

Migratory Metaorganisms: Gut Microbiome Structure and Function in Yellowstone's Elk

Eli Grossman

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Kartzinel Laboratory

Institute at Brown for Environment and Society;
Dept. of Ecology, Evolution, and Organismal Biology
Brown University

Thesis Advisor: Dr. Tyler Kartzinel

Second Reader: Dr. Daniel Weinreich

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May the work presented in this thesis one day find some use in the important process of building more just and sustainable relationships between all the human and non-human communities who share these lands today.

* <https://www.usgs.gov/observatories/yvo/news/land-burning-ground-history-and-traditions-indigenous-people-yellowstone>

** <https://www.nps.gov/yell/learn/historyculture/associatedtribes.htm>

*** <https://landacknowledgment.brown.edu/land-acknowledgment-statement>

Abstract

While understudied in migratory mammalian herbivores, the gut microbiome plays essential roles in ungulate nutrition, including energy harvest, plant metabolite detoxification, and vitamin synthesis. Here, we present evidence that the Yellowstone elk's migratory process is associated with a distinct elk gut microbial community composition characterized by metabolic changes relevant to host physiology. These changes include shifts in the gut microbiome's capacity to produce energy for the host in the form of short chain fatty acids, detoxify important plant defense compounds such as catechols and phenolics, and synthesize essential micronutrients including the vitamin thiamine. We use a neutral community model to identify sets of candidate keystone microbial taxa within migratory and non-migratory elk microbiomes, documenting common functions associated with these taxa while also tracking changes in microbial community structure between migratory and non-migratory host states. We propose the gut microbiome is a novel factor ecologists should consider when attempting to understand the nutritional ecology of elk migrations.

Keywords: migration, microbiome, elk, neutral community models, nutritional ecology

Introduction

Like many wild ungulate populations that support a mixture of migratory and non-migratory individuals, the Yellowstone elk exhibit a “partial migration” (Chapman, et al. 2011a; Chapman, et al. 2011b; Berg, et al. 2019; Zuckerman, et al. 2023). Classically, the evolutionary persistence of elk’s often risky and energetically costly migration strategy has been explained by individuals’ ability to access higher quality forage and avoid predators (Fryxell, et al. 1988). However, the proportion of Yellowstone elk migrating into higher elevation summer grazing ranges has declined in recent decades (Middleton, et al. 2013; Cole, et al. 2015). As Yellowstone’s most abundant large herbivore, dwindling elk migrations present the possibility of major impacts on the greater Yellowstone ecosystem, including changes to trophic structure (Henden, et al. 2014), nutrient transfer (Hobbs 1996), habitat connection (Shaw 2016), and disease spillover dynamics (Rayl, et al. 2021). Better understanding the Yellowstone elk migration is therefore a point of urgent conservation concern in a system of great scientific, cultural, and economic importance for diverse communities (Massey, et al. 2013).

Many factors such as genetics, physiology, social experience, predation, and environmental cues have been considered in modeling shifting elk migrations (Fryxell and Holt 2013; Berg, et al. 2019; Rickbeil, et al. 2019; Zuckerman, et al. 2023), but the role of the gut microbiome in the nutritional ecology of the migratory process has remained unexamined. This omission represents a potentially serious lacuna in our knowledge, especially given that ungulate herbivores rely on their microbiome for a suite of vital metabolic processes including energy harvest (Bergman 1990; Van Soest 1994), amino acid production (Newsome, et al. 2020), plant

secondary metabolite detoxification (Allison, et al. 1990; Allison, et al. 1992; Kohl, et al. 2014; Miller, et al. 2014; Kohl and Dearing 2016; Kohl, et al. 2016), and vitamin synthesis (Dearing and Kohl 2017). Remarkably, microbially derived short-chain fatty acids constitute up to 70% of host caloric intake in some ungulates (Bergman 1990). Evidence has emerged suggesting mammalian herbivore gut microbiomes help hosts adapt to changing food availabilities (Amato, et al. 2015; Sun, et al. 2016, Mallott, et al. 2022) and may even cue host foraging preferences (Trevelline and Kohl 2022). Conversely, observations that gut microbial communities exhibit a form of ecological “memory,” growing more efficient at utilizing nutrients upon repeated exposure, raises the possibility that a change in diet composition may impose a transient metabolic cost on the host via its gut microbial community (Tracy, et al. 2006; Letourneau, et al. 2022). Renewed appreciation for elk’s migratory flexibility (Eggeman, et al. 2016; Zuckerman, et al. 2023) and observations in other ungulates that gut community composition correlates with host grazing range (Wolf, et al. 2021) raises the prospect that the gut microbiome may be an important factor in both the initiation and the success of migratory behavior.

While prior microbiome studies in elk have documented community censuses (Kim, et al. 2019), responses to supplemental feeding (Couch, et al. 2021), and diagnostic uses for estimating host characteristics (Pannoni, et al. 2022), this study represents the first attempt to understand the host-microbiome interaction during a key part of an elk’s natural life history. Mammalian gut microbial communities are often thought to be structured by niche interactions (Jeraldo, et al. 2012; Harris, et al. 2017; Li, et al. 2018), and changing diets (Littleford-Colquehoun, unpublished data) in addition to other physiological/environmental differences (Berg, et al. 2019) associated with migration are likely to shift the distribution of niches within the elk gut. For example, prior observations of positive correlations between host diet and microbiome

dissimilarity (Muegge, et al. 2011, Kartzinel, et al. 2019, Littleford-Colquehoun, unpublished) seem to support the classic “nutrient niche hypothesis” (Freter, et al. 1983; Pereira and Berry 2017) in the context of changing wild ungulate diets. Thus, we hypothesize:

Environmental differences and host diets involved in migration will result in taxonomic (hypothesis 1) and functional differentiation (hypothesis 2) between the gut microbiomes of migratory and non-migratory elk.

Motivated by these initial hypotheses, we set out to better understand the community assembly of elk gut microbiomes and how this process changes based on host migration state. Leveraging both our taxa abundance/frequency data and our taxa-level functional predictions, we employed neutral community models (NCMs) (Hubbell 1979, 2001; Sloan, et al. 2006; Sieber, et al. 2019) to identify candidate keystone taxa dynamics within the elk gut microbiome.

"Keystone taxa," a concept originally coined in the macroecological literature (Paine 1969; Cottee-Jones and Whittaker 2012), has mainly been articulated in the microbial ecology context as describing species with an outsize influence on the structure and function of the microbial community (Banerjee, et al. 2018; Tudela, et al. 2021). Identifying taxa that meet this definition often requires manipulative experiments (Gutiérrez and Garrido 2019; Chng, et al. 2020) or *in silico* network analyses that can be complicated by competitive interactions between co-occurring species (Levy and Borenstein 2013; Banerjee, et al. 2019; Röttjers and Faust 2019; Blanchet, et al. 2020). Inspired by other definitions of keystone species that emphasize the impact of a species relative to its abundance (Power, et al. 1996; Cottee-Jones and Whittaker 2012), we used a simple “neutral” model of community assembly that assumes all species are

ecologically equivalent to identify outlier groups that likely participate in strong ecological interactions relative to their abundance.

Prior studies have speculated that these non-neutral “scarce but frequent” (SBF) taxa might participate in important species-specific interactions with the host or other microbial community members. While these authors have analyzed specific taxa identities (Sieber, et al. 2019; Heys, et al. 2020), phylogenetic distribution (Burns, et al. 2016), or overall community composition (Adair, et al. 2018) of this taxa fraction, our study presents the first attempt to systematically apply metabolic functional predictions to these taxa. We reasoned that the SBF taxa would include species occupying specialized metabolic niches that require significant energy investment while also performing important roles in community recruitment and function, thereby explaining these taxa’s low relative abundance and high occurrence frequency. In particular, we hypothesize:

Migration-associated changes in the selective environment of the gut microbiome will lead migratory and non-migratory communities to have distinct sets of taxa deviating from the neutral model (hypothesis 3).

These “scarce but frequent” taxa will be associated with metabolic functions involved in ecological processes that depart from neutral assumptions, such as primary complex polysaccharide degradation (hypothesis 4a) and cross-feeding interactions (hypothesis 4b).

Methods

Study Species and System

Our study area encompassed the northern and central regions of the Greater Yellowstone ecosystem (Figure S6). The non-migratory elk summer ranges consist of low-lying grasslands and shrublands near the park boundaries on a mixture of public and private lands (Whipple 2001; Gigliotti, et al. 2022). These grazing areas are characterized by anthropogenically modified environments including irrigated agriculture (Wilmers and Levi 2013) and introduced invasive species such as desert alyssum (*Alyssum desertorum*) (Whipple 2001). In the spring, migratory individuals follow a “green wave” of freshly emerging vegetation into higher elevation alpine meadows and lodgepole pine forests within the core of the park, remaining there until fall when they return to their winter range (Hebblewhite, et al. 2008; Merkle, et al. 2016; Middleton, et al. 2013; Rickbeil, et al. 2019; Zuckerman, et al. 2023).

Sample Collection and Laboratory Analyses

In collaboration with the National Park Service’s Yellowstone bison team , elk fecal samples from non-migratory (N = 41) and migratory individuals (N = 23) were collected opportunistically in late July and early August 2022. Non-migratory individuals were sampled around the towns of Mammoth (N = 19) and Gardiner (N = 22) at the northern entrance to the park, whereas migratory individuals were sampled throughout the park’s core, including Yellowstone Lake (N = 10), Hayden Valley (N = 4), Gibbons Meadows (N = 8), and Fire Hole (N = 1) (see Supplementary Fig S6, Supplementary Table 3). For each fecal sample, 3-5 pellets were collected and thoroughly mixed before being stored in sterile containers and frozen at -20°C until further analysis at Brown University (Providence, RI). DNA was extracted from

samples using Zymo Quick-DNA™ Fecal/Soil Microbe Miniprep Kit using the manufacturer's protocol (Zymo Research), along with four extraction blank controls.

Sequencing and Microbial Taxa Identification

We performed PCR targeting the V4 hyper-variable region of the bacterial 16S gene (Kozich, et al. 2013; Thompson, et al. 2017), Supplementary Text B). Amplicons were then sequenced on an Illumina MiSeq platform with 2 x 250bp chemistry at the University of Rhode Island's RI-INBRE Molecular Informatics Core. The resulting reads were demultiplexed and adapter sequences were removed for analysis via BaseSpace using standard Illumina FASTQ generation pipelines. Reads were subsequently processed using QIIME2 (Bolyen, et al. 2019), including denoising by DADA2 (Callahan, et al. 2016) and ASV phylogeny assignment by a classifier pre-trained on the SILVA rRNA database (Quast, et al. 2012; Bokulich, et al. 2018). Features only present in a single sample were removed prior to subsequent analyses, and features that were non-bacterial (archaea, chloroplasts, and mitochondria) were also removed, leaving 1656 ASVs across 64 samples. We consolidated feature tables, sample metadata, and taxonomic information into R using phyloseq objects (McMurdie and Holmes 2013; R Team 2023), then rarefied the ASV counts to even depth across all 64 samples (N = 8666 reads per sample for a total of 554,624 reads in our dataset covering 1635 unique ASVs).

Functional Predictions

Functional predictions of taxa and community-level metabolic pathway abundance were performed using the PICRUSt2 default pipeline (Douglas, 2020). Briefly, ASV representative sequences were aligned to a default reference database (Markowitz, et al. 2012) using HMMER (<http://www.hmmer.org>) and placed into a phylogenetic tree using EPA-NG (Barbera, et al.

2018) and gappa (Czech, et al. 2020). Subsequent analysis excluded all ASVs with a Nearest Sequenced Taxon Index (NSTI) of greater than 2 ($N = 6$), resulting in a sample-wide average NSTI of 0.36. The resulting tree contains both fully sequenced genomes of reference organisms and our sample ASVs. We then used this tree to predict ASV gene content using Castor (Louca and Doebeli 2017). To infer MetaCyc pathways based on Enzyme Commission (E.C.) gene families, PICRUSt2 uses MinPath (Ye and Doak 2009; Caspi, et al. 2015). The procedure calls pathways based either on total sample predicted gene content (“unstratified”) or on within-ASV predicted gene content (“stratified”). Across all samples, a total of 378 pathways were identified. Individual pathways were then placed into an eight-level pathway classification structure using a custom mapping file constructed from the MetaCyc database (see Supplementary Text A, Supplementary Table 1).

We performed differential abundance testing on the unstratified pathway feature table using Maaslin2’s log-transformed linear model approach on total-sum scaled normalized pathway counts (Mallick, et al. 2021). Prior to model estimation, we applied prevalence (30%) and relative abundance thresholds (0.1%), leading to the removal of 78 pathways. P-values were assigned based on the null-hypothesis of a zero pathway model coefficient, and we controlled the false discovery rate using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995). Differential abundance testing was performed at every pathway class level (Supplementary Table 1).

Diversity Analyses

We conducted alpha and beta diversity analyses on rarefied taxa and pathway data using the vegetarian (Charney and Record 2012) and vegan (Oksanen, et al. 2022) packages in R.

Unstratified pathway feature counts were rounded prior to rarefaction. We employed a suite of Hill number-based alpha diversity metrics (D) to quantify the effective number of species or pathways with varying sensitivity to relative abundance (p_i) (Hill 1973; Jost 2006, 2007):

$$\text{Richness } (^0D): \sum_{i=1}^S p_i^0$$

$$\text{Shannon's Diversity } (^1D): \exp(-\sum_{i=1} S p_i \ln p_i)$$

$$\text{Simpson's Diversity } (^2D): \frac{1}{\sum_{i=1}^S p_i^2}$$

Beta diversity was assessed by computing sample pairwise Bray-Curtis dissimilarities (Bray and Curtis 1957), where

$$d_{jk} = \frac{\sum_i \|x_{ij} - x_{ik}\|}{\sum_i (x_{ij} + x_{ik})}$$

For statistical evaluation of the differences between the compositional variance within and between migratory and non-migratory samples, PERMANOVAs using the adonis2 function (Anderson 2001; Anderson and Walsh 2013) and dispersion tests using the betadisper function (Anderson 2006) with 999 permutations were performed on the Bray-Curtis dissimilarity matrix. For visualization purposes, we used NMDS ordinations (Kruskal 1964).

Neutral Model

To explore microbial community assembly dynamics, we employed a neutral community model (Sloan, et al. 2006) implemented in Python (Sieber, et al. 2019) with custom modifications for comparison of two metacommunities. Briefly, the model assumes a birth-death process in a community of fixed population size (N), where individual deaths occur at a constant rate and are

replaced by either migration from a metacommunity source pool with probability (m) or reproduction of a local individual with probability (1-m). For our purposes, we assumed migratory and non-migratory samples constituted distinct host metacommunities. In the neutral assumption of ecological equivalence between species (Hubbell 1979, 2001), each species in the local community is selectively equivalent and the probability of migration is only proportional to that species' total relative abundance across the metacommunity (p_i), yielding the following transition probabilities:

$$P(N_i \rightarrow N_i + 1) = \frac{N - N_i}{N} (mp_i + (1 - m)\frac{N_i}{N - 1})$$

$$P(N_i \rightarrow N_i - 1) = \frac{N_i}{N} (m(1 - p_i) + (1 - m)\frac{N - N_i}{N - 1})$$

$$P(N_i \rightarrow N_i) = \frac{N - N_i}{N} (m(1 - p_i) + (1 - m)\frac{N - N_i - 1}{N - 1}) + \frac{N_i}{N} (mp_i + (1 - m)\frac{N_i - 1}{N - 1})$$

Assuming the community is at migration-drift equilibrium ($dp_i/dt = 0$ for all species), a diffusion approach has shown that each species' frequency in a local community should be described by a beta distribution parameterized by its overall relative abundance, p_i , and the community-wide migration rate, m (Sloan, et al. 2006):

$$\phi_i(x_i, N, p_i, m) = c(1 - x_i)^{Nm(1-p_i)-1} x_i^{Nmp_i-1}, c = \frac{\Gamma(Nm)}{\Gamma(Nm(1-p_i))\Gamma(Nmp_i)m}$$

which yields the truncated cumulative probability density function describing the probability that species i is observed in a given local community:

$$\mathbb{P}_i(\text{observed}) = \int_{\frac{1}{N}}^1 \phi_i(x_i, N, p_i, m) dx$$

We fit the remaining free parameter, m , to the empirical abundance and occurrence frequencies of our rarefied taxa data using non-linear least squares minimization (Newville, et al. 2016).

Goodness of fit was then assessed using the standard coefficient of determination:

$$R^2 = 1 - \frac{\sum_i (f_i - \Phi_i)^2}{\sum_i (f_i - \bar{f})^2}, \text{ where } \Phi_i \text{ is the expected occurrence frequency from the best-fit neutral model.}$$

We classified a species as a “non-neutral taxon” if it fell above or below the 95% confidence interval we constructed using a bootstrap procedure with 100 iterations. Non-neutral taxa were further partitioned into taxa whose occurrence frequency was greater than expected based on their total relative abundance, described elsewhere as the “above partition” fraction (Burns, et al. 2016; Adair, et al. 2018) and here as the “scarce but frequent” (“SBF”) taxa fraction.

Functions Associated with SBF Taxa

We developed a novel statistic to identify pathways that were enriched in specific members of an SBF taxa fraction (see Figure S7). Using the stratified PICRUSt2 output and lists of neutral and non-neutral taxa, we first calculated the relative pathway abundance contributed by SBF and non-SBF taxa for each pathway. We then divided each taxa group’s contribution by the number of taxa that contributed, helping to control for the greater numbers of taxa present in the neutral fraction. The resulting average per-taxa pathway abundance (C) for SBF contributors and non-SBF contributors were then used to compute that pathway’s “SBF Taxa Contribution Index” as:

$$\text{STCI} = \frac{C_{\text{SBF}} - C_{\text{non-SBF}}}{C_{\text{SBF}} + C_{\text{non-SBF}}}, \in [-1, 1]$$

Thus, a pathway predicted to be highly enriched only in a select subset of the SBF taxa should have an $\text{STCI} > 0$. For our discussion of SBF-associated functions, we choose to focus on pathways with an $\text{STCI} > 0.1$ based on the overall distribution of pathway STCI values (Figure S5).

Results

Microbiome Taxonomic and Metabolic Diversity Analyses

Consistent with hypothesis (1), there was a significant difference in microbial taxa composition between migratory and non-migratory sample groups (Fig 1a, PERMANOVA, pseudo- $F_{1,62} = 5.02$, $R^2 = 0.07$, $P \leq 0.001$). While some authors have suggested that increased taxonomic dispersion may be associated with disruptions in host-microbiome homeostasis (Zaneveld, et al. 2017), no significant differences in taxonomic dispersion between migratory and non-migratory communities were observed ($F = 0.33$, $P = 0.582$). Taxonomic richness does not differ significantly between the groups (Welch's T-test, $P = 0.275$), though non-migratory samples had a minor but significant increase in both Shannon diversity ($P = 0.045$) and Simpson diversity ($P = 0.039$), indicative that non-migratory samples have a more even distribution of their taxa (see Fig S1). Within both the migratory and non-migratory groups, sampling location explained an additional significant amount of variance (Fig S2bc, PERMANOVA, migratory: pseudo- $F_{2,19} = 2.85$, $R^2 = 0.23$, $P \leq 0.001$, non-migratory: pseudo- $F_{1,39} = 2.66$, $R^2 = 0.06$, $P = 0.001$), suggesting that factors such as host population structure and/or environmental heterogeneity within the migratory ranges or between the towns of Mammoth and Gardiner also contribute to between-host differences in gut microbiota composition.

The overall metabolic profile appears less differentiated than the taxonomic profile, mainly characterized by a dispersion increase in migratory pathway profiles (Fig 1b). While our PERMANOVA test returned a significant value (pseudo- $F_{1,62} = 4.06$, $R^2 = 0.06$, $P = 0.032$), the differences in dispersion ($F = 4.43$, $P = 0.031$, mean sample distance to group median, migratory: 0.097, non-migratory: 0.064) coupled with the uneven sampling scheme can increase

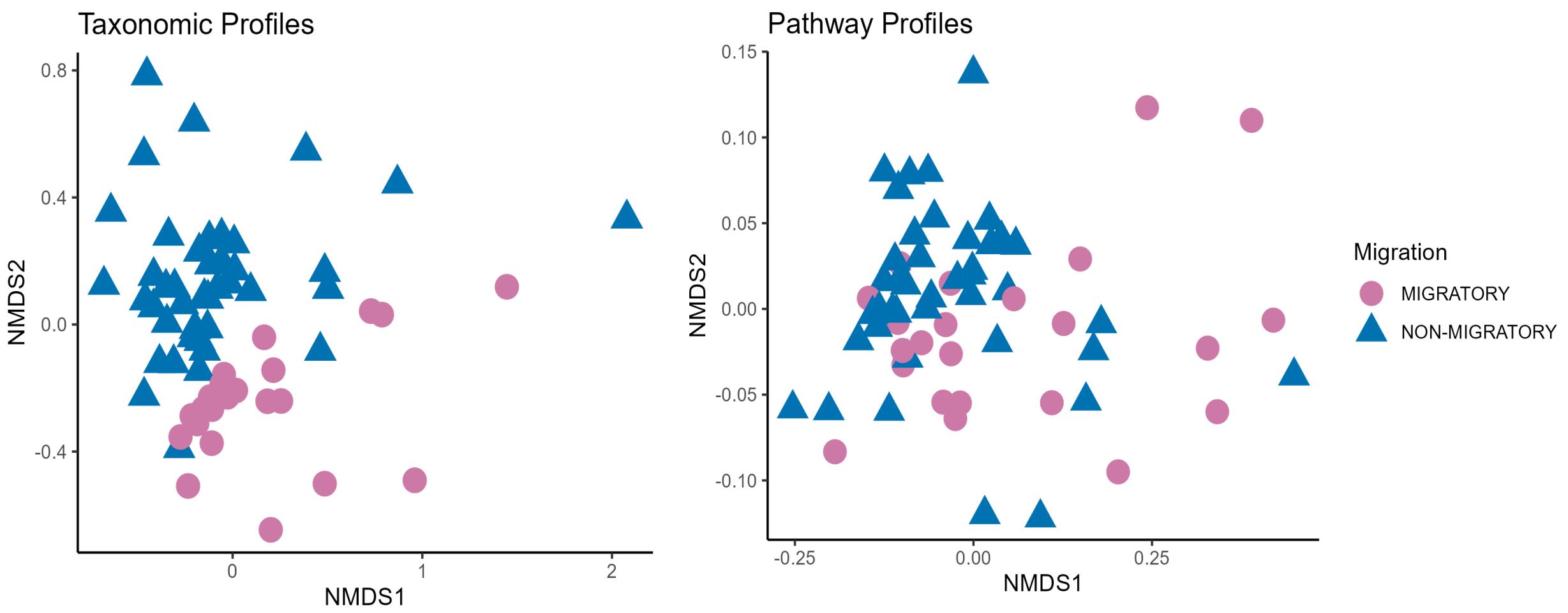


Figure 1. Migration Associated Differentiation of Elk Gut Microbiome Taxonomic and Metabolic Pathway Profiles. Bray-Curtis dissimilarities were calculated on sample taxa counts (A) and predicted metabolic pathway counts (B). NMDS ordinations were performed on the dissimilarity matrix (A: stress = 0.1671938, B: stress = 0.05103952). PERMANOVA tests found a significant effect of migration at both the taxonomic ($\text{pseudo-}F_{1,62} = 5.02$, $R^2 = 0.07$, $P \leq 0.001$) and metabolic level ($\text{pseudo-}F_{1,62} = 4.06$, $R^2 = 0.06$, $P = 0.032$), but dispersion differences likely explain most of the observed effect in the pathway case.

PERMANOVA's Type I error rate (Anderson and Walsh 2013). Migratory microbiomes were also predicted to have a modest but significant increase in pathway richness (Welch's Two-Sample T-Test, $P = 0.015$), Shannon diversity ($P = 0.025$), and Simpson diversity ($P = 0.033$) (Fig S1def). Similar to our observations in the taxonomic data, we found strong effects of sublocation on functional profiles within migratory samples (FigS2f, PERMANOVA: pseudo- $F_{2,19} = 9.66$, $R^2 = 0.50415$, $P \leq 0.001$). However, non-migratory samples did not exhibit the same trend (FigS2e, PERMANOVA: pseudo- $F_{1,39} = 2.22$, $R^2: 0.05$, $P = 0.096$).

Metabolic Pathways Associated with Migration State

While our diversity analyses suggest a lack of overall metabolic differentiation, these analyses aggregate hundreds of pathways, many of which likely indicate shared properties of gut microbial communities. To further understand the functional impacts of migration-associated taxa-level changes, we next explored how specific pathway classes differed between migratory and non-migratory groups. Consistent with hypothesis (2), we observed a suite of significantly differentially abundant pathways between the migratory and non-migratory samples, including pathways with likely impacts on host health and adaptation (Supplementary Table 1). For discussion in the main text, we choose to focus on pathway classes with an expected False Discovery Rate of less than one percent and clear host-relevant properties documented in the literature (Fig 2). All pathway class trends' direction and significance were robust to whether pathway calls were allowed between taxa in a sample ("unstratified," Fig 2) or only within single organism predicted genomes ("stratified," Fig S3).

In terms of known host-relevant pathways, we found migratory microbiomes had significantly higher predicted aromatic compound degradation (Fig2a, MetaCyc pathway class

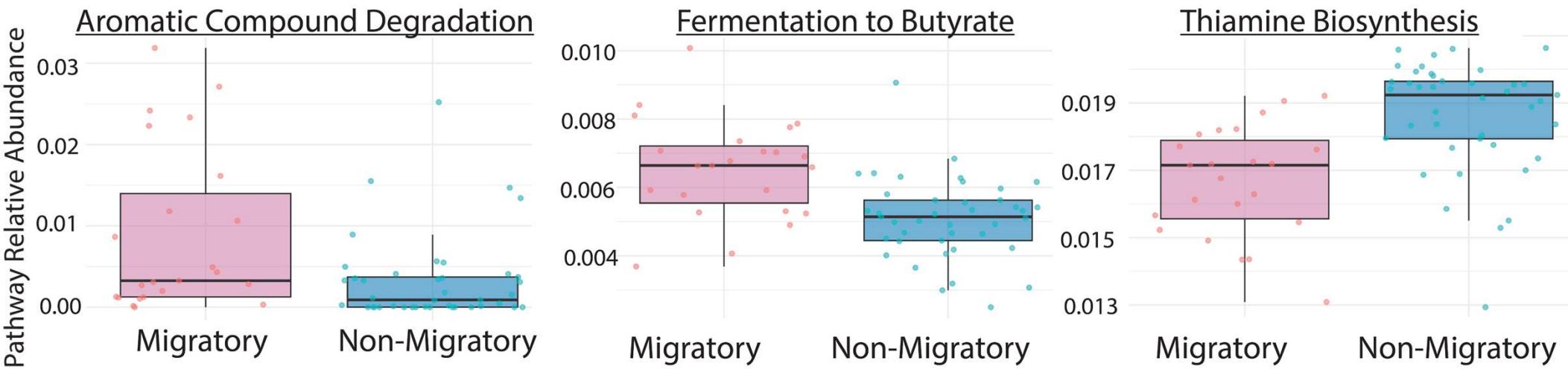


Figure 2: Host-Relevant Pathways Differ Between Migration Strategies. PICRUSt2 predictions of (A) Aromatic Compound Degradation (MetaCyc pathway class level 2, $P = 0.0024$, $q = 0.0098$), (B) Fermentation to Butyrate (MetaCyc pathway class level 4, $P = 0.0000036$, $q = 0.0004$), and (C) Thiamine Biosynthesis (MetaCyc pathway class level 4, $P = 0.000043$, $q = 0.0022$) relative pathway abundance.

level 2, $P = 0.0024$, $q = 0.0098$), including classes of compounds often associated with plant anti-herbivory secondary metabolites such as catechols (FigS4a, MetaCyc pathway class level 3, $P = 0.0019$, $q = 0.0145$) and phenolics (Fig S4b, MetaCyc pathway class level 3, $P = 0.0483$, $q = 0.1042$) (Kohl, et al. 2014; Divekar, et al. 2022). We also observed a significant increase in predicted butyrate production capacity in migratory samples (Fig2b, FigS4d, MetaCyc pathway class level 4, $P = 0.0000036$, $q = 0.0004$), an important pathway implicated in the efficiency of the fermentation process and subsequent body fat accumulation in ungulates (Shabat, et al. 2016; Peng, et al. 2021). In the non-migratory group, we observed an increase in microbial thiamine (Vitamin B1) biosynthesis (Fig 2c, Fig S4e, MetaCyc class level 4, $P = 0.000043$, $q = 0.0022$), a key nutrient source for ruminants in addition to their dietary thiamine intake (Schwab, et al. 2006; Pan, et al. 2018). Each of these trends were driven by multiple, but not always all, sub-locations within the migratory or non-migratory samples (Fig S4cde).

Microbial Community Assembly Dynamics

We grouped samples into metacommunities based on migration status, then ran a neutral community model (Sloan, et al. 2006; Sieber, et al. 2019) to identify taxa deviating from the neutral theory's predicted frequency/abundance relationship within a given metacommunity (Fig 3). Overall, we found the neutral model explained a significant amount of the observed taxa frequency/abundance patterns (Migratory $R^2 = 0.61$, Non-Migratory $R^2 = 0.69$), consistent with observations in the gut microbiomes of wild flies (Adair, et al. 2018) and juvenile zebrafish (Burns, et al. 2016), though not as high as the R^2 values reported for other, longer-lived mammals such as mice (Sieber, et al. 2019).

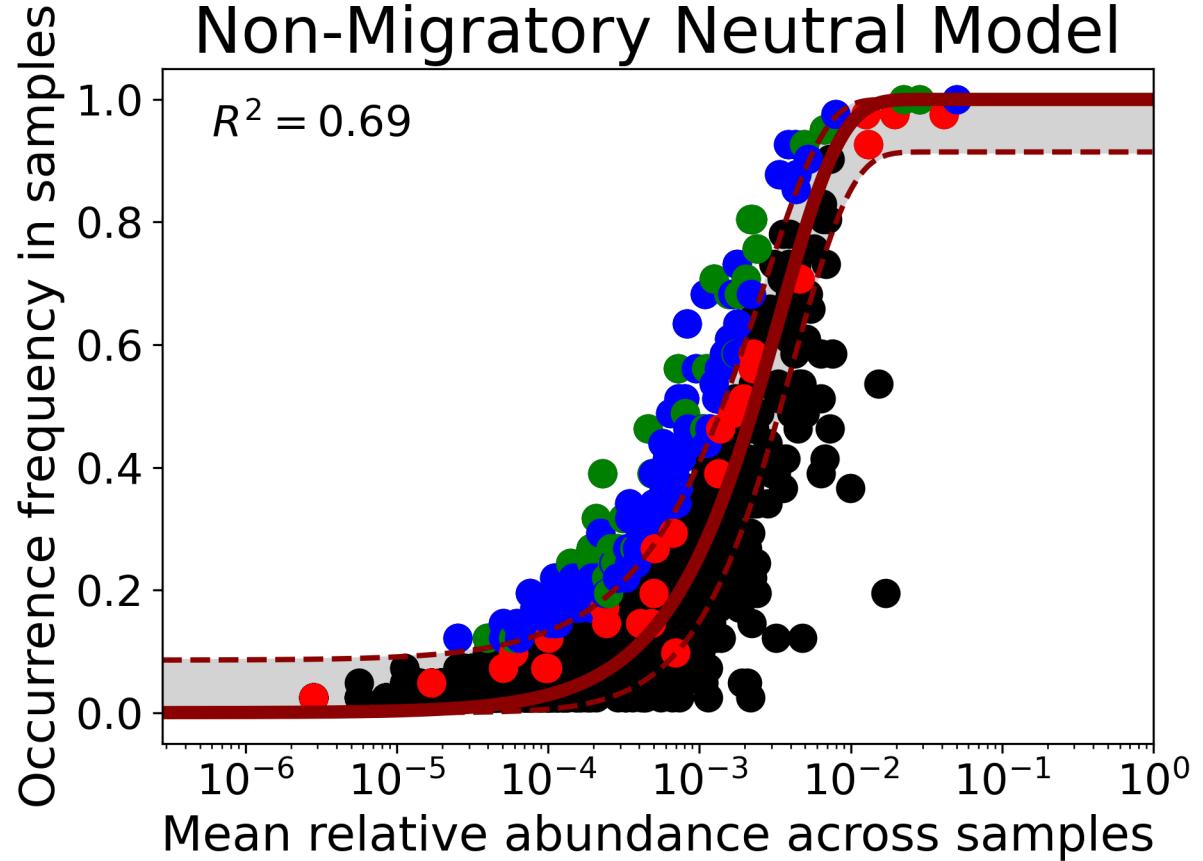
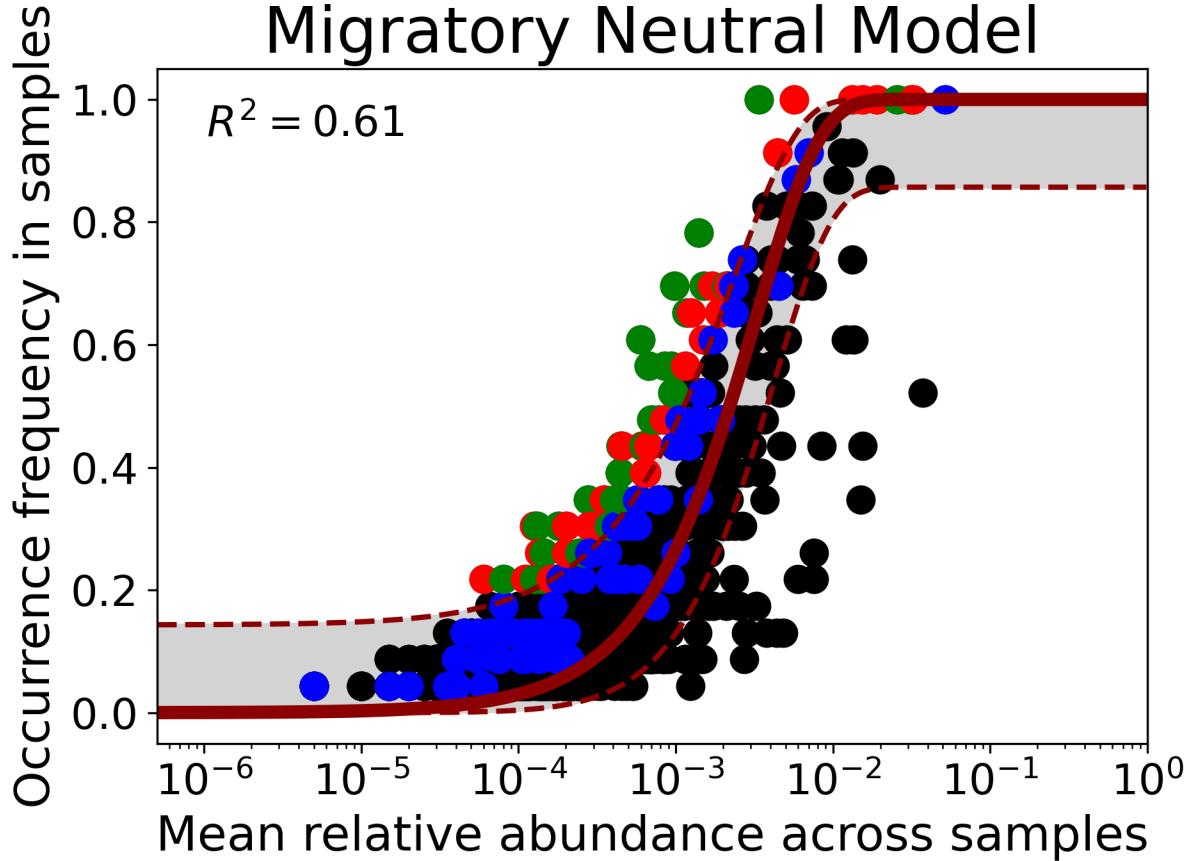


Figure 3: Neutral Community Model Results. (A) Migratory elk gut communities (fitted migration rate, $m = 0.0083$, $R^2 = 0.61$). (B) Non-Migratory elk gut communities ($m = 0.0079$, $R^2 = 0.69$). Red - taxa only scarce but frequent (SBF) in migratory samples, Blue - taxa only SBF in non-migratory samples, Green - taxa present in both sample groups' SBF fraction.

We then further investigated the fraction of taxa that occurred more frequently than expected given their overall relative abundance in the metacommunity, described here as “scarce but frequent” (SBF) taxa (Supplementary Table 2). We identified thirty-five taxa that fit this category in both migratory and non-migratory samples. Consistent with hypothesis (3), we also found an additional thirty SBF taxa in the migratory group and one hundred and two taxa in the non-migratory group that were not present in the other migration group’s SBF fraction.

Functional Profiles of Non-Neutral Taxa

We next scanned our pathways for functions associated with specific SBF taxa by computing the SBF Taxa Contribution Index (STCI) statistic (see Methods) for both the migratory and non-migratory SBF fractions. While most pathways are not associated (Figure S5), we identified a set of pathways uniquely enriched in migratory and/or non-migratory SBF taxa (average STCI of > 0.1 , Figure 4, Figure S8), including many relevant to proposed species-specific ecological interactions (hypothesis 4) that might produce a deviation from the neutral model.

Consistent with the keystone degrader hypothesis (4a), we find polysaccharide degradation pathway classes enriched in SBF taxa from both migratory and non-migratory samples, including starch degradation (Ze, et al. 2012) and mannan degradation (Moreira and Filho 2008; Solden, et al. 2018; Mafa and Malgas 2023). Following the cross-feeding hypothesis (4b) (Culp and Goodman 2023), we find aromatic amino acid biosynthesis pathways enriched in both SBF taxa groups, albeit in the form of slightly different pathways. Non-migratory SBF taxa are associated with pathways for tyrosine and phenylalanine biosynthesis, whereas migratory SBF are associated with pathways synthesizing the aromatic amino acid precursor chorismate

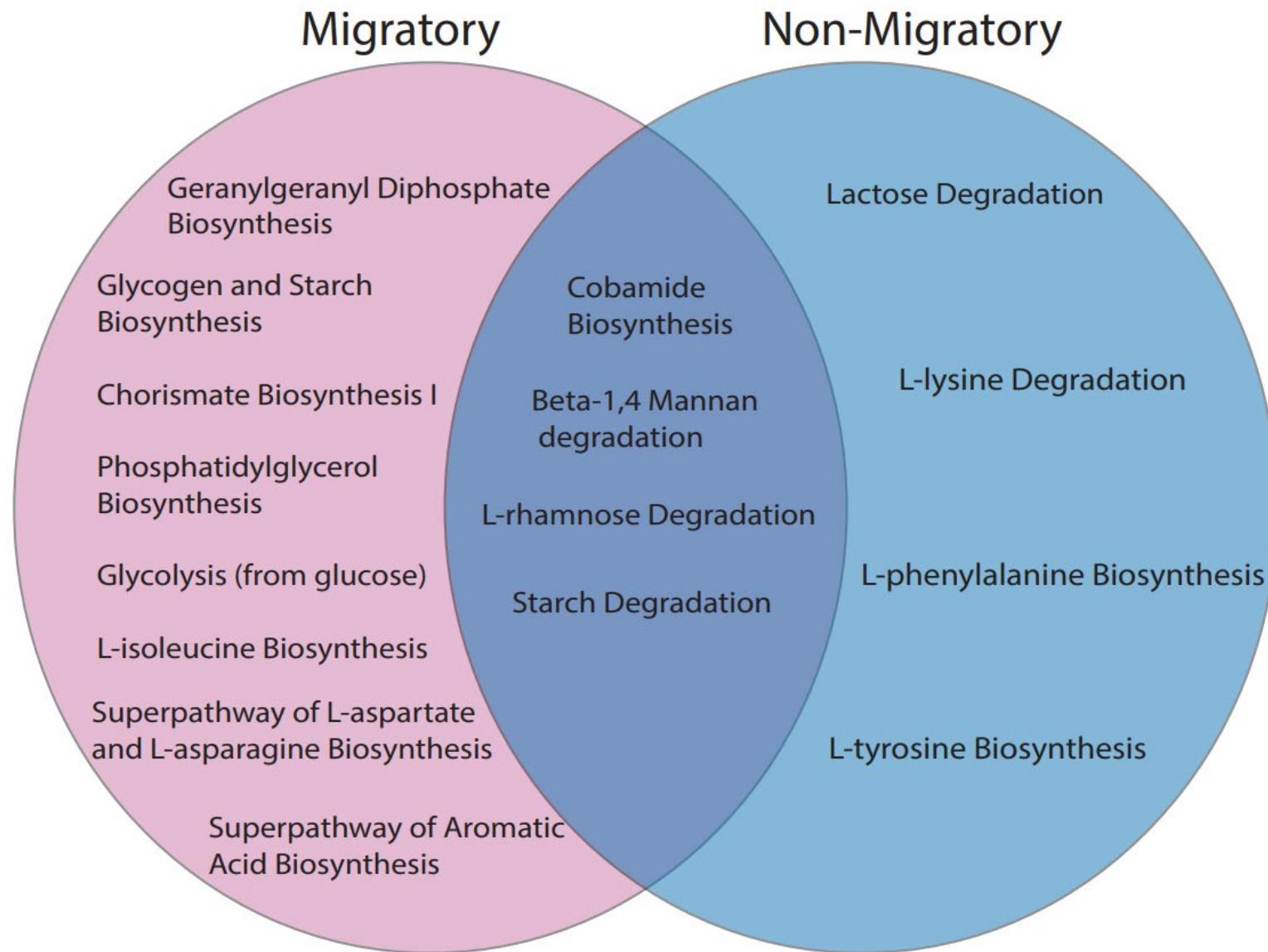


Figure 4: Pathway Classes Associated with SBF Taxa. MetaCyc pathway class level 4 features with average STCI scores greater than 0.1 for at least one migration group's SBF taxa fraction. Shared pathways were additionally required to have positive STCI values in the other migration group as well. For all pathway class STCI scores, see Fig S8.

and the MetaCyc superpathway “aromatic amino acid biosynthesis,” which includes tyrosine, phenylalanine, and tryptophan biosynthesis pathways (see Supplementary Text A). Similarly, another pathway associated with SBF taxa in both groups is cobalamin (Vitamin B12), an essential micronutrient thought to be required by many gut microbes (Degnan, et al. 2014; Patrick, et al. 2014).

Despite the high numbers of unique SBF taxa in each migratory group, there are comparatively few unique pathways associated only with one group. We do find isoleucine, aspartate, and asparagine biosynthesis uniquely associated with migratory SBF taxa, suggesting additional unique auxotrophies may be at play in the migratory microbiome. The upregulation of geranylgeranyl, a key precursor for terpenoid synthesis (Yamada, et al. 2015) might imply a role for microbial secondary metabolites in migratory non-neutral dynamics, while glycogen biosynthesis has been linked to organisms better adapted to short-term fluctuating environmental conditions (Strange 1968; Sekar, et al. 2020).

When comparing differentially abundant pathways to SBF taxa-associated pathways, we do not find a clear trend suggesting that the unique functions associated with a migration group’s SBF taxa are either more or less likely to be generally upregulated in that sample group (Figure S5b, Figure S8). These results suggest that the shifting SBF taxa fraction cannot directly account by itself for the changing functional profiles of the microbial communities.

Discussion

A Functionally Distinct Migratory Elk Gut Microbiome

Consistent with hypotheses (1) and (2), this study demonstrates that the Yellowstone elk migration is associated with a distinct elk gut microbial community composition characterized by metabolic changes relevant to host physiology. We therefore illustrate the stakes of understanding the ecology of the elk gut microbiome to the broader study of elk migration. While we focus our discussion below on microbiome functions of existing interest to the mammalian herbivore nutritional ecology literature, we also note that we have identified several additional differentially abundant pathways (Supplement S1) whose impact on host health remains to be explored.

One major constraint on herbivore foraging strategies are plant secondary metabolites (PSMs) (Freeland and Janzen 1974; Dearing, et al. 2000; Dearing, et al. 2005), and mammalian herbivores often rely on their gut microbiome's metabolic diversity to help process these complex toxins (Allison, et al. 1990; Allison, et al. 1992; Kohl, et al. 2014; Miller, et al. 2014; Kohl and Dearing 2016; Kohl, et al. 2016). In migratory elk, aromatic compound degradation pathways are enriched, including important plant PSM components such as catechols and phenols (Fig2a, FigS4ab, Van Soest 1994). Thus, migratory elk may rely on their gut microbiota to adapt to changing diet inputs on their summer ranges. More generally, migratory microbiomes are characterized both by modest increases in functional richness (FigS1) and greater variation between samples ("dispersion," Fig1b), consistent with microbiomes responding to their migratory hosts' increased environmental heterogeneity with greater functional diversity.

The connection between body fat and migration has also been well-established in the literature (Middleton, et al. 2013a, b) and more recent speculation has even linked ungulate gut microbiome composition to both host range and body fat (Wolf, et al. 2021; Pannoni, et al. 2022). Our finding that migration increases the predicted butyrate production capacity of elk gut microbiomes (Fig 2b) provides additional evidence that migratory hosts rely on their microbiomes to maximize the nutritional rewards of an otherwise taxing and risky migration strategy. By routing the flow of diet-derived carbon towards host-usable short-chain fatty acids and away from wasteful methane production, microbial communities with increased butyrate production have been associated with increased feed efficiency and subsequent host body fat accumulation (Turnbaugh, et al. 2006; Shabat, et al. 2016; Peng, et al. 2021). Additionally, butyrate production has been linked to gut homeostasis, as gut colonocytes rely on them as their primary source of energy (Dallas, et al. 2011). Given that the success of the Yellowstone elk's migration strategy relies at least partly upon maximizing feed efficiency while on the summer range (Hebblewhite, et al. 2008), our results suggest microbial butyrate production may be an important mechanism maintaining migratory behavior in these populations.

Following calls in the mammalian herbivore literature to consider microbiome services beyond caloric intake (Rabenheimer, et al. 2009; Dearing and Kohl 2017), our identification of thiamine biosynthesis as a pathway class enriched in non-migratory animals suggests migration-associated changes in the microbiome may also affect the ability of the elk host to access key micronutrients. Studies in dairy cows show that ruminants rely on microbially produced thiamine for 60-90% of their dietary intake (Schwab, et al. 2006; Pan, et al. 2018), indicating that changes in microbial thiamine production linked to migratory status may have non-trivial impacts on host health. Given the important role of microbially synthesized micronutrients to the ruminant host

(Dearing and Kohl 2017), our results suggest that integrating microbiome insights along with plant functional data will be a critical component of understanding the changing nutritional profiles of these migratory animals.

Scarce but Frequent Taxa as Candidate Keystone Species

Having established the potential importance of gut microbes to understanding elk migration, we attempted to better understand the community ecology of these microbiomes using neutral community models (NCMs). Our functional profiling of non-neutral taxa supports both our primary degrader (4a) and cross-feeding (4b) hypotheses, providing additional evidence that specific species-species ecological interactions drive some of the SBF taxa's deviation from neutral expectations. As such, our study should help inform future efforts to understand how important low-abundance community members impact the structure and function of the elk gut microbiome (Jousset, et al. 2017).

Primary degraders of recalcitrant plant polymers form a distinct trophic level in the gut that regulates downstream community structure (Soden, et al. 2018; Gralka, et al. 2020; Culp and Goodman 2023). Some authors suggest that specialist primary degraders play keystone roles in gut microbial communities by breaking down complex polysaccharides and releasing simple sugars to primary fermenters, creating a variety of exploitative, commensal, and even mutualistic interactions with other community members (Ze, et al. 2012; Rakoff-Nahoum, et al. 2016; Cartmell, et al. 2018; Feng, et al. 2022). Our finding (Fig 4) that SBF-taxa in both migratory and non-migratory elk are associated with the degradation of important plant polysaccharides supports our hypothesis (4a) that this taxa fraction includes these primary degrader specialists. For example, one SBF-associated pathway was mannan degradation, a key component of

hemicellulose (Moreira and Filho 2008) and found to be the second most abundant polysaccharide in the winter forage of wild moose (Soden, et al. 2018). Likewise, one of the first uses of “keystone species” in the gut microbial ecology literature was to describe starch degradation (Ze, et al. 2012), another function we found to be associated with SBF taxa.

Other cross-feeding interactions involving prototrophs and auxotrophs for various amino acids and key micronutrients like vitamins have also been found to play important roles in gut microbial community assembly (Escriva, et al. 2022, Culp and Goodman 2023). Supporting the relevance of these interactions to the SBF taxa fraction (hypothesis 4b), we find aromatic amino synthesis is associated with SBF taxa in both migratory and non-migratory hosts. Given aromatic amino acids’ high expected synthesis cost (Akashi and Gojobori 2002) and widespread auxotrophy among gut microbiota (Starke, et al. 2023), it is not unreasonable to expect that a community’s prototrophs may be more likely to be found at lower abundance yet also remain critical for that community’s function. Similarly, cobalamin (Vitamin B12) biosynthesis is metabolically expensive, constricted to only certain microbes, and associated with SBF-taxa from both migration groups (Degnan, et al. 2014; Patrick, et al. 2014; Magnúsdóttir, et al. 2015; Culp and Goodman 2023).

Distinct Microbiome Community Compositions: Dispersal Limitation or Ecological Selection?

Overall, our functional and modeling results are consistent with host migration introducing the elk gut microbiome to a novel selective environment that reshapes the gut microbial community via deterministic changes in species’ fitnesses (Vellend and Agrawal 2010; Vellend 2016).

Migration-induced dispersal limitation coupled with ecological drift could also theoretically lead to community differentiation at the taxa-level (Antwis, et al. 2018; Couch, et al. 2020; Stothart, et al. 2021). However, our evidence of distinct functional differences between migratory and non-migratory microbiomes suggests that ecological selection also plays a role in the observed differences in community composition. There is no evidence in the mammalian gut microbiome literature suggesting that drift divergence can result in community-level functional differences, consistent with reports of widespread metabolic redundancy amongst members of the species-rich gut microbial community (Turnbaugh, et al. 2009; Burke, et al. 2011; Levy and Borenstein 2013; Louca, et al. 2016; Louca and Doebeli 2017; Zhou and Ning 2017; Louca, et al. 2018). Thus, previously observed diet-microbiome taxa composition correlations in our dataset (BL unpublished) may be at least partially due to ecological selection as opposed to artifacts of dispersal limitation-related spatial (auto)correlation.

The large numbers of unique taxa in each of the migratory and non-migratory SBF taxa fractions support the hypothesis that distinct ecological interactions occur depending on host migration status (hypothesis 3, Fig 3). While the migratory and non-migratory SBF taxa fractions share many important functions (see above), they are also associated with unique functions as well. These unique SBF-associated functions suggest that some axes of strong ecological interactions only occur in certain community states, indicating a larger migration-associated shift in microbial community structure. Thus, NCMs coupled with functional data provide further evidence of migration-associated changes in the ecological interactions structuring the microbial community.

Ecological Selection: Environmental Filtering vs. Biotic Interactions

We carefully note the difference between the (supported) claim that functional differentiation accompanying taxonomic composition indicates the action of ecological selection and the (unsupported) claim that new biotic interactions arising from these taxa changes are responsible for the observed functional differentiation. Instead, functional changes in microbiomes have often been interpreted through the functional guild framework (*sensu* Louca, et al. 2018), where shifting environmental physicochemical filters control the presence and abundance of large groups of functionally equivalent taxa groupings (Levy and Borenstein 2013, 2014; Vieira-Silva, et al. 2016). In the context of the gut microbiome, such changes might look like new diet inputs altering the available nutrient supply/stoichiometry (“Nutrient Niche Hypothesis,” Freter, Brickner, Botney, et al. 1983; Pereira and Berry 2017) or host passage rates shifting the thermodynamic balance of different metabolic strategies (Vieira-Silva, et al. 2016).

Indeed, microbial neutral models were originally proposed in the functional guild framework (Sloan, et al. 2006), and the relatively good fits for our neutral models within each migratory/non-migratory metacommunity have been used by other workers to indicate that environmental filtering is the dominant form of ecological selection (Sieber, et al. 2019). However, recent work has shown that high density species-species interactions can also produce “neutral” frequency-abundance patterns (Chisholm and Pacala 2010; Zapién-Campos, et al. 2022), and so our NCM should better be understood as a useful taxa identification strategy rather than proving that the neutral taxa fraction is not participating in important, even “keystone,” species-species interactions that impact overall community function.

Future Directions

Further work will be needed to parse out the relative roles of dispersal-limitation/drift and ecological selection in driving the taxonomic and functional differentiation associated with elk migration. Similarly, whether that ecological selection takes the form of environmental filtering (Levy and Borenstein 2013; Louca, et al. 2018; Sieber, et al. 2019) or a variety of species-species interactions such as predation by protozoa (Wildschutte, et al. 2004), infection by phage (Manrique, et al. 2016; Louca and Doebeli 2017), colonization/competition trade-offs (Livingston, et al. 2012; Yawata, et al. 2014; Adair, et al. 2018; Custer, et al. 2022; Wetherington, et al. 2022; Ontiveros, et al. 2023), niche construction (Bittleston, et al. 2020; Gralka, et al. 2020; Estrela, et al. 2022), priority effects (Freter, Brickner, Fekete, et al. 1983; Burke, et al. 2011; Levy and Borenstein 2013; Furman, et al. 2020), host immune suppression (Coyte, et al. 2015), or cross-feeding (Solden, et al. 2018; Goyal, et al. 2021; Culp and Goodman 2023) remains an open question, as is how these changing biotic interactions lead to functional differentiation. For example, to what extent do the changing unique interactions associated with SBF taxa result from, lead to, or are orthogonal with host-relevant functions that are enriched in a particular migratory host state?

Employing more advanced metrics designed to quantify the relative contribution of ecological selection compared to dispersal-limitation/drift (Stegen, et al. 2012; Stegen, et al. 2013; Zhou and Ning 2017; Gibert and Escarguel 2019; Ning, et al. 2019) should help further clarify the dominant ecological forces re-shaping the elk gut microbiome during migration. Likewise, leveraging metagenomic and metabolomic functional data in experimental designs explicitly testing a selective pressure (Krizsan, et al. 2018; Solden, et al. 2018; Peng, et al. 2021) will shed more light into understanding how and to what extent elk gut microbiomes respond to different selective pressures associated with their host's migratory strategy.

Conclusion

By studying gut microbial assembly processes in the context of functional changes relevant to a wildlife behavior of conservation concern, we aimed to contribute to the development of microbial ecology into a predictive science (Zhou and Ning 2017) capable of informing conservation practice (Trevelline, et al. 2019). From a microbial community ecology perspective, this study has shown that neutral models from macro-ecology can be used to identify groups of microbial taxa likely to be involved in strong species-species interactions, including specialist primary degraders and prototrophs of key micronutrients required by other members of the gut community. From an ungulate ecology perspective, our study adds the gut microbiome as a novel player to the long list of genetic, physiological, social, and environmental factors (Berg, et al. 2019) ecologists should consider when attempting to understand how elk populations support partial migrations. In sum, the Yellowstone elk provide an excellent model to synthesize microbial and macro-ecological approaches into a more complete understanding of metaorganism migration.

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Supplementary Methods Text

A. MetaCyc Pathway Class Assignment

To conduct functional analysis at multiple levels of abstraction, we assigned each metabolic pathway predicted by PICRUST2 to an eight-level classification structure based on that pathway's MetaCyc annotations. This scheme allowed us to compare biologically relevant pathway classes ("Fermentation to Butyrate" or "Aromatic Compound Degradation") to other classes at a similar level of functional abstraction for the purposes of differential abundance testing or other analyses. However, it should be noted that MetaCyc's native pathway class structure is not strictly analogous to a taxonomic phylogeny. Thus, we made several modifications and assumptions in order to create a quasi-taxonomic scheme for the metabolic pathways.

First, not all MetaCyc pathways are assigned to the same number of parent classes. Currently, MetaCyc's pathways have at most seven parent classes, hence our eight-level classification structure. Some pathways do not have any other parent classes beyond the most abstract levels (i.e. "Biosynthesis") due to their unique metabolic functions. To assign these pathways to lower levels of functional classes, we created a monotypic class at each subsequent level where the pathway did not have an annotated parent class in MetaCyc (Supplementary Table 1). For example, the pathway "Choline Biosynthesis III" (PWY3561) is translated from MetaCyc as follows:

Original MetaCyc Annotations:

"Biosynthesis", "Polyamine-Biosynthesis: Amide, Amidine, Amine, and Polyamine Biosynthesis", "Choline-Biosynthesis", "choline biosynthesis III"

New Pathway Scheme (added monotypic classes underlined):

“Biosynthesis”, “Polyamine-Biosynthesis: Amide, Amidine, Amine, and Polyamine Biosynthesis”, “Choline-Biosynthesis”, “Choline Biosynthesis - choline biosynthesis III”, “choline biosynthesis III”

We found class levels three and four were often the most biologically informative, controlling for multiple different pathways with similar effects while remaining specific enough to provide functional insight. For the number of instances in each class level for our dataset, see Supplement Table 1.

Second, MetaCyc will sometimes assign pathways to multiple pathway classes (analogous to assigning a species to two different genera or a single metabolic reaction to two different E.C. numbers). For example, the pathway pyridoxal 5'-phosphate biosynthesis I (ID: PYRIDOXSYN-PWY) is classified in MetaCyc as both:

Classification Chain 1: “Biosynthesis,” “Cofactor, Carrier, and Vitamin Biosynthesis,” “Enzyme Cofactor Biosynthesis,” “Vitamin B6 Biosynthesis,” “pyridoxal 5'-phosphate biosynthesis I”

Classification Chain 2: “Biosynthesis,” “Cofactor, Carrier, and Vitamin Biosynthesis,” “Vitamin Biosynthesis,” “Vitamin B6 Biosynthesis,” “pyridoxal 5'-phosphate biosynthesis I”

Pathways were restricted to only one classification chain in our analyses, with the longer, more detailed chain chosen as a default. If both classification schemes for the pathway were equally detailed (as above), we chose one at random.

Third, MetaCyc treats pathways and “superpathways” (pathways composed of a base pathway plus additional reactions or pathways) as identical levels of objects, each with unique pathway ID numbers (Caspi, et al. 2015). This means that even the base unit

“pathway” called by PICRUST2 sometimes varies in abstraction. Additionally, PICRUST2’s pathway calling procedure uses an older version of the MetaCyc database than this paper’s compiled map files (accessed 1/11/2024), so a few pathways were not properly annotated (‘not found’) since their pathway ID numbers have been changed.

The pathway mapping structure proposed here can be applied to any MetaCyc pathway data analysis, including pathway counts inferred directly from metagenomic data (ie Beghini, et al. 2021). Indeed, most previous studies using MetaCyc functional data only focus on either extremely abstract functional categories (i.e. “Biosynthesis” or “Amino Acid Degradation”) or very specific pathways (Brown, et al. 2020; Karp, et al. 2022; Brown, et al. 2023). Thus, our technique for analyzing MetaCyc pathway changes across an extended range of functional abstraction may be useful for a wide range of future studies seeking to leverage MetaCyc functional data from PICRUST2, metagenomics, or other sources. While sufficient for our present analyses, subsequent work will be required to curate the MetaCyc mapping files constructed using this paper’s rough and ready rules-based approach into a comprehensive pathway classification system akin to E.C. numbers for genes or the Linnaean taxonomy for species.

B. PCR Protocol

We used standard V4 metabarcoding primers (Caporaso, et al. 2011; Kozich, et al. 2013) to perform PCR using AccuPrime Pfx Supermix (Thermofisher). We included three negative (nuclease-free water) and three positive PCR controls (ZymoBIOMICS Microbial Community DNA Standard). To enable subsequent sequencing, primers were modified slightly to include Illumina Nextera Transposase adapters. Prior to sequencing, we checked amplicons on 1.5%

agarose gels for contamination during extraction and PCR. Amplicons were then multiplexed using the Illumina P5/P7 adapters in a second round of PCR prior to sequencing at the RI-INBRE Molecular Informatics Core. Samples in each sequencing batch were pooled into a sequencing library at equimolar concentrations and sequenced on the Illumina MiSeq platform with 2 x 250 bp paired-end chemistry.

Forward Primer (515F): (5' GTGCCAGCMGCCGCGGTAA 3')

Reverse Primer (806R): (5' GGACTACHVGGGTWTCTAAT 3')

Reactions:

Reagent	Desired Final Concentration 1X	
Accuprime Master Mix	2 U/rxn	17.0 ul
515F (10uM)	0.2 uM	2.0
806R (10uM)	0.2uM	2.0
DNA template	1-500 ng	1.0
Total Rxn		22.0

Thermocycler Protocol:

Step #	Temp (°C)	Time
1 cycle	95	5'
35 cycles	95	30"
--	55	30"
--	72	30"
1 cycle	72	10'
HOLD	4	HOLD

Supplementary Methods References

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Supplementary Figures

Taxonomic Richness

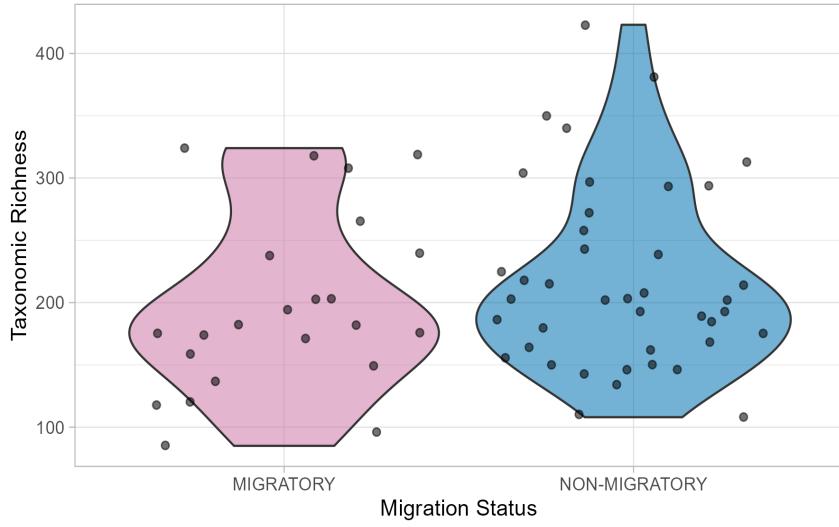


Figure S1a

Taxonomic Shannon's H

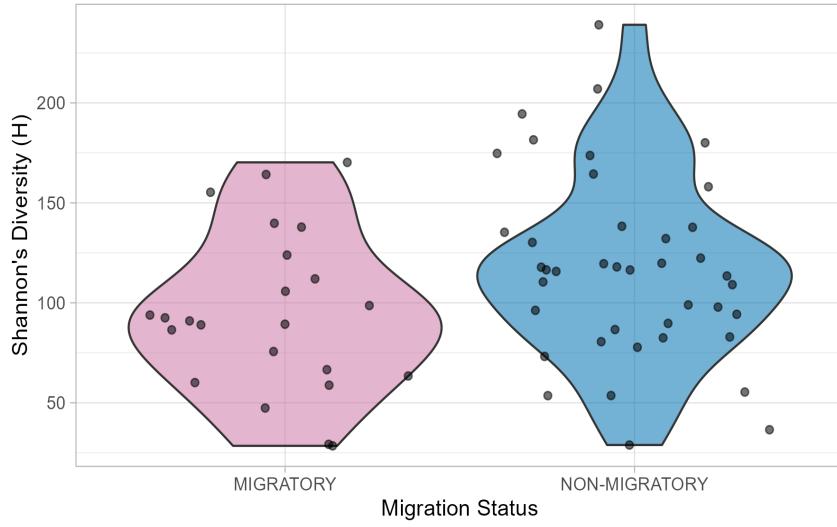


Figure S1b

Taxonomic Simpson's D

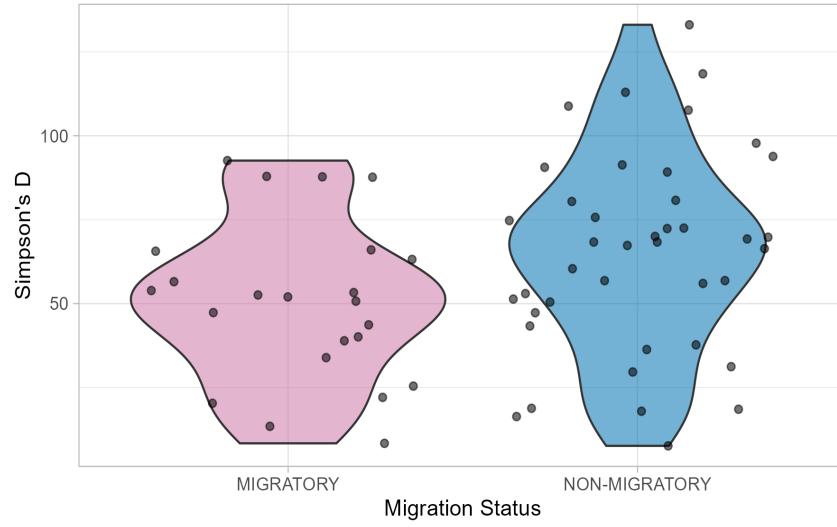


Figure S1c

Pathway Richness

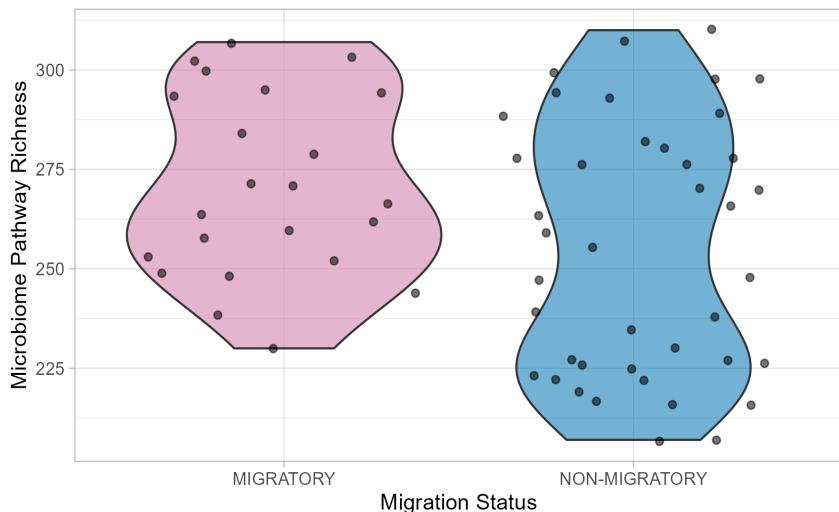


Figure S1d

Pathway Shannon's H

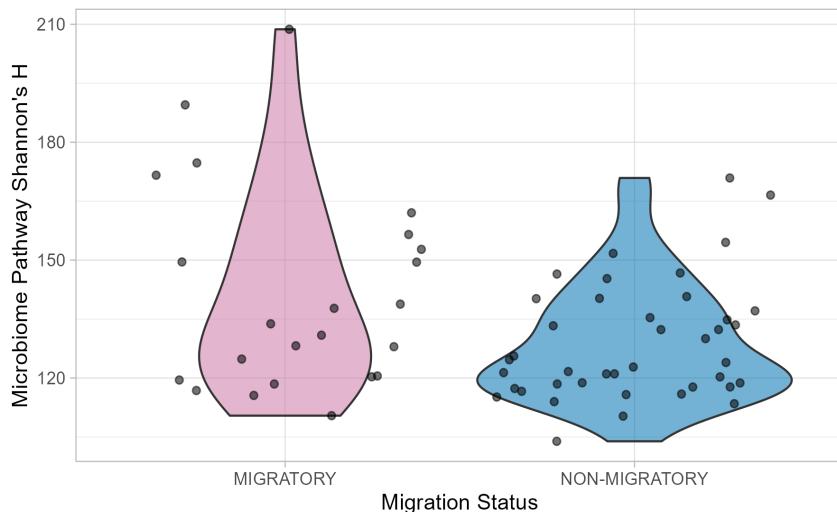


Figure S1e

Pathway Simpson's D

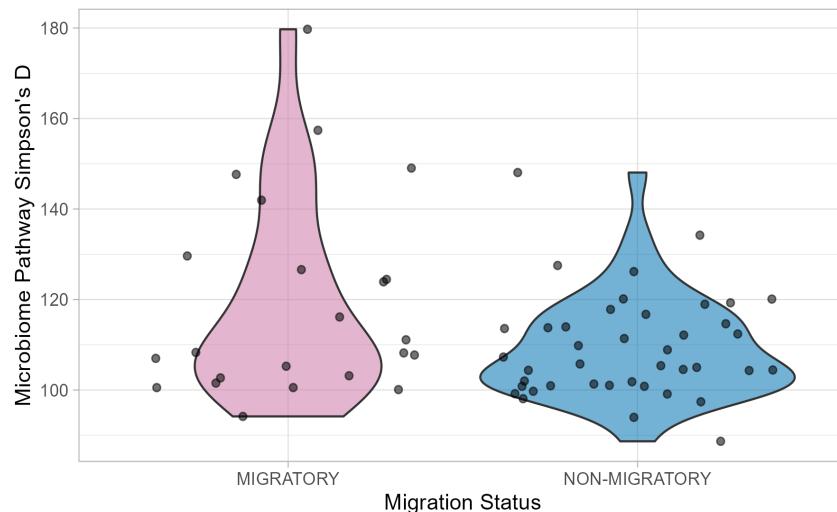


Figure S1f

Figure S1: Alpha Diversity Metrics for Taxa (top) and Pathway (bottom) Composition. Taxonomic richness does not differ significantly between the groups (a, Welch's T-test, $P = 0.275$), but non-migratory samples exhibit an increase in both Shannon's diversity (b, $P = 0.045$) and Simpson's diversity (c, $P = 0.039$). For predicted pathways, migratory samples had a modest but significant increase in pathway richness (d, Welch's Two-Sample T-Test, $P = 0.015$), Shannon diversity (e, $P = 0.025$), and Simpson diversity (f, $P = 0.033$).

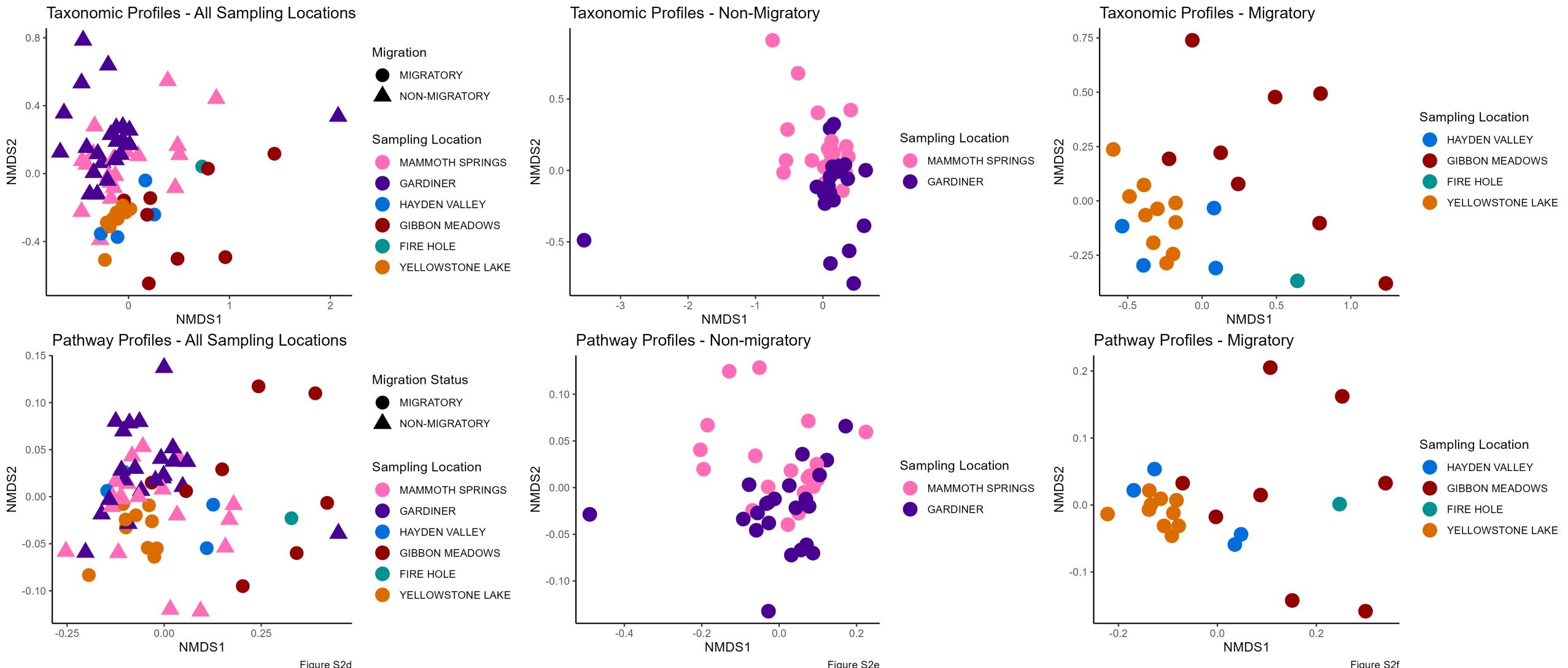


Figure S2: Sampling Location Association with Sample Taxonomic (Top) and Pathway (Bottom) Composition. (a,d) Ordinations depicted in Figure 1 annotated with additional sampling location information (a, ordination stress: 0.1671938, d, stress: 0.05103952). (b,c) Testing for a location effect within non-migratory (b) and migratory (c) samples revealed collecting location had a significant correlation with a sample's taxonomic profile ((b) stress: 0.1152754, dispersion: $F = 0$, $p = 0.999$, PERMANOVA: $R^2 = 0.06389$, $F_{39,1} = 2.6617$, $p = 0.001$, (c) stress: 0.1069645, dispersion: $F = 8.238$, $p = 0.003$, PERMANOVA: $R^2 = 0.23075$, $F_{19,2} = 2.8496$, $p = 0.000999$). (e,f) Sampling location did not have a significant association with pathway profiles for non-migratory samples (e, stress: 0.04879845, dispersion: $F = 0.0653$, $p = 0.866$, PERMANOVA: $R^2 = 0.05379$, $F_{39,1} = 2.217$, $p = 0.096$), but was significant for migratory samples (f, stress: 0.02650014, dispersion: $F = 20.943$, $p = 0.001$, PERMANOVA: $R^2 = 0.50415$, $F_{19,2} = 9.6591$, $p = 0.001$). All statistics for migratory samples excluded Fire Hole ($n = 1$).

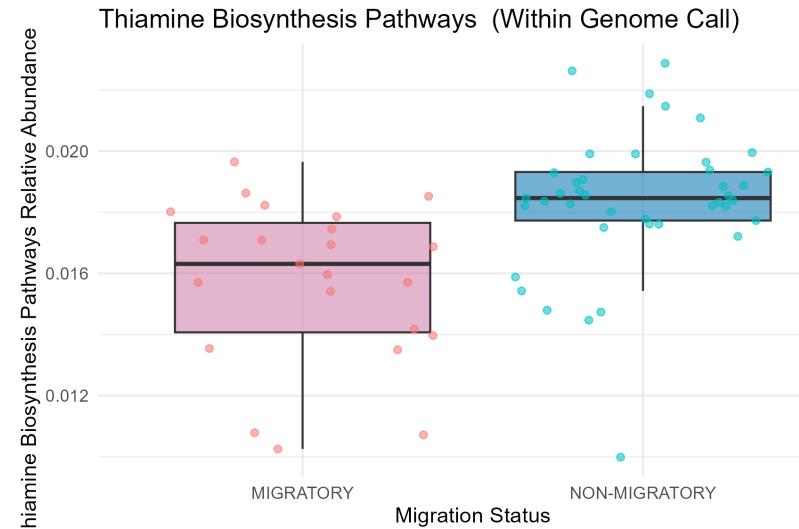
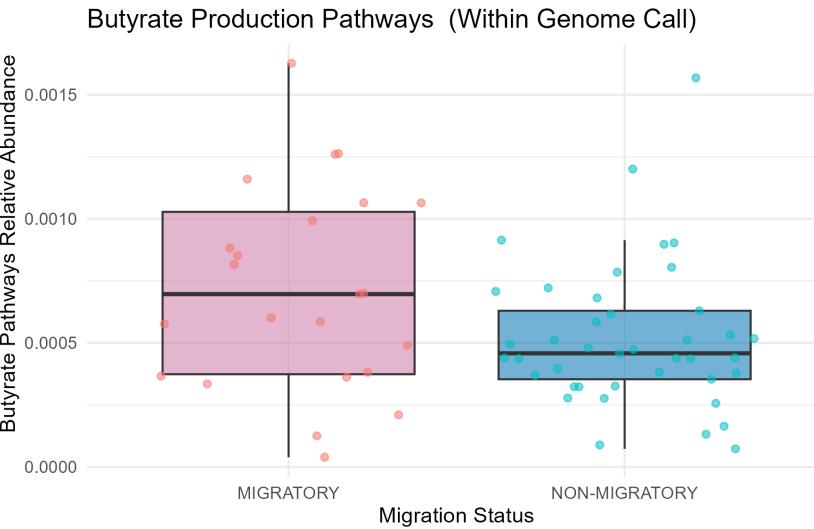
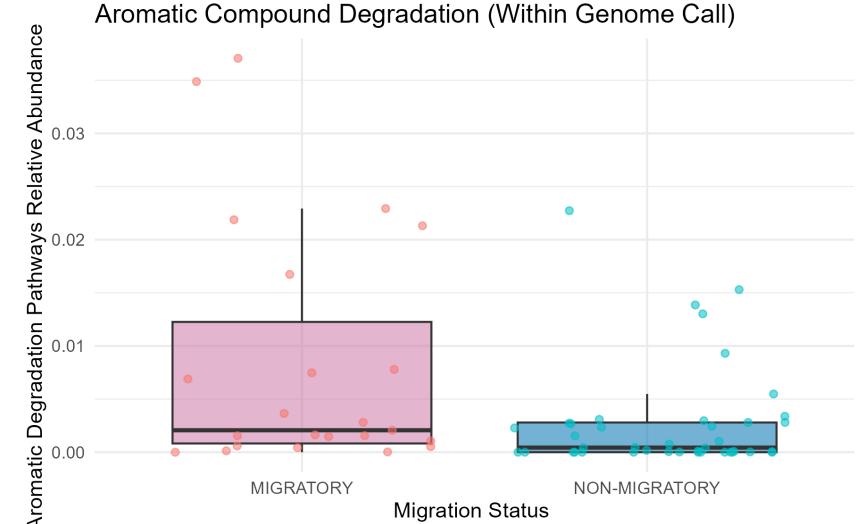


Figure S3: Host-Relevant Pathways Differ Between Migration Strategies (“stratified” data). PICRUST2 predictions of (A) Aromatic Compound Degradation ($p\text{-value}: 0.0029, q = 0.021$) (B) Fermentation to Butyrate ($p\text{-value}: 0.0101, q = 0.061$), and (C) Thiamine (Vitamin B1) Biosynthesis ($p\text{-value}: 0.0002, q = 0.005$) relative pathway abundance. Pathway counts were restricted to pathways present within a single taxa’s predicted genome.

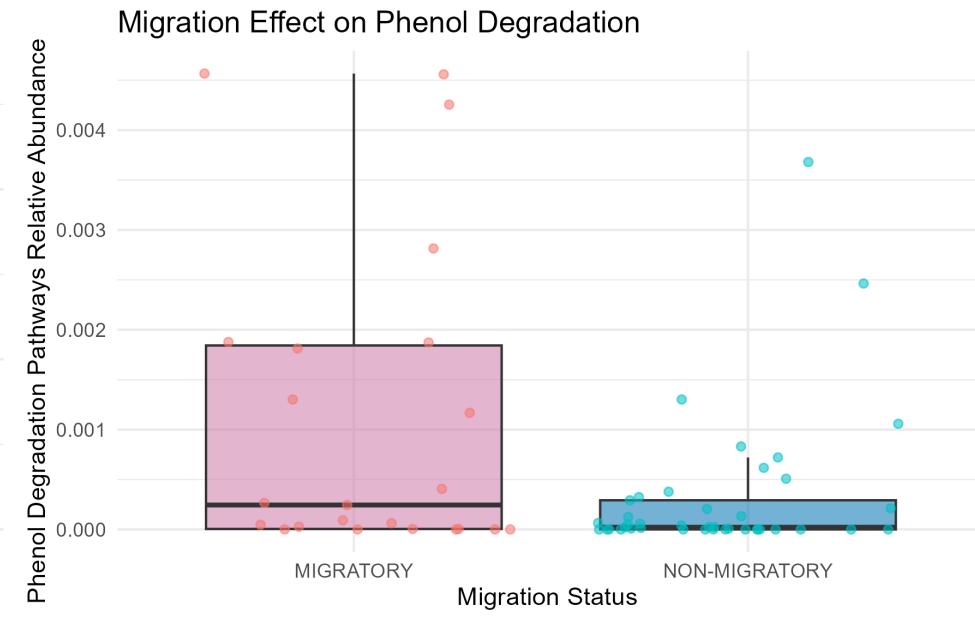
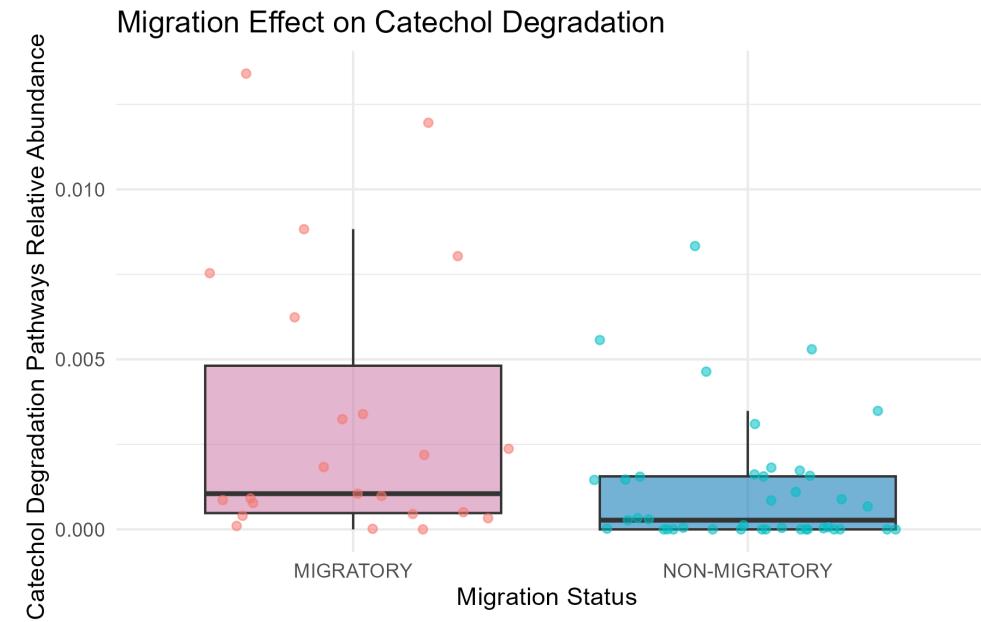


Figure S4a

Figure S4b

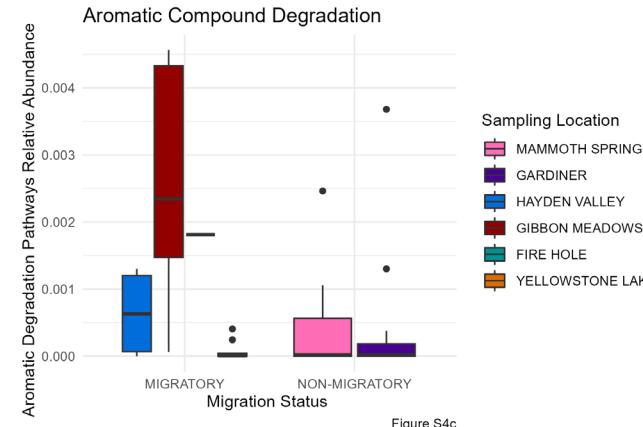


Figure S4c

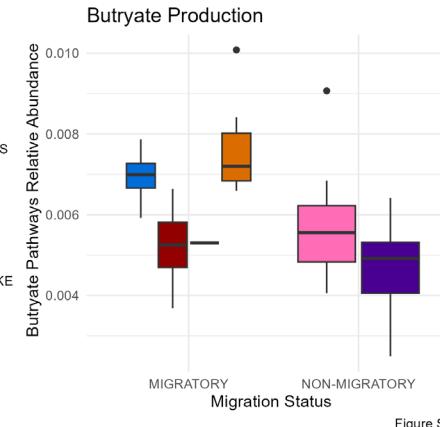


Figure S4d

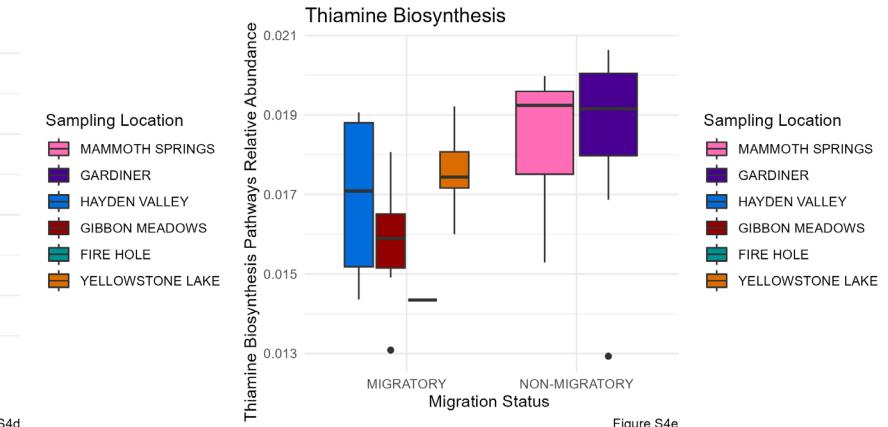


Figure S4e

Figure S4: Host Relevant Differentially Abundant Pathways – Supplement. (a,b) Key plant secondary metabolite degradation pathway classes within the aromatic compound degradation class include catechols (a, MetaCyc pathway class level 3, $p = 0.0019$, $q = 0.015$) and phenolics (b, MetaCyc pathway class level 3, $p = 0.0483$, $q = 0.104$). (c-e) Differences between migratory and non-migratory samples are driven by multiple, but not always all sampling, locations within each migration group.

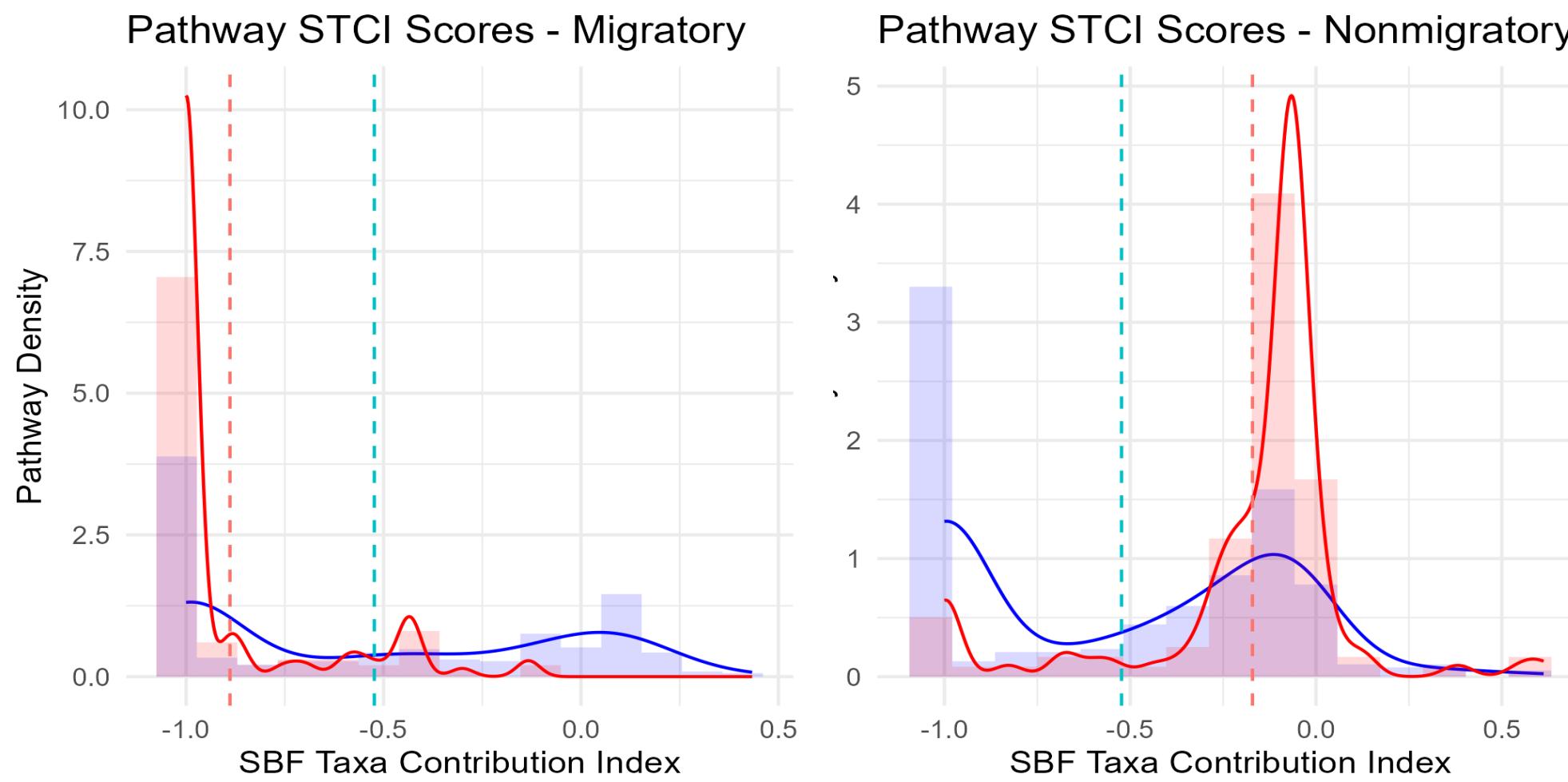


Figure S5: Distribution of Pathway Associations with SBF Taxa. Each histogram count represents the average STCI value of one pathway (Migratory Differentially Abundant (A, red): $N = 97$, Migratory Total Pathways (A, blue): $N = 322$, Non-Migratory Differentially Abundant (B, red): $N = 104$, Non-Migratory Total Pathways (B, blue): $N = 334$). Histogram counts are normalized to the kernel density estimate, and dotted lines represent mean STCI value for either total or differentially abundant pathways. Medians of differentially abundant and total pathway distributions differed significantly in both groups (Wilcoxon Rank-Sum: $p < 1 E-10$), albeit in opposite directions. Pathways were classified as differentially abundant using an FDR cut-off of 25%.

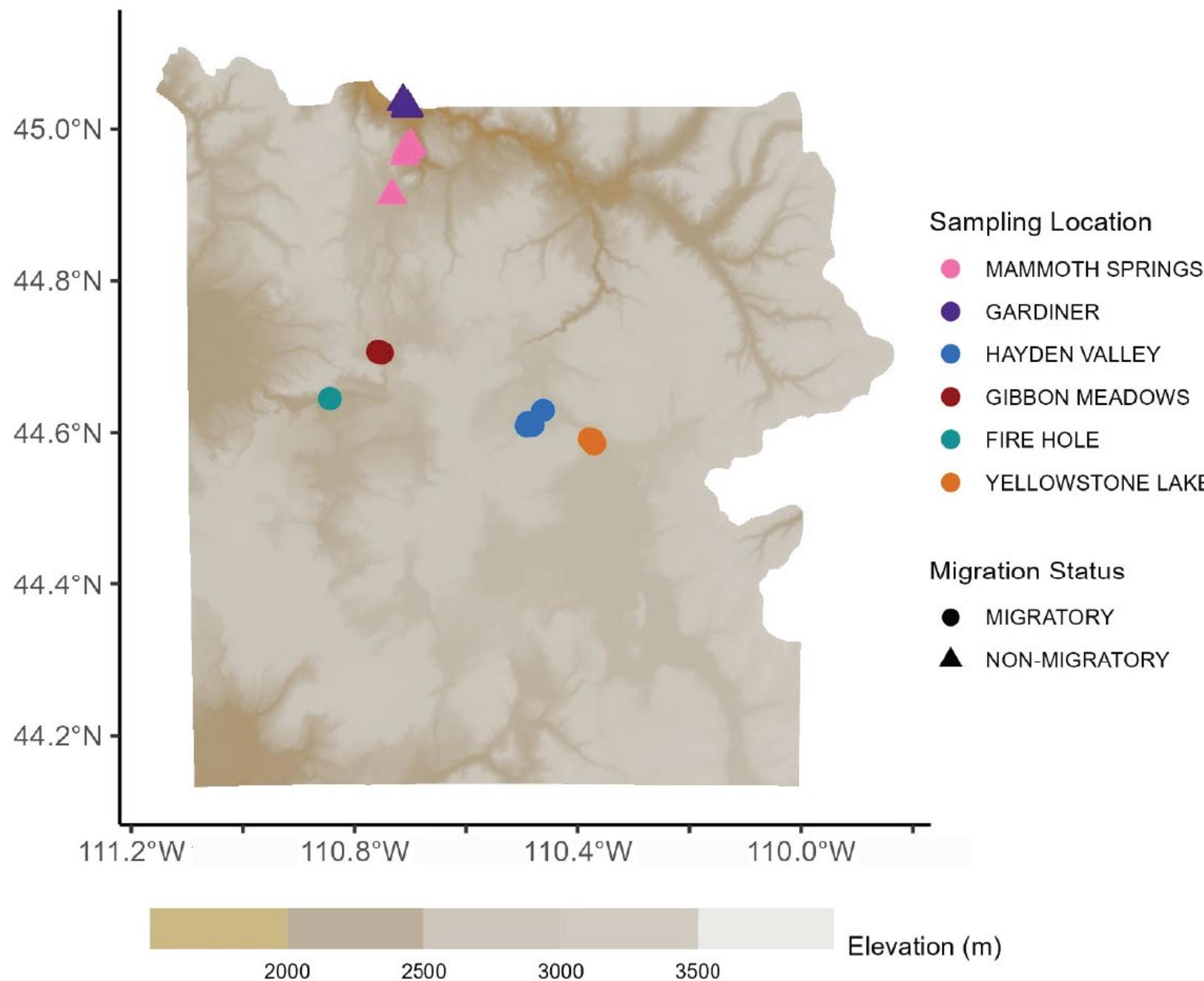


Figure S6: Sampling Locations within Yellowstone National Park

Supplementary Figure 7: Calculating SBF Taxa Contribution Index Values Using Stratified PICRUST2 Output

Step 1: Group row entries (ASV x function x sample) by each ASV's SBF status within each function x sample, calculating both:

- cumulative proportional contribution to a given function in a sample ("norm_taxon_function_contrib")
- number of taxa contributing from the SBF or non-SBF taxa fraction ("taxon_num")

Step 2: Multiply each sample/function/taxa group's normalized contribution by that corresponding pathway's count in that sample

Step 3: Divide taxa group's contribution by the number of taxa in each group

Step 4: Use these counts to calculate the STCI statistic.

sample	function	taxon	norm_taxon_function_contrib
1	1CMET2-PWY	00f59509ac0a78eb860ad258ecfcfb58	0.0020785979
2	1CMET2-PWY	012c9b238bc78fd4a9d8e470e2393a0d	0.0153816242
3	1CMET2-PWY	03f1ede76e9cdc34f986f85d191ec67a	0.0116401480
4	1CMET2-PWY	041b408292d9023346f354c632a96559	0.0014550185
5	1CMET2-PWY	0c88e015cdcbc6913e91cb9e32d05d4da	0.0135048102

Stratified PICRUST Output - Taxa Contributions

Step 1

pathway	YNP521	YNP523
1 1CMET2-PWY	4810.935385	4961.047443
2 3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	0.000000	0.000000
3 ALL-CHORISMATE-PWY	0.000000	130.948905
4 ANAEROFRUCAT-PWY	62.616085	635.958011
5 ANAGLYCOLYSIS-PWY	8559.366690	7624.073659

Stratified Overall Pathway Counts Per Sample

sample	function	Mig_SBF_Status	Mig_Status	norm_taxon_function_contrib	taxon_num	Weighted_Path_Class_Contrib	Weighted_Contrib_Per_Taxa
1	1CMET2-PWY	MIGRATORY NON-SBF	MIGRATORY	0.923634389	63	4590.246923	72.861062
2	1CMET2-PWY	MIGRATORY SBF	MIGRATORY	0.076365611	15	379.519231	25.301282

Output from step (1)

Step 2

Step 3

Step 4

sample	function	Mig_SBF_Status	Mig_Status	norm_taxon_function_contrib	taxon_num	Weighted_Path_Class_Contrib	Weighted_Contrib_Per_Taxa
1	1CMET2-PWY	MIGRATORY NON-SBF	MIGRATORY	0.923634389	63	4590.246923	72.861062
2	1CMET2-PWY	MIGRATORY SBF	MIGRATORY	0.076365611	15	379.519231	25.301282

sample	Mig_Status	ta9	Test_Stat
1	MIGRATORY	1CMET2-PWY	-0.48450127

$$STCI = \frac{C_{SBF} - C_{non-SBF}}{C_{SBF} + C_{non-SBF}}$$

Functions Associated with Scarce But Frequent Taxa						
Feature	Maaslin2 Differential Abundance Testing				SBF Taxa Contributions	
	coef	stderr	pval	qval	STCI _{mig}	STCI _{nonmig}
<i>Shared</i>						
Cobamide Biosynthesis	0.140848673	0.097692268	0.154402784	0.332701236	0.235935802	0.080805101
Starch Degradation	0.240078379	0.169131241	0.160767631	0.342340485	0.241874524	0.038948126
Beta-1,4 Mannan degradation	0.32151719	0.285268137	0.264056043	0.442538369	0.37296568	0.381286393
L-rhamnose Degradation	0.189350693	0.176049044	0.286293387	0.471082755	0.244089322	0.127356775
<i>Migratory</i>						
Glycogen and Starch Biosynthesis	0.189618082	0.129326527	0.147648594	0.321980668	0.227689321	-0.000917507
Superpathway of aromatic amino acid biosynthesis	0.009271099	0.058137122	0.873817341	0.917056297	0.176501416	-0.025624451
Chorismate Biosynthesis I	0.011718671	0.0589694	0.843128398	0.897683765	0.166546964	-0.022462554
Phosphatidylglycerol Biosynthesis	0.00032422	0.058528804	0.995597936	0.995843018	0.141604034	-0.029952154
Glycolysis (from glucose)	0.20965068	0.09060979	0.024009246	0.092461137	0.128565827	-0.068874781
Superpathway of L-aspartate and L-asparagine biosynthesis	0.005768991	0.052673433	0.913140831	0.934995009	0.125040549	-0.010339745
L isoleucine Biosynthesis	0.100657471	0.050017621	0.048522627	0.136250757	0.109802899	-0.049267008
Geranylgeranyl Diphosphate Biosynthesis	0.196559575	0.092840412	0.038263816	0.117385604	0.101043325	-0.123063207
<i>Non-Migratory</i>						
Lactose Degradation	1.57131745	0.62822783	0.015033033	0.076642591	-1	0.612407862
L-tyrosine Biosynthesis	0.357952049	0.465767698	0.445095546	0.570881795	-0.569320359	0.402172378
L-lysine Degradation	0.193604581	0.357846903	0.590428141	0.689467701	-0.165086134	0.377488607
L-phenylalanine Biosynthesis	-0.470632359	0.54673449	0.392661613	0.518771912	-0.688713023	0.214889159

Figure S8: Pathway Classes Associated with SBF Taxa (Table Form of Figure 4). MetaCyc pathway class level 4 features with average STCI scores > 0.1 for at least one migration group's SBF taxa fraction. For all pathway class STCI scores, see Supplementary Table 1.

Supplementary Table List

- Table 1: Pathway Maaslin2 Differential Abundance Results/STCI Scores
- Table 2: Neutral Community Model Results
- Table 3: Sample Metadata

Available at: <https://drive.google.com/drive/folders/1lfPDRk4lMpdjaE9tkyuKPCrPeG7QJ-U4?usp=sharing>