Sciencexpress Reports

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 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 contributing to nitrogen cycling and a reproducible network of decomposers that emerge on predictable timescales. The results show 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 that it offers unique opportunities for forensic investigations.

The process of decay and decomposition in mammalian and other vertebrate taxa is a key process 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 co-evolution of microbial decomposers with the availability of vertebrate cadavers over the past 400 million years would be expected to lead to conservation of key biochemical metabolic pathways and cross-kingdom ecological interactions for efficient recycling of nutrient reserves. Although in most ecosystems mammalian corpses likely represent a relatively small component of the detritus pool (*2*, *3*) their role in nutrient cycling and community dynamics may be disproportionately large relative to input size due to the high nutrient content of cadavers (*3*, *4*) and their rapid rates of decomposition [e.g., up to three orders of magnitude faster than plant litter (*2*)]. These qualities make cadavers 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 [see (*5*)], which are associated with a reproducible microbial succession across mice (*7*), swine (*8*), and human cadavers (*9*). Yet microbial metabolism and successional ecology underpinning decomposition is still poorly understood. We do not understand: whether microbial taxa that drive decomposition are ubiquitous across environment, season, and host phylogeny, whether microbes that drive decomposition derive primarily from the host or from the environment, or 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? From where does the microbial decomposer community originate? How does mammalian decomposition affect the metabolic capacity of microbial communities? To answer these questions, we used mouse cadavers under laboratory settings and human donors in outdoor settings. Mice were decomposed on three contrasting soil types under constant temperature and humidity, with insects excluded. We sampled microbial communities on the skin, abdominal cavity, and gravesoil (soils associated with de-

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composition) 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 cadavers were conducted at the Sam Houston State University 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 subjects, two of which were placed in the winter and two in 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 16S rRNA gene (archaeal and bacterial community), 18S rRNA gene (microbial eukaryotic community), and ITS region (fungal community) to characterize the full microbial diversity associated with decomposition (figs. S2 to S5).

A mammalian carcass 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 microbial communities 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 Forest regression model trained on our microbial data resulted in estimates of the postmortem interval (PMI) with errors ~2-3 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 subjects within season (Fig. 1B and fig. S8), and 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 confirmed that microbial succession was predictable across soil types, seasons, and across 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 colonization of mice (Fig. 2A). To identify the potential sources of decomposer microbial communities, we deeply sequenced 16S 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 approximately 40% of microbial decomposers were detected in soils at the start of experiments, but at very low relative abundances (supplementary text) (Fig. 2B). To understand the extent to which the blow fly, a common postmortem insect, may contribute to the microbial decomposer community, we also sequenced the bacterial and archaeal communities on 79 blow fly tarsi (supplementary materials), and discovered they were a potential source for microbial decomposers, particularly in the spring human model experiment (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 bloating of the carcass, and eventually to its 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 upon 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 while fungi in Hypocreales appear dependent upon 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 by 16S rRNA data using PICRUSt (Fig. 3B) (*11*), particularly for Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology group Metabolism (Fig. 3C). Indeed, 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 cadaver rupture, ammonia-rich fluids permeate the soil resulting in extreme and significant effects on nitrogen concentrations and pH of gravesoil (Fig. 4A, fig. S11, and table S13). This rich source of nutrients and marked change to soil chemistry initiate a marked 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 (e.g., Fig. 4C). Surprisingly, although soil nitrate concentrations and processes that consume nitrate increase

(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 cadaver 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 striking changes in functional potential during decomposition. The large and rapid taxonomic changes in microbial communities, and their subsequent effect on the predicted metabolic capacity of both the cadaver (Fig. 3) and its surrounding environment (Fig. 4 and fig. S13) during decomposition may be a competitive strategy by microbes to outcompete insects and scavengers for an ephemeral, nutrient-rich resource. The dramatic change 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 having implications for biogeochemical cycling and forensic science.

These data are important in the context of ecosystem function. Decomposition is a fundamental microbial function spanning terrestrial ecosystems and, while plant inputs are the dominant source of organic matter, vertebrate corpse inputs can be significant resources (*5*, *6*). For example, one rain forest in Panama was estimated to receive 750 kg in mammal cadavers annually per km^2 (*12*). While this represents less than 1% of the mass of plant litter received by another Panamanian rain forest (*13*), cadaver 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, our understanding of the role cadaver inputs play in larger-scale biogeochemical cycling (e.g., global carbon and nitrogen cycling) and in supporting specific communities and microbial diversity remains limited (*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: evidence of the time that has elapsed since death (postmortem interval) and the location of death scenes. Our observation that postmortem microbial communities changed in a clock-like manner that provided an estimate of absolute postmortem interval is similar to using the development of fly larvae to estimate postmortem interval. However, the fly larvae PMI proxy is limited by corpse accessibility and season, which tend to result in estimates of PMI in the range of weeks, months, and 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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19 August 2015; accepted 25 November 2015
Published online 10 December 2015
10.1126/science.aad2646

ACKNOWLEDGMENTS

The data reported in this paper are available on the QIITA database (http://qiita.ucsd.edu/, accessions 10141-10143, 10321) and European Nucleotide Archive (EBI www.ebi.ac.uk/ena, accessions ERP012866, ERP012879, ERP012880, and ERP012894). We thank the donors and their family for their contribution to scientific research. We thank STAFS at SHSU and the MCDB Transgenic facility at CU Boulder for providing the space and opportunity for this research. We thank Noah Fierer, Jane Zelikova, and Jon Leff for assistance with project logistics and data processing. We thank the Mountain Research Station and Shortgrass Steppe Long Term Ecological Research for permission to collect soils. Mice were euthanized humanely under approved protocol #08-04-ACK-01, PI Gail Ackermann. This research was funded by the Office of Justice Programs National Institute of Justice Awards NIJ-2011-DN-BX-K533 (JLM, DOC, RK) and NIJ-2012-DN-BX-K023 (SRB and AML). Research capacity and infrastructure at Chaminade University of Honolulu is supported by NIH-BRIC P789097-876. W.V.T. and S.J.W. were supported by the National Human Genome Research Institute Grant# 3 R01 HG004872-03S2, and the National Institute of Health Grant# 5 U01 HG004866-04. Use of trade, product, or firm names is for information purposes only and does not constitute an endorsement by the U.S. Government. Joseph Petrosino: "CSO and Founder of Diversigen"; Catherine Nicholas: "miRagen Therapeutics"; Rob Knight: "Biota Technology, Inc.: Chief Science Officer and Employee Temasek Life Sciences Laboratory: Member of the Scientific Advisory Panel Nestec Ltd., Nestle Research Center.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S19
Tables S1 to S20
References (*16*–*29*)

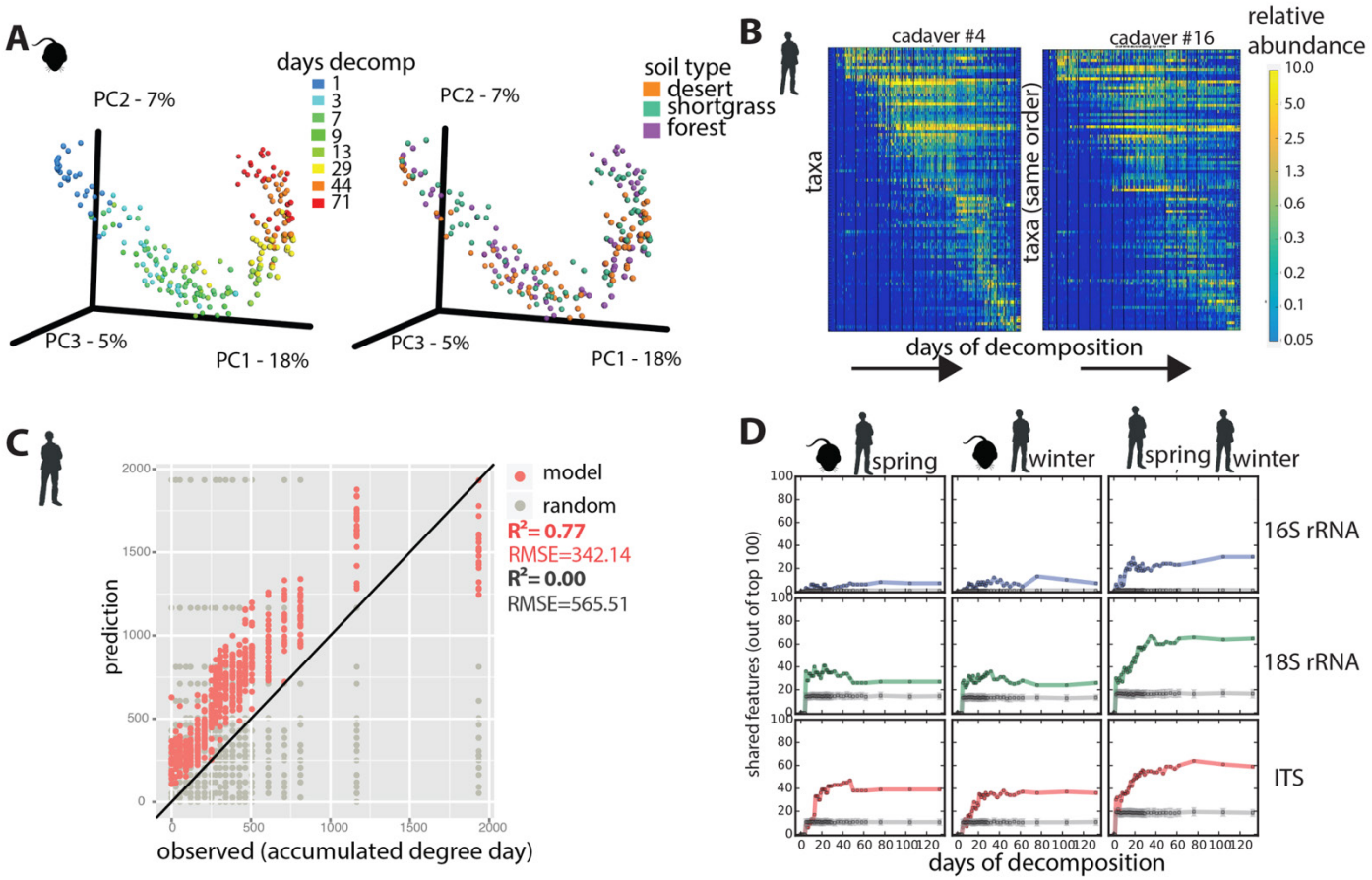

Fig. 1. Microbial decomposer communities are similar across environments. (**A**) Principal Coordinates Analysis (PCoA) based on unweighted UniFrac distances for mouse skin bacterial and archaeal communities with samples colored by (left) days of decomposition and (right) soil type. (**B**) A log scale heat map of 16S rRNA OTUs colonizing the skin of human cadavers. (**C**) A 16S rRNA-based Random Forests (RF) model using winter season skin and soil data set to train the model and predict the PMI of human body in spring season. Each point indicates a sample collected at a certain PMI, with RF predicted PMI in red and randomly guessed PMI in gray. (**D**) Percentage of shared features (out of top 100 PMI regression features) from each experiment and marker type.

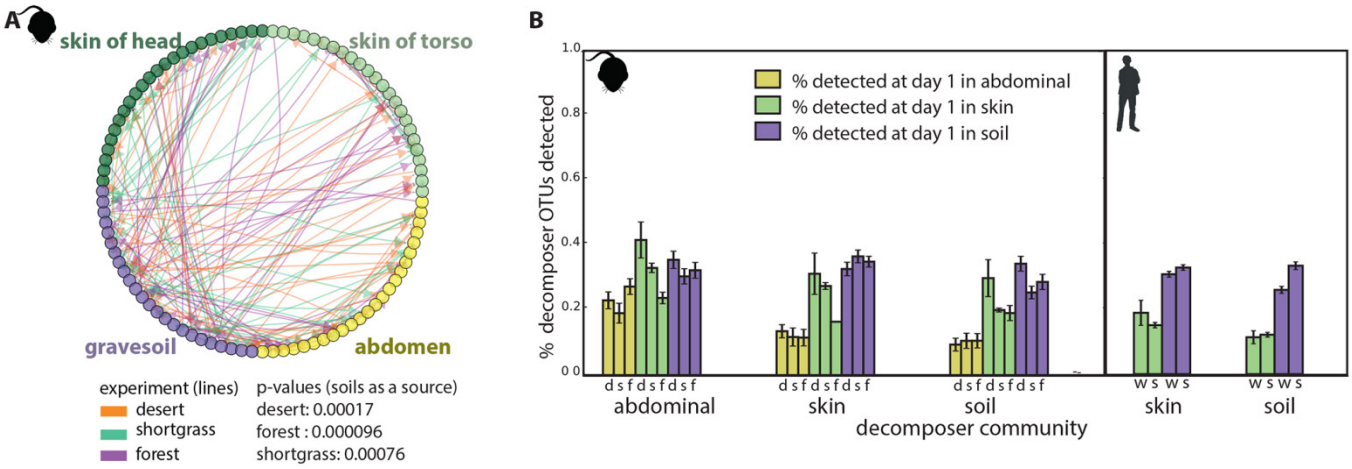

Fig. 2. Bacterial and archaeal decomposers emerge from multiple environments, but primarily soil. (**A**) Dynamic Bayesian Inference Networks: Neural Information Flow Network of microbial taxa during decomposition shows soils as the most common source. (**B**) y-axis is the proportion of abdominal, skin, 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), short grass (s), and forest (f), or season: winter (w), and spring (s). Decomposers were detected in soils more frequently than in the abdomen in every comparison (Mann-Whitney U $p < 0.05$).

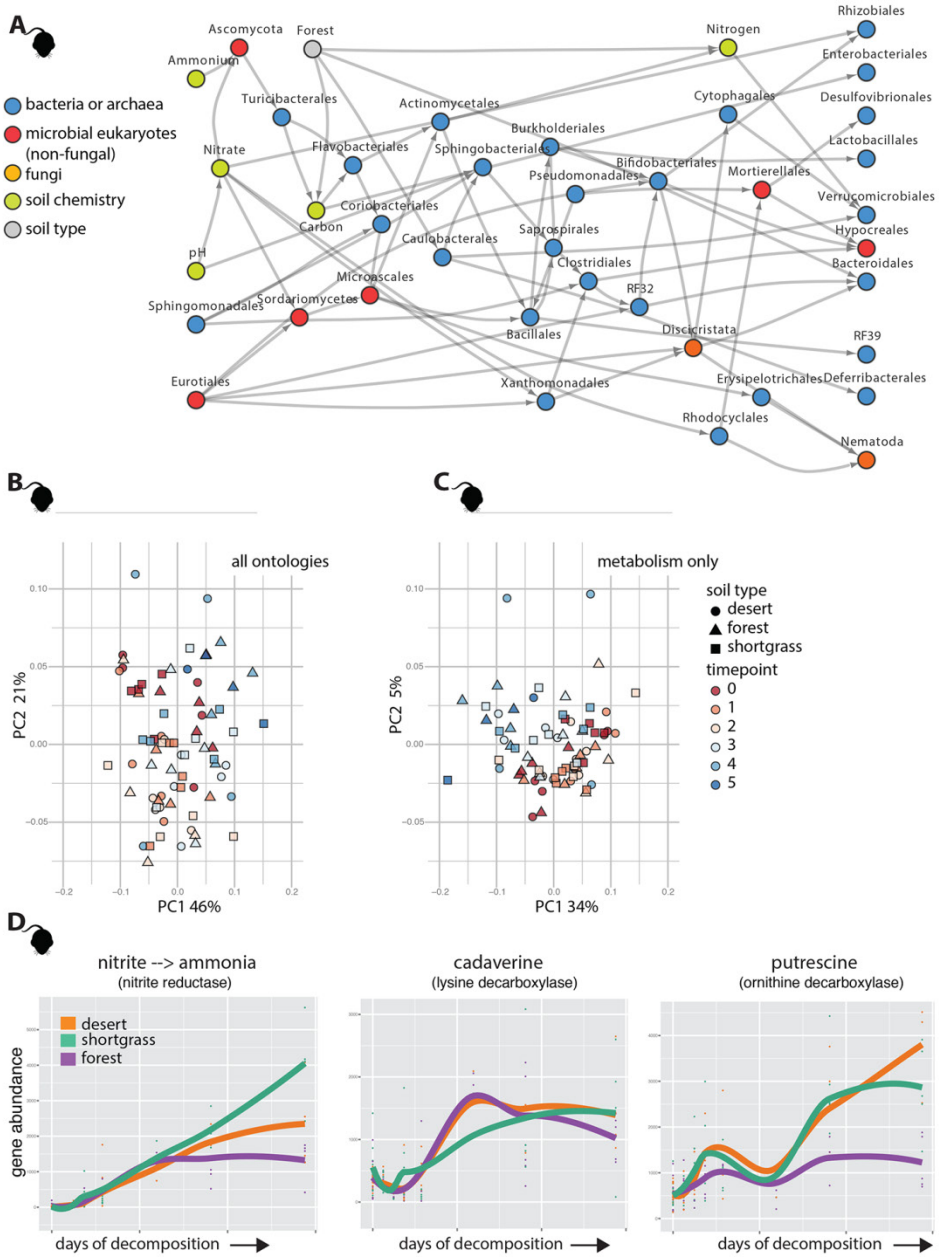

Fig. 3. Succession of decomposer communities in the abdominal cavity. (**A**) Dynamic Bayesian Network of interactions between archaeal, bacterial, microbial eukaryotes, and environmental abundance measurements during decomposition. Arrows indicate direction of causality, and the network is arranged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

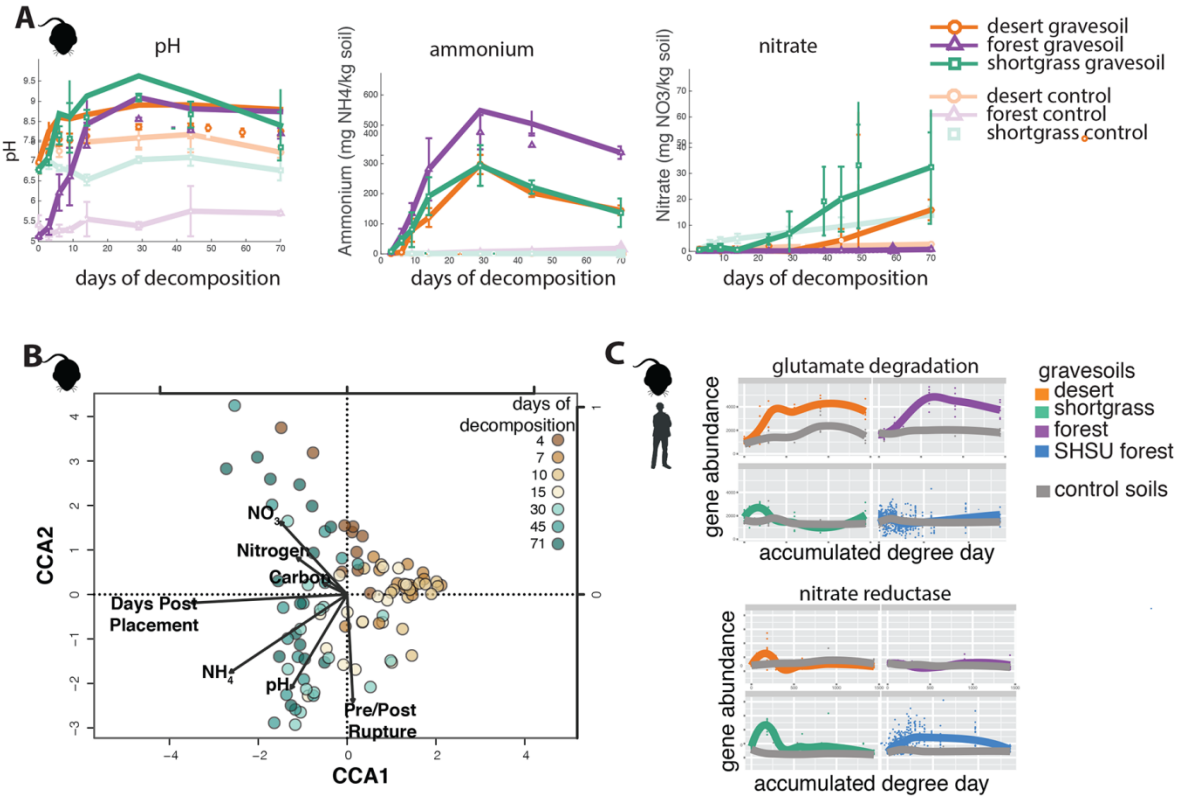

Fig. 4. The effect of mammalian decomposition on soils. (A) pH, total nitrogen, and ammonium concentrations in mouse gravesoils and control soils. (B) canonical correspondence analysis (CCA) of gravesoil bacterial predicted gene ontologies during decomposition. PICRUSt predicted function data are based on KO with only genes classified as "Metabolism included. (C) Predicted gene abundances of glutamate dehydrogenase and nitrate reductase in soils during decomposition.