Genes, geology, and germs: gut microbiota across a primate hybrid zone are explained by site soil properties, not host species

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Supplementary information

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Supplementary Methods and Results

1. Ruling out soil contamination

Because our samples were collected from the ground, we could not exclude the possibility that some samples were contaminated with microbes from the soil. However, fecal samples from all 14 baboon populations in our data set were compositionally similar to those of other primates such that Firmicutes and Bacteroidetes are the most common phyla [1-3]. In contrast, around the globe and in Western Kenya, soil communities are typically dominated by Acidobacteria, Chloroflexi, Proteobacteria, Planctomycetes, and Verrucomicrobia [2, 4-6].

In addition to these broad compositional differences, soil contamination is unlikely to explain our results for three main reasons. First, mammalian feces contain one of the densest microbial communities on earth, with approximately 10^{11} microbes per gram [7]. In contrast, a typical soil sample contains 10^{8} microbes per gram [8]. If a 4 g fecal sample was coated with ~200 mg of soil (a generous estimate), the soil would contribute ~0.005% of the microbial cells in the sample (2×10^{7} cells in 200 mg of soil / 4×10^{11} cells in a 2 g fecal sample). These contaminating cells might represent rare taxa in baboon gut microbiota, if they differ from microbes already present in the gut, or they might contribute to read counts of taxa already present in the gut, especially to microbes ingested incidentally along with the baboons' food. If they fall into the first category, we find that our results are robust to the removal of rare taxa, both in terms of the percent of samples a microbe is found (prevalence) in and mean microbial abundance across samples (see Applying BEDASSLE to microbial presence/absence data). These results imply that contamination from rare microbes are not disproportionately contributing to the patterns we see in our data

Second, if the contaminating microbes overlap with (i.e. are not different from) those already present in the gut, then their addition should not substantially alter the observed proportions of these microbes in our data. In other words, if fecal samples are contaminated by soil after defecation, the microbes in the soil should be similar to those that the baboons are ingesting and thus should not distort the proportions. Specifically, the upper bound of a possible difference in abundance is around the 0.005% contribution of soil microbial reads to the overall read pool, estimated above (note, this assumes that all contaminating reads belonged to the same OTU, which is conservative and extremely unlikely). However, the mean difference in microbial abundance between populations was 0.037%, an order of magnitude larger than 0.005%. Our

effect sizes are hard to attribute to the addition of such a small number of reads. For beta diversity, the Mantel Pearson's r correlations for significant soil traits were very large, indicating that 59% of the variation could be explained by exchangeable sodium, 36% by soil pH, or 18% by geology. In Figure 3, it's hard to explain such large shifts in beta diversity (weighted UniFrac ranges from 0.3 to 0.5) with the addition of a small number of contaminating reads. For alpha diversity, a shift from the least to most salty soils (1% to 23% exchangeable sodium) led to a gain of 323 OTUs, which is striking, given that the median number of OTUs in our samples was 414 OTUs.

Third, it is theoretically possible that differences in soil microbe abundance are so great between populations that even a small amount of contamination could falsely point to between-population differences. However, even if the host-microbe abundances did not differ between populations at all, the relative proportion of contaminant microbes is so small (<0.005%) that between-population differentiation in the soil microbes would have to be enormous to generate the results we observe. For example, even if the abundance of an OTU contaminant differed between populations by 10 standard deviations (i.e., the abundance of the OTU in soil from any given population was completely non-overlapping with the abundance in any other population), we would never observe significant between-population differences with only a 0.005% contribution to the read pool (based on conservative simulation using ANOVA).

2. Ruling out technical differences between Amboseli and other populations

In our dataset, we found that baboons sampled from Amboseli had gut microbiota that were distinct from samples from other populations. We were concerned that this difference was attributable to differences in the time to sample preservation in Amboseli versus the other populations. Specifically, fecal samples from Amboseli baboons were collected and preserved within 15 minutes of defecation, while fecal samples from some of the other populations were collected from a few hours to two days after defecation. Exposure to the environment could affect microbiota composition through exposure to environmental microbes or microbial growth before preservation. Two prior studies have found that time to preservation does not explain significant variation in gut microbiota measured in fecal samples [9, 10]. However, to confirm that time to preservation did not drive between-population differences between Amboseli and

other populations, we ran a time series experiment to test the effects of exposure time on gut microbiome alpha and beta diversity.

Methods

We collected one fecal sample from each of 10 Amboseli baboons and exposed equal-sized aliquots of these samples to natural environmental conditions in Amboseli over 5 time points: 15 min, 1 h, 8 h, 24 h, and 48 h after defecation. After exposure, each aliquot was preserved in 95% ethanol. One sample was misplaced before shipment to the US, leaving a total of n = 49 time point samples. Although DNA extraction and 16S rRNA gene sequencing of the 49 timepoint samples was performed separately from the 191 samples described in the main text, the timepoint samples are taxonomically similar to the other Amboseli samples (Fig. S13). For these 49 samples, we obtained 78,750 ± 19,820 (median ± sd) reads per sample and 604 ± 121 (median ± sd) OTUs per sample.

To test whether time to preservation predicted gut microbial alpha diversity, we ran linear mixed models in the *lme4* [11] and *lmerTest* packages [12], with OTU richness as the response variable, per sample read count and time point as fixed effects, and baboon identity as a random effect.

To test whether time to preservation predicted gut microbial beta diversity, we ran a PERMANOVA with weighted UniFrac as the response variable and baboon identity and time point as the predictor variables in the *vegan* package [13].

To test whether time to preservation predicted the relative abundance of individual gut microbial phyla in each sample, we ran linear mixed models with the per-sample relative abundance of each phylum as the response variable, per sample read count and time point as fixed effects, and baboon identity as a random effect. We repeated the analysis at the levels of class, order, family, and genus for each taxon not listed as ‘Other’ or ‘Unknown’, and then corrected for multiple hypothesis testing using the false discovery rate approach of Benjamini-Hochberg [14]. There were 2,423 OTUs in the time series data that were identified to a genus or higher taxonomic level in QIIME. These OTUs were collapsed into 641 taxon-level classifications, excluding OTUs listed as unassigned or other.

Results

We found no evidence that time to preservation predicted gut microbial composition. OTU richness did not change with exposure time ($p = 0.85$; Fig. S1A), and exposure time explained only 4.6% of the variance in microbial weighted UniFrac ($r = 0.046$, $p = 0.051$; Fig. S1B). Further, in taxon specific analyses, only 8 of 641 taxa changed in abundance based on time until sample preservation (5% FDR threshold). These included the class Coriobacteriia and, within it, the order Coriobacteriales and family Coriobacteriaceae; the order Lactobacillales and, within it, the family Lactobacillaceae and genus *Lactobacillus*; the family Mogibacteriaceae; and the genus *Faecalibacterium*. However, each of these taxa comprised < 3% of reads in most samples and 0.4 – 3.8% of OTUs per sample. They also changed by <1% over the 48-hour sampling period.

3. Quantifying environmental differences between populations

We characterized environmental differences between all 14 populations using 13 environmental variables extracted from maps (Tables S2, S3; Fig. S2; [15-17]). All of these variables are potential predictors of the availability of baboon foods and/or microbial exposures from the environment, and the majority are uncorrelated with each other (Table S3; Mantel R (mean ± s.d.) = 0.089 ± 0.207; range=-0.241 – 0.882). For each population, we measured each environmental variable based on a 28 km^2 circle (6 km diameter) surrounding the sampling location (Fig. S2) using ArcMap 10.2.2 [18]. We chose a 28 km^2 circle because 6 km corresponds to the largest observed core home range diameter (i.e. 75% of usage time) of baboons in Amboseli (Grieneisen et al., unpublished data), making 28 km^2 a generous estimate of range size.

Vegetation. The composition of vegetation predicts the availability of plant-based foods, which comprise the vast majority of baboon diets [19]. We used a 1 km^2 grid cell resolution vegetation map to measure the relative abundance of 10 vegetation types within each sampling circle (e.g. montane forest, closed deciduous forest, sparse grassland, etc.; Table S2; Fig. S2A; [17] [19]). We then calculated a single vegetation Bray-Curtis dissimilarity matrix between populations using *vegan* [13].

Soil traits. We chose ten soil traits that could influence plant and microbial communities for each population: bulk density, cation exchange capacity, clay cation exchange capacity,

drainage, exchangeable sodium percentage, pH, sand percentage, total carbon, total nitrogen, and SOTER Soil Units (SOil and TERrain) Soil Units (Tables S2, S3; [20-23]). SOTER Soil Units are a standardized soil taxonomy system that groups soils by their geological genesis, terrain, weathering, and chemical properties. SOTER Soil Units are not tightly correlated with the other 9 soil characteristics (Table S3), in part because they are based on the top 20-100 cm of soil, while the other 9 soil metrics are derived from the topsoil alone (i.e., the top 0-20 cm). All 10 soil traits were obtained from a high-resolution soil and terrain map of Kenya divided into polygons (Fig. S2B [15]). We calculated a single weighted mean value for each soil trait, except for SOTER Soil Units, at each population based on the relative area of each polygon in the sampling circle and then created a between-population distance matrix for each trait. For SOTER Soil Units, we calculated a Bray-Curtis dissimilarity matrix between populations

Geology. The underlying geology of a population predicts its soil properties, especially nutrients and minerals, which in turn affect plant and microbial community composition [24]. Geological composition for each population was determined from a fine-scale (1 cm represents 10 km) geology map (Fig. S2C; [16]). There were 9 geological types across the 14 baboon populations (Table S2). To calculate between-population differences in geological types, we used geological type composition to calculate a Bray-Curtis dissimilarity matrix.

Elevation. Elevation is a strong proxy for rainfall and ambient temperature in Kenya, both of which can affect the composition of plant and microbial communities [25]. We pulled elevation data for each population using the strm elevation model [26]. Elevation ranged from 623 – 1,797 m above sea level (median = 1,227 m).

4. Characterizing gut microbiota in each baboon fecal sample

DNA extraction and 16S rRNA gene sequencing. DNA was extracted from each fecal sample using the Powersoil DNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) [27]. We amplified the V4 region of the bacterial 16S rRNA gene via polymerase chain reaction using barcoded primers 515F and 806R [28]. Multiplexed libraries were subjected to paired-end sequencing (301 bp per sequence) on the Illumina HiSeq 2000 platform, yielding 31,521,814 paired raw sequencing reads.

Bioinformatics pipeline. Read merging, quality filtering, OTU (operational taxonomic unit) clustering, and taxonomic assignments were conducted using a QIIME-based pipeline (Fig.

S3 [29]). Five of 196 samples were removed during quality filtering, yielding a final dataset of 191 samples from 14 populations (mean=14 samples; range=1 – 27; Table S1). We retained 26,458,080 reads after quality filtering (mean=138,524; range=30,700–430,465), which clustered into 2,711 OTUs overall (mean per population=1,496; range=727–2,050), and a mean of 791 OTUs per sample (range=133–1,152).

Alpha diversity, measured as per-sample OTU richness, was calculated in QIIME. We normalized the OTU table using cumulative sum scaling in *metagenomeSeq* to account for variation in the number of reads per sample [30], and then calculated weighted UniFrac to measure microbiome composition [31].

5. Applying BEDASSLE to microbial presence / absence data

The BEDASSLE model assumes that populations are at migration-drift equilibrium such that allele presence/absence can be modeled based on geographic distance between populations [32]. Here, we generalized BEDASSLE to model between-population variation in OTU presence/absence instead of host allele frequencies at unlinked genetic loci. We also confirmed that its results are robust to modeling correlated OTUs (since it was originally designed to work with unlinked variants) and low read count (i.e. rare) OTUs ([33]). Our inputs for BEDASSLE were: (1) a matrix of OTU prevalence (analogous to bi-allelic count data), in which each row is a population, each column is an OTU (locus), and each cell gives the number of times the OTU is observed in each population (i.e., the number of baboons who have the OTU), and (2) a matrix of sample sizes, in which the rows and columns are the same as in the first matrix but each cell gives the total number of baboons (analogous to chromosomes) sampled in each population [33]. We tested the two major ways in which microbial presence/absence data could vary from genotype data under the assumptions of the BEDASSLE model.

First, the absence of an allelic variant in an individual likely represents true absence of the allele. This assumption is reasonable because genotype data are generally treated as if they were known without error, and each allele in a diploid organism represents 50% of the signal/read depth in most genotyping approaches. Rare OTUs, however, could go undetected during sampling because they represent a very small fraction of the total microbe pool; OTUs that are rare in all individuals in a population sample might therefore also be missed in the population as a whole, and therefore indicate a false negative. To test if rare or missing OTUs

had an undue influence on the model output, we limited the dataset to more common OTUs based on two natural breaks in OTU abundance (Fig. S4). Specifically, we excluded the rarest 27.4% of OTUs (those found in < 2.1% of samples; 742 OTUs excluded) and ran BEDASSLE using the same MCMC control parameters as in the full dataset. We also ran the model excluding the rarest 40.3% of OTUs (those found in < 4.7% of samples; 1,093 OTUs excluded; Fig. S4). We ran 2 MCMC replicates for each subsampled dataset, and the results were consistent across replicates. The outputs from both the 27.4% and 40.3% models were consistent with the full dataset, suggesting that the BEDASSLE results are robust to the presence of rare OTUs (Table S4; Fig. S5-S7).

Second, BEDASSLE assumes that the input data are uncorrelated (i.e., that variants in the original model are not in linkage disequilibrium). Violating the assumption of independence between estimates is not expected to bias parameter estimation, but it can make the posterior credible intervals artificially narrow. To determine if the OTUs in our dataset violated the assumption of non-linkage, we ran two sets of analyses. First, we calculated the Phi coefficient of association using the rcorr function in *Hmisc* [34] on sample-level presence / absence for all possible pairs of OTUs. Few OTUs demonstrated strong correlations (0.39% of OTU pairs exhibited correlations > 0.5), and this effect was further weakened when Amboseli was excluded (0.2% of OTU pairs exhibited correlations > 0.5; Fig. S8A, S8B).

We additionally ran a sensitivity analysis to break up any potentially correlated sets of OTUs. Following the rare OTU analysis, we randomly excluded 27.4% of OTUs from the full dataset (n = 742 OTUs) and ran BEDASSLE on the random subset. We repeated the analysis on 4 additional random subsets of 27.4% of OTUs removed. We also ran BEDASSLE on 5 random subsets of the data in which 40.3% of OTUs (n = 1,093 OTUs) were excluded. Outputs from 9 models (one model failed to run) revealed that, although the actual parameter estimates fluctuated, the qualitative ordering of effect sizes was consistent across fairly substantial perturbations of the dataset (Table S4; Fig. S5-S7).

Taken together, our results suggest that BEDASSLE is a reliable method to test predictors of microbial OTU distributions across geographically disparate populations. Future applications of the BEDASSLE model to microbiome data could explore extensions that incorporate uncertainty in OTU detection as a function of abundance patterns.

BEDASSLE settings specific to our dataset

We ran BEDASSLE using the beta-binomial model (code available on the Archie Lab github: https://github.com/ArchieLab/grieneisen_etal_2019_PRSB), which prevents outlier populations from having an undue influence on the effect size estimates of the predictor variables [32] (Amboseli was a clear outlier in our analysis). We ran the final model for 10 million generations with an 8 million generation burn-in, and then ran 1,000 posterior predictive sample replicates to evaluate model fit (Fig. S9).

Figure S1. (A) Time to sample collection did not influence OTU richness. (B) A PCoA showing that time to sample collection did not predict gut microbiome composition (weighted UniFrac).

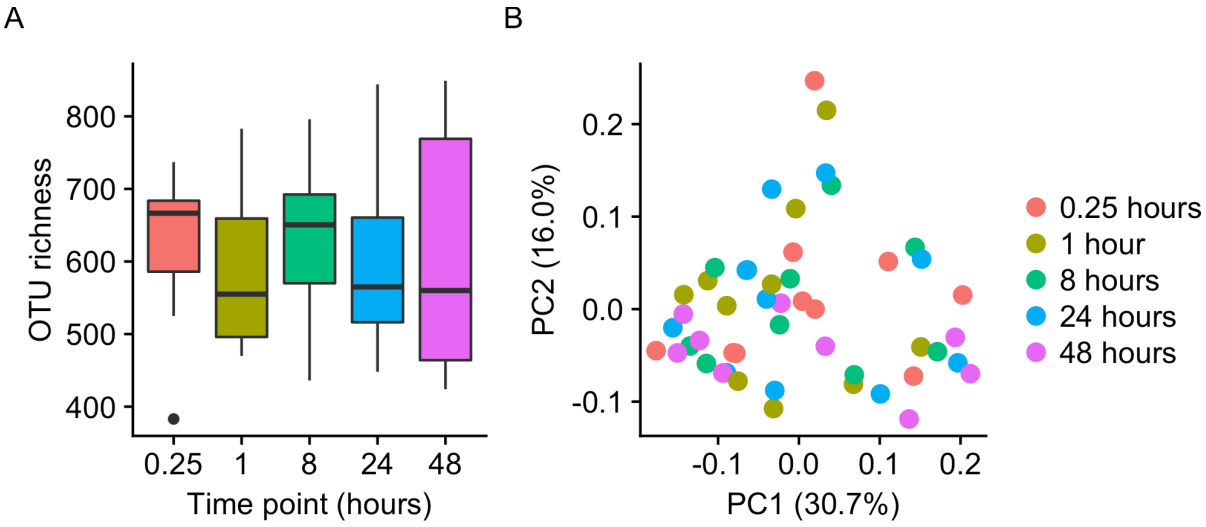


Figure S2. Maps show the 6 km diameter buffers drawn around 3 of the 14 baboon populations. (A) Vegetation types indicated by different colors at a 1 km^2 grid resolution [17]. (B) All ten soil traits were obtained from a high-resolution soil and terrain map of Kenya divided into polygons, where each polygon was assigned a soil trait value. Soil traits are reflected in different colors and are given on a numeric scale; the example soil trait shown here is soil pH. Polygon construction is detailed in [15]. (C) Geology types indicated by different colors [16]. All 3 maps extract data at the scale of 1 km or finer resolution.

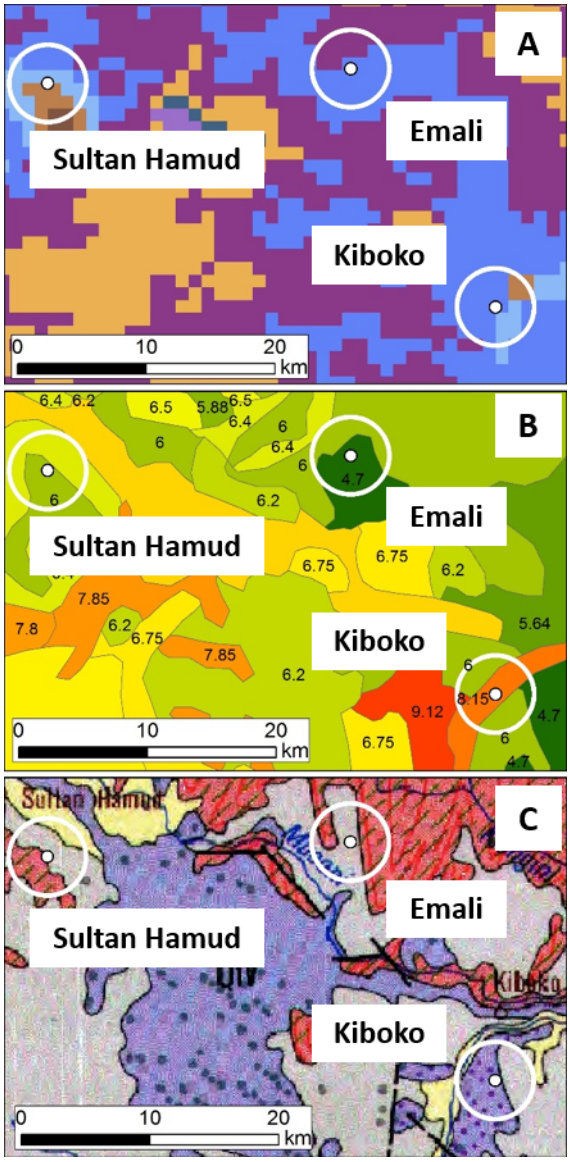


Figure S3. Bioinformatics pipeline. Green boxes indicate read counts, grey boxes are conceptual steps, and white boxes indicate specific computational steps. We used USEARCH v8.0.1623_i86osx32 [35, 36], cutadapt v1.6 [37], greengenes v13.5 [38], and the fasttree method of tree construction [39] as implemented in MacQIIME 1.8.0-20140103 [29, 40, 41].

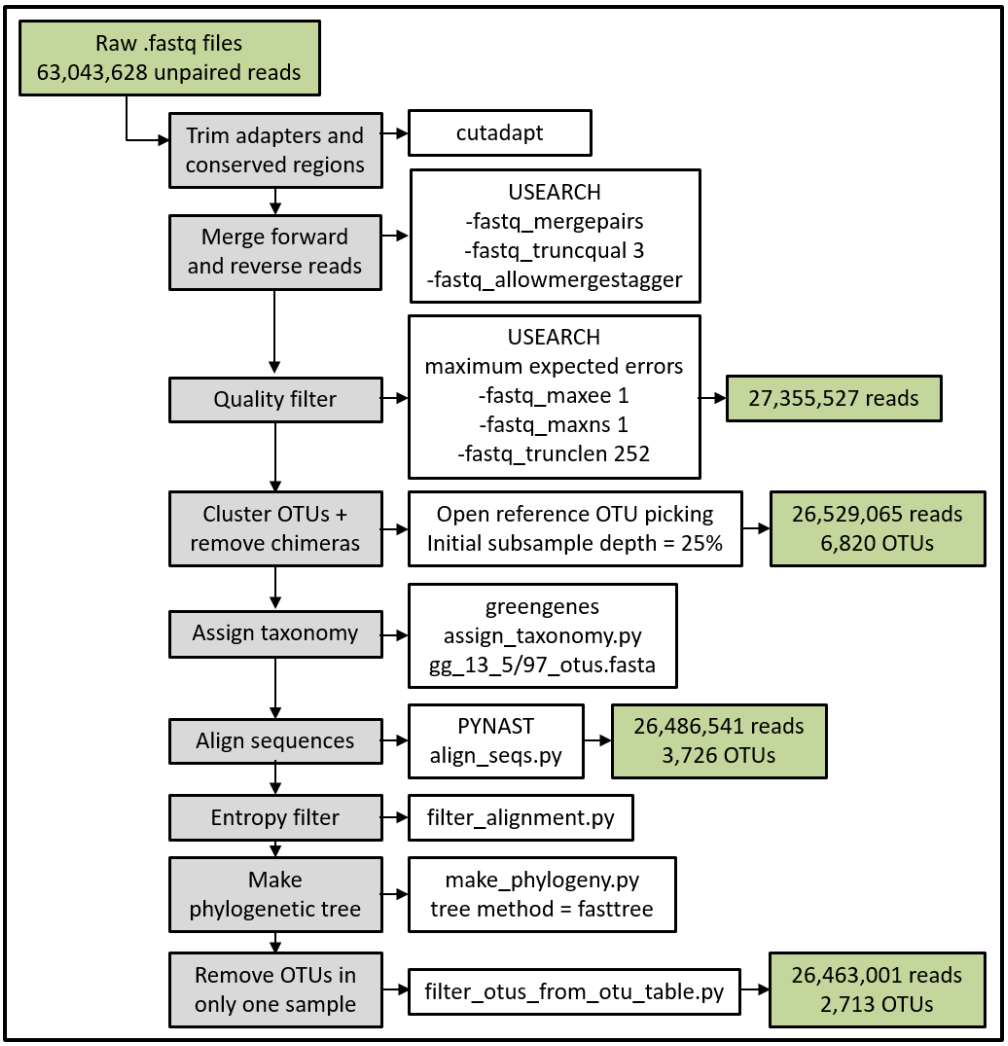


Figure S4. OTU presence / absence distribution across the dataset. The red and blue lines indicate the natural breaks in the data used to run the rare and random OTU BEDASSLE models.

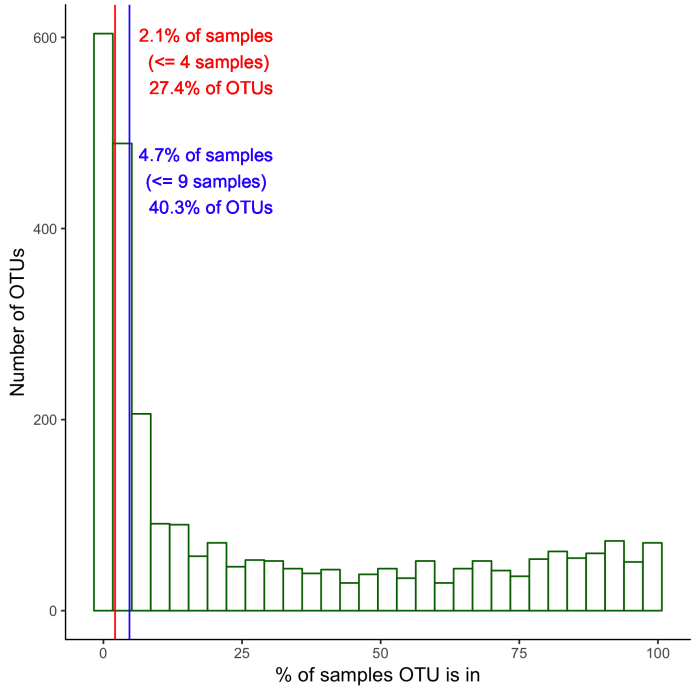


Figure S5. BEDASSLE output graphs of the geographic distance equivalent (km) per 1 unit geology Bray-Curtis across rare and random OTU subset models. After an 80% burn-in, the models all demonstrated a 'fuzzy caterpillar' pattern that indicates a well-mixed model [33]. Black lines indicate the mean geographic distance equivalent (km) per 1 unit geology Bray-Curtis, highlighting that the average value fluctuates between models, but is qualitatively similar.

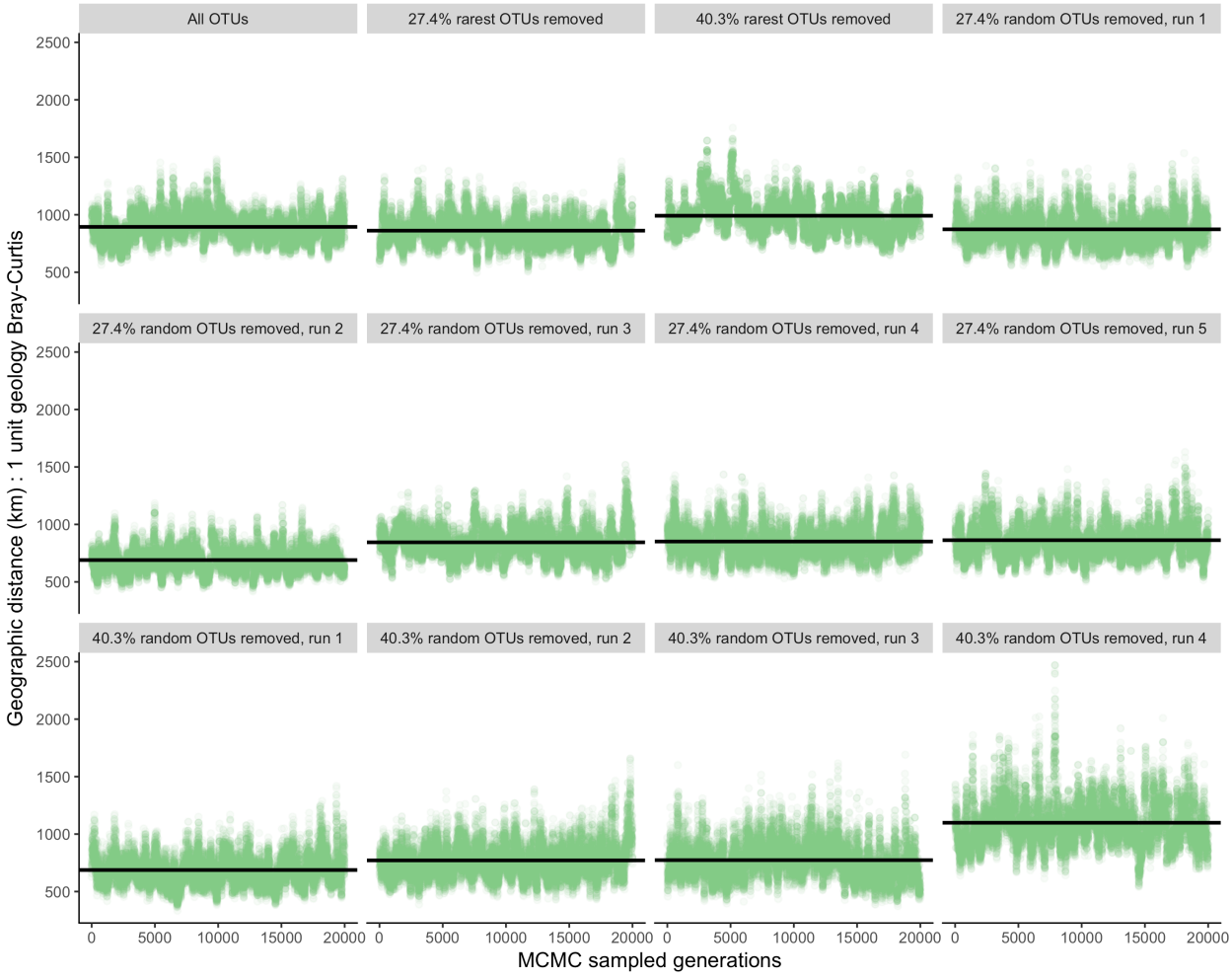


Figure S6. BEDASSLE output graphs of the geographic distance equivalent (km) per 1 unit soil PC1 across rare and random OTU subset models. After an 80% burn-in, the models all demonstrated a 'fuzzy caterpillar' pattern that indicates a well-mixed model [33]. Black lines indicate the mean geographic distance equivalent (km) per 1 unit soil PC1, highlighting that the average value fluctuates between models, but is qualitatively similar.

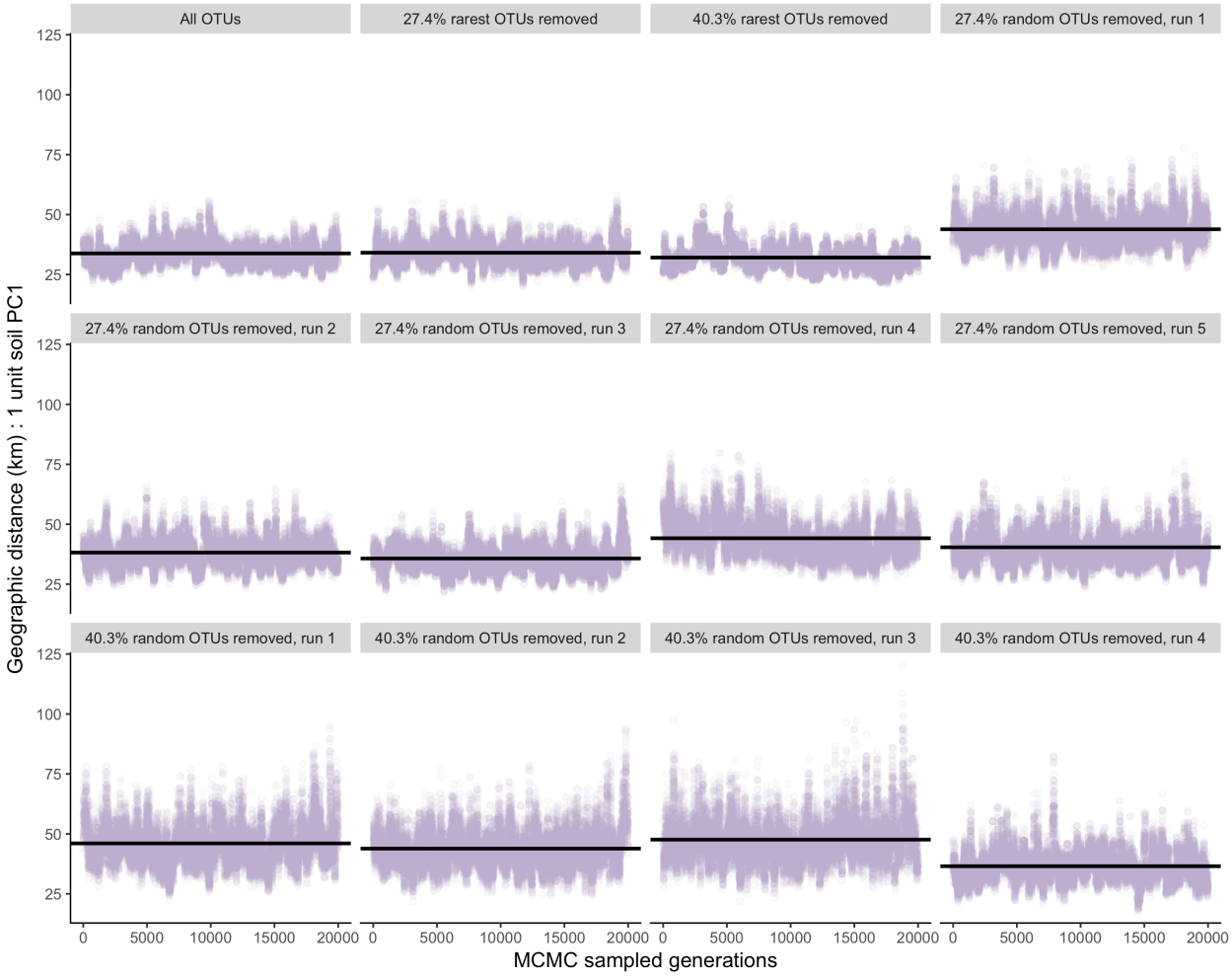


Figure S7. BEDASSLE output graphs of the geographic distance equivalent (km) per 1 unit genetic F_{ST} across rare and random OTU subset models. After an 80% burn-in, the models all demonstrated a 'fuzzy caterpillar' pattern that indicates a well-mixed model [33]. Black lines indicate the mean geographic distance equivalent (km) per 1 unit genetic F_{ST}, highlighting that the average value fluctuates between models, but is qualitatively similar.

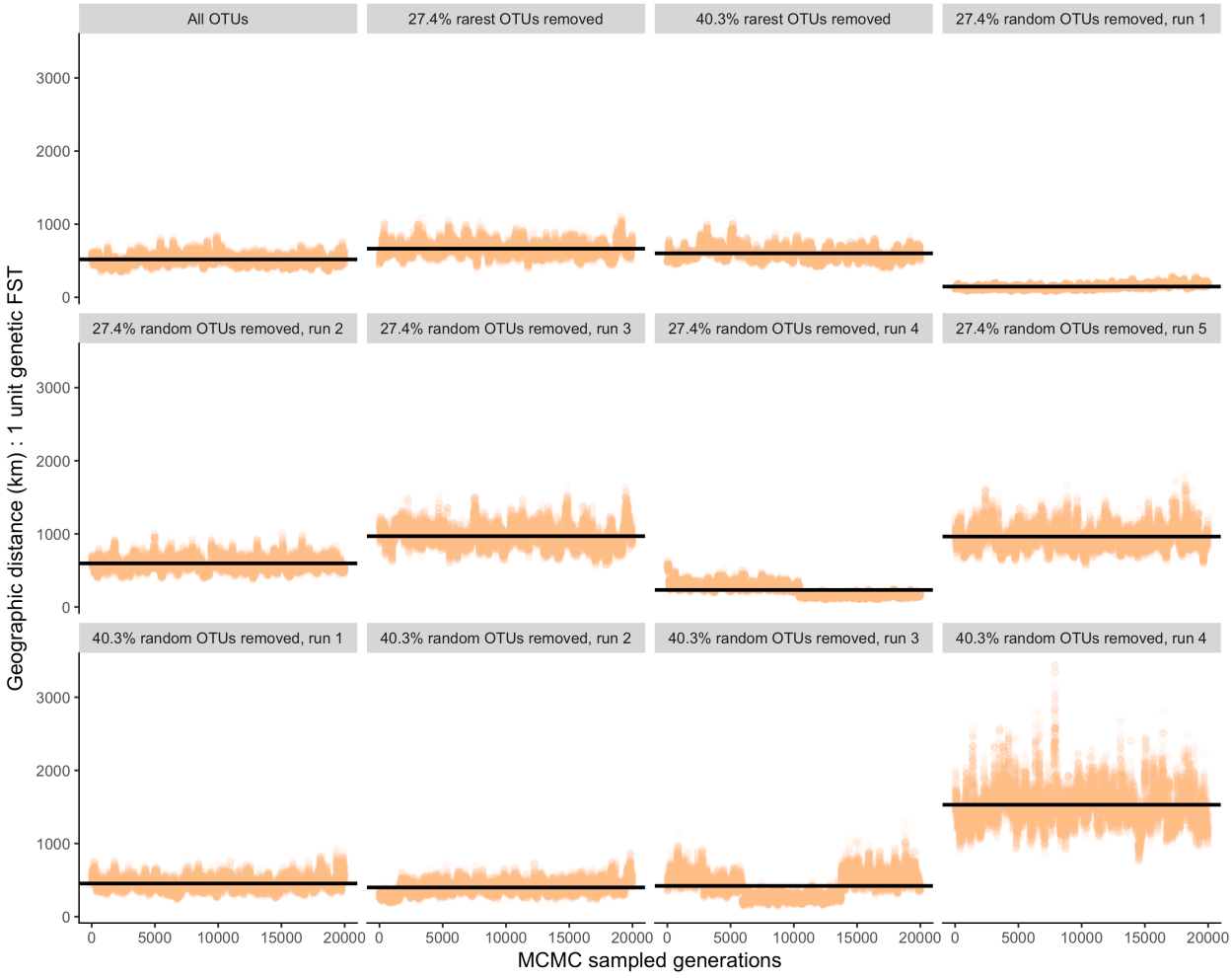


Figure S8. Correlation heatmap for OTU presence (A) for the full dataset and (B) excluding Amboseli. Each column and row represents an OTU. Cell values are phi correlations ranging from -1 (OTU pair was never found in the same individuals) to +1 (OTU pair was always found in the same individuals).

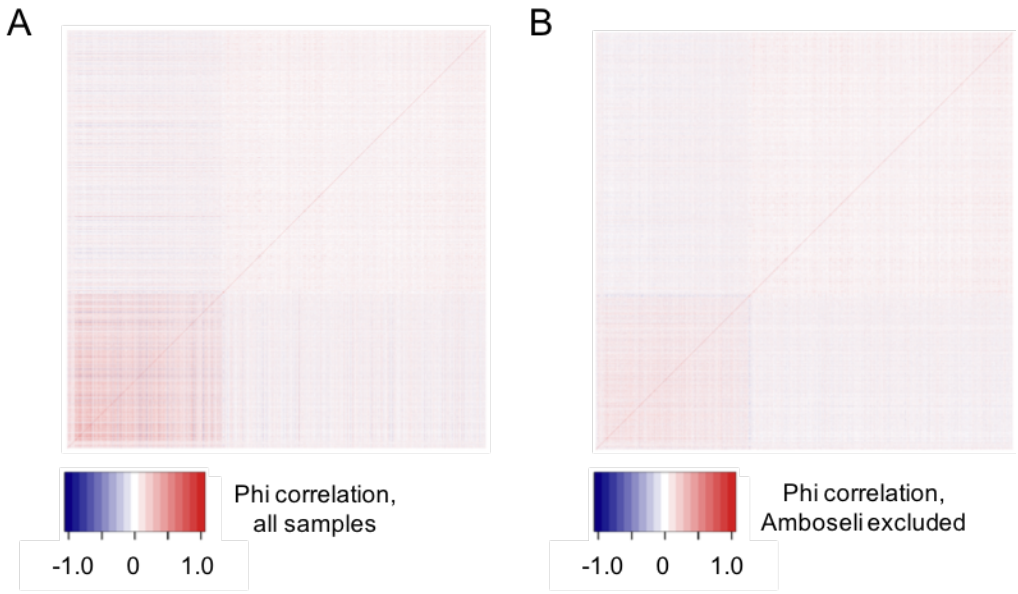


Figure S9. BEDASSLE output graphs. The BEDASSLE model was run with geographic distance and genetic F_{ST} normalized by their standard deviations. (A) After an 80% burn-in, the predictor variables all demonstrated a 'fuzzy caterpillar' pattern that indicates a well-mixed model [33]. (B) We ran 1,000 posterior predictive samples to determine that the model was a good fit for the data. Red dots are the observed values and black streaks are the distribution of predicted values from the model, indicating that the model did an accurate job predicting the data distribution.

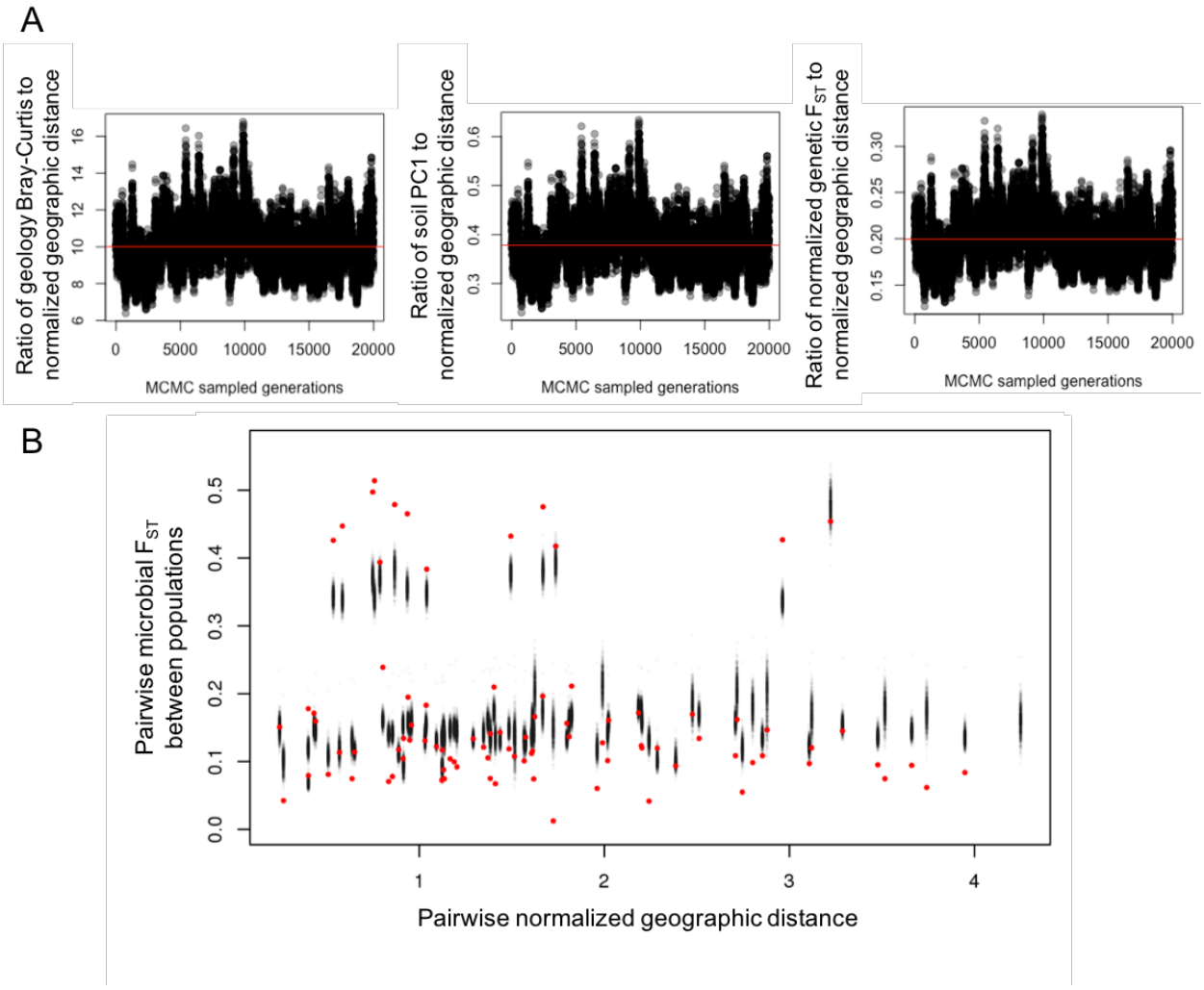


Figure S10. (A) PCoA of weighted UniFrac dissimilarities colored by baboon genetic ancestry. (B) Genetic ancestry did not predict the residuals of OTU richness, correcting for read count and significant environmental variables. (C) Pairwise genetic relatedness between baboons did not predict gut microbial dissimilarity, controlling for population co-residency. Each dot represents one pair of samples.

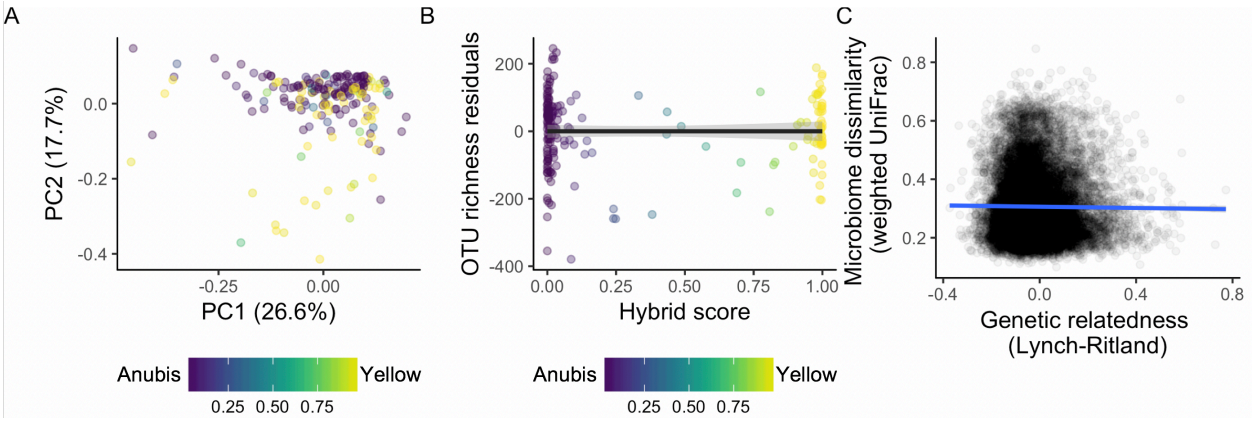


Figure S11. For genera with many OTUs, OTU prevalence is not explained by host ancestry. Specifically, for the 13 genera represented by at least 10 OTUs in the dataset, some OTUs were found in only unadmixed yellow or unadmixed anubis baboons, but the majority were found in baboons of various degrees of hybrid ancestry. For the purposes of this figure, anubis, yellow, and hybrid baboons are defined following [42], such that a hybrid score < 0.02 is categorized as anubis, 0.02 – 0.98 is categorized as hybrid, and > 0.98 is categorized as yellow.

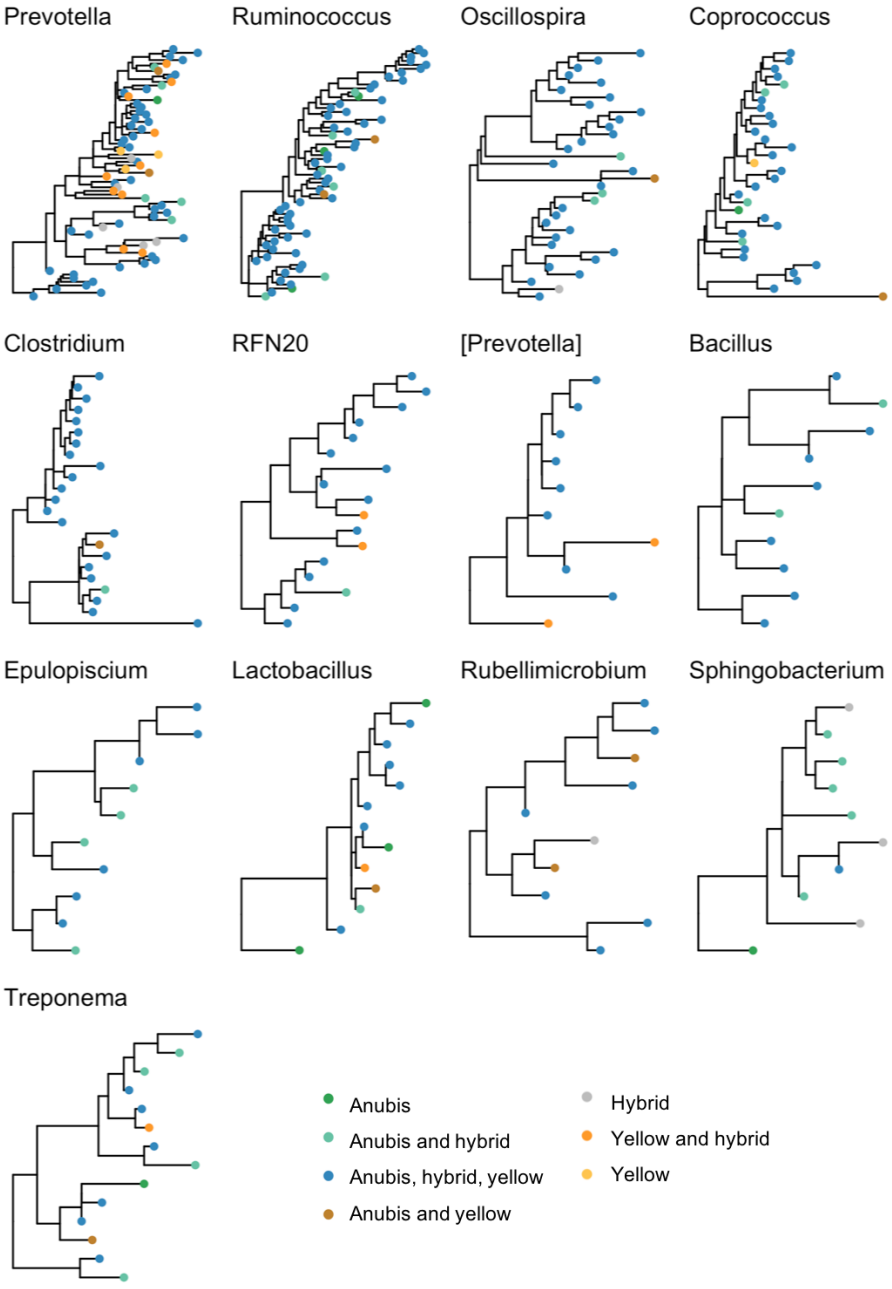


Figure S12. (A) The presence of microbial OTUs across populations follows a U-shaped distribution: some OTUs were found in all populations (light grey), while the remainder were not (dark grey). (B) OTUs found in some populations were enriched for an aerobic lifestyle and depleted for an anaerobic lifestyle relative to OTUs found in all populations. (C) OTUs found in all populations tended to be depleted for sporulation. In (B) and (C), the proportion of OTUs found in all populations (light grey) versus some populations (dark grey) per lifestyle trait is shown on each bar. The black line indicates the percentage of OTUs found in some but not all populations, and hence the background expectation for OTUs in each lifestyle class.

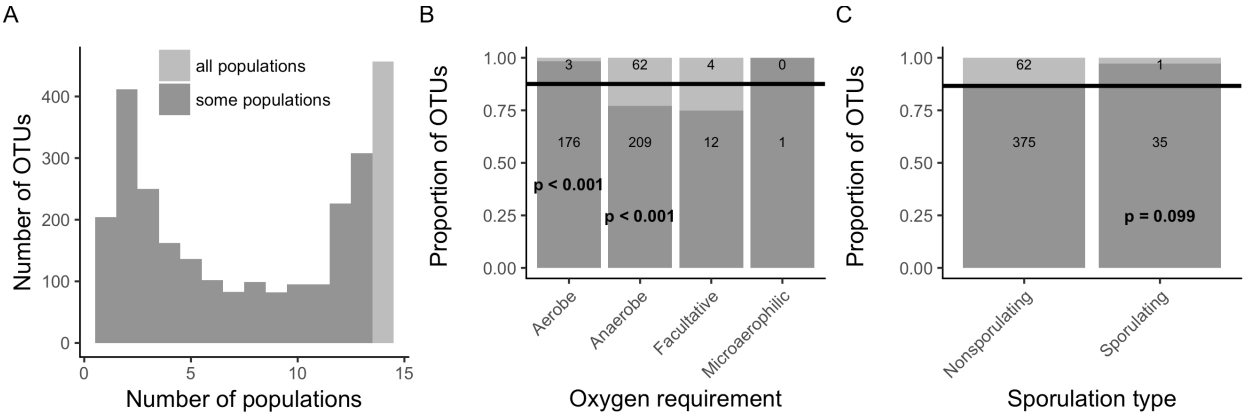
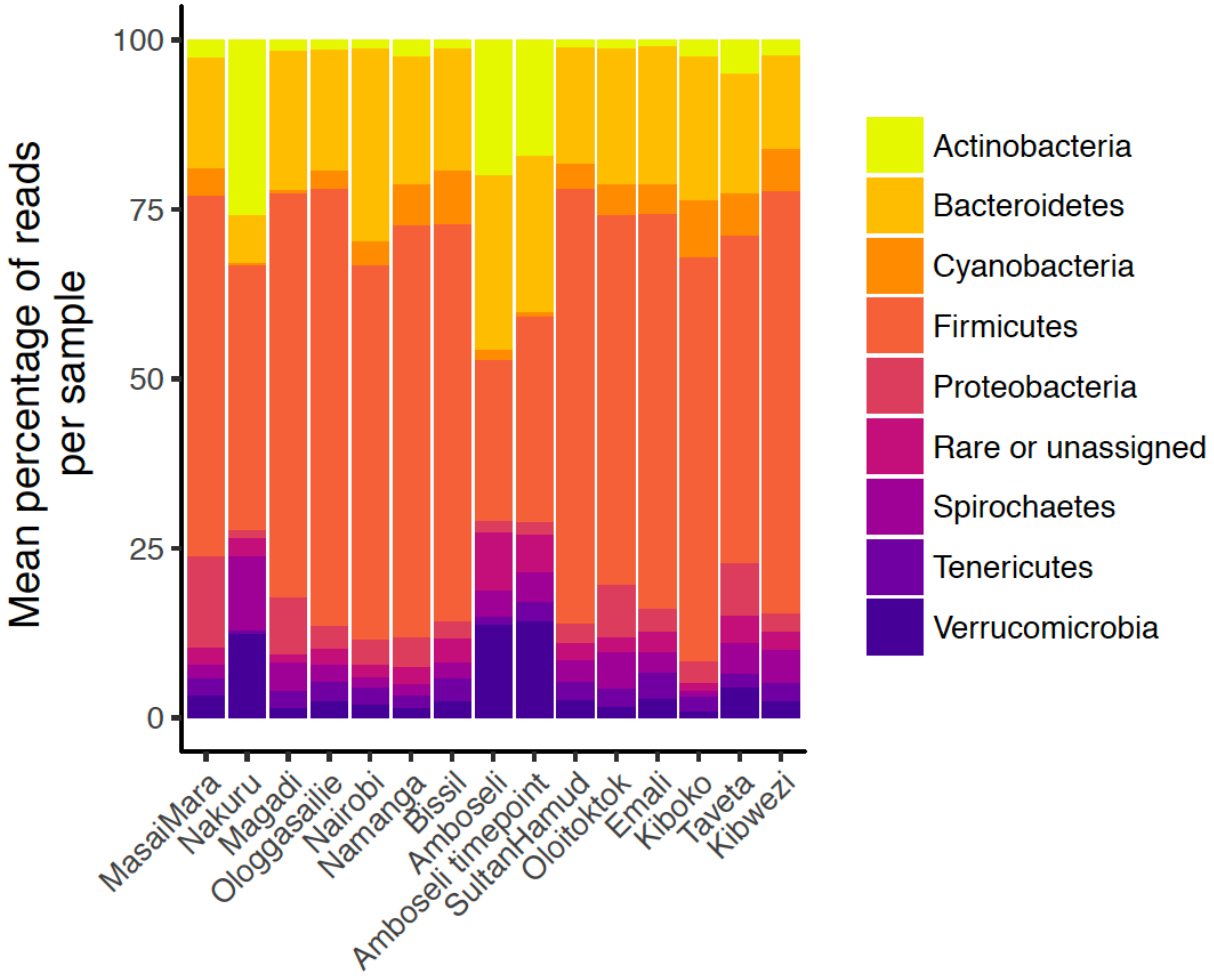


Figure S13. Relative abundance of microbial phyla averaged per population and across all Amboseli timepoint samples. Populations are listed from west to east. The Amboseli timepoint samples closely resemble the main dataset Amboseli samples.



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