**Title: Bacterial dispersal and drift drive microbiome diversity patterns within a population of feral hindgut fermenters**

**Running Title:** Feral horse microbiome ecology

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**Abstract**

Studies of microbiome variation in wildlife often emphasize host physiology and diet as proximate selective pressures acting on host-associated microbiota. In contrast, microbial dispersal and ecological drift are more rarely considered. Using amplicon sequencing, we characterized the bacterial microbiome of adult female ($n$ = 86) Sable Island horses (Nova Scotia, Canada) as part of a detailed individual-based study of this feral population. Using data on sampling date, horse location, age, parental status, and local habitat variables, we contrasted the ability of spatiotemporal, life history, and environmental factors to explain microbiome diversity among Sable Island horses. We extended inferences made from these analyses with both phylogeny-informed and phylogeny-independent null modeling approaches to identify deviations from stochastic expectations. Phylogeny-informed diversity measures were correlated with spatial and local habitat variables, but null modelling results suggested that heterogeneity in ecological drift, rather than differential selective pressures acting on the microbiome, was responsible for these correlations. Conversely, phylogeny-independent diversity measures were best explained by host spatial and social structure, suggesting that taxonomic composition of the microbiome was shaped most strongly by bacterial dispersal. Parental status was important but correlated with measures of β-dispersion rather than β-diversity (mares without foals had lower alpha diversity and more variable microbiomes than mares with foals). Our results suggest that between host microbiome variation within the Sable Island horse population is driven more strongly by bacterial dispersal and ecological drift than by differential selective pressures. These results emphasize the need to consider alternative ecological processes in the study of microbiomes.

***Keywords:*** Microbial Ecology, Mammal, Null Models, Phylogenetic Ecology, Social Microbiome, Wildlife

## 1 | Introduction

Nascent recognition of the physiological, ecological, and evolutionary importance of host-associated microbial communities (microbiomes) has inspired growing interest in microbial applications towards human health, domestic animal production, and wildlife conservation (Arias-Sánchez, Vessman, & Mitri, 2019; Gilbert et al., 2018; Trevelline, Fontaine, Hartup, & Kohl, 2019). But to effectively manipulate microbiomes we must first understand predictors of microbiome variation and acknowledge the full scope of ecological processes which underly the assembly of biological communities (selection, ecological drift, dispersal; Vellend, 2010). However, selection, drift, and dispersal are all influenced by the artificial laboratory conditions from which much of our understanding of host-associated microbiomes are derived (Greyson-Gaito et al., 2020). Therefore, there is value in supplementing highly controlled laboratory experiments with observations from wild systems. To date, research on microbiome variation in wild systems have most heavily emphasized host or environmental factors thought to exert divergent selective pressures microbiome (i.e. host physiology: Amato et al., 2014; Stothart, Palme, & Newman, 2019; Suzuki et al., 2019; host diet: Kartzinel, Hsing, Musili, Brown, & Pringle, 2019; Teyssier et al., 2020). More recently, researchers have speculated as to the ecological and evolutionary importance of ecological drift and microbiota dispersal in shaping microbiome variation in nature (Adair & Douglas, 2017; Kohl, 2020; Miller, Svanbäck, & Bohannan, 2018; Sarkar et al., 2020), however, few empirical estimates of these processes have been made outside of the laboratory.

While host physiology and diet clearly shape wildlife-microbiome variation, the ability of microbiota to disperse between host intestinal tracts arguably supersede the importance of either in governing microbiome diversity (Miller et al., 2018). Correlations between microbiome composition and social networks in gregarious hosts illustrate the importance of microbial community connectivity and bacterial dispersal on microbiome diversity (savannah baboons [*Papio cynocephalus]*, Tung et al., 2015; Sarkar et al., 2020). Bacterial dispersal between hosts can occur through grooming (rhesus macaques [*Macaca mulatta*], Balasubramaniam et al., 2018), coprophagy (domestic horses [Equus ferus caballus], ,

shared environments (humans [*Homo sapiens*], Rothschild et al., 2018), or copulation (black legged kittiwakes [*Rissa tridactyla*], White et al., 2010). Therefore, we would expect rates of bacterial dispersal to decrease as a function of the time and space separating hosts. An effect of spatial separation on the microbiome has been demonstrated at large spatial scales between (sub)populations of red squirrels (*Tamiasciurus hudsonicus*; ~7km; Ren et al., 2017), bighorn sheep (~150 km; Couch et al., 2020), house mice (*Mus musculus*; ~1100 km; Linnenbrink et al., 2013), American pikas (*Ochotona princeps*; ~1400 km; Kohl, Varner, Wilkening, & Dearing, 2018), red colobus (*Procolobus rufomitratus*; ~1100km; Mccord et al., 2014), and between pairs of predator and prey species (~12100 km; Moeller et al., 2017).

The affects of spatial separation on microbial dispersal between social groups of host individuals within populations are more rarely considered. One study of a single focal population of house mice (*Mus musculus domesticus*) found a greater importance of fine-scale habitat heterogeneity than spatial separation (Goertz et al., 2019). Conversely, spatial structuring of the microbiome has been reported among a contiguous moose population spanning 150 km (Fountain-Jones et al., 2020). Similar effects of spatial proximity have been observed among semi-feral ponies (40 $km^2$; Antwis, Lea, Unwin, & Shultz, 2018), but were limited to comparisons between three large social groups (bands). Regardless of the spatial scale considered, many studies do not control for local environmental variation. Conversely, studies which consider environmental terms often do not consider spatial processes, which is problematic given an expectation that environmental conditions are spatially autocorrelated. Therefore, relationships between microbiome beta-diversity and host spatial distribution can derive from underlying environmental selective pressures, or higher rates of microbiota dispersal between hosts in close-proximity—parsing these mechanisms is important but challenging.

Greater rates of microbiota dispersal between co-occurring hosts can drive microbiome similarity, but strong dispersal limitation can cause greater than expected divergence between communities and unpredictable β-diversity patterns. In a meta-population context, dispersal between communities are thought to stabilize populations (Crowley, 1981), so long as dispersal is not so high as to drive spatial

synchrony (Yaari, Ben-Zion, Shnerb, & Vasseur, 2012). Conversely, dispersal limitation among isolated biological communities increases the strength of ecological drift and heightens the risk of local extinctions (Vellend, 2010). Hosts disconnected from the broader meta-community of conspecific microbiomes (Miller et al., 2018)—those in low density populations, at the fringes of populations, or experiencing social isolation—may be at greater risk of stochastic microbiome dysregulation. These concerns have been raised with respect to wildlife in captivity (McKenzie et al., 2017; Trevelline et al., 2019); although, this effect remains to be explicitly tested in free-living settings.

Dispersal limitation can feed ecological drift but so too can dietary and physiological factors which are often assumed to be deterministic. For example, different diets can exert divergent selective pressures, but can also differ in the energy made accessible to the microbiome and the diversity of metabolic niche space supported. Labile high energy diets may fail to support fibrolytic and cellulolytic niche-space in the microbiome (Oliphant & Allen-Vercoe, 2019) and can destabilize microbial communities in a process similar to the paradox of enrichment (Coyte, Schluter, & Foster, 2015; Rosenzweig, 1971). Similarly, while different host physiological states might select for different microbial functions (Foster, Schluter, Coyte, & Rakoff-Nahoum, 2017), a loss of host homeostatic control among physiologically stressed hosts might result in community instability and greater stochastic variation (Zaneveld, McMinds, & Vega Thurber, 2017). Microbiome β-dispersion, a measure of microbiome variance, is one indication of the relative strength of stochasticity. A second indication of stochasticity is the failure of communities to deviate from predictions made by null modelling approaches. Despite past misuse (for an overview see: Narwani, Matthews, Fox, & Venail, 2015), phylogenetic null modelling methods are valuable to consider alongside conventional β-diversity metrics, as traditional diversity metrics can be influenced by system gamma diversity and imbalances in alpha diversity between communities (Chase, Kraft, Smith, Vellend, & Inouye, 2011; Gering & Crist, 2002; Zhou & Ning, 2017). However, we stress that the results of null modelling approaches are exploratory, rather than definite measures of ecological processes underlying community assembly.

Here we directly contrast the ability of host life history, habitat heterogeneity, and spatial measures to explain variation in the faecal bacterial microbiome of feral horses using 86 adult females from the closed population of Sable Island (Nova Scotia, Canada). Building on a comprehensive, long-term, detailed individual-based study of ecology and evolution for this population (Richard, Simpson, Medill, & Mcloughlin, 2014), we apply a combination of conventional diversity analyses and null modeling approaches to evaluate the evidence for drift, dispersal, and niche-based processes. If environmental conditions and host life history (a proxy for physiology) are more similar within populations than between populations or between species, then microbial dispersal patterns and ecological drift might play comparably large roles in shaping inter-individual microbiome variation within populations. Specifically, we predicted that phylogeny-independent diversity measures would be most strongly influenced by spatial and social variables, reflecting microbial dispersal patterns. Conversely, we predicted that host life history and local habitat heterogeneity would better explain variation in phylogeny-weighted metrics of microbiome diversity—reflecting different selective pressures imposed on host-associated microbiota between host physiological states or diets. These predictions are predicated on the presumption that microbial niche-spaces are phylogenetically conserved, a pattern which we indirectly test. Our study represents one of the first direct comparisons between environmental and spatial effects on host-associated microbiomes in the wild at a within population scale, with consideration offered to alternative ecological processes.

## 2 | Methods

### 2.1 | Study area and population

Sable Island National Park Reserve, a crescent-shaped emergent sand bar located 175 km off the east coast of Nova Scotia (Canada), spans ~49 km (east-west) but is only ~1.2 km at its widest point (Figure 1). The treeless island is dominated by marram grass (*Ammophila breviligulata*), a common species in

early-successional grasslands, occurring both in pure swards and in mixed communities alongside other species such as red fescue (*Festuca rubra*), beach pea (*Lathyrus japonicus* var. *maritimus*), and forbs such as meadow rue (*Thalictrum pubescens*) or pearly everlasting (*Anaphalis margaritacea*). These grasslands comprise the most common vegetation community (Contasti, Tissier, Johnstone, & McLoughlin, 2012). Sheltered by 10–30-m high dunes, in the interior of the island grasslands give way to late-successional mixed heath communities characterized by shrubs (e.g. common juniper [*Juniperus communis* var. *megistocarpa*], lowbush blueberry [*Vaccinium angustifolium*], northern bayberry [*Myrica pensylvanica*]), and the presence of an organic soil layer (Catiling, Lucas, & Freedman, 2009; Tissier, Mcloughlin, Sheard, & Johnstone, 2013). Dune height and vegetated landcover decrease as the island tapers towards its longitudinal extremes, where beach pea and seaside goldenrod (*Solidago sempervirens*) are co-dominant with marram grass and the semi-succulent forb sandwort (*Honckenya peploides*) dominates at the edges of dunes (Catiling et al., 2009; Tissier et al., 2013). Sandwort is an important component of the Sable Island horse diet (Contasti et al., 2012) and a nutritional outlier, being lower in fibre and higher in crude protein compared to other types of forage on Sable Island (personal communication K. Johnsen; Lee, 2018))

Introduced to the island circa 1750 and studied intensively by our research group since 2007 (Contasti, Van Beest, Vander Wal, & McLoughlin, 2013; Gold et al., 2019), the feral horses are the only terrestrial mammal found on the island (Freedman, 2016). Since their introduction, the horses have remained unmanaged with very limited introgression from mainland domestic stock (most recently a single adult male in the 1930s; Welsh, 1975). The horse population (550 individuals in 2014) declines sharply in density from west to east (Marjamäki, Contasti, Coulson, & Mcloughlin, 2013). A polygynous mating system exists, characterized by mixed-sex social bands guarded by (usually) a single dominant adult male (stallion) against mating attempts by other males. Females in the population invariably segregate across these mixed-sex social bands which are comprised of the dominant stallion, adult females (mares), and subadult (<3 years of age) offspring (Regan et al., 2019). Bands can therefore be as

small as 2 horses (one adult male and one female), although bands as large as 16 horses have been observed (Manning & McLoughlin, 2017). Band memberships are stable across years but 67% of adult females have been observed to disperse to a different social band at least once during a 7-year period (Debeffe, Richard, Medill, Weisgerber, & McLoughlin, 2015). Outside of social dispersal events, social bands traverse the landscape together but very rarely stray farther than 4000 m in either direction from the centre of their home-range during the summer. Most bands constrain their movements to <2000 m from their home-range's centre (Rozen-Rechels et al., 2015). Bacterial dispersal between horses is expected to occur primarily between members of the same—or interacting—social bands and be facilitated by grooming, coprophagy, interactions with faecal territorial markers (stud piles), or the use of shared resources (Figure 2). Social dispersal of horses might likewise facilitate bacterial transmission between social bands over longer distances.

### 2.2 | Location and life history data

Location data are collected during annual systematic surveys conducted between the months of July–September. Each day (weather permitting) one of seven sections is surveyed on foot by one or multiple observers, and adjacent sections are not surveyed on consecutive days. Consequently, each section is typically surveyed once per week over a 6 to 8 weeks period. When horses are encountered, identifying photos are taken alongside location to the nearest 5 m using a handheld GPS device. Every year, each horse is sighted 5 ± 2 times ($\bar{x}$ ± SD; Rozen-Rechels et al., 2015). Annual surveys across years allow us to track the birth, age, change in reproductive status, death, and social parentage of every individual.

### 2.3 | Sample collection and storage

Faecal samples are collected using sterile nitrile gloves which are inverted, sealed, and kept in insulated bags containing icepacks until returning to the laboratory on the same day (max ~6 hrs). Samples are collected immediately upon defecation but only if the sample has not been disturbed or environmentally contaminated, and only portions of the faecal pile not in contact with the ground or vegetation are

collected. Subsamples (~1–2 grams each) are stored in cryotubes at −20°C while on the island before transfer to long-term storage at −80°C on the mainland at the end of each field season. For the present study we selected 86 fecal samples collected in 2014 between mid-July and early-September from 86 different adult females (mares) spanning 52 social bands (1–4 samples/band) which ranged from 3–12 horses in size. Each mare represented in the dataset was only sampled once. Ages ranged from 3–9+; mares classed as 9-years of age might be older than 9 years, as they were adults before the inaugural field season of the long-term study.

### 2.4 | Habitat Classification

Habitat classifications were developed using Light Detection And Ranging (LiDAR) surveys and high-resolution aerial photo in 2009 by the Applied Geomatics Research Group (Nova Scotia Community College, Middleton, Nova Scotia; van Beest et al. 2014). Non-vegetated habitat classes included bare sand, ocean, human structures (buildings with fenced perimeters), and freshwater ponds. Vegetated habitat classes were characterized by their dominant plant species: grassland (marram grass), heath (mixed juniper, crowberry, and blueberry), sandwort, and beach pea. Vegetated classes subcategorized into ‘sparse’ or ‘dense’ (e.g. sparse grassland and dense grassland) in original classification efforts were combined in our analyses. The distribution of vegetated habitats on Sable Island is stable across years (van Beest et al., 2014), and so classifications made from the 2009 remote sensing data are thought to accurately reflect habitat heterogeneity in 2014 (the year faecal samples were collected).

To quantify variation in an individual’s local environment, we calculated the area of habitat classes overlapping a 150-m radius circular buffer centred on the location of sample collection in R (v3.5.1). A 150-m radius buffer corresponds approximately to the observed median daily movement of horses in 2014 (positive skewed distribution, median: 108 m/day; mean 317 m/day), and so is expected to coarsely reflect the types of environment, and therefore forage, encountered during the 24 hours preceding defecation. Habitat class variables were calculated as the area of a given habitat class relative to

the total occupiable terrestrial area included within an individual's buffer ($Area_{Buffer} - Area_{Building} - Area_{Ocean}$). Sandwort abundance was zero-inflated and non-normally distributed. Further, resource selection analysis of Sable Island horse foraging behaviour suggests horses actively select for sandwort when it is present, while other vegetated habitat classes are used in proportion to their abundance on the local landscape (personal communication K. Johnsen). For these statistical and biological reasons, sandwort was parameterized as 'present' or 'absent' in our analyses. Only vegetated habitat classes were parameterized in analyses to limit model inflation and limit collinearity between terms.

### 2.5 | Sequencing and Bioinformatics

Using 2 mL bead beating tubes (0.7 mm Dry Garnet) and a Vortexed-Genie 2 fitted with Qiagen's Vortex Adapter (cat. No. 13000-V1-24), we homogenized 0.20-gram sub-samples of horse fecal material. We extracted DNA from homogenized fecal samples using QIAgen's QIAamp PowerFecal DNA Kits, following manufacturer recommendations outlined in the Qiagen PowerFecal DNA handbook. Notably, we used a single tube extraction protocol (rather than a 96-well format) and randomized the order in which samples were extracted. In the final step, we eluted DNA from the spin columns using 100 µl of $ddH_2O$ pre-warmed to 60°C. Prior to sequencing, we quantified the DNA in eluted extracts using a Qubit dsDNA BR Assay Kit and standardized DNA concentration to 20 ng/uL prior to PCR amplification. We PCR amplified the v3–v4 region of the 16S rRNA gene using the 341f forward and 805r reverse universal primers. PCR products were sequenced on an Illumina MiSeq platform (v3 chemistry: 2 x 300 base-pair read pairs) at the University of Calgary Centre for Health Genomics and Informatics.

*Cutadapt* v1.16 was used to remove 341f and 805r primers or discard untrimmed reads (Martin, 2011). Trimmed reads were processed in dada2 v1.6 using a standard pipeline (https://benjjneb.github.io/dada2/tutorial_1_6.html; Callahan et al., 2016). In brief, sequences with a maximum expected error of two or greater, PhiX spike-ins, and bases with a quality score of <2 were discarded using the *filterAndTrim* command. Forward and reverse sequences were truncated to lengths of

250 and 200, respectively. The remaining commands were conducted using default parameters unless otherwise noted. Filtered sequences were used to create an error model using the *learnErrors* command and were subsequently dereplicated using the *derepFastq* command. Error correction was performed using the *dada* command, at which point, forward and reverse sequences were merged using the *mergePairs* command with the *trimOverhang* parameter set to "TRUE". Chimeras were removed using the "consensus" method with the *removeBimeraDenovo* command. Taxonomic assignment of amplicon sequence variants (ASVs) was performed using implementation of the naïve Bayesian classifier (Wang et al. 2007) and v132 of the SILVA database (Yilmaz et al., 2014) using the command *assignTaxonomy*. To further conservatively filter sequencing errors and possible extraction kit contaminants, as well as to reduce singleton noise prior to analysis, ASVs which were not represented by at least 1 count in 4 samples were removed from the dataset (Knowles, Eccles, & Baltrūnaitė, 2019). Additionally, reads classified as mitochondria or chloroplasts were likewise removed. ASV sequences were aligned using MUSCLE with default parameters (Edgar, 2004) and a relaxed neighbour-joining method was used to construct a phylogenetic tree using the mothur implementation of clearcut (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013; Sheneman, Evans, & Foster, 2006).

Two negative controls, but not field controls, representing DNA extraction kit blanks were processed and sequenced as described above. Sequencing recovered 3412 and 3015 paired-end reads per negative control, which were represented by only 20 ASVs. ASVs found observed in the negative controls were absent from horse fecal samples and therefore removed prior to data analysis.

### 2.6 | Diversity Analysis

We used the number of observed ASVs (ASV richness) from a rarefied microbiome dataset as a measure of within-host microbial diversity (α-diversity). While α-diversity indicates within-host diversity, β-diversity indicates pair-wise differences in community composition between hosts. We analyzed two β-diversity metrics: Euclidean distances from a centred log-ratio transformed ASV dataset (Gloor,

Macklaim, Pawlowsky-Glahn, & Egozcue, 2017) and weighted UniFrac distances (Lozupone, Hamady, Kelley, & Knight, 2007) from a rarefied ASV dataset (34,280 reads/sample; rarefaction curve: Figure S1, Supporting information). Both β-diversity metrics weight differences in the ASV composition and relative abundance of ASVs between communities, but the weighted UniFrac measure differs by simultaneously weighting the phylogenetic relatedness of ASVs. Finally, we also considered β-dispersion, calculated as the distance from each sample to the sample-set centroid in Euclidean or weighted UniFrac space (Anderson, Ellingsen, & McArdle, 2006).

We evaluated the ability of spatiotemporal (day of year, longitude, and distance from the population's midpoint), host life history (using age and parental status as proxies of host physiology), and habitat class relative areas to predict patterns in the described microbiome diversity measures. Given an east-west orientation of Sable Island's linear landmass, longitude is a good 1-dimensional measure of location on the island. Distance from the population midpoint was calculated as the longitudinal distance separating an individual at the time of fecal sample collection from the average horse longitude in 2014 (5166 sightings total). We theorized that individuals further from the population's core might be less well connected by microbial dispersal to the rest of the population. Day of year, longitude, and longitudinal distance from the population's centre were scaled to a mean of 0 and a standard deviation of 1 prior to analysis. Age was coded as continuous data in 1-year increments, with a linear and $2^{nd}$ order polynomial fit considered in analyses, given a curvilinear relationship between gut microbiome diversity and age among humans (Yatsunenko et al., 2012). Parental status, shown to affect the microbiome in other systems (Amato et al., 2014), was coded as a dichotomous variable based on whether adult females were nursing a foal (<1 year old offspring) during the 2014 field survey.

For univariate diversity measures (α-diversity and β-dispersion), we used a multi-model inference approach implemented in the R package MuMIn v1.43.6 (Bartoń, 2009). A starting global general linear model was parameterized with the spatiotemporal, life history, and environmental terms described above, without interactions. We determined parameter estimates and significance from conditional AICc

averaging of models which had a Δ AICc < 3 (Burnham & Anderson, 2002; Grueber, Nakagawa, Laws, & Jamieson, 2011). Patterns in β-diversity were analyzed using a backwards selection approach from PERMANOVA outputs, with the global model outputs reported in the Supporting information (vegan R package v2.5-6, *adonis2* function, by = 'margin'; Oksanen et al., 2019). Additionally, we ran a Mantel test to test for a correlation between spatial separation and β-diversity measures, and a separate univariate PERMANOVA to test for an effect of social band membership.

### 2.7 | Testing for a phylogenetic signal

Inferences made from phylogeny-informed null modeling approaches are predicated on the existence of a positive phylogenetic signal in species niche-space (Webb, Ackerly, McPeek, & Donoghue, 2002). A positive phylogenetic signal is a pattern wherein closely related species possess similar suites of traits or occupy similar niches (Tucker, Davies, Cadotte, & Pearse, 2018). We tested for a phylogenetic signal with respect to abundance in the presence of sandwort using the R package phylosignal v1.2.1 (Keck, Rimet, Bouchez, & Franc, 2016). To approximate an ASVs association with a (putatively) sandwort-based diet, we estimated the ecological niche space of each ASV based on its average relative abundance within horses for which sandwort was present or absent. Briefly, for each ASV, sequence counts within a given horse in a rarefied dataset was divided by the total sequence count of that ASV summed across all samples. Relative abundance estimates among horses with access to sandwort were multiplied by 1 and relative abundances among horses without sandwort access were multiplied by −1. The sum of these values within each ASV were assigned as a 'niche-score' for each ASV which varied continuously between 1 (ASV only present in horses with access to sandwort) and -1 (ASV only present in horses without access to sandwort).

Sandwort was chosen as the focal environmental variable since: 1) dietary components are expected to vary in their polysaccharide composition, thereby selecting for different microbial metabolic functions (Julliand & Grimm, 2017), 2) sandwort has a very different nutritional profile than all other

components of the Sable Island horse diet (lower fibre, higher crude protein; personal communication K. Johnsen; Lee, 2018) and 3) sandwort presence was observed in preceding analyses to be an important correlate of phylogeny-informed and phylogeny-independent β-diversity.

Again, we emphasize that we inferred phylogenetic conservatism of bacterial niche-space based on ecological associations, rather than making direct measurements of functional traits. The phenomenon of lateral gene transfer (LGT) has raised concerns that traits will not be phylogenetically conserved among bacteria (Boucher et al., 2003). Despite theoretical concerns, reconstructed ancestral gene contents of archaea and proteobacteria suggests that vertical transmission is more influential than LGT (Snel, Bork, & Huynen, 2002). Further, large-scale analyses of thousands of publicly archived prokaryotic genomes indicate that functional traits (especially those related to carbohydrate substrate utilization) are often shallowly phylogenetically conserved (Berlemont & Martiny, 2013; Jain, Rodriguez-R, Phillippy, Konstantinidis, & Aluru, 2018; A. C. Martiny, Treseder, & Pusch, 2013; Martiny, Jones, Lennon, & Martiny, 2015; Van Assche et al., 2017). Counterintuitively, LGT may even reinforce trait conservatism over shallow phylogenetic distances, since rates of LGT are higher between closely related bacteria than between more distant relatives (Jeong, Arif, Caetano-Anollés, Kim, & Nasir, 2019).

### 2.8 | Null modelling within communities

Like macro-ecological communities, the bacterial microbiome can be shaped by deterministic processes (selection), stochastic processes (ecological drift), and dispersal (Adair & Douglas, 2017). If a given community is strongly shaped by selection acting on microbial traits, and microbial traits are phylogenetically conserved, then the phylogenetic structure of this community is expected to deviate from communities assembled through chance (Webb et al., 2002). Conversely, if a community is strongly influence by ecological drift, then phylogenetic structure of this community is not expected to deviate greatly from null expectations.

To evaluate evidence for the strength of stochastic and deterministic processes in the Sable Island horse microbiome, we first calculated mean nearest taxon distances (MNTDs) using the *ses.mntd* function from the R package picante v1.8 (Kembel et al., 2010). MNTD is a measure of the average phylogenetic distance separating every taxon (in this instance ASV) in a community to its nearest neighbour on a phylogenetic tree—this emphasizes diversity at the tips of a phylogenetic tree. For each horse microbiome, a MNTD null distribution was generated via 9999 randomly assembled communities of ASV richness equal to that of the observed community. Randomized communities were generated by re-shuffling taxa labels and relative abundances across a fixed phylogenetic tree comprising the pool of gamma diversity observed across the entire sample-set. MNTDs were effect size-standardized ($MNTD_{ses}$) relative to the mean and standard deviation of the null distribution for a given community (Stegen, Lin, Konopka, & Fredrickson, 2012). A $MNTD_{ses}$ value smaller than -2 or greater than 2 indicate that a community is more phylogenetically clustered or over-dispersed than expected by chance, respectively. While these thresholds have historically been used to make inferences about the relative strength of competition versus environmental filtering (Cavender-Bares, Kozak, Fine, & Kembel, 2009), thought experiments and mixed results from the literature demonstrate such cut-offs are overly simplistic and can lead to a misattribution of patterns to specific ecological process (Mayfield & Levine, 2010). We instead considered only the magnitude of phylogenetic departure from stochastic expectations ($|MNTD_{ses}|$) in a mixed model inference.

### 2.9 | Null modelling between communities

The same principles which underlie the use of phylogenetic null modelling within a given community, can be used to infer possible mechanisms for the variation observed between communities (for a schematic overview of the interpretation of measures in this section, refer to diagram 3 in Zhou & Ning 2017). In the context of between community comparisons, nearest taxon distances are instead calculated between an ASV in one microbiome and its closest relative in a second microbiome (β mean nearest taxon distance [βMNTD]; Stegen et al., 2012), using the *ses.comdistnt* function from the R package

MicEco v0.9.4 (Russel, 2019). For every community pair we standardized βMNTD by the mean and standard deviation of a null distribution created via 999 randomly assembled community pairs ($\beta MNTD_{ses}$). Positive $\beta MNTD_{ses}$ values >2 indicate that two communities are more phylogenetically disparate than expected by community pairs assembled through random sampling of a defined pool of gamma diversity. Conversely, negative $\beta MNTD_{ses}$ <-2 indicate that two communities are more phylogenetically similar than expected by chance. Assuming taxa niche-spaces and phylogenies are correlated, then positive and negative $\beta MNTD_{ses}$ values can indicate that the differences or similarities observed between two communities might be the result of differential or similar selective pressures, respectively (Stegen et al., 2012). $|\beta MNTD_{ses}|$ values of < 2 are conventionally considered to indicate that inter-community differences might be more strongly the result of dispersal patterns or ecological drift, as phylogenetic patterns observed between communities do not differ greatly from those of randomly assembled community pairs. We analyzed $\beta MNTD_{ses}$ using a PERMANOVA parameterized identically to the β-diversity analyses described above. Additionally, we ran a mantel test, to test for a correlation between spatial separation and $\beta MNTD_{ses}$ values, and a univariate PERMANOVA to test for an effect of social band. For all nearest taxon analyses, we used a phylogenetic tree made ultrametric ($\lambda = 1$) using the *chronos* function from the R package ape v5.3 (Paradis & Schliep, 2019).

Finally, we also used a phylogeny-independent extension of this null modeling framework by calculating Raup-$Crick_{Bray}$ ($RC_{bray}$) values (Chase et al., 2011; Richter-Heitmann et al., 2020; Stegen et al., 2013). Rather than consider greater- or less- than-expected phylogenetic similarities between communities, $RC_{bray}$ values indicate whether taxa co-occur at similar abundances more or less often than expected independent of their phylogenetic relatedness (Lowe & McPeek, 2014; Stegen et al., 2013). Among communities which do not show strong phylogenetic deviations from null expectations, $RC_{bray}$ estimates < -0.95 indicate that taxa co-occur at similar abundances between communities more frequently than would be expected by chance, an indication of homogenizing dispersal. $RC_{bray}$ estimates > 0.95 indicate that taxa co-occur between communities less often than would be expected given random

expectations, indicating dispersal limitation. Finally, $|RC_{bray}| < 0.95$ indicate that rates of taxa co-occurrence do not differ from null expectations, suggesting possible ecological drift. The null distributions used to make comparisons were created via 9999 community pairs created through randomization. Like $\beta MNTD_{ses}$, $RC_{bray}$ values were analyzed via PERMANOVA and a partial Mantel test was used to test for a correlation between $RC_{bray}$ values and longitudinal separation, after controlling for $\beta MNTD_{ses}$ values.

## 3 | Results

### 3.1 | Summary of the Sable Island Horse Microbiome

We used a 16S amplicon sequencing approach to characterize the bacterial microbiome of faecal samples collected from 86 adult females of the Sable Island feral horse population. Sequencing resulted in an average of 51,480 quality assembled reads per sample (rarefied to 34,280 reads for all analyses other than those which used centred-log ratio transformed count tables). A total of 3,767 ASVs were detected in the population, although the average horse hosted 817 ± 11 SE ASVs, and only 2 ASVs were observed in all 86 horses.

The average Sable Island horse microbiome was comprised of Ruminococcaceae (15% ± 4% SD mean relative abundance), Lachnospiraceae (13% ± 3%), Prevotellaceae (10% ± 2%), Spirochaetaceae (9% ± 3%), Fibrobacteriaceae (9% ± 4%), Rikenellaceae (8% ± 3%), and three Bacteroidales families (p-251-o5: 9% ± 4%, F082: 3% ± 2%, RF16: 2% ± 1%). An additional 56 families comprised 13% ± 3% of rarefied reads, while the remaining 9% ± 2% of sequences could not be assigned to family; almost half of these unassigned reads were identified as members of the order WCHB1-41 within the newly described class, Kiritimatiellae (Figure S2, Supporting information). Alpha diversity (ASV richness) decreased from west to east (-45 ASVs ± 12 SE per 1 standard deviation change in longitude; $p < 0.01$). Horses with access to sandwort also had 137 ± 35 SE fewer ASVs than those without access to sandwort ($p < 0.01$),

while mares without foals had 52 ± 20 SE fewer ASVs than mares with foals ($p = 0.01$; Figure 3). The full model averaging output can be found in Table S1 of the Supporting Information.

### 3.2 | Phylogeny-Independent β-Diversity

Euclidean distance, a phylogeny-independent β-diversity distance measure, was significantly correlated with day of year ($R^2 = 0.02$, $p < 0.01$), longitude ($R^2 = 0.02$, $p < 0.01$), distance from the population's centre ($R^2 = 0.02$, $p < 0.01$), and sandwort availability ($R^2 = 0.02$, $p < 0.01$). The full PERMANOVA output is reported in Table S2 of the Supporting Information. Sandwort presence appeared to underlie the primary ecological gradient in these communities based on PCA visualization (Figure 4A). Furthermore, Euclidean distances were correlated with the longitudinal distance separating horses ($r_{pearson} = 0.37$, $p < 0.01$; Figure 4B). Additionally, in a univariate PERMANOVA, social band membership was significantly correlated with Euclidean distances ($R^2 = 0.66$, $p < 0.01$); although, this result should be treated with caution, since the number of social groups (52) relative to our sample size likely lead us to over-estimate the explanatory power of social band membership. Multi-model inference analysis of β-dispersion, a measure of β-diversity between an individual horse's microbiome and the horse population's theoretical average microbiome, indicated a negative correlation with longitude (west-east; $p = 0.01$) and a positive correlation with distance from the centre of the population ($p = 0.03$; Table S3, Supporting Information).

### 3.3. | Phylogeny-Weighted β-Diversity

Phylogeny weighted β-diversity (weighted UniFrac distance) was significantly correlated with day of year ($R^2 = 0.02$, $p = 0.01$), longitude ($R^2 = 0.03$ , $p = 0.01$), sandwort presence ($R^2 = 0.03$, $p < 0.01$), and beach pea availability ($R^2 = 0.02$, $p = 0.02$; Table S4, Supporting Information). A positive correlation was again observed between the longitudinal distance separating horses and weighted UniFrac distance (Mantel test: $r_{pearson} = 0.33$, $p < 0.01$). Similarly, in a univariate PERMANOVA, band membership was found to be significantly correlated with weighted UniFrac distance ($R^2 = 0.69$, $p < 0.01$). Log-transformed weighted UniFrac β-dispersion was greater among horses with access to sandwort ($p < 0.01$) but negatively correlated with beach pea abundance ($p = 0.01$, Table S5, Supporting information). Of note, β-dispersion

in weighted UniFrac space trended towards being higher among mares with foals than those without, although this effect was marginally non-significant ($p = 0.06$).

### 3.4 | Ecological Null Modeling

We detected a positive phylogenetic signal over short distances with respect to sandwort availability ($p < 0.05$, $r = 0.02$; Figure S3, Supporting Information). Of the life history, environmental, and spatial terms considered, only parental status ($p = 0.03$) was associated with non-random patterns of null modelling estimates of phylogenetic dispersion. Namely mares with foals had higher $|MNTD_{ses}|$ values (Table S6, Supporting information). Overall, based on between-sample comparisons, communities were more often phylogenetically conserved ($\beta MNTD_{ses} < 0$) than they were phylogenetically disparate ($\beta MNTD_{ses} > 0$) but usually did not deviate in expected phylogenetic similarity from pairs of randomly assembled communities ($|\beta MNTD_{ses}| < 2$).

$\beta MNTD_{ses}$ values were correlated with day of year ($R^2 = 0.03$, $p < 0.01$), sandwort presence ($R^2 = 0.07$, $p < 0.01$), beach pea availability ($R^2 = 0.03$, $p = 0.02$), heathland availability ($R^2 = 0.03$, $p = 0.01$), and grassland availability ($R^2 = 0.02$, $p = 0.04$; Table S7, Supporting Information). In the absence of sandwort, $\beta MNTD_{ses}$ values appeared to be negatively correlated with average grassland availability (Figure 5), but positively correlated with average heath availability (Figure S4A, Supporting Information); conversely, $\beta MNTD_{ses}$ values were greater when sandwort was present for at least one horse in pairwise comparisons and appeared to be negatively correlated with average day of year. In contrast, the absolute magnitude of phylogenetic deviation from stochastic expectations ($|\beta MNTD_{ses}|$) was correlated with beach pea availability ($R^2 = 0.05$, $p < 0.01$), longitude ($R^2 = 0.05$, $p = 0.04$), and parental status ($R^2 = 0.03$, $p = 0.03$; Table S8, Supporting Information). Specifically, $|\beta MNTD_{ses}|$ values appeared to be positively correlated with beach pea availability (Figure S4B, Supporting Information) as well as average longitude, and greater among mares with foals than mares without foals (Figure S4C, Supporting Information). No significant correlation was observed between longitudinal separation and $\beta MNTD_{ses}$

after controlling for sandwort presence (partial Mantel test: $p = 0.68$). Similarly, no effect of band membership on $\beta MNTD_{ses}$ was observed (PERMANOVA: $p = 0.45$).

Approximately 14% of $\beta MNTD_{ses}$ were beyond 2 standard deviations of the randomized null distributions. Of the remaining ~86% of pairwise comparisons, ~97% had corresponding $RC_{bray}$ values exceeding 0.95, which signals greater ASV turnover than expected under ecological drift alone (a pattern suggestive of dispersal limitation). Based on PERMANOVA analyses, $RC_{bray}$ values were correlated with longitude ($R^2 = 0.02$, $p < 0.01$), distance from the centre of the population ($R^2 = 0.02$, $p < 0.01$), sandwort presence ($R^2 = 0.01$, $p = 0.04$), beach pea availability ($R^2 = 0.01$, $p = 0.04$), and day of year. ($R^2 = 0.02$, $p < 0.01$; Table S9, Supporting Information). Additionally, in a univariate PERMANOVA, band membership was significantly correlated with $RC_{bray}$ values ($R^2 = 0.65$, $p < 0.01$). Furthermore, $RC_{bray}$ values were positively correlated with the longitudinal distance separating horses even after controlling for $\beta MNTD_{ses}$ values (partial Mantel test: $r_{pearson} = 0.17$, $p < 0.01$; Figure 6A), but negatively correlated with average longitude and lower among members of the same band than between members of different bands (Figure 6B).

## 4 | Discussion

Accounting for spatial processes in our system was integral to explaining observed patterns of microbiome variation. Longitude, a proxy for horse location on the island, explained variation in almost every microbiome diversity measure considered. Unmeasured environmental variables across the island may account for these patterns; however, plant communities representing the Sable Island horses' primary forage were present in our analyses. Furthermore, if environmental selective pressures acting on the microbiome were spatially autocorrelated, we would have expected co-occurring horse microbiomes to be more phylogenetically similar, and spatially distant pairs of horses to have microbial communities which were more phylogenetically disparate, than expected by chance. Pairwise weighted UniFrac distances, but

notably not $\beta MNTD_{ses}$ were correlated with the longitudinal distance separating horses. This disagreement suggests that the correlation between the longitudinal distance separating horses and weighted UniFrac distances may be the result of differences in α-diversity, rather than disparate selective pressures. Pairs of communities with low diversity are less likely to share phylogenetic branch lengths by chance, and thus, can have larger weighted UniFrac distances (Cadotte & Davies, 2016). Rather than divergent selective pressures, the consistent effect of longitude on measures of microbiome diversity may therefore derive from more frequent microbial transmission between co-occurring individuals. Concomitantly, factors that affect ecological drift or stability of the microbiome could contribute to the effect of longitude. For example, both longitude and distance from the population centre were correlated with Euclidean beta-dispersion in the microbiome relative to the population mean. The significance of spatial terms in PERMANOVA analyses may therefore derive partly from correlations with community variance rather than differences in average community structure.

Host genetics, and thus the physiological environment with which microbes directly interact, might explain some of the spatial variation in microbiome variance. Based on microsatellite data, Sable Island horse genetic heterozygosity is higher in the east (Lucas, McLoughlin, Coltman, & Barber, 2009) which is where we also observed lower microbiome alpha diversity and beta-dispersion when compared to horses in the west. Evidence from captive and wild mammalian systems has shown microbiome alpha diversity to be negatively correlated with host heterozygosity (Grosser et al., 2019; Wadud Khan, Zac Stephens, Mohammed, Round, & Kubinak, 2019). Similarly, an effect of population-level heterozygosity has been reported on the bacterial microbiome of free-living bighorn sheep (Couch et al., 2020). The homozygosity implicit of inbred hosts might restrict their immunological complexity (Potts & Wakeland, 1993; Reid, Arcese, & Keller, 2003), thereby also restricting the dexterity with which host's recruit and "leash" their microbial communities (Foster, Schluter, Coyte, & Rakoff-Nahoum, 2017), perhaps allowing for greater stochastic variation between individuals. Alternatively, $F_{st}$ values in Sable Island horses suggest population sub-structuring between the east and west (Lucas et al., 2009), therefore genetic differences between horses

might also explain why the microbiome differs across the island's length. For example, among free-living house mice, genetic relatedness along a latitudinal gradient was a better predictor of microbiome similarity than spatial proximity (Suzuki et al., 2019). Genetic variation among Sable Island horses expressed as phenotypic variation could therefore drive microbiome variance across the island's longitude (Alberdi, Aizpurua, Bohmann, Zepeda-Mendoza, & Gilbert, 2016).

While we cannot rule out a role for host genetics, in the present absence of data informative for testing this, bacterial dispersal limitation between horses provides the most parsimonious explanation of observed patterns. For example, we observed an apparent positive correlation between the proximity of horses and similarity of their microbiome in Euclidean space (independent of local habitat composition). A similar relationship was observed with respect to weighted UniFrac distances however, no positive relationship was observed among phylogeny-informed null modeling approaches ($\beta MNTD_{ses}$). Assuming bacterial niche space and phylogeny are non-independent, these patterns suggest that the decrease in microbiome similarity with spatial separation was not due primarily to differences in selective pressures across space. Conversely, the positive relationship between spatial separation and $RC_{bray}$ values suggests dispersal limitation may occur over relatively short spatial scales. Evidence for dispersal limitation may be unsurprising given a zero-inflated ASV count table. Of 3767 ASVs, only 2 were detected in all horses and only 441 were present in at least half of the horses.

In addition to a positive correlation with spatial separation, $RC_{bray}$ values were negatively correlated with the average longitude of horse pairs, suggesting greater dispersal limitation among horses in the west than the east. This was unexpected since horse population density, which could facilitate bacterial dispersal between individuals, decreases from west to east (Marjamäki et al., 2013). However, while multiple above-ground ponds can be found in the west, horses in the east must crater through sand to access freshwater (Contasti et al., 2012). Horse-excavated wells are semi-permanent within a season and visited by multiple social bands but are only accessible to 1–2 horses at a time (Figure 2D). Prolonged occupancy of an area of social band overlap, and bottlenecked access to a communal

consumable resource, could catalyze bacterial dispersal despite low population densities in the east. Similar host aggregation due to patchy resource distribution on urban landscapes facilitates disease transmission in wildlife (Bradley & Altizer, 2007); the same aggregative effect could as easily facilitate transmission of commensal and mutualistic microbiota.

Bacterial dispersal between horses undoubtedly occurs; however, it may be largely restricted to between individuals within the same, or closely interacting, social bands; although, we lack the resolution in social data to directly test the latter assertion beyond reporting the effect of spatial proximity (a proxy for overlap in social band territories). Social band membership was correlated with both Euclidean and weighted UniFrac β-diversity; however, microbiome phylogenetic diversity ($\beta MNTD_{ses}$) was no more similar between members of the same band than between members of different bands (when compared to null expectations), offering little support for homogenizing selection as the mechanism for the effect of band membership on the microbiome. $RC_{bray}$ values, which were lower between members of the same band than between horses of different bands, suggests bacterial dispersal limitation as a primary cause for the observed effect of social band. This interpretation is consistent with Antwis, Lea, Unwin, & Shultz (2018) who report an effect of band identity and inter-band connectivity on microbiome β-diversity among three large social bands of feral Welsh ponies. Similar differences in band connectivity might explain why, above and beyond parameterized environmental terms, distance from the population's centre was correlated with Euclidean β-diversity and β-dispersion. No relationship was observed with respect to $\beta MNTD_{ses}$ but, $RC_{bray}$ values were positively correlated with the average distance of horse pairs from the centre of the population. Horses on the edges of the population—those more poorly connected within the population's microbiome meta-community (Miller et al., 2018)—might be vulnerable to erosion of microbiome diversity through microbial extinctions and exacerbated ecological drift. Together these results support recent theorization that inter-host microbial dispersal is an important mechanism which shapes the microbiome variation observed in free-living wildlife populations (Sarkar et al., 2020).

Phylogeny-informed measures of diversity were generally better explained by local plant community composition than spatial terms. Horses with sandwort in their 150-m radius buffer had lower alpha diversity and differed in both phylogeny-independent (Euclidean) and phylogeny-informed (weighted UniFrac) β-diversity measures. The intuitive explanation is that local plant communities reflect dietary composition, and dietary components differ in their polysaccharide composition, and thus, the microbial functions required to fully metabolize (David et al., 2014; Julliand & Grimm, 2017). However, among pairwise comparisons in which sandwort was present for at least one horse, microbiomes were no more phylogenetically disparate than expected by chance ($\beta MNTD_{ses}$ values close to 0). By comparison, the microbiomes of horses without access to sandwort tended to be more phylogenetically similar. Conversely, average grassland and beach pea habitat class covers were negatively correlated with $\beta MNTD_{ses}$, while heath (only present where sandwort was absent) appeared to be positively correlated with $\beta MNTD_{ses}$. Therefore, phylogenetic patterns most consistent with homogenizing selection acting on the microbiome were observed when sandwort and heath were absent, but beach pea and marram grass were abundant. Under reversed conditions, phylogenetic similarities did not deviate far from stochastic expectations.

Increased evidence for stochasticity in the presence of sandwort and heathland may stem from the fact that sandwort, as well as the forbs and small graminoids which comprise the primary horse forage in heathland habitats, possess lower neutral detergent fibre (NDF) when compared to beach pea and marram grass (personal communication K. Johnsen; Lee, 2018). NDF is a coarse measure of plant lignin, hemicellulose, and cellulose (Mongeau & Brassard, 1982)—compounds which many herbivores are obligately reliant upon their gastrointestinal microbiota to metabolize (Costa & Weese, 2012). The low NDF characteristic of sandwort and heathland forbs may alleviate the horses' reliance on their intestinal microbiota, allowing them to directly absorb nutrients from a relatively labile diet. Loss of dietary complexity constrains fibrolytic and cellulolytic niche-space in the microbiome which can manifest as reductions in bacterial gene richness (Cotillard et al., 2013) or alpha diversity (Schnorr et al., 2014).

Conversely, high fibre forage (e.g. marram grass and beach pea) can facilitate complex microbial symbioses in which different species specialize on metabolizing different biochemical compounds, and in doing so, create by-products to be absorbed by the host or further metabolized by other microbiota (Oliphant & Allen-Vercoe, 2019). The reduction in alpha diversity observed in horses with access to sandwort mirrors the effects of low dietary fibre manipulations in domestic horses (Julliand & Grimm, 2017). When compared to marram grass and beach pea, sandwort might represent a reduction in the carbon source complexity accessible to the microbiome, a property thought to have a stabilizing effect on the microbiome (Coyte et al., 2015). A diet containing sandwort might not select for different microbial functions, so much as fail to support the full diversity of fibrolytic niche-space created by high fibre diets, leading to species extirpation and greater ecological drift within individual host microbiomes (Deehan & Walter, 2016). This could also explain the greater variability in weighted UniFrac β-diversity among horses with access to sandwort and the decrease in dispersion in response to beach pea availability. These results highlight how dietary derived microbiome variation might not always be the result of strong differential selective pressures between communities; the relationship between dietary complexity and ecological drift must also be considered (Adair & Douglas, 2017; Zhou & Ning, 2017).

Parental status was more strongly correlated with measures of microbiome variance, rather than mean community structure. Specifically, mares with foals had microbiomes which were a) more diverse, b) marginally less variable in weighted UniFrac space, c) less randomly phylogenetically dispersed (higher $|MNTD_{ses}|$), and d) further from phylogenetic null expectations of random community assembly (higher $|\beta MNTD_{ses}|$) when compared to mares without foals. Effects of parturition and maternal status on microbiome alpha and β-diversity have been observed in livestock (Lima et al., 2015) and wildlife (Amato et al., 2014). Although, to our knowledge, a difference in β-dispersion between parental states has not previously been reported. Myriad changes to maternal physiology during pregnancy and parturition are likely partly responsible for microbiome differences during birth and child-rearing (Huang et al., 2019; Nuriel-Ohayon, Neuman, & Koren, 2016). In addition to these physiological changes, maternal care

among mammals (especially lactation) saddles mothers with a heavy energetic burden (Dufour & Sauther, 2002; Scantlebury, Russell, McIlrat, Speakman, & Clutton-Brock, 2002). To meet higher energetic demands, hosts may become increasingly reliant on their microbiomes (Amato et al., 2014); especially in species such as horses, which are obligately reliant on their gut microbiomes for nutrient uptake (Costa & Weese, 2012). Therefore, during periods of high energetic demand hosts might enforce stronger control on the microbiome to maximize metabolic efficiency. For example, in laboratory mice, post-partum dampening of bi-directionality in the host-microbiome relationship is evidenced by attenuated bacterial driven immunomodulation (Mu et al., 2019). We suggest that hosts facing a high energetic burden might keep their microbial constituents on a "tighter leash" than those with a lower energetic demand (Foster et al., 2017). Within host species, host physiological variation might in many cases act to facultatively constrain β-dispersion, rather than drive changes in mean β-diversity, although patterns of the former are often overlooked (Zaneveld et al., 2017). The reverse causal relationship could also explain the patterns observed, whereby a diverse microbiome under tight host control signals better host health and therefore greater likelihood of carrying a foal to term. We also note that our inference is limited by our inability to confidently assess the pregnancy status of mares without foals at the time of sampling.

The inferences we derive from null modelling results—and therefore our interpretation of spatial, environmental, and life history effects—are likewise limited, predicated as they are on the assumption that bacterial niche-spaces are shallowly phylogenetically conserved. That bacterial traits are likely phylogenetically conserved in our system is broadly supported by re-analysis of archived bacterial genomes (Berlemont & Martiny, 2013; Jain, Rodriguez-R, Phillippy, Konstantinidis, & Aluru, 2018; A. C. Martiny, Treseder, & Pusch, 2013; Martiny, Jones, Lennon, & Martiny, 2015; Van Assche et al., 2017), and more specifically, by the positive phylogenetic signal we detected with respect to sandwort. Nonetheless, future shotgun metagenomic sequencing and *de novo* genome assembly will be required to empirically demonstrate phylogenetic conservatism in the Sable Island horse microbiome. In the absence of this data, we cannot unreservedly conclude that the failure of communities to deviate from null

637 expectations is the result of weakened deterministic processes. Nonetheless, these results help to generate
638 new hypotheses which can be directly tested in future research.

639 Overall, the bacterial microbiome of Sable Island horses is dominated by clades of fibrolytic taxa,
640 including Ruminococcaceae, Lachnospiraceae, Prevotellaceae, and Fibrobacteraceae (Biddle, Stewart,
641 Blanchard, & Leschine, 2013; Esquivel-Elizondo, Ilhan, Garcia-Peña, & Krajmalnik-Brown, 2017; Spain,
642 Forsberg, & Krumholz, 2011). Spirochaetaceae and Kiritimatiellae are also present at modest relative
643 abundances; however, their metabolic niches are currently less well characterized. These results are
644 consistent with findings from domestic, feral, and wild horse systems (Antwis et al., 2018; Costa et al.,
645 2015; Metcalf et al., 2017) and a comprehensive comparison of wild and domestic equid species
646 (Edwards et al., 2020). Unlike previous studies, however, we detected no effect of age, likely because we
647 constrained sampling to horses of at least 3 years of age, and the horse microbiome appears to reach
648 maturation after ~1 year (Antwis et al., 2018; De La Torre et al., 2019; Metcalf et al., 2017).

649 We characterized the bacterial microbiome of 86 mares from the feral horse population of Sable
650 Island (Nova Scotia, Canada) and contrasted the ability of spatiotemporal, life history, and diet-linked
651 environmental variables to explain microbiome variation. Phylogeny-independent measures of diversity
652 were best explained by spatial variables while phylogeny-informed measures were generally better
653 characterized by measures of local habitat heterogeneity and host life history (parental status); however,
654 despite statistical significance, these variables explained only nominal variation in overall β-diversity.
655 Only the longitudinal distance separating horses and social band membership explained what could be
656 considered substantive variation, and yet, much of the variation in the Sable Island horse microbiome
657 remained unexplained. In context, our results suggest a predominant importance of bacterial dispersal and
658 ecological drift in shaping faecal microbiome variation among Sable Island horses. Our findings are
659 relevant to the study of wildlife microbiome variation: clearly data on the spatial distribution of hosts
660 should be collected, even at the within-population scale, alongside metrics of individual-based

environmental variation. Further, when a response of the microbiome to environmental or physiological variation is observed, deterministic processes must not be assumed as the sole causal process.

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## Data Accessibility

- Sample metadata, R scripts, and bioinformatic pipelines: https://doi.org/10.5061/dryad.stqjq2c27
- DNA Sequences: NCBI SRA (BioProject accession number: PRJNA674675)

## Author Contributions

JP, PDM and AJW secured research funding. JP and PDM led sample collection and laboratory analysis. AH and SG completed bioinformatic processing. JP and MRS designed the study. MRS completed analyses and led the writing efforts. RJG contributed habitat classification data, created map visualization, and wrote the description and discussion of the island's vegetation. All authors contributed to the writing of the final submitted manuscript.

## Figures

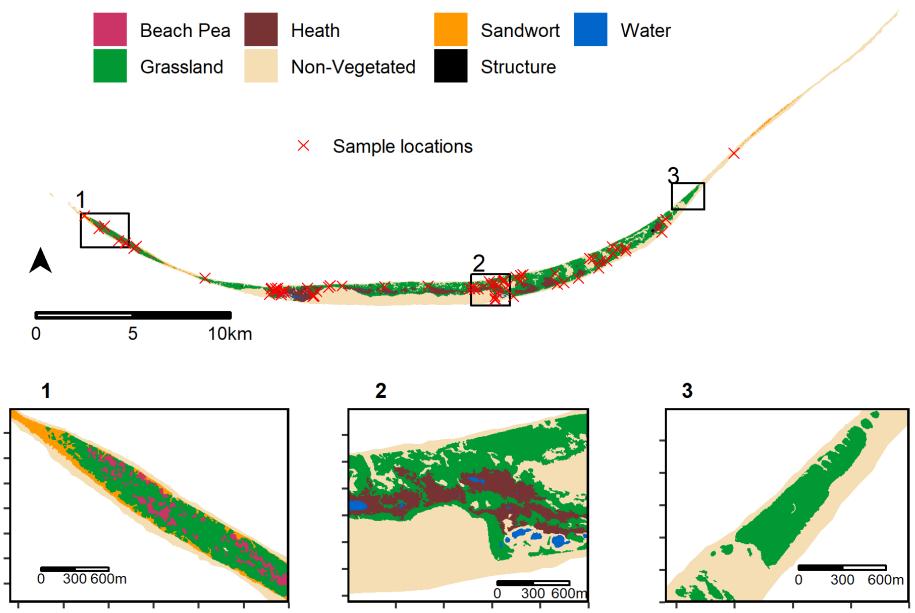


Figure 1: A map of Sable Island National Park Reserve, Nova Scotia (Canada). Habitat classes were delineated through a combination of Light Detection And Ranging (LiDAR) surveys, high-resolution aerial photography, and ground truthing. X marks the spot of collection for the faecal samples used in this study. Insets 1, 2, and 3 demonstrate habitat class heterogeneity across the island's length.


Figure 2: Putative mechanisms of bacterial dispersal between Sable Island horses: (A) social grooming [pictured: social band stallion (left) and mare (right) engaged in reciprocal grooming], (B) coprophagy, the consumption of faeces [pictured: a foal (foreground) consuming the faeces of its mother (background)], (C) interactions with the faeces of band members or faecal territory markers (stud piles) [pictured: band stallion scenting faeces from a social band mare], (D) aggregation of social bands at communal resources [pictured: horses standing in—and drinking from—an excavated freshwater well (background) immediately adjacent to a fecal stud pile (foreground)]. Photos ©Mason R. Stothart.

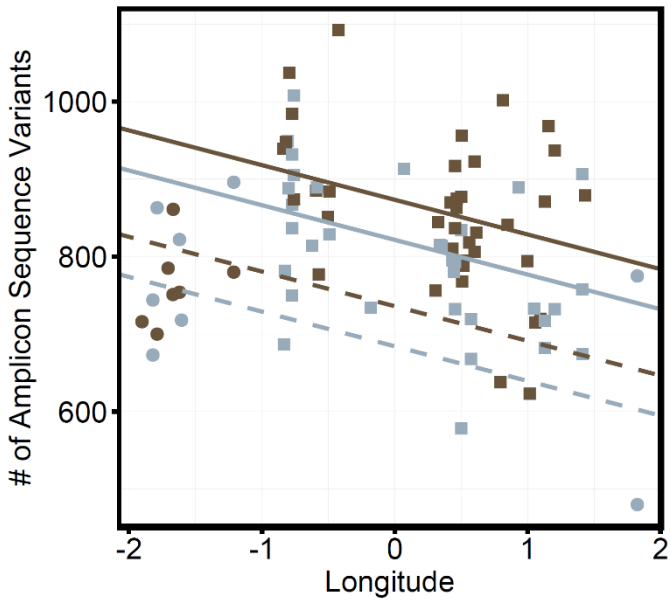


Figure 3: A scatter plot of Sable Island horse microbiome ASV richness versus standardized longitude coloured by parental status and shaped by sandwort presence in a 150-m radius buffer surrounding the point of sample collection. Points are shaped by whether sandwort is present (circle: ●) or absent (square: ■) and coloured by whether mares were with a foal (brown: ◙) or without a foal (light blue: ◙). Lines are parameterized by estimates from multi-model inference model averaging, typed by sandwort presence (present, dashed: ---; absent, solid: —) and coloured by parental status (with a foal, brown: —; without a foal, light blue: —).

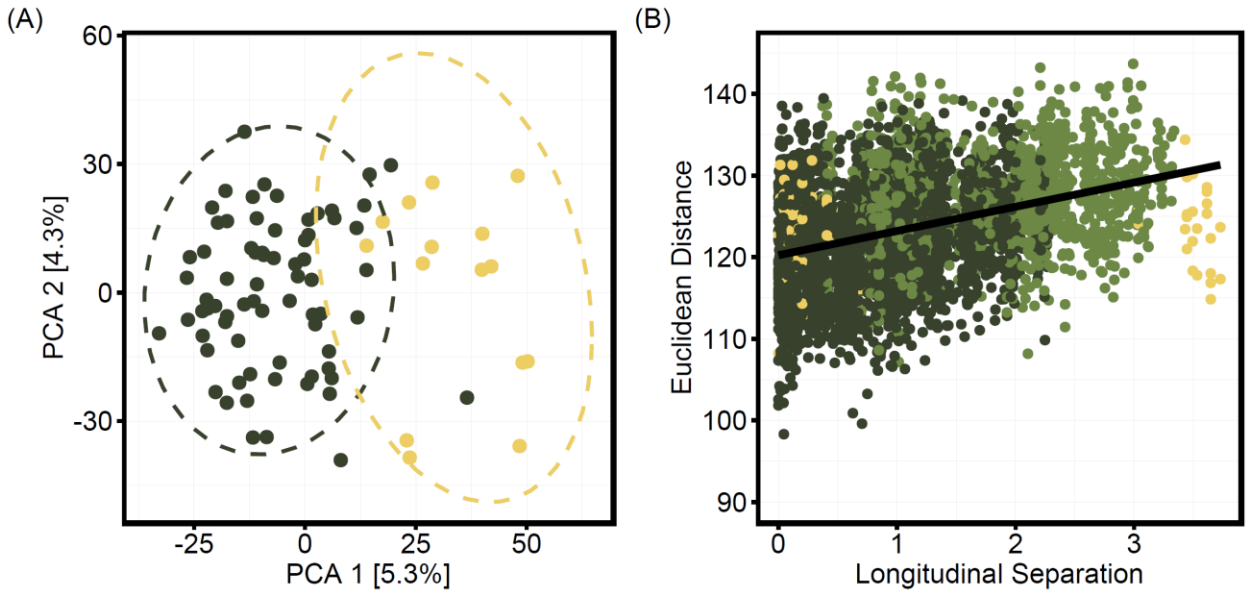


Figure 4: The Sable Island horse faecal microbiome β-diversity (Euclidean distance centred log-transformed counts) visualized in (A) a PCA coloured by sandwort availability in 150-m radius buffers surrounding the point of sample collection (absent, dark green: ●, present, gold: ●) and (B) a scatterplot of Euclidean distance and the longitudinal distance separating pairs of horses, points coloured depending on whether 150-m spatial buffer contained sandwort for neither horse (dark green: ●), only one horse (green: ●), or both horses (gold: ●). For ease of plot visualization, a single point was omitted from panel '(B)' corresponding to two horses of the same social band sampled at the same location (longitudinal separation = 0, Euclidean distance = 64).

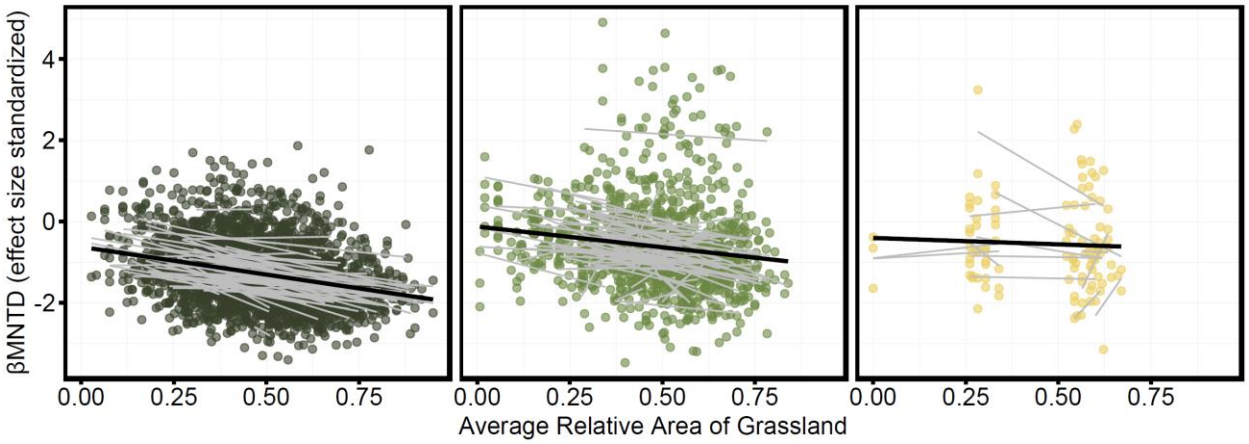


Figure 5: Scatterplot of pairwise average relative area of grassland within 150-m radius buffers centred on point of sample collection versus effect size standardized β mean nearest taxon distance between pairs of horses. Plot facetted by sandwort presence within 150-m radius buffer (absent for both horses, dark green: ●; present for only one horse, green: ●; present for both horses, gold: ●). Black lines denote the lines of best fit, grey lines are lines of best fit group by individual one of the pairwise comparisons.

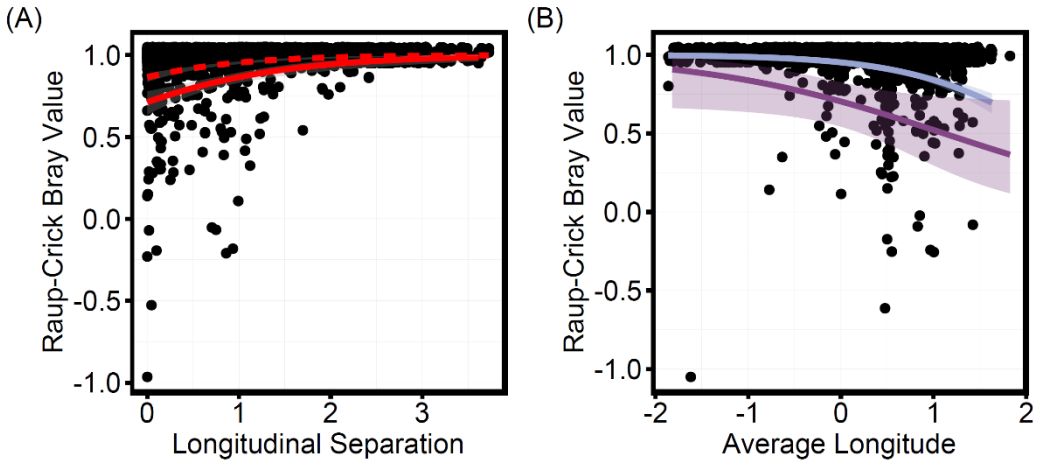


Figure 6: Scatterplot of Raup-Crick$_{bray}$ values versus (A) longitudinal separation of horses with best fit binomial regression grouped by whether the corresponding β mean nearest taxon distance did (dashed: --- ) or did not (coded as "0", solid: — ) deviate from null phylogenetic expectations and (B) average longitude with best fit binomial regression coloured by whether comparisons were made between members of the same (dark purple: —) or different (light purple: —) social bands. Shading represent 95% confidence intervals. Binomial regressions were fit to a binary dataset, in which Raup-Crick$_{bray}$ were categorized as >0.95 ("1") or <0.95 ("0").