

1 **METAGENOMIC ANALYSIS OF COPROLITES FROM THREE LATE**
2 **PLEISTOCENE MEGAHERBIVORES FROM THE SOUTHWESTERN UNITED**
3 **STATES.**

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23 desiccation.

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25 **1. SUPPLEMENTARY INFORMATION**

26 **COPROLITE COLLECTION SITES**

27 **Bison sp.:** Mammoth Alcove is a medium-sized shelter at 1188 m elevation with a south-
28 southeast exposure, whereas Grobot Grotto and Hooper's Hollow are both large south-facing
29 alcoves close together, located at 1189 m and 1204 m elevation, respectively (Mead and
30 Agenboard, 1989).

31 **Shasta ground sloth:** Two locations in Arizona, Muav and Rampart Caves, discovered in the
32 early 1930s at an elevation of approximately 426 m and 530 m, and found to contain rich
33 deposits of Late Pleistocene fossils and fecal material (Martin, Sabels and Shutler, 1961;
34 Long and Martin, 1974; Hansen, 1978; Martin, Thompson and Long, 1985; Schmidt,
35 Duszynski and Martin, 1992; Poinar *et al.*, 1998, 2003; Hofreiter *et al.*, 2000).

36 **Mammoth:** Bechan cave is a large sandstone grotto at 1280 m elevation, 52.8 m in depth,
37 and identified in 1982 as containing a blanket layer of dry dung (Davis *et al.*, 1984; Mead *et*
38 *al.*, 1986; Martin, 1987; Agenbroad and Mead, 1989; Mead and Agenboard, 1989).

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2. SUPPLEMENTARY TABLES AND FIGURES

Species	Storage site	NPS* Accession number	Collection sites	Study identification numbers	Radiocarbon dates \pm error	95% probability date ranges (ybp)
Shasta ground sloth (<i>Nothrotheriops shastensis</i>)	Grand Canyon National Park Museum	4597	Rampart Cave, Mohave, AZ	GRCA-59574	10,927 \pm 27	10,946 – 10,808
					11,240 \pm 27	11,225 – 11,147
					10,873 \pm 26	10,884 – 10,797
					11,351 \pm 27	11,353 – 11,222
					11,338 \pm 26	11,351 – 11,217
		4712	Muav cave, Mohave, AZ	GRCA-59511	11,180 \pm 27	11,214 – 11,136
		GRCA-4712	11,446 \pm 27	11,470 – 11,289		
				11,232 \pm 27	11,221 – 11,147	
				11,412 \pm 28	11,155 – 10,979	
				11,412 \pm 28	11,396 – 11,231	
				11,093 \pm 27	11,146 – 10,976	
				11,231 \pm 28	11,221 – 11,146	
Paleontological bison (<i>Bison sp.</i>)	Museum of Northern Arizona	82	Mammoth Alcove, Kane, UT	GLCA-821	18,611 \pm 34	20,688 – 20,425
			Grobot Grotto, Kane, UT	GLCA-877	18,806 \pm 35	20,982 – 20,592
			Hooper's Hollow, Kane, UT	GLCA-900	15,479 \pm 30	16,903 – 16,772
Columbian mammoth (<i>Mammuthus columbi</i>)	Museum of Northern Arizona	81	Bechan Cave, Kane, UT	GLCA-372	12,304 \pm 29	12,856 – 12,143
				GLCA-370	12,456 \pm 29	12,992 – 12,383
				GLCA-367	12,430 \pm 30	12,923 – 12,352
				GLCA-2578	12,401 \pm 30	12,896 – 12,315
				GLCA-382	12,426 \pm 30	12,915 – 12,349
				GLCA-2627	12,427 \pm 29	12,915 – 12,351

42 **Table S1.** Summary of collection information, study identification numbers, AMS radiocarbon dates, as well as the number of reads and queries
43 obtained from paleontological coprolite samples included in the study: Shasta ground sloth, paleontological bison, and Columbian mammoth
44 (top to bottom). Multiple samples were run for Shasta ground sloth coprolites. The 95% date ranges were calculated using OxCAL version 4.4
45 (Ramsey, 2009) and atmospheric data from Reimer *et al.*, (2020).

P1: Kmer query size		X	P2: No edits		X	P3: Binning mode			X	P4: Minimum threshold for USH*									
36	50		3	5		Fast	Efficient	Sensitive		0	200	400	600	800	100	1200	1400	1600	1800

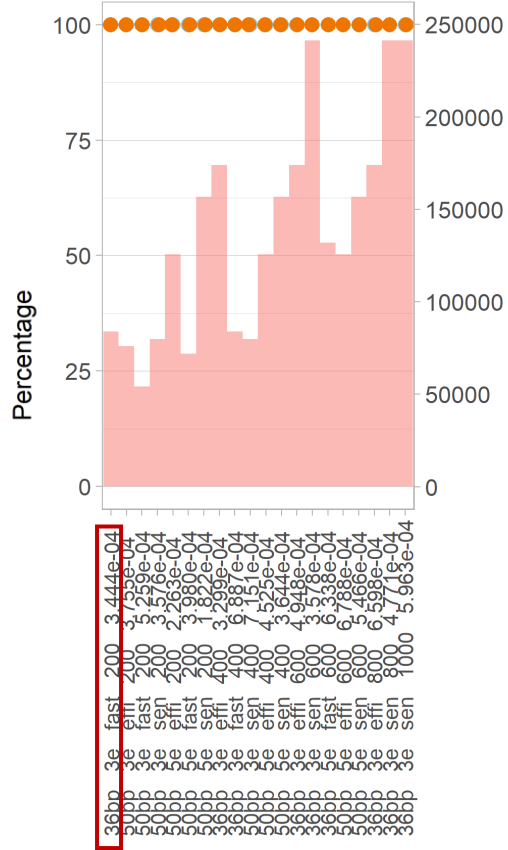
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47 **Table S2.** The combination of parameters (P1-4) used for MTSv runs on 1,000,000 simulated gargammel aDNA reads. Kmer query length refers
48 to the size of the deduplicated queries generated by the binning-analysis pipeline. Edits represents the threshold number of mismatches between
49 a query and reference. Binning mode represents the combination of parameters (seed size, minimum seeds, and seed gap; parameter order
50 respective hereafter) used by the assignment algorithm during binning. We used three binning modes during this study: fast (17, 5, 2), efficient
51 (14, 4, 2), and sensitive (11, 3, 1). (* the USH threshold was converted into a proportion of the total USH generated by the MTSv run).

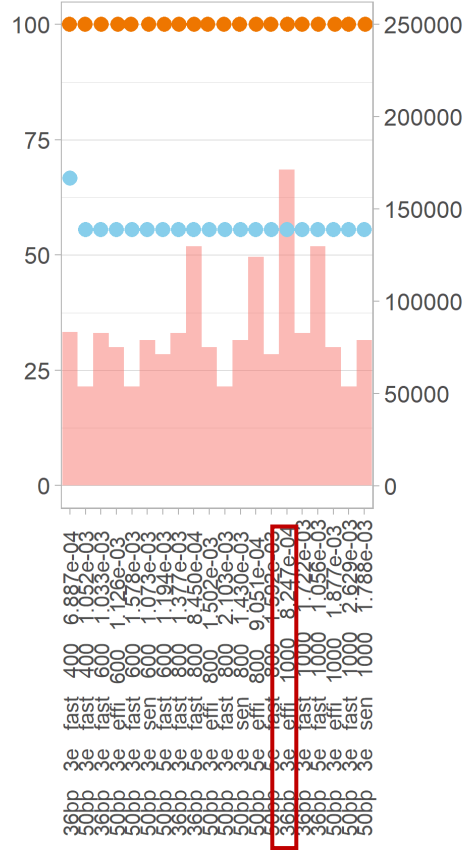
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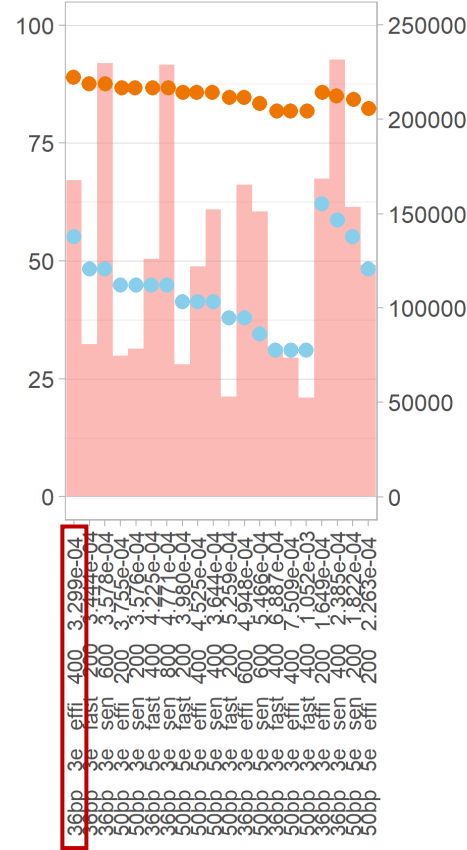
Class:



Order:



Family:



Total USH used

MTSv classification performance metrics:

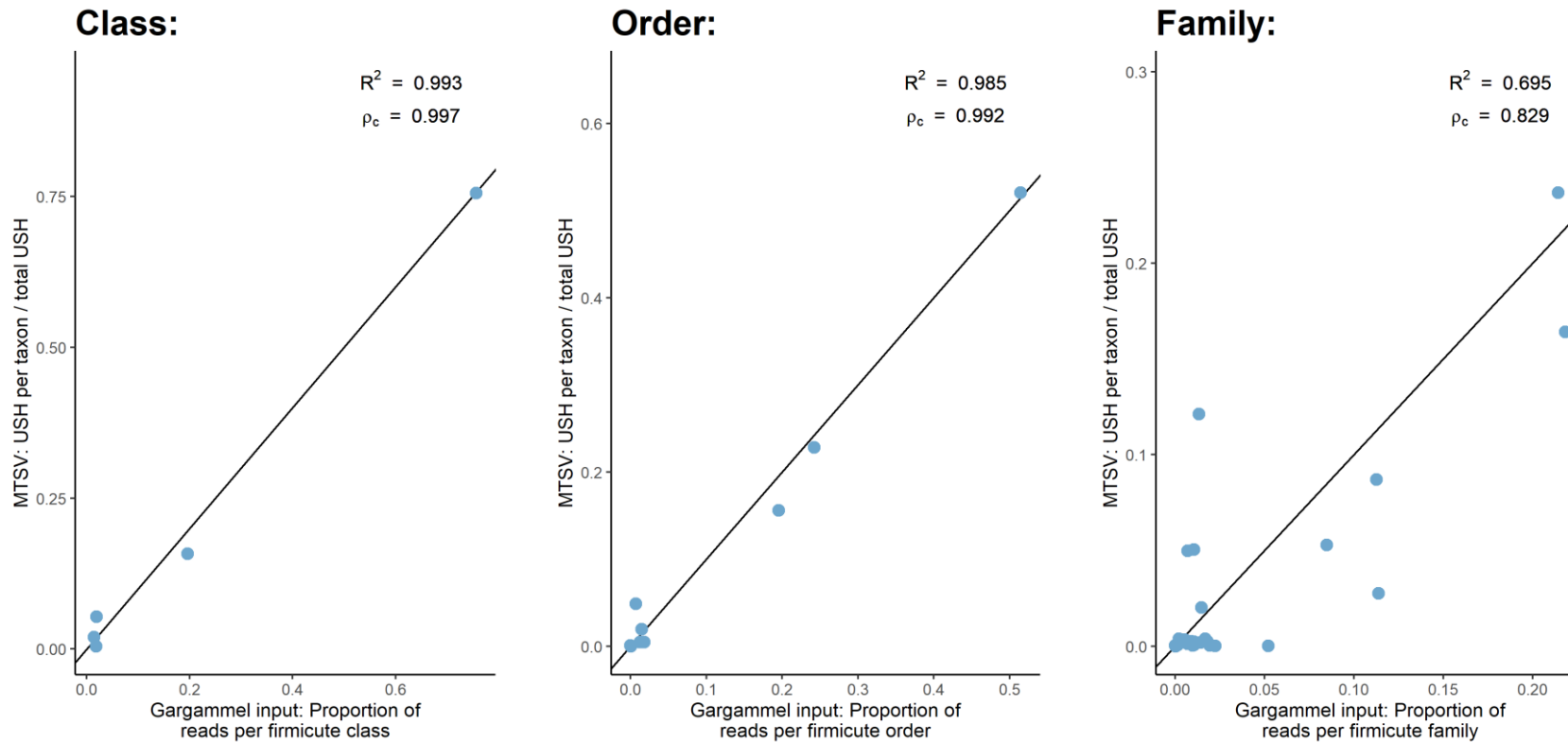
- Identification positive predictive value
- Identification sensitivity

MTSv output:

- Unique signature hits (USH)

Parameters (query length; number of edits permitted; alignment sensitivity; number and proportion of USH threshold)

72 **Figure S1.** The top 20 MTSv runs in identifying the classes, orders and families associated with the reads from 200 firmicute species processed
73 by gargammel to include age-related changes. These runs were ranked in descending order of positive predictive values (orange points) and
74 sensitivity scores (blue points). MTSv runs differed in the query lengths, number of edits permitted between a query and reference, the
75 sensitivity of alignment (determined by alignment parameters summarized in Table S2), and the minimum number of USH for taxon inclusion
76 (converted to a proportion of the total USH), as well as the total number of USH used by the classifier (pale pink histogram). Highlighted MTSv
77 runs indicate the parameter combination selected for further downstream analyses of coprolites (36bp query length, 3 edits between query and
78 reference and efficient alignment, with USH thresholds varying for different taxonomic levels).



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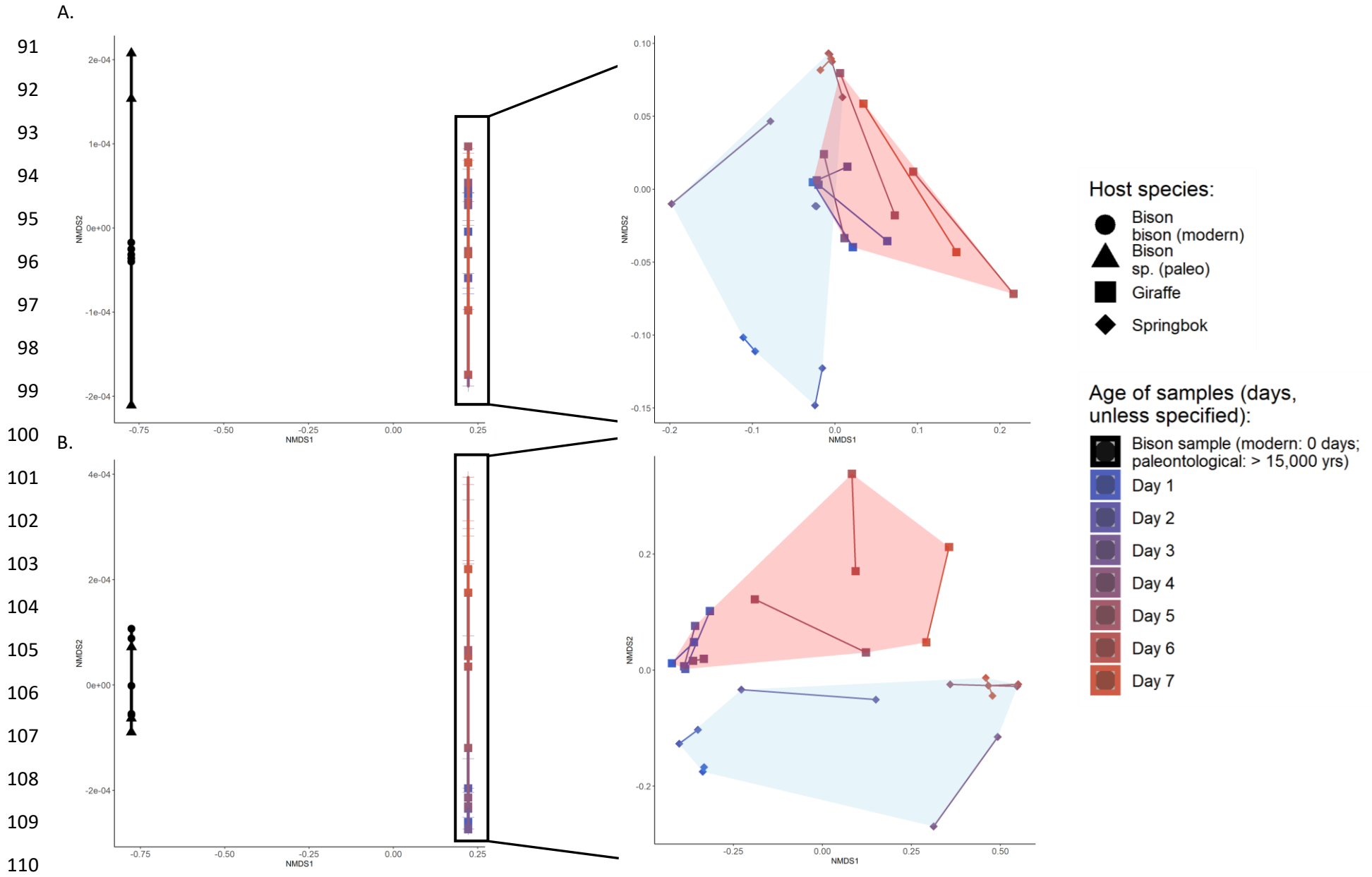
80 **Figure S2.** The relative abundance of gargammel reads from each taxon versus the proportion of USH identified by MTSv at the class, order,
 81 and family levels. The points displayed came from the MTSv run parameterized for a 36 bp query length, 3 edits and an efficient alignment
 82 mode. Each comparison shows the R^2 value of the points away from a 1-to-1 line as well as Lin's concordance correlation coefficient (ρ_c). The
 83 figure highlights that MTSv USHs are a good indicator of bacterial abundance at taxonomic levels above family.

Species	Kingdom	Phylum: percentage
<i>Bison bison</i> (modern)	Archaea	Euryarchaeota: 13.1743890722925
	Bacteria	Firmicutes: 67.5520625769282, Bacteroidetes: 7.44743119898517, Actinobacteria: 4.20642352093039, Proteobacteria: 2.64228434768255
	Eukaryota	Streptophyta: 2.98733840850779
<i>Bradypus variegatus</i>	Archaea	Euryarchaeota: 7.82385380631297
	Bacteria	Verrucomicrobia: 35.3748023500362, Bacteroidetes: 34.9505239153169, Firmicutes: 9.5333940320368, Proteobacteria: 8.00912507747584, Lentisphaerae: 2.60800869647104, Actinobacteria: 1.09013993949783
	Eukaryota	Streptophyta: 5.96165209225422
<i>Loxodonta africana</i>	Archaea	Euryarchaeota: 1.53448308845536
	Bacteria	Proteobacteria: 47.1217352111144, Bacteroidetes: 16.5815399968847, Firmicutes: 16.1444151957561, Verrucomicrobia: 3.49460616137364, Actinobacteria: 3.46800601395302, Spirochaetes: 1.36889802527466, Tenericutes: 1.02575840554082
	Eukaryota	Streptophyta: 5.96165209225422
<i>Bison sp.</i> (paleontological)	Bacteria	Actinobacteria: 49.9242291884496, Proteobacteria: 34.2753071984631, Firmicutes: 15.4635984544028
<i>Mammuthus columbi</i>	Bacteria	Actinobacteria: 51.66045226374, Proteobacteria: 44.5132985506531, Firmicutes: 3.47683619907833
<i>Nothrotheriops shastensis</i>	Bacteria	Actinobacteria: 39.8043818948311, Firmicutes: 37.8428912509215, Proteobacteria: 14.7871067207959, Bacteroidetes: 6.25992283764298

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86 **Supplementary Table 3.** The percentages of the dominant phyla (>1% USH) per kingdom
87 for each paleontological and modern species. The majority of USHs from paleontological
88 samples were assigned to Actinobacteria and Proteobacteria, with a high percentage of
89 Firmicutes detected in the coprolites of *N. shastensis*.

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111 **Figure S3.** The left-hand NMDS plots show the dissimilarity between paleontological bison, modern bison, and desiccated samples from Menke
112 et al (2015) at the phylum (A) and class (B) levels. For each taxonomic level, the right-hand NMDS plots show the Menke et al (2015) giraffe
113 and springbok samples (pink and blue convex hulls, respectively) after ordination was repeated without the bison samples. This was done to
114 show the separation of points along a continuum from fresh to desiccated.

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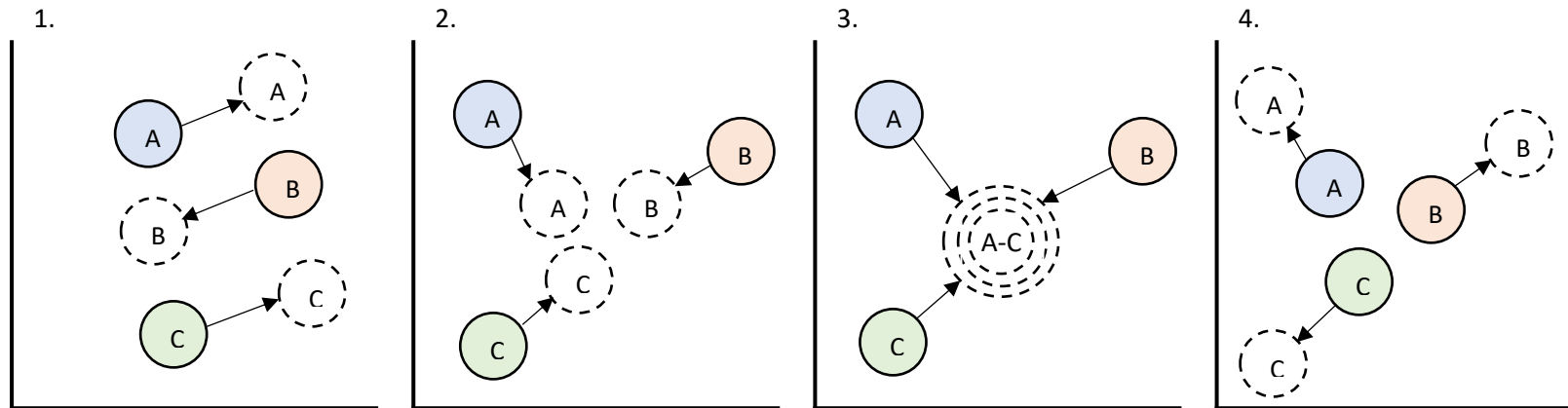
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125 **Figure S4.** Hypothetical ordination plots showing how the distinctiveness of microbiomes in fresh fecal samples from different host species
126 (clusters represented as solid shaded circles A - C) might change during desiccation studies (clusters as dashed circles A - C). The four plots
127 represent situations during desiccation in which the beta diversity of the three samples: 1) does not change, 2) converges somewhat, 3) converges
128 completely, and 4) increases. In plots 1, 2, and 4, the original microbiome may be inferred from the desiccated samples. However, the complete
129 convergence of desiccated microbiomes in plot 3 would prevent this.

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