MICROBIOME

Microbial community assembly and metabolic function during mammalian corpse decomposition

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Vertebrate corpse decomposition provides an important stage in nutrient cycling in most terrestrial habitats, yet microbially mediated processes are poorly understood. Here we combine deep microbial community characterization, community-level metabolic reconstruction, and soil biogeochemical assessment to understand the principles governing microbial community assembly during decomposition of mouse and human corpses on different soil substrates. We find a suite of bacterial and fungal groups that contribute to nitrogen cycling and a reproducible network of decomposers that emerge on predictable time scales. Our results show that this decomposer community is derived primarily from bulk soil, but key decomposers are ubiquitous in low abundance. Soil type was not a dominant factor driving community development, and the process of decomposition is sufficiently reproducible to offer new opportunities for forensic investigations.

The process of decay and decomposition in mammalian and other vertebrate taxa is a key step in biological nutrient cycling. Without the action of vertebrate and invertebrate scavengers, bacteria, archaea, fungi, and protists, chemical decomposition of animal waste would proceed extremely slowly and lead to reservoirs of biochemical waste (*1*). The coevolution of microbial decomposers with the availability of vertebrate corpses over the past 400 million years is expected to result in conservation of key biochemical metabolic pathways and cross-kingdom ecological interactions for efficient recycling of nutrient reserves. Although mammalian corpses likely represent a relatively small component of the detritus pool (*2*, *3*) in most ecosystems, their role in nutrient cycling and community dynamics may be disproportionately large relative to input size, owing to the high nutrient content of corpses (*3*, *4*) and their rapid rates of decomposition [e.g., up to three orders of magnitude faster than plant litter (*2*)]. These qualities make corpses a distinct and potentially critical driver of terrestrial function (*5*, *6*).

When a mammalian body is decomposing, microbial and biochemical activity results in a series of decomposition stages (*5*) that are associated with a reproducible microbial succession across mice (*7*), swine (*8*), and human corpses (*9*). Yet the microbial metabolism and successional ecology underpinning decomposition are still poorly understood. At present, we do not fully comprehend (i) whether microbial taxa that drive decomposition are ubiquitous across environment, season, and host phylogeny; (ii) whether microbes that drive decomposition derive primarily from the host or from the environment; and (iii) whether the metabolic succession of microbial decomposition is conserved across the physicochemical context of decay and host phylogeny.

Several questions arise: Are microbial decomposer communities ubiquitous? What is the origin of the microbial decomposer community? How does mammalian decomposition affect the metabolic capacity of microbial communities? To answer these questions, we used mouse corpses in laboratory settings and human donors in outdoor settings (see supplementary materials and methods). We observed mouse decomposition on three different soil types under constant temperature and humidity, with insects excluded. We sampled microbial communities on the skin, abdominal cavity, and gravesoil (soils associated with decomposition) by destructively sampling five mice per soil type per time point every 3 days for the first 2 weeks and less frequently thereafter over 71 days of decomposition (fig. S1). Outdoor experiments on human corpses were conducted at the Sam Houston State University (SHSU) Southeast Texas Applied Forensic Science (STAFS) Facility (a willed-body donation facility), where human bodies were exposed to all natural elements, including invertebrate and vertebrate scavengers. We sampled the skin and gravesoil associated with four decomposing human bodies—two of which were placed in the winter and two in the spring—over 143 days and 82 days, respectively (fig. S1). Human donors were sampled either daily or every other day during the first month and less frequently thereafter. We used high-throughput amplicon-based sequencing of 16*S* ribosomal RNA (rRNA) genes (archaeal and bacterial community), 18*S* rRNA genes (microbial eukaryotic community), and internal transcribed spacer regions (fungal community) to characterize the full microbial diversity associated with decomposition (figs. S2 to S5).

A mammalian corpse is a disturbance habitat that selects for a specialized microbial community capable of decomposing a highly concentrated source of proteins and lipids, rather than the plant-derived polysaccharides from which most detritus is derived. Our results show that microbial communities change significantly during decomposition (tables S1 to S12) and become more similar to each other across body sites and gravesoils (supplementary materials). Although mice were decomposed on soils with different chemical properties (table S13), soil type was not a major driver of skin decomposer bacterial structure (Fig. 1A). A Random Forests regression model trained on our microbial data resulted in estimates of the postmortem interval (PMI) with errors ~2 to 3 days over first 2 weeks of decomposition (fig. S6). Additionally, estimates of PMI remained accurate when bacterial data associated with one soil type were used to train a regression model and predict PMI for samples associated with other soil types (fig. S7). In our human experiments, we also observed a reproducible succession of microbes across bodies within the same season (Fig. 1B and fig. S8), as well as accurate estimates of PMI across seasons and host species (Fig. 1C and fig. S9). We discovered that important features (i.e., microbes) in our experiment-specific regression models were similar across experiments (Fig. 1D). Together these results confirm that microbial succession was predictable across soil types, seasons, and host species.

The microbial decomposer community may emerge from multiple environments in which decomposer organisms are often rare (low abundance) before decomposition begins. For the mouse experiment, we used dynamic Bayesian inference neural information flow networks, which revealed that soil was significantly more likely to be a source of bacteria and archaea for

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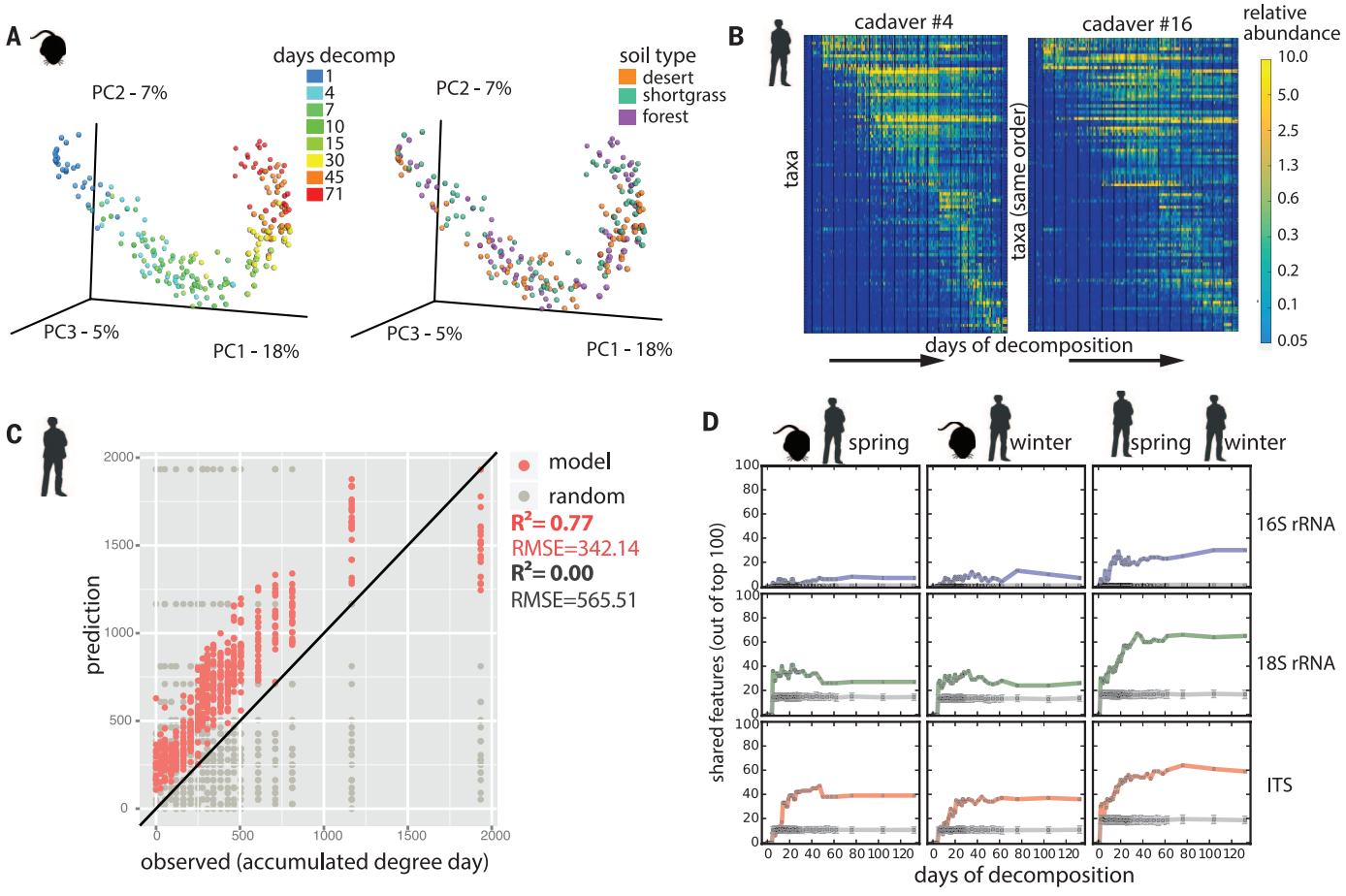

Fig. 1. Microbial decomposer communities are similar across environments. (**A**) Results of principal coordinates analysis (PCoA) based on unweighted UniFrac distances for mouse skin bacterial and archaeal communities. Samples are colored by days of decomposition (left) and soil type (right). (**B**) Log scale heat map of 16S rRNA operational taxonomic units (OTUs) colonizing the skin of human corpses. (**C**) A 16S rRNA–based Random Forests (RF) model using our winter-season skin-and-soil data set to train the model and predict the PMI of human bodies in the spring. Each point indicates a sample collected at a certain PMI, with RF-predicted PMIs shown in red and randomly guessed PMIs in gray. RMSE, root mean square error. (**D**) Percentage of top 100 PMI regression features from each environment that were shared (colored lines) versus number of shared features from randomly selected subsets of size 100 (gray lines). ITS, internal transcribed spacer.

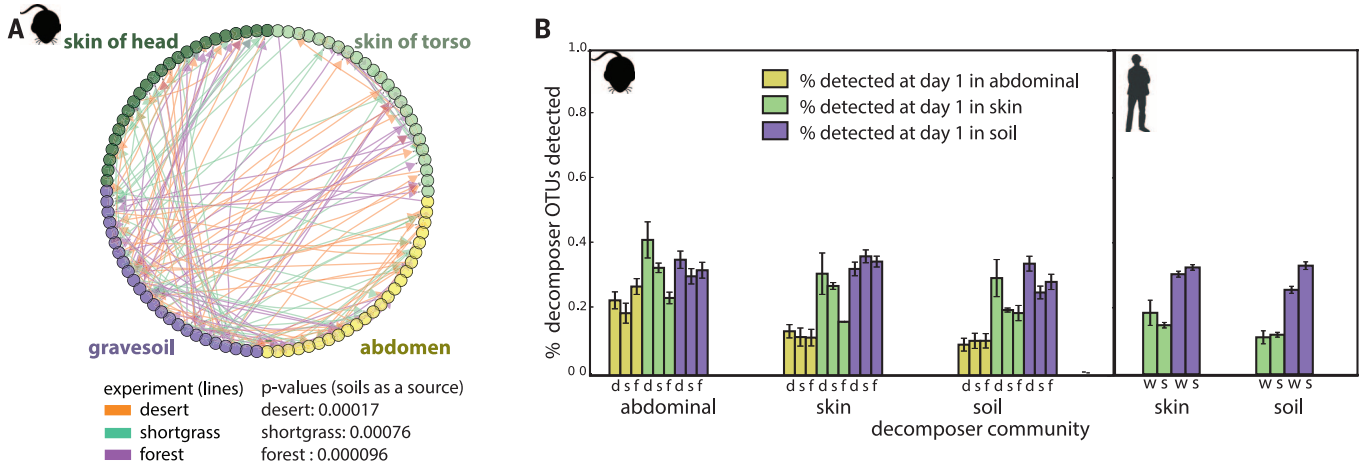

Fig. 2. Bacterial and archaeal decomposers emerge from multiple environments, but primarily from soil. (**A**) Dynamic Bayesian inference networks: A neural information flow network of microbial taxa during decomposition shows soils as the most common source of decomposers. (**B**) Results from deeply sequencing 16S rRNA amplicons from samples collected on the first day of each experiment. The *y* axis indicates the proportion of abdominal, skin, and soil decomposer OTUs (*x* axis) detected in each environment at the start of the experiment. Bars with standard error are ordered by soil type [desert (d), shortgrass (s), and forest (f)] (left) or season [winter (w) and spring (s)] (right). Decomposers were detected in soils more frequently than in the abdomen in every comparison (Mann-Whitney *U* test: $P < 0.05$).

the colonization of mice (Fig. 2A). To identify the potential sources of decomposer microbial communities, we deeply sequenced 16*S* rRNA amplicons from samples collected on the first day of each experiment. We searched these deeply sequenced data for decomposers, which we defined as microbes that differentially increased during decomposition, and found that ~40% of microbial decomposers were detected at very low relative abundances in soils at the start of experiments (supplementary text) (Fig. 2B). To understand the extent to which the blow fly, a common postmortem scavenger insect, may contribute to the microbial decomposer community, we also sequenced the bacterial and archaeal communities on 79 blow

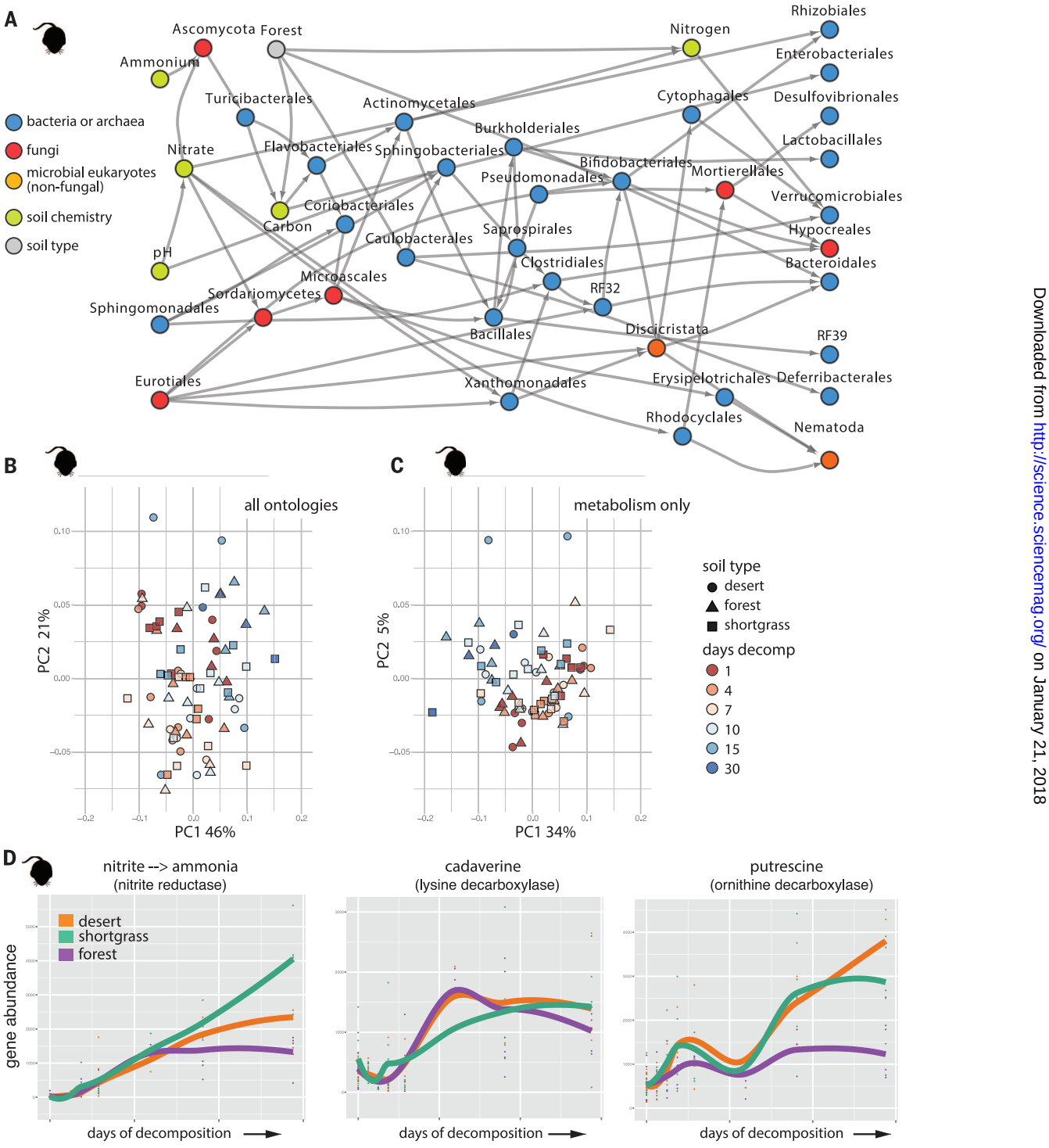

Fig. 3. Succession of decomposer communities in the abdominal cavity. (**A**) Dynamic Bayesian network of interactions between archaea, bacteria, microbial eukaryotes, and environmental abundance measurements during decomposition. Arrows indicate the direction of causality, and the network is arranged hierarchically so that it is a proxy for succession. (**B** and **C**) Results of PCoA of cecum, with all of the PICRUSt-predicted KEGG orthologies (KOs) (B) or KOs only classified as "metabolism" in KEGG functional hierarchies (C). (**D**) PICRUSt-predicted nitrite reductase, lysine decarboxylase, and ornithine decarboxylase enzyme-level genes in the mouse abdominal cavity during decomposition.

fly tarsi (supplementary materials) and discovered that they were a potential source for microbial decomposers, particularly in the human model experiment that occurred in the spring (fig. S10). Our results show that soil may be the main source of the microbial decomposer community, even though soil type is not important.

When a mammal dies, its immune system no longer functions and its internal temperatures change (*10*), radically altering the environment for microbial colonization and growth. Most endogenous mammalian microbes reside in the gastrointestinal tract, and postmortem changes in the gut microbial community lead to corpse bloating and, eventually, rupture (*5*). To investigate the microbial community dynamics of the abdominal cavity during decomposition, we used longitudinal data from the mouse abdomen samples to construct a dynamic Bayesian network of interactions between different taxa and several soil environmental factors (as a proxy for the abdominal environment). Nematodes are dependent on the actions of fungi and bacteria, with kinetoplastids (Discicristata) playing a key role in community succession (Fig. 3A). Fungi in the groups Eurotiales and Ascomycota are strong drivers of community structure, whereas fungi in Hypocreales appear to depend on the presence of bacteria for colonization of the abdomen. These shifts in microbial taxa are associated with large shifts in functional gene abundances, as predicted from 16*S* rRNA data analysis using the PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved state) software (Fig. 3B) (*11*), particularly for Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology group "metabolism" (Fig. 3C). We detected predicted increases in genes related to nitrogen cycling and amino acid degradation, including those required for the breakdown of lysine and arginine into the foul-smelling decomposition by-products cadaverine and putrescine (Fig. 3D).

After corpse rupture, ammonia-rich fluids permeate the soil, resulting in extreme and significant effects on the nitrogen concentration and pH of gravesoil (Fig. 4A, fig. S11, and table S13). This rich source of nutrients and the marked changes to soil chemistry initiate a clear ecological succession of soil microbial organisms with increased capacity for nitrogen cycling and tolerance for the altered soil chemical environment (Fig. 4B and fig. S12). Predicted functions of bacterial communities increased in relative abundance of genes for amino acid degradation and subsequent ammonia production (Fig. 4C). Surprisingly, although we observed increases in soil nitrate concentrations and processes that consume nitrate (figs. S13 and S14), we did not see genetic signs of increased nitrification rates (figs. S13 and S14). This suggests that nitrification pulses induced by vertebrate decomposition may occur on finer spatial or temporal scales or, alternatively, that the PICRUSt reference database lacks genomes from the vertebrate corpse microbial nitrifier community (e.g., fungal genomes). Taken together, analysis of the full community of predicted metabolism-related functional genes, in association with the PMI and soil chemistry data, revealed marked changes in functional potential during decomposition. The large and rapid taxonomic changes in microbial communities—as well as their subsequent effect on the predicted metabolic capacity of both the corpse (Fig. 3) and its surrounding environment (Fig. 4 and fig. S13) during decomposition—may be part of a microbial strategy to outcompete insects and scavengers for an ephemeral, nutrient-rich resource. The dramatic changes in community structure and function may also reflect the selective pressures applied by the biogeochemical hotspot formed during corpse decomposition (Fig. 4A) (*5*). As a consequence, microbial succession during decomposition appears to be a predictable process that has implications for biogeochemical cycling and forensic science.

These data are important in the context of ecosystem function. Decomposition is a fundamental

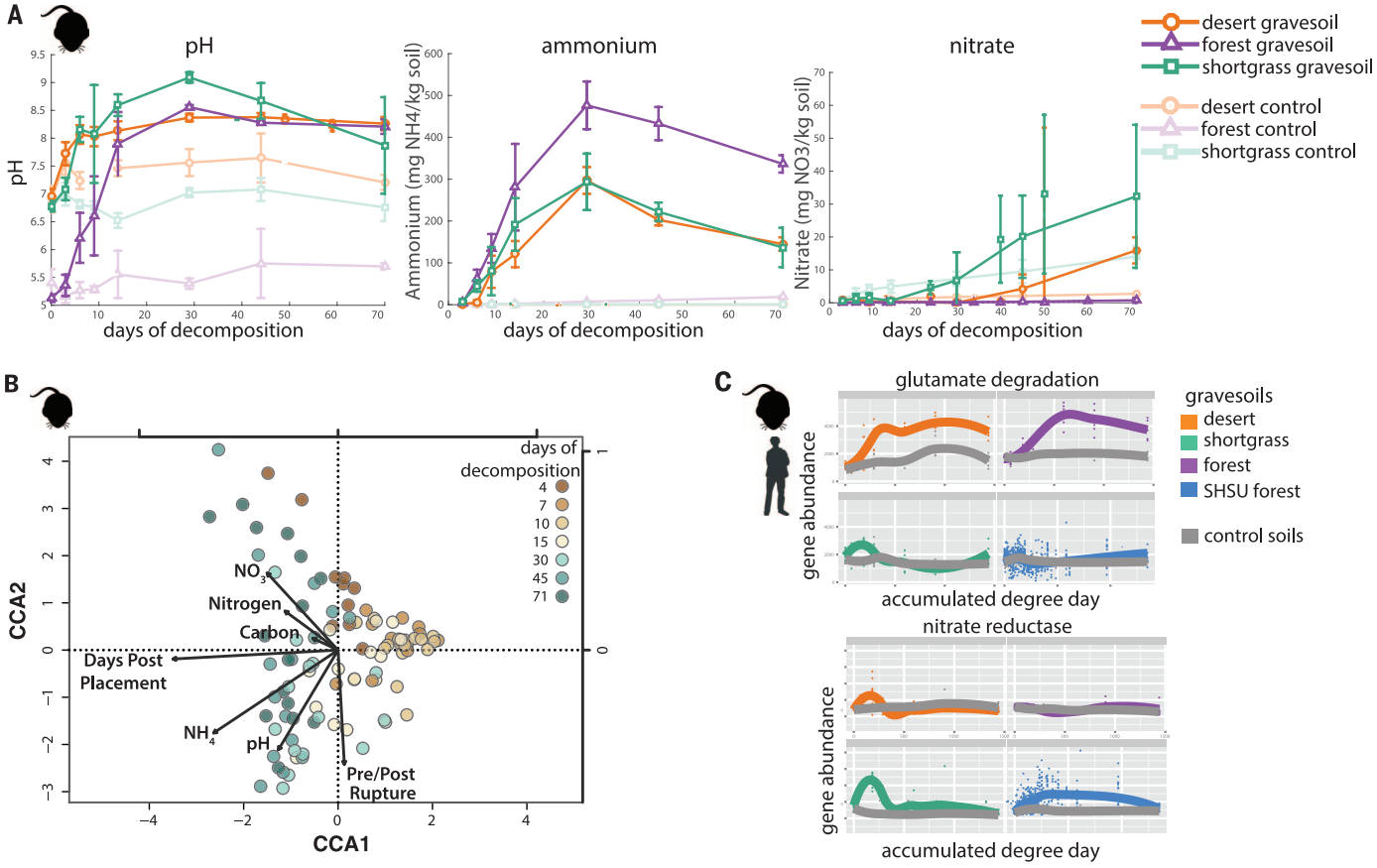

Fig. 4. Effect of mammalian decomposition on soils. (**A**) pH, ammonium, and nitrate concentrations in mouse gravesoils and control soils. Error bars indicate 1 SD from the mean of five sample measurements. (**B**) Canonical correspondence analysis (CCA) of gravesoil bacterial predicted gene ontologies during decomposition. PICRUSt-predicted function data are based on KOs, with only genes classified as "metabolism" included in this analysis. (**C**) Predicted gene abundances of glutamate dehydrogenase and nitrate reductase in soils during decomposition.

microbial function spanning terrestrial ecosystems, and though plant inputs are the dominant source of organic matter, vertebrate corpse inputs can be important resources (*5*, *6*). For example, one rain forest in Panama was estimated to receive 750 kg in mammal corpses annually per square kilometer (*12*). Although this represents less than 1% of the mass of plant litter received by another Panamanian rain forest (*13*), corpse nutrient sources can be an order of magnitude more concentrated than plant litter (*5*), and direct comparisons between plant and animal decomposition resources are rare (*14*). Thus, much is still unclear about the role of corpse inputs in larger-scale biogeochemical cycling (e.g., global carbon and nitrogen cycling) and in supporting specific communities and microbial diversity (*14*), and our results provide an important microbial perspective.

A societal impact of these results is the value of microbial data as physical evidence in medicolegal death investigation. We show that decomposer microbial communities could potentially serve as temporal (succession-based) and spatial (origin-based) (supplementary text) forms of physical evidence, such as the time elapsed since death (PMI) and the location of death. Our observation that postmortem microbial communities changed in a clock-like manner that provided an estimate of absolute PMI is similar to using the development of fly larvae to estimate PMI. However, the fly larvae PMI proxy is limited by corpse accessibility and season, resulting in PMI estimates in the range of weeks, months, and even years (*15*). Taken together, our findings demonstrate that postmortem microorganisms can provide both spatial and temporal insight into the events surrounding death.

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The data reported in this paper are available in the Qiita database (http://qiita.ucsd.edu/) (accession numbers 10141 to 10143 and 10321) and the European Bioinformatics Institute European Nucleotide Archive (www.ebi.ac.uk/ena) (accession numbers ERP012866, ERP012879, ERP012880, and ERP012894). We thank the donors and their families for their contribution to scientific research; the STAFS Facility at SHSU and the Molecular, Cellular, and Developmental Biology Transgenic Facility at the University of Colorado, Boulder, for providing the space and opportunity for this research; N. Fierer, J. Zelikova, and J. Leff for assistance with project logistics and data processing; and the Mountain Research Station and Shortgrass Steppe Long Term Ecological Research for permission to collect soils. Mice were euthanized humanely under approved protocol no. 08-04-ACK-01 (principal investigator G.A.). This research was funded by the Office of Justice Programs National Institute of Justice Awards NIJ-2011-DN-BX-K533 (J.L.M., D.O.C., R.K.) and NIJ-2012-DN-BX-K023 (S.R.B. and A.M.L.). Research capacity and infrastructure at Chaminade University of Honolulu is supported by NIH Building Research Infrastructure and Capacity Program P789097-876. W.V.T. and S.W. were supported by the National Human Genome Research Institute grant 3 R01 HG004872-03S2, and NIH grant 5 U01 HG004866-04. J.L.M. was partially supported by a Templeton Foundation grant (R.K. and V. McKenzie). Use of trade, product, or firm names is for informational purposes only and does not constitute an endorsement by the U.S. government. J.F.P. is Chief Scientific Officer and Founder of Diversigen; C.N. is an employee of miRagen Therapeutics; and R.K. is Chief Science Officer and employee of Biota Technology, a member of the Scientific Advisory Panel at Temasek Life Sciences Laboratory, and a speaker at Nestec, Nestle Research Center.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/351/6269/158/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S19
Tables S1 to S20
References (*16*–*29*)

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ANCIENT MICROBIOME

The 5300-year-old *Helicobacter pylori* genome of the Iceman

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The stomach bacterium *Helicobacter pylori* is one of the most prevalent human pathogens. It has dispersed globally with its human host, resulting in a distinct phylogeographic pattern that can be used to reconstruct both recent and ancient human migrations. The extant European population of *H. pylori* is known to be a hybrid between Asian and African bacteria, but there exist different hypotheses about when and where the hybridization took place, reflecting the complex demographic history of Europeans. Here, we present a 5300-year-old *H. pylori* genome from a European Copper Age glacier mummy. The "Iceman" *H. pylori* is a nearly pure representative of the bacterial population of Asian origin that existed in Europe before hybridization, suggesting that the African population arrived in Europe within the past few thousand years.

The highly recombinant pathogen *Helicobacter pylori* has evolved to live in the acidic environment of the human stomach (*1*). Today, this Gram-negative bacterium is found in approximately half the world's human population, but fewer than 10% of carriers develop disease that manifests as stomach ulcers or gastric carcinoma (*2*, *3*). Predominant intrafamilial transmission of *H. pylori* and the long-term association with humans has resulted in a phylogeographic distribution pattern of *H. pylori* that is shared with its host (*4*, *5*). This observation suggests that the pathogen not only accompanied modern humans out of Africa (*6*), but that it has also been associated with its host for at least 100,000 years (*7*). Thus, the bacterium has been used as a marker for tracing complex demographic events in human prehistory (*4*, *8*, *9*). Modern *H. pylori* strains have been assigned to distinct populations according to their geographic origin (hpEurope, hpSahul, hpEastAsia, hpAsia2, hpNEAfrica, hpAfrica1, and hpAfrica2) that are derived from at least six ancestral sources (*4*, *5*, *8*). The modern *H. pylori* strain found in most Europeans (hpEurope) putatively originated from recombination of the two ancestral populations Ancestral Europe 1 and 2 (AE1 and AE2) (*6*). It has been suggested that AE1 originated in Central Asia, where it evolved into hpAsia2, which is commonly found in South Asia. On the other hand, AE2 appears to have evolved in northeast Africa and hybridized with AE1 to become hpEurope (*4*). However, the precise hybridization zone of the parental populations and the true origin of hpEurope are controversial. Early studies observed a south-to-north cline in AE2/AE1 frequency in Europe (*4*, *6*). This finding has been attributed to independent peopling events that introduced these ancestral *H. pylori* components, which eventually recombined in Europe since the Neolithic period. More recently, it has been suggested that the AE1/AE2 admixture might have occurred in the Middle East or Western Asia between 10,000 and 52,000 years ago and that recombinant strains were introduced into Europe with the first human recolonizers after the last glacial maximum (*7*).

Science

Microbial community assembly and metabolic function during mammalian corpse decomposition

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Decomposition spawns a microbial zoo

The death of a large animal represents a food bonanza for microorganisms. Metcalf *et al.* monitored microbial activity during the decomposition of mouse and human cadavers. Regardless of soil type, season, or species, the microbial succession during decomposition was a predictable measure of time since death. An overlying corpse leaches nutrients that allow soil- and insect-associated fungi and bacteria to grow. These microorganisms are metabolic specialists that convert proteins and lipids into foul-smelling compounds such as cadaverine, putrescine, and ammonia, whose signature may persist in the soil long after a corpse has been removed.

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