	13.0457	**0.001**
	Site	1.2569	0.0819	3.1646	**0.001**	0.3476	0.06641	1.6598	**0.023**
	Sex	0.0839	0.00546	0.6335	0.83	0.0605	0.01156	0.867	0.505
	Age	0.1249	0.00814	0.9434	0.489	0.0729	0.01392	1.0441	0.302
Weighted unifrac	Species	3.6992	0.21817	12.5477	**0.001**	0.8903	0.24793	7.7369	**0.001**
	Site	0.5485	0.03235	1.2403	0.251	0.1711	0.04765	0.9913	0.464
	Sex	0.0864	0.0051	0.5864	0.695	0.0297	0.00827	0.516	0.857
	Age	0.2398	0.01414	1.6266	0.14	0.0833	0.0232	1.4479	0.155
Dissimilarity Index	Variable	Colon				Rectum			
		Sum of squares	R2	F	*p*	Sum of squares	R2	F	*p*
Jaccard	Species	7.588	0.22193	12.1555	**0.001**	4.1466	0.22154	6.7272	**0.001**
	Site	1.593	0.0466	1.7016	**0.003**	1.3082	0.0699	1.4149	**0.027**
	Sex	0.312	0.00912	0.9994	0.324	0.3179	0.01699	1.0316	0.287
	Age	0.352	0.01031	1.1291	0.222	0.3081	0.01646	0.9996	0.36
Bray-Curtis	Species	7.967	0.23028	12.8502	**0.001**	4.386	0.23187	7.1296	**0.001**
	Site	1.718	0.04966	1.8474	**0.002**	1.2794	0.06764	1.3865	**0.025**
	Sex	0.318	0.0092	1.0268	0.349	0.3038	0.01606	0.9877	0.411
	Age	0.414	0.01198	1.3367	0.112	0.3349	0.01771	1.0889	0.262
Unweighted unifrac	Species	3.4537	0.36371	24.7898	**0.001**	1.811	0.3439	12.5438	**0.001**
	Site	0.4477	0.04714	2.1421	**0.005**	0.3408	0.06472	1.5738	**0.033**
	Sex	0.0633	0.00666	0.9085	0.43	0.084	0.01596	1.1641	0.238
	Age	0.0978	0.0103	1.4034	0.148	0.0706	0.01341	0.9779	0.355
Weighted unifrac	Species	1.5127	0.21585	11.5832	**0.001**	0.9121	0.23594	7.1953	**0.001**
	Site	0.256	0.03652	1.3066	0.156	0.2055	0.05316	1.0807	0.336
	Sex	0.0706	0.01007	1.0811	0.361	0.0851	0.022	1.342	0.192
	Age	0.0756	0.01079	1.1578	0.286	0.0645	0.01668	1.017	0.418

Table S17 PERMANOVA results comparing gut regions among species, all species included. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine				Cecum			
		Sum of squares	R2	F	*p*	Sum of squares	R2	F	*p*
Jaccard	Species	4.95	0.14003	13.5642	**0.001**	3.3089	0.21426	10.9643	**0.001**
	Site	1.84	0.05206	1.6809	**0.001**	1.2758	0.08261	1.4092	**0.023**
	Sex	0.415	0.01173	1.1363	0.175	0.3062	0.01983	1.0145	0.335
	Age	0.409	0.01158	1.1218	0.186	0.2915	0.01888	0.9661	0.397
Bray-Curtis	Species	6.535	0.19063	19.7864	**0.001**	3.6814	0.24221	12.8325	**0.001**
	Site	1.829	0.05336	1.8461	**0.002**	1.1799	0.07763	1.3709	0.07
	Sex	0.341	0.00995	1.0325	0.345	0.2409	0.01585	0.8397	0.647
	Age	0.474	0.01384	1.4365	0.093	0.3433	0.02258	1.1966	0.178
Unweighted unifrac	Species	2.3594	0.17321	18.5568	**0.001**	1.4552	0.33364	20.5476	**0.001**
	Site	1.3134	0.09643	3.4434	**0.001**	0.3451	0.07911	1.6241	**0.037**
	Sex	0.1645	0.01208	1.2937	0.202	0.0665	0.01526	0.9396	0.401
	Age	0.1209	0.00888	0.951	0.419	0.0869	0.01992	1.2268	0.204
Weighted unifrac	Species	2.5086	0.17325	17.2415	**0.001**	0.61768	0.23025	11.7861	**0.001**
	Site	0.6107	0.04218	1.3991	0.151	0.15268	0.05691	0.9711	0.491
	Sex	0.0762	0.00527	0.524	0.758	0.03135	0.01169	0.5982	0.751
	Age	0.226	0.01561	1.5531	0.153	0.09912	0.03695	1.8913	0.076
Dissimilarity Index	**Variable**	**Colon**				**Rectum**			
		Sum of squares	**R2**	**F**	***p***	**Sum of squares**	**R2**	**F**	***p***
Jaccard	Species	6.5373	0.20839	20.8669	**0.001**	3.1497	0.20371	10.0046	**0.001**
	Site	1.6034	0.05111	1.706	**0.002**	1.3012	0.08416	1.3777	**0.036**
	Sex	0.3157	0.01006	1.0076	0.349	0.3155	0.02041	1.0022	0.347
	Age	0.3575	0.0114	1.141	0.208	0.3057	0.01977	0.9709	0.432
Bray-Curtis	Species	6.8858	0.21806	22.3311	**0.001**	3.357	0.21663	10.8262	**0.001**
	Site	1.7597	0.05573	1.9023	**0.002**	1.2777	0.08246	1.3736	0.051
	Sex	0.3103	0.00983	1.0065	0.335	0.2964	0.01913	0.9558	0.451
	Age	0.421	0.01333	1.3654	0.112	0.3324	0.02145	1.0719	0.261
Unweighted unifrac	Species	2.9769	0.32936	39.5733	**0.001**	1.4584	0.33571	20.2105	**0.001**
	Site	0.4531	0.05013	2.0077	**0.012**	0.3535	0.08137	1.6328	**0.035**
	Sex	0.0789	0.00873	1.0489	0.295	0.0774	0.01783	1.0732	0.313
	Age	0.1134	0.01254	1.5068	0.115	0.0736	0.01695	1.0203	0.335
Weighted unifrac	Species	1.2944	0.21858	21.7946	**0.001**	0.69639	0.2371	12.1151	**0.001**
	Site	0.2423	0.04092	1.3599	0.14	0.23924	0.08146	1.3874	0.134
	Sex	0.0276	0.00466	0.4642	0.881	0.04199	0.0143	0.7305	0.595
	Age	0.0813	0.01373	1.3695	0.203	0.06257	0.0213	1.0886	0.348

Table S18 Pairwise PERMANOVA results comparing gut regions between *A. speciosus* and *M. rufocanus*. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine				Cecum			
		Sum of squares	R2	F	*p*	Sum of squares	R2	F	*p*
Jaccard	Species	0.7782	0.07587	2.7271	**0.001**	0.7785	0.0732	2.7953	**0.001**
	Site	1.1868	0.11571	1.3864	**0.001**	1.2264	0.11532	1.4678	**0.001**
	Sex	0.2901	0.02828	1.0166	0.381	0.2949	0.02773	1.059	0.275
	Age	0.2974	0.02899	1.0422	0.329	0.2581	0.02427	0.9268	0.687
Bray-Curtis	Species	0.816	0.07714	2.735	**0.001**	1.2237	0.11463	4.5261	**0.001**
	Site	1.0608	0.10028	1.1851	0.095	1.0294	0.09644	1.2692	**0.048**
	Sex	0.282	0.02666	0.9451	0.574	0.2152	0.02016	0.7961	0.832
	Age	0.3632	0.03433	1.2172	0.153	0.3658	0.03427	1.3531	0.099
Unweighted unifrac	Species	0.18585	0.07569	2.7162	**0.001**	0.20173	0.08141	3.1259	**0.001**
	Site	0.26102	0.1063	1.2716	**0.027**	0.2635	0.10634	1.361	**0.005**
	Sex	0.07048	0.0287	1.0301	0.418	0.07092	0.02862	1.0989	0.282
	Age	0.09076	0.03696	1.3264	0.075	0.0704	0.02841	1.0909	0.326
Weighted unifrac	Species	0.10315	0.04071	1.4155	0.194	0.23371	0.09595	3.7704	**0.003**
	Site	0.22207	0.08765	1.0158	0.422	0.20773	0.08529	1.1171	0.3
	Sex	0.13933	0.055	1.912	0.085	0.04129	0.01695	0.6661	0.68
	Age	0.10138	0.04002	1.3913	0.182	0.15533	0.06377	2.5058	**0.04**
Dissimilarity Index	**Variable**	**Colon**				**Rectum**			
		Sum of squares	**R2**	**F**	***p***	**Sum of squares**	**R2**	**F**	***p***
Jaccard	Species	0.7655	0.05248	2.661	**0.001**	0.7782	0.07587	2.7271	**0.001**
	Site	1.3609	0.09331	1.5769	**0.001**	1.1868	0.11571	1.3864	**0.001**
	Sex	0.3077	0.0211	1.0698	0.216	0.2901	0.02828	1.0166	0.381
	Age	0.3568	0.02446	1.2402	**0.042**	0.2974	0.02899	1.0422	0.329
Bray-Curtis	Species	0.8344	0.05535	2.8639	**0.001**	0.816	0.07714	2.735	**0.001**
	Site	1.4731	0.09772	1.6854	**0.001**	1.0608	0.10028	1.1851	0.095
	Sex	0.3313	0.02198	1.1373	0.245	0.282	0.02666	0.9451	0.574
	Age	0.49	0.0325	1.6817	**0.014**	0.3632	0.03433	1.2172	0.153
Unweighted unifrac	Species	0.2388	0.06516	3.4343	**0.001**	0.18585	0.07569	2.7162	**0.001**
	Site	0.3698	0.10091	1.7728	**0.001**	0.26102	0.1063	1.2716	**0.027**
	Sex	0.0796	0.02172	1.145	0.2	0.07048	0.0287	1.0301	0.418
	Age	0.1257	0.03431	1.8081	**0.005**	0.09076	0.03696	1.3264	0.075
Weighted unifrac	Species	0.1422	0.03478	1.8977	0.098	0.10315	0.04071	1.4155	0.194
	Site	0.3285	0.08039	1.4619	0.128	0.22207	0.08765	1.0158	0.422
	Sex	0.201	0.04917	2.6828	**0.028**	0.13933	0.055	1.912	0.085
	Age	0.344	0.08418	4.5928	**0.005**	0.10138	0.04002	1.3913	0.182

Table S19 Pairwise PERMANOVA results comparing gut regions between *A. speciosus* and *A. argenteus*. Bolded text indicates significance.

Dissimilarity Index	Variable	Small Intestine				Cecum			
		Sum of squares	R2	F	*p*	Sum of squares	R2	F	*p*
Jaccard	Species	1.8038	0.0873	4.8339	**0.001**	2.1867	0.24652	7.5102	**0.001**
	Site	1.9785	0.09576	1.7674	**0.001**	1.4027	0.15813	1.6058	**0.015**
	Sex	0.3675	0.01779	0.9848	0.452	0.2921	0.03293	1.0033	0.346
	Age	0.4659	0.02255	1.2486	0.076	0.3303	0.03724	1.1345	0.237
Bray-Curtis	Species	2.1944	0.10802	6.2356	**0.001**	2.3726	0.26921	8.4506	**0.001**
	Site	1.928	0.0949	1.8262	**0.001**	1.3737	0.15586	1.6309	**0.024**
	Sex	0.3143	0.01547	0.8931	0.603	0.258	0.02928	0.9191	0.476
	Age	0.7461	0.03672	2.12	**0.002**	0.3168	0.03595	1.1285	0.259
Unweighted unifrac	Species	0.8717	0.12151	6.905	**0.001**	0.96941	0.37484	14.7491	**0.001**
	Site	0.6244	0.08704	1.6487	**0.011**	0.42496	0.16432	2.1552	**0.009**
	Sex	0.1134	0.01581	0.8987	0.522	0.05945	0.02299	0.9046	0.425
	Age	0.1359	0.01894	1.0762	0.329	0.08078	0.03123	1.229	0.248
Weighted unifrac	Species	1.8437	0.17247	10.5428	**0.001**	0.45296	0.28928	8.8961	**0.001**
	Site	0.6754	0.06318	1.2873	0.239	0.1951	0.1246	1.2772	0.196
	Sex	0.1019	0.00953	0.5828	0.651	0.03483	0.02225	0.6841	0.685
	Age	0.5494	0.0514	3.1419	**0.024**	0.06826	0.04359	1.3405	0.22
Dissimilarity Index	**Variable**	**Colon**				**Rectum**			
		Sum of squares	**R2**	**F**	***p***	**Sum of squares**	**R2**	**F**	***p***
Jaccard	Species	2.1993	0.13112	6.7826	**0.001**	2.134	0.22679	7.0295	**0.001**
	Site	1.6035	0.0956	1.6484	**0.001**	1.453	0.15442	1.5955	**0.009**
	Sex	0.3091	0.01843	0.9531	0.517	0.3226	0.03428	1.0625	0.275
	Age	0.3401	0.02027	1.0488	0.308	0.3392	0.03605	1.1172	0.208
Bray-Curtis	Species	2.2328	0.13488	7.098	**0.001**	2.1719	0.23115	7.3834	**0.001**
	Site	1.7422	0.10525	1.8462	**0.001**	1.5346	0.16332	1.7389	**0.001**
	Sex	0.2932	0.01771	0.9322	0.556	0.3198	0.03403	1.0871	0.26
	Age	0.3319	0.02005	1.0552	0.304	0.3692	0.03929	1.2552	0.145
Unweighted unifrac	Species	1.0477	0.24827	15.1989	**0.001**	0.96079	0.34494	12.9127	**0.001**
	Site	0.4091	0.09694	1.9782	**0.006**	0.39258	0.14094	1.7587	**0.025**
	Sex	0.0627	0.01485	0.9092	0.502	0.08852	0.03178	1.1896	0.228
	Age	0.0811	0.01923	1.1772	0.228	0.07858	0.02821	1.0561	0.31
Weighted unifrac	Species	0.46014	0.15965	8.8899	**0.001**	0.56929	0.3133	11.7362	**0.001**
	Site	0.31747	0.11015	2.0445	**0.018**	0.26719	0.14704	1.8361	**0.034**
	Sex	0.0359	0.01246	0.6936	0.669	0.04746	0.02612	0.9784	0.401
	Age	0.10174	0.0353	1.9657	0.071	0.10852	0.05972	2.2372	**0.044**

Table S20 Pairwise PERMANOVA results comparing gut regions between *A. argenteus* and *M. rufocanus*. Bolded text indicates significance.

Species	Small Intestine	Cecum	Colon	Rectum	Average
A. speciosus	0	0	0	1	0.25
A. argenteus	4	13	5	17	9.75
M. rufocanus	0	1	0	0	0.25
Average	1.33	4.67	1.67	6	

Table S21 Number of more abundant genera found in each gut region based on LEfSe analysis when all regions (whole gut) were included.

	CE - SI		RC - SI		CL - SI		CE – CL		CE - RC		RC - CL	
Species	**CE**	**SI**	**RC**	**SI**	**CL**	**SI**	**CE**	**CL**	**CE**	**RC**	**RC**	**CL**
A. speciosus	47	23	44	19	47	22	4	4	5	5	7	5
A. argenteus	39	10	35	7	33	8	1	0	2	2	1	0
M. rufocanus	48	9	53	10	50	20	6	0	1	3	12	0
Average	44.67	14	44	12	43.33	16.67	3.67	1.33	2.67	3.33	6.67	1.67

Table S22 Number of more abundant genera found in each gut region based on pairwise LEfSe analysis we SI is small intestine, CE is cecum, CL is colon, and RC is the rectum.

	A. speciosus	*A. argenteus*	*M. rufocanus*
Small Intestine	17	8	10
Cecum	14	9	15
Colon	20	8	17
Rectum	15	6	16
Average	16.5	7.75	14.5

Table S23 Number of more abundant genera when gut region is compared among species based on LEfSe analysis when all species were included.

	A. speciosus* – *A. argenteus		***A. speciosus* – *M. rufocanus***		***A. argenteus* – *M. rufocanus***	
	A. speciosus	***A. argenteus***	***A. speciosus***	***M. rufocanus***	***A. argenteus***	***M. rufocanus***
Small Intestine	6	28	26	21	41	7
Cecum	9	7	24	30	22	28
Colon	12	1	32	31	18	23
Rectum	8	3	22	32	17	22
Average	8.75	9.75	26	28.5	24.5	20

Table S24 Number of more abundant genera when gut region is compared among species based on pairwise LEfSe analysis.

Chapter 3

Species specific changes in the gut microbial communities of two sympatric species of rodent associated with an altered diet in an urban ecosystem

Abstract

Cities represent the most extreme form of anthropogenic ecosystem modification and the urbanization processes exerts profound effects on the wildlife that inhabit them. Access to human derived food resources within cities may alter a species' diet, in turn shaping the microbial community of the gastrointestinal tract and thereby affecting the host's health and survival. Plasticity of the gut microbiome may thus promote the survival and adaptability of some species in urban ecosystems. In this study I investigated the effects of urbanization on the diet and gut microbiota of two sympatric species of rodents, the omnivorous large Japanese field mouse (*Apodemus speciosus*) and the more herbivorous grey red-backed vole (*Myodes rufocanus*), using stable isotope analysis of hair and gut microbiome analysis across four gut regions of the gastrointestinal tract. Both species exhibited an expanded dietary niche width that may be attributable to novel anthropogenic food resources or increased competition. Furthermore, there was a dietary shift where urban *A. speciosus* were consuming more terrestrial animals and *M. rufocanus* more herbaceous plants while both species were consuming less C3 fruits and nuts. These alterations in diet may be associated with specific changes in the gut microbial community such as a decrease in *Alistipes* in the cecum and colon in *M. rufocanus*. There was also an increase in abundance of the probiotic *Lactobacillus* in the small intestine of urban *A. speciosus* and potentially pathogenic *Helicobacter* in the colon of *M. rufocanus* suggesting that *A. speciosus* may be maintaining a healthier gut microbiota within the modified environment.

Introduction

The global trend of increasing urbanization, particularly in biodiverse regions, has a profound effect on the ecology and life history of wildlife that inhabit these areas (Güneralp et al., 2020; Seto et al., 2012; Shochat et al., 2006). The extreme level of ecosystem modification through habitat fragmentation (McKinny, 2008), artificial feeding (Pagani-Núñez et al., 2019), and pollution (Isaksson, 2015) can negatively impact the health and survival of animals (MH Murray et al., 2019). Importantly, the high degree of human-wildlife interactions within urban areas not only alters animal behavior (Bateman & Fleming, 2014; Ditchkoff et al., 2006) but increases transmission risk of zoonotic parasites and diseases such as *Echinococcus*, hantavirus, and Lyme disease (Bradley & Altizer, 2006; Mackenstedt et al., 2014). Therefore, proper management of urban wildlife is important not only from a conservation point of view, but also from a public health perspective.

One area of recent but rapidly growing interest is how urbanization affects the gut microbiota of wildlife (Fuirst et al., 2018; Littleford-Colquhoun et al., 2019; M. H. Murray et al., 2020; Phillips et al., 2018; Stothart et al., 2019; Teyssier et al., 2018, 2020). The gut microbiome of vertebrates plays a pivotal role in development (Fraune & Bosch, 2010), nutritional uptake (Hooper et al., 2002), and general immune system function (Schluter et al., 2020; Sekirov & Finlay, 2009). Therefore, any disruption to the gut microbial community, a condition known as dysbiosis, can adversely impact numerous aspects of the host's physiology and life history ultimately affecting their health and survival (Logan et al., 2016). For example, diet simplification due to forest fragmentation can decrease gut microbiome diversity (Amato et al., 2013; Barelli et al., 2015) negatively affecting immune system function (de Paiva et al., 2016). A shift in diet of urban animals through artificial feeding (Maureen Murray et al., 2015), if composed of low quality food, may cause a detrimental shift in the microbial community structure within the gut (Singh et al., 2017) that can induce numerous health issues such as obesity or inflammatory bowel disease (Chin et al., 2000; Turnbaugh et al., 2006). Furthermore, urbanization can lead to higher levels of both acute and chronic stress due to chemical, noise and light pollution that can negatively impact the gut microbiome and host health (Gao et al., 2018; Isaksson, 2015).

Not all species suffer under the effects of anthropogenic ecosystem modification, as urban exploiters such as pigeons (Kark et al., 2007) and squirrels (Bateman & Fleming, 2014) indeed thrive. While their success is in part due to behavioral adaption (Bateman & Fleming, 2014; Ditchkoff et al., 2006) or a wide dietary niche (Palacio, 2020), plasticity in what constitutes a healthy gut microbial community may help facilitate adaption to a rapidly changing environment (Alberdi et al., 2016; Hauffe & Barelli, 2019). Symbiotic gut microbes may aid in the acquisition of energy and nutrition from novel food items (Bäckhed et al., 2004; David et al., 2014) or facilitate the breakdown of toxins that would otherwise cause adverse health effects (Kohl et al., 2014; Sasaki et al., 2005). Therefore, it is imperative that we understand how urbanization affects the gut microbial communities of animals and how it impacts their health and survival to better anticipate management targets.

To date, studies investigating the gut microbial communities of urban animals have all demonstrated some degree of change in composition and diversity as compared to conspecifics in less urbanized environments. Interestingly, while lower microbial alpha diversity was found in the guts of urban house sparrows and herring gulls (Fuirst et al., 2018; Teyssier et al., 2018, 2020), the opposite was found in white-crowned sparrows (Phillips et al., 2018), reptilian water dragons (Littleford-Colquhoun et al., 2019), and coyotes (Sugden et al., 2020). These differences may be related to the diversity of habitat in which the animals forage or their dietary resources (Fuirst et al., 2018; Littleford-Colquhoun et al., 2019; Phillips et al., 2018; Teyssier et al., 2020). However, with only a handful of studies so far, most of which investigated such effects in birds, our current understanding of the wildlife gut microbiome within the context of urbanization is rudimentary. Furthermore, because all previous investigations were conducted within different cities spread across three different continents, the specific factors of urbanization are likely to be vastly different making them difficult to compare. No study has looked at the gut microbiota of multiple animal species within the same urban areas.

In this study I investigated the gut microbial community in four regions (i.e. small intestine, cecum, colon, and rectum) of the gastrointestinal tract (GIT) of two sympatric species of rodents in urban environments as compared to conspecifics in a more natural ecosystem. Specifically, I was interested in if a dietary change in response to novel anthropogenic food resources could be linked to changes in the gut microbiota. The large Japanese field mouse

(*Apodemus speciosus*) and the grey red-backed vole (*Myodes rufocanus*) occupy the same habitat patches throughout the island of Hokkaido, Japan (Kaneko et al., 1998; Saitoh et al., 2007). While both species are omnivorous, *A. speciosus* preferentially consumes seeds, nuts, and insects, while *M. rufocanus* has a diet predominantly composed of bamboo and herbaceous plants (Kaneko et al., 1998; Tatsukawa & Murakami, 1976). Therefore, they are likely to exhibit differential responses when exposed to the same environmental pressures of urbanization, both in what dietary items they select and how wide the overall niche of the population may be. I investigated if the more omnivorous *A. speciosus* was more likely to exhibit an expanded dietary niche than *M. rufocanus*. I assessed the different resource use patterns of these species in urban and natural ecosystems using stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), providing a foundation for interpreting how changes in diet may be mediated by local environmental changes, resulting in altered microbial communities.

I predicted that an expanded dietary niche width in urban *A.* speciosus as is typical of omnivorous species with access to anthropogenic food sources (Palacio, 2020) would have a positive effect on gut microbiome alpha diversity in all four regions of the GIT as compared to natural conspecifics. I expected the largest impact in the lower GIT (i.e. cecum, colon, and rectum) where fermentation of plant polysaccharides occurs and the host immune system has a diminished role in shaping the microbial community (Donaldson et al., 2016). Little to no change in alpha diversity of the microbial community of *M. rufocanus* was anticipated as their primary food source (i.e. herbaceous plants) would be unlikely to change and are less likely to use anthropogenic resources. Furthermore, I expected a change in the microbial community composition, particularly in *A. speciosus* because novel food items are likely to require a shift in the digestive capabilities of the microbial flora (Carmody et al., 2015; David et al., 2014). Even if the primary dietary source (i.e. insects or plants) does not change in the urban environment, species specific items will as plant and insect communities are known to be impacted by urbanization (Beninde et al., 2015; Faeth et al., 2011). To our knowledge, this is the first study investigating multiple gut regions and species to investigate the effects of urbanization on the gut microbiome of wildlife.

Methods

Field and gut content sampling

In October, 2019, two sympatric species of rodents, the grey red-backed vole *Myodes rufocanus* and the large Japanese field mouse *Apodemus speciosus,* were captured in two urban parks (i.e. Kaguraoka Koen and Shunkodai koen) in the city of Asahikawa and one park (i.e. Maruyama koen) in the city of Biei, as well as four natural sites (i.e. Shirakkeyama, Chitoseyama, Harushinai, and Mukoyama) within the surrounding Kamikawa Chubu National forest in central Hokkaido, Japan (Fig. S1, Table S1). A detailed description of the microbial communities and how they differ among gut region and species of the individuals from the national forest can be found in chapter 2. The urban parks are surrounded by residential areas with Kaguraoka koen notably situated next to the central built up of area of Asahikawa city. All parks are actively managed and heavily used by the public. The natural sites were located in the middle of the forest at least 1km from any agriculture or built-up areas. These sites were higher in elevation from any potential pollution runoff but below 500m in order to avoid altitudinal variation in the gut microbiome (T. A. Suzuki et al., 2018). Both the national forest and managed forest fragments within the urban parks were primarily composed of deciduous trees such as Birch, Oak, and Walnut while the underbrush was mostly dwarf bamboo (*Sasa kurilensis*) with various small leafy plants mixed in. At each site, two or three trap grids of Sherman traps (H.B. Sherman Traps, Inc.) baited with oatmeal and placed in a four by 10 grid pattern with each trap 10m apart were utilized for capturing animals. Traps were checked within one hour after sunrise for two to three consecutive days, and any trap containing an animal was replaced with a fresh one.

Animals were transported to the Department of Parasitology at Asahikawa Medical University in Asahikawa where they were euthanized, identified, and morphometric measurements collected. I determined body condition by dividing the log of body weight by the log of body length (Labocha et al., 2014). The gastrointestinal tract was removed, and gut content was collected from the ileum in small intestine, the cecum, and the descending colon of the large intestine, as well as fecal matter from the rectum using a small steel spatula. Samples were placed in a -80^{o} C freezer within 1 hour of collection where they were stored until transferred to the Laboratory of Parasitology in the Faculty of Veterinary Medicine at Hokkaido

University, Sapporo, Japan for DNA processing. Gut content was collected using clean laboratory techniques and a more detailed description of the methods can be found in chapter 2. Finally, hair was collected from the outer hind legs and dried under a fume hood for 48 hours for use in stable isotope analysis.

Stable isotope analysis

Stable isotope analysis is widely applied in the reconstruction of trophic interactions within ecosystems (Baltensperger et al., 2015; Ben-David & Flaherty, 2012). Stable isotopes of carbon ($\delta^{13}C$) can reflect changes in the type of vegetation consumed such as C3 and C4 plants, and nitrogen ($\delta^{15}N$) can discern movement up a food chain, being enriched with each stage of consumption from herbivore to carnivore (Ben-David & Flaherty, 2012). Combining $\delta^{13}C$ and $\delta^{15}N$ values can delimit an individual's coordinates within an overall community isotopic niche space and can be further analyzed at the population level to compare dietary flexibility and foraging behaviors (Baltensperger et al., 2015; Ben-David & Flaherty, 2012; Layman & Post, 2008). Here, I analyzed stable isotope values of hair as a proxy for long term resource use to determine the effect of dietary habits on the gut microbial community. Before analysis, hair was washed using a chloroform:methanol solution (2:1 v/v) for removal of surface oils, then rinsed in distilled water and dried in an oven at 60^{o} C for 24 to 48 hours. I wrapped 0.5 mg of hair from each individual in tin capsules and analyzed stable isotope ratios using an elemental analyzer (Flash EA 1112, Thermofisher) coupled to an isotopic ratio mass spectrometer (IRMS, Delta V Plus, Thermofisher). Standardization of isotopic ratios are based on Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and Air (atmospheric nitrogen) for $\delta^{15}N$ and presented as parts per mil (‰).

Extraction, PCR, and metagenomics

Following Hayakawa et al. (2018), DNA was extracted from gut content and fecal samples using the QIAamp fast DNA stool mini kit (Qiagen) after bead beading for three minutes with 1mg of 0.1mm and four 3mm silica/zirconia beads. PCR amplification of the V3-V4 region of the 16S rRNA gene was performed using the 314F/805R universal primers (Klindworth et al., 2013). DNA extraction and PCR amplification were done under sterile

conditions and a negative control was included in each batch of samples. Finally, metagenomic analysis was done on an Illumina Miseq 300bp paired-end platform using a v3 reagent kit after library preparation using a Nextera XT DNA v2 set A, B, C, or D following the manufacturer's instructions. A more detailed description of the methods can be found in chapter 2.

Data analysis

To compare $\delta^{13}C$ and $\delta^{15}N$ values between species and ecosystem, a pairwise Wilcoxon rank sum test was utilized as the data deviated from normality. I then investigated the $\delta^{13}C$ and $\delta^{15}N$ isotopic niche space occupied by urban and natural populations by calculating standard ellipse area (small sample size corrected; SEAc) for each host species in the R package SIBER (Jackson et al., 2011). A Bayesian multivariate distribution was fit to each host species in urban and natural habitats using Gibbs sampling technique over 20,000 iterations with a burn in of 1000 implemented in the R package rjags (Plummer, 2019). The niche size of urban and natural populations was compared using both maximum likelihood estimates of SEAc and by calculating the posterior distribution of the covariance matrix generating Bayesian SEA (SEAb). For pairwise comparisons between the ellipse sizes of the different species and ecosystems, I calculated the probability that a given ellipse had a larger posterior distribution using paired posterior draws where the proportion of draws that were larger serves as a proxy for probability. I also calculated the proportional overlap of maximum likelihood fitted standard ellipses to quantify the degree of niche space overlap between the two species or ecosystems, where 0 indicates no overlap and 1 complete overlap in isotopic niche space.

I quantified the differential contributions of resource groups to both host species in each habitat type using a stable isotope mixing model in the R package SIMMR (Parnell, 2020). Because no specific trophic enrichment factors are available for these host species, I used an average value of rodent hair isotopic offsets ($\delta^{15}N$ 2.7 ± 1.67‰ and $\delta^{13}C$ 2.4 ± 1.01‰) as utilized in a study of similar focal taxa (Baltensperger et al., 2015). Isotopic values of potential food items are from previously published data from Hokkaido of various C3 plants ($\delta^{13}C$ -31.65 ± 0.62‰ and $\delta^{15}N$ -1.98 ± 1.32‰; Osaki et al., 2019), C3 fruits and nuts including acorns ($\delta^{13}C$ -28.15 ± 1.12‰ and $\delta^{15}N$ -2.13 ± 0.33‰; Osaki et al., 2019), C4 corn ($\delta^{13}C$ -10.19 ± 0.04‰ and $\delta^{15}N$ -2.13 ± 0.33‰; Matsubayashi et al., 2014), and terrestrial animals including

herbivorous mammals and insects ($\delta^{13}C$ -26.3 ± 0.5‰ and $\delta^{15}N$ 3.7 ± 1.5‰; Matsubayashi et al., 2014). Where necessary, $\delta^{13}C$ values were corrected for the Suess effect of atmospheric carbon depletion using a year-specific correction value to 2019 values (Long et al., 2005). Model fit was evaluated through assessment of Gelman diagnostics of MCMC convergence and a posterior predictive check.

Metagenomic data was demultiplexed and merged, then denoised and quality filtered using the DADA2 pipeline in QIIME2 version 2020.2 (Bolyen et al., 2019; Callahan et al., 2016), producing a table of amplicon sequence variants (ASVs). Potential contaminant sequences were identified using the frequency method with a threshold of 0.1 in the Decontam package in R version 4.0.2 (Davis et al., 2018; Core RvTeam, 2020) with each identified sequence being manually checked against our negative controls and subsequently removed using sequence identifiers in QIIME2. The SILVA classifier (release n138) was used for taxonomic classification of each ASV (Bokulich et al., 2018). Those identified as eukaryotes, archaea, chloroplastida, mitrochondria, and any sequences not identified to Phylum level were also removed. Using the FastTree method in the MAFFT plugin in QIIME2 (Price et al., 2010), a rooted phylogenetic tree was generated.

Based on alpha rarefaction analysis, samples were rarified to a sampling depth of 10,000 reads for diversity analysis. A total of six samples (three small intestine and one feces from *M. rufocanus* and two small intestine from *A. speciosus*) with low sequence count were excluded from diversity analysis. Alpha diversity was quantified using Shannon diversity, Faith's phylogenetic diversity (PD), Pielou's evenness, and the number of ASVs. To analyze beta-diversity unweighted and weighted unifrac dissimilarity matrices were generated in Qiime2.

To investigate whether alpha diversity of the gut microbiome of rodents is altered within the urban environment, a linear mixed effects model was used where the response variable was log transformed alpha diversity, the random effect was site, and the explanatory variables were ecosystem (i.e. urban or natural), sex, age (adult or sub-adult), $\delta^{13}C$, and $\delta^{15}N$ using the nlme package in R (Pinheiro et al., 2020). This was repeated for each gut region for each host species. Body condition was not included in the models because I found no difference between urban and natural populations in either *A. speciosus* ($F = 0.186$, $p = 0.667$) nor *M. rufocanus* ($F = 0.333$, $p = 0.565$). Beta-diversity was first visualized using principle coordinate analysis (PCoA) plots for

each gut region in each species performed in the R package phyloseq (McMurdie & Holmes, 2013). PERMANOVA analysis was then conducted to determine the impact of ecosystem type, sex, age, $\delta^{13}C$, and $\delta^{15}N$ on beta-diversity within each gut region of both rodent species using the adonis2 function with the margin="by" option to determine the marginal effect of each in the vegan package in R (Oksanen et al., 2007). Within ecosystem variation of the gut microbial community within each gut region was analyzed using PERMDISP with 999 permutations in the vegan package in R for both unweighted and weighted unifrac dissimilarity matrices(Oksanen et al., 2007).

Linear discriminant analysis effect size (LEfSe) was used to compare relative abundances of the different microbial genera between urban and natural populations of both *A. speciosus* and *M. rufocanus*. This was done for each gut region separately within each species using the Huttenhower lab Galaxy pipeline where class was ecosystem (Segata et al., 2011). I selected those genera identified as being significantly more abundant in either the urban or natural habitats with either known probiotic or pathogenic species characteristics, or to have an association with a high protein diet to further explore what variables may be influencing their abundances. I used a generalized linear mixed effects model (GLMM) with negative binomial distribution to investigate if age, sex, $\delta^{13}C$, and $\delta^{15}N$ (i.e. fixed effect variables) had any effect on their abundances (i.e. response variable) with site as a random effect and ecosystem added as an additional fixed effect to confirm the results of LEfSe analysis. The models were run using the NBZIMM package in R (Zhang et al., 2018). The association between our explanatory variables and relative abundance was then analyzed in natural and urban populations separately to determine potential within ecosystem specific interactions.

Results

Host and gut content

I captured 42 and 41 *A. speciosus*, and 43 and 50 *M. rufocanus* from natural and urban sites respectively (Table S1). A total of 245 gut content and fecal matter samples were collected from *A. speciosus* consisting of 81 from the small intestine (42 natural, 39 urban), 43 from the cecum (27 natural, 16 urban), 80 from the colon (41 natural, 39 urban), and 41 from the rectum

(25 natural, 16 urban). From *M. rufocanus*, 92 gut content samples were collected from the small intestine (43 natural, 49 urban), 31 from the cecum (14 natural, 17 urban), 83 from the colon (38 natural, 45 urban), as well as 32 rectum samples (16 natural, 16 urban) comprising a total of 238. After quality filtering, 21,820,759 high quality reads were obtained with 11,264,730 (average of 45978 ± 795 SEM per sample) from *A.* speciosus and 10,556,029 (average of 44167 ± 1070 SEM per sample) from *M. rufocanus*.

Stable isotope

I found significant differences in $\delta^{15}N$ ($p < 0.001$) between natural and urban populations of both species, but not $\delta^{13}C$ (Fig. 1). There was a significant difference in both $\delta^{13}C$ and $\delta^{15}N$ values between species in the urban areas ($p < 0.001$), but only $\delta^{13}C$ was significantly different within the natural sites (Fig. 1, Table S2).

Isotopic niche width estimated using SEAc found that urban *A. speciosus* (SEAc = 5.78) had an isotopic niche almost twice as large as those in the natural ecosystem (SEAc = 2.9) with a 99.88% probability of the urban ellipse being larger than the natural based on paired posterior draws of the Bayesian distribution (Fig. 2, S2, Table S3). *M. rufocanus* also exhibited a larger niche width in the urban parks, although less so, with an SEAc of 3.01 and 2.68 in the urban and natural areas respectively, and a probability of 71.38% that the urban ellipse was larger (Fig. 2, S2). Furthermore, the pairwise overlap between natural and urban ecosystems was larger for *M. rufocanus* (0.59) than *A. speciosus* (0.39). *A. speciosus* had larger SEA_b values than *M. rufocanus* in both the urban (99.93% probability) and natural (66.45% probability) ecosystems. Estimating the proportion of each food item in the diet of both species of rodent surprisingly found that terrestrial animals made up a marginally larger portion of the diet of *M. rufocanus* (32.2%) than *A. speciosus* (28.7%) within the natural ecosystem (Table 1). *A. speciosus*, shifted to consuming more terrestrial animal protein within the urban parks (43.2% of their diet) as compared to their natural conspecifics (28.7%) with a slight shift away from C3 plants (60.4% to 52.4% in natural and urban respectively; Table 1). Urban *M. rufocanus* exhibited the opposite trend with shift towards C3 plants (35.9% to 60.6% in natural and urban respectively) while consumption of terrestrial animal protein slightly decreased from 32.2% to 28.26% (Table 1).

Both species were consuming less C3 fruits and nuts in the urban parks as compared to their natural conspecifics (Table 1).

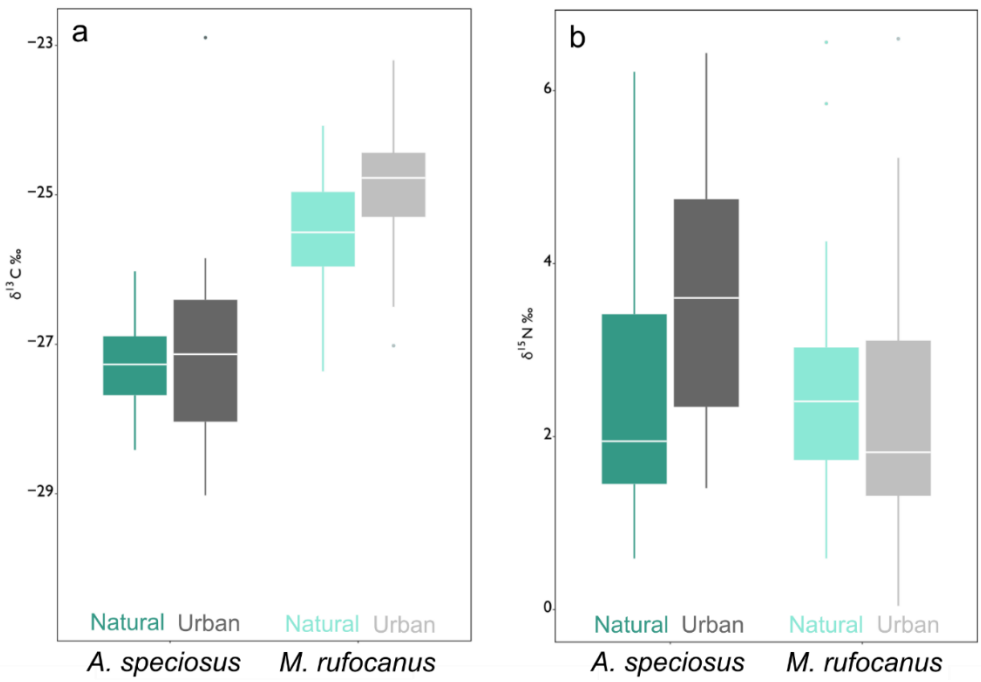


Fig. 1 Comparison of $\delta^{13}C$ and $\delta^{15}N$ between species and populations in urban and natural areas.

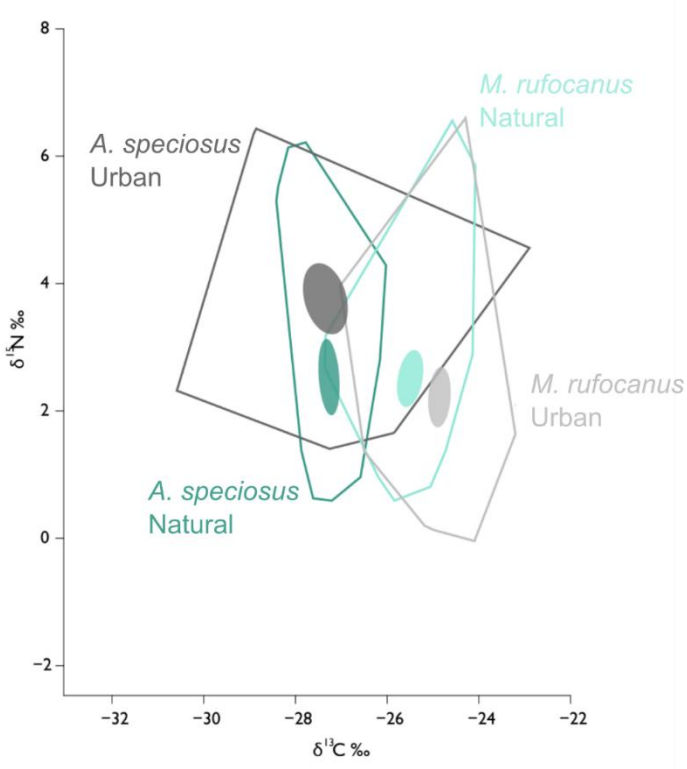


Fig. 2 Total area convex hulls (solid lines) and 95% confidence intervals around bivariate means (filled ovals) for urban (gray) and natural (green) populations of *A. speciosus* and *M. rufocanus*, as calculated in SIBER.

	A. speciosus		***M. rufocanus***	
	Natural	**Urban**	**Natural**	**Urban**
C3 Plants	60.4 ± 3.9	52.4 ± 4.2	35.9 ± 9.8	60.6 ± 3.9
C3 Fruits & Nuts	9.1 ± 4.8	3.4 ± 2.4	25.5 ± 11.6	8.9 ± 4.8
C4 Corn	1.8 ± 0.8	1.0 ± 0.6	6.5 ± 2.2	1.9 ± 0.9
Terrestrial Animals	28.7 ± 3.7	43.2 ± 3.9	32.2 ± 3.4	28.6 ± 3.7

Table 1 The average proportion of each food item in the diet of *A. speciosus* and *M. rufocanus* in natural and urban populations (percent ± standard deviation).

Natural vs. urban gut microbial alpha and beta diversity

I found that ecosystem had no effect on alpha diversity for any diversity index in any gut region for either host species, following our prediction for *M. rufocanus* but not *A. speciosus* (all $p < 0.05$; Fig. 3, S3, Table S4 to S7). Interestingly, neither $\delta^{13}C$ nor $\delta^{15}N$ significantly impacted gut microbiome alpha diversity in any gut region of the omnivorous *A. speciosus*, despite there being significantly higher $\delta^{15}N$ values in the fur of urban individuals (Table S4 to S7). In the more herbivorous *M. rufocanus*, $\delta^{13}C$ had a significantly negative impact on Faith's PD and the number of ASVs in the cecum and rectum, with a negative effect in the colon for Faith's PD, and in the small intestine for the number of ASVs (all $p < 0.05$; Table S5, S7). On the other hand, $\delta^{15}N$ had a significantly positive effect on Faith's PD and the number of ASVs in both the colon and rectum (all $p < 0.05$; Table S5, S7). Sex and age were not the focus of this study and will not be discussed at length. In short, sex significantly affected alpha diversity in the cecum, colon and rectum of *A. speciosus*, but not in the small intestine nor any gut region in *M. rufocanus* (Table S4 to S7). Age, on the other hand, significantly impacted alpha diversity in *M. rufocanus*, particularly in the colon and small intestine, but had no effect in *A. speciosus* (Table S4 to S7).

Visualization of the gut microbial community composition using PCoA plots exhibited a high degree of overlap in the clustering of urban and natural populations in all gut regions in both host species, though not entirely (Fig. 4, 5). Interestingly, the cluster of individuals from the urban populations had the same cluster area size or were slightly larger than the cluster from urban parks in the cecum, colon, and rectum of both species for both unweighted and weighted unifrac PCoA plots (Fig. 4, 5). This was also the case in the small intestine of *A. speciosus*, but not *M. rufocanus* where the cluster area of individuals from the urban parks was smaller than those from the national forest (Fig. 4, 5). Furthermore, the relationship between PCoA 1 and PCoA 2 for unweighted unifrac is inverted in the cecum and rectum of urban *A. speciosus* as compared to their natural conspecifics, but only in the rectum according to weighted unifrac (Fig. 4). This trend was mostly unconfirmed by PERMDISP as a significant difference in dispersion was only found in the small intestine of *M.* rufocanus for unweighted unifrac ($F = 4.027$, $p = 0.039$; Table S8).

Using PERMANOVA I found a significant effect of ecosystem on beta diversity in in all four gut regions of both *A. speciosus* and *M. rufocanus* for unweighted unifrac (all $p < 0.05$;

Table S9, S10). There was also a significant effect in the colon and small intestines (both $p < 0.05$) but not the cecum or rectum (both $p > 0.05$) in *A. speciosus* based on weighted unifrac (Table S9). In *M. rufocanus* there was a significant effect of ecosystem in all gut regions except the rectum ($F = 2.054$, $p = 0.064$; Table S10) according to weighted unifrac. Similar to alpha diversity, $\delta^{13}C$ and $\delta^{15}N$ values had a greater effect on gut microbiome beta-diversity in *M. rufocanus* than *A. speciosus*. Specifically, $\delta^{13}C$ significantly impacted beta-diversity in all four gut regions based on unweighted unifrac, but only in the small intestine according to weighted unifrac (all $p < 0.05$; Table S10). $\delta^{15}N$ also significantly impacted beta-diversity in the cecum, colon, and rectum of *M. rufocanus* based on unweighted unifrac (all $p < 0.05$), but no effect in any gut region for weighted unifrac (all $p > 0.05$; Table S10). In *A. speciosus*, only in the colon was there a significant effect of $\delta^{13}C$ on gut microbiome beta-diversity according to unweighted unifrac alone ($F = 1.769$, $p = 0.005$), however, $\delta^{15}N$ had no effect in any gut region (all $p > 0.05$; Table S9). Unlike alpha diversity, sex had a minimal impact on beta-diversity in either host species with it only being significant in the colon of *A. speciosus* as well as the rectum and colon of *M. rufocanus* (all $p < 0.05$; Table S9, S10). Age significantly impacted beta-diversity in the colon of *A. speciosus* for both unweighted and weighted unifrac, while it was significant in the cecum, colon, and small intestine of *M. rufocanus* based on unweighted unifrac (all $p < 0.05$; Table S9, S10).

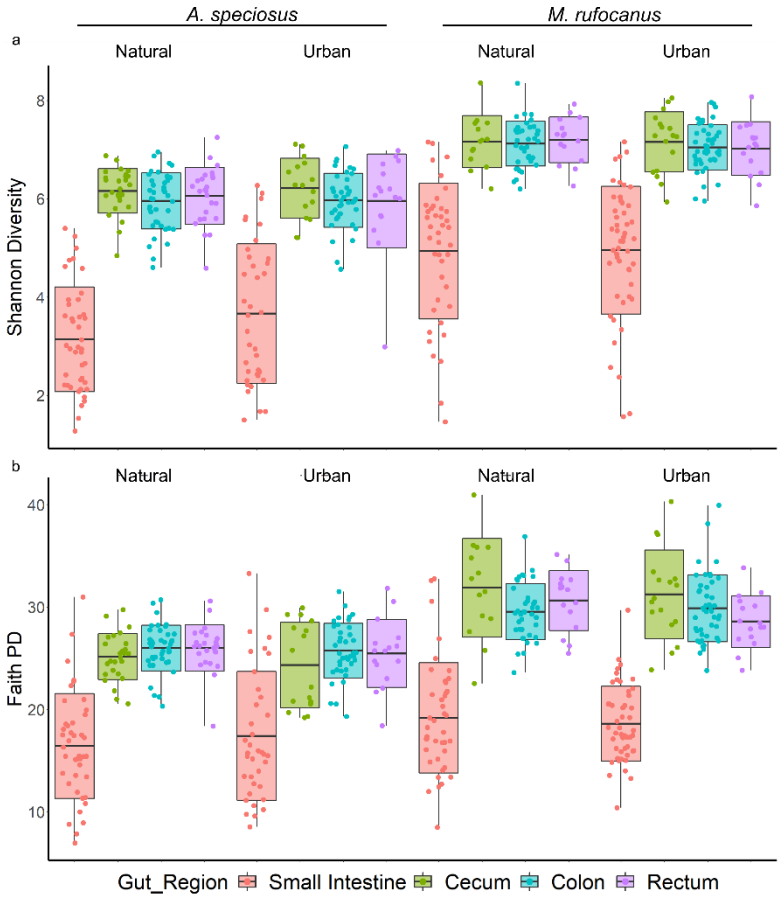


Fig. 3 Alpha diversity along the gastrointestinal tract of *A. speciosus* and *M. rufocanus* in natural and urban populations according to a) Shannon diversity and b) Faith's PD, c) Pielou's evenness, and d) the number of ASVs.

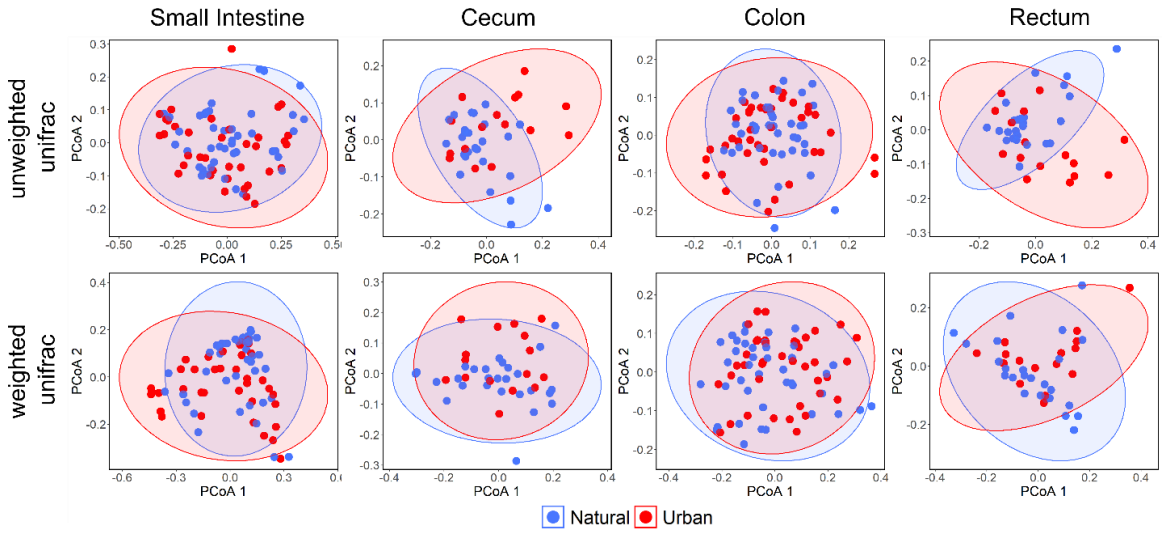


Fig. 4 PCoA plots of the gut microbial community along the gastrointestinal tract of *A. speciosus* based on unweighted and weighted unifrac dissimilarity matrices. Blue are individuals from the natural areas and red are urban.

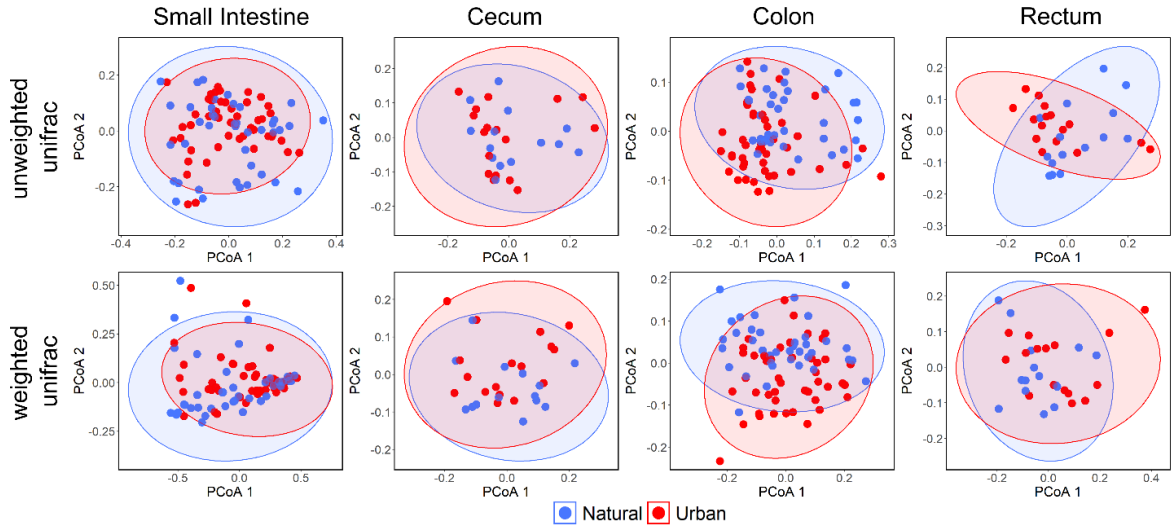


Fig. 5 PCoA plots of the gut microbial community along the gastrointestinal tract of *M. rufocanus* based on unweighted and weighted unifrac dissimilarity matrices. Blue are individuals from the natural areas and red are urban.

Relative abundances of taxonomic groups

Firmicutes and Bacteroidota were the two most abundant phyla all three regions of the lower GIT of both host species comprising 80% of all microbes in *A. speciosus* and 90% in *M. rufocanus* in both the natural and urban populations (Fig. 6). Campilobacterota was the third most abundant in these same gut regions in *A. speciosus* except in the rectum of urban animals where Proteobacteria was the third most dominant phylum. Spirochaetota was the third most abundant phylum in the cecum, colon, and rectum of *M. rufocanus* with little variation in relative abundance between natural and urban populations (Fig. 6). Interestingly, although Firmicutes, Bacteroidota, and Proteobacteria were the three most abundant phyla in the small intestine of *A. speciosus*, Firmicutes was more abundant in the natural ecosystem with 71% as opposed to 58% in the urban parks, while Bacteroidota showed the opposite trend with 13% and 25% in the natural and urban ecosystems respectively (Fig. 6). A similar but weaker trend was found in *M. rufocanus* where Bacteroidota made up 41% and 54% in the natural and urban sites respectively, while Firmicutes comprised 34% and 26% of all microbes in the natural and urban sites respectively (Fig. 6).

LEfSe analysis at the genus level found that urban *A. speciosus* exhibited more genera in significantly higher abundance throughout the GIT as compared to those in the national forest rather than vice versa with 10 and 6 more abundant genera in the small intestine, 4 and 4 in the cecum, 7 and 5 in the colon, and 6 and 4 in the rectum for urban and natural populations respectively (Fig. S4). Interestingly, *M. rufocanus* showed the opposite trend with 3 and 13 more abundant genera in the small intestine, 5 and 7 in the cecum, 6 and 12 in the colon, and 1 and 7 in the rectum of urban and natural populations respectively (Fig. S5). While many of these genera are known to be associated with the breakdown of food items such as plant polysaccharides, there were several genera of note. In particular, the probiotic groups *Lactobacillus* (Liu et al., 2010) in the small intestine, *Butyricicoccus* (Boesmans et al., 2018) in the cecum and colon, and *Bifidobacterium* (Meddah et al., 2001) in both the small intestine and colon had significantly higher relative abundance in urban *A. speciosus* (Fig. S4). While I didn't find any probiotic bacterial genera with higher relative abundance in urban *M. rufocanus*, I did find significantly higher abundance of the potentially pathogenic *Helicobacter* in the colon (Chin et al., 2000; Yang et al., 2013). Curiously, the opposite was found in the small intestine with

higher abundance of *Helicobacter* in individuals in the national forest as compared to the urban populations (Fig. S5). Although LEfSe analysis specifically tests for higher abundance of microbes, the opposite can be inferred. For example, the significantly higher relative abundance of *Alistipes* in both the cecum and colon in the rectum of *M. rufocanus* from the natural ecosystem means there is lower abundance in the urban parks (Fig. S5).

In *A. speciosus*, only the higher relative abundance of *Butyricicoccus* in the colon of urban individuals was confirmed GLMM ($b = 1.108 \pm 0.355$, $p = 0.026$), while *Butyricicoccus* in the cecum, and *Lactobacillus* in the small intestine were not (all $p < 0.05$; Table S11). Furthermore, none of the model variables exhibited similar association with relative abundance of any genus within the natural and urban habitats. Specifically, $\delta^{15}N$ had a significantly positive effect on *Lactobacillus* abundance in natural populations while $\delta^{13}C$ had a negative effect in the urban animals (both $p < 0.05$; Table S11). There was also a negative effect of $\delta^{13}C$ values on *Butyricicoccus* abundance in both the cecum and colon of animals in the national forest (both $p < 0.05$), but no effect in the urban individuals (both $p > 0.05$; Table S11). Similarly in *M. rufocanus*, only the lower abundance of *Helicobacter* in the small intestine of urban animals ($b = -3.2 \pm 1.13$, $p = 0.037$) was confirmed by the GLMM, though higher relative abundance in the colon was nearly significant ($b = 0.793 \pm 0.314$, p – 0.053; Table S12). Only *Alistipes* was significantly affected by $\delta^{15}N$ values in the cecum of *M. rufocanus* in the national forest ($b = 1.035 \pm 0.392$, $p = 0.046$; Table S12).

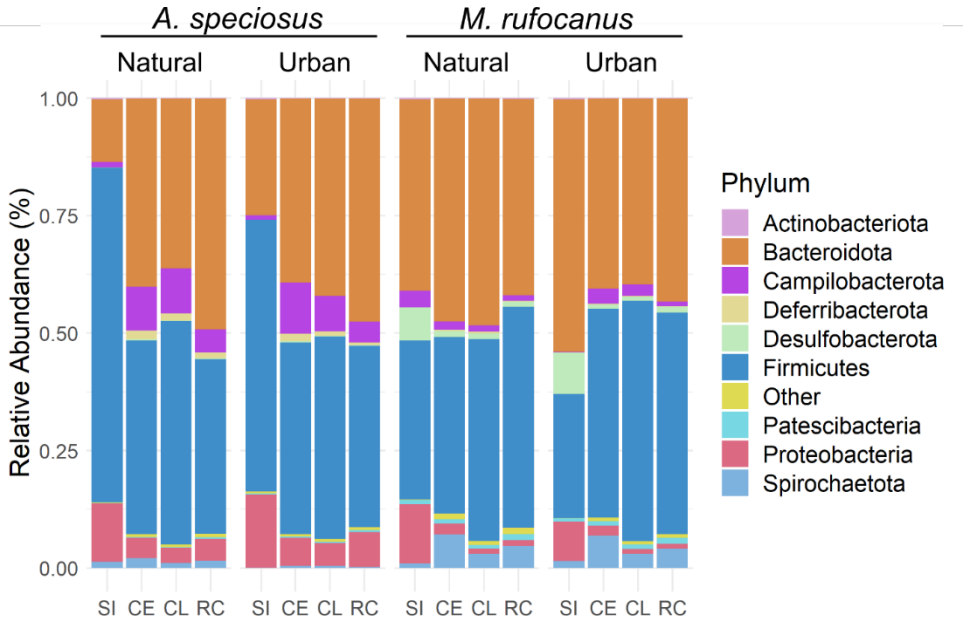


Fig. 6 Relative abundance of the nine most abundance phyla in each gut region in each host species from natural and urban ecosystems. SI is the small intestine, CE the cecum, CL the colon, and RC the rectum.

Discussion

Increased dietary niche width in urban populations

I found that both species of rodents are experiencing a dietary niche expansion within the urban parks, but to a much larger degree for the omnivorous *A. speciosus* (Fig. 2, S2). Furthermore, there was less overlap in the isotopic niche space between natural and urban populations of *A. speciosus* suggesting that they are more likely to utilize novel food resources and diverge from their natural dietary habits than the more herbivorous *M. rufocanus*. A similar trend has been reported in a wide range of omnivorous species occupying urban habitats (Larson et al., 2020; Pagani-Núñez et al., 2019), although the opposite was found in water dragons in Australia (Littleford-Colquhoun et al., 2019) and invasive rats in Madagascar (Dammhahn et al., 2017).

The increased proportion of animal protein consumed by urban *A. speciosus* as compared to their conspecifics in the national forest could come from the consumption of human provided animal products. The urban sites in this study are heavily used parks by local citizens with barbequing and picnics being popular activities during late spring to early autumn. Trash was also commonly seen throughout the forest fragments (personal observation). Leftover scraps of meat and other food trash may be opportunistically consumed by *A. speciosus* as an easy energy source (Larson et al., 2020; Pagani-Núñez et al., 2019). However, *A.* speciosus could simply be consuming more insects as the isotopic mixing model could not distinguish between the two (Table 1). Within the national forest, the population size of *A. speciosus* and *M. rufocanus* were inversely related, but in the urban parks they were more even in number (Table S1), possibly due to forest fragmentation limiting dispersal even when population density is high (Sato et al., 2014). Therefore, increased inter-specific competition for food resources may push each species to preferentially consume food items for which they are more specialized (i.e. insects for *A. speciosus* and herbaceous plants for *M. rufocanus*) despite the presence of artificial feeding, This could also explain why *M. rufocanus* has shifted towards eating more C3 plants within the urban parks. This is in contrast to more free roaming animals such as coyotes and birds that have access to a much wider array of habitats and can more readily take advantage of anthropogenic resources within cities and avoid inter-specific competition (Larson et al., 2020; Pagani-Núñez et al., 2019; Phillips et al., 2018).

No change in alpha diversity in urban populations

The altered diet of urban animals has been implicated as a proponent of change in alpha diversity of the gut microbiome whether it be lower in response to a simplified or low quality diet (Fuirst et al., 2018; Teyssier et al., 2020), or higher due to access to more diverse or novel resources (Littleford-Colquhoun et al., 2019; Phillips et al., 2018). Lower alpha diversity of the gut microbial community is typically associated with dysbiosis (Logan et al., 2016). Therefore, it is important to understand what aspects of urbanization can impact it, both positively and negatively. In the present study, no change in gut microbial alpha diversity was found in any gut region within the urban populations of either rodent species as compared to those in the natural habitat despite the wider dietary niche (Fig. 3, S3,, Table S4 to S7). It is possible that other

factors not tested for such as pollution or stress have a large enough impact on alpha diversity in these animals to mask any positive effect of diet (Gao et al., 2018; Han et al., 2014; Isaksson, 2015). On the other hand, a more plausible explanation is that intra-individual dietary diversity remains consistent regardless of habitat type, but that inter-individual variation is much greater in the urban parks due to access to novel human associated food items.

Microbial community composition affected by both diet and ecosystem type

Unlike alpha diversity, gut microbial beta-diversity was highly affected by ecosystem type throughout the GIT of both rodent species (Table S9, S10). Although the larger cluster area within the lower GIT of urban individuals in the PCoA plots suggests higher inter-individual variation in the gut microbial community, particularly for *A. speciosus*, this was largely unconfirmed by PERMDISP (Fig. 4, 5, Table S8). Therefore, shifts in the gut microbial community composition are at the population level rather than an individual response to the urban environment.

Diet is one of the major factors affecting gut microbial community structure (Wu et al., 2011). The dietary shift of the urban populations in the present study is likely the main culprit affecting beta-diversity and overall microbial community structure as $\delta^{13}C$ and $\delta^{15}N$ values had a large impact throughout the GIT of *M. rufocanus* as well as in the colon of *A. speciosus* (Table S11, S12). For example, *Bifidobacterium* was only found in the lower GIT of urban *A. speciosus* and was non-existent in individuals from the national forest and could be a human associated microbe. *Bifidobacterium* is typically found in fermented foods such as natto, miso, and yogurt (Fujisawa et al., 2006) that could be consumed by *A. speciosus*. In *M. rufocanus*, I found lower abundance of *Alistipes* in the colon and cecum of urban individuals. Both genera are associated with the consumption of animal protein and their lower abundance fits with the shift in diet away from terrestrial animals, although the shift is small and the change in relative abundance was not confirmed by GLMM (David et al., 2014).

It is interesting that no effect of stable isotope values was found on beta-diversity in the small intestine, cecum, or rectum of *A. speciosus* despite the significant difference of $\delta^{15}N$ values between natural and urban populations. This does not exclude the effect of diet, however, as the

proportion of fats, protein, and carbohydrates that can have a profound effect on microbial community structure (Caesar et al., 2015; Dominika et al., 2011; Singh et al., 2017) could not be estimated using stable isotope analysis as I did not identify specific food items. For example higher relative abundance of *Lactobacillus* in the small intestine of urban *A. speciosus* could be explained by the increased consumption of animal food products provided by humans as increased protein consumption has been shown to positively affect *Lactobacillus* abundance (Dominika et al., 2011). However, I was unable to confirm the relationship between increased consumption of terrestrial animals and higher *Lactobacillus* abundance using GLMM. It may instead be related to consumption of more acorns, a preferred food item of *A. speciosus*, as *L. apodemi* has been isolated from rodent species and is known to aid in the breakdown of tannins (Sasaki et al., 2005). However, shift away from consuming C3 fruits and nubs by urban *A. speciosus* suggests that this is unlikely the case.

A similar increase in abundance of *Lactobacillus* within urban populations as compared to those outside of city limits has been reported in house sparrows in Europe (Teyssier et al., 2018, 2020) and water dragons in Australia (Littleford-Colquhoun et al., 2019). Why I did not see a similar increase in *M. rufocanus* is an important question that must be investigated further. *Lactobacillus* is a probiotic genus of bacteria that provide a wide range of benefits to their host including reduction of intestinal inflammation (Liu et al., 2010) and regulation of immune system function (Sekirov & Finlay, 2009), Therefore, they may aid in the successful adaption of animals to the urban environment. Furthermore, wildlife management practices that promote their abundances could be useful in preventing the spread of zoonotic pathogens.

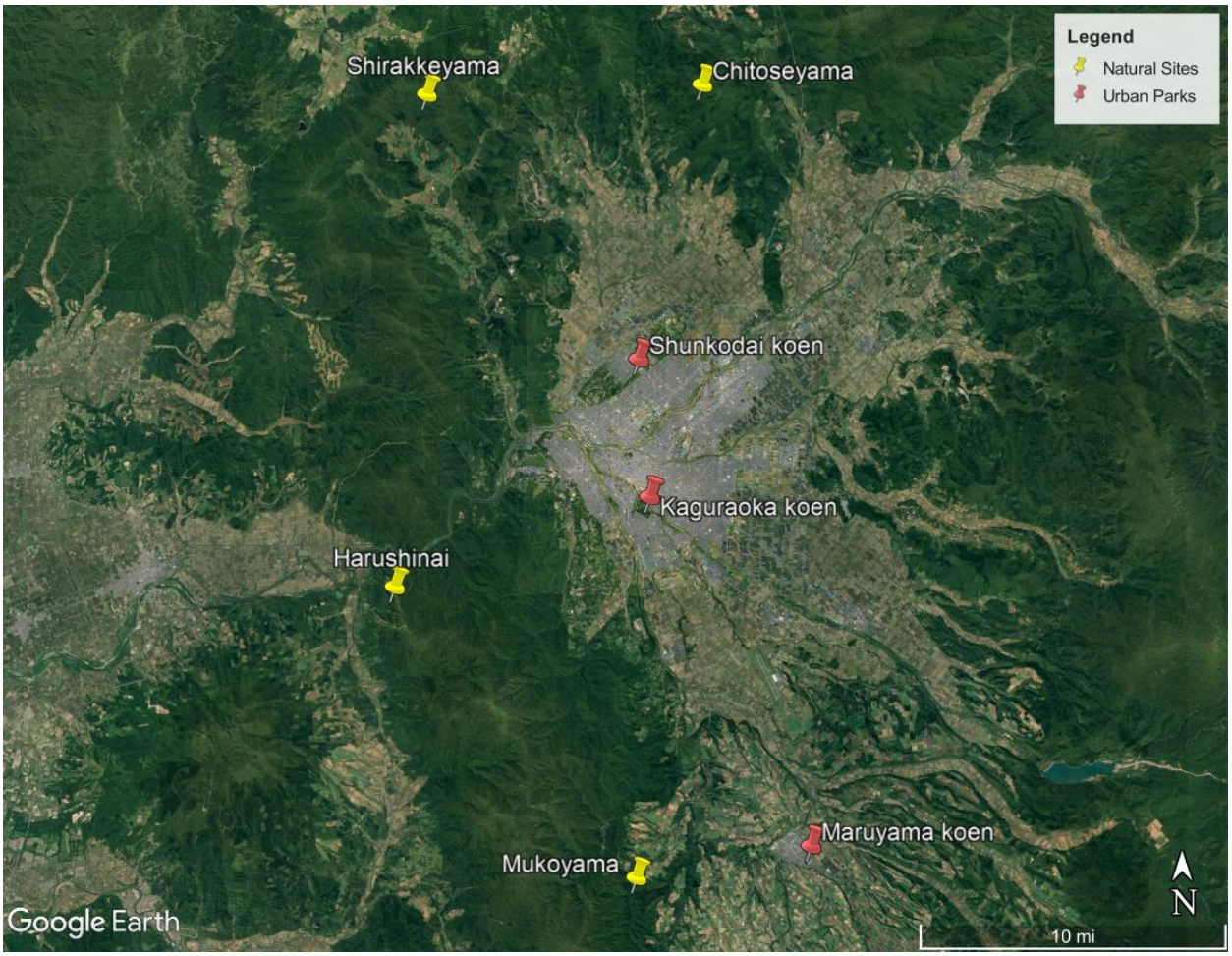
Curiously, there was lower abundance of *Helicobacter* in the small intestine, but higher abundance in the colon of *M. rufocanus* in the urban parks as compared to those in the national forest. The differential abundance was confirmed by GLMM in the small intestine and was nearly significant in the colon though neither were associated with diet (Table S12). Most species of *Helicobacter* thrive within the low pH environment of the stomach and small intestine, and many species are pathogenic in both humans and animals (On et al., 2015). Those species that have been isolated from the lower GIT such as *H. hepaticus* are known to induce inflammatory bowel disease (IBD) in immunocompromised animals and are associated with a markedly different microbial community of the cecum and colon (Chin et al., 2000; Yang et al.,

2013). Although I did not test immune system function directly, there was less inter-individual variation in the gut microbiome of the small intestine within the urban populations due species membership (Fig. 5, Table S8). The small intestine is the most immunologically active location in the entire body and the host immune system plays a pivotal role in shaping the gut microbial community (Bevins & Salzman, 2011). Therefore, a similar and strong immunological response to the urban environment due to elevated stress (Gao et al., 2018; Isaksson, 2015) or pollution (Isaksson, 2015; Rashed & Soltan, 2005) could cause a convergence in the gut microbial community structure and leave them more susceptible to pathogens in the lower GIT such as *Helicobacter*. However, a lower body condition would be expected in diseased animals, yet I found no difference between urban and natural populations. Future studies should investigate species specific immune response to urbanization and how this may affect their gut microbiome and their susceptibility to pathogens.

Conclusion

The results of this study have shown species specific response in the gut microbial communities of two sympatric species of rodents in urban areas as compared to conspecifics in a more natural habitat. Much of the change can be associated with a dietary shift in urban populations that is in line with their life history and may be influenced by the consumption of novel anthropogenic food resources. Specifically, the consumption of more terrestrial animal protein may have caused increased abundance of *Lactobacillus* in the small intestine of *A. speciosus* while the shift towards a larger proportion of herbaceous plants in the diet of *M. rufocanus* may be associated with a decrease in *Alistipes* in the lower GIT. Furthermore, while I did not find a clear indication of dysbiosis in either species, the increase in homogenization of the gut microbiome in the small intestine of *M. rufocanus* may be related to immunodeficiency and infection with the pathogenic *Helicobacter*. Future studies should identify specific food items associated with higher abundance of both probiotic and pathogenic microbes in these species.

Appendices



Harushinai

Kaguraoka koen

Maruyama koen

Fig. S1 A satellite image showing the location of field sites and pictures of three of the sites (two urban and one natural)

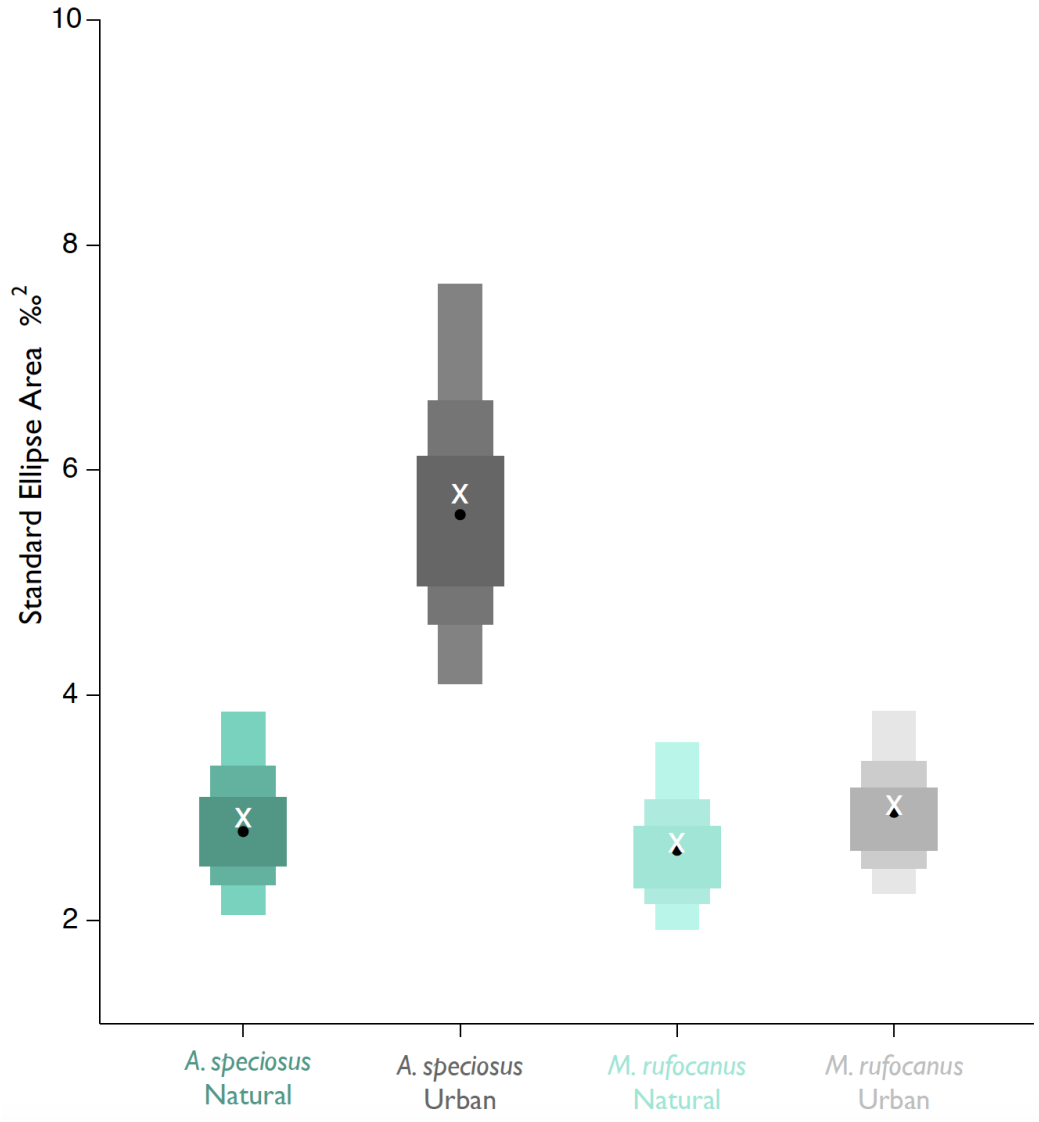


Fig. S2 Bayesian standard ellipse area (SEA_b) for each group after 20,000 posterior draws where the boxes indicate 50%, 75%, and 95% confidence intervals and the white X is the maximum likelihood estimate of the small sample size corrected standard ellipse area (SEA_c).

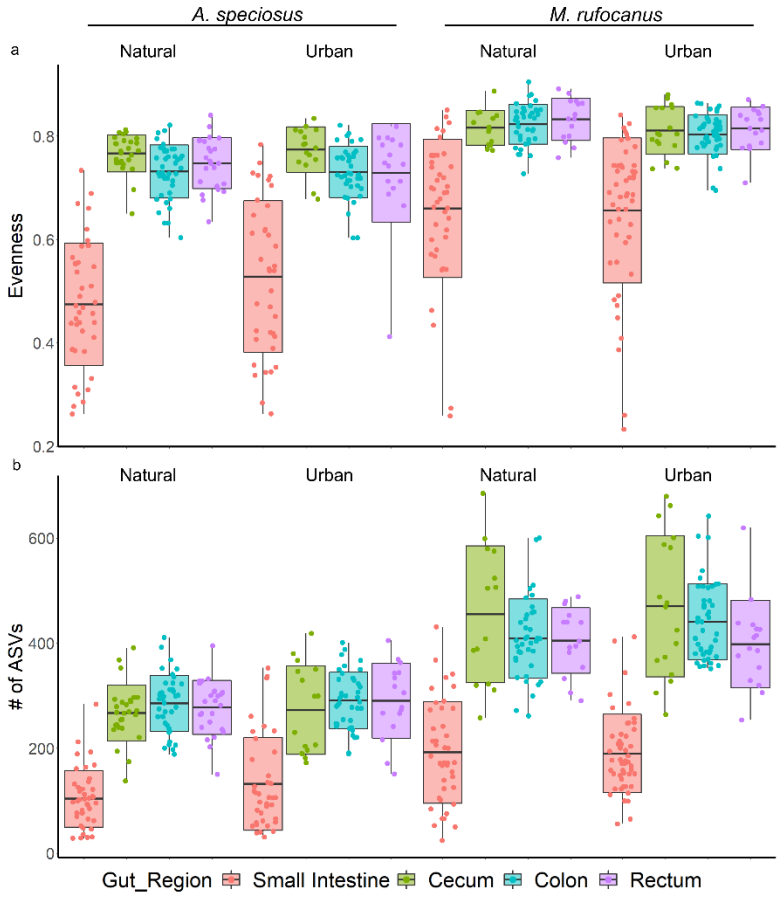


Fig. S3 Alpha diversity along the gastrointestinal tract of *A. speciosus* and *M. rufocanus* in natural and urban populations according to a) Pielou's evenness and b) the number of ASVs.

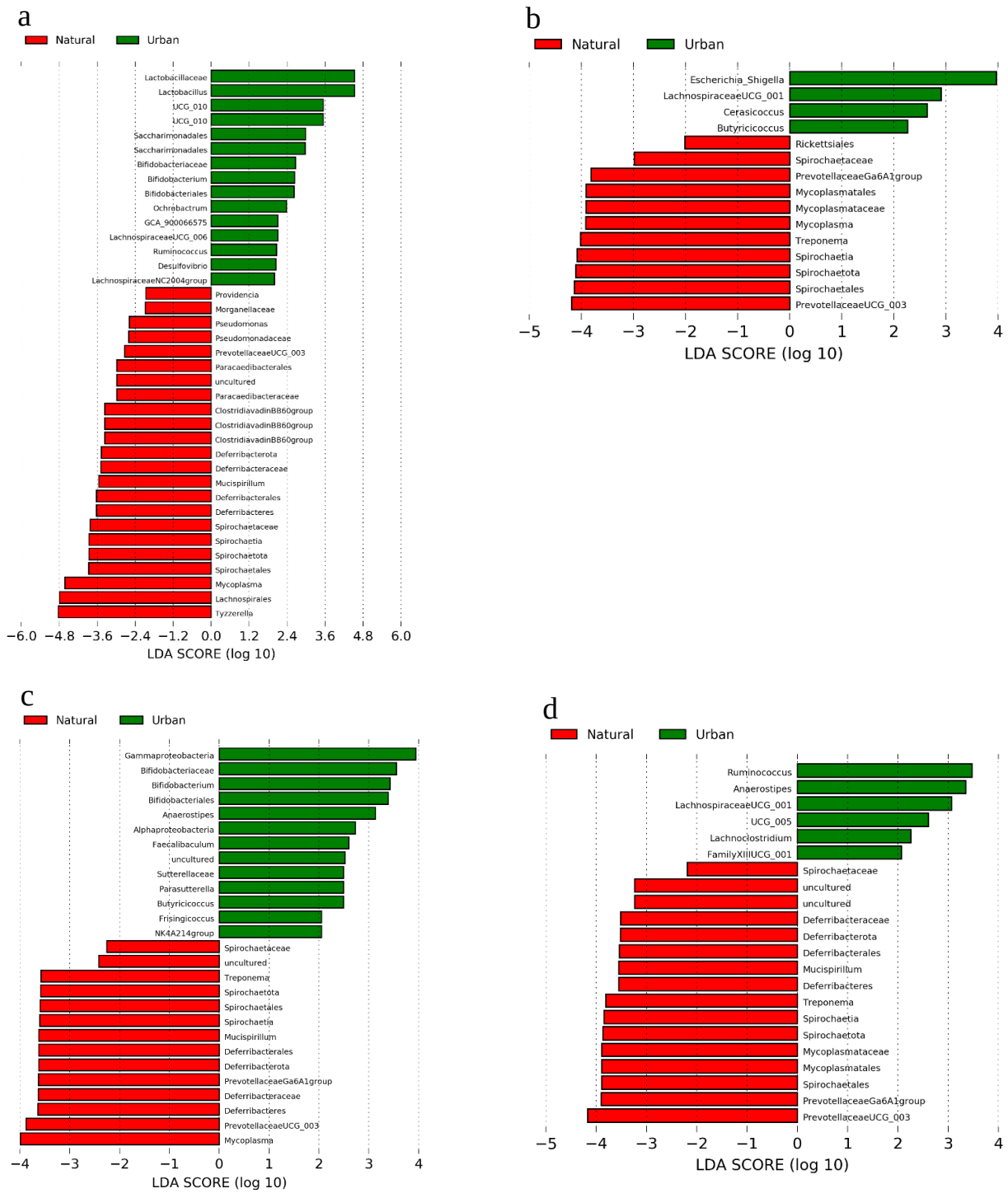


Fig. S4 More abundant microbial taxon at the genus level and up in the a) small intestine, b) cecum, c) colon, d) and rectum of *A. speciosus* in the natural and urban ecosystems based on LEfSe analysis.

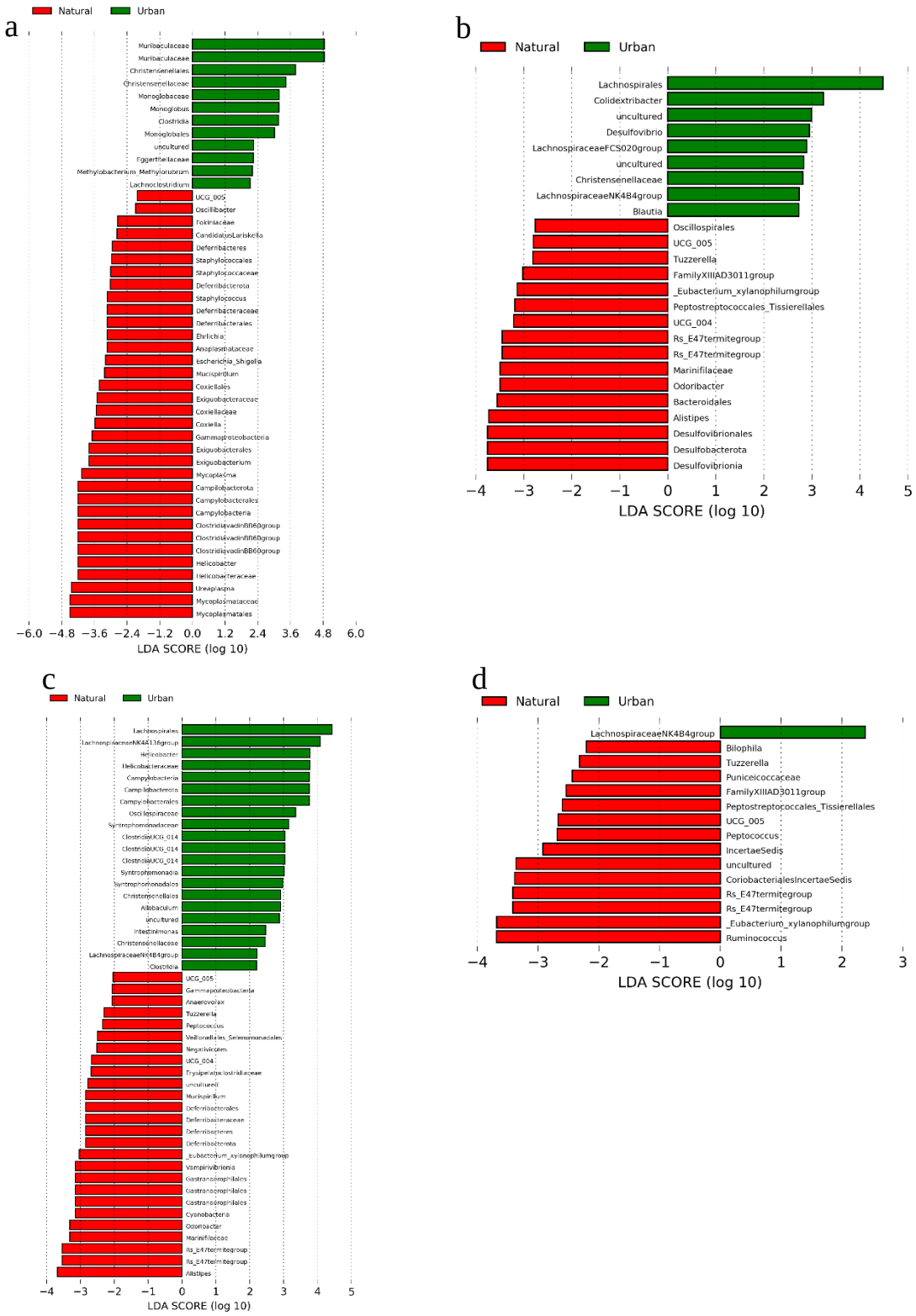


Fig. S5 More abundant microbial taxon at the genus level and up in the a) small intestine, b) cecum, c) colon, d) and rectum of *M. rufocanus* in the natural and urban ecosystems based on LEfSe analysis.

Chapter 4

Altered intestinal helminth – gut microbiota interactions in urban environments

Abstract

The gut microbiota is pivotal for maintaining host health as it aids in digestion, nutritional uptake, and immune system regulation. Microbes are not the only organisms residing within the gastrointestinal tract (GIT), as helminths are ubiquitous throughout nature. Many helminths are host species specific and have been co-evolving with both the host and their associated microbes over millions of years. Therefore, there is likely complex interactions both directly in competition for food resources and indirectly through modulation of the host immune system, and alterations to one will likely impact the other. Urbanization not only affects animals, but also alters their gut microbiota and intestinal helminths. In this study, I investigated gut microbiota-intestinal helminth interaction across the GIT (i.e. small intestine, cecum, colon, and rectum) in two sympatric species of rodents (*Apodemus speciosus* and *Myodes rufocanus*) within natural and urban ecosystems to determine how their relationship may be altered and if helminths can be used as a therapeutic tool to remedy urban induced dysbiosis. I found the nematode *Heligmosomoides kurilensis* from *A. speciosus* and Cestoda spp. from *M. rufocanus* were almost non-existent within the urban parks despite being commonly found in the natural areas. Prevalence and abundance of all helminth species were associated with significant changes in both alpha diversity and community composition in both rodent species. However, the gut region in which associations were found differed between natural and urban areas. Furthermore, helminth presence had a larger impact on both microbial alpha diversity and community composition within the urban populations, particularly for *A. speciosus*, than in the natural areas. Interestingly, the nematode *H. spumosa* may promote gut homeostasis within human modified environments by increasing microbial alpha diversity and decreasing the abundance of *Helicobacter* in the lower GIT. It is challenging to predict complex host-microbe-helminth-environment interactions within modified ecosystems and further studies are needed.

Introduction

The gut microbiota of vertebrates is pivotal for maintaining the health of individuals through complex host-microbe interactions (McFall-Ngai et al., 2013). Disruption of the microbial community structure, a condition known as dysbiosis (Logan et al., 2016), will invariably impact the host's well-being by affecting nutritional uptake (Turnbaugh et al., 2006), suppressing immune system activity (de Paiva et al., 2016), and inducing conditions such as inflammatory bowel disease (Broadhurst et al., 2012). However, microbes are not the only organisms inhabiting the gastrointestinal tract (GIT), as intestinal helminths (i.e. cestodes, trematodes, and nematodes) are ubiquitous throughout nature (Leung & Poulin., 2008). While they are widely considered to be detrimental to the host, low to moderate abundances are often tolerated without any clinical signs of infection (Bilbo et al., 2011; Kutzer & Armitage, 2016).

Both helminths and microbes have evolved ways to immunomodulate their host to avoid detection and create a more favorable environment for their persistence (Gause & Maizels, 2016; Midha et al., 2017). Because the immune system helps shape the helminth and microbial communities (Bevins & Salzman, 2011; Donaldson et al., 2016), modifying its functionality will ultimately impact which organisms can remain within the gut, thereby necessitating complex indirect helminth-host-microbiota interactions (Gause & Maizels, 2016; Midha et al., 2017). Such relationships have been implicated in both laboratory inoculation experiments (Broadhurst et al., 2012; Cattadori et al., 2016; Holm et al., 2015; Rausch et al., 2013; Reynolds et al., 2014; Walk et al., 2010) as well as several field studies (Aivelo & Norberg, 2018; Kreisinger et al., 2015; Newbold et al., 2017). Interestingly, it has even been found that helminth infection is associated with changes in microbial alpha diversity and community composition within gut regions they do no reside in (Holm et al., 2015; Kreisinger et al., 2015; Newbold et al., 2017; Rausch et al., 2013). Helminth-gut microbiota interactions may also be more direct as nematodes produce their own anti-microbial compounds while species such as *Trichuris muris* and *T. suis* require the presence of specific microbes to be present in order for the eggs to successfully hatch (Midha et al., 2017; Vejzagić et al., 2015).

Recently, interest has been growing regarding the use of intestinal helminths as a therapeutic tool to remedy dysbiosis and improve animal health. For example, the nematode *T. trichuria* has been used to cure chronic dysentery and restore a healthy microbial community in captive macaques (Broadhurst et al., 2012), while infection with *Heligmosomoides polygyrus*

bakeri in laboratory mice reduced symptoms of allergic asthma through promoting the growth of microbes with higher production of short chain fatty acids (Zaiss et al., 2015). Therefore, intestinal helminths are a natural component of the ecosystem within the gut that may help promote gut homeostasis and could be used to remedy dysbiosis in wildlife (Broadhurst et al., 2012; Kreisinger et al., 2015), particularly those living in environments heavily impacted by human activities. No study to date has investigated the role that intestinal helminths may play in maintaining gut homeostasis in wildlife affected by ecosystem modification.

The most extreme form of ecosystem modification is urbanization, impacting wildlife through forest fragmentation (McKinney, 2008), pollution (Isaksson, 2015; Rashed & Soltan, 2005), and artificial feeding (Larson et al., 2020; Pagani-Núñez et al., 2019). Both intestinal helminths and the gut microbiota of wildlife are significantly altered within urban populations as compared to conspecifics in more undisturbed habitat (Anders et al., 2019; Sugden et al., 2020; Teyssier et al., 2018, 2020; Werner & Nunn, 2020). It is important to understand how the altered macro- and micro- organism communities within the GIT affect their interactions and the health of the host. Specifically, can the persistence of helminths help prevent human induced dysbiosis or will it compound any negative impacts of urbanization on the gut microbiota?

In this study, I first analyzed the relationship between intestinal helminths and the gut microbial community along the GIT (i.e. small intestine, cecum, colon, rectum) in two sympatric species of rodents (i.e. *Apodemus speciosus* and *Myodes rufocanus*) within forests minimally impacted by human activity. I then investigate if those same relationships are maintained within urban populations. Specifically, I wanted to know if changes in alpha and beta diversity of the gut microbial community associated with the presence/absence or abundance of each helminth species would be similar in both natural and urban ecosystems. Furthermore, I was interested in exploring if helminths naturally infecting these hosts could be used as a therapeutic tool for remedying dysbiosis induced by urbanization. Both *A. speciosus* and *M. rufocanus* harbor unique helminth communities with many of the species parasitizing each host being distinctly different (Asakawa, 2005). However, the generalist nematode *Heterakis spumosa* is known to reside within the colon of both rodents and was commonly found in the present study (Asakawa et al., 1994). Therefore, special attention will be given to this helminth with a focus on if a similar relationship with the gut microbiota can be found within each host species.

Methods

Field survey and sample processing

A detailed description of the field survey as well as laboratory methods related to stable isotope and microbiome analysis can be seen in chapters 2 and 3 of the present thesis and will only be described here in brief. Both rodent species were live trapped in three urban parks (two in Asahikawa city, one in Biei town) and four natural sites located within the surrounding national forest in central Hokkaido, Japan (Table S1). Trapping occurred over two to three consecutive nights at each site using Sherman traps baited with oatmeal in October 2019. The animals were transported to the Department of Parasitology at Asahikawa Medical University in the city of Asahikawa. After cervical dislocation, the intestinal tract was removed and separated into three parts corresponding to the small intestine, cecum, and large intestine. Gut content was collected from the ileum, cecum, and ascending colon, as well as fecal matter from the rectum using a small steel spatula and sterile laboratory techniques.

Collection and identification of intestinal helminths was performed in the Graduate School of Environmental Science, Hokkaido University, Sapporo Japan from intestinal tracts preserved in 70% ethanol. The intestines of each individual were dissected under a stereo microscope (Olympus SZX10), helminths were identified morphologically, and their abundance tabulated.

DNA was extracted from gut content and fecal matter samples using the QIAamp fast DNA Stoll Mini Kit from Qiagen after bead beating following Hayakawa et al. (2018). PCR amplification of the V3-V4 region of the 16S rRNA gene was performed using the 341F-805R universal primers (Chapter 2, Klindworth et al., 2013). An Illumina MiSeq 300bp paired end platform was used with a v3 Reagent Kit for high-throughput sequencing of all PCR products after library preparation with Nextera XT DNA Index Kit v2. Sequence data was demultiplexed, paired-end reads merged, then quality filtered using the DADA2 pipeline in Qiime2 version 2020.2 (Bolyen et al., 2019; Callahan et al., 2016). Contaminant sequences in the resulting Amplicon Sequence Variants (ASV) table were identified and removed using the package Decontam in R version 4.0.2 (Davis et al., 2018; Core R Team, 2020) and taxonomic classification was performed using the SILVA classifier (release 138) in Qiime2 (Bokulich et al., 2018). Sequences not identified to phylum level as well as those determined to be archaea, eukaryotes, mitochondria, and chloroplastida were removed from the ASV table and a

phylogenetic tree was generated by the FastTree method in Qiime2 (Price et al., 2010). After alpha rarefaction to a sampling depth of 10,000 reads per sample, microbial alpha diversity was quantified using Shannon diversity, Faith's phylogenetic diversity (PD), Pielou's evenness, and the number of ASVs (i.e. richness), while dissimilarity matrices were generated for beta-diversity analysis using unweighted and weighted unifrac methods.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes are widely used for characterizing diet and trophic interactions as $\delta^{13}C$ and $\delta^{15}N$ values in animal tissue are derived from their food sources (Ben-David & Flaherty, 2012). Not only does diet heavily influence the gut microbial community, but dietary habits influence foraging behavior and exposure to parasitic helminths. In the present study, stable isotope analysis was performed on fur samples collected from the hind legs of each individual after a 2:1 (v/v) choloform:methanol wash to remove surface oils. Tin caps containing 500mg of fur were analyzed for $\delta^{13}C$ and $\delta^{15}N$ ratios using a continuous flow isotopic ratio mass spectrometer (IRMS, Delta V Plus, Thermofisher) coupled to a FLASH EA 1112 (Thermofisher) elemental analyzer. Isotopic ratios are presented as parts per mil (‰) after standardization of isotopic ratios in relation to Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

Statistical analysis

Differences in helminth prevalence between natural and urban populations was analyzed using a generalized linear model (GLM) with binomial distribution using the R package lme4 (Bates et al., 2015) where prevalence was the response variable, and ecosystem, sex, and age (adult / sub-adult) were fixed effects. Due to the large number of zeros (i.e. uninfected individuals) in helminth abundance data, a zero-inflated negative binomial model (ZINB) in the R package pscl (Zeileis et al., 2008) was utilized for analyzing differences in abundance between ecosystem type with the same fixed effects as the GLM . Because of 100 percent prevalence of *Heligmonoides speciosus* in *A. speciosus*, a GLM with negative binomial distribution was used instead.

Linear mixed effects models (LME) with gaussian distribution were utilized for analyzing the effect of parasite presence/absence and abundance of each species on log transformed alpha diversity in each gut region of both host species using the R package nlme (Pinheiro et al., 2020). The model was first performed using individuals from natural populations

where age, sex, $\delta^{13}C$, $\delta^{15}N$, and presence/absence or abundance of each helminth species were fixed effects and site was the random effect. It was then repeated for individuals from the urban parks to determine if any similarities in the effect of helminths on microbial alpha diversity could be identified. Analysis of the effect of parasite prevalence and abundance on beta-diversity in each gut region in both host species was performed using permutational multivariate analysis of variance (PERMANOVA) on both unweighted and weighted unifrac dissimilarity matrices with the vegan package in R (Oksanen et al., 2007). Similar to alpha diversity analysis, PERMANOVA was performed on natural and urban populations separately with age, sex, $\delta^{13}C$, $\delta^{15}N$, and prevalence or abundance of each helminth species as explanatory variables. *Syphacia emileromani* from *A. speciosus* and *H. speciosus* from *M. rufocanus* as well as *B. asawakawai* from both species were excluded from diversity analysis due to exceedingly rare or accidental infections (Table S2). Furthermore, Cestoda spp. was excluded from urban population analysis of *M. rufocanus* due to only a single individual being infected (Table S2). *H. speciosus* prevalence in *A. speciosus* was also excluded from the models due to 100 percent of individuals being infected (Table S2).

The change in relative abundance of microbial taxonomic genera in relation to infection (i.e. presence or absence) of each helminth species was analyzed using linear discriminant analysis effect size (LEfSe) in the Huttenhower laboratory Galaxy pipeline (Segata et al., 2011). This was performed on each gut region of each host species within each ecosystem separately. Because LEfSe analysis cannot include interaction terms nor multiple helminth species simultaneously, analysis was performed separately for each species.

Results

Hosts, helminths, and gut content samples

A total of 83 *A. speciosus* (42 natural and 41 urban) and 83 *M. rufocanus* (43 natural and 50 urban) individuals were captured in this study. Seven nematode species, one trematode species, as well as Cestode spp. were collected from both hosts (Table S2). The nematode *H. spumosa*, the trematode *B. asakawai*, and Cestode spp. were collected from the colon, cecum, and small intestine respectively in both hosts (Table S2). The remaining helminths were host species dependent. Specifically, *Syphacia agraria* was found in the cecum, while *S. emileromani*, *Heligmosomoides kurilensis*, and *H. speciosus* were in the small intestine of *A.*

speciosus (Table S2). *Syphacia montana* and *Heligmosomum yamagutii* were collected from the cecum and small intestine respectively in *M. rufocanus* (Table S2). *H. speciosus* is typically only found in the small intestine of *A.* speciosus and *Apodemus argenteus* (Asakawa, 2005). Therefore, the single worm found from *M. rufocanus* was likely an accidental infection.

For the microbiome analysis I collected 42 and 39 gut content samples from the small intestine, 27 and 16 from the cecum, 41 and 30 from the colon, as well as 25 and 16 fecal matter samples from the rectum of *A. speciosus* in the natural and urban ecosystems respectively. From *M. rufocanus*, 43 and 49 gut content samples were collected from the small intestine, 14 and 17 from the cecum, 38 and 45 from the colon, as well as fecal matter from the rectum of 16 and 16 individuals from populations in the national forest and urban parks respectively. A total of 21,820,759 high quality reads were obtained from high-throughput sequencing, 11,264,730 of which were from *A. speciosus* (average of 45978 ± 795 SEM per sample) and 10,55,029 (average of 44167 ± 1070 SEM per sample) from *M. rufocanus*.

Altered parasite community in urban populations

Only *H. kurilensis* was found to be significantly less prevalent in urban *A. speciosus* as compared to the natural populations while both *H. spumosa* and Cestoda spp. were less prevalent in urban *M. rufocanus* although *H. spumosa* prevalence remained high (all $p < 0.01$; Fig 1a, c, Table S2 to S4). However, *H. spumosa* was significantly more abundant in the urban populations of *M. rufocanus* ($b = 0.873 \pm 0.32$, $p = 0.006$), while no difference was found between urban and natural populations of *A. speciosus* ($b = 0.435 \pm 0.486$, $p = 0.371$; Fig. 1b, d, Table S3, S4). Cestoda spp. were significantly higher in abundance in urban *A. speciosus* ($b = -3.862 \pm 1.001$, $p < 0.001$) but no difference was found in *M. rufocanus* ($b = -2.995 \pm 2.792$, $p = 0.283$; Fig. 1b, d, Table S3, S4). Both *H. kurilensis* and *H. speciosus* in *A. speciosus* as well as *S. montana* in *M. rufocanus* were significantly less abundant in the urban parks as compared to the national forest (all $p < 0.05$; Fig. 1b, d, Table S3, S4).

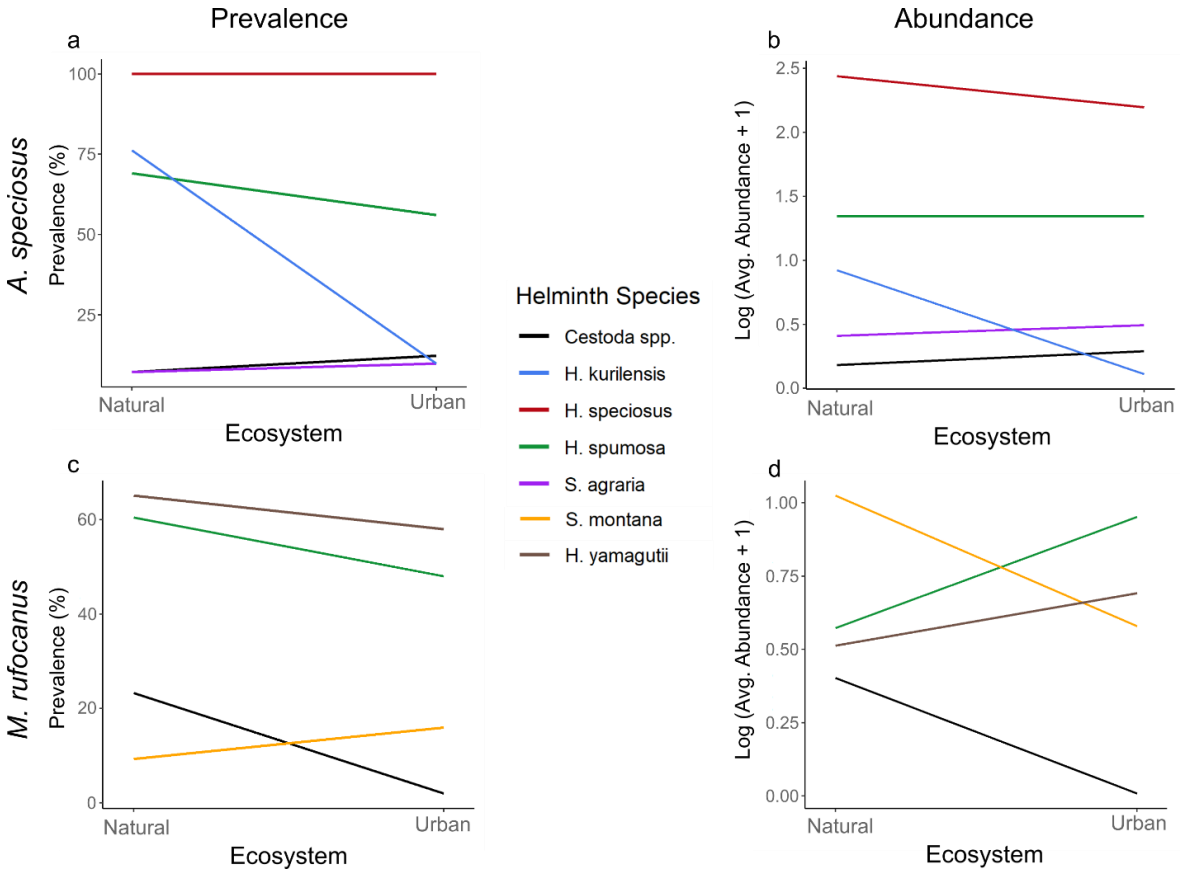


Fig. 1 Helminth prevalence (a, c) and abundance (b, d) in natural and urban ecosystems for *A. speciosus* (a, b) and *M. rufocanus* (c, d).

Helminths - gut microbiome alpha diversity

Interestingly, helminth presence/absence was associated with more changes in gut microbial alpha diversity of *A. speciosus* within the urban areas than in the national forest. (Table S5 to S8). Furthermore, helminth presence/absence was mostly associated with significantly lower alpha diversity within the natural areas, but higher alpha diversity in the urban parks except for Cestoda spp. which was always associated with lower diversity in both ecosystems (Table S5 to S8). Specifically, Cestoda spp. was associated with significantly lower Shannon diversity and evenness in the colon of *A. speciosus* in the natural areas, but lower evenness in the cecum and rectum as well as the number of ASVs in colon of urban individuals (all $p < 0.05$; Table S5, S7, S8). *H. kurilensis* was associated with significantly lower Shannon diversity and

number of ASVs in the small intestines of natural individuals, but higher alpha diversity in the cecum of those from the urban parks for all alpha diversity metrics except evenness (Table S5 to S8). While significant associations were also found between *H. spumosa* and higher Shannon diversity, Faith's PD, and evenness in the cecum of urban animals, no relationships were found within the natural populations.

Associations between the abundance of each helminth species and microbial diversity within *A. speciosus* was highly inconsistent in terms of which gut region the relationship was found among alpha diversity metrics or between natural and urban populations (Table S9 to S12). Furthermore, the effect size of each potential interaction was exceedingly small. I found that Cestoda spp. abundance was associated with significantly lower evenness in the colon of individuals from the national forest, but higher Faith's PD and number of ASVs in the small intestine of urban *A. speciosus* (all $p < 0.05$; Table S10 to S12). On the other hand, *H. spumosa* was found to be associated with significantly higher Faith's PD and number of ASVs in the small intestine but lower number of ASVs in the colon of animals from the national forest (all $p < 0.05$; Table S10, S12). However, in urban *A. speciosus*, the same helminth was associated with marginally lower evenness in the colon ($b = 0.0004 \pm 0.0002$, $p = 0.017$; Table S11).

Fewer associations between helminth presence/absence and gut microbial alpha diversity were found in *M. rufocanus* than in *A. speciosus*, however, associations were more consistent between natural and urban individuals (Table S13 to S16). Specifically, the nematode *S. montana* was found to be associated with significantly lower Shannon diversity and the number of ASVs in the cecum of both natural and urban individuals (all $p < 0.05$; Table S13, S14). It was also associated with lower evenness and the number of ASVs in the rectum of those from the national forest, but lower Faith's PD in the cecum of urban animals (all $p < 0.05$; Table S12 to S16). *H. yamagutii* exhibited a similar trend across ecosystem type as it was associated with significantly higher number of ASVs in the cecum of both natural and urban individuals, although no other associations were found (Table S13 to S16). Similar to what was found in *A. speciosus*, the presence of *H. spumosa* was associated with higher evenness in the colon and number of ASVs in the cecum of individuals from the national forest, as well as higher evenness in the rectum of urban animals (all $p < 0.05$; Table S15, S16).

Analogous to what was found in *A. speciosus*, there was more inconsistency in the associations between helminth abundance and microbial alpha diversity in terms of gut region,

alpha diversity metric, and ecosystem type in *M. rufocanus* than there were for presence/absence (Table S17 to S20). For example, *H. yamagutii* abundance was associated with higher Faith's PD in the small intestine and colon but lower evenness in the cecum of natural individuals, as well as lower evenness in the rectum of urban individuals (all $p < 0.05$; Table S18, S19). Despite the consistent relationships between *S. montana* presence and lower alpha diversity in both ecosystem types, its abundance was associated with significantly higher evenness in the cecum of natural individuals (Table S19). However, this helminth was also associated with lower alpha diversity throughout the lower GIT of urban individuals (Table S17 to S20). It should also be noted that *H. spumosa* was associated with lower evenness in the cecum of *M. rufocanus* from the natural areas but higher evenness in the rectum of individuals from the urban parks (both $p < 0.05$; Table S19).

Helminth - gut microbiota Beta-diversity

Helminth presence of any species had little effect on the gut microbial community structure along the gastrointestinal tract of *A. speciosus* within the natural areas with only *H. kurilensis* having a significant association in the small intestine based on both unweighted and weighted unifrac (Table S21). There were far more significant associations in all gut regions of urban individuals (Table S22). *H. spumosa* in particular had a high number of associations as its presence had a significant effect on beta-diversity in all four gut regions according to unweighted unifrac as well as in the small intestine and colon for weighted unifrac (all $p < 0.05$; Table S22). *H. kurilensis* also showed a significant effect on beta-diversity throughout the lower GIT of urban *A. speciosus*, but only for unweighted unifrac (all $p < 0.05$; Table S22).

More significant associations were found between *H. spumosa* abundance and gut microbial beta diversity in *A. speciosus* than any other helminth species in both the natural and urban populations (Table S23, S24). Specifically, significant associations were found within the small intestine, colon, and rectum of individuals from the national forest based on unweighted unifrac as well as in the cecum and colon according to weighted unifrac (all $p < 0.05$; Table S23). *H. spumosa* also had a significant effect in the small intestine and colon of urban individuals for both unweighted and weighted unifrac (all $p < 0.05$; Table S24). Few significant associations were found between the abundance of the other helminth species and beta-diversity

in either the natural or urban populations of *A. speciosus* with no consistency in terms of which gut region they were found (Table S23, S24).

I found few significant associations between prevalence or abundance of any helminth species and beta-diversity along the GIT of *M. rufocanus* in either the natural or urban ecosystems (Table S25 to S28). In fact, *S. montana* was the only helminth in which prevalence was found to have a significant effect within individuals from the natural areas, and only within the colon and rectum based on weighed unifrac alone (both $p < 0.05$; Table S25). However, in the urban parks, significant associations were found for *H. spumosa* presence in the colon based on unweighted unifrac as well as *H. yamagutii* in the rectum according to weighted unifrac (both $p < 0.05$; Table S26). There was no effect of helminth abundance on beta-diversity in any gut region of *M. rufocanus* from the natural areas while only a few were found within the urban individuals (Table S27, S28). Specifically, *H. spumosa* abundance had a significant effect on beta-diversity in both the cecum and rectum according to weighted unifrac while a significant association was found for *S. montana* in the cecum based on unweighted unifrac, as well as *H. yamagutii* in the rectum for weighted unifrac (all $p < 0.05$; Table S28).

Helminth infection impacts microbial relative abundance

Infection with each species of helminth was associated with changes in relative abundance of multiple microbial genera across the GIT in both host species regardless of ecosystem type (Table S29, S30). The presence of *H. spumosa* was correlated with the largest number of more abundant genera within *A. speciosus* although the relationship was not consistent between natural and urban areas (Table S29). Specifically, uninfected hosts in the national forest were found to have more genera in higher abundance in uninfected hosts but the opposite was true in urban parks with more genera with increased abundances were found in infected hosts (Table S29). Interestingly, the largest degree of change occurred within the small intestine, and the number of affected genera gradually decreased along the GIT toward the colon where *H. spumosa* resides (Table S29). In *M. rufocanus*, *H. yamagutii*, Cestoda spp., *S. montana*, and *H. spumosa* were all associated with changes in relative abundance of a similar number of genera although the most affected gut region varied depending on helminth species (Table S30).

There was a remarkable lack of consistency in which microbial genera exhibited higher abundance in relation to the presence or absence of each helminth species with most significant

relationships being host, gut region, and ecosystem specific (Fig. S1 to S9). There were a couple of notable exceptions, however. For example, higher abundance of *Fusobacterium* in one or more gut regions of urban *A. speciosus* is associated with infection by *H. kurilensis*, Cestoda spp., and *S. agraria* as well as higher abundance in the small intestine of urban *M. rufocanus* infected with *H. spumosa* (Fig. S1 to S3, S9). This relationship was not found in natural populations despite the presence of *Fusobacterium*. The most notable helminth-microbe interaction in *M. rufocanus* is between the helminth *H. spumosa* and the potentially pathogenic microbial genus *Helicobacter*. Infection with *H. spumosa* in the natural areas is associated with higher abundance of *Helicobacter* in the small intestine, but higher abundance of *Helicobacter* in the cecum, colon, and rectum was found in urban individuals uninfected with *H. spumosa* (Fig. 2, a, b, S9). There was also significantly higher abundance of *Butyricicoccaceae* UCG_009 throughout the lower GIT of urban individuals infected with *H. spumosa* although this relationship was not found in the natural areas despite the presence of this microbial genus (Fig. 2 c, d, S9).

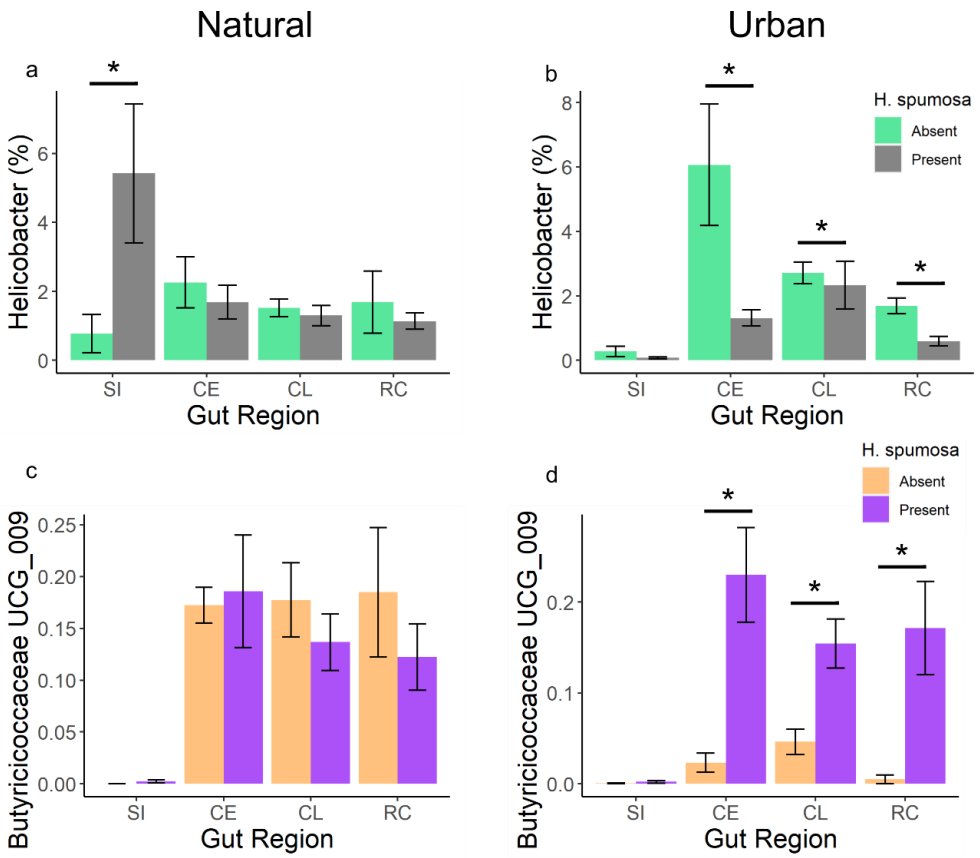


Fig. 2 Relative abundance of *Helicobacter* (a, b) and *Butyricicoccaceae* UCG_009 (c, d) throughout the GIT of *M. rufocanus* infected with the helminth *H. spumosa* in Natural (a, b) and urban (b, d) ecosystems. The small intestine is SI, cecum is CE, colon is CL, and the rectum is RC.

Discussion

Altered intestinal helminth communities of urban individuals

I found that multiple helminth species exhibited significantly different prevalence and abundance within the urban populations of both host species as compared to their natural conspecifics (Fig. 1, Table S2 to S4). In particular, there was almost no *H. kurilensis* in found urban *A. speciosus* despite the high prevalence within the national forest (76%; Fig. 1a, b, Table 2, 3). This is in contrast to what was found in the city Obihiro, Hokkaido where both prevalence and abundance were exceedingly high in an urban park (Anders et al., 2019). These distinctly different trends could be in response to city specific differences in fertilizers or pesticides used

for management practices that affect the survival rate of the larval stage before infecting the host (Sures, 2004). Another possibility is the difference in rodent community structure within urban parks (i.e. a more equal number of *A. speciosus* and *M. rufocanus*) as compared to the natural areas (i.e. one rodent species is dominant at each site) could affect the helminths chances to come into contact with a proper host.

The near non-existence of Cestoda spp. in urban *M. rufocanus* is of no surprise as this is a common occurrence for parasites with a complex life-cycle (Fig, 1c, d; Werner & Nunn, 2020). Cestodes require an intermediate host, typically an arthropod, to be consumed by the final host for transmission to occur (Goater et al., 2014). Therefore, the shift away from consuming terrestrial animal protein as shown in chapter 3 of the present thesis could explain this trend although the shift was small. The loss of important intermediate hosts could also be the cause as urbanization is known to alter insect communities, thereby preventing the persistence of cestodes (McKinney, 2008).

Intestinal helminths and changes in gut microbial alpha diversity

In this study I found that prevalence and abundance of all intestinal helminth species were associated with significant changes in alpha diversity in both species of rodents (Table S5 to S20). However, these relationships were mostly inconsistent among gut regions or between natural and urban populations, particularly in *A. speciosus*, suggesting a high degree of context dependence. Furthermore, the effect size of helminth abundance was exceedingly small compared to that of presence/absence indicating that infection status is more important than worm burden.

Interestingly, there were more associations between helminth presence and changes in gut microbiota alpha diversity in urban *A. speciosus* as compared to those in the natural areas suggesting altered helminth – gut microbiota interactions. Nematodes in particular were associated with higher alpha diversity in the cecum despite both *H. kurilensis* and *H. spumosa* residing elsewhere in the gut. Therefore, these potential interactions are likely to be indirect through immunomodulation such as the up regulation of Th cytokines or the increased production of mucins that microbes are able to utilize as a food source, thereby impacting which microbial species can persist as well as their abundances (Fricke et al., 2015; Gause & Maizels, 2016; Midha et al., 2017).

Because both *H. kurilensis* and *H. spumosa* were associated with higher alpha diversity in urban individuals, despite the extremely low prevalence of *H. kurilensis*, they may help offset any negative impacts of urbanization on the gut microbiota and maintain gut homeostasis (Broadhurst et al., 2012; Kreisinger et al., 2015). This may partially explain why no differences in microbial alpha diversity was found between natural and urban areas in chapter 3 of this thesis. *H. spumosa* may be especially important in this regard as its presence was similarly associated with higher alpha diversity throughout the lower GIT of *M. rufocanus*, and both prevalence and abundance remained high in urban populations. However, because diet is a major factor affecting gut microbiota composition (David et al., 2014) as well as foraging habits affecting exposure to helminths, it is possible these are not true helminth – gut microbiota interactions. On the contrary, if dietary habits are the underlying cause of this association, I would expect to see a similar relationship within the national forest as well as a larger impact of stable isotope values on alpha diversity. Future laboratory inoculation experiments would be ideal for determining causality.

Although most helminth – gut microbiota alpha diversity associations were inconsistent between natural and urban areas in regard to gut region and helminth species involved, there were a couple of notable exceptions that remained consistent across ecosystem type. Specifically, *S. montana* prevalence and abundance in *M. rufocanus* in particular was almost always associated with lower gut microbial alpha diversity regardless of gut region, diversity metric, or ecosystem type. Because these associations are entirely within the lower GIT, especially within the cecum where the helminth resides, these potential interactions are likely to be more direct (Brosschot & Reynolds, 2018; Midha et al., 2017). For example, *S. montana* could be excreting anti-microbial compounds to kill off microbial competitors for limited food resources (Midha et al., 2017). In *A. speciosus*, Cestoda spp. prevalence and abundance were associated with lower alpha diversity in the colon of both natural and urban individuals although the number of associations were few and the alpha diversity metric differed. Cestodes occupy a large portion of the small intestine of these animals and absorb a significant amount of the food resources that the host ingests, thereby limiting the quantity that reaches the cecum where microbial abundance is usually highest and reducing the number of microbes that can be supported (Donaldson et al., 2016; Goater et al., 2014). However, the opposite trend was found in *M. rufocanus* from the natural areas where Cestoda spp. prevalence and abundance were associated with higher alpha

diversity in the cecum. Perhaps each host species is exhibiting a specific immunological response to cestode infection that differentially impacts the microbial diversity within the cecum.

Differential effect of helminths on gut microbial community structure

I found that helminth presence/absence and abundance was associated with more changes in gut microbial community composition in *A. speciosus* than in *M. rufocanus* in both the natural and urban ecosystems (Table S21 to S28). In fact, neither helminth prevalence nor abundance had much of an effect in any gut region of *M. rufocanus* in either the natural or urban areas (Table S25 to S28). Therefore, it is likely that the impact of helminths on community composition is minimal within this species. It is also plausible that the effect of other factors such as diet are large enough to make it difficult to detect any influence that helminths may have if the effect is small.

Although few helminth – gut microbiota associations were found in *M. rufocanus*, the nematode *H. spumosa* was associated with the largest number of changes in microbial community structure within both host species (Table S21 to S28). Interestingly, most associations in *A. speciosus* in the natural areas were with *H. spumosa* abundance but presence/absence within the urban parks suggesting altered interactions (Table S21 to S24). Furthermore, only in urban individuals of *M. rufocanus* were associations found between *H. spumosa* and the gut microbiota community composition although they were limited in number (Table S25 to S28). Because the microbial community composition is altered within the urban populations (chapter 3), perhaps the urban associated microbes are more strongly effected by the chemical compounds excreted by the nematode or by the modulated immune system activity (Gause & Maizels, 2016; Midha et al., 2017). In such a case, the mere presence of the helminth would induce a strong enough effect that worm burden is unimportant. However, animals must cope with numerous other environmental factors within the urban ecosystem such as pollution and stress that can impact immune system function (Gao et al., 2018; Isaksson, 2015). Therefore, there may be a synergistic effect of human associated environmental stressors and helminth mediated immuno-modulation that induces a unique immune response by the host that the gut microbes must cope with.

Helminth prevalence associated with changes in microbial genera relative abundance

Numerous microbial genera were found to have a change in relative abundance associated with the presence or absence of each helminth species (Fig. S1 to S9). However, there was little consistency in terms of which microbes in each gut region or ecosystem type were associated with each helminth species. Even within the same gut region the microbial genera found to be in higher abundance in individuals harboring each helminth species were not the same between urban and natural populations. However, there were a few notable exceptions. Specifically, higher abundance of *Fusobacterium* in one or more gut regions was associated with infection of several different helminth species within the urban parks. *Fusobacterium* is a common gut microbe of both humans and animals that forms colonies on the mucosal surface, and high abundances have been associated with numerous diseases such as inflammatory bowel disease (Allen-Vercoe et al., 2011). Therefore, infection with helminths may disrupt the mucosal barrier allowing *Fusobacterium* to proliferate. However, higher abundances were in gut regions other than where the associated helminth resides and may be associated with other factors or the correlation was purely chance (Fig. S1 to S9).

The most notable finding was the relationship between the potentially pathogenic *Helicobacter* and the helminth *H. spumosa* in *M. rufocanus* (Fig. 2). Many species of *Helicobacter* induce negative effects such as inflammatory bowel disease (Chin et al., 2000; Yang et al., 2013). Infection with *H. spumosa* was associated with higher abundance of *Helicobacter* in the small intestine of individuals in the natural areas, but lower abundance throughout the lower GIT in urban *M. rufocanus* (Fig. 2a, b). There was also significantly higher abundance of *Bityricicoccaceae* UCG_009 throughout the lower GIT of urban individuals infected by *H. spumosa* (Fig. 2c, d). Members of *Butyricicoccaceae* have recently been identified as “next-generation probiotics” that may be used to cure dysbiosis as they produce the chemical compound butyrate that reduces intestinal inflammation and improves the epithelial defense barrier (Boesmans et al., 2018). In chapter 3, I reported higher abundance of *Helicobacter* in the lower GIT of urban *M. rufocanus*. Therefore, *H. spumosa* may provide a protection against *Helicobacter* proliferation within the colon, cecum, and rectum through the promotion of the probiotic *Butyricicoccaceae*, thereby reducing the effects of urbanization induced dysbiosis. Although I did not quantify dysbiosis in this study, such an effect is similar to how infection with *Trichuris trichuria* cured dysbiotic inflammatory bowel disease in captive macaques by inducing

a T_H2-type immune response that likely caused the expulsion of pathogenic bacteria and promoted healing of the mucosal layer (Broadhurst et al., 2012). Why the opposite trend was seen within the small intestine of individuals in the natural areas is uncertain, but the species of *Helicobacter* in the upper and lower GIT are typically not the same (Chin et al., 2000; Schulz et al., 2015). Therefore, interactions with *H. spumosa* are likely to be different.

Conclusion

I found that helminth – gut microbiota interactions differed between natural and urban habitats. Specifically, the gut regions in which associations were found were not the same and that helminth presence/absence was associated with more changes in alpha diversity within the urban populations than in the natural areas, especially for *A. speciosus*. There was also little consistency between natural and urban populations in regard to which microbial genera exhibited higher abundance in response to infection by each species of helminth. In general, it is challenging to determine complex host-microbe-helminth-environment interactions that may be altered in a modified ecosystem as other factors such as a dietary shift or pollution may have a larger effect that masks the influence of helminths. The most compelling finding of this study is the role that *H. spumosa* may have in maintaining gut homeostasis. Its presence was found to be associated with higher gut microbial alpha diversity in both host species, but especially within urban *A. speciosus*. This nematode may also promote the probiotic *Butyricicoccaceae* within the lower GIT of *M. rufocanus*, thereby reducing the pathogenic *Helicobacter* in urban individuals. *H. spumosa* is a cosmopolitan species that is known to parasitize numerous species of rodents including but not limited to *Rattus spp.* and *Mus musculus* (Pakdeenarong et al., 2014; Pakdel et al., 2013), *Apodemus spp.* (Dwużnik et al., 2017), and *Mycromys minutus* (Kim et al., 2015). Rodents are ubiquitous throughout human modified environments and are known to carry numerous zoonotic disease that are of constant concern for public health (Meerburg et al., 2009). Therefore, *H. spumosa* could potentially be used as a management tool to help a wide range of rodent species maintain a healthy gut microbiota in the presence of human activities and improve their and ability to defend against pathogens that could be passed to humans. Future studies should further explore this relationship between *H. spumosa* and the gut microbiota of rodents.

Appendices

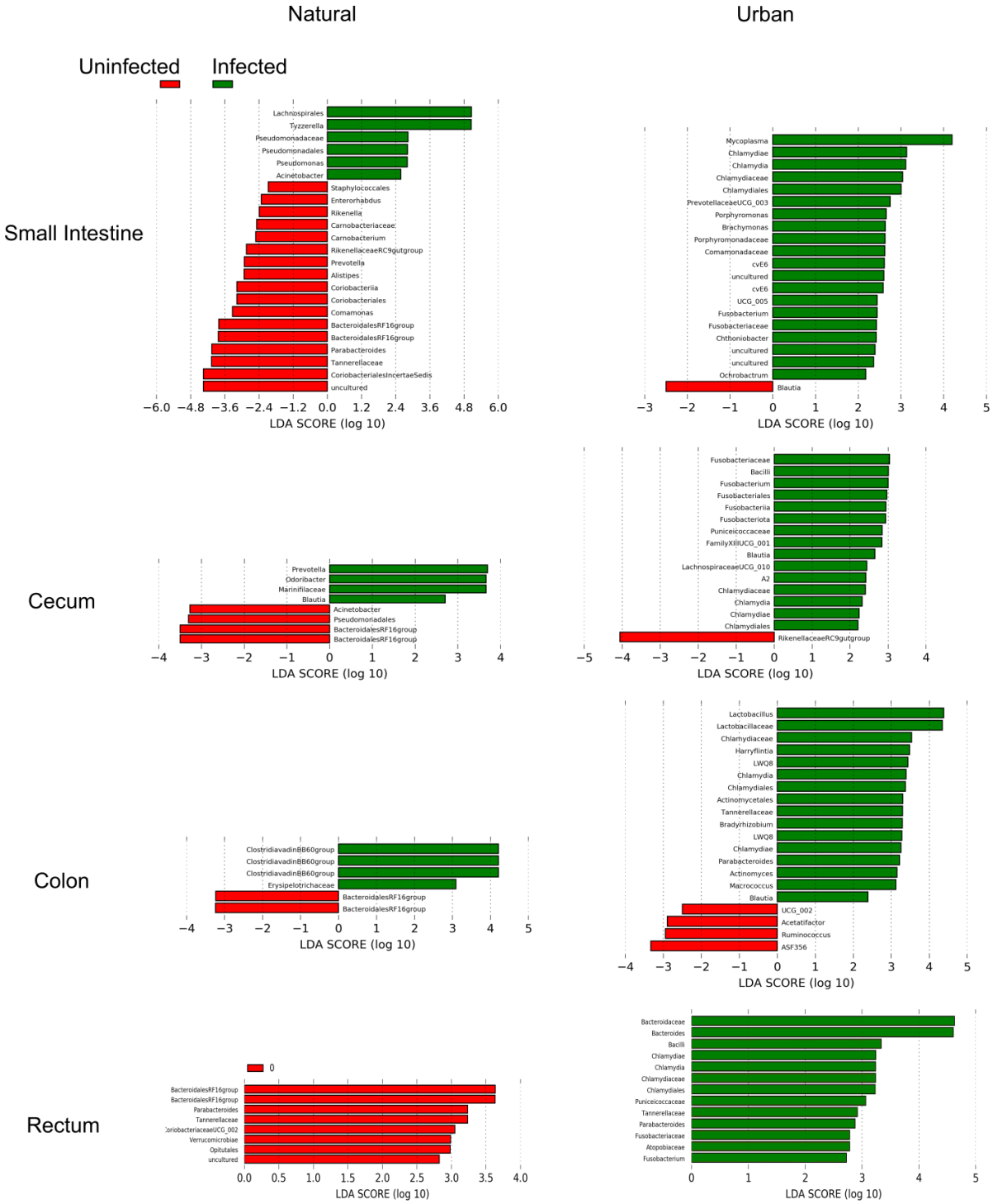


Fig. S1 More abundant microbial taxon along the gastrointestinal tract of *A. speciosus* infected (green) and uninfected (red) with the helminth *H. kurilensis* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

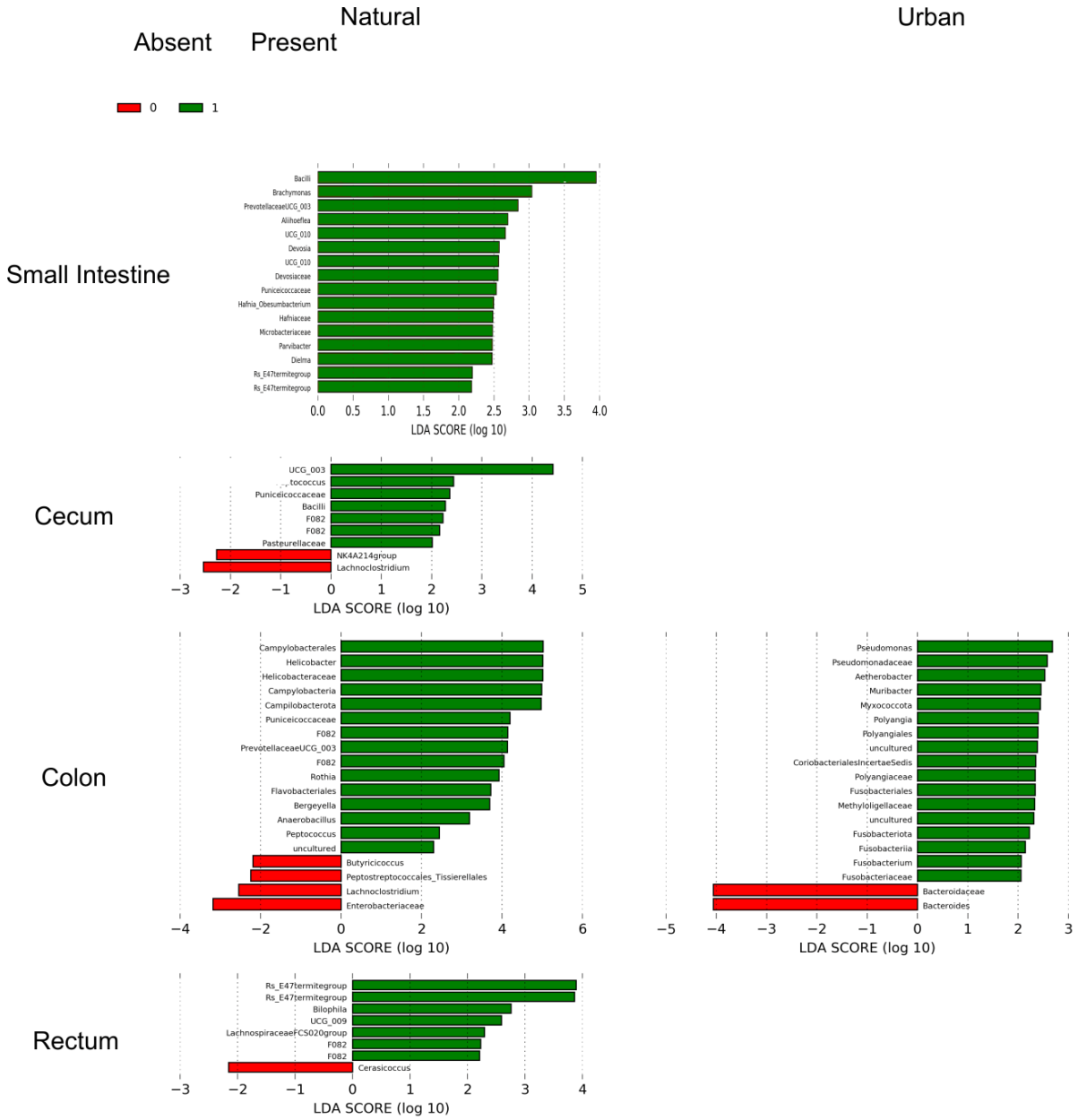


Fig. S2 More abundant microbial taxon along the gastrointestinal tract of *A. speciosus* infected (green) and uninfected (red) with the helminth Cestoda spp in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

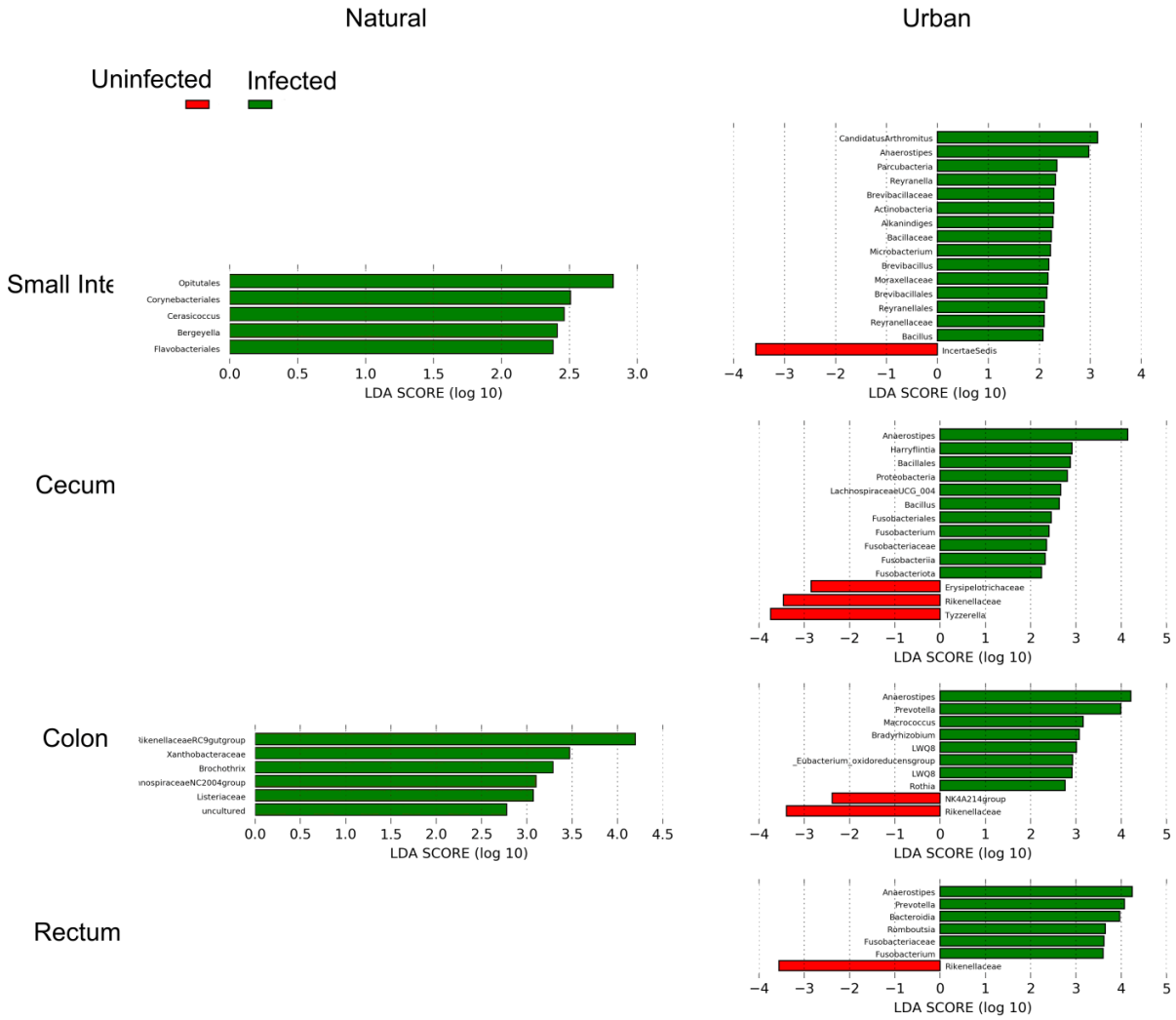


Fig. S3 More abundant microbial taxon along the gastrointestinal tract of *A. speciosus* infected (green) and uninfected (red) with the helminth *S. agraria* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

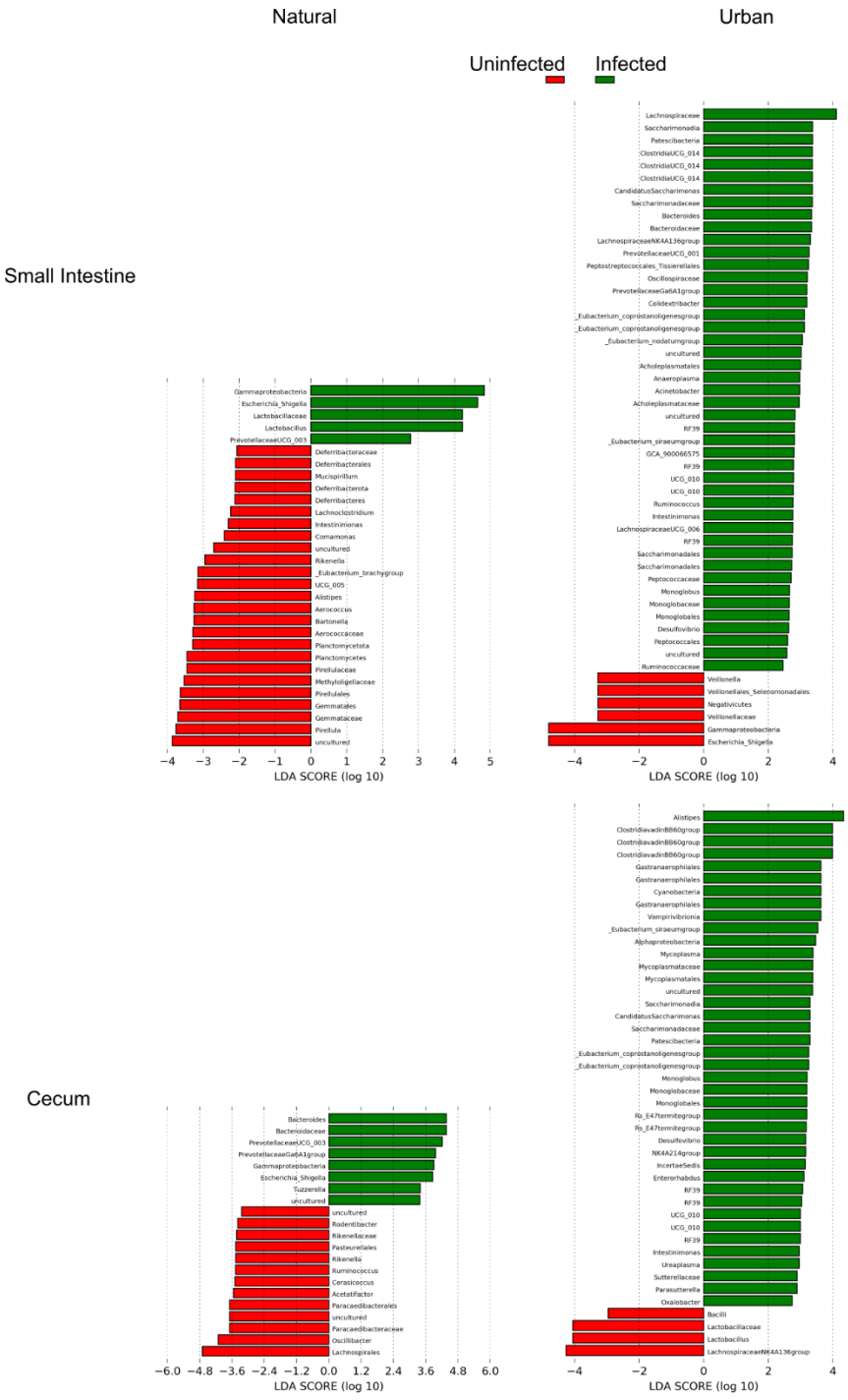


Fig. S4 More abundant microbial taxon in the small intestine and cecum of *A. speciosus* infected (green) and uninfected (red) with the helminth *H. spumosa* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

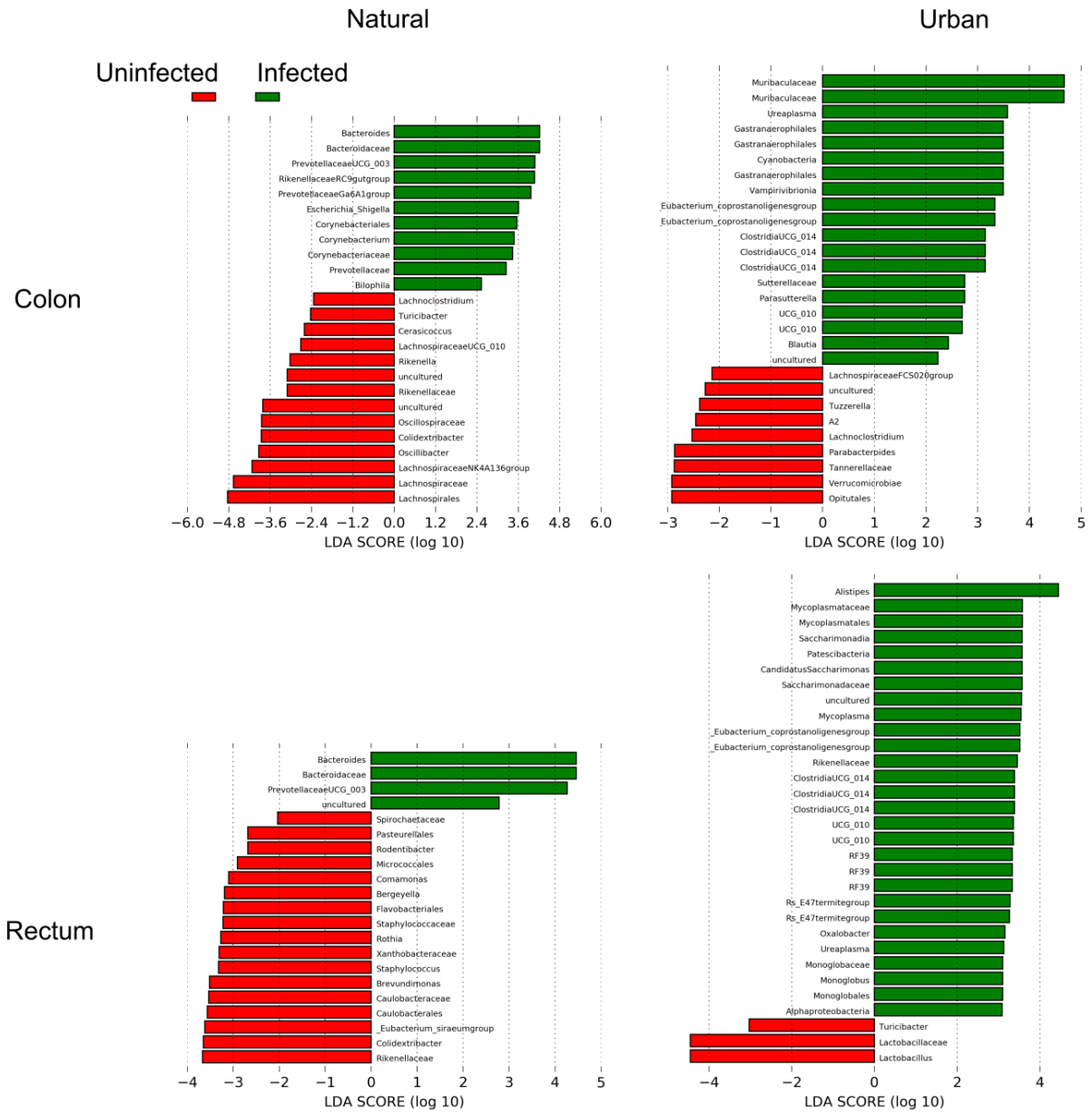


Fig. S5 More abundant microbial taxon in the colon and rectum of *A. speciosus* infected (green) and uninfected (red) with the helminth *H. spumosa* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

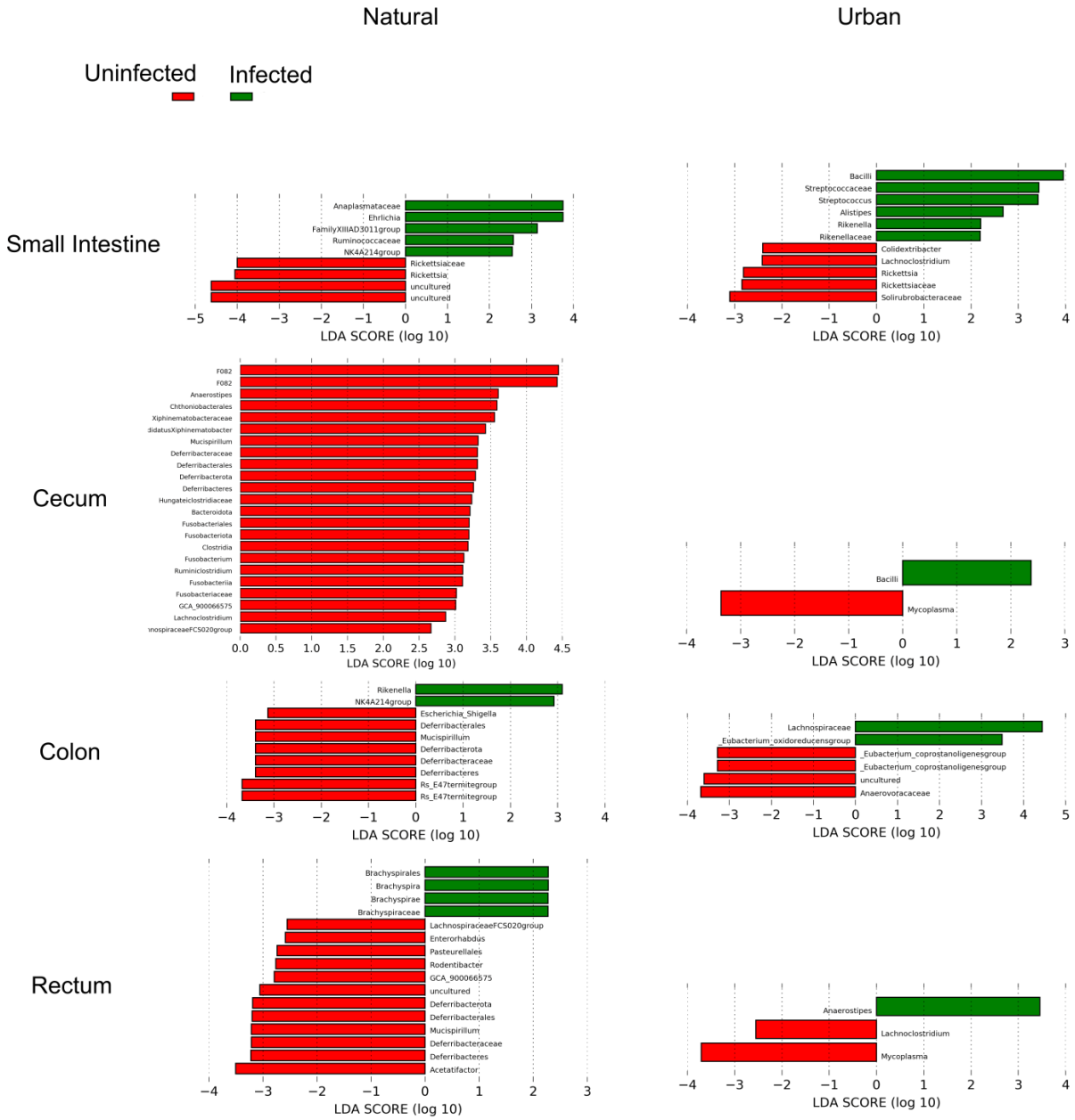


Fig. S6 More abundant microbial taxon along the gastrointestinal tract of *M. rufocanus* infected (green) and uninfected (red) with the helminth *H. yamagutii* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

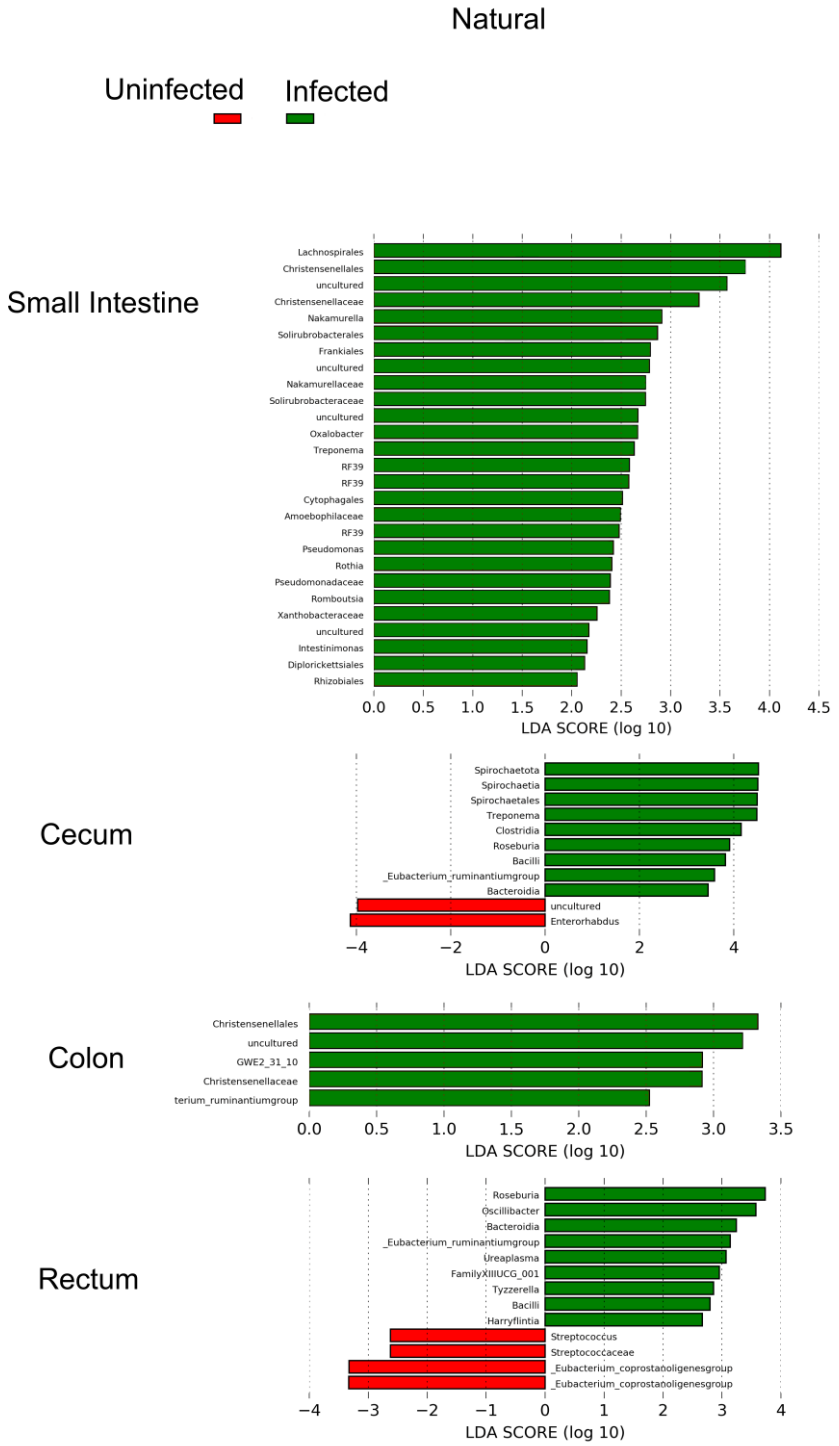


Fig. S7 More abundant microbial taxon along the gastrointestinal tract of *M. rufocanus* infected (green) and uninfected (red) with the helminth Cestoda spp. in the natural ecosystem only. Results based on LEfSe analysis.

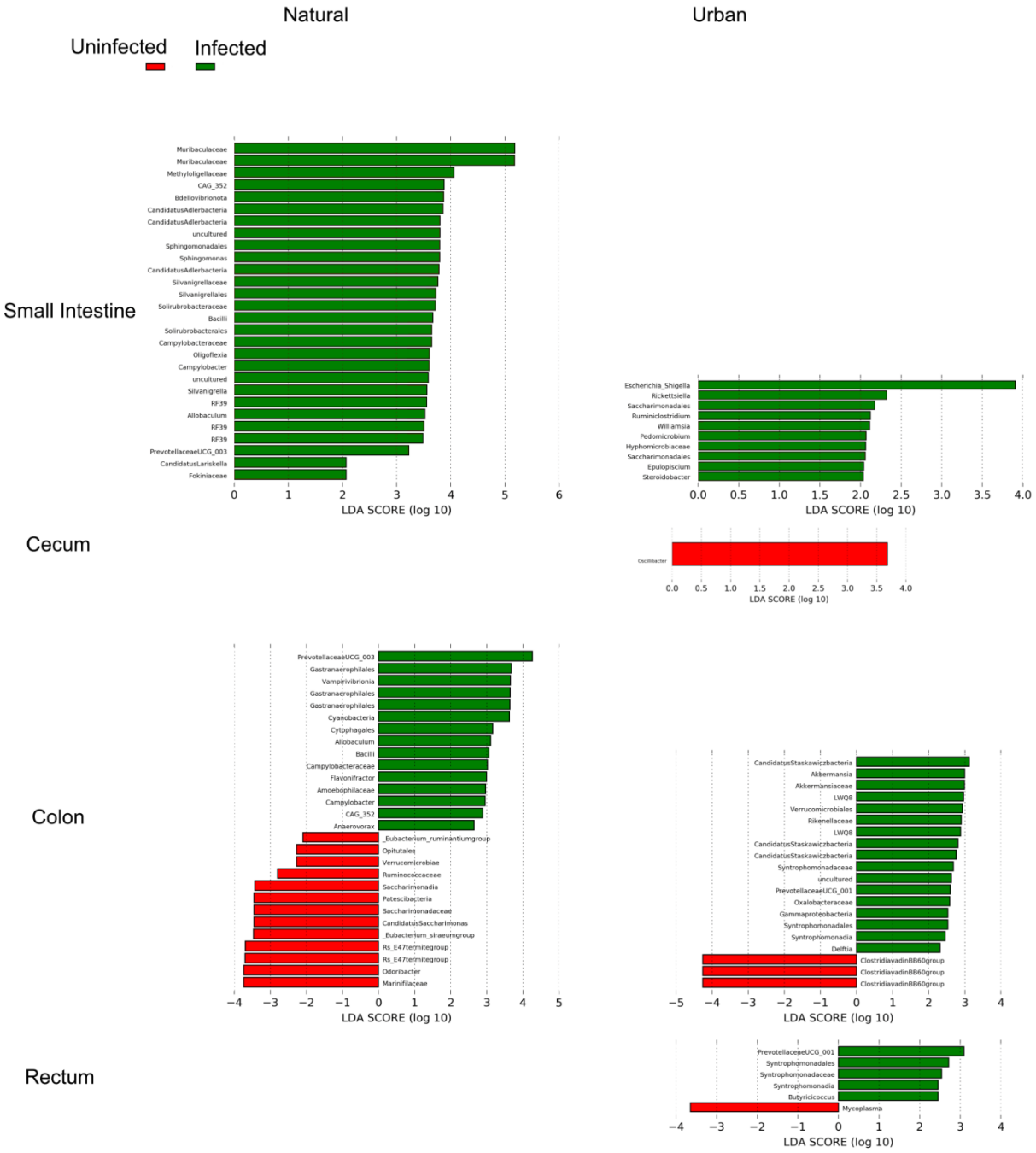


Fig. S8 More abundant microbial taxon along the gastrointestinal tract of *M. rufocanus* infected (green) and uninfected (red) with the helminth *S. montana* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

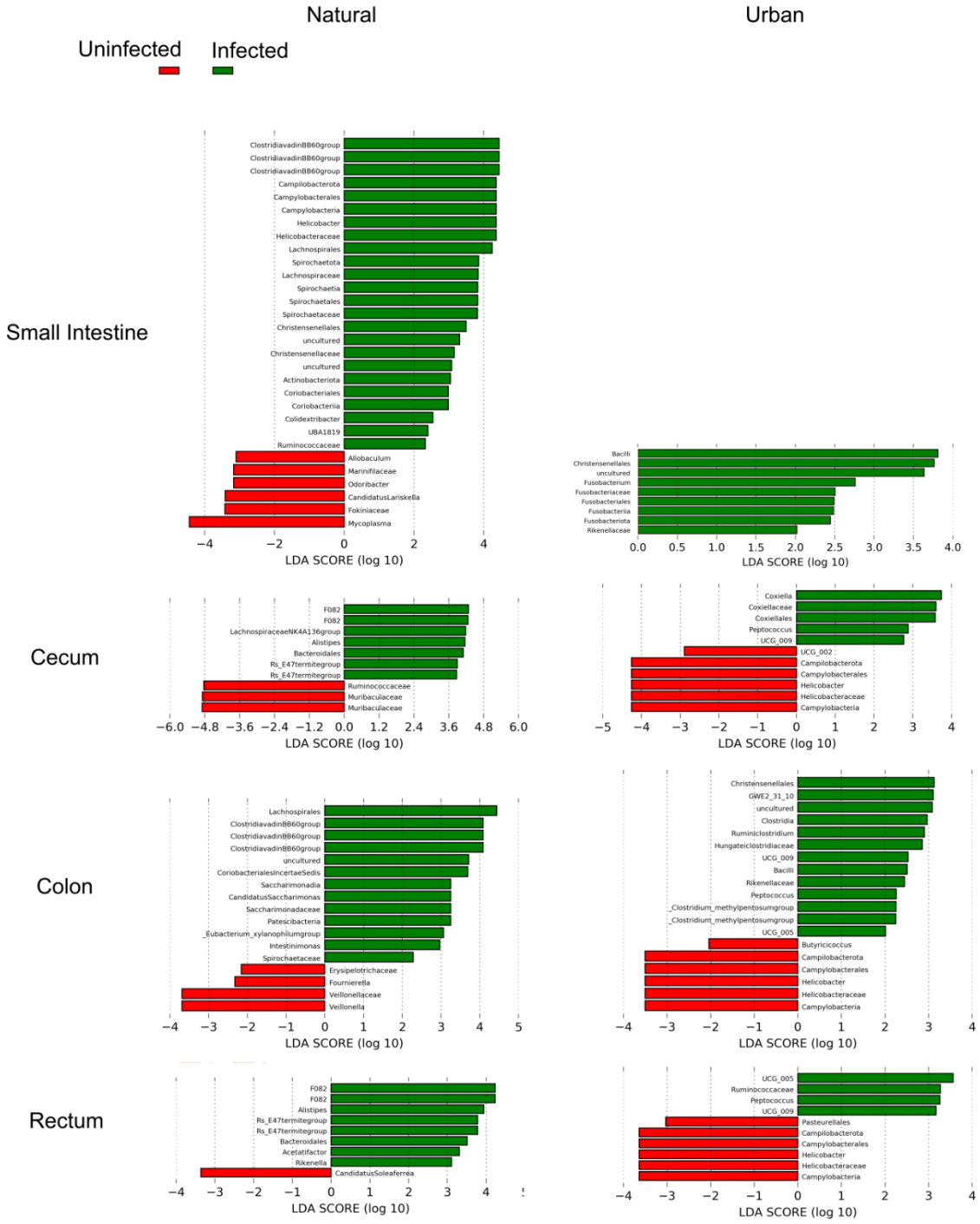


Fig. S9 More abundant microbial taxon along the gastrointestinal tract of *M. rufocanus* infected (green) and uninfected (red) with the helminth *H. spumosa* in the natural (left column) and urban (right column) ecosystems. Results based on LEfSe analysis.

Ecosystem	Site	Latitute	Longitude	*A. speciosus*	*M. rufocanus*
Natural	Chitoseyama	43° 56' 30.64"	142° 24' 10.09"	6	16
	Harushinai	43° 42' 19.82"	142° 12' 08.28"	5	20
	Mukoyama	43° 34' 07.41"	142° 21' 34.57"	11	3
	Shirakkeyama	43° 56' 14.15"	142° 13' 21.56"	20	4
Urban	Kaguraoka koen	43° 44' 52.24"	142° 22' 05.94"	19	13
	Shunkodai koen	43° 48' 45.38"	142° 21' 40.40"	16	29
	Maruyama koen	43° 35' 00.68"	142° 28' 23.54"	6	8
Total				83	93

Table S1 Field sites with capture data for each host species

A. speciosus

Helminth	Natural		Urban	
	Prevalence (%)	**Average (min - max)**	**Prevalence (%)**	**Average (min - max)**
Heterakis spumosa	69.05	21.21 (0 - 299)	56.1	21.2 (0 - 361)
Syphacia agraria	7.14	1.57 (0 - 45)	9.76	2.12 (0 - 58)
Syphacia emileromani	4.76	3.31 (0 - 138)	0	0
Heligmosomoides kurilensis	76.19	7.38 (0 - 41)	9.76	0.29 (0 - 4)
Heligmonoides speciosus	100	274.55 (1 - 1731)	100	156.37 (22 - 578)
Brachylaima asakawai	2.38	0.024 (0 - 1)	0	0
Cestoda spp.	7.14	0.52 (0 - 18)	12.2	0.95 (0 - 22)

M. rufocanus

Helminth	Natural		Urban	
	Prevalence (%)	**Average (min - max)**	**Prevalence (%)**	**Average (min - max)**
Heterakis spumosa	60.47	2.74 (0 - 21)	48	7.96 (0 - 81)
Syphacia montana	9.3	9.6 (0 - 274)	16	2.8 (0 - 47)
Heligmosomum yamagutii	65.12	2.26 (0 - 12)	58	3.92 (0 - 36)
Heligmonoides speciosus	2.32	0.02 (0 - 1)	0	0
Brachylaima asakawai	0	0	2	0.04 (0 - 2)
Cestode spp.	23.26	1.53 (0 - 53)	2	0.02 (0 - 1)

Table S2 Intestinal helminths and their prevalence and abundances in each host species in the natural and urban areas.

Host: *A. speciosus*

Helminth species	Variable	Prevalence Est.	Prevalence SE	Prevalence *p*	Abundance Est.	Abundance SE	Abundance *p*
Heterakis spumosa	Ecosystem (urban)	-0.725	0.543	0.182	0.435	0.486	0.371
	Age	-2.354	0.582	**< 0.001**	-1.724	0.7	**0.014**
	Sex	-0.282	0.549	0.607	-1.514	0.41	**< 0.001**
Syphacia agraria	Ecosystem (urban)	0.401	0.839	0.633	0.415	0.609	0.496
	Age	-17.407	2035.3	0.993	-0.002	–	–
	Sex	1.771	1.12	0.114	6.464	1.248	**< 0.001**
Heligmosomoides kurilensis	Ecosystem (urban)	-3.841	0.754	**< 0.001**	-1.282	0.55	**< 0.001**
	Age	-1.812	0.754	**0.016**	-1.378	0.46	**0.003**
	Sex	0.706	0.69	0.306	-0.126	0.308	0.683
Heligmonoides speciosus	Ecosystem (urban)	–	–	–	-0.502	0.166	**0.002**
	Age	–	–	–	-0.71	0.181	**< 0.001**
	Sex	–	–	–	0.169	0.168	0.313
Cestoda spp.	Ecosystem (urban)	0.291	0.827	0.725	-3.862	1.001	**< 0.001**
	Age	-0.895	1.135	0.431	0.069	1.308	0.958
	Sex	1.813	1.11	0.102	-4.581	1.289	**< 0.001**

Table S3 Comparison of prevalence and abundance of each helminth in natural vs. urban populations of *A. speciosus*. Prevalence was analyzed using a GLM with binomial distribution and abundance using ZINB except for *H. speciosus* where a GLM with negative binomial distribution was used. Bolded values indicate significance.

Host: M. rufocanus

Helminth species	Variable	Prevalence Est.	Prevalence SE	Prevalence *p*	Abundance Est.	Abundance SE	Abundance *p*
Heterakis spumosa	Ecosystem (urban)	-3.506	1.159	**0.002**	0.873	0.32	**0.006**
	Age	-2.086	0.802	**0.009**	-1.177	0.339	< **0.001**
	Sex	-0.61	0.831	0.463	-0.458	0.373	0.22
Syphacia montana	Ecosystem (urban)	0.368	0.679	0.587	-2.194	1.026	**0.032**
	Age	-0.73	0.673	0.278	-0.155	0.87	0.859
	Sex	-1.046	0.843	0.215	-0.848	1.343	0.528
Heligmosomum yamagutii	Ecosystem (urban)	-0.439	0.446	0.324	0.118	0.477	0.805
	Age	-0.594	0.495	0.231	-1.453	0.456	**0.001**
	Sex	-0.191	0.464	0.681	0.144	0.355	0.685
Cestoda spp.	Ecosystem (urban)	-3.506	1.159	**0.002**	-2.995	2.792	0.283
	Age	-2.086	0.802	**0.009**	-3.34	0.552	< **0.001**
	Sex	-0.61	0.831	0.463	3.786	0.344	< **0.001**

Table S4 Comparison of prevalence and abundance of each helminth in natural vs. urban populations of *M. rufocanus*. Prevalence was analyzed using a GLM with binomial distribution and abundance using ZINB. Bolded values indicate significance.

Shannon's diversity

Host: *A. speciosus*

Gut Region	Variable	Prevalence					
		Natural			Urban		
		Est.	**SE**	***p***	**Est.**	**SE**	***p***
Small Intestine	Sex	0.2960	0.1278	**0.029**	-0.0083	0.1481	0.956
	Age	-0.2255	0.1929	0.253	0.2666	0.1768	0.144
	$\delta^{13}C$	-0.0064	0.0958	0.947	0.0384	0.0747	0.611
	$\delta^{15}N$	-0.0415	0.0418	0.330	0.0222	0.0580	0.706
	Cestoda spp.	-0.0907	0.2145	0.676	-0.1791	0.2762	0.523
	H. spumosa	-0.1568	0.1917	0.421	0.2742	0.1608	0.100
	S. agraria	-0.1588	0.2198	0.477	-0.0810	0.3626	0.825
	H. kurilensis	-0.2969	0.1390	**0.042**	0.1382	0.2475	0.581
Cecum	Sex	0.0788	0.0334	**0.033**	0.0720	0.0199	**0.023**
	Age	0.0172	0.0520	0.745	0.1228	0.0292	**0.014**
	$\delta^{13}C$	-0.0203	0.0275	0.472	0.0112	0.0099	0.320
	$\delta^{15}N$	-0.0035	0.0114	0.761	0.0162	0.0080	0.111
	Cestoda spp.	-0.0347	0.0521	0.517	0.0450	0.0593	0.491
	H. spumosa	-0.0249	0.0461	0.598	0.2050	0.0258	**0.001**
	S. agraria	-0.0364	0.0606	0.558	0.0888	0.0365	0.072
	H. kurilensis	0.0502	0.0379	0.206	0.0962	0.0286	**0.028**
Colon	Sex	0.0452	0.0362	0.222	0.0727	0.0260	**0.009**
	Age	0.0243	0.0546	0.660	0.0298	0.0303	0.333
	$\delta^{13}C$	-0.0193	0.0271	0.482	0.0206	0.0161	0.213
	$\delta^{15}N$	0.0072	0.0118	0.547	0.0241	0.0112	**0.040**
	Cestoda spp.	-0.1382	0.0607	**0.031**	-0.0659	0.0473	0.174
	H. spumosa	-0.0783	0.0542	0.161	0.0060	0.0300	0.844
	S. agraria	0.0656	0.0622	0.301	-0.0004	0.0554	0.994
	H. kurilensis	0.0128	0.0393	0.748	0.0530	0.0448	0.247
Rectum	Sex	0.1039	0.0442	**0.037**	0.1353	0.0690	0.107
	Age	0.0827	0.0710	0.267	0.1537	0.1011	0.189
	$\delta^{13}C$	-0.0503	0.0363	0.191	0.1234	0.0342	**0.015**
	$\delta^{15}N$	0.0128	0.0139	0.374	0.0872	0.0276	**0.025**
	Cestoda spp.	0.0136	0.0873	0.879	-0.4304	0.2054	0.090
	H. spumosa	-0.0577	0.0581	0.340	0.1850	0.0894	0.093
	S. agraria	-0.0683	0.0759	0.386	-0.0999	0.1263	0.465
	H. kurilensis	0.0279	0.0500	0.585	0.0521	0.0990	0.622

Table S5 The effect of intestinal helminth prevalence on Shannon's diversity in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

Faith's PD

Host: *A. speciosus*

		Prevalence					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.2058	0.1143	0.083	-0.1016	0.1248	0.423
	Age	-0.3152	0.1715	0.078	-0.0229	0.1488	0.879
	$\delta^{13}C$	-0.0257	0.0916	0.781	-0.0333	0.0633	0.603
	$\delta^{15}N$	-0.0187	0.0372	0.620	-0.0639	0.0490	0.203
	Cestoda spp.	0.1054	0.1895	0.583	0.0706	0.2334	0.765
	H. spumosa	-0.2544	0.1713	0.150	0.2536	0.1362	0.074
	S. agraria	0.1403	0.1998	0.489	0.1892	0.3055	0.541
	H. kurilensis	-0.2543	0.1243	0.051	0.0557	0.2088	0.792
Cecum	Sex	0.0872	0.0430	0.062	0.0059	0.0422	0.895
	Age	-0.0191	0.0644	0.771	0.0669	0.0548	0.289
	$\delta^{13}C$	0.0071	0.0336	0.835	-0.0215	0.0223	0.390
	$\delta^{15}N$	-0.0014	0.0148	0.928	0.0124	0.0163	0.490
	Cestoda spp.	0.0078	0.0683	0.911	0.2609	0.1340	0.123
	H. spumosa	-0.0077	0.0581	0.896	0.2796	0.0745	**0.020**
	S. agraria	-0.0507	0.0790	0.531	0.2447	0.0784	**0.036**
	H. kurilensis	0.0125	0.0486	0.801	0.1986	0.0623	**0.033**
Colon	Sex	0.0693	0.0346	0.056	0.0252	0.0318	0.436
	Age	0.0152	0.0522	0.774	-0.0046	0.0371	0.902
	$\delta^{13}C$	0.0046	0.0259	0.861	0.0080	0.0194	0.685
	$\delta^{15}N$	0.0066	0.0113	0.563	0.0199	0.0136	0.153
	Cestoda spp.	-0.0028	0.0581	0.962	-0.1171	0.0579	0.053
	H. spumosa	-0.0365	0.0519	0.488	0.0595	0.0358	0.108
	S. agraria	0.0485	0.0595	0.423	0.0088	0.0679	0.898
	H. kurilensis	0.0099	0.0376	0.795	0.0354	0.0549	0.525
Rectum	Sex	0.0665	0.0546	0.247	0.1071	0.0643	0.157
	Age	0.0324	0.0882	0.720	0.0668	0.0942	0.510
	$\delta^{13}C$	0.0116	0.0433	0.793	0.0330	0.0318	0.347
	$\delta^{15}N$	0.0013	0.0172	0.941	0.0311	0.0257	0.280
	Cestoda spp.	0.0829	0.1072	0.454	-0.1398	0.1914	0.498
	H. spumosa	-0.0114	0.0714	0.876	0.1832	0.0832	0.079
	S. agraria	-0.0303	0.0942	0.753	0.0483	0.1176	0.698
	H. kurilensis	-0.0038	0.0604	0.951	-0.0188	0.0922	0.846

Table S6 The effect of intestinal helminth prevalence on Faith's PD in natural and urban populations of *A. speciosus* using an on LME. Bolded values indicate significance.

Evenness

Host: *A. speciosus*

Gut Region	Variable	Prevalence					
		Natural			Urban		
		Est.	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.1828	0.1029	0.087	0.0295	0.1038	0.779
	Age	-0.0916	0.1553	0.560	0.2388	0.1239	0.065
	$\delta^{13}C$	-0.0064	0.0771	0.935	0.0450	0.0524	0.398
	$\delta^{15}N$	-0.0314	0.0336	0.359	0.0416	0.0407	0.316
	Cestoda spp.	-0.0322	0.1726	0.853	-0.1862	0.1937	0.345
	H. spumosa	-0.0543	0.1543	0.728	0.1806	0.1128	0.121
	S. agraria	-0.1699	0.1769	0.346	-0.1348	0.2543	0.601
	H. kurilensis	-0.1597	0.1119	0.166	0.1100	0.1735	0.532
Cecum	Sex	0.0374	0.0219	0.110	0.0451	0.0101	**0.011**
	Age	0.0126	0.0339	0.716	0.0959	0.0138	**0.002**
	$\delta^{13}C$	-0.0142	0.0179	0.442	0.0217	0.0052	**0.014**
	$\delta^{15}N$	-0.0021	0.0075	0.784	0.0190	0.0040	**0.009**
	Cestoda spp.	-0.0177	0.0343	0.615	-0.0891	0.0317	**0.048**
	H. spumosa	-0.0199	0.0302	0.520	0.1130	0.0159	**0.002**
	S. agraria	-0.0210	0.0399	0.608	0.0287	0.0188	0.202
	H. kurilensis	0.0344	0.0249	0.189	0.0332	0.0151	0.093
Colon	Sex	0.0182	0.0254	0.479	0.0495	0.0198	**0.019**
	Age	0.0024	0.0384	0.951	0.0204	0.0231	0.386
	$\delta^{13}C$	-0.0124	0.0191	0.519	0.0191	0.0123	0.132
	$\delta^{15}N$	0.0057	0.0083	0.497	0.0181	0.0085	**0.042**
	Cestoda spp.	-0.1241	0.0427	**0.007**	-0.0261	0.0361	0.475
	H. spumosa	-0.0657	0.0382	0.097	0.0032	0.0229	0.891
	S. agraria	0.0601	0.0437	0.181	-0.0027	0.0423	0.950
	H. kurilensis	-0.0003	0.0277	0.992	0.0422	0.0342	0.227
Rectum	Sex	0.0634	0.0274	**0.039**	0.0816	0.0460	0.136
	Age	0.0519	0.0431	0.253	0.1221	0.0673	0.130
	$\delta^{13}C$	-0.0308	0.0244	0.231	0.1124	0.0228	**0.004**
	$\delta^{15}N$	0.0065	0.0085	0.461	0.0747	0.0184	**0.010**
	Cestoda spp.	-0.0004	0.0548	0.995	-0.4056	0.1368	**0.031**
	H. spumosa	-0.0467	0.0365	0.225	0.1459	0.0595	0.058
	S. agraria	-0.0601	0.0463	0.219	-0.1113	0.0841	0.243
	H. kurilensis	0.0119	0.0316	0.713	0.0489	0.0659	0.492

Table S7 The effect of intestinal helminth prevalence on Evenness in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

# of ASVs

Host: *A. speciosus*

Gut Region	Variable	Prevalence					
		Natural			Urban		
		Est.	**SE r**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.4929	0.1807	**0.011**	-0.1920	0.2372	0.426
	Age	-0.5738	0.2728	**0.045**	0.1426	0.2830	0.619
	$\delta^{13}C$	-0.0049	0.1354	0.972	-0.0265	0.1196	0.827
	$\delta^{15}N$	-0.0453	0.0591	0.450	-0.0884	0.0929	0.350
	Cestoda spp.	-0.2544	0.3033	0.409	0.0370	0.4423	0.934
	H. spumosa	-0.4765	0.2711	0.091	0.4820	0.2575	0.073
	S. agraria	0.0321	0.3109	0.919	0.2469	0.5806	0.674
	H. kurilensis	-0.5812	0.1966	**0.007**	0.1233	0.3963	0.758
Cecum	Sex	0.2262	0.0987	**0.038**	0.0765	0.0913	0.449
	Age	0.0429	0.1486	0.777	0.1482	0.1196	0.283
	$\delta^{13}C$	-0.0471	0.0776	0.553	-0.0600	0.0479	0.279
	$\delta^{15}N$	-0.0093	0.0341	0.788	-0.0120	0.0354	0.752
	Cestoda spp.	-0.0806	0.1568	0.615	0.7862	0.2888	0.053
	H. spumosa	-0.0390	0.1338	0.775	0.4262	0.1574	0.054
	S. agraria	-0.0764	0.1815	0.680	0.3913	0.1694	0.082
	H. kurilensis	0.0659	0.1118	0.565	0.4731	0.1350	**0.025**
Colon	Sex	0.1503	0.0744	0.054	0.1322	0.0548	**0.023**
	Age	0.1209	0.1123	0.292	0.0552	0.0639	0.395
	$\delta^{13}C$	-0.0379	0.0557	0.503	0.0016	0.0335	0.961
	$\delta^{15}N$	0.0081	0.0243	0.741	0.0326	0.0234	0.174
	Cestoda spp.	-0.0820	0.1249	0.517	-0.2180	0.0996	**0.037**
	H. spumosa	-0.0702	0.1116	0.535	0.0219	0.0619	0.726
	S. agraria	0.0324	0.1280	0.802	0.0136	0.1167	0.908
	H. kurilensis	0.0727	0.0809	0.378	0.0637	0.0944	0.505
Rectum	Sex	0.2294	0.1016	**0.043**	0.2960	0.1363	0.082
	Age	0.1815	0.1640	0.290	0.1859	0.2001	0.396
	$\delta^{13}C$	-0.0527	0.0806	0.526	0.0495	0.0699	0.510
	$\delta^{15}N$	0.0220	0.0319	0.503	0.0651	0.0549	0.289
	Cestoda spp.	0.0846	0.1993	0.679	-0.0606	0.4220	0.891
	H. spumosa	-0.0430	0.1327	0.751	0.2187	0.1887	0.299
	S. agraria	-0.1065	0.1752	0.555	0.0873	0.2541	0.745
	H. kurilensis	0.0738	0.1123	0.523	0.0431	0.1994	0.838

Table S8 The effect of intestinal helminth prevalence on number of ASVs in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

Shannon's diversity

Host: *A. speciosus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***p***	**Est.**	**SE**	***p***
Small Intestine	Sex	0.4088	0.1401	**0.007**	0.0363	0.1495	0.810
	Age	0.0555	0.1664	0.742	0.3080	0.1642	0.072
	$\delta^{13}C$	-0.0991	0.1273	0.444	0.1264	0.0764	0.111
	$\delta^{15}N$	-0.0639	0.0434	0.153	0.0349	0.0547	0.529
	Cestoda spp.	0.0082	0.0204	0.692	0.0491	0.0306	0.122
	H. spumosa	0.0025	0.0013	0.077	0.0012	0.0012	0.365
	S. agraria	-0.0114	0.0081	0.170	-0.0541	0.0269	0.055
	H. kurilensis	-0.0066	0.0097	0.501	0.0492	0.0682	0.478
	H. speciosus	-0.0001	0.0002	0.764	0.0013	0.0006	**0.041**
Cecum	Sex	0.0636	0.0350	0.092	0.0739	0.0491	0.230
	Age	-0.0175	0.0465	0.712	0.0873	0.0595	0.239
	$\delta^{13}C$	-0.0176	0.0326	0.599	0.0270	0.0250	0.358
	$\delta^{15}N$	0.0001	0.0122	0.997	0.0061	0.0199	0.779
	Cestoda spp.	0.0017	0.0044	0.711	0.0688	0.2503	0.801
	H. spumosa	-0.0003	0.0003	0.287	0.0007	0.0011	0.544
	S. agraria	-0.0021	0.0017	0.241	-0.0049	0.0144	0.754
	H. kurilensis	-0.0055	0.0035	0.139	0.0334	0.0202	0.196
	H. speciosus	-0.0001	0.0001	0.226	0.0002	0.0003	0.591
Colon	Sex	0.0271	0.0364	0.463	0.0534	0.0270	0.058
	Age	-0.0066	0.0442	0.882	0.0048	0.0302	0.875
	$\delta^{13}C$	0.0087	0.0314	0.785	0.0233	0.0145	0.120
	$\delta^{15}N$	0.0050	0.0114	0.663	0.0208	0.0105	0.058
	Cestoda spp.	-0.0103	0.0053	0.064	-0.0058	0.0058	0.321
	H. spumosa	-0.0007	0.0003	0.059	-0.0005	0.0002	0.066
	S. agraria	-0.0011	0.0021	0.615	-0.0003	0.0014	0.827
	H. kurilensis	-0.0056	0.0025	**0.037**	0.0151	0.0130	0.254
	H. speciosus	-0.0001	0.0001	0.163	-0.0001	0.0001	0.618
Rectum	Sex	0.0909	0.0431	0.059	0.1429	0.0809	0.152
	Age	0.0613	0.0605	0.332	0.1221	0.1098	0.329
	$\delta^{13}C$	-0.0209	0.0363	0.577	0.1315	0.0441	**0.041**
	$\delta^{15}N$	0.0157	0.0132	0.260	0.0747	0.0317	0.078
	Cestoda spp.	-0.0184	0.0799	0.823	0.1645	0.4742	0.746
	H. spumosa	-0.0005	0.0004	0.163	-0.0005	0.0018	0.812
	S. agraria	-0.0012	0.0051	0.819	-0.0408	0.0253	0.182
	H. kurilensis	-0.0093	0.0040	**0.040**	0.0328	0.0367	0.423
	H. speciosus	0.0000	0.0001	0.581	0.0003	0.0005	0.546

Table S9 The effect of intestinal helminth abundance on Shannon's diversity in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

Faith's PD

Host: *A. speciosus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.3506	0.1223	**0.008**	-0.0947	0.1252	0.456
	Age	0.0196	0.1435	0.892	0.0224	0.1376	0.872
	$\delta^{13}C$	-0.1563	0.1138	0.182	0.0308	0.0640	0.635
	$\delta^{15}N$	-0.0381	0.0376	0.320	-0.0609	0.0459	0.197
	Cestoda spp.	0.0153	0.0178	0.396	0.0548	0.0256	**0.043**
	H. spumosa	0.0024	0.0012	**0.050**	0.0008	0.0010	0.465
	S. agraria	0.0035	0.0071	0.623	-0.0117	0.0225	0.609
	H. kurilensis	-0.0049	0.0084	0.566	0.0137	0.0571	0.813
	H. speciosus	-0.0001	0.0002	0.514	0.0013	0.0005	**0.015**
Cecum	Sex	0.1144	0.0456	**0.026**	0.0269	0.0893	0.783
	Age	-0.0086	0.0606	0.890	0.0027	0.1084	0.982
	$\delta^{13}C$	-0.0227	0.0424	0.602	0.0006	0.0453	0.990
	$\delta^{15}N$	-0.0105	0.0159	0.520	-0.0064	0.0361	0.871
	Cestoda spp.	0.0032	0.0057	0.582	-0.0061	0.4566	0.990
	H. spumosa	0.0004	0.0004	0.314	0.0009	0.0020	0.688
	S. agraria	-0.0023	0.0022	0.319	0.0119	0.0262	0.680
	H. kurilensis	-0.0018	0.0045	0.705	0.0434	0.0368	0.323
	H. speciosus	0.0000	0.0001	0.712	0.0003	0.0005	0.608
Colon	Sex	0.0876	0.0349	**0.019**	0.0304	0.0388	0.439
	Age	0.0160	0.0425	0.709	-0.0149	0.0434	0.734
	$\delta^{13}C$	-0.0089	0.0302	0.770	0.0065	0.0204	0.752
	$\delta^{15}N$	-0.0038	0.0110	0.729	0.0217	0.0150	0.160
	Cestoda spp.	-0.0003	0.0051	0.957	-0.0003	0.0083	0.970
	H. spumosa	0.0001	0.0003	0.831	0.0002	0.0003	0.542
	S. agraria	-0.0003	0.0020	0.883	-0.0004	0.0020	0.846
	H. kurilensis	-0.0038	0.0024	0.133	0.0147	0.0186	0.435
	H. speciosus	0.0000	0.0001	0.636	0.0000	0.0002	0.885
Rectum	Sex	0.0863	0.0514	0.121	0.0653	0.0591	0.331
	Age	0.0418	0.0727	0.577	0.0540	0.0803	0.539
	$\delta^{13}C$	0.0178	0.0430	0.686	0.0689	0.0345	0.116
	$\delta^{15}N$	0.0013	0.0157	0.934	0.0171	0.0232	0.502
	Cestoda spp.	-0.0365	0.0955	0.710	-0.1582	0.3413	0.667
	H. spumosa	-0.0001	0.0004	0.847	-0.0013	0.0014	0.407
	S. agraria	0.0018	0.0062	0.775	-0.0107	0.0189	0.602
	H. kurilensis	-0.0092	0.0047	0.074	-0.0074	0.0274	0.801
	H. speciosus	0.0001	0.0001	0.224	0.0007	0.0004	0.123

Table S10 The effect of intestinal helminth abundance on Faith's PD in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

Evenness

Host: *A. speciosus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.2460	0.1100	**0.035**	0.0725	0.1064	0.502
	Age	0.0529	0.1307	0.689	0.2586	0.1169	**0.036**
	$\delta^{13}C$	-0.0516	0.0998	0.609	0.1043	0.0544	0.067
	$\delta^{15}N$	-0.0474	0.0341	0.176	0.0528	0.0390	0.187
	Cestoda spp.	0.0050	0.0160	0.758	0.0279	0.0218	0.213
	H. spumosa	0.0015	0.0010	0.173	0.0009	0.0009	0.347
	S. agraria	-0.0089	0.0063	0.175	-0.0464	0.0191	**0.023**
	H. kurilensis	-0.0043	0.0076	0.576	0.0380	0.0485	0.441
	H. speciosus	0.0000	0.0002	0.887	0.0008	0.0004	0.088
Cecum	Sex	0.0233	0.0228	0.325	0.0581	0.0242	0.096
	Age	-0.0158	0.0303	0.612	0.0838	0.0288	0.062
	$\delta^{13}C$	-0.0032	0.0212	0.882	0.0319	0.0123	0.081
	$\delta^{15}N$	0.0011	0.0080	0.891	0.0110	0.0098	0.345
	Cestoda spp.	0.0007	0.0029	0.804	-0.0078	0.1208	0.953
	H. spumosa	-0.0003	0.0002	0.132	0.0006	0.0005	0.336
	S. agraria	-0.0011	0.0011	0.352	-0.0078	0.0071	0.349
	H. kurilensis	-0.0045	0.0023	0.070	0.0128	0.0098	0.281
	H. speciosus	0.0000	0.0000	0.559	0.0001	0.0001	0.414
Colon	Sex	0.0114	0.0268	0.673	0.0343	0.0194	0.088
	Age	-0.0084	0.0321	0.795	-0.0041	0.0217	0.853
	$\delta^{13}C$	-0.0004	0.0239	0.988	0.0230	0.0104	**0.036**
	$\delta^{15}N$	0.0037	0.0083	0.664	0.0148	0.0076	0.060
	Cestoda spp.	-0.0082	0.0039	**0.045**	-0.0038	0.0042	0.368
	H. spumosa	-0.0004	0.0003	0.152	-0.0004	0.0002	**0.017**
	S. agraria	-0.0010	0.0015	0.504	-0.0003	0.0010	0.760
	H. kurilensis	-0.0041	0.0019	**0.036**	0.0076	0.0093	0.423
	H. speciosus	-0.0001	0.0000	0.057	-0.0001	0.0001	0.459
Rectum	Sex	0.0654	0.0303	0.054	0.1022	0.0582	0.154
	Age	0.0552	0.0414	0.209	0.1017	0.0790	0.267
	$\delta^{13}C$	-0.0260	0.0262	0.342	0.1140	0.0320	**0.024**
	$\delta^{15}N$	0.0070	0.0094	0.469	0.0645	0.0228	**0.047**
	Cestoda spp.	-0.0008	0.0564	0.989	0.1615	0.3401	0.660
	H. spumosa	-0.0002	0.0003	0.460	0.0002	0.0013	0.873
	S. agraria	-0.0023	0.0036	0.526	-0.0369	0.0182	0.113
	H. kurilensis	-0.0053	0.0030	0.105	0.0331	0.0265	0.279
	H. speciosus	0.0000	0.0000	0.934	0.0001	0.0004	0.719

Table S11 The effect of intestinal helminth abundance on Pielou's evenness in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

# of ASVs

Host: *A. speciosus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.7046	0.2042	**0.002**	-0.1872	0.2360	0.435
	Age	0.0160	0.2481	0.949	0.2327	0.2592	0.378
	$\delta^{13}C$	-0.1942	0.1765	0.282	0.1033	0.1206	0.400
	$\delta^{15}N$	-0.0751	0.0640	0.252	-0.0849	0.0864	0.336
	Cestoda spp.	0.0132	0.0299	0.663	0.1001	0.0483	**0.049**
	H. spumosa	0.0043	0.0019	**0.035**	0.0014	0.0020	0.472
	S. agraria	-0.0093	0.0116	0.430	-0.0351	0.0425	0.417
	H. kurilensis	-0.0104	0.0142	0.470	0.0460	0.1077	0.673
	H. speciosus	-0.0004	0.0003	0.173	0.0026	0.0010	**0.012**
Cecum	Sex	0.2248	0.1040	**0.050**	0.0815	0.1528	0.631
	Age	-0.0109	0.1384	0.938	0.0233	0.1853	0.908
	$\delta^{13}C$	-0.0803	0.0969	0.422	-0.0208	0.0776	0.806
	$\delta^{15}N$	-0.0088	0.0363	0.812	-0.0266	0.0618	0.696
	Cestoda spp.	0.0049	0.0131	0.712	0.4454	0.7802	0.608
	H. spumosa	-0.0001	0.0009	0.952	0.0005	0.0034	0.886
	S. agraria	-0.0058	0.0051	0.275	0.0133	0.0448	0.786
	H. kurilensis	-0.0049	0.0104	0.644	0.1156	0.0629	0.163
	H. speciosus	-0.0002	0.0001	0.138	0.0002	0.0008	0.801
Colon	Sex	0.0960	0.0719	0.194	0.1084	0.0631	0.097
	Age	0.0185	0.0873	0.834	0.0524	0.0706	0.465
	$\delta^{13}C$	0.0216	0.0621	0.731	-0.0037	0.0333	0.912
	$\delta^{15}N$	0.0064	0.0225	0.779	0.0334	0.0244	0.182
	Cestoda spp.	-0.0101	0.0105	0.347	-0.0108	0.0135	0.431
	H. spumosa	-0.0016	0.0007	**0.030**	-0.0001	0.0006	0.808
	S. agraria	-0.0014	0.0041	0.732	-0.0001	0.0033	0.986
	H. kurilensis	-0.0089	0.0050	0.087	0.0424	0.0302	0.172
	H. speciosus	0.0000	0.0001	0.990	0.0001	0.0003	0.843
Rectum	Sex	0.2099	0.0976	0.055	0.2230	0.1274	0.155
	Age	0.1244	0.1381	0.387	0.1218	0.1726	0.519
	$\delta^{13}C$	-0.0001	0.0816	0.999	0.0923	0.0674	0.243
	$\delta^{15}N$	0.0231	0.0299	0.455	0.0549	0.0499	0.333
	Cestoda spp.	-0.1100	0.1813	0.557	0.0203	0.7509	0.980
	H. spumosa	-0.0010	0.0008	0.260	-0.0037	0.0028	0.262
	S. agraria	0.0040	0.0117	0.737	-0.0201	0.0394	0.638
	H. kurilensis	-0.0172	0.0089	0.079	-0.0010	0.0572	0.987
	H. speciosus	0.0002	0.0001	0.233	0.0011	0.0008	0.236

Table S12 The effect of intestinal helminth abundance on the number of ASVs in natural and urban populations of *A. speciosus* using an LME. Bolded values indicate significance.

Shannon diversity

Host: *M. rufocanus*

		Prevalence					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***p***	**Est.**	**SE**	***p***
Small Intestine	Sex	0.0755	0.1285	0.562	0.0988	0.1145	0.394
	Age	-0.2405	0.2003	0.240	-0.3215	0.1346	**0.022**
	$\delta^{13}C$	-0.1734	0.1030	0.104	-0.0007	0.0774	0.993
	$\delta^{15}N$	0.0183	0.0639	0.777	-0.0115	0.0428	0.790
	Cestoda spp.	-0.1310	0.1893	0.495	–	–	–
	H. spumosa	0.0115	0.1489	0.939	-0.1352	0.1381	0.334
	S. montana	0.2075	0.2357	0.386	0.0271	0.1647	0.870
	H. yamagutii	0.0962	0.1329	0.475	0.0395	0.1103	0.722
Cecum	Sex	0.0019	0.0082	0.854	-0.0527	0.0356	0.190
	Age	-0.0103	0.0107	0.511	0.0258	0.0423	0.564
	$\delta^{13}C$	-0.0119	0.0060	0.300	-0.0964	0.0228	**0.006**
	$\delta^{15}N$	0.0304	0.0027	0.057	0.0434	0.0166	**0.040**
	Cestoda spp.	-0.0196	0.0104	0.309	–	–	–
	H. spumosa	0.0305	0.0079	0.163	0.0956	0.0436	0.071
	S. montana	-0.1567	0.0116	**0.047**	-0.1892	0.0512	**0.010**
	H. yamagutii	0.0308	0.0126	0.247	0.0215	0.0436	0.640
Colon	Sex	-0.0006	0.0218	0.979	-0.0427	0.0242	0.086
	Age	-0.0105	0.0422	0.806	-0.0469	0.0285	0.109
	$\delta^{13}C$	-0.0189	0.0228	0.415	-0.0239	0.0149	0.118
	$\delta^{15}N$	0.0170	0.0107	0.124	0.0002	0.0092	0.980
	Cestoda spp.	-0.0011	0.0379	0.978	–	–	–
	H. spumosa	0.0486	0.0282	0.098	-0.0060	0.0295	0.839
	S. montana	-0.0520	0.0403	0.210	-0.0439	0.0319	0.177
	H. yamagutii	0.0076	0.0232	0.747	-0.0231	0.0223	0.308
Rectum	Sex	0.0283	0.0415	0.566	-0.0747	0.0320	0.067
	Age	0.0082	0.0516	0.889	0.0642	0.0423	0.189
	$\delta^{13}C$	0.0268	0.0242	0.383	-0.0567	0.0231	0.058
	$\delta^{15}N$	0.0191	0.0106	0.213	0.0181	0.0133	0.234
	Cestoda spp.	0.0677	0.0438	0.262	–	–	–
	H. spumosa	0.0129	0.0395	0.775	0.0961	0.0405	0.064
	S. montana	-0.2281	0.0574	0.058	-0.0757	0.0595	0.260
	H. yamagutii	-0.0093	0.0367	0.824	-0.0157	0.0356	0.677

Table S13 The effect of intestinal helminth prevalence on Shannon's diversity in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

Faith's PD

Host: *M. rufocanus*

Gut Region	Variable	Prevalence					
		Natural			Urban		
		Est.	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.0097	0.0978	0.921	0.0004	0.0653	0.995
	Age	-0.0771	0.1525	0.617	-0.0284	0.0767	0.713
	$\delta^{13}C$	-0.0681	0.0784	0.393	-0.0445	0.0451	0.329
	$\delta^{15}N$	0.0083	0.0487	0.866	0.0096	0.0243	0.695
	Cestoda spp.	0.1962	0.1442	0.185	–	–	–
	H. spumosa	0.0900	0.1133	0.434	0.0151	0.0801	0.851
	S. montana	-0.1231	0.1795	0.499	-0.0876	0.0965	0.370
	H. yamagutii	0.1157	0.1012	0.263	0.0991	0.0626	0.122
Cecum	Sex	0.0861	0.0783	0.470	0.0132	0.0538	0.815
	Age	-0.0250	0.1030	0.848	-0.0398	0.0639	0.556
	$\delta^{13}C$	-0.0678	0.0550	0.434	-0.1778	0.0345	**0.002**
	$\delta^{15}N$	0.0669	0.0262	0.238	0.0345	0.0251	0.219
	Cestoda spp.	0.0293	0.0989	0.817	–	–	–
	H. spumosa	0.2010	0.0766	0.232	0.1094	0.0659	0.148
	S. montana	-0.2282	0.1147	0.297	-0.3212	0.0774	**0.006**
	H. yamagutii	0.2131	0.1144	0.314	0.0911	0.0659	0.216
Colon	Sex	-0.0069	0.0288	0.813	-0.0458	0.0373	0.228
	Age	-0.0363	0.0483	0.460	-0.0529	0.0440	0.238
	$\delta^{13}C$	-0.0219	0.0250	0.390	-0.0622	0.0231	**0.011**
	$\delta^{15}N$	0.0414	0.0139	**0.007**	0.0193	0.0142	0.184
	Cestoda spp.	0.0453	0.0484	0.359	–	–	–
	H. spumosa	0.0080	0.0329	0.810	0.0280	0.0455	0.543
	S. montana	-0.0874	0.0530	0.112	-0.0672	0.0492	0.181
	H. yamagutii	0.0446	0.0302	0.153	-0.0018	0.0344	0.958
Rectum	Sex	-0.0320	0.0619	0.657	-0.0185	0.0454	0.700
	Age	0.0194	0.0798	0.831	0.0375	0.0658	0.593
	$\delta^{13}C$	-0.0363	0.0370	0.431	-0.0917	0.0316	**0.034**
	$\delta^{15}N$	0.0632	0.0195	0.084	0.0128	0.0206	0.559
	Cestoda spp.	0.0334	0.0668	0.667	–	–	–
	H. spumosa	0.0006	0.0723	0.994	0.1121	0.0559	0.101
	S. montana	-0.1053	0.0989	0.398	-0.0399	0.0702	0.595
	H. yamagutii	-0.0940	0.0558	0.234	0.0045	0.0524	0.935

Table S14 The effect of intestinal helminth prevalence on Faith's PD in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

Evenness

Host: *M. rufocanus*

		Prevalence					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	0.0936	0.0923	0.320	0.0851	0.0942	0.372
	Age	-0.2151	0.1438	0.146	-0.2609	0.1107	**0.024**
	$\delta^{13}C$	-0.1104	0.0739	0.147	0.0267	0.0637	0.677
	$\delta^{15}N$	0.0029	0.0459	0.950	-0.0160	0.0352	0.653
	Cestoda spp.	-0.1681	0.1360	0.227	–	–	–
	H. spumosa	-0.0330	0.1069	0.760	-0.1562	0.1135	0.177
	S. montana	0.2112	0.1693	0.223	0.0458	0.1354	0.737
	H. yamagutii	0.0473	0.0954	0.624	0.0452	0.0907	0.621
Cecum	Sex	-0.0364	0.0091	0.156	-0.0316	0.0301	0.334
	Age	-0.0133	0.0119	0.465	0.0239	0.0358	0.529
	$\delta^{13}C$	-0.0104	0.0066	0.360	-0.0318	0.0193	0.151
	$\delta^{15}N$	0.0181	0.0030	0.105	0.0328	0.0141	0.059
	Cestoda spp.	-0.0464	0.0115	0.154	–	–	–
	H. spumosa	-0.0311	0.0088	0.176	0.0670	0.0369	0.119
	S. montana	-0.0940	0.0130	0.087	-0.0478	0.0433	0.312
	H. yamagutii	-0.0233	0.0138	0.339	-0.0295	0.0369	0.455
Colon	Sex	0.0006	0.0147	0.970	-0.0256	0.0189	0.185
	Age	-0.0013	0.0294	0.964	-0.0337	0.0223	0.141
	$\delta^{13}C$	-0.0146	0.0161	0.376	-0.0102	0.0117	0.388
	$\delta^{15}N$	0.0047	0.0072	0.526	-0.0040	0.0072	0.587
	Cestoda spp.	-0.0187	0.0259	0.477	–	–	–
	H. spumosa	0.0495	0.0195	**0.018**	-0.0141	0.0231	0.546
	S. montana	-0.0179	0.0272	0.517	-0.0257	0.0250	0.310
	H. yamagutii	-0.0018	0.0156	0.908	-0.0150	0.0175	0.397
Rectum	Sex	0.0353	0.0254	0.299	-0.0398	0.0213	0.120
	Age	0.0093	0.0312	0.795	0.0433	0.0280	0.183
	$\delta^{13}C$	0.0346	0.0146	0.142	-0.0222	0.0154	0.208
	$\delta^{15}N$	0.0029	0.0063	0.690	0.0161	0.0088	0.129
	Cestoda spp.	0.0354	0.0266	0.314	–	–	–
	H. spumosa	0.0276	0.0235	0.361	0.0733	0.0270	**0.042**
	S. montana	-0.1504	0.0344	**0.049**	-0.0469	0.0396	0.290
	H. yamagutii	0.0042	0.0223	0.867	-0.0301	0.0236	0.259

Table S15 The effect of intestinal helminth prevalence on Pielou's evenness in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

# of ASVs

Host: *M. rufocanus*

Gut Region	Variable	Prevalence					
		Natural			Urban		
		Est.	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	-0.0650	0.2194	0.769	0.0611	0.1272	0.634
	Age	-0.1772	0.3418	0.608	-0.3268	0.1494	**0.035**
	$\delta^{13}C$	-0.3184	0.1758	0.081	-0.1489	0.0883	0.100
	$\delta^{15}N$	0.0817	0.1091	0.460	0.0292	0.0474	0.542
	Cestoda spp.	0.1793	0.3232	0.584	–	–	–
	H. spumosa	0.1792	0.2541	0.487	0.0934	0.1568	0.555
	S. montana	-0.0666	0.4024	0.870	-0.0852	0.1891	0.655
	H. yamagutii	0.2487	0.2268	0.282	-0.0423	0.1220	0.731
Cecum	Sex	0.2227	0.0033	**0.009**	-0.1326	0.0838	0.165
	Age	0.0112	0.0043	0.233	0.0022	0.0995	0.983
	$\delta^{13}C$	-0.0122	0.0024	0.125	-0.3917	0.0537	**< 0.001**
	$\delta^{15}N$	0.0744	0.0011	**0.009**	0.0643	0.0392	0.152
	Cestoda spp.	0.1433	0.0041	**0.018**	–	–	–
	H. spumosa	0.3591	0.0032	**0.006**	0.1620	0.1026	0.166
	S. montana	-0.3704	0.0046	**0.008**	-0.8629	0.1205	**< 0.001**
	H. yamagutii	0.3180	0.0050	**0.010**	0.3125	0.1027	**0.023**
Colon	Sex	-0.0081	0.0619	0.897	-0.1039	0.0520	0.054
	Age	-0.0531	0.1038	0.613	-0.0810	0.0613	0.196
	$\delta^{13}C$	-0.0225	0.0537	0.679	-0.0848	0.0322	**0.013**
	$\delta^{15}N$	0.0782	0.0299	**0.015**	0.0257	0.0198	0.204
	Cestoda spp.	0.1248	0.1040	0.242	–	–	–
	H. spumosa	-0.0255	0.0707	0.721	0.0488	0.0635	0.447
	S. montana	-0.2070	0.1138	0.081	-0.1108	0.0686	0.116
	H. yamagutii	0.0701	0.0649	0.291	-0.0498	0.0480	0.307
Rectum	Sex	-0.0456	0.1018	0.698	-0.2050	0.0839	0.058
	Age	0.0313	0.1294	0.831	0.1320	0.1174	0.312
	$\delta^{13}C$	-0.0377	0.0607	0.598	-0.2084	0.0591	**0.017**
	$\delta^{15}N$	0.0971	0.0280	**0.074**	0.0098	0.0368	0.800
	Cestoda spp.	0.1807	0.1092	0.240	–	–	–
	H. spumosa	-0.0876	0.1043	0.490	0.1640	0.1043	0.177
	S. montana	-0.4604	0.1481	**0.090**	-0.1194	0.1430	0.442
	H. yamagutii	-0.0992	0.0913	0.391	0.0623	0.0960	0.545

Table S16 The effect of intestinal helminth prevalence on the number of ASVs in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

Shannon diversity

Host: *M. rufocanus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***p***	**Est.**	**SE**	***p***
Small Intestine	Sex	0.0663	0.1296	0.613	0.0889	0.1147	0.443
	Age	-0.1894	0.1938	0.337	-0.2855	0.1177	**0.020**
	$\delta^{13}C$	-0.1713	0.1086	0.126	-0.0247	0.0732	0.738
	$\delta^{15}N$	0.0183	0.0707	0.797	-0.0021	0.0382	0.955
	Cestoda spp.	-0.0064	0.1170	0.957	–	–	–
	H. spumosa	0.0003	0.0236	0.991	-0.0046	0.0060	0.447
	S. montana	0.0009	0.0021	0.664	-0.0018	0.0053	0.736
	H. yamagutii	-0.0001	0.0309	0.998	0.0064	0.0128	0.617
Cecum	Sex	-0.0142	0.0071	0.296	-0.0622	0.0415	0.184
	Age	-0.0068	0.0106	0.637	-0.0257	0.0389	0.534
	$\delta^{13}C$	-0.0335	0.0116	0.212	-0.0421	0.0207	0.088
	$\delta^{15}N$	0.0333	0.0043	0.082	0.0341	0.0204	0.147
	Cestoda spp.	-0.0445	0.0134	0.186	–	–	–
	H. spumosa	0.0045	0.0025	0.321	0.0012	0.0017	0.504
	S. montana	-0.0009	0.0002	0.120	-0.0050	0.0016	**0.020**
	H. yamagutii	0.0035	0.0021	0.343	-0.0007	0.0040	0.875
Colon	Sex	-0.0035	0.0242	0.887	-0.0372	0.0236	0.125
	Age	-0.0469	0.0343	0.185	-0.0311	0.0226	0.178
	$\delta^{13}C$	-0.0175	0.0176	0.330	-0.0196	0.0141	0.176
	$\delta^{15}N$	0.0226	0.0128	0.089	0.0024	0.0080	0.762
	Cestoda spp.	0.0012	0.0019	0.527	–	–	–
	H. spumosa	-0.0009	0.0037	0.806	0.0005	0.0011	0.686
	S. montana	-0.0003	0.0004	0.373	-0.0017	0.0010	0.099
	H. yamagutii	-0.0020	0.0055	0.716	-0.0006	0.0024	0.818
Rectum	Sex	0.0230	0.0249	0.453	-0.1114	0.0151	**0.001**
	Age	-0.0303	0.0312	0.435	0.0049	0.0138	0.738
	$\delta^{13}C$	0.0566	0.0246	0.148	-0.0411	0.0069	**0.002**
	$\delta^{15}N$	0.0040	0.0095	0.719	0.0106	0.0068	0.183
	Cestoda spp.	0.0552	0.0239	0.147	–	–	–
	H. spumosa	-0.0075	0.0067	0.378	0.0009	0.0005	0.156
	S. montana	-0.0005	0.0005	0.436	-0.0047	0.0005	< **0.001**
	H. yamagutii	0.0089	0.0051	0.223	-0.0022	0.0013	0.148

Table S17 The effect of intestinal helminth abundance on Shannon's diversity in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

Faith's PD

Host: *M. rufocanus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**SE**	***P***	**Est.**	**SE**	***P***
Small Intestine	Sex	-0.0110	0.0915	0.906	-0.0089	0.0642	0.891
	Age	-0.2924	0.1500	0.062	-0.0797	0.0691	0.256
	$\delta^{13}C$	-0.0074	0.0906	0.936	-0.0274	0.0421	0.518
	$\delta^{15}N$	-0.0490	0.0513	0.348	-0.0038	0.0219	0.864
	Cestoda spp.	0.0529	0.0848	0.539	–	–	–
	H. spumosa	-0.0293	0.0178	0.111	-0.0052	0.0034	0.130
	S. montana	0.0009	0.0015	0.544	-0.0009	0.0032	0.785
	H. yamagutii	0.0586	0.0233	**0.018**	0.0119	0.0072	0.105
Cecum	Sex	-0.0088	0.0333	0.835	0.0456	0.0627	0.495
	Age	0.0026	0.0489	0.966	-0.1373	0.0524	**0.040**
	$\delta^{13}C$	-0.1883	0.0500	0.165	-0.1045	0.0309	**0.015**
	$\delta^{15}N$	0.0794	0.0193	0.152	0.0320	0.0294	0.318
	Cestoda spp.	-0.2077	0.0589	0.176	–	–	–
	H. spumosa	0.0263	0.0108	0.249	0.0016	0.0022	0.482
	S. montana	-0.0029	0.0008	0.165	-0.0067	0.0025	**0.039**
	H. yamagutii	0.0413	0.0095	0.144	0.0012	0.0052	0.824
Colon	Sex	-0.0224	0.0291	0.449	-0.0412	0.0349	0.246
	Age	-0.0710	0.0412	0.098	-0.0575	0.0334	0.094
	$\delta^{13}C$	-0.0258	0.0211	0.234	-0.0510	0.0209	**0.020**
	$\delta^{15}N$	0.0411	0.0153	**0.013**	0.0160	0.0118	0.183
	Cestoda spp.	-0.0009	0.0023	0.699	–	–	–
	H. spumosa	-0.0036	0.0044	0.425	0.0021	0.0017	0.210
	S. montana	-0.0006	0.0004	0.210	-0.0024	0.0015	0.107
	H. yamagutii	0.0144	0.0065	**0.037**	-0.0029	0.0036	0.431
Rectum	Sex	-0.0388	0.0607	0.588	-0.0362	0.0404	0.411
	Age	-0.0657	0.0790	0.493	-0.0290	0.0390	0.491
	$\delta^{13}C$	-0.0097	0.0543	0.875	-0.0784	0.0189	**0.009**
	$\delta^{15}N$	0.0419	0.0239	0.222	0.0116	0.0187	0.561
	Cestoda spp.	0.0481	0.0580	0.494	–	–	–
	H. spumosa	-0.0116	0.0160	0.542	0.0011	0.0016	0.516
	S. montana	0.0003	0.0012	0.822	-0.0033	0.0014	0.066
	H. yamagutii	0.0019	0.0126	0.892	-0.0002	0.0037	0.966

Table S18 The effect of intestinal helminth abundance on Faith's PD in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

Evenness

Host: *M. rufocanus*

		Abundance					
		Natural			Urban		
Gut Region	**Variable**	**Est.**	**Std. Error**	***P***	**Est.**	**Std. Error**	***P***
Small Intestine	Sex	0.0917	0.0942	0.339	0.0796	0.0955	0.410
	Age	-0.1141	0.1408	0.425	-0.2171	0.0983	**0.033**
	$\delta^{13}C$	-0.1119	0.0789	0.168	-0.0026	0.0611	0.966
	$\delta^{15}N$	0.0218	0.0514	0.674	-0.0074	0.0318	0.817
	Cestoda spp.	-0.0100	0.0850	0.907	–	–	–
	H. spumosa	0.0033	0.0171	0.849	-0.0043	0.0050	0.388
	S. montana	0.0007	0.0015	0.647	-0.0003	0.0045	0.947
	H. yamagutii	-0.0179	0.0224	0.431	0.0055	0.0106	0.607
Cecum	Sex	-0.0243	0.0006	**0.017**	-0.0443	0.0272	0.155
	Age	-0.0166	0.0009	**0.036**	0.0053	0.0266	0.849
	$\delta^{13}C$	0.0242	0.0010	**0.028**	-0.0139	0.0137	0.349
	$\delta^{15}N$	0.0109	0.0004	**0.022**	0.0340	0.0137	**0.048**
	Cestoda spp.	0.0190	0.0012	**0.040**	–	–	–
	H. spumosa	-0.0085	0.0002	**0.017**	0.0016	0.0012	0.221
	S. montana	0.0002	0.0000	**0.050**	-0.0024	0.0010	0.060
	H. yamagutii	-0.0066	0.0002	**0.018**	-0.0028	0.0028	0.350
Colon	Sex	0.0028	0.0173	0.872	-0.0204	0.0187	0.281
	Age	-0.0239	0.0261	0.370	-0.0192	0.0179	0.291
	$\delta^{13}C$	-0.0055	0.0150	0.715	-0.0084	0.0112	0.456
	$\delta^{15}N$	0.0077	0.0092	0.410	-0.0019	0.0063	0.763
	Cestoda spp.	0.0005	0.0014	0.704	–	–	–
	H. spumosa	0.0002	0.0027	0.940	0.0002	0.0009	0.851
	S. montana	-0.0002	0.0003	0.405	-0.0009	0.0008	0.264
	H. yamagutii	-0.0028	0.0041	0.503	-0.0004	0.0019	0.833
Rectum	Sex	0.0183	0.0199	0.455	-0.0709	0.0094	**0.001**
	Age	-0.0194	0.0251	0.520	0.0044	0.0086	0.636
	$\delta^{13}C$	0.0343	0.0195	0.221	-0.0111	0.0043	**0.050**
	$\delta^{15}N$	-0.0040	0.0077	0.654	0.0112	0.0043	**0.047**
	Cestoda spp.	0.0090	0.0191	0.682	–	–	–
	H. spumosa	-0.0017	0.0053	0.775	0.0009	0.0003	**0.049**
	S. montana	-0.0005	0.0004	0.336	-0.0035	0.0003	**0.000**
	H. yamagutii	0.0054	0.0041	0.315	-0.0029	0.0008	**0.016**

Table S19 The effect of intestinal helminth abundance on Pielou's evenness in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

# of ASVs

Host: *M. rufocanus*

Gut Region	Variable	Abundance					
		Natural			Urban		
		Est.	Std. Error	*P*	Est.	Std. Error	*P*
Small Intestine	Sex	-0.1027	0.2163	0.639	0.0345	0.1246	0.784
	Age	-0.4148	0.3234	0.211	-0.3605	0.1286	**0.008**
	$\delta^{13}C$	-0.3104	0.1812	0.098	-0.1304	0.0797	0.110
	$\delta^{15}N$	-0.0107	0.1180	0.928	0.0319	0.0416	0.448
	Cestoda spp.	0.0044	0.1952	0.982	–	–	–
	H. spumosa	-0.0163	0.0393	0.682	-0.0017	0.0065	0.796
	S. montana	0.0011	0.0036	0.766	-0.0083	0.0058	0.161
	H. yamagutii	0.0877	0.0515	0.100	0.0051	0.0139	0.718
Cecum	Sex	0.0621	0.0380	0.349	-0.1070	0.1546	0.515
	Age	0.0475	0.0560	0.552	-0.2927	0.1207	0.052
	$\delta^{13}C$	-0.3155	0.0595	0.119	-0.2464	0.0755	**0.017**
	$\delta^{15}N$	0.1268	0.0225	0.112	0.0075	0.0706	0.919
	Cestoda spp.	-0.3529	0.0693	0.123	–	–	–
	H. spumosa	0.0716	0.0128	0.112	-0.0025	0.0050	0.636
	S. montana	-0.0062	0.0009	0.091	-0.0189	0.0065	**0.027**
	H. yamagutii	0.0578	0.0109	0.119	0.0107	0.0117	0.397
Colon	Sex	-0.0414	0.0636	0.522	-0.1014	0.0497	**0.049**
	Age	-0.1263	0.0902	0.175	-0.0728	0.0475	0.135
	$\delta^{13}C$	-0.0400	0.0462	0.395	-0.0689	0.0298	**0.027**
	$\delta^{15}N$	0.0814	0.0335	**0.023**	0.0267	0.0168	0.121
	Cestoda spp.	-0.0002	0.0050	0.969	–	–	–
	H. spumosa	-0.0101	0.0097	0.304	0.0018	0.0024	0.460
	S. montana	-0.0009	0.0009	0.346	-0.0048	0.0021	**0.028**
	H. yamagutii	0.0232	0.0143	0.118	-0.0010	0.0051	0.853
Rectum	Sex	0.0296	0.0326	0.460	-0.2329	0.0592	**0.011**
	Age	-0.0687	0.0407	0.234	0.0006	0.0572	0.992
	$\delta^{13}C$	0.1282	0.0326	0.059	-0.1847	0.0277	**0.001**
	$\delta^{15}N$	0.0487	0.0125	0.060	0.0014	0.0274	0.963
	Cestoda spp.	0.2732	0.0313	**0.013**	–	–	–
	H. spumosa	-0.0336	0.0088	0.062	0.0003	0.0023	0.902
	S. montana	0.0001	0.0006	0.939	-0.0068	0.0021	**0.021**
	H. yamagutii	0.0206	0.0067	0.091	0.0037	0.0054	0.524

Table S20 The effect of intestinal helminth abundance the number of ASVs in natural and urban populations of *M. rufocanus* using an LME. Bolded values indicate significance.

Host: *A. speciosus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.2021	0.0421	1.6928	**0.038**	0.0986	0.0467	1.1846	0.169
	Age	0.1450	0.0302	1.2141	0.181	0.0838	0.0398	1.0076	0.428
	$\delta^{13}C$	0.1426	0.0297	1.1944	0.197	0.0705	0.0335	0.8478	0.747
	$\delta^{15}N$	0.0905	0.0188	0.7575	0.823	0.0742	0.0352	0.8917	0.651
	Cestoda spp.	0.1523	0.0317	1.2758	0.166	0.0737	0.0349	0.8852	0.660
	H. spumosa	0.1701	0.0355	1.4248	0.093	0.1069	0.0507	1.2848	0.127
	S. agraria	0.1337	0.0279	1.1199	0.288	0.0692	0.0328	0.8311	0.720
	H. kurilensis	0.2297	0.0479	1.9235	**0.024**	0.1016	0.0482	1.2211	0.152
weighted unifrac	Sex	0.1984	0.0586	2.3116	**0.037**	0.0525	0.0338	0.9014	0.462
	Age	0.0916	0.0270	1.0671	0.377	0.0793	0.0511	1.3626	0.206
	$\delta^{13}C$	0.0676	0.0199	0.7872	0.551	0.0635	0.0409	1.0912	0.332
	$\delta^{15}N$	0.0580	0.0171	0.6763	0.653	0.0409	0.0263	0.7023	0.673
	Cestoda spp.	0.0738	0.0218	0.8596	0.489	0.0537	0.0346	0.9222	0.445
	H. spumosa	0.0896	0.0264	1.0435	0.405	0.1002	0.0645	1.7210	0.104
	S. agraria	0.0368	0.0109	0.4290	0.873	0.0557	0.0358	0.9565	0.397
	H. kurilensis	0.2274	0.0671	2.6501	**0.020**	0.0951	0.0612	1.6332	0.105

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	**p**	**SS**	**R2**	**F**	**p**
unweighted unifrac	Sex	0.0874	0.0268	1.0273	0.402	0.1098	0.0544	1.2596	0.119
	Age	0.0871	0.0267	1.0242	0.392	0.0853	0.0423	0.9790	0.464
	$\delta^{13}C$	0.1133	0.0347	1.3324	0.060	0.1093	0.0542	1.2544	0.117
	$\delta^{15}N$	0.0882	0.0270	1.0368	0.377	0.0798	0.0396	0.9159	0.613
	Cestoda spp.	0.0755	0.0231	0.8874	0.629	0.0750	0.0372	0.8603	0.674
	H. spumosa	0.0856	0.0262	1.0063	0.452	0.0865	0.0429	0.9925	0.450
	S. agraria	0.0573	0.0176	0.6738	0.973	0.0594	0.0295	0.6816	0.915
	H. kurilensis	0.1014	0.0311	1.1919	0.169	0.0916	0.0454	1.0511	0.331
weighted unifrac	Sex	0.0797	0.0296	1.2265	0.255	0.1354	0.0852	2.2233	**0.024**
	Age	0.1143	0.0424	1.7579	0.079	0.1028	0.0647	1.6880	0.103
	$\delta^{13}C$	0.0759	0.0282	1.1677	0.265	0.0646	0.0407	1.0613	0.361
	$\delta^{15}N$	0.0895	0.0332	1.3768	0.181	0.0927	0.0583	1.5219	0.168
	Cestoda spp.	0.1264	0.0469	1.9435	0.067	0.0632	0.0398	1.0376	0.376
	H. spumosa	0.0307	0.0114	0.4724	0.916	0.0515	0.0324	0.8459	0.553
	S. agraria	0.0636	0.0236	0.9785	0.430	0.0380	0.0239	0.6236	0.743
	H. kurilensis	0.1676	0.0622	2.5781	**0.018**	0.1101	0.0693	1.8083	0.082

Table S21 The effect of intestinal helminth prevalence on beta-diversity in natural populations of *A. speciosus* using an PERMANOVA. Bolded values indicate significance.

Host: *A. speciosus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.1306	0.0269	0.9863	0.422	0.0779	0.0520	1.0313	0.374
	Age	0.1397	0.0288	1.0552	0.336	0.0579	0.0387	0.7666	0.791
	$\delta^{13}C$	0.1064	0.0219	0.8038	0.686	0.1356	0.0905	1.7934	**0.029**
	$\delta^{15}N$	0.1360	0.0280	1.0268	0.372	0.0753	0.0503	0.9960	0.463
	Cestoda spp.	0.0743	0.0153	0.5613	0.977	0.1468	0.0980	1.9425	**0.020**
	H. spumosa	0.2918	0.0601	2.2041	**0.014**	0.1764	0.1177	2.3335	**0.001**
	S. agraria	0.1273	0.0262	0.9613	0.459	0.0930	0.0621	1.2308	0.208
	H. kurilensis	0.1146	0.0236	0.8656	0.568	0.1683	0.1124	2.2266	**0.005**
weighted unifrac	Sex	0.0841	0.0209	0.7464	0.553	0.0473	0.0548	1.1151	0.360
	Age	0.1766	0.0438	1.5679	0.162	0.0267	0.0310	0.6302	0.727
	$\delta^{13}C$	0.0942	0.0234	0.8366	0.493	0.0776	0.0899	1.8282	0.092
	$\delta^{15}N$	0.1263	0.0313	1.1212	0.291	0.1275	0.1478	3.0046	**0.008**
	Cestoda spp.	0.0888	0.0221	0.7887	0.525	0.0745	0.0864	1.7561	0.117
	H. spumosa	0.3044	0.0755	2.7021	**0.029**	0.0633	0.0734	1.4922	0.174
	S. agraria	0.0773	0.0192	0.6861	0.616	0.0515	0.0597	1.2129	0.311
	H. kurilensis	0.0911	0.0226	0.8088	0.505	0.0824	0.0955	1.9409	0.073

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.0985	0.0290	1.1909	0.178	0.1096	0.0697	1.3220	0.115
	Age	0.0995	0.0293	1.2027	0.172	0.0711	0.0452	0.8574	0.696
	$\delta^{13}C$	0.1554	0.0457	1.8789	**0.004**	0.1245	0.0792	1.5027	**0.070**
	$\delta^{15}N$	0.1015	0.0299	1.2272	0.159	0.0974	0.0620	1.1756	0.219
	Cestoda spp.	0.0793	0.0233	0.9581	0.492	0.1542	0.0981	1.8610	**0.015**
	H. spumosa	0.1349	0.0397	1.6314	**0.025**	0.1475	0.0938	1.7793	**0.019**
	S. agraria	0.0944	0.0278	1.1412	0.268	0.0894	0.0568	1.0782	0.325
	H. kurilensis	0.1434	0.0422	1.7334	**0.019**	0.1943	0.1236	2.3440	**0.003**
weighted unifrac	Sex	0.0956	0.0352	1.4729	0.154	0.0404	0.0394	0.8062	0.585
	Age	0.0813	0.0299	1.2528	0.227	0.0346	0.0338	0.6904	0.697
	$\delta^{13}C$	0.1439	0.0529	2.2160	**0.023**	0.1671	0.1630	3.3325	**0.011**
	$\delta^{15}N$	0.0892	0.0328	1.3735	0.161	0.1755	0.1713	3.5017	**0.003**
	Cestoda spp.	0.0478	0.0176	0.7361	0.671	0.1304	0.1272	2.6008	**0.041**
	H. spumosa	0.1310	0.0482	2.0177	**0.038**	0.0904	0.0882	1.8034	0.100
	S. agraria	0.0664	0.0244	1.0222	0.371	0.0338	0.0330	0.6749	0.687
	H. kurilensis	0.0997	0.0367	1.5364	0.113	0.0660	0.0644	1.3156	0.203

Table S22 The effect of intestinal helminth prevalence on beta-diversity in urban populations of *A. speciosus* using an PERMANOVA. Bolded values indicate significance.

Host: *A. speciosus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.3181	0.0663	2.6814	**0.002**	0.1023	0.0485	1.2836	0.111
	Age	0.0762	0.0159	0.6426	0.932	0.0663	0.0314	0.8320	0.749
	$\delta^{13}C$	0.1486	0.0310	1.2529	0.173	0.0719	0.0341	0.9028	0.599
	$\delta^{15}N$	0.1129	0.0235	0.9514	0.498	0.0889	0.0422	1.1159	0.259
	Cestoda spp.	0.1101	0.0230	0.9285	0.484	0.0699	0.0331	0.8769	0.600
	H. spumosa	0.1962	0.0409	1.6540	**0.035**	0.1112	0.0527	1.3955	0.080
	S. agraria	0.2597	0.0541	2.1893	**0.017**	0.0851	0.0403	1.0676	0.325
	H. kurilensis	0.1021	0.0213	0.8603	0.612	0.1402	0.0665	1.7588	**0.011**
	H. speciosus	0.1543	0.0321	1.3006	0.141	0.1147	0.0544	1.4395	0.080
weighted unifrac	Sex	0.2867	0.0846	3.2117	**0.004**	0.0553	0.0356	1.1268	0.317
	Age	0.0593	0.0175	0.6647	0.653	0.0697	0.0448	1.4196	0.194
	$\delta^{13}C$	0.0782	0.0231	0.8761	0.510	0.0370	0.0238	0.7532	0.615
	$\delta^{15}N$	0.1143	0.0337	1.2804	0.263	0.0533	0.0343	1.0860	0.336
	Cestoda spp.	0.0292	0.0086	0.3276	0.857	0.0247	0.0159	0.5032	0.773
	H. spumosa	0.1217	0.0359	1.3633	0.225	0.1588	0.1022	3.2359	**0.016**
	S. agraria	0.0802	0.0237	0.8988	0.420	0.0660	0.0424	1.3443	0.261
	H. kurilensis	0.1022	0.0302	1.1450	0.323	0.0791	0.0509	1.6130	0.124
	H. speciosus	0.1124	0.0332	1.2587	0.262	0.0706	0.0454	1.4381	0.189

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.0853	0.0261	1.0320	0.388	0.1105	0.0548	1.3618	0.082
	Age	0.0842	0.0258	1.0182	0.419	0.0765	0.0379	0.9427	0.574
	$\delta^{13}C$	0.0983	0.0301	1.1890	0.186	0.0892	0.0442	1.0988	0.289
	$\delta^{15}N$	0.1088	0.0333	1.3157	0.082	0.0952	0.0472	1.1734	0.196
	Cestoda spp.	0.0641	0.0196	0.7748	0.813	0.0764	0.0379	0.9418	0.559
	H. spumosa	0.1282	0.0393	1.5499	**0.023**	0.1223	0.0607	1.5075	**0.044**
	S. agraria	0.0865	0.0265	1.0465	0.343	0.0693	0.0343	0.8535	0.680
	H. kurilensis	0.0804	0.0247	0.9729	0.513	0.1317	0.0653	1.6224	**0.030**
	H. speciosus	0.1115	0.0342	1.3480	0.074	0.0981	0.0486	1.2082	0.197
weighted unifrac	Sex	0.0380	0.0141	0.6507	0.727	0.0920	0.0579	1.6875	0.092
	Age	0.0792	0.0294	1.3561	0.213	0.0664	0.0418	1.2175	0.254
	$\delta^{13}C$	0.1355	0.0503	2.3192	**0.022**	0.0253	0.0159	0.4648	0.925
	$\delta^{15}N$	0.1467	0.0545	2.5117	**0.024**	0.0711	0.0448	1.3048	0.222
	Cestoda spp.	0.0651	0.0242	1.1141	0.330	0.0263	0.0165	0.4816	0.868
	H. spumosa	0.2580	0.0958	4.4152	**0.001**	0.0833	0.0524	1.5285	0.161
	S. agraria	0.0923	0.0342	1.5789	0.120	0.0305	0.0192	0.5598	0.802
	H. kurilensis	0.0589	0.0219	1.0084	0.398	0.1206	0.0759	2.2132	**0.033**
	H. speciosus	0.1362	0.0506	2.3306	**0.034**	0.0595	0.0375	1.0922	0.347

Table S23 The effect of intestinal helminth abundance on beta-diversity in natural populations of *A. speciosus* using an PERMANOVA. Bolded values indicate significance.

Host: *A. speciosus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.1183	0.0244	0.9572	0.432	0.0797	0.0532	0.8017	0.738
	Age	0.1526	0.0314	1.2347	0.193	0.0838	0.0559	0.8430	0.675
	$\delta^{13}C$	0.1309	0.0270	1.0595	0.332	0.1381	0.0922	1.3897	0.101
	$\delta^{15}N$	0.1403	0.0289	1.1349	0.272	0.0642	0.0428	0.6455	0.933
	Cestoda spp.	0.2216	0.0456	1.7929	**0.020**	0.0776	0.0518	0.7804	0.739
	H. spumosa	0.1485	0.0306	1.2013	0.225	0.0790	0.0528	0.7950	0.758
	S. agraria	0.1627	0.0335	1.3166	0.137	0.0812	0.0542	0.8167	0.705
	H. kurilensis	0.1283	0.0264	1.0380	0.341	0.1502	0.1003	1.5108	0.090
	H. speciosus	0.3418	0.0704	2.7658	**0.003**	0.0494	0.0330	0.4971	0.993
weighted unifrac	Sex	0.0455	0.0113	0.4421	0.832	0.0374	0.0434	0.6520	0.708
	Age	0.2080	0.0516	2.0195	0.092	0.0314	0.0364	0.5463	0.808
	$\delta^{13}C$	0.1491	0.0370	1.4472	0.207	0.0778	0.0901	1.3544	0.242
	$\delta^{15}N$	0.1528	0.0379	1.4836	0.169	0.0865	0.1003	1.5068	0.171
	Cestoda spp.	0.0799	0.0198	0.7760	0.504	0.0213	0.0247	0.3712	0.942
	H. spumosa	0.3538	0.0878	3.4352	**0.015**	0.0457	0.0530	0.7958	0.613
	S. agraria	0.1882	0.0467	1.8268	0.129	0.0186	0.0215	0.3235	0.964
	H. kurilensis	0.1809	0.0449	1.7567	0.122	0.0632	0.0733	1.1012	0.339
	H. speciosus	0.1666	0.0414	1.6179	0.140	0.0151	0.0175	0.2629	0.980

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.0892	0.0262	1.0842	0.302	0.1086	0.0691	1.1274	0.307
	Age	0.0978	0.0288	1.1890	0.201	0.0910	0.0579	0.9452	0.526
	$\delta^{13}C$	0.1345	0.0396	1.6346	**0.017**	0.1235	0.0786	1.2821	0.154
	$\delta^{15}N$	0.0826	0.0243	1.0037	0.435	0.0880	0.0560	0.9138	0.588
	Cestoda spp.	0.0626	0.0184	0.7604	0.784	0.0881	0.0560	0.9147	0.612
	H. spumosa	0.1345	0.0396	1.6345	**0.046**	0.0965	0.0614	1.0018	0.446
	S. agraria	0.1030	0.0303	1.2512	0.175	0.0789	0.0502	0.8188	0.747
	H. kurilensis	0.1950	0.0573	2.3690	**0.003**	0.1812	0.1153	1.8817	**0.014**
	H. speciosus	0.0763	0.0225	0.9277	0.576	0.0690	0.0439	0.7160	0.883
weighted unifrac	Sex	0.0480	0.0177	0.7610	0.684	0.0290	0.0283	0.5109	0.867
	Age	0.0860	0.0316	1.3621	0.184	0.0441	0.0430	0.7773	0.613
	$\delta^{13}C$	0.1636	0.0602	2.5915	**0.012**	0.1493	0.1456	2.6299	**0.036**
	$\delta^{15}N$	0.0544	0.0200	0.8616	0.548	0.1131	0.1103	1.9921	0.084
	Cestoda spp.	0.0736	0.0271	1.1668	0.305	0.0433	0.0423	0.7635	0.615
	H. spumosa	0.1715	0.0631	2.7173	**0.012**	0.0847	0.0826	1.4920	0.147
	S. agraria	0.0646	0.0238	1.0232	0.372	0.0281	0.0274	0.4951	0.857
	H. kurilensis	0.1011	0.0372	1.6022	0.118	0.0526	0.0513	0.9261	0.467
	H. speciosus	0.0581	0.0214	0.9210	0.518	0.0334	0.0326	0.5889	0.785

Table S24 The effect of intestinal helminth abundance on beta-diversity in urban populations of *A. speciosus* using an PERMANOVA. Bolded values indicate significance.

Host: *M. rufocanus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.1028	0.0204	0.8297	0.675	0.0772	0.0668	0.8422	0.727
	Age	0.1337	0.0266	1.0791	0.324	0.1050	0.0909	1.1460	0.231
	$\delta^{13}C$	0.1656	0.0329	1.3371	0.115	0.1167	0.1011	1.2736	0.119
	$\delta^{15}N$	0.1560	0.0310	1.2598	0.180	0.0928	0.0804	1.0128	0.398
	Cestoda spp.	0.1434	0.0285	1.1580	0.271	0.0887	0.0768	0.9675	0.497
	H. spumosa	0.1588	0.0316	1.2820	0.162	0.0784	0.0679	0.8559	0.705
	S. montana	0.1378	0.0274	1.1125	0.275	0.0778	0.0674	0.8490	0.735
	H. yamagutii	0.1513	0.0301	1.2218	0.182	0.0959	0.0831	1.0469	0.349
weighted unifrac	Sex	0.1942	0.0270	1.1347	0.291	0.0300	0.0526	0.6588	0.729
	Age	0.1298	0.0180	0.7584	0.498	0.0242	0.0425	0.5320	0.854
	$\delta^{13}C$	0.4986	0.0693	2.9129	**0.035**	0.0528	0.0925	1.1582	0.323
	$\delta^{15}N$	0.0283	0.0039	0.1654	0.983	0.0251	0.0440	0.5504	0.846
	Cestoda spp.	0.2158	0.0300	1.2604	0.260	0.0960	0.1683	2.1071	0.066
	H. spumosa	0.0723	0.0100	0.4224	0.769	0.0487	0.0854	1.0690	0.396
	S. montana	0.4409	0.0613	2.5754	0.064	0.0583	0.1022	1.2801	0.278
	H. yamagutii	0.1892	0.0263	1.1054	0.290	0.0491	0.0861	1.0776	0.414

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.0711	0.0229	0.8447	0.784	0.0972	0.0833	1.1585	0.229
	Age	0.0814	0.0262	0.9663	0.506	0.0831	0.0712	0.9906	0.465
	$\delta^{13}C$	0.1118	0.0361	1.3280	0.083	0.1003	0.0859	1.1954	0.196
	$\delta^{15}N$	0.1157	0.0373	1.3738	0.071	0.1002	0.0858	1.1942	0.222
	Cestoda spp.	0.0686	0.0221	0.8151	0.798	0.0983	0.0842	1.1715	0.221
	H. spumosa	0.1186	0.0382	1.4079	0.052	0.0686	0.0587	0.8172	0.764
	S. montana	0.1124	0.0363	1.3352	0.070	0.0719	0.0615	0.8564	0.699
	H. yamagutii	0.0875	0.0282	1.0392	0.362	0.0994	0.0851	1.1844	0.234
weighted unifrac	Sex	0.0523	0.0295	1.0811	0.325	0.0523	0.0295	1.0811	0.325
	Age	0.0192	0.0108	0.3963	0.954	0.0192	0.0108	0.3963	0.954
	$\delta^{13}C$	0.0208	0.0117	0.4292	0.951	0.0208	0.0117	0.4292	0.951
	$\delta^{15}N$	0.0620	0.0350	1.2811	0.217	0.0620	0.0350	1.2811	0.217
	Cestoda spp.	0.0484	0.0273	1.0009	0.409	0.0484	0.0273	1.0009	0.409
	H. spumosa	0.0391	0.0221	0.8088	0.537	0.0391	0.0221	0.8088	0.537
	S. montana	0.1665	0.0939	3.4417	**0.012**	0.1665	0.0939	3.4417	**0.012**
	H. yamagutii	0.0642	0.0362	1.3275	0.224	0.0642	0.0362	1.3275	0.224

Table S25 The effect of intestinal helminth prevalence on beta-diversity in natural populations of *M. rufocanus* using an PERMANOVA. Bolded values indicate significance.

Host: *M. rufocanus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.1456	0.0268	1.3212	0.100	0.0875	0.0616	1.1589	0.246
	Age	0.1273	0.0234	1.1551	0.233	0.0746	0.0525	0.9879	0.429
	$\delta^{13}C$	0.2466	0.0454	2.2385	**0.001**	0.2005	0.1411	2.6558	**0.001**
	$\delta^{15}N$	0.0665	0.0123	0.6039	0.980	0.0833	0.0586	1.1033	0.281
	H. spumosa	0.1082	0.0199	0.9818	0.465	0.0981	0.0690	1.2996	0.145
	S. montana	0.1093	0.0201	0.9920	0.453	0.1078	0.0759	1.4280	0.090
	H. yamagutii	0.1413	0.0260	1.2828	0.123	0.0849	0.0597	1.1242	0.253
weighted unifrac	Sex	0.1439	0.0193	0.9043	0.427	0.0483	0.0768	1.6661	0.109
	Age	0.4347	0.0583	2.7312	**0.041**	0.0577	0.0917	1.9892	0.071
	$\delta^{13}C$	0.0869	0.0116	0.5458	0.699	0.0660	0.1050	2.2767	**0.040**
	$\delta^{15}N$	0.0617	0.0083	0.3878	0.874	0.0623	0.0991	2.1485	**0.042**
	H. spumosa	0.2058	0.0276	1.2928	0.264	0.0311	0.0494	1.0716	0.375
	S. montana	0.0958	0.0128	0.6021	0.684	0.0251	0.0400	0.8663	0.548
	H. yamagutii	0.1400	0.0188	0.8794	0.432	0.0520	0.0827	1.7939	0.100

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.1296	0.0335	1.5661	**0.013**	0.0975	0.0740	1.2881	0.105
	Age	0.0976	0.0252	1.1787	0.149	0.0724	0.0549	0.9559	0.495
	$\delta^{13}C$	0.1856	0.0480	2.2423	**0.002**	0.1820	0.1381	2.4027	**0.001**
	$\delta^{15}N$	0.0764	0.0198	0.9230	0.609	0.0798	0.0605	1.0532	0.352
	H. spumosa	0.1108	0.0287	1.3391	**0.050**	0.1019	0.0774	1.3458	0.130
	S. montana	0.0745	0.0193	0.9002	0.667	0.0850	0.0645	1.1218	0.234
	H. yamagutii	0.0829	0.0215	1.0022	0.456	0.0734	0.0557	0.9689	0.466
weighted unifrac	Sex	0.0379	0.0204	0.9304	0.450	0.1136	0.1718	3.3847	**0.020**
	Age	0.0464	0.0250	1.1395	0.302	0.0323	0.0489	0.9630	0.413
	$\delta^{13}C$	0.0839	0.0452	2.0592	**0.042**	0.0667	0.1009	1.9872	0.109
	$\delta^{15}N$	0.0352	0.0190	0.8650	0.503	0.0371	0.0561	1.1048	0.340
	H. spumosa	0.0757	0.0408	1.8585	0.063	0.0369	0.0558	1.0994	0.347
	S. montana	0.0209	0.0113	0.5141	0.870	0.0339	0.0513	1.0099	0.382
	H. yamagutii	0.0437	0.0235	1.0728	0.334	0.0912	0.1379	2.7166	**0.047**

Table S26 The effect of intestinal helminth prevalence on beta-diversity in urban populations of *M. rufocanus* using an PERMANOVA. Bolded values indicate significance.

Host: *M. rufocanus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.0929	0.0185	0.7247	0.841	0.0725	0.0628	0.8254	0.764
	Age	0.2247	0.0447	1.7525	**0.022**	0.0927	0.0803	1.0556	0.345
	$\delta^{13}C$	0.1866	0.0371	1.4549	0.079	0.1150	0.0996	1.3094	0.104
	$\delta^{15}N$	0.1675	0.0333	1.3059	0.138	0.0960	0.0831	1.0924	0.287
	Cestoda spp.	0.0991	0.0197	0.7728	0.786	0.0846	0.0732	0.9627	0.479
	H. spumosa	0.1107	0.0220	0.8636	0.650	0.0889	0.0770	1.0124	0.446
	S. montana	0.1085	0.0216	0.8463	0.640	0.0921	0.0798	1.0487	0.364
	H. yamagutii	0.1438	0.0286	1.1218	0.295	0.1016	0.0880	1.1567	0.207
weighted unifrac	Sex	0.1999	0.0278	1.1151	0.304	0.0262	0.0459	0.5760	0.828
	Age	0.3012	0.0419	1.6801	0.186	0.0198	0.0347	0.4351	0.919
	$\delta^{13}C$	0.4069	0.0565	2.2698	0.082	0.0332	0.0582	0.7299	0.688
	$\delta^{15}N$	0.0527	0.0073	0.2941	0.903	0.0246	0.0431	0.5406	0.838
	Cestode spp.	0.0727	0.0101	0.4057	0.814	0.0248	0.0434	0.5448	0.842
	H. spumosa	0.0497	0.0069	0.2772	0.934	0.0535	0.0938	1.1765	0.307
	S. montana	0.0512	0.0071	0.2855	0.919	0.0725	0.1272	1.5952	0.144
	H. yamagutii	0.1429	0.0199	0.7970	0.459	0.0886	0.1554	1.9484	0.073

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.0680	0.0219	0.7887	0.878	0.0801	0.0686	0.9095	0.555
	Age	0.1115	0.0360	1.2945	0.086	0.0723	0.0619	0.8202	0.710
	$\delta^{13}C$	0.1171	0.0378	1.3591	0.060	0.0872	0.0747	0.9896	0.437
	$\delta^{15}N$	0.1011	0.0326	1.1735	0.196	0.0906	0.0776	1.0288	0.370
	Cestoda spp.	0.0807	0.0260	0.9367	0.577	0.0770	0.0659	0.8741	0.622
	H. spumosa	0.0994	0.0320	1.1532	0.196	0.0635	0.0544	0.7210	0.916
	S. montana	0.0921	0.0297	1.0685	0.318	0.0625	0.0535	0.7092	0.913
	H. yamagutii	0.0890	0.0287	1.0335	0.380	0.0908	0.0777	1.0304	0.391
weighted unifrac	Sex	0.0462	0.0261	0.8396	0.501	0.0462	0.0261	0.8396	0.501
	Age	0.0513	0.0289	0.9318	0.416	0.0513	0.0289	0.9318	0.416
	$\delta^{13}C$	0.0211	0.0119	0.3830	0.961	0.0211	0.0119	0.3830	0.961
	$\delta^{15}N$	0.0327	0.0185	0.5946	0.804	0.0327	0.0185	0.5946	0.804
	Cestoda spp.	0.0202	0.0114	0.3677	0.960	0.0202	0.0114	0.3677	0.960
	H. spumosa	0.0456	0.0257	0.8282	0.504	0.0456	0.0257	0.8282	0.504
	S. montana	0.0179	0.0101	0.3250	0.975	0.0179	0.0101	0.3250	0.975
	H. yamagutii	0.0230	0.0130	0.4181	0.939	0.0230	0.0130	0.4181	0.939

Table S27 The effect of intestinal helminth abundance on beta-diversity in natural populations of *M. rufocanus* using an PERMANOVA. Bolded values indicate significance.

Host: *M. rufocanus*		Small Intestine				Cecum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.1454	0.0268	1.3164	0.104	0.0881	0.0620	1.1661	0.215
	Age	0.1630	0.0300	1.4753	**0.050**	0.1050	0.0739	1.3900	0.096
	$\delta^{13}C$	0.2847	0.0524	2.5765	**0.001**	0.2271	0.1598	3.0062	**0.001**
	$\delta^{15}N$	0.0827	0.0152	0.7489	0.856	0.0997	0.0701	1.3198	0.133
	H. spumosa	0.1095	0.0202	0.9915	0.441	0.0840	0.0591	1.1118	0.256
	S. montana	0.1041	0.0192	0.9425	0.509	0.1209	0.0851	1.6007	**0.046**
	H. yamagutii	0.0976	0.0180	0.8838	0.634	0.0736	0.0518	0.9749	0.433
weighted unifrac	Sex	0.1288	0.0173	0.7827	0.526	0.0577	0.0918	2.1309	**0.049**
	Age	0.3514	0.0471	2.1349	0.088	0.0746	0.1187	2.7546	**0.016**
	$\delta^{13}C$	0.0716	0.0096	0.4350	0.833	0.0861	0.1370	3.1804	**0.009**
	$\delta^{15}N$	0.0911	0.0122	0.5533	0.733	0.1136	0.1807	4.1956	**0.002**
	H. spumosa	0.0895	0.0120	0.5438	0.729	0.0660	0.1050	2.4374	**0.032**
	S. montana	0.1136	0.0152	0.6902	0.627	0.0439	0.0698	1.6194	0.163
	H. yamagutii	0.0685	0.0092	0.4164	0.833	0.0524	0.0833	1.9337	0.089

		Colon				Rectum			
Dissimilarity Index	**Variable**	**SS**	**R2**	**F**	***p***	**SS**	**R2**	**F**	***p***
unweighted unifrac	Sex	0.146	0.038	1.769	**0.003**	0.095	0.072	1.244	0.171
	Age	0.120	0.031	1.446	**0.032**	0.107	0.081	1.397	0.082
	$\delta^{13}C$	0.219	0.057	2.640	**0.001**	0.258	0.196	3.381	**0.001**
	$\delta^{15}N$	0.097	0.025	1.174	0.159	0.081	0.061	1.062	0.321
	H. spumosa	0.080	0.021	0.963	0.527	0.060	0.046	0.789	0.793
	S. montana	0.098	0.025	1.183	0.203	0.121	0.092	1.584	0.086
	H. yamagutii	0.084	0.022	1.018	0.391	0.065	0.050	0.855	0.629
weighted unifrac	Sex	0.047	0.025	1.128	0.302	0.127	0.192	4.340	**0.013**
	Age	0.040	0.022	0.972	0.394	0.064	0.096	2.177	0.071
	$\delta^{13}C$	0.088	0.047	2.120	**0.042**	0.086	0.131	2.947	**0.037**
	$\delta^{15}N$	0.061	0.033	1.461	0.148	0.104	0.158	3.554	**0.019**
	H. spumosa	0.034	0.018	0.817	0.525	0.108	0.163	3.674	**0.018**
	S. montana	0.035	0.019	0.853	0.503	0.037	0.056	1.254	0.235
	H. yamagutii	0.040	0.022	0.967	0.404	0.099	0.149	3.364	**0.021**

Table S28 The effect of intestinal helminth abundance on beta-diversity in urban populations of *M. rufocanus* using an PERMANOVA. Bolded values indicate significance.

Host: *A. speciosus*

	H. kurilensis				**Cestoda spp.**			
	Natural		**Urban**		**Natural**		**Urban**	
Gut region	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
Small intestine	8	3	8	1	0	9	0	0
Cecum	1	3	1	5	2	1	0	0
Colon	0	0	4	8	2	6	0	4
Rectum	2	0	0	4	1	0	0	0
	S. agraria				***H. spumosa***			
	Natural		**Urban**		**Natural**		**Urban**	
	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
Small intestine	2	0	0	6	10	3	2	13
Cecum	0	0	1	0	6	5	2	11
Colon	0	3	1	5	5	7	4	5
Rectum	0	0	0	4	7	1	2	6

Table S29 The number of genera with significantly higher abundance in each gut region associated with infection (Inf.) or lack of infection (Uninf.) of each helminth species in *A. speciosus*.

Host: *M. rufocanus*

	H. yamagutii				**Cestoda spp.**			
	Natural		**Urban**		**Natural**		**Urban**	
Gut region	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
Small intestine	1	3	3	3	6	0	–	–
Cecum	7	0	1	0	1	2	–	–
Colon	2	2	0	0	0	1	–	–
Rectum	6	1	2	1	1	6	–	–
	S. montana				***H. spumosa***			
	Natural		**Urban**		**Natural**		**Urban**	
	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
Small intestine	0	5	0	7	3	4	0	1
Cecum	0	0	1	0	0	1	2	3
Colon	1	5	0	3	2	1	2	5
Rectum	0	0	1	2	0	3	2	3

Table S30 The number of genera with significantly higher abundance in each gut region associated with infection (Inf.) or lack of infection (Uninf.) of each helminth species in *M. rufocanus*.

Chapter 5

General discussion

The aim of this thesis was to investigate the differential response to urbanization of the gut microbiota in two sympatric species of rodents and what factors may underlie the observed changes. First, I found that the small intestine harbored a unique microbiota as compared to the cecum, colon, and rectum in *Apodemus speciosus*, *A. argenteus*, and *Myodes rufocanus*. Among species, the largest differences were found between *M. rufocanus* and *Apodemus spp.*, but the gut microbiota was not identical between *A. speciosus* and *A. argenteus*, thereby confirming that phylosymbiosis is occurring. Second, both *A. speciosus* and *M. rufocanus* are experiencing an expanded dietary niche within the urban environment as compared to conspecifics in the natural ecosystem with a larger degree of expansion for the omnivorous *A. speciosus*. Furthermore, each species of rodent exhibited a shift in dietary preference within the urban parks in agreement with their life histories as the herbivorous *M. rufocanus* were consuming more C3 plants while *A. speciosus* were eating more terrestrial animals. This change in diet was associated with host species-specific alterations in the gut microbial community but could not explain all differences in the urban populations as compared to the natural ecosystem. Third, while potential interactions between intestinal helminths and the gut microbiota were identified, association were not consistent across ecosystem type. Specifically, the gut region in which associations were found or the microbial genera involved were different. Despite this, infection with the helminth *Heterakis spumosa* was associated with an increase in microbial alpha diversity in both host species in the natural and urban areas. It may even lower the abundance of the potentially pathogenic *Helicobacter* in the lower gastrointestinal tract (GIT) of urban *M. rufocanus*. This thesis highlighted the importance of investigating the gut microbiota of multiple animal species living within the same urban environment to provide a deeper understanding of what factors allow certain species to successfully adapt.

Importance of investigating the gut microbiota of multiple species in the same urban environments

Interest in how urbanization impacts the gut microbiota of wildlife has seen a recent and rapid surge (Fuirst et al., 2018; Littleford-Colquhoun et al., 2019; M. H. Murray et al., 2020; Phillips et al., 2018; Sugden et al., 2020; Teyssier et al., 2018, 2020) because it is essential for maintaining health and may aid in the successful adaption of animals to urban environments (Hauffe & Barelli, 2019). However, previous studies have only focused on a single animal species and were conducted in different cities spread across three continents, thereby making them difficult to compare. In chapter 2, I demonstrated that three sympatric species of rodents (i.e. *Apodemus speciosus*, *A. argenteus*, and *Myodes rufocanus*) in Hokkaido, Japan harbor their own unique microbial community within the GIT. Microbes, like animals, are unlikely to respond to ecosystem modification in a similar fashion due to species-specific characteristics. Therefore, how the gut microbiota of wildlife responds to urbanization will depend upon the microbial species harbored by the host and how they respond directly to urbanization, as well as how host species-specific life history traits are impacted, thereby modifying the environment within the gut and which microbes can persist. This complex host-microbiota-environment interaction is likely to affect how each animal species and their associated microbiota respond to ecosystem modification, even when faced with the same environmental pressures within a single urban city. By investigating the dietary habits, gut microbiota, and intestinal helminths in two sympatric species of rodents, I was able to characterizes species-specific responses to the same degree of urbanization.

Species-specific response to urbanization

In chapter 3 I found that *A. speciosus* and *M. rufocanus* as well as their associated gut microbes are differentially responding to urbanization and that *A. speciosus* may be a better urban adapter. The larger dietary niche expansion with less overlap in isotopic niche space between natural and urban populations of *A. speciosus* as compared to *M. rufocanus* suggests the field mouse more readily utilizes novel food resources. This is not surprising as *A. speciosus* is more omnivorous (Tatsukawa & Murakami, 1976), and therefore, is less choosey in the food items it consumes whether it be artificial feeding or invasive plants and insects. This likely

allows *A. speciosus* to be more competitive in urban forest fragments where dispersal is limited (Sato et al., 2014).

Urban *A. speciosus* may also be maintaining a healthier gut microbiota as higher abundance of several probiotic microbial genera (i.e. *Lactobacillus*, *Butyricicoccaceae*, and *Bifidobacterium*) were found throughout the GIT as compared to natural conspecifics. These genera are known to decrease bowel inflammation (Liu et al., 2010), help regulate immune system function (Sekirov & Finlay, 2009), and defend against pathogens (Boesmans et al., 2018), thereby potentially helping this species to adapt and remain healthy in the face of negative environmental impacts associated with the extreme ecosystem modification of cities.

M. rufocanus on the other hand may be experiencing dysbiosis. For example, there was a degree of inter-individual convergence in the gut microbiota of the small intestine within the urban parks. It is plausible that factors associated with urbanization such as pollution, stress, or a poor diet are negatively impacting immune system function of *M. rufocanus*, thereby shaping the microbial community of the small intestine where immune activity is typically highest (Bowcutt et al., 2014). Furthermore, *Helicobacter* was found in higher abundance within the lower GIT of urban individuals. *M. rufocanus* may simply be more susceptible to infection with *Helicobacter* or it could be a non-pathogenic species as higher abundance was found in the small intestine of individuals from the natural areas as compared to the urban parks. However, species found in the upper and lower GITs are usually different with most being pathogenic and associated with dysbiosis (Chin et al., 2000; Schulz et al., 2015). If urban *M. rufocanus* are experiencing a dysbiotic gut microbiota it could be a public health concern as it may leave them more susceptible to infection with zoonotic diseases. For instance, about 25% prevalence of *Echinococcus multilocularis* has been found in *M. rufocanus* from one of the urban parks (i.e. Shunkodai koen); an extremely high infection rate considering no infected individuals were found in the national forest and prevalence is typically less than 1% in Hokkaido (unpublished data). Although humans can only be infected by the helminth eggs deposited in the feces of the final host such as foxes (Saitoh & Takahashi, 1998), *M. rufocanus* is serving as a reservoir for this zoonotic parasite that is of increasing concern for public health throughout the northern hemisphere (Liccioli et al., 2015)..

Many questions still remain regarding how these animals and their associated gut microbiota are adapting to the urban environment. For example, in this thesis I found that the populations sizes of *A. speciosus* and *M. rufocanus* are inversely related at each of the sites within the national forest, but they are more even in number within the urban parks. It's been found that different species of animals inhabiting the same habitat patches share a portion of their gut microbiota (Moeller et al., 2017). Future studies should investigate if the altered rodent community structure within the urban parks increases inter-specific interactions, thereby increasing the chance of sharing microbes through direct transmission. Urbanization also impacts the soil microbiota (Bray & Wickings, 2019; McGuire et al., 2013), thereby influencing which microbial species the animals come into contact with and become established in the gut as they explore and forage for food. Finally, *M. rufocanus* is more timid than *A. speciosus* (personal observation), therefore, noise and light pollution in the urban areas may have a larger impact on the stress levels of the vole (Isaksson, 2015). Each species may also be differentially affected by chemical pollution from urban run-off (Isaksson, 2015). Both chemical pollutants and stress induce changes in the gut microbial community by altering host immune system function (Gao et al., 2018; Mutlu et al., 2018; Rosenfeld, 2017). Future studies should investigate species-specific immune response to urbanization and how it impacts the gut microbiota by measuring cortisol levels as well as immunoglobulin A, natural killer cells, neutrophils, and other immunological factors.

Heterakis spumosa: the gut microbiota protector?

It may be possible to cure or prevent dysbiosis in wild rodents effected by human activities through the use of intestinal helminths as a management tool. In chapter 4, I found that infection with *H. spumosa* was associated with higher alpha diversity of the gut microbiota in both *A. speciosus* and *M. rufocanus* and may reduce *Helicobacter* abundance in the lower GIT of urban *M. rufocanus*. Therefore, *H. spumosa* may help maintain gut homeostasis and improve the health of these rodents within human modified environments. Importantly, *H. spumosa* is a cosmopolitan helminth that is known to parasitize numerous rodent species such as *Rattus spp.* and *Mus musculus* (Pakdeenarong et al., 2014; Pakdel et al., 2013), *Apodemus spp.* (Dwużnik et al., 2017), and *Mycromys minutus* (Kim et al., 2015) among others. Because rodents are

ubiquitous throughout human modified environments and are known to carry numerous zoonotic diseases (Meerburg et al., 2009), it's imperative that management practices aim to maintain a healthy gut microbiota in these animals to improve their defense against pathogens and reduce their transmission to humans. *H. spumosa* could potentially be used as a management tool to help a wide range of rodent species maintain a healthy gut microbiota in modified ecosystem, although further research is needed. Future studies should determine the causality of such a relationship as well is if a similar trend can be found in the other host species.

Concluding remarks

The conversion of land into urbanized cities is increasing at a rapid rate that is unlikely to abate during the next several decades (Güneralp et al., 2020; Seto et al., 2012). Understanding how this extreme form of ecosystem modification affects wildlife is of critical importance not only for the preservation of biodiversity, but also from a public health perspective as human-wildlife conflicts can lead to the transmission of zoonotic diseases (Bradley & Altizer, 2006). Because the gut microbiota is essential for the health and survival of animals, it is an important target for management practices (Hauffe & Barelli, 2019). In this thesis I have shown the importance of considering the life history of each animal species, their diet and helminth assemblages in particular, when considering the impact of urbanization on the gut microbiota and their ability to adapt successfully. Because the gut microbiota of each species is likely to exhibit a unique response to human activities, a large variety of management practices will likely be necessary. Despite this, it may be possible to develop techniques that can be applied to a wide range of species such as the use of therapeutic generalist helminths like *H. spumosa* to help maintain gut homeostasis, thereby improving their health.

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