

The social microbiome: the relationship between the microbiome and sociality in a wild mammal

Abstract

The microbiome has a well-documented relationship with host behavior. Greater microbial diversity and specific microbes have been associated with improved fitness, though these associations have been studied almost exclusively in captive animals with implications for human health. In wild populations, the microbiome has important fitness consequences, such as being associated with mass gain and body condition, and specifically, microbial diversity has been associated with increased individual stability and resistance to over-domination of specific microbes. An individual's social behavior also has important fitness consequences for acquiring food resources and avoiding predation. While both factors individually influence fitness, the relationship between the microbiome and sociality is understudied. Here we use social network analysis to quantify the relationship between microbial diversity and a variety of attributes of social relationships in a wild, free-living population of yellow-bellied marmots (*Marmota flaviventer*). We found no significant associations between microbial diversity and social network measures, suggesting that in facultatively social mammals, microbial diversity does not explain variation in the social relationship structure. Further research must be completed to examine the impact of social behavior on microbial composition and diversity.

Introduction

The collection of microorganisms in the gut of vertebrates, known as the gut microbiome, has co-existed and evolved alongside their animal hosts for millions of years (Moeller et al., 2016; Groussin et al., 2020; Ley et al., 2008). However, only within the last two decades has the technology been available to study the potential role microbes play in host physiology and behavior (Neish, 2009; Cryan et al., 2019). The symbiotic microorganisms that live in the host's gut, for example, has been shown to influence host activity and behavior, including various aspects of metabolic processes and energy balance (Turnbaugh et al., 2006; Xiao and Kang, 2020), immune system maturation (Fung et al., 2017), and nutrient intake (Berding et al., 2018; Sarkar et al., 2017). Microbial diversity is also associated with fitness, improving stability of both individuals and groups (Stressmann et al., 2012; Garcia-Garcia et al., 2019). This is due to the inverse relationship between diversity and the dilution of dominant microbes in the microbial metacommunity, causing higher stability and resistance to over-dominance of particular microbes (Erkus et al., 2013, Shade et al., 2013). Erkus et al. demonstrated that diversity strengthens an individual's ability to avoid facing the fitness consequences of loss or damage to a particular microbe. In addition, having a diverse microbe has been associated with numerous benefits, including reducing susceptibility to disease and improving immunity (Neff, 2013; Shapira, 2016).

Recent studies have shown that the microbiome can also influence behavior through the brain-gut axis - the communication between the central and enteric nervous systems. Particularly, the microbiome has been implicated in stress modulation (Fung et al., 2017), infant health and metabolism (Jašarević et al., 2015, 2018), and has been identified as a key factor that influences the likelihood of stress-related disorders and diseases (Foster et al., 2017). Behavioral changes have also been correlated to variation in the gut microbiota. In humans, gut microbiome variation has been linked to personality differences (Johnson, 2020) and animal models have exhibited the brain-gut axis' relationship with individual emotions and learning (Diaz Heijtz et al., 2011) and social behavior (Sarkar, 2020). Arentsen et al. (2015) found microbiome deficient mice experienced depressed fitness in relation to wild-type mice, performing different social and repetition behaviors. However, most of these studies have been done in captivity, where animals are consuming different diet profiles and experiencing variable stress responses to predators than their natural scenarios (Bolnick, 2014; Uenishi, 2007). To better our understanding of the relative importance of the microbiome's ecological implications, interactions in and amongst wild populations must be adequately studied.

Social behaviors and group-living in wild species have long been associated with fitness through the ability to find mates, food acquisition, and protection from predators (Janson and Goldsmith, 1995; Alexander, 1974). These interactions can influence how populations and territories form and change over time (Makagon et al., 2012). Over the last decade, social network analysis (SNA) has been increasingly used to examine and quantify patterns and attributes of social interactions (Hasenjager and Dugatkin, 2015). These attributes of sociality can range from simple measurements, such as the number of social partners, to more complex measures such as embeddedness - how well an individual is integrated into a group. SNA is a powerful tool to quantify the attributes of sociality because it builds on previous models in network mathematics and allows ecologists to quantify group structures, network traits, and types of interactions within the same framework (Kraus, 2009). SNA has been used to trace disease transmission in a population of Tasmanian devils (*Sarcophilus harrisii*) (Hamede et al., 2009) and quantify group instability through grooming and aggressive behaviors in primates (McCowen et al., 2008). By using SNA, we can begin to address questions about population-level patterns, such as specific social consequences of variation in microbiome composition.

While studies have shown that both the microbiome and social behavior contribute to fitness, less is known about how they relate to each other. Recent studies have shown promise in determining how the microbiome may influence sociality, such as a study that found microbial composition influenced the likelihood of mice to exhibit submissive or dominant behavior (Agranyoni et al., 2021). However, further study is needed to quantify the relationship between attributes of sociality and the microbiome in wild populations.

We studied the relationship between microbial diversity and social network attributes in a yellow-bellied marmot (*Marmota flaviventer*) population located in and around the Rocky

Mountain Biological Laboratory, which have been under continuous study since 1962. Marmots are a good model to address the sociality-microbiome relationship due to their variation in social behavior and the large dataset consisting of social observations and microbiome samples. Additionally, results can be framed in context because social attributes have been shown to influence survival (Yang et al. 2016; Montero et al., 2020), alarm call propensity (Fuong et al. 2015), reproductive success (Wey and Blumstein 2012), and longevity (Blumstein et al. 2018). We fitted linear mixed effect models to determine the relationship between microbial diversity (measured using Shannon Weiner and Faith's phylogenetic diversity indices), and specific social attributes.

In marmots, social connectivity can be detrimental to fitness, with strong affiliative interactions being associated with reduced life longevity and a higher likelihood of dying over the winter hibernation period (Blumstein et al., 2018; Yang et al., 2017; Wey and Blumstein 2012; Fuong et al. 2015). However, microbial diversity has been associated with increased fitness in this system. Specifically, higher levels of *Firmicutes* microbes relate to faster mass gain, which is essential for a successful hibernation (Johnson et al., 2021). In addition, gut composition in other mammals are associated with social group membership, independent of an individual's diet (Tung et al., 2015). However, the mechanism of the brain-gut axis is understudied and exploration into this mechanism is necessary to properly predict the extent of this relationship. Thus, our *a priori* hypothesis is that microbial diversity will be associated with social network position, but the mechanism of this association and the drivers of this relationship requires further study to make an accurate prediction.

Methods

Site specifics and data collection

We studied yellow-bellied marmots from 2015 to 2020 at the Rocky Mountain Biological Laboratory in the Upper East River Valley in Gothic, Colorado, USA (38°57'N, 106°59'W; ca. 2900 m elevation). Marmots were individually marked and studied at the same sites annually. These study sites consisted of two core areas designated as “up valley” or “down valley”. These two areas do not geographically overlap and social interactions do not occur between the two areas. The up-valley area is higher in elevation and experiences more harsh weather than the down valley area (Fig 1; Van Vuren and Armitage 1991; Blumstein et al. 2006; Maldonado-Chaparro et al. 2015).

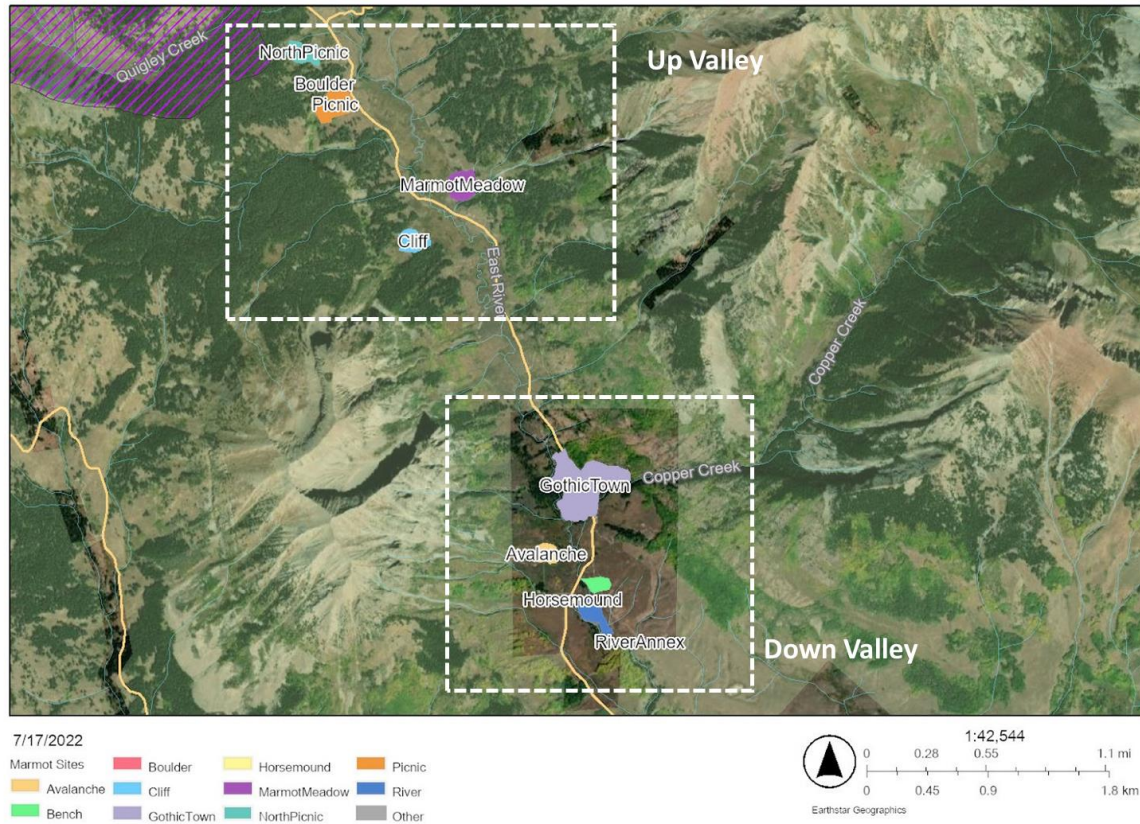


Fig. 1. Colony sites where data was collected; up valley and down valley sites are distinguished using white boxes.

To individually mark individuals and collect microbiome samples, Tomahawk live traps were placed near burrows. Trapped individuals were transferred to a cloth handling bag to quantify morphological features, such as mass, sex, reproductive status, and hindfoot length. They were also given a unique metal ear tag (Monel self-piercing fish tags #3, National Band and Tag, Newport, KY) and dorsal fur mark with non-toxic Nyanzol-D dye (Greenville Colorants, Jersey City, NJ) to facilitate identification from afar. Fecal samples were collected from the traps or during handling and immediately put on ice, before being transferred to a -20 °C freezer to await process back at UCLA.

Behavioral observations were conducted using binoculars and spotting scopes from 20-150 m away. Distances vary to limit observer effect but maximize visibility and accuracy of data (Blumstein et al., 2009). These observations were conducted during peak marmot activity (0700 – 1000 and 1600 – 1900 hours; Armitage, 1962). All social interactions were recorded and classified as either affiliative or agonistic. The initiator and the recipient of each interaction were recorded, as well as the date, time, and location of each interaction.

Social Network Measures

We constructed weighted and directed social networks based on affiliative interactions using the R (version 4.1.2) package “igraph” (Csardi and Nepusz 2006; version 1.2.11). We filtered out social interactions in three ways. First, we excluded interactions with unknown recipients and initiators so we could calculate directed interactions during analysis. While most interactions occurred between identified individuals (81.34% of observed interactions), the initiator and/or recipient couldn’t be identified in 8.29% of interactions due to the interaction already occurring when the observer arrived or visual obstructions (e.g., tall vegetation, marmot orientation to observer). Social structure should not be significantly influenced by the exclusion of these undirected interactions between unidentified individuals (Silk et al. 2015). Secondly, we excluded pups from our measure of social interactions, since they emerge halfway through the year and typically interact exclusively with their mother. Finally, we excluded any individuals that were seen or trapped fewer than 5 times. These individuals were considered transients or dispersing, meaning their interactions were not considered a part of the social group (Wey and Blumstein 2012; Fuong et al. 2015; Yang et al. 2017; Blumstein et al. 2018).

These affiliative networks consisted of 13,511 total social interactions between 192 unique individuals in 37 different social groups. 43.38% of these interactions occurred down valley and 56.62% occurred up valley. We calculated seven social network measures: degree, closeness, strength, betweenness, eigenvector centrality, embeddedness, and density (Table 1). The relatively low rate of unknown individuals in our observations, which occurred over the entire active season of these marmots, facilitates the reliability of our social network measures (Silk et al. 2015; Davis et al. 2018; Sánchez Tójar et al. 2018). We selected each measure based on its biological importance and connection to the individual nature of microbiome data. Justifications for each are as follows.

Social Measure	Description	References
Individual-level measures		
Degree	Number of social partners an individual has	Flack et al., 2006; Blumstein, Wey, and Tang, 2009
Closeness	How centralized an individual is in a network calculated by the reciprocal of the sum of the shortest path lengths between a focal individual and all other individuals in its network	Wey and Blumstein, 2010; Rimbach et al., 2015

Strength	Number of social interactions an individual has, including repeated interactions	Montero, 2020
Betweenness	Number of times an individual lies on the shortest path between each other	Wey and Blumstein, 2012
Eigenvector centrality	A measure of how connected an individual's social mates are	Montero 2020
Embeddedness	A measure of how many individuals need to be separated in order to completely isolate an individual	Blumstein 2009; Wey and Blumstein, 2012; Montero, 2020
Network-level measures		
Density	Describes how connected a network is as a measure of the number of interactions observed as a fraction of all possible interactions	Burt 1992; Philson et al. 2021; Brent et al., 2013

Table 1. Descriptions of social network measures and their classifications

Microbiome data processing

Our data collection and selection criteria specifics follow the protocol found in the sample section methodology from Johnson et al. (2021). Bacterial DNA samples were extracted from 207 samples, taken from 51 unique individuals, and underwent PCR in order to develop a library of microbial DNA. The library was examined using QIIME2 (v. 2019.9) in the Hoffman2 supercomputer terminal. Raw sequencing data of both forward and reverse reads were imported, and visualized to determine ideal cutoffs for truncation. The cutoff was determined as where the number of reads began to decrease below 1000 reads. Using this, quality control was completed using the QIIME2 DADA2 denoising tool. All samples were truncated by 250 reads in order to generalize read length. The resulting data were summarized in a feature table and used to determine community diversity indices. Alpha diversity was solely chosen to examine diversity due to its ability to assign diversity to each individual. From the list of alpha diversity metrics, Shannon Wiener diversity and Faith's phylogenetic diversity were calculated, using a phylogenetic tree generated from the feature table for the Faith's diversity measurement. Amplicon sequence variance (ASV) was also used to identify taxonomic assignment at varying levels, with phyla and family groupings excluding the most "unknown" categories. Diversity

measures for each sample were then cross referenced with UID, pairing up individuals to their calculated diversity metric.

Data Analysis

We fitted linear mixed-effects models using *lme4* (Bates et al., 2015) to analyze the relationship between microbial diversity and SNA. Fourteen models were fitted to account for all seven network measures in addition to the two separate diversity metrics chosen, Shannon Weiner's diversity index and Faith's diversity index. Network measures were the dependent variables and microbial diversity metrics were the independent variables. Based on histogram visualizations and normality tests, all social measures were transformed to meet model assumptions. Age class, valley position, and group size were included as additional fixed effects, and marmot ID and year were fitted as random effects. We checked residuals using the *performance* package (Lüdtke, 2021). Marginal and conditional part R^2 were calculated using the "partR2" package in R (Nakagawa and Schielzeth 2013; Stoffel et al., 2021; version 0.9.1). Marginal R^2 gives an estimate of the variance explained by all fixed effects whereas the conditional R^2 gives an estimate of the variance explained by all the fixed effects plus all random effects. We also calculated marginal semi-partial R^2 , which gives an estimate of the variance explained by each fixed effect. We calculated 95% confidence intervals for our R^2 and semi-partial R^2 values using 100 parametric bootstrap iterations.

Results

Microbiome Abundance and Distribution

Results for microbial abundance are identical to those found in Johnson et al. (2021). *Firmicutes* and *Bacteroidetes* phyla accounted for 90% of all gut microbes at the phyla level and less than 10% accounted for the rest of all microbes. The dominance of two phyla across multiple individuals and social groups suggests low overall diversity of gut microbiota in this wild population of marmots.

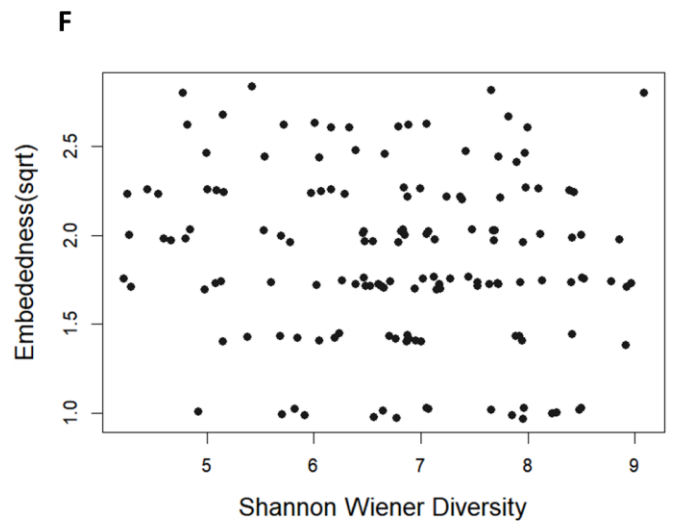
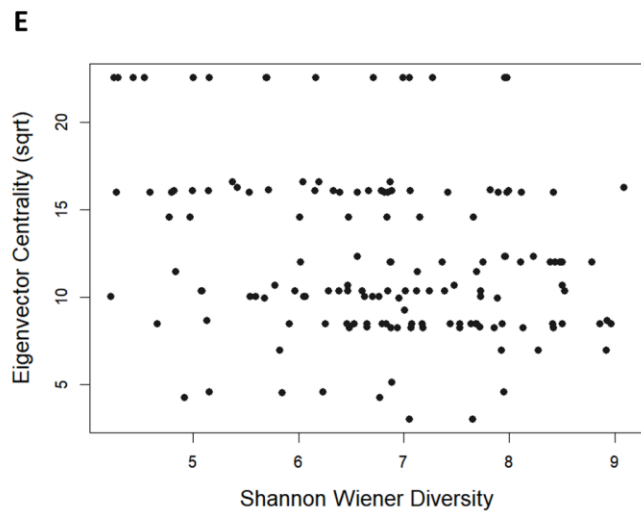
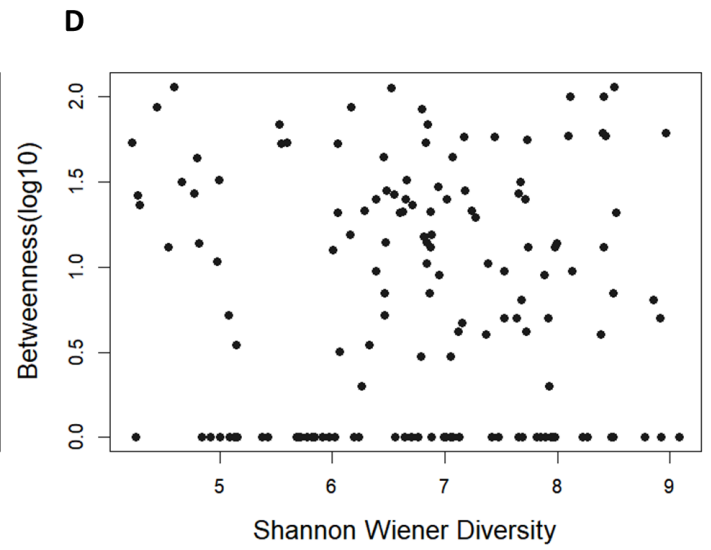
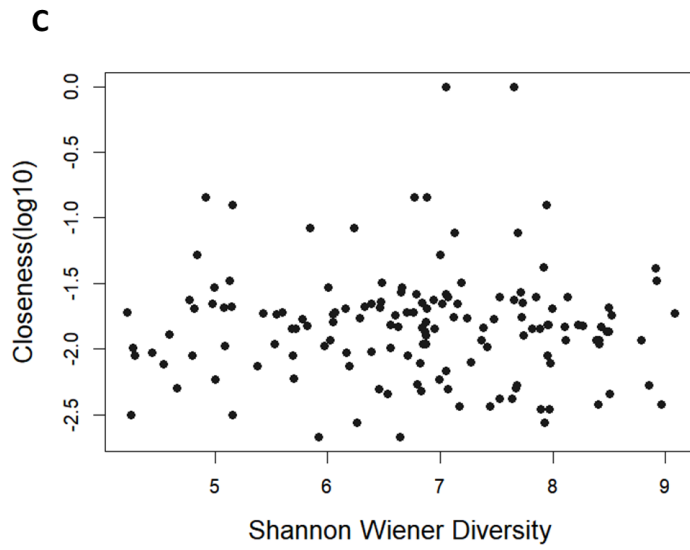
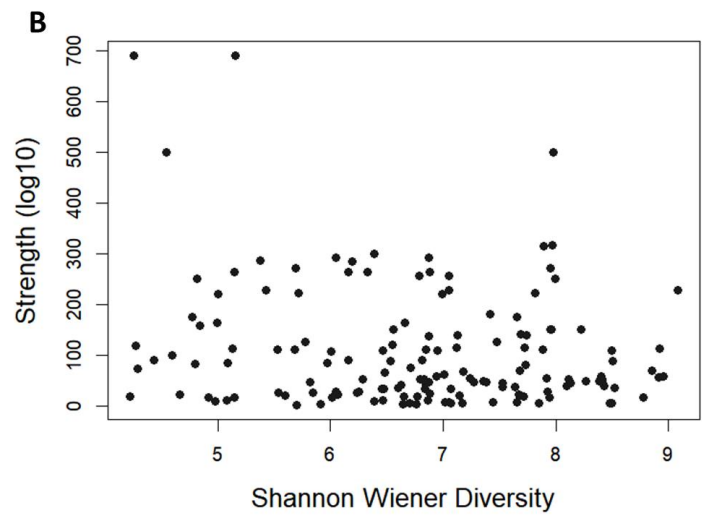
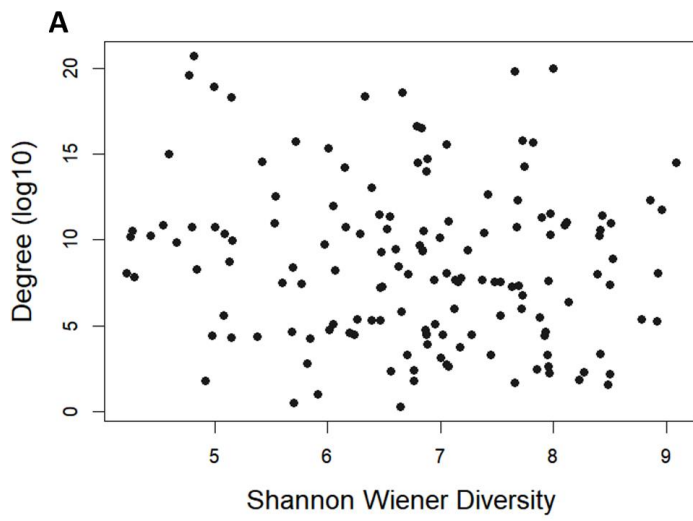
Gut Microbes Explain Variation in Some Social Network Measures

Seven social measures were fitted to linear mixed effect models as the dependent variable against Shannon Weiner diversity index for each individual, along with four covariates, which have been known to influence social behavior (Kroeger, Blumstein, and Martin, 2021; Van Vuren and Armitage 1991; Maldonado-Chaparro, Hubbard). Results indicated that fixed effects (marginal R^2) explained 39% of variation and the entire model (conditional part R^2) explained 81% of variation, indicating the confidence in the model to accurately explain the observed variation in the Shannon Weiner diversity index.

	Degree (log10)	Strength (log)	Closeness (log10)	Between- ness (log10)	Eigenvector Centrality (sqrt)	Embedded ness (sqrt)	Density (sqrt)
Shannon entropy	Est. 0.011 Part R ² : M. 0.000 C. 0.498	Est. 0.001 Part R ² : M. 0.000 C. 0.637	Est. -0.789 Part R ² : M. 0.000 C. 0.149	Est. -0.004 Part R ² : M. 0.000 C. 0.466	Est. -0.214 Part R ² : M. 0.009 C. 0.566	Est. 0.011 Part R ² : M. 0.000 C. 0.494	Est. -0.002 Part R ² : M. 0.000 C. 0.131
Age class (yearling)	Est. 0.234*** Part R ² : M. 0.192 C. 0.694	Est. 0.857*** Part R ² : M. 0.076 C. 0.713	Est. 0.003 Part R ² : M. 0.000 C. 0.146	Est. -0.014 Part R ² : M. 0.000 C. 0.466	Est. 0.431 Part R ² : M. 0.000 C. 0.557	Est. 0.410*** Part R ² : M. 0.159 C. 0.695	Est. 0.018 Part R ² : M. 0.000 C. 0.130
Valley location (up valley)	Est. 0.183** Part R ² : M. 0.104 C. 0.605	Est. -0.403 Part R ² : M. 0.020 C. 0.657	Est. 0.195*** Part R ² : M. 0.022 C. 0.172	Est. -0.028 Part R ² : M. 0.000 C. 0.464	Est. -3.025*** Part R ² : M. 0.075 C. 0.632	Est. 0.435*** Part R ² : M. 0.159 C. 0.656	Est. 0.094*** Part R ² : M. 0.000 C. 0.133
Group size	Est. 0.283*** Part R ² : M. 0.121 C. 0.623	Est. -0.302 Part R ² : M. 0.004 C. 0.641	Est. -1.00*** Part R ² : M. 0.547 C. 0.697	Est. 0.859*** Part R ² : M. 0.142 C. 0.609	Est. 5.303*** Part R ² : M. 0.111 C. 0.668	0.531*** Est. 0.531*** Part R ² : M. 0.138 C. 0.632	-0.615*** Est. -0.615*** Part R ² : M. 0.717 C. 0.853

Table 2. Parameter estimates of fixed effects from linear mixed effect models with semi-partial marginal and conditional R², with Shannon Weiner Diversity Index as the independent variable. Significance code: ‘***’ < 0.001 ‘**’ < 0.01 ‘*’ < 0.05 ‘.’ < 0.1.

After controlling for variation explained by other fixed effects, and while not statistically significant, Shannon Weiner diversity was negatively associated with four out of the six measures, with most indirect measures having negative associations (betweenness, eigenvector centrality) and most direct measures have positive associations (degree, strength). The average of variation explained by the Shannon Wiener diversity index across all social network measures was very modest (marginal semi-partial part R² = 0.01%). Fixed effects explained more of the variation among social network measures (Table 2; age class marginal semi-partial part R² = 6.6%; valley location marginal semi-partial part R² = 5.4%), with group size explaining the most amount of variation (marginal semi-partial part R² = 25.4%).



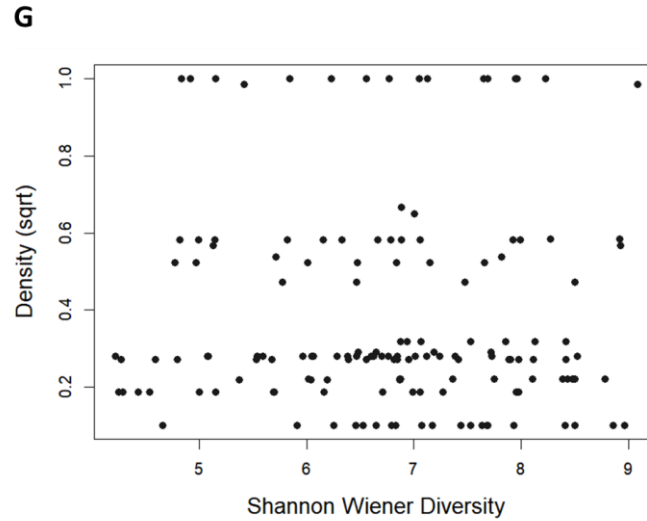


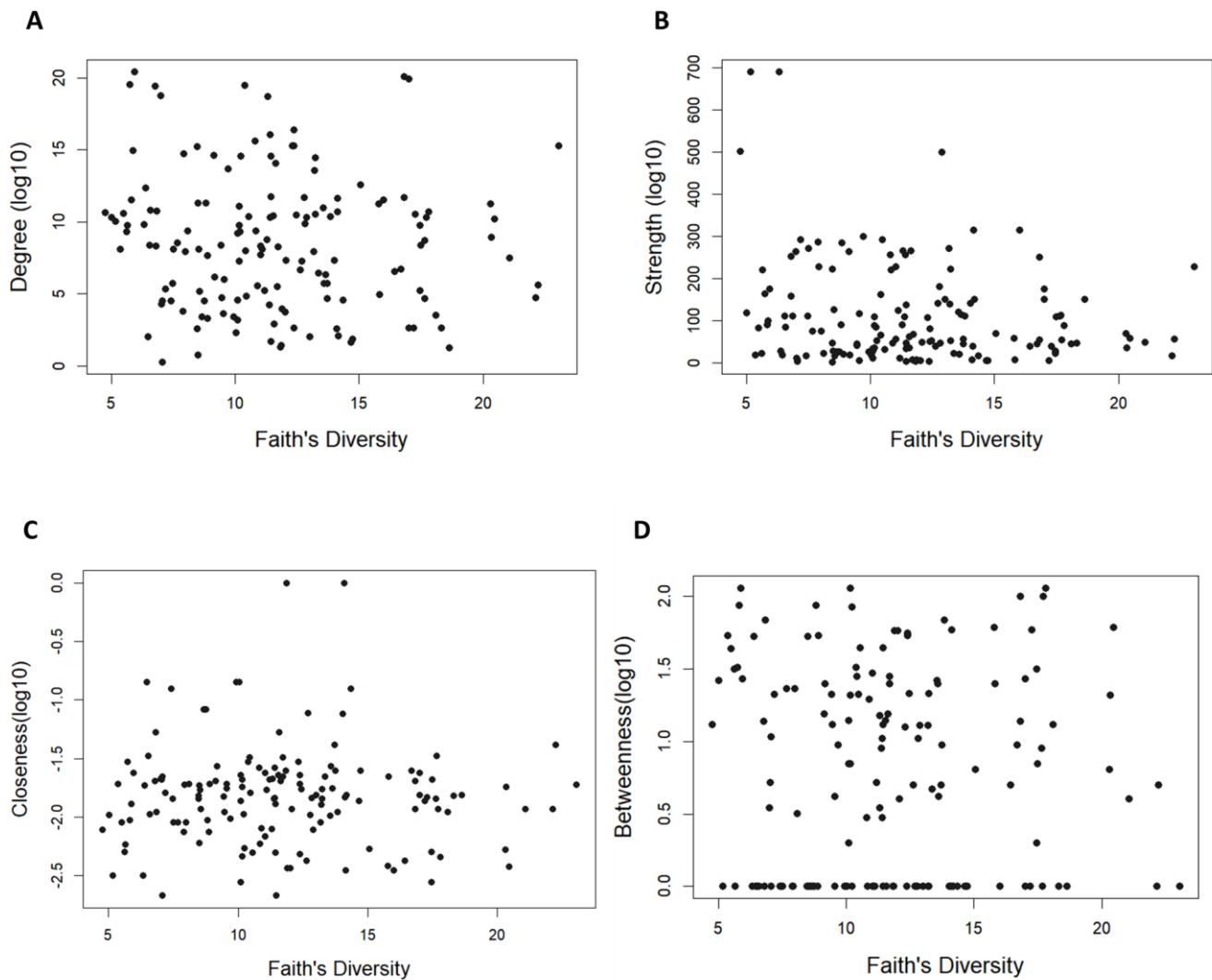
Fig. 2. The relationship between microbial diversity (Shannon Wiener diversity index) and social network position.

	Degree (log10)	Strength (log)	Closeness (log10)	Between- ness (log10)	Eigenvector Centrality (sqrt)	Embedded -ness (sqrt)	Density (sqrt)
Faith's Phylogenetic Diversity	Est. 0.234 Part R ² : M. 0.000 C. 0.496	Est. 0.002 Part R ² : M. 0.000 C. 0.637	Est. -0.004 Part R ² : M. 0.000 C. 0.141	Est. 0.001 Part R ² : M. 0.000 C. 0.465	Est. 0.048 Part R ² : M. 0.003 C. 0.564	Est. 0.004 Part R ² : M. 0.000 C. 0.493	Est. 0.000 Part R ² : M. 0.000 C. 0.135
Age class (yearling)	Est. 0.004*** Part R ² : M. 0.192 C. 0.690	Est. 0.857*** Part R ² : M. 0.192 C. 0.690	Est. 0.035 Part R ² : M. 0.000 C. 0.140	Est. 0.010 Part R ² : M. 0.000 C. 0.465	Est. 0.405 Part R ² : M. 0.000 C. 0.560	Est. 0.411*** Part R ² : M. 0.198 C. 0.694	Est. 0.018 Part R ² : M. 0.000 C. 0.132
Valley location (up valley)	Est. 0.181** Part R ² : M. 0.102 C. 0.600	Est. -0.406 Part R ² : M. 0.020 C. 0.657	Est. 0.198** Part R ² : M. 0.023 C. 0.167	Est. -0.028 Part R ² : M. 0.000 C. 0.463	Est. -3.023*** Part R ² : M. 0.076 C. 0.636	Est. 0.431*** Part R ² : M. 0.157 C. 0.653	Est. 0.097*** Part R ² : M. 0.000 C. 0.135
Group size	Est. 0.286*** Part R ² : M. 0.125 C. 0.623	Est. -0.301 Part R ² : M. 0.004 C. 0.641	Est. -1.00*** Part R ² : M. 0.550 C. 0.694	Est. 0.860*** Part R ² : M. 0.000 C. 0.609	Est. 5.249*** Part R ² : M. 0.012 C. 0.667	Est. 0.533*** Part R ² : M. 0.138 C. 0.633	Est. -0.615*** Part R ² : M. 0.717 C. 0.853

Table 3. Parameter estimates, marginal and conditional R² of fixed effects from linear mixed effect models explaining variation in network measures, with Faith's Phylogenetic Diversity Index as the independent variable. Significance code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Similar to the Shannon Wiener Diversity index, and after explaining variation accounted for by other fixed effects and recognizing that none of these relationships had a significant association,

Faith's phylogenetic diversity metric was negatively associated with closeness and eigenvector centrality. Betweenness and density, which were negatively associated in Shannon Wiener results, are slightly positively related when considering phylogenetic groupings in Faith's Diversity index. The average of variation explained by the Faith's phylogenetic diversity index across all social network measures was very modest (marginal semi-part part $R^2 = 0.05\%$). Fixed effects accounted for most of the variation in social network data (age class marginal semi-partial part $R^2 = 6.6\%$; valley location marginal semi-partial part $R^2 = 5.4\%$), with group size being highly significant for almost all measures and explaining most of the variation in the data (Table 3; marginal semi-partial part $R^2 = 24.2\%$).



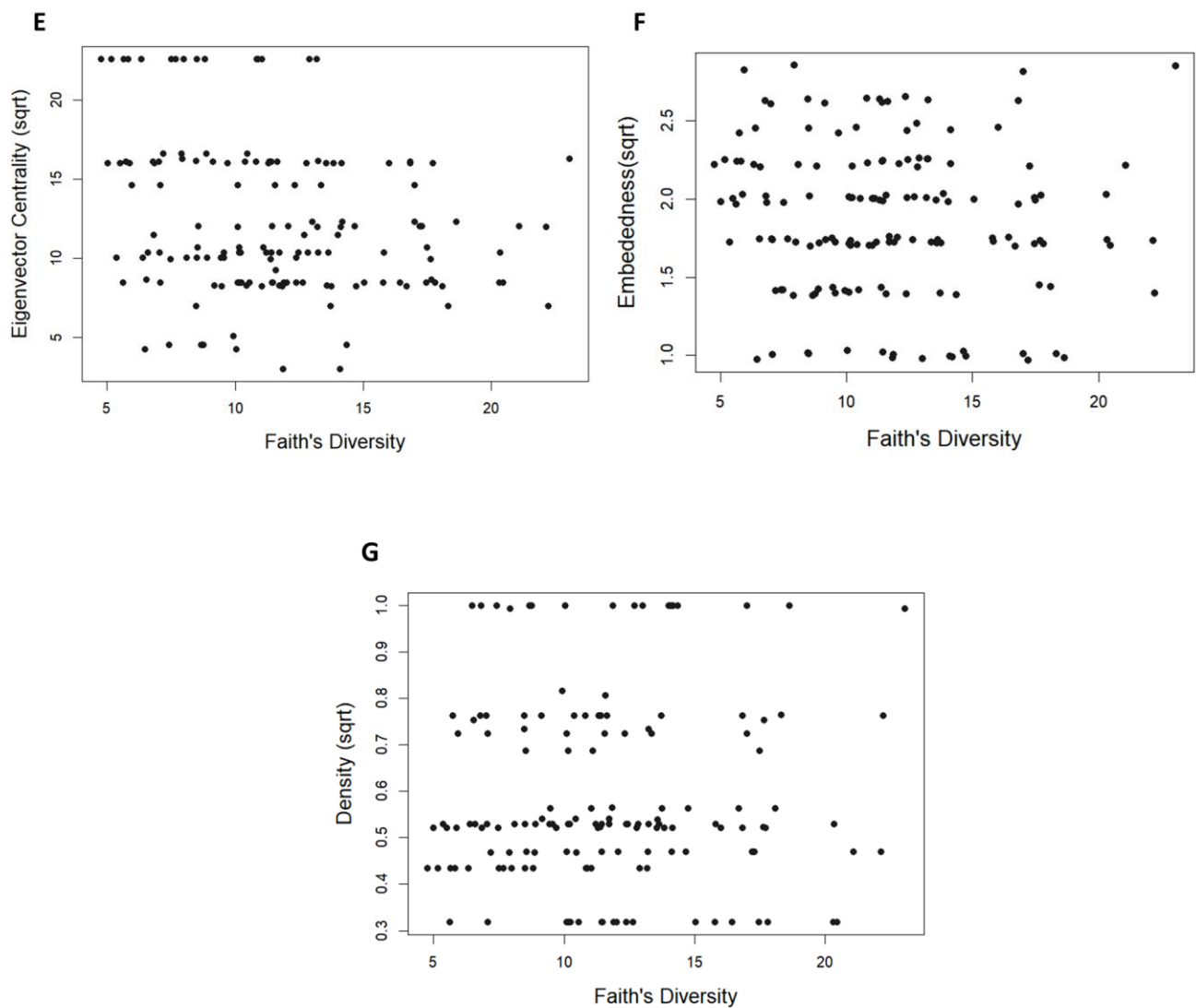


Fig. 3. The relationship between microbial diversity (Faith's phylogenetic diversity index) and social network position.

Discussion

In all, we found no significant interactions between microbial diversity and social network measures (both individual and network-level), indicating that microbial diversity does not drive sociality.

Previous studies have shown links between the relative abundance of particular microbes and fitness proxies in wild mammals, such as mass gain, defense against pathogens, and heat tolerance (Johnson et al., 2021, Oliver et al., 2003; Moeller et al., 2020). A relationship between

social behavior and fitness is also prevalent in marmots (Armitage, 2011; Blumstein, Wey, and Tang, 2009). However, marmots are facultative, not obligatory, social species (Armitage, 2019). Therefore, living in a social group is not always beneficial, and can be detrimental to marmot reproductive success and life longevity (Blumstein et al., 2018). Social behavior has been shown to be associated with external pressures, such as harsher physical environments and predation (Firman et al., 2020, Rubenstein, 1978). Our results corroborating these findings, indicating that an individual's propensity to be involved in social behavior is likely associated with an individual's external pressures, not internal composition.

These results also provide a wide variety of exploratory options for future research. For example, in order to understand the full extent of the relationship between the microbiome and social behavior, it will be crucial to test if microbial diversity is a consequence of social behavior. Thereby studying the opposing direction of the brain-gut axis. Social group living and kinship typically leads to individuals having a shorter range and decreases the likelihood of dispersal, and therefore, more socially connected individuals will be likely eating similar food resources (Blumstein, Wey, and Tang, 2009). Since diet is an important predeterminer of microbial composition and diversity, it will be important to understand this relationship to fully examine drivers of microbial content in marmot populations.

Finally, microbial composition, not diversity, has been shown to influence fitness in marmots and may also influence and be influenced by social behavior. Taking into account relative abundance data, and the abundance of key microbes that are known to influence behavior will also be crucial in determining this relationship. Previous studies have shown that *Lactobacillus* sp., *Bacteroides fragilis*, in addition to microbial families, *Lachnospiraceae* and *Ruminococcaceae*, influence social behavior in other species (Sharon et al., 2010; Hsiao et al., 2014; Gacias et al., 2016). In marmots, Firmicutes and Bacteriodes phyla, as well as *Ruminococcaceae*, *Lachnospiraceae*, *Muribaculaceae*, and *Rikenellaceae*, have been associated with fitness in marmots (Johnson et al., 2021). These microbes have been shown to have relative phyla dominance in marmot microbiomes, indicating low diversity at the phyla level (Johnson et al., 2021). However, using more specific levels such as family and genus of microbial taxonomic classification will provide more insight into general microbial composition in marmots, but also provide a clearer understanding of microbial diversity in marmots. Determining the abundance of microbes that have been shown to influence sociality can be calculated through Qiime2 and will allow us to quantify the effects of certain microbes on social network measures. Future research can also focus on these microbes as a baseline for what may influence other fitness correlates. In addition, this study compiled sequencing data from 2015 to 2018 and including more data, from the existing data from 2018 to 2021, may provide a more comprehensive understanding this relationship and take into account a greater range of individuals. Therefore, testing how relative abundance of these microbes might influence propensity to engage in sociality on a more comprehensive dataset will be vital to understanding the associations between these two fitness correlates. Furthermore, our results are purely correlative, meaning that experiments (Stevenson,

Buck, & Duddleston, 2014; Sommer et al., 2016) will be needed to understand the causation of these patterns. Future endeavors in this project will expand on these results and provide more concrete conclusions on how the brain-gut axis functions in marmots and provide more insight into the role of microbes in a facultative species.

Overall, gut microbes play important roles in life history and behavioral traits. Understanding these relationships in a wild species allows us to observe these changes over time and further our understanding of the brain-gut axis, and specifically, its relationship to fitness. Gaining insight into how microbes are changing due to external pressures allows us to further understand animal adaptations to different social scenarios, and by proxy, environmental conditions, both of which can aid in future conservation management plans of facultatively social species. Symbiotic gut microbes are crucial for all species and understanding the extent and importance of these interactions is necessary to further understanding of what influences fitness in a wild population.

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