



Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Overview of samples, study design and quality

measures to validate the authenticity of the palaeofaeces. **a**, Schematic of gene-catalogue and genome-reconstruction pipelines. **b**, Samples used in this study, archaeological sites and ^{14}C dating. Data were obtained from this study (Mexico) and previous studies: Fiji³¹, Peru⁴, Madagascar¹³, Tanzania⁵⁷, USA^{4,55}, Denmark⁵⁶ and Spain⁵⁶. Map data are from Google Maps (2021 Google, INEGI). **c**, Scanning electron microscopy images of dietary remains in the palaeofaeces. Zape1, maize pollen grains (more than 191,000 grains per gram) (top) and agave phytoliths (middle and bottom). Zape2, *U. maydis* spores (hundreds of millions per gram). Zape3, Chenopod or amaranth foliage and/or buds (smaller pollen) and squash (larger pollen with spines). UT30.3, druse phytoliths, annular xylem vessel secondary wall thickenings and epidermis of Cactaceae. A complete description is provided in Supplementary Information section 2. Reproducibility and independently repeated experiments are described in the Methods. **d**, Principal component analysis of the species composition of palaeofaeces, soil samples and publicly available archaeological sediment samples^{70,71}. Species were identified by MetaPhlAn2²⁰. **e**, Prediction of source of microbial communities by SourceTracker2⁷² using the species abundance matrix from MetaPhlAn2 as input. Archaeological sediment

samples included three soil samples collected in this study, seven Holocene human-associated sediments from CoproID⁷¹ and 40 Pleistocene sediment samples⁷⁰. **f**, The percentage of reads aligned to the MetaPhlAn2 database per sample (HMP, $n = 146$; Mexican, $n = 22$; Fijian, $n = 174$; palaeofaeces, $n = 8$; soil, $n = 3$) (Supplementary Information section 4). **g**, aDNA damage levels of Firmicutes and Bacteroidetes genomes for medium-quality and high-quality pre-filtered and filtered bins (two-tailed Wilcoxon rank-sum test; pre-filtered bins Bacteroidetes, $n = 69$ MAGs; pre-filtered bins Firmicutes, $n = 359$ MAGs; filtered bins Bacteroidetes, $n = 24$ MAGs; filtered bins Firmicutes, $n = 161$ MAGs) (Supplementary Information section 5). 5p, 5' end; 3p, 3' end. **h**, Abundances of VANISH²¹ and BloSSUM²² families as identified by MetaPhlAn2²⁰ (palaeofaeces $n = 8$; non-industrial $n = 370$; industrial $n = 418$). In **f-h**, data are presented as box plots (middle line, median; lower hinge, first quartile; upper hinge, third quartile; upper whisker extends from the hinge to the largest value no further than $1.5 \times$ the interquartile range from the hinge; lower whisker extends from the hinge to the smallest value at most $1.5 \times$ the interquartile range from the hinge; data beyond the end of the whiskers are individually plotted outlying points).