The Effects of Urbanization on *Peromyscus sonoriensis*: A Microbiome Analysis

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Abstract

Microbiome diversity is one of many intersecting factors serving as a key indicator of wildlife and ecosystem health. Gut microbiomes are intricately linked with host organism health and behavior, and can be influenced by environmental factors such as diet or exposure to new microorganisms. Here, we investigated a range of urban and rural habitats around Spokane, Washington, with the aim of assessing microbiome diversity of both soil and the western deer mouse, *Peromyscus sonoriensis*. Soil microbiome composition was compared to that of mice in order to study the relationship between environment and organism. In this field-based observational study, deer mice were live-trapped at 10 different parks over a two-week period in early summer of 2023. Fecal pellets (57 total samples from 43 unique mice) and soil samples (51 total, n = 3-9 per location) were collected and analyzed for microbial diversity using Nanopore MinION sequencing technology. Results showed that urbanization did not affect the deer mouse microbiome at the phylum level, but did impact soil microbiome composition. Soil samples exhibited higher overall diversity while fecal samples consisted of three main phyla. Additional measures of habitat characteristics and wildlife health were also collected in the field, and future research will investigate potential associations among microbiome composition, habitat, and other health metrics. These results provide an important baseline to establish detailed physiological impacts of habitat alteration on deer mouse health.

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Introduction

Urbanization is a leading cause of the current biodiversity crisis. As humans continue to expand urban and suburban communities, many species are faced with rapid changes in habitat and resource availability. Specifically, habitat fragmentation, degradation, and overall loss have a significant negative impact on the health of animals living in close proximity to humans.[1] As demonstrated in environmental studies, organism health declines as external stressors increase, and in severe cases entire populations can be lost.[1] The reason why changes to habitat are so detrimental is difficult to understand, as organism health is multifaceted and can be influenced by the environment in many ways. Small mammal species such as deer mice serve as good model organisms for studying wildlife, as they are found in a wide variety of habitats and their health metrics are well-defined. One important variable related to both the environment and deer mice health is the gut microbiome.

The composition of microbial species that colonize the gastrointestinal tract, also known as the gut microbiome, plays a critical role in a host organism's health and well-being.[2] Changes in microbiome composition can lead to changes in overall health and behavior of a host organism. The mechanisms by which microorganisms are able to influence behavior are not fully understood, but studies have shown that the populations of microbes in the gut directly correlate with certain psychiatric conditions.[3] *Peromyscus maniculatus* (eastern deer mouse) that develop large-nest-building behaviors (LNB) have distinctly different microbiomes when compared to normal mice.[3] LNB is a condition analogous to obsessive-compulsive disorder in humans, suggesting a connection between microbiome composition and detrimental mental states.[3] This could potentially be due to gut microorganisms interacting directly with the body, influencing its immune system and ability to respond to stressors. The mice that presented with LNB had several types of bacteria that are associated with inflammatory conditions and injury, for example *Aestuariispira*.[3] Investigating how environmental stressors interact with microbiome composition is a key component of understanding organism health because the gut microbiome has a significant impact on the well-being of host organisms.

Beyond intrinsic factors such as organism age or sex, there are a variety of ways that the composition of the gut microbiome can be altered by the environment.[4,5] Habitat conditions, social interactions, stress, and diet are a few of many variables that can change the distribution of species in the microbiome.[5] While microbiome composition is relatively stable within an individual, differences between individuals are common.[5] Studies show that organisms living in different environments can have significantly different microbiomes.[4,5] For example, Diaz et al. found that the gut microbiome of eastern deer mice differed between undeveloped, urban, zoo, and laboratory environments.[5] The biggest differences were reported between mice living in captivity and those living in the wild, but variation was observed across all environments studied.[5] Studies on similar organisms, specifically *Sciurus carolinensis* (eastern grey squirrel), suggest that the gut microbiome is indirectly influenced by the environment of the host.[4]

For squirrels, the hypothalamic-pituitary-adrenal axis, a measure of host physiology, was a better predictor of microbiome composition than environment.[4] Specifically, the physiological changes the squirrels underwent in response to the environment (urban or forest) changed which microorganisms were able to survive in the gut.[4] Stothart et al. predicted that unaccounted differences in the gut microbiome might be indirectly linked to the environment by way of diet.[4] Whether the relationship between the environment and the composition of the gut microbiome is direct or indirect, evidence shows that the gut microbiome is susceptible to change by external factors. Ultimately, the complicated interactions between host physiology, environment, and gut microbiome composition contribute to how organisms respond to external stressors such as urbanization.

Microbiome composition can be studied using Nanopore sequencing technology. Nanopore is a third-generation sequencing method designed to read direct DNA products by measuring changes in an engineered electrochemical gradient created as DNA moves through nanopores.[6] It is a versatile sequencing tool that can gather a substantial amount of data (several Gb in a single instrument run) and generates long sequence reads of up to several kb. Nanopore can be used for a variety of applications including whole genome sequencing, species identification, or DNA library construction. One of the reasons why Nanopore technology is so adaptable is its use of barcoding, which allows for separate DNA samples to be run simultaneously and separated later by a computer program. For example, Nanopore can be used in conjunction with 16S PCR barcoding for efficiency. The 16S rRNA gene is highly conserved across bacterial species, meaning that it can be used to identify specific bacteria within a broader community.[7] Total DNA extractions can be run through the 16S PCR protocol, barcoded, and then multiplexed together on Nanopore flow cells.[6] This allows for many samples to be analyzed at once, making studies of microbiome composition more efficient and cost-effective.

The intricate relationship between gut microbiota and their host organisms means that studying the microbiome provides insight into the effects of urbanization on wildlife well-being. Current studies have shown how organisms that live in different environments have different microbiomes, and that microbiome composition can serve as an indicator of organism health.[3,4,5] The focus of this study was to investigate the effects of urbanization on the health of *Peromyscus sonoriensis* (western deer mouse) through the lens of gut microbiome composition. Fecal samples, used as a proxy for the gastrointestinal tract, were compared between mice living in urban and rural environments. The composition of soil microbiomes was also explored to provide information about habitat differences at the microbial level and serve as an indicator of what mice might be exposed to in their respective habitats.

Materials & Methods

Sample Collection

P. sonoriensis fecal and environmental soil samples were collected from 10 sites in Spokane, WA over the course of two weeks: May 22-June 2, 2023. The sites included

parks located both inside the city and in county regions. The relative locations of these sites are shown in **Figure 1**. Polly Judd, High Bridge, Palisades, Finch, and Lincoln represented an urban environment while Fisk, McLellen, Monk, Wilson, and Turnbull were considered rural (based on city proximity). At most parks, two to three collection sites were established approximately one mile apart to investigate different mouse populations living within the same environment. Exceptions included Polly Judd and Monk, which were smaller than the other locations.

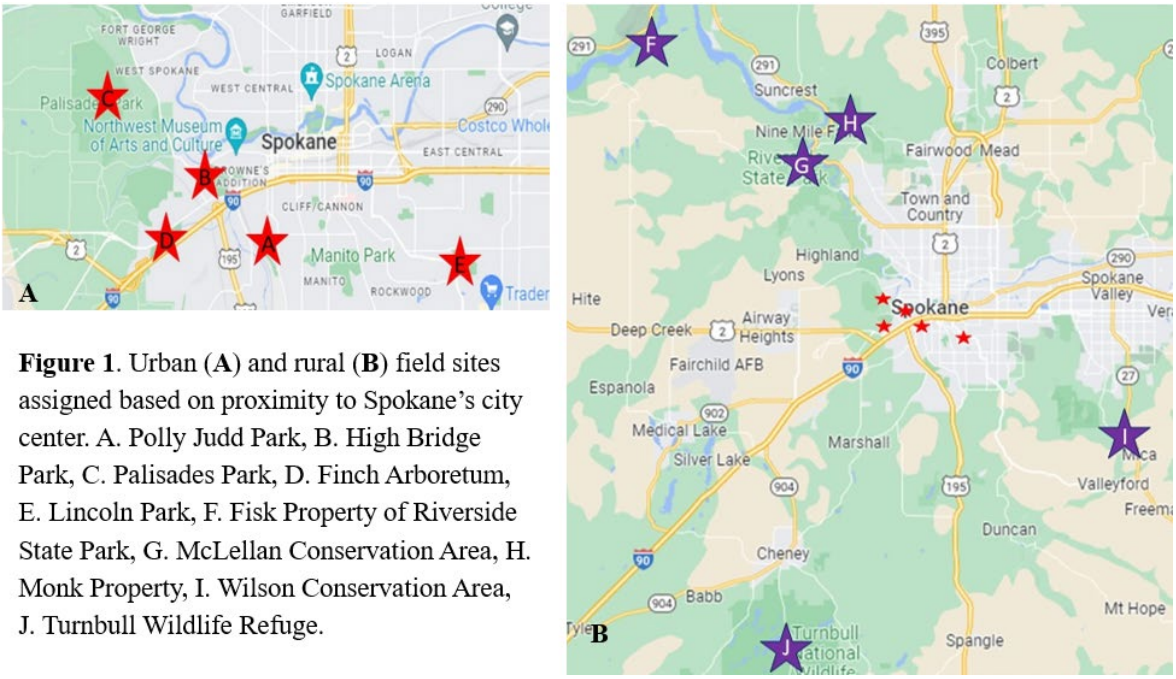

Figure 1. Urban (**A**) and rural (**B**) field sites assigned based on proximity to Spokane's city center. A. Polly Judd Park, B. High Bridge Park, C. Palisades Park, D. Finch Arboretum, E. Lincoln Park, F. Fisk Property of Riverside State Park, G. McLellan Conservation Area, H. Monk Property, I. Wilson Conservation Area, J. Turnbull Wildlife Refuge.

Sherman live traps, developed in the 1920s by H. B. Sherman for use with small mammals, were used to catch the mice.[8] Fifty traps per site were set in the evening and checked a maximum of four hours later, whereupon any mice caught were transferred to a second trap. This was to account for potential effects of containment on short-term stress measures. The traps were collected the following morning. All mice, including the ones that had been transferred to new traps, were processed at the collection sites. Information was gathered about the animals' weight, length, sex, age, and overall health (general body condition, reproductive status, and ectoparasites) via a physical examination. Blood, hair, and fecal samples were then collected before the mice were released. Blood samples were used for white blood cell counts and preserved for flow cytometry to determine overall immune cell composition (University of Portland). A small patch of hair was shaved off the hindquarters of captured mice and analyzed at the University of Portland for evidence of cortisol, a marker of long-term stress. Fecal samples were collected via ethanol-sterilized forceps into autoclaved Eppendorf tubes and used for microbiome analysis (Carroll College) and a glucocorticoid metabolites assay to assess short-term stress (Gonzaga University). Each site was trapped for two nights, and additional fecal samples were collected from any recaptured mice. A total of

61 individual deer mice (not including recaptures) were trapped during the collection period.

Three soil samples from each individual trapping site were collected into sterile centrifuge tubes for a total of 3-9 replicates depending on the size of the park. Environmental data were also taken at each site for each trap capture. This included vegetation structure, diversity, and organic litter cover, all measured via 1 x 1 m grids divided into 10 x 10 cm grids. Information on arthropod diversity (collected via pitfall traps) and evidence of human presence (assessed via counts over a specified time period) was also collected. This information will be used by Gonzaga University to develop an urbanization gradient for the sites.

DNA Extraction, Amplification, and Sequencing

Fecal samples were divided between Gonzaga and Carroll, with 57 samples from 43 mice being used for microbiome analysis. A total of 10 mice provided multiple replicates: five mice had both before and after four-hour replicates as well as recapture replicates, three mice had before and after four-hour replicates, one mouse had a recapture replicate, and one mouse had enough fecal matter to split into two technical replicates.

From 17 individual trapping sites, 51 soil samples were also collected for analysis. DNA was extracted from fecal and soil samples using the DNeasy® PowerSoil® Pro Kit (QIAGEN 47016) following the manufacturer's instructions. The concentration and quality of DNA was checked with a Nanodrop Lite from ThermoFisher, and all samples contained enough high-quality DNA to be sequenced. A 16S Polymerase Chain Reaction (16S PCR) was performed using the 16S Barcoding Kit 1-24 from Nanopore (SQK-16S024) following manufacturer's instructions. This process amplified full-length 16S rRNA genes, highly conserved across bacterial species, from the extracted DNA. Each sample was tagged with a barcoded primer for the prokaryotic 16S rRNA gene to allow for multiplexing of up to 24 samples on the sequencer. The products were then cleaned on PCR KleenSpin purification columns (Bio-Rad 7326300) according to manufacturer's instructions. Amplified and cleaned PCR products were pooled in groups of 24 barcodes, loaded onto the Nanopore MinION flow cell (R9), and run for 48 hours. Five flow cells total were run to encompass all of the samples. After sequencing, fast basecalling was performed in MinKNOW to assign nucleotide sequences to the recorded signals from the flow cell runs.

Statistical Analysis

Once basecalled, DNA sequences from each flow cell run were uploaded to Nanopore EPI2ME software to identify taxonomies via the Fastq16S pipeline and then downloaded as csv files. This software assigned NCBI taxonomy IDs (taxid numbers) to the sequences in each sample, which were used to aggregate taxonomic groups. Aggregation was performed at the phylum level across samples (to observe a broad-level resolution of microbiome composition) with a custom R script employing the taxonomizr

package. A data input file for microbiome analysis was compiled, combining the phyla count matrix (number of sequence reads belonging to each phylum from each sample) with categorical data describing the soil and fecal samples. For the soil samples, variables of interest included site location and soil type (data collected from the United States Department of Agriculture Web Soil Survey, https://websoilsurvey.nrcs.usda.gov/app/). Site location was also considered for the mice, along with age and sex. All data were analyzed using the Microbiome package in RStudio, which includes a variety of methods to visualize data and make comparisons between groups.[9] Relative abundance analysis was used to visualize phyla distribution within the soil and mice samples. A Shannon diversity metric was used to describe the alpha diversity within samples. Ordination analysis using multidimensional scaling assessed diversity between samples using the Bray-Curtis dissimilarity metric. Finally, PERMANOVA and Wilcoxon t-tests were performed as statistical analyses to assess the association of variables with microbiome composition. Statistical significance was defined by p-values less than 0.05. All customized R scripts are available upon request.

Results

Sequence Read Results

A total of 31,712,246 successfully classified 16S sequence reads were obtained, with an average of 332,139 classified sequence reads per mouse sample and 257,107 reads per soil sample.

Overall Microbiome Composition

Relative abundance charts for both the mouse and soil samples showed the overall composition of bacterial phyla in the sample populations. Phyla that constituted more than 0.5% of all the sample reads were shown as distinct groups, with the remaining phyla being categorized as “other”. A total of 44 unique phyla were identified across both soil and fecal samples (44 for soil samples and 40 for fecal samples). Overall, the distribution of phyla varied more in the soil samples than in the mice samples (**Figure 2**), as demonstrated by a more refined analysis of core taxa (defined as a minimum of 0.1% relative abundance across 75% of all samples). Ten core phyla were specific to soil, only one was specific to mice, and three core phyla were shared among all samples. As shown in **Figure 3A**, the most abundant phyla in the soil samples were Pseudomonadota, Acidobacteriota, Bacillota, and Bacteroidota. As shown in **Figure 3B**, the mice samples contained three main bacterial groups: Bacillota, Bacteroidota, and Campylobacterota with Bacillota predominating. Campylobacterota was only detected at a high abundance in the rodent samples, but Bacillota and Bacteroidota were found in both mice and soil samples.

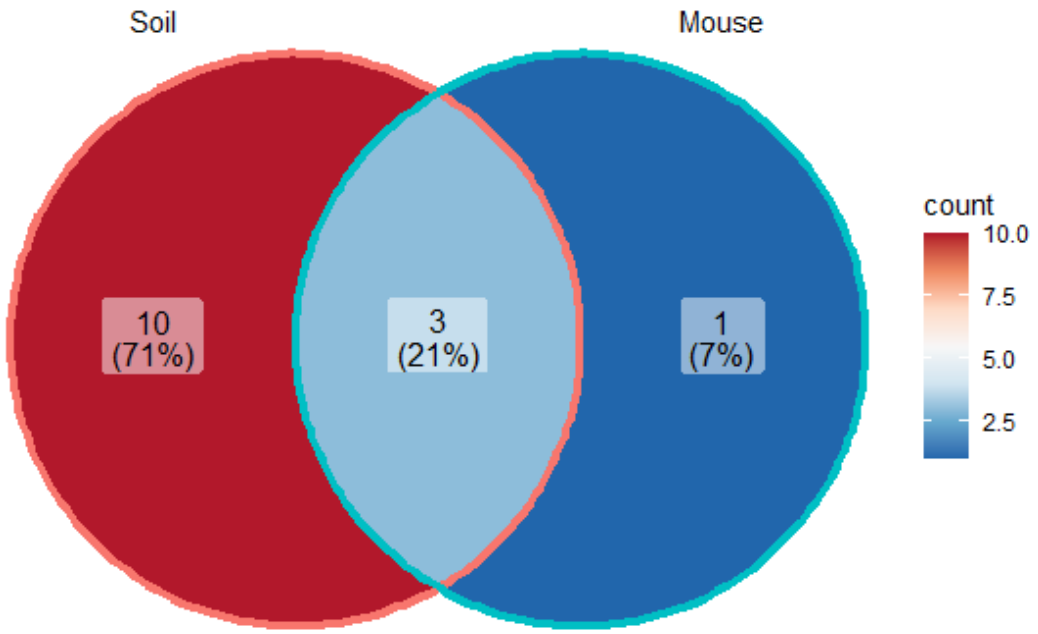

Figure 2. Venn diagram showing the distribution of core phyla across soil and mouse samples. A phylum was identified as a "core" group if it comprised at least 0.1% relative abundance across 75% of samples.

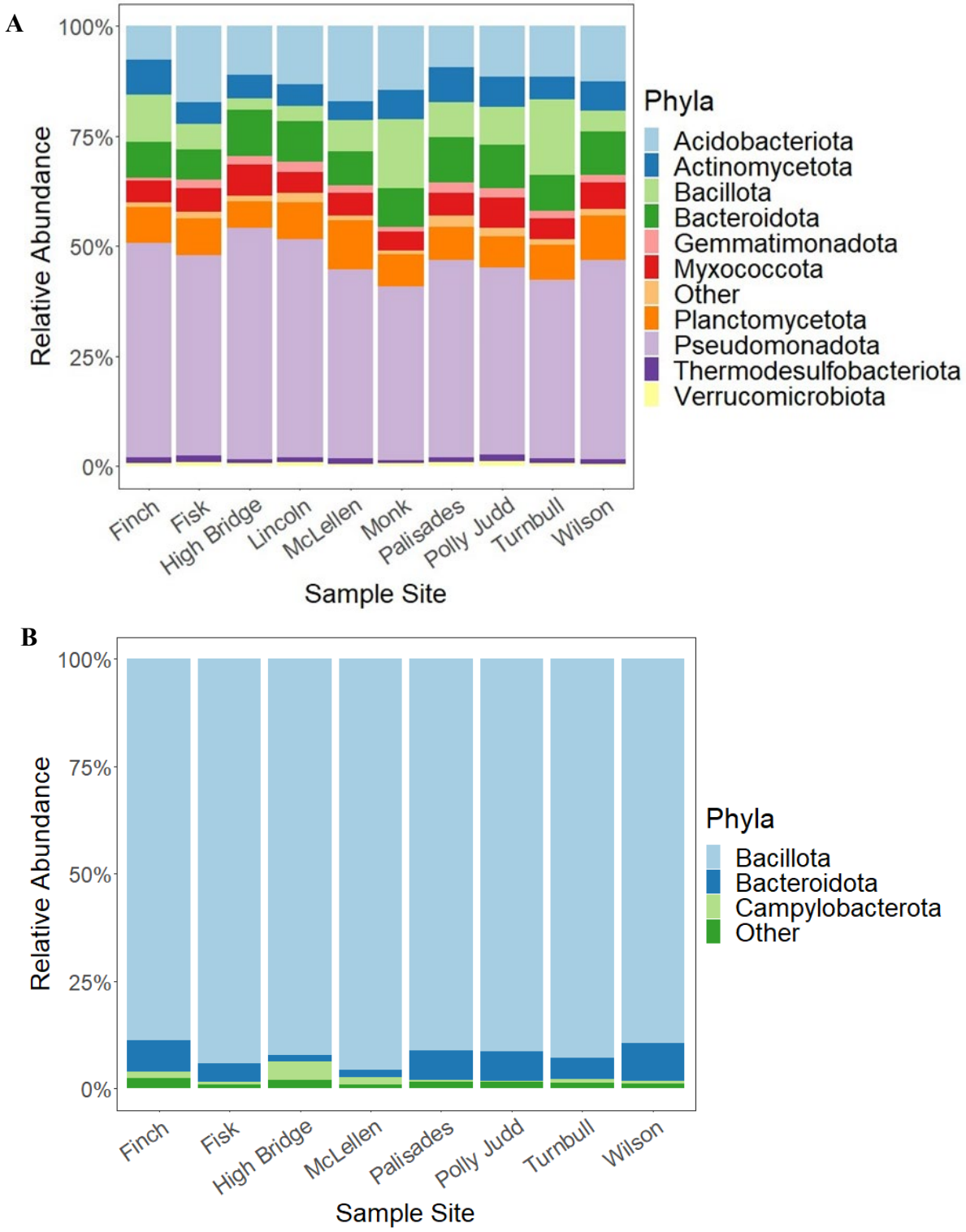

Figure 3. Relative abundance of top phyla in soil samples **(A)** and mice samples **(B)** averaged by site location. Phyla that represented less than 0.5% of the total composition were grouped into "Other". (n = 3-9 for soil; for mice, n varied based upon individual mice

from each site: Finch = 24, Fisk = 2, High Bridge = 1, McLellen = 2, Palisades = 14, Polly Judd = 1, Turnbull = 10, Wilson = 3).

Alpha Diversity Analysis

To investigate microbiome diversity within sample sets based on location, soil and mice samples were analyzed by site group. Shannon diversity measures, accounting for both species richness and evenness, illustrated the range of microbiome diversity within individual soil or mice samples from a given site location. The Shannon diversity values were much higher for soil samples when compared to mouse samples, as can be seen by the difference in y-axis ranges in **Figure 4.** Wilcoxon tests were then used to determine statistically significant differences between the site locations. The mice samples did not show any significant variation when separated by site group. For the soil samples, High Bridge soil was significantly different ($p < 0.05$) from Wilson, Turnbull, Monk, and Polly Judd samples. Fisk soil was also significantly different ($p < 0.05$) from Turnbull and Lincoln.

Beta Diversity Analysis

Beta diversity analysis allowed for investigation into microbiome composition differences between groups. Samples were first separated by sample type and urbanization category, then analyzed using Bray-Curtis dissimilarity metrics. PERMANOVA tests were performed to investigate statistical differences in microbiome composition based on Bray-Curtis dissimilarity. PERMANOVA is a non-parametric, multivariate version of ANOVA that compares the average values of multiple groups and allows for the separation of samples based on specific variables. For this analysis, the groups that showed significant differences ($p < 0.05$) were visualized further using ordination techniques. Using Bray-Curtis dissimilarity for input, p-values were calculated with 10,000 permutations. PERMANOVA results indicated that there were differences between mice and soil ($p = 9.999 \times 10^{-5}$), and soil samples separated by both site group (p-value = 0.0144) and urbanization ($p = 0.0046$). Non-significant variables included age, sex, soil type, and urbanization for mice. **Table 1** reports p-values for the PERMANOVA tests.

Table 1. PERMANOVA results for variables of interest. PERMANOVA tests were based on Bray-Curtis dissimilarity input using 10,000 permutations.

Sample Group	*Variable*	*p-value*
Mice	Urbanization	0.1812
Mice	Age	0.1260
Mice	Sex	0.8568
Soil	Soil Type	0.2269
Soil	Site Group	0.0144
Soil	Urbanization	0.0046
Mice and Soil	Sample Type	9.999×10^{-5}

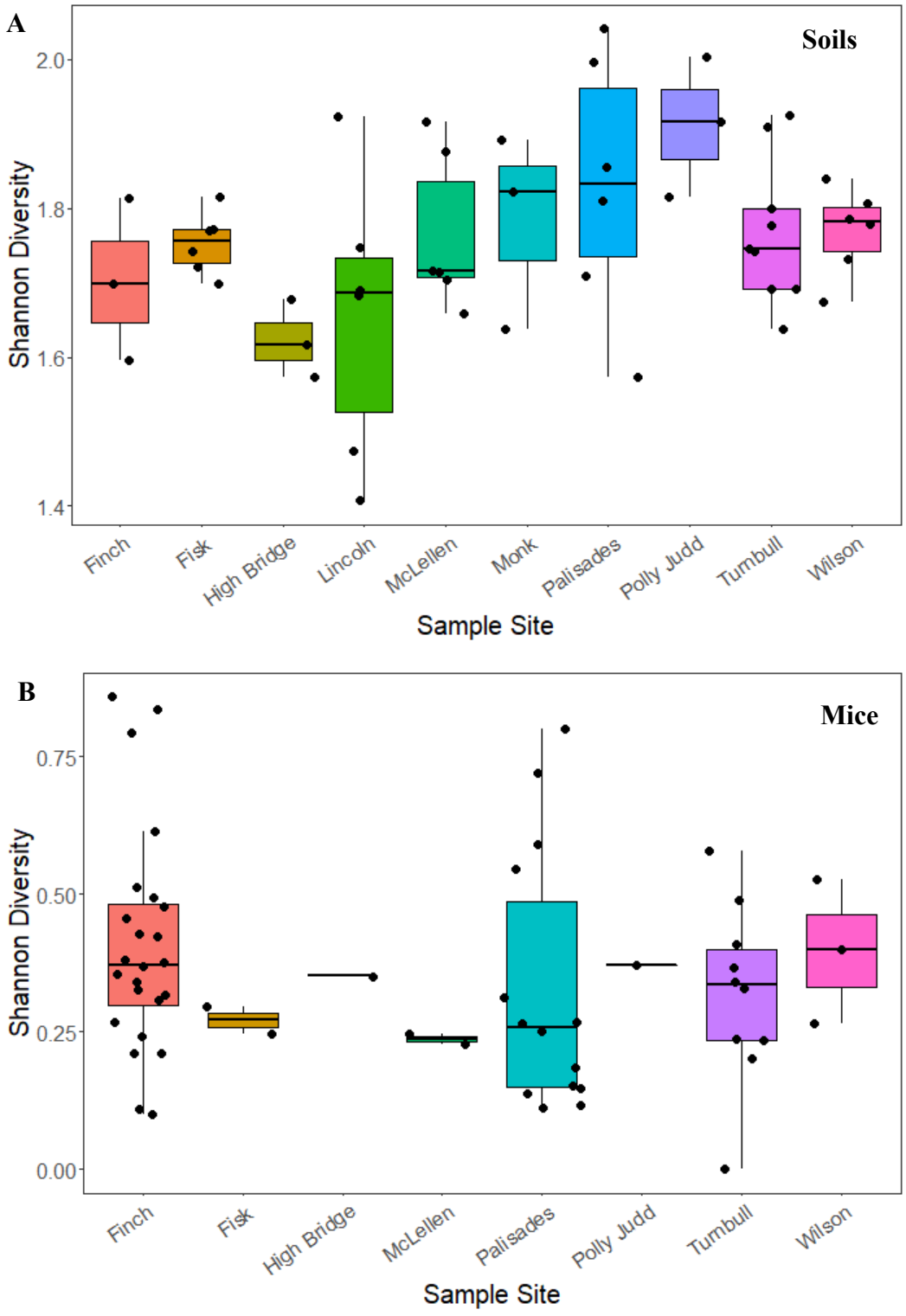

Figure 4. Shannon diversity plots of soil samples (**A**) and fecal samples (**B**) grouped by site location. Replicates ranged from 3-9 for soil samples. Each mouse was sampled individually (Finch = 24, Fisk = 2, High Bridge = 1, McLellen = 2, Palisades = 14, Polly

Judd = 1, Turnbull = 10, Wilson = 3). Dots represent individual replicates and whiskers encompass the range of each data set excluding outliers.

Ordination analysis using multidimensional scaling was then performed to visualize significant differences between groups. As shown in **Figure 5**, mice and soil samples were highly dissimilar ($p < 0.0001$, **Table 2**); the ordination axes account for 86.3% of the sample variation. Within the mouse group, urbanization did not differentiate samples ($p > 0.05$, **Table 2**). Within the soil group, urbanization did influence microbiome composition ($p < 0.01$, **Table 2**). While there is overlap between urban and rural soil samples as seen in **Figure 6**, the urban samples (shown in blue) cluster more densely than rural samples (shown in red) which demonstrates the dissimilarity of the groups. Ordination axes in **Figure 6** account for 70.3% of the variation in microbiome composition.

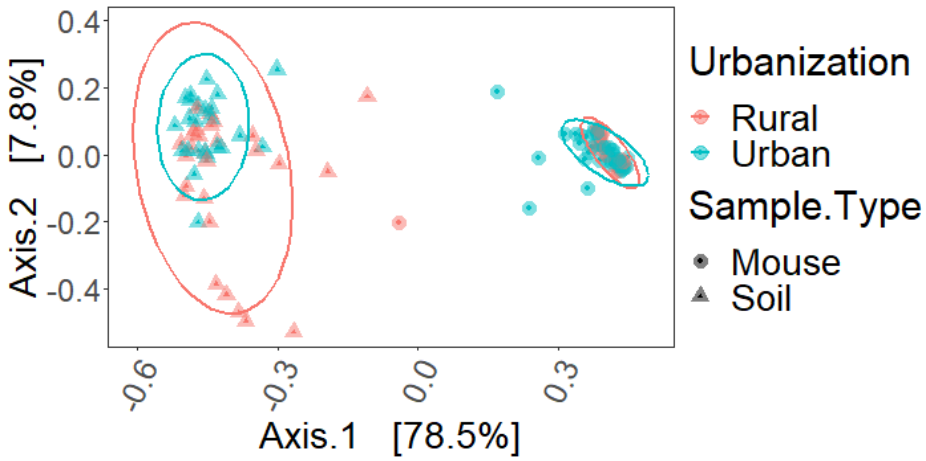

Figure 5. Clustering of samples by urbanization category and sample type as shown by multidimensional scaling ordination analysis using Bray-Curtis dissimilarity. Ellipses are shown at a 95% confidence level. Each point maps an individual sample; red represents rural samples while blue represents urban samples. Circles indicate mice and triangles indicate soils.

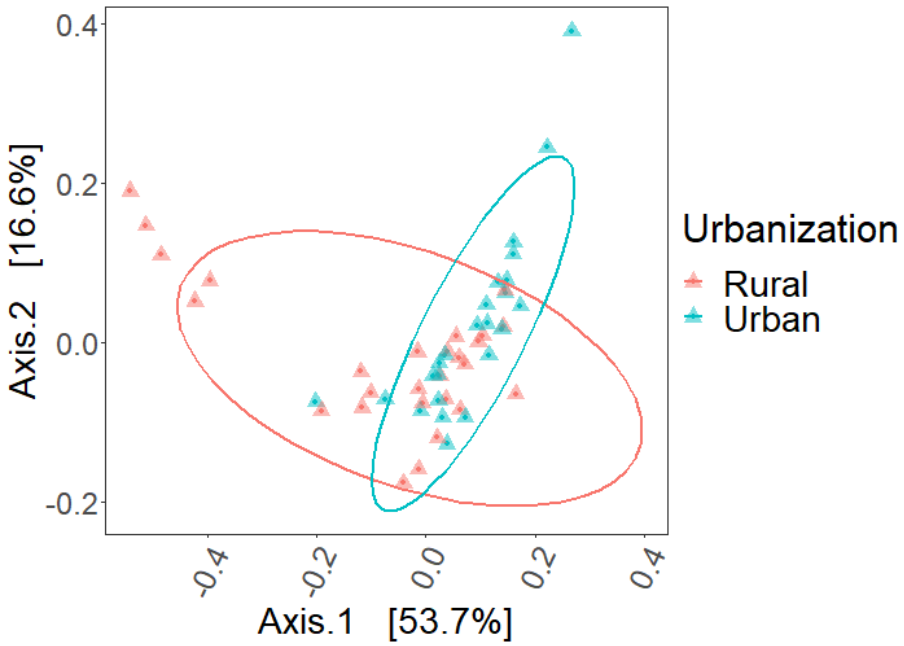

Figure 6. Clustering of soil samples by urbanization category as shown by multidimensional scaling ordination analysis using Bray-Curtis dissimilarity. Ellipses are shown at a 95% confidence level. Each point maps an individual sample; red represents rural samples while blue represents urban samples.

To further illustrate the variability within soil samples and visualize the differences between different sample locations, ordination based on site group was also performed (**Figure 7**). Despite the overlap among all of the sites, differences in microbiome composition were apparent for several site groups, for example when comparing Lincoln and Turnbull soils, and warrant further investigation in conjunction with environmental data collected.

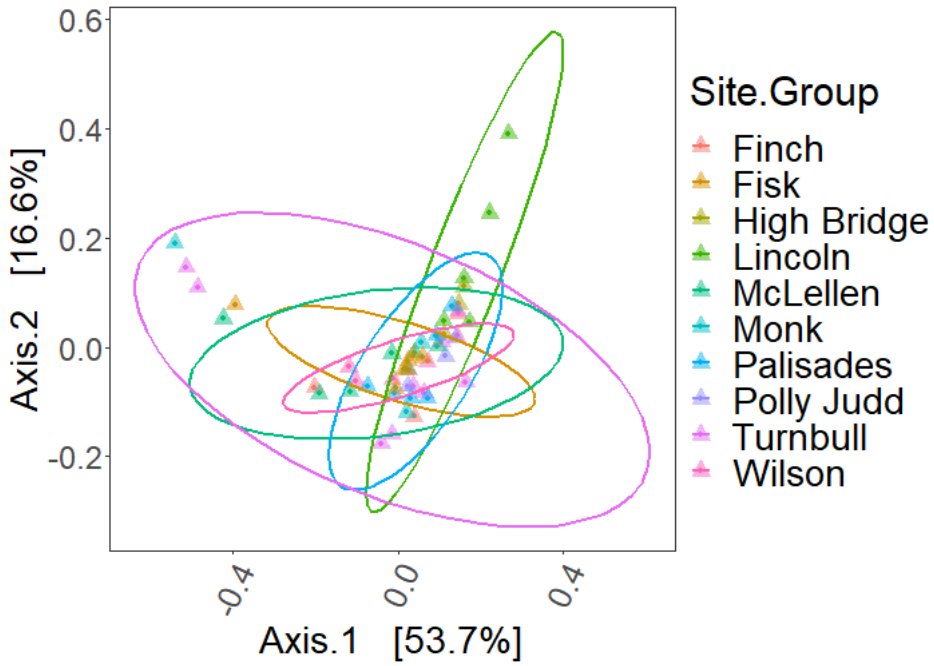

Figure 7. Clustering of soil samples by site group, shown by multidimensional scaling ordination analysis using Bray-Curtis dissimilarity. Ellipses are shown at a 95% confidence level. Each point represents an individual sample.

Discussion

A total of 108 samples were analyzed for microbiome composition from 10 different parks around Spokane, including 51 soil samples and 57 fecal samples. After DNA extraction, 16S PCR amplification, PCR cleanup, and sequencing, an average of 332,139 classified sequence reads per mouse sample and 257,107 reads per soil sample were obtained, providing more than sufficient sequencing depth to represent each community.

At the phylum level, soil samples contained a higher level of microbial diversity than the mouse samples. As shown by **Figure 2**, soil samples had a total of 13 core phyla, each of which constituted over 0.1% of total composition for over 75% of all the samples. Three of those phyla were also found in the mouse samples, which had a total of four core phyla overall (**Figure 2**). **Figure 3** expanded on this general categorization by visualizing the overall distribution of phyla. This reduced diversity in the gut microbiome was expected, since organisms such as mice typically have a few core phyla while soils are very diverse.[3,11] Alpha and beta diversity analyses also demonstrated that the soil samples showed more microbial diversity than the mouse samples (**Figures 4-5**). Significant differences in microbiome composition between sites were found in the soil samples using alpha diversity analysis (**Figure 4**) and visualized in **Figures 5-7**. A high percentage of variation between samples could be explained by the variables investigated using multidimensional scaling ordination (70.3% for soil samples, **Figures 6** and **7**), accounting for a large portion of the variation in microbiome composition between

samples. Differences in microbiome composition were not found in the mouse samples (**Table 1**).

Soil Microbial Communities

The soil microbiome consisted of 13 core phyla with a total of 44 unique phyla overall. This increased diversity compared to the mice was shown by both relative abundance and Shannon diversity analyses; soil samples contained a greater number of phyla than mouse samples (**Figure 3**) and larger Shannon diversity values (**Figure 4**). Pseudomonadota (formerly Proteobacteria) formed the most abundant phylum, which aligns with common findings among soil bacteria.[10] Both site group and urbanization category significantly affected soil microbiome composition ($p < 0.01$, **Table 2**). Soil microbiome diversity is malleable, heavily dependent on temperature, and can vary widely between seasons.[11,12] If changes are drastic enough, the 'framework' of microbial species in a given area might shift completely from year to year.[11] Soils serve as the foundation for entire ecosystems, with microbes playing important roles in feeding pathways, nitrogen fixation, and organismal communication.[11] Changes in the soil microbiome can lead to macroscopic changes in the environment.[11,12] In order to fully understand soil microbiome composition and continue to relate it to the gut microbiome of deer mice, soil sampling should continue to be a part of future studies. Soil data can be compared between each collection year to see if microbial communities are changing or if they are relatively fixed across years.

The two sites that showed the widest range in soil diversity were Lincoln Park and Palisades Park which were both urban locations. During the field collection period, high levels of human and dog traffic were noticed at these two parks. This activity could be contributing to the increased soil microbiome diversity, as visitors likely came from a variety of places around Spokane and introduced different bacterial species. The development of a quantitative urbanization gradient for future studies will help investigate this observation. Environmental data (vegetation cover, human presence, arthropod diversity, etc.) gathered during the collection period can be used to construct this gradient, allowing for each sample site to be given an urbanization score.[13] The current distinction between urban and rural environments was made based on the proximity of sample sites to Spokane's city center (i.e., inside or outside city limits). This categorization system might not be representative of the actual impact of human activity on those environments. For example, one collection site in the Turnbull Wildlife Refuge was surrounded by hiking trails. Even though the refuge is distant from Spokane, some areas still experience high human traffic which has the potential to impact the environment. A quantitative urbanization scoring system can help incorporate all aspects of human activity and habitat alteration into a model of urbanization influence. This will allow for a more accurate understanding of the relationship between mouse populations, health metrics, and urbanization levels.

Mouse Gut Microbial Communities

The mice fecal samples consisted predominantly of three main phyla: Bacillota, Bacteroidota, and Campylobacterota. There were no differences in microbiome composition for the mice based on any of the tested variables, including urbanization category, site group, sex, and age (**Table 2** and **Figures 4** and **5**). For deer mice, most microorganisms fall into the phyla Bacteroidota (formerly Bacteroidetes) and Bacillota (formerly Firmicutes) as reported by a previous study with *P. maniculatus* in different environments.[3] Our study corroborates this finding, with Bacillota being the most abundant phylum in the mouse samples. One of the biggest limitations of this study was a lack of replicates amongst the mouse samples. For Fisk, High Bridge, McLellen, and Polly Judd, the low number of total captures meant that only one or two fecal samples from those sites were analyzed for microbiome composition. Additionally, splitting samples between microbiome and glucocorticoid metabolites analyses reduced the number of samples available. The Monk Property and Lincoln Park were not represented in our analysis of the mouse samples. The site with the most captures, Finch Arboretum, showed the highest variability out of all the mice samples. This indicates that individual mice do have biological variability within their gut microbiomes, though they do share the same core taxa. Studies indicate that differences between the composition of microbiomes are easier to elucidate when explored at the genus level.[3,4,5] Examining bacterial genera and species would provide a more specific distribution of microorganisms, making differences clearer. Future research aims both to collect more samples from mice and analyze sequencing data at the genus level; additionally, the yield from DNA extractions indicated that most fecal samples provided enough material for both microbiome analysis and the glucocorticoid metabolites assay, providing another way to increase sample size.

As highlighted in a study on squirrels in urban and forested areas by Stothart et al., environmental factors may not change the gut microbiome directly.[4] Instead, an organism's response to differing environments leads to changes in physiological features that have the ability to influence the gut microbiome. Stothart et al. proposed that physiological changes impact the permeability of a host's 'bacterial filters', potentially allowing new types of bacteria to colonize the gut. Several factors that correlated with microbiome composition in the study included cortisol and lymphocytes. Cortisol serves as a measure of long-term stress, while blood lymphocytes are an important aspect of immune system strength. In the study by Stothart et al., urban squirrels had higher stress levels and more significant immune responses.[4] This observation might also be made in deer mice, as urban environments include increased human presence and novel threats (for example house cats as predators). Stothart et al. investigated the connection between physiological measures and microbial composition by focusing on specific bacterial families.[4] Nearest taxon index, principal component analyses, and general linear modeling were used to compare the relative abundance of a bacterial family to cortisol measurements and immune cell ratios.[4] Future studies should incorporate physiological data into the comparisons between urban and rural mouse populations. These types of

analyses (associating the presence of dominant bacterial species with numerical measurements) can be used to integrate data collected on immune function and stress hormones and determine how physiological conditions impact the mouse gut microbiome.

Soil and Gut Microbiome Intersection

While physiological changes likely have a more significant impact on gut microbes, factors such as diet or microbe exposure can also influence microbiome composition. Soils are complex habitats and have a significant impact on overall ecosystem health.[11] The composition of soil microbiomes is important to understand because deer mice are exposed to soil microbes through foraging and burrowing activities.[5] Soil microbiomes can also influence vegetation type and diversity, contributing to differences in food sources between habitats.[11] Investigating soil microbiomes and comparing them to mouse gut microbiomes can reveal what kinds of microbes mice might be picking up from their environment. In this study, the mice shared three core phyla with the soils: Bacillota and Bacteroidota (most abundant) as well as Pseudomonadota (less abundant; **Figures 2** and **3**). Notably, the most abundant member of Bacillota in the fecal samples was the genus *Lactobacillus*, a common gut microbe known to facilitate digestion and serve as a defense against some pathogens.[14] Future tracking of *Lactobacillus* levels may provide more insight into mice health.

Conclusion

Though there were no significant differences found in mouse samples, the increased variability that was observed with higher numbers of replicates suggests there are differences that could not be elucidated at the phylum level. It is also possible that mice have a consistent microbiome that is resistant to environmental influence. Future studies will increase mice replicates to continue exploring diversity in the mouse gut microbiome and generate a more comprehensive data set for all the sample sites. Studies will also include the urbanization gradient, stress measures, and immune health measures as part of the analysis to investigate the relationships between urbanization, organism health, and microbiome composition more specifically. These analyses will be done at the genus and species level to examine microbial diversity within mouse microbiomes, as the mice shared the core taxa of Bacillota, Bacteroidota, and Campylobacterota across environments. Overall, this study demonstrates how gut microbiome analysis provides a lens into organism health and is an important indicator of the effects (or lack thereof) of urbanization on the well-being of creatures such as deer mice. As humans continue altering the environment we share with wildlife, an improved understanding of health impacts is crucial to help us better steward and preserve earth's biodiversity.

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