DR. CHRISTOPHER CM BAKER (Orcid ID : 0000-0002-2675-1078)
DR. JESSICA ANNE CASTILLO VARDARO (Orcid ID : 0000-0001-9580-6987)

Article type : Original Article

SPATIAL PATTERNING OF SOIL MICROBIAL COMMUNITIES CREATED BY FUNGUS-FARMING TERMITES

Running head: Spatial patterning of soil microbes

Christopher C. M. Baker[1,2,3,*], Jessica A. Castillo Vardaro[1,2,4], Daniel F. Doak[2,5], Johan Pansu[1,6,7], Jérémy Puissant[8], Robert M. Pringle[1,2], and Corina E. Tarnita[1,2]

[1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton NJ 08544, USA

[2] Mpala Research Centre, PO Box 555, Nanyuki 10400, Kenya

[3] Present address: Department of Organismic and Evolutionary Biology, Harvard University, Cambridge MA 02138, USA

[4] Present address: Department of Biological Sciences, San José State University, San José CA 95192, USA

[5] Environmental Studies Program, University of Colorado, Boulder CO 80309, USA

[6] Present address: CSIRO Ocean & Atmosphere, Lucas Heights NSW 2234, Australia

[7] Present address: Station Biologique de Roscoff, UMR 7144 CNRS-Sorbonne Université, 29688 Roscoff, France

[8] Centre for Ecology and Hydrology, Wallingford, UK

[*] Corresponding author: bakerccm@gmail.com

Keywords: African savannas, spatial structure, termites, soil microbial communities, DNA metabarcoding, nitrogen cycling

Abstract

Spatially overdispersed mounds of fungus-farming termites (Macrotermitinae) are hotspots of nutrient availability and primary productivity in tropical savannas, creating spatial heterogeneity in communities and ecosystem functions. These termites influence the local availability of nutrients in part by redistributing nutrients across the landscape, but the links between termite ecosystem engineering and the soil microbes that are the metabolic agents of nutrient cycling are little understood. We used DNA metabarcoding of soils from *Odontotermes montanus* mounds to examine the influence of termites on soil microbial communities in a semi-arid Kenyan savanna. We found that bacterial and fungal communities were compositionally distinct in termite-mound topsoils relative to the surrounding savanna, and that bacterial communities were more diverse on mounds. The higher microbial alpha and beta diversity associated with mounds created striking spatial patterning in microbial community composition, and boosted landscape-scale microbial richness and diversity. Selected enzyme assays revealed consistent differences in potential enzymatic activity, suggesting links between termite-induced heterogeneity in microbial community composition and the spatial distribution of ecosystem functions. We conducted a large-scale field experiment in which we attempted to simulate termites' effects on microbes by fertilizing mound-sized patches; this altered both bacterial and fungal communities, but in a different way than natural mounds. Elevated levels of inorganic nitrogen, phosphorus, and potassium may help to explain the distinctive fungal communities in termite-mound soils, but cannot account for the distinctive bacterial communities associated with mounds.

Introduction

Spatial heterogeneity is an important contributor to the productivity, diversity, and robustness of many ecological communities (Tilman 1994; Loreau *et al.* 2003; Bonachela *et al.* 2015). In tropical savannas, fungus-farming termites (Macrotermitinae) act as ecosystem engineers and are major sources of spatial heterogeneity (Bignell & Eggleton 2000; Jouquet *et al.* 2006; Jouquet *et al.* 2011; Muvengwi *et al.* 2017). Within their subterranean nests (henceforth mounds), termites locally increase macro- and micronutrient concentrations (Holdo & McDowell 2004; Seymour *et al.* 2014) and modify soil texture and moisture (Wood 1988; Holt &

Lepage 2000) in ways that alter plant assemblages and enhance primary productivity (Sileshi *et al.* 2010) compared to the surrounding savanna (henceforth matrix). These edaphic changes are likely to both involve and produce changes in soil microbial communities; yet to date, there is limited information about how fungus-farming termites influence soil microbes.

In East African vertisol savannas, spatially overdispersed mounds of the fungus-farming termite *Odontotermes montanus* Harris 1960 form a regularly patterned template that influences numerous aspects of the ecosystem (Table S1; Palmer 2003; Pringle *et al.* 2010; Brody *et al.* 2010). Mature mounds are up to 10–20 m diameter and 1-2 m deep (Figure S1), containing hundreds of chambers in which the termites cultivate a symbiotic *Termitomyces* fungus (Darlington 2005). Vegetation on mounds has elevated foliar nutrients (Fox-Dobbs *et al.* 2010) and is visibly greener than vegetation in the matrix during rainy seasons (Brody *et al.* 2010). Plant community composition also differs on mounds compared to the matrix; for example, *Odontotermes* mounds in central Kenya are dominated by the grass *Pennisetum stramineum* and rarely support woody vegetation, whereas matrix plant assemblages are more diverse in plant species and life-forms (Brody *et al.* 2010; Fox-Dobbs *et al.* 2010; Palmer 2003; Odadi *et al.* 2007; Riginos & Grace 2008).

These spatial patterns in the vegetation reflect the influence of termites on soil physical and chemical properties around mounds. Like those of other African fungus-farming termites (Sileshi *et al.* 2010), *O. montanus* mounds are enriched in total nitrogen and phosphorus, nitrate , and total and organic carbon (Brody *et al.* 2010; Fox-Dobbs *et al.* 2010). *Odontotermes* mounds are also lower in clay and higher in sand content than the surrounding clay-heavy vertisols, owing to translocation of particles from lower soil horizons (Brody *et al.* 2010). This tends to improve water infiltration and aeration on termite mounds, aided by the presence of termite galleries (Bignell 2006) and a network of shallow cracks promoted by bioturbation (DeCarlo & Caylor 2019).

But it remains unclear how such local environmental modifications affect free-living microbial communities, their biogeochemical functions, and their distribution across the landscape. We hypothesized that the enrichment of soil macronutrients close to mounds causes localized changes in soil microbial communities, creating landscape-scale spatial patterns in

community composition and microbe-driven ecosystem functions. Nutrient limitations play a key role in the ecology of tropical savannas (Pellegrini 2016). Furthermore, studies conducted over large spatial scales across a wide range of ecosystems and climates have documented changes in microbial community composition in response to anthropogenic nutrient addition (Ramirez *et al.* 2010; Fierer *et al.* 2011). Investigating the patterns and drivers of spatial heterogeneity in soil microbes is an important step towards understanding heterogeneity throughout the savanna biome, because soil microbes play important roles in nutrient cycling, including the conversion of nutrients between forms that differ in availability to plants.

Here, we used DNA metabarcoding to examine spatial patterning in free-living soil microbial communities created by *O. montanus* termite mounds in central Kenya. We anticipated two broad trends. First, we predicted that mound communities would have lower richness and evenness (alpha diversity; Whittaker 1972) than matrix communities. We reasoned that the lower diversity of plants and the greater abundance of simple, accessible nutrients (e.g., nitrates, Fox-Dobbs *et al.* 2010) on mounds would favor a limited number of microbial taxa with high growth rates, whereas the higher diversity of plants and of complex, recalcitrant molecules (e.g., celluloses, lignins) in the matrix would promote a greater diversity of microbes that specialize in breaking down these different substrates. Second, we predicted that mound communities would differ in composition from matrix communities (beta diversity), generating large-scale spatial structure patterned on the overdispersed template of termite mounds. Specifically, we expected mounds to have higher relative abundances of copiotrophic microbes that thrive in nutrient-rich environments (e.g., the bacterial groups Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Bacteroidetes) and lower relative abundances of oligotrophic groups that perform better in nutrient-poor conditions (e.g., Acidobacteria and Deltaproteobacteria) compared to the matrix. The placement of these microbial groups along a copiotrophic-oligotrophic spectrum has found empirical support in a range of environments, albeit with substantial heterogeneity among taxa within the groups (Fierer *et al.* 2007; Ramirez *et al.* 2010; Campbell *et al.* 2010; Leff *et al.* 2015).

As an initial attempt to probe the mechanisms responsible for the predicted differences in microbial community composition between mounds and matrix habitats, we conducted a large-

scale field experiment involving the repeated addition of inorganic nitrogen-potassium-phosphorus (NPK) fertilizer to replicated mound-sized patches. Although the addition of these three major macronutrients is a crude way to imitate the effects of termites—mound soils are characteristically elevated in a wide range of macro- and micronutrients, in addition to differing in pH and physical structure (Sileshi *et al.* 2010; Seymour *et al.* 2014)—N, P, and K frequently have strong influences on both plant and microbial communities (Güsewell 2004; Pan *et al.* 2014) and could thus account directly and/or indirectly for a major part of the effects of mounds on microbes. Accordingly, we expected microbial communities in fertilized patches to be more similar to those on mounds, with lower alpha diversity and greater relative abundances of copiotrophic bacterial groups compared to control patches.

Finally, to complement our metabarcoding data, we explored microbial community function by measuring extracellular enzyme activities linked to carbon, nitrogen, and phosphorus cycles in a subset of our mounds. Extracellular enzyme activity is commonly viewed as a proxy for microbial nutrient demand, since these enzymes catalyze nutrient acquisition from complex organic matter (Sinsabaugh *et al.* 2008). We expected nutrient demand to be lower on mounds than in the matrix because microbes should take advantage of more readily accessible simple nutrients close to mounds (Allison & Vitousek 2005).

Materials and Methods

Here we provide an overview of our materials and methods, including all essential information necessary to understand the results. Additional technical details are provided in Document S1.

Field site

We conducted fieldwork in July 2016 and May–June 2017 at the Mpala Research Centre and Conservancy (MRC) in the Laikipia Highlands of Kenya (0.2924°N 36.8980°E, ~1800 m elevation). *Odontotermes montanus* mounds occur in poorly drained, clay-rich vertisols, known locally as 'black cotton', which are found across large areas of MRC (Pringle *et al.* 2016) and elsewhere across Laikipia (Ahn & Geiger 1987) and East Africa more broadly. Mature mounds at MRC are characteristically overdispersed at spatial scales of <100 m (Pringle *et al.* 2010; Bonachela *et al.* 2015; Tarnita *et al.* 2017).

Field sampling: transects between termite mounds

To examine the effects of termite mounds on soil microbes, we sampled soil in 2016 from 21 transects grouped in 3 clusters (north, central, and south; Figures S2a-d). Each transect began at a focal termite mound and ran into the matrix towards a neighboring mound; six transects spanned the entire distance between two neighboring mounds, and the remainder spanned half the distance. At 2.5-m intervals within the first 10 m along each transect and at 5-m intervals thereafter, we took soil cores from the top ~10 cm, recorded the dominant grass species (that with the highest areal cover within ~0.5 m of the sampling point) and classified each point as being located on a mound or in the matrix. We measured moisture content and pH for each soil sample and extracted extracellular DNA for metabarcoding. Subsamples from seven mound samples and seven matrix samples were used for measurements of enzymatic activity (see *Extracellular enzyme activity assays* below).

We resampled parts of these transects in 2017 to assess consistency over time and for comparison with experimental samples from 2017 (see *Field sampling: fertilization experiment* below). Instead of resampling entire transects, we only sampled from the center of the focal mound and the midpoint between the focal mound and its neighbor.

Field sampling: fertilization experiment

In 2015, we established an experiment in which we semiannually fertilized a set of termite-mound-sized patches with inorganic NPK fertilizer in 96 patches of 10-m diameter (see Document S1 *Field sampling: fertilization experiment* for further details). These fertilized patches were arranged in 6 plots of 16 patches each; the plots, in turn, were arranged in 3 blocks of 2 plots each (north, central, and south; see Figures S2a and S2g-i). All three blocks were located between 0.5 and 2.3 km from the north cluster of transects (for comparison, the north and south transect clusters were ~3.4 km apart). In May–June 2017, after the experiment had been running for 2 years (and ~5 months after the most recent fertilization), we sampled topsoil from the centers of 84 fertilized patches across all 6 plots (14 patches per plot). For each of these fertilized samples, we also collected a nearby control sample from the unfertilized area within the experimental plot but between treatment patches. These samples were processed in the same way as the transect samples.

DNA metabarcoding

We used DNA metabarcoding to characterize microbial community composition in each soil sample. Extracellular DNA was extracted from each 15-g soil subsample using the methods in Taberlet *et al.* (2012). We PCR amplified a ~258 bp fragment of the bacterial 16S ribosomal RNA gene (Fliegerova *et al.* 2014) and a ~185 bp fragment covering the fungal internal transcribed spacer I region (Epp *et al.* 2012) using tagged primers. We then constructed multiplexed amplicon libraries and sequenced them using paired-end reads of 400 (bacteria) or 350 (fungi) cycles on an Illumina MiSeq. We processed our sequence data using the OBITools pipeline (Boyer *et al.* 2016) and then used Sumatra for clustering using a 97% similarity threshold (Mercier 2015).

We rarefied our OTU tables to 1500 reads per sample to minimize statistical artifacts arising from sequencing depth variation (Hughes & Hellmann 2005; Goodrich *et al.* 2014; Weiss *et al.* 2017). We chose our rarefaction depth to strike a balance between excluding samples with low read counts and retaining adequate read depth. From our initial 452 samples, this led us to drop 36 bacterial samples and 54 fungal samples, leaving us with 416 samples and 398 samples in our bacterial and fungal datasets, respectively.

Analysis of site characteristics

We compared pH and soil moisture between mounds and matrix, and between fertilized and control sampling points, using linear mixed-effects models and likelihood-ratio (LR) tests. We compared grass species richness between mounds and matrix, and between fertilized and control sites, using Wilcoxon rank-sum tests. We used permutational χ^2 tests to compare the frequency distributions of dominant grass species between mounds and matrix, and between fertilized and control sites.

Analysis of microbial alpha and beta diversity

We compared OTU richness and Shannon diversity between mounds and matrix, and between fertilized and control samples, using linear mixed-effects models and LR tests. We visualized microbial beta diversity using non-metric multidimensional scaling (NMDS) ordinations of Bray-Curtis dissimilarities (Anderson *et al.* 2006). We tested for differences between mound and matrix samples, and between fertilized and control samples, using

permutational MANOVA (Anderson 2001; McArdle & Anderson 2001). We used Kruskal-Wallis tests to compare pairwise dissimilarities between mound and fertilized samples to pairwise dissimilarities between matrix and control samples. We likewise compared pairwise dissimilarities between mound and fertilized samples to pairwise dissimilarities between mound and control samples. We used Mantel tests to evaluate the Pearson correlation between Bray-Curtis dissimilarities and pairwise differences in pH, and we visualized the relationship between pH and community composition by plotting the position of each sample along the first NMDS axis against sample pH.

To explore the effects of termite mounds on particular bacterial and fungal groups, we calculated median read counts for phyla, classes, and fungal orders that accounted for >1% of reads from the 2016 transect samples. We used Wilcoxon rank-sum tests to compare read counts for each of these taxonomic groups between mound and matrix samples, and between fertilized and control patches.

To examine the effects of termites and fertilization on individual bacterial and fungal taxa, we used an Analysis of Microbial Communities (ANCOM) (Mandal *et al.* 2015) to identify OTUs showing a strong association with mounds or fertilized patches. We employed the ANCOM method on a set of 'core OTUs' that we defined by taking OTUs that were (i) present in ≥50% of bacterial samples or ≥20% of fungal samples, and (ii) in the top decile of bacterial OTUs by total reads or the top two deciles of fungal OTUs. Document S1 provides further details of the definition and analysis of these core OTUs.

Analysis of the spatial extent of mound influence

To visualize the spatial extent of the termite mounds' influence on soil bacterial and fungal communities, we first plotted the Bray-Curtis compositional dissimilarities (based on all OTUs) between each of the 2016 transect samples and the centroid of the 2016 mound samples as a function of distance from the mound edge. Next, we described the relationship between microbial community dissimilarity and distance from the mound edge by using the R function `nls` to fit the model

$$dissimilarity = \alpha + \beta e^{\gamma . distance}.$$

We chose this functional form, with free parameters α, β, and γ, for its ability to generate a curve that approximated the shape of our observed data. We visualized these results across a representative portion of the landscape as described in Document S1.

Analysis of microbial gamma diversity

Microbial diversity at the landscape scale (gamma diversity) is a function of diversity at smaller spatial scales. Having already compared the diversity of individual mound and matrix samples (alpha diversity), we next compared beta diversity between pairs of mound and matrix samples, both within and among transects. First, we compared mound-sample pairs and matrix-sample pairs from the same transect. For this analysis, we calculated for each transect the Bray-Curtis dissimilarities between 0 m and 5 m (i.e., from the center and toward the edge of the same mound) and between 25 m and 30 m (i.e., matrix samples separated by the same distance as those on the mound); then, taking all transects together, we used Kruskal-Wallis tests to compare the dissimilarities between the mound pairs and the matrix pairs. Second, we examined among-transect beta diversity by using permutational MANOVA to compare the pairwise Bray-Curtis dissimilarities between all mound samples at 0 m (i.e. mound centers) to the pairwise Bray-Curtis dissimilarities between all matrix samples at 25 m from the mound center. Third, we used sample-wise rarefaction curves to evaluate the overall effect of termite mounds on soil microbial gamma diversity (as described in detail in Document S1).

Assays of extracellular enzyme activity

We measured hydrolytic extracellular enzyme activity associated with carbon, nitrogen, and phosphorus cycles (Table 1) in seven mound samples and seven matrix samples collected in 2016. We also measured fluorescein diacetate hydrolysis to estimate overall soil microbial activity (Green *et al.* 2006). Enzyme assays were performed according to Marx *et al*. (2001) with the modifications of Puissant *et al*. (2015) as detailed in Document S1. Enzyme activities were calculated in nanokatal (nmol of product per second) normalized by dry soil mass. We also calculated ratios between the measured activities of different enzymes to assess relative resource allocation to the acquisition of carbon (β-glucosidase), nitrogen (leucine-aminopeptidase), and phosphorus (phosphatase) (Sinsabaugh *et al.* 2008; Stone *et al.* 2013). To compare enzyme activity and activity ratios between mound and matrix samples, we fitted

mixed-effects models with location (mound versus matrix) as a fixed effect and transect as a random effect. We used LR tests to compare these models to random effects models with transect as a random effect.

Results

Site characteristics

Mound samples had higher pH than matrix samples (Figures 1a and S3a). In contrast, fertilized samples had lower pH than control samples (Figure 1b). Both termite mounds and experimental fertilization tended to reduce soil moisture, but there was also substantial inter-sample variation (Figures S3b-d).

The median species richness of grasses at sampling points on mounds was 1 in both 2016 and 2017, compared to 3 in 2016 and 2 in 2017 in the matrix (2016: W=671, n_{mounds}=49, n_{matrix}=141, p<0.001; 2017: W=93.5, n_{mounds}=21, n_{matrix}=22, p=0.001). The relative frequencies of dominant grass species also differed between mound and matrix locations. *P. stramineum* was the dominant grass species for almost all of the mound sampling points, whereas we recorded *Brachiaria lachnantha* and *P. mezianum* as the most common dominant species in the matrix in 2016 and 2017 respectively (Figures S3e,f). Like real mounds, sampling points in experimentally fertilized patches had lower grass species richness than control samples in 2017 (median 1 species in fertilized patches; median 2 species in control patches; W=2617, $n_{fertilized}$=$n_{control}$=84, p=0.001). *P. mezianum* was the most common dominant species for both fertilized and control sampling points in 2017, although *P. stramineum* was more frequently dominant in fertilized than control patches, possibly reflecting a trend towards increased vegetation similarity between fertilized patches and mounds (Figure S3f).

Microbial alpha diversity

Contrary to our prediction, mound soils had higher bacterial OTU richness (Figure 1c) and Shannon diversity (Figure S4a) than matrix soils; the richness and diversity of fungal OTUs did not differ significantly between mounds and matrix (Figures 1c and S4). In contrast, experimental fertilization decreased both bacterial and fungal richness by ~20% relative to control samples (Figure 1d) and likewise suppressed bacterial and fungal diversity

(Figure S4b). Thus, although this effect of fertilization was consistent with our prediction, NPK addition and termite mounds had opposing effects on microbial diversity.

Microbial beta diversity

The most common bacterial phyla across both transect and experimental samples were Actinobacteria and Proteobacteria (Figure S5a; median 64% and 18% of reads per sample respectively). In the fungal dataset, the phylum Ascomycota accounted for the majority of reads (Figure S5b; median 81% of reads per sample), of which Sordariomycetes and Dothideomycetes were the most abundant classes.

Mound samples had distinctive bacterial and fungal communities compared to matrix samples (Figures 2a,b; see also Figure S6). Likewise, experimental fertilization produced distinctive bacterial and fungal communities relative to controls (Figures 2c,d; see also Figure S7).

Termites and fertilization had contrasting effects on bacterial, but not fungal, community composition (Figures 2e,f; see also Figure S8). Although both bacterial and fungal communities differed subtly between matrix and control samples (Figures 2e,f), possibly reflecting minor differences in background soil conditions between the transect and experimental plot locations, the pairwise dissimilarities in bacterial communities were significantly greater between mound and fertilized-patch samples than they were between matrix and control samples (Figure S9a). Moreover, experimental fertilization caused soil bacterial communities to become more dissimilar to those on termite mounds than either matrix or control samples (Figures 2e and S9b). In contrast to the bacterial patterns, the pairwise dissimilarities in fungal communities were not significantly greater between mound and fertilized samples than they were between matrix and control samples (Figure S9a), and fertilization tended to make fungal communities more similar to those on termite mounds, although mound samples still formed a largely discrete cluster in the NMDS ordination (Figures 2f and S9b).

Pairwise dissimilarities in both bacterial and fungal communities were positively correlated with pairwise differences in pH between samples (bacteria: $r=0.36$, $p=0.001$; fungi: $r=0.094$, $p=0.001$), suggesting that soil pH (Figures 1a,b) might explain some of the differences in microbial communities observed among mound, matrix, fertilized, and control samples.

Variation in pH corresponded to variation in bacterial community composition as indexed by the first NMDS axis from the combined transects and experimental fertilization datasets, with samples to the left of the ordination (lower values on the NMDS axis) having higher pH than those on the right (Figure 3a). For fungal communities, the samples on the left of the NMDS ordination comprised both mound samples (high pH) and fertilized samples (low pH), such that pH did not correspond well to the variation in community composition among sample types (Figure 3b).

Several taxonomic groups occurred at higher or lower relative abundances in mound samples compared to the matrix (Table S2). Among bacterial phyla, Actinobacteria and Verrucomicrobia were less abundant on mounds than in the matrix, whereas Proteobacteria, Bacteroidetes, and Chloroflexi were more abundant on mounds than in the matrix. Among fungi, Ascomycota were less abundant on mounds than in the matrix, whereas Basidiomycota were more abundant on mounds than in the matrix. At the level of individual OTUs, ANCOM analysis identified 139 bacterial core OTUs and 30 fungal core OTUs with significantly different relative abundances on mounds compared to the matrix, or in fertilized relative to control samples (Table S3).

Although previous studies (e.g., Jouquet *et al.* 2005) have speculated that *Termitomyces* fungi might contribute to soil communities around fungus-farming termite nests, we found little evidence for this. *Termitomyces* OTUs were present in only 4 of 76 mound samples, comprising just 0.01%, 0.5%, 0.8%, and 3.0% of reads in those samples.

Spatial extent of mound influence

The effects of mounds on soil bacterial and fungal communities extended several meters beyond the mound edge. Communities >10 m from mound edges were markedly dissimilar to those at mound centers, but the dissimilarities saturated as distance increased beyond 10 m (Figure 4a). Termites' relatively localized influence on soil microbial communities thus scales up to generate regular spatial patterning in community composition across the landscape, corresponding to the spatially overdispersed mounds (Figures 4b-d).

Microbial gamma diversity

Sample-to-sample turnover of bacterial OTUs within transects was higher on mounds than in the matrix, but this was not true of fungal OTUs (Figure S10a). The mean Bray-Curtis dissimilarity in bacterial communities between 0 m and 5 m mound samples on the same transect was significantly higher than between 25 m and 30 m matrix samples. For fungal communities, however, Bray-Curtis dissimilarities did not differ significantly between mound and matrix sample pairs.

Bacterial OTU turnover between transects was also higher among mound samples than among matrix samples, but this was not true of fungal OTUs (Figure S10b). Pairwise Bray-Curtis dissimilarities in bacterial communities among 0 m samples (i.e., mound centers) were higher than dissimilarities among 25 m samples (i.e., matrix) on different transects. For fungal communities, however, OTU turnover across transects did not differ significantly between mound and matrix.

Sample-based rarefaction curves illustrate the extent to which termite mounds increase soil bacterial and fungal diversity at the landscape scale. Bacterial OTU richness was estimated to be 6.3% higher in 100 mixed mound/matrix samples than in 100 matrix-only samples, and fungal OTU richness was estimated to be 8.0% higher (Figures 4e,f). Bacterial and fungal Shannon diversity were estimated to be 2.2% and 2.1% higher, respectively, in mixed samples compared to matrix-only samples (Figure S11).

Measured extracellular enzyme activity

Overall microbial activity potential was lower on mounds than in the matrix (Figure 5a). β-glucosidase, chitinase, and phosphatase activity were all lower on mounds; leucine aminopeptidase activity, however, was higher on mounds than in the matrix (Figure 5a). Enzyme C:N (β-glucosidase:leucine-aminopeptidase) was lower on mounds than in matrix samples, whereas both enzyme C:P (β-glucosidase:phosphatase) and enzyme N:P (leucine-aminopeptidase:phosphatase) were higher on mounds than in the matrix (Figure 5b).

Discussion

In this study, we used DNA metabarcoding in conjunction with observational and experimental sampling to explore the effects of *O. montanus* termites on free-living soil microbial

communities. Using samples from transects, we showed that bacterial and fungal communities differed in composition between mounds and matrix, and that bacterial (but not fungal) communities were more diverse on mounds. Our analyses indicate that the relatively localized effects of termites on microbes scale up to create regular spatial pattern in community composition, and that this patterning increases the total microbial diversity of the savanna landscape. A field manipulation designed to simulate the effects of termites on the concentrations of three major nutrients altered the diversity and composition of bacterial and fungal communities, but in different ways. Experimental fertilization caused fungal communities to become more similar to those on real termite mounds, but caused bacterial communities to become markedly more dissimilar to those on real mounds.

Although fungus-farming termites have dramatic effects on the structure and functioning of tropical savannas (Davies *et al.* 2016; Pringle *et al.* 2010; Sileshi *et al.* 2010), and these effects are undoubtedly mediated to some degree by microbial activity, few studies have characterized the influence of termites on soil microbes. Our study extends previous work that has reported distinctive mound-associated soil microbial communities on the basis of community fingerprinting methods (automated ribosomal intergenic spacer analysis, Jouquet *et al.* 2005) or metabarcoding of a limited number of soil samples (Makonde *et al.* 2015). Our study also begins to explore the potential mechanisms underlying termite-driven heterogeneity in soil microbial communities by coupling observational sampling with manipulative experimentation. Our results show that elevated levels of inorganic N, P, and K cannot account for the elevated microbial diversity and distinctive bacterial communities associated with *O. montanus* mounds, although these nutrients may contribute to the patterns observed in fungal community composition.

There are many potential reasons why the application of inorganic NPK fertilizer did not replicate the effects of mounds on bacterial communities. We briefly review the available evidence below. We conclude with a consideration of how future work might further clarify the ecological roles of fungus-farming termites in African savannas.

Potential mechanisms of mound-induced shifts in microbial community composition

Soil pH is likely to act in conjunction with soil nutrients to determine microbial responses to termite mounds and experimental fertilization. While fertilization lowered soil pH relative to controls, pH was higher on mounds relative to the matrix, consistent with a previous study of *O. montanus* mounds in our study area (Petipas & Brody 2014). pH is frequently cited as a major driver of bacterial communities at spatial scales ranging from tens of meters (Baker *et al.* 2009), to tens of kilometers (Bru *et al.* 2011) and beyond (Fierer *et al.* 2009; Fierer & Jackson 2006; Chu *et al.* 2010; Lauber *et al.* 2009). The mechanisms underlying the influence of pH on bacterial communities are not well understood, but may include differences in salinity or nutrient availability, altered microbial enzyme activity, interference with microbial metabolisms, and changes to the thermodynamics and kinetics of redox reactions (Jin & Kirk 2018a, b). Studies have also suggested that soil fungi may be less responsive than bacteria to pH (Lauber *et al.* 2008; Rousk *et al.* 2010). Indeed, we found that differences in pH were strongly correlated with dissimilarities in bacterial community composition, but less so for fungal communities (Figure 3). Thus, we propose that the contrasting effects of termites and experimental fertilization on soil pH may help explain the strongly divergent responses of bacteria, but not fungi, to these influences.

Termite-driven changes to soil nutrients might select directly for microbes able to exploit those resources. We attempted to replicate this using inorganic NPK fertilizer in our experiment because these nutrients are generally elevated on termite mounds (Sileshi *et al.* 2010) and are probably limiting nutrients for savanna grasses (Ries & Shugart 2008), at least in the matrix. However, our metabarcoding data provided very little evidence that inorganic NPK availability is a major driver of the distinctive mound-associated communities. Of the 96 bacterial and 27 fungal OTUs that were significantly elevated or depressed on mounds relative to matrix in our ANCOM results, only 4 bacterial and 5 fungal OTUs showed a significant response in the same direction to experimental fertilization.

Semi-annual fertilization over the two years prior to sampling was adequate to induce consistent changes in soil microbial communities. We think it likely that any strong transient short-term effects of fertilization would have passed within a couple of months after fertilizer application and thus would not have been evident when we sampled five months later (e.g., see

review by Geisseler & Scow 2014). On the other hand, the two-year duration of our treatment is short relative to the age of termite mounds, which may persist for centuries (Darlington 1985). It is possible that repeated fertilizer applications over much longer periods might produce stronger (or even qualitatively different) effects on soil microbial communities. Studies have suggested that soil nutrient levels may take decades to come to equilibrium following changes in land use or nutrient inputs (e.g., Sebilo *et al.* 2013; Oberholzer *et al.* 2014). Furthermore, chronic nutrient inputs may lead to 'permanent' state shifts in microbial community composition by stimulating evolutionary change (e.g., in N-fixing rhizobial bacteria, Weese *et al.* 2015). It would be useful to assess such long-term trajectories by tracking soil microbial community composition through time under repeated fertilization.

Another limitation to our experimental inferences is that we employed only a single fertilization regime and did not measure soil-nutrient content in fertilized patches to evaluate the extent to which our treatment resulted in levels of N, P, and K similar to those on real mounds. We selected the rate of fertilization with the goal of replicating the direction and approximate magnitude of the nutrient gradient on termite mounds. We benchmarked our treatment regime against local agricultural practices and previous nutrient manipulation studies, and used a small pilot to verify that our treatment elevated the foliar nitrogen content of trees in fertilized patches by an amount roughly comparable to the difference between mound and matrix trees (see Document S1 *Field sampling: fertilization experiment* for further details). However, a detailed accounting of soil nutrients on both mounds and fertilized patches would have been helpful in clarifying nutrient dynamics and identifying specific similarities and differences between mounds and our experiment.

Although our results did not provide much evidence that mound-associated microbial communities are driven by inorganic NPK availability, they do not rule out the possibility that microbes respond to the elevated availability of other nutrients on *O. montanus* mounds. Organic carbon, for example, is elevated on *O. montanus* mounds (Palmer 2003; Brody *et al.* 2010), and this is also likely to be the case for other complex organic substrates. Organic nutrient additions can alter soil microbial community composition (Yao *et al.* 2006; Pérez-Piqueres *et al.* 2006; Cline *et al.* 2018) and may produce different outcomes than inorganic

nutrient additions, as the microbes best able to exploit these resources may differ. Although many microbes probably use the inorganic nutrients available on mounds in preference to complex organic substrates (Allison & Vitousek 2005), certain microbial taxa may thrive in mound soils by degrading complex substrates to obtain nutrients that remain limiting for microbial growth. The elevated leucine-aminopeptidase activity that we measured on mounds may reflect such use of complex substrates to obtain nitrogen, as this enzyme is involved in breaking down proteins and peptides. In contrast, lower β-glucosidase and phosphatase activity on mounds suggests less use of complex substrates to obtain carbon or phosphorus. The lower ratio of β-glucosidase to leucine-aminopeptidase activity on mounds compared to the matrix, and the higher ratio of leucine-aminopeptidase to phosphatase activity, in turn suggest that nitrogen (but not carbon or phosphorus) remains limiting on mounds, despite elevated soil nitrogen levels, as costly enzyme production should only be favored where nutrients cannot be adequately obtained in more accessible inorganic forms.

Although the effects of termites and fertilization on soil microbes could in principle be mediated by plant community composition (O'Donnell *et al.* 2001; de Vries *et al.* 2012), our data suggest that this was not a prevailing mechanism. We defined mound edges based on the extent of the distinctive mound-associated grass community dominated by *P. stramineum*, and this boundary was usually sharply visible. Yet our data showed that mound-associated soil microbial communities persist 5-10 m beyond that edge, suggesting that the effects of termites on soil microbes may be best understood in terms of resources that can either diffuse (perhaps aided by the greater porosity and extensive shallow cracking of mound soils; DeCarlo & Caylor 2019) or be transported (e.g., by termites moving soil particles) beyond mound edges.

Other edaphic properties might also contribute independently and interactively to shaping microbial communities. Soil moisture, for example, can affect microbes (Lipson 2007; Lauber *et al.* 2013) and was lower both on mounds compared to the matrix and in fertilized patches compared to controls. The lowering of moisture by both termites and fertilization implies that moisture cannot entirely explain the contrasting responses of bacterial communities to these two influences. However, we cannot rule out a role for soil moisture in explaining the fungal community results or in contributing to the bacterial responses. The contrasting effects of

termites and fertilization on bacterial communities could also be explained if the effect of nutrient-enrichment on microbes interacts with physical characteristics that differ between mound and matrix soils, such as altered porosity (Brody *et al.* 2010; Neumann *et al.* 2013), compaction, and cracking (DeCarlo & Caylor 2019).

Conclusions

We have shown that the topsoil of *O. montanus* mounds has distinctive bacterial and fungal communities compared to the surrounding matrix, and we report evidence from an initial experimental inquiry into the potential mechanisms behind these patterns. The assembly of these different microbial communities may be influenced heavily by the redistribution of organic nutrients by termites, and/or by subsequent changes in pH, but for the most part appear not to be driven directly by inorganic macronutrient availability. Microbial communities remain similar to those on mounds for several meters beyond the mound edge, but past that distance the effect of mound proximity is minimal. Termites thus generate spatial heterogeneity in the composition and function of free-living soil microbes across the landscape, which mirrors the regular patterning of the mounds themselves.

Such regular patterning of spatially overdispersed social-insect nests is observed in ecosystems worldwide (Pringle & Tarnita 2017) and can be explained theoretically by territorial competition between neighboring colonies (Tarnita *et al.* 2017; see also Korb & Linsenmair 2001). A key outstanding question is how these patterns influence other ecosystem properties and processes (Pringle & Tarnita 2017). A previous study from our system found that the regular patterning of *O. montanus* mounds boosted net productivity across the landscape (Pringle *et al.* 2010). Here, we found that termite mounds influenced the alpha diversity of soil microbiota, and that the spatially patterned template of termite mounds created pronounced beta diversity, such that the overall richness and diversity of soil bacteria and fungi was greater in the real landscape than in simulated landscapes lacking mounds. As soil microbes are agents of nutrient cycling, decomposition, and other ecosystem functions, these results deepen our understanding of mechanisms that generate spatial heterogeneity in tropical savanna ecosystems, paralleling previous work on the influence of *O. montanus* termites on symbiotic nitrogen fixation (Fox-Dobbs *et al.* 2010).

We suggest that future work proceed by investigating the roles of nutrient availability and pH in shaping the distinctive soil microbial communities associated with fungus-farming termite mounds. It would be helpful to begin with a more detailed accounting of specific organic and inorganic nutrients. Stable-isotope tracers, manipulative experiments, metatranscriptomic sequencing, and measurements of microbial biomass and activity could all help to understand the effects of different nutrients in different forms, as well as the relationships between soil metabolic activity and pH. Developing a more detailed understanding of the soil microbial ecology will contribute to a clearer picture of how termite-induced spatial heterogeneity in microbial communities influences other aspects of the ecosystem, and the ways in which such connections might apply in other ecosystems.

Acknowledgements

We are grateful for help with fieldwork from Onesmus Kibiwott, James Kiplang'at, Echakan Nairobi, Paul Seketeti, and John Mwihaki (MRC), and Marissa Dyck (Ohio University). As always, the staff at MRC made our stay there both fun and productive. Special thanks to MRC Executive Director Dino Martins for encouraging our research. This work could not have been conducted without the support of the National Museums of Kenya (NMK) and the Kenya Wildlife Service. We are most grateful to Laban Njoroge, Esther Kioko, and Richard Bagine (NMK), and Tyler Kartzinel (Brown University), for their assistance and helpful suggestions. We thank Sylvia Ochanda and the staff at Nature Kenya for invaluable logistics support. The computational parts of this work were run on the Della computing cluster supported by the Princeton University Research Computing Group and the FASRC Cannon cluster supported by the FAS Division of Science Research Computing Group at Harvard University. This work was supported by NSF grant DEB-1355122 to Tarnita and Pringle, and NSF grant DEB-1353781 to Doak. Funding was also provided by a Grand Challenges grant from the Princeton Environmental Institute to Tarnita and Pringle.

Data accessibility

Data from sequencing and enzyme assays are available through Dryad, along with code for bioinformatic processing with ObiTools and statistical analysis in R (Baker *et al.* 2020)

https://datadryad.org/stash/share/IICLl2teEvVh-98uoHmjUcXQ2e7I7ETRK5P0YqTVHew (doi:10.5061/dryad.mw6m905th).

Author contributions

C.C.M.B. and C.E.T. conceived the study, and all authors contributed to research design through discussions. C.C.M.B. conducted sampling, generated molecular data, and performed analyses. J.Puissant conducted enzyme assays. C.C.M.B. wrote the manuscript with the contribution of all co-authors.

References

Ahn PM, Geiger LC (1987) Kenya Soil Survey: Soils of Laikipia District. Ministry of Agriculture, National Agricultural Laboratories.

Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* **37**, 937-944.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**, 32-46.

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. *Ecol Lett* **9**, 683-693.

Baker CC, Castillo Vardaro JA, Doak DF*, et al.* (2020) Data from: Spatial patterning of soil microbial communities created by fungus-farming termites, v3. Dryad.

Baker KL, Langenheder S, Nicol GW*, et al.* (2009) Environmental and spatial characterisation of bacterial community composition in soil to inform sampling strategies. *Soil Biology and Biochemistry* **41**, 2292-2298.

Bignell DE (2006) Termites as Soil Engineers and Soil Processors. In: *Intestinal Microorganisms of Termites and Other Invertebrates* (eds. König H, Varma A), pp. 183-220. Springer Berlin Heidelberg, Berlin, Heidelberg.

Bignell DE, Eggleton P (2000) Termites in Ecosystems. In: *Termites: Evolution, Sociality, Symbioses, Ecology* (eds. Abe T, Bignell DE, Higashi M), pp. 363-387. Kluwer Academic Publishers, Dordrecht.

Bonachela JA, Pringle RM, Sheffer E*, et al.* (2015) Termite mounds can increase the robustness of dryland ecosystems to climatic change. *Science* **347**, 651-655.

Boyer F, Mercier C, Bonin A, *et al.* (2016) OBITOOLS: a UNIX-inspired software package for DNA metabarcoding. *Molecular Ecology Resources* **16**, 176-182.

Brody AK, Palmer TM, Fox-Dobbs K, Doak DF (2010) Termites, vertebrate herbivores, and the fruiting success of *Acacia drepanolobium*. *Ecology* **91**, 399-407.

Bru D, Ramette A, Saby NPA, *et al.* (2011) Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. *ISME Journal* **5**, 532-542.

Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environmental Microbiology* **12**, 1842-1854.

Chu H, Fierer N, Lauber CL, *et al.* (2010) Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environmental Microbiology* **12**, 2998-3006.

Cline LC, Huggins JA, Hobbie SE, Kennedy PG (2018) Organic nitrogen addition suppresses fungal richness and alters community composition in temperate forest soils. *Soil Biology and Biochemistry* **125**, 222-230.

Darlington JPEC (1985) Lenticular soil mounds in the Kenya highlands. *Oecologia* **66**, 116-121.

Darlington JPEC (2005) Termite nest structure and impact on the soil at the Radar Site, Embakasi, Kenya (Isoptera: Termitidae). *Sociobiology* **45**, 521-542.

Davies AB, Baldeck CA, Asner GP (2016) Termite mounds alter the spatial distribution of African savanna tree species. *Journal of Biogeography* **43**, 301-313.

de Vries FT, Manning P, Tallowin JRB, *et al.* (2012) Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters* **15**, 1230-1239.

DeCarlo KF, Caylor KK (2019) Biophysical effects on soil crack morphology in a faunally active dryland vertisol. *Geoderma* **334**, 134-145.

Epp LS, Boessenkool S, Bellemain EP, *et al.* (2012) New environmental metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. *Molecular Ecology* **21**, 1821-1833.

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* **88**, 1354-1364.

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences* **103**, 626-631.

Fierer N, Lauber CL, Ramirez KS*, et al.* (2011) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME Journal* **6**, 1007-1017.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. *Ecology Letters* **12**, 1238-1249.

Fliegerova K, Tapio I, Bonin A*, et al.* (2014) Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe* **29**, 80-84.

Fox-Dobbs K, Doak DF, Brody AK, Palmer TM (2010) Termites create spatial structure and govern ecosystem function by affecting N_2 fixation in an East African savanna. *Ecology* **91**, 1296-1307.

Geisseler D, Scow KM (2014) Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biology and Biochemistry* **75**, 54-63.

Goodrich Julia K, Di Rienzi Sara C, Poole Angela C*, et al.* (2014) Conducting a Microbiome Study. *Cell* **158**, 250-262.

Green VS, Stott DE, Diack M (2006) Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biology & Biochemistry* **38**, 693-701.

Güsewell S (2004) N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist* **164**, 243-266.

Harris WV (1960) Further records of East African Termites - III. *Proceedings of the Royal Entomological Society of London. Series B, Taxonomy* **29**, 17-21.

Holdo RM, McDowell LR (2004) Termite mounds as nutrient-rich food patches for elephants. *Biotropica* **36**, 231-239.

Holt JA, Lepage M (2000) Termites and Soil Properties. In: *Termites: Evolution, Sociality, Symbioses, Ecology* (eds. Abe T, Bignell DE), pp. 389-407. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hughes JB, Hellmann JJ (2005) The application of rarefaction techniques to molecular inventories of microbial diversity. *Methods Enzymol* **397**, 292-308.

Jin Q, Kirk MF (2018a) pH as a Primary Control in Environmental Microbiology: 1. Thermodynamic Perspective. *Frontiers in Environmental Science* **6**.

Jin Q, Kirk MF (2018b) pH as a Primary Control in Environmental Microbiology: 2. Kinetic Perspective. *Frontiers in Environmental Science* **6**.

Jouquet P, Dauber J, Lagerlöf J, Lavelle P, Lepage M (2006) Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. *Applied Soil Ecology* **32**, 153-164.

Jouquet P, Ranjard L, Lepage M, Lata JC (2005) Incidence of fungus-growing termites (Isoptera, Macrotermitinae) on the structure of soil microbial communities. *Soil Biology and Biochemistry* **37**, 1852-1859.

Jouquet P, Traoré S, Choosai C, Hartmann C, Bignell D (2011) Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology* **47**, 215-222.

Korb J, Linsenmair KE (2001) The causes of spatial patterning of mounds of a fungus-cultivating termite: results from nearest-neighbour analysis and ecological studies. *Oecologia* **127**, 324-333.

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. *Applied and Environmental Microbiology* **75**, 5111-5120.

Lauber CL, Ramirez KS, Aanderud Z, Lennon J, Fierer N (2013) Temporal variability in soil microbial communities across land-use types. *The Isme Journal* **7**, 1641-1650.

Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* **40**, 2407-2415.

Leff JW, Jones SE, Prober SM*, et al.* (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences* **112**, 10967-10972.

Lipson DA (2007) Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients. *FEMS Microbiology Ecology* **59**, 418-427.

Loreau M, Mouquet N, Gonzalez A (2003) Biodiversity as spatial insurance in heterogeneous landscapes. *Proceedings of the National Academy of Sciences* **100**, 12765-12770.

Makonde HM, Mwirichia R, Osiemo Z, Boga HI, Klenk HP (2015) 454 pyrosequencing-based assessment of bacterial diversity and community structure in termite guts, mounds and surrounding soils. *Springerplus* **4**, 471.

Mandal S, Van Treuren W, White RA*, et al.* (2015) Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health and Disease* **26**, 10.3402/mehd.v3426.27663.

Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology & Biochemistry* **33**, 1633-1640.

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology* **82**, 290-297.

Mercier C (2015) *Développements méthodologiques autour de l'analyse des données de metabarcoding ADN*.

Muvengwi J, Witkowski ETF, Davies AB, Parrini F (2017) Termite mounds vary in their importance as sources of vegetation heterogeneity across savanna landscapes. *Journal of Vegetation Science* **28**, 1008-1017.

Neumann D, Heuer A, Hemkemeyer M, Martens R, Tebbe CC (2013) Response of microbial communities to long-term fertilization depends on their microhabitat. *FEMS Microbiology Ecology* **86**, 71-84.

O'Donnell AG, Seasman M, Macrae A, Waite I, Davies JT (2001) Plants and fertilisers as drivers of change in microbial community structure and function in soils. *Plant and Soil* **232**, 135-145.

Oberholzer HR, Leifeld J, Mayer J (2014) Changes in soil carbon and crop yield over 60 years in the Zurich Organic Fertilization Experiment, following land-use change from grassland to cropland. *Journal of Plant Nutrition and Soil Science* **177**, 696-704.

Odadi WO, Young TP, Okeyo-Owuor JB (2007) Effects of wildlife on cattle diets in Laikipia rangeland, Kenya. *Rangeland Ecology & Management* **60**, 179-185.

Palmer TM (2003) Spatial habitat heterogeneity influences competition and coexistence in an African acacia ant guild. *Ecology* **84**, 2843-2855.

Pan Y, Cassman N, de Hollander M*, et al.* (2014) Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *FEMS Microbiology Ecology* **90**, 195-205.

Pellegrini AFA (2016) Nutrient limitation in tropical savannas across multiple scales and mechanisms. *Ecology* **97**, 313-324.

Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. *Soil Biology and Biochemistry* **38**, 460-470.

Petipas RH, Brody AK (2014) Termites and ungulates affect arbuscular mycorrhizal richness and infectivity in a semiarid savanna. *Botany* **92**, 233-240.

Pringle RM, Doak DF, Brody AK, Jocque R, Palmer TM (2010) Spatial Pattern Enhances Ecosystem Functioning in an African Savanna. *PLoS Biology* **8**.

Pringle RM, Prior KM, Palmer TM, Young TP, Goheen JR (2016) Large herbivores promote habitat specialization and beta diversity of African savanna trees. *Ecology* **97**, 2640-2657.

Pringle RM, Tarnita CE (2017) Spatial Self-Organization of Ecosystems: Integrating Multiple Mechanisms of Regular-Pattern Formation. *Annu Rev Entomol* **62**, 359-377.

Puissant J, Cecillon L, Mills RTE*, et al.* (2015) Seasonal influence of climate manipulation on microbial community structure and function in mountain soils. *Soil Biology & Biochemistry* **80**, 296-305.

Ramirez KS, Lauber CL, Knight R, Bradford MA, Fierer N (2010) Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* **91**, 3463-3470.

Ries LP, Shugart HH (2008) Nutrient limitations on understory grass productivity and carbon assimilation in an African woodland savanna. *Journal of Arid Environments* **72**, 1423-1430.

Riginos C, Grace JB (2008) Savanna tree density, herbivores, and the herbaceous community: Bottom-up vs. top-down effects. *Ecology* **89**, 2228-2238.

Rousk J, Bååth E, Brookes PC*, et al.* (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *The Isme Journal* **4**, 1340.

Sebilo M, Mayer B, Nicolardot B, Pinay G, Mariotti A (2013) Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences* **110**, 18185-18189.

Seymour CL, Milewski AV, Mills AJ*, et al.* (2014) Do the large termite mounds of *Macrotermes* concentrate micronutrients in addition to macronutrients in nutrient-poor African savannas? *Soil Biology & Biochemistry* **68**, 95-105.

Sileshi GW, Arshad MA, Konaté S, Nkunika POY (2010) Termite-induced heterogeneity in African savanna vegetation: mechanisms and patterns. *Journal of Vegetation Science* **21**, 923-937.

Sinsabaugh RL, Lauber CL, Weintraub MN*, et al.* (2008) Stoichiometry of soil enzyme activity at global scale. *Ecology Letters* **11**, 1252-1264.

Stone MM, Plante AF, Casper BB (2013) Plant and nutrient controls on microbial functional characteristics in a tropical Oxisol. *Plant Soil* **373**, 893-905.

Taberlet P, Prud'Homme SM, Campione E*, et al.* (2012) Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology* **21**, 1816-1820.

Tarnita CE, Bonachela JA, Sheffer E*, et al.* (2017) A theoretical foundation for multi-scale regular vegetation patterns. *Nature* **541**, 398-401.

Tilman D (1994) Competition and Biodiversity in Spatially Structured Habitats. *Ecology* **75**, 2-16.

Weese DJ, Heath KD, Dentinger BTM, Lau JA (2015) Long-term nitrogen addition causes the evolution of less-cooperative mutualists. *Evolution* **69**, 631-642.

Weiss S, Xu ZZ, Peddada S*, et al.* (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **5**.

Whittaker RH (1972) Evolution and Measurement of Species Diversity. *Taxon* **21**, 213-251.

Wood TG (1988) Termites and the soil environment. *Biology and Fertility of Soils* **6**, 228-236.

Yao S, Merwin IA, Abawi GS, Thies JE (2006) Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. *Soil Biology and Biochemistry* **38**, 587-599.

Table 1. Extracellular enzymes assessed for potential activity (see Figure 5)

Enzyme	Enzyme Commission number	Substrate	Function
β-glucosidase	EC 3.2.1.21	4-methylumbelliferyl-β-D-glucopyranoside	release of glucose from cellulose
phosphatase	EC 3.1.3.1	4-methylumbelliferyl-phosphate	phosphorus mineralization
chitinase	EC 3.2.1.52	4-methylumbelliferyl-N-acetyl-β-D-glucosaminide	degradation of chitin compounds
leucine-aminopeptidase	EC 3.4.11.1	L-leucine-7-amino-4-methylcoumarin	degradation of protein into amino acid

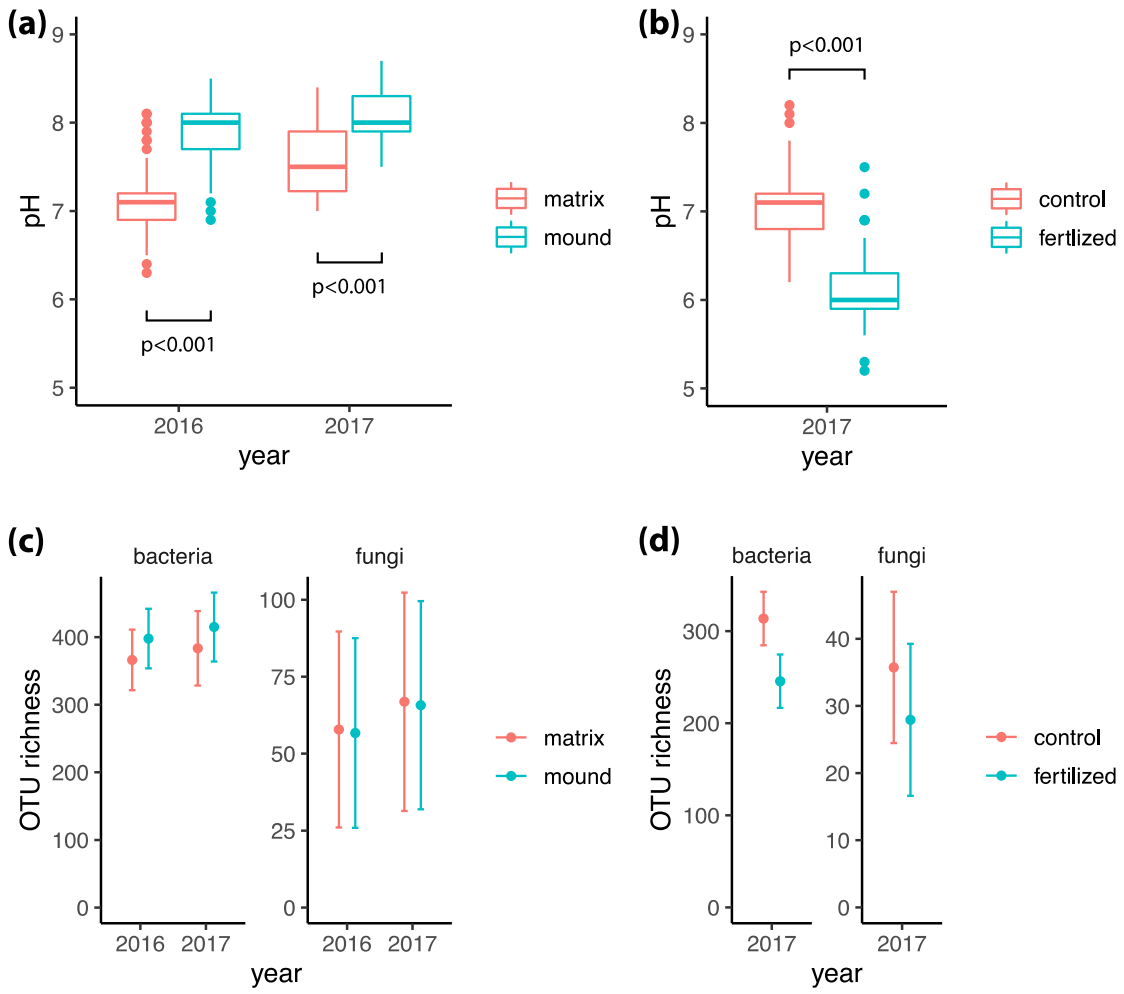

Figure 1. **Effects of termite mounds and experimental fertilization on soil pH and microbial community richness. (a)** In the transect samples, termite mounds had higher soil pH than the surrounding matrix (2016: χ^2=202.7, df=1, n=258, p<0.001; 2017: χ^2=19.4, df=1, n=43, p<0.001). **(b)** In the experimental samples, fertilized patches had lower pH than control samples (χ^2=202.7, df=1, n=168, p<0.001). **(c)** Estimated OTU richness for transect samples at 1:10 PCR template dilution. Mounds had higher bacterial OTU richness than matrix soils, but similar fungal OTU richness (bacteria: χ^2=11.1, df=1, n=250, p<0.001; fungi: χ^2=0.10, df=1, n=241, p=0.76). **(d)** Estimated OTU richness for experimental samples. Experimental fertilization decreased bacterial and fungal OTU richness relative to control samples (bacteria: χ^2=46.3, df=1, n=166, p<0.001; fungi: χ^2=8.71, df=1, n=156, p=0.003). Error bars in (c) and (d) show 95% confidence intervals.

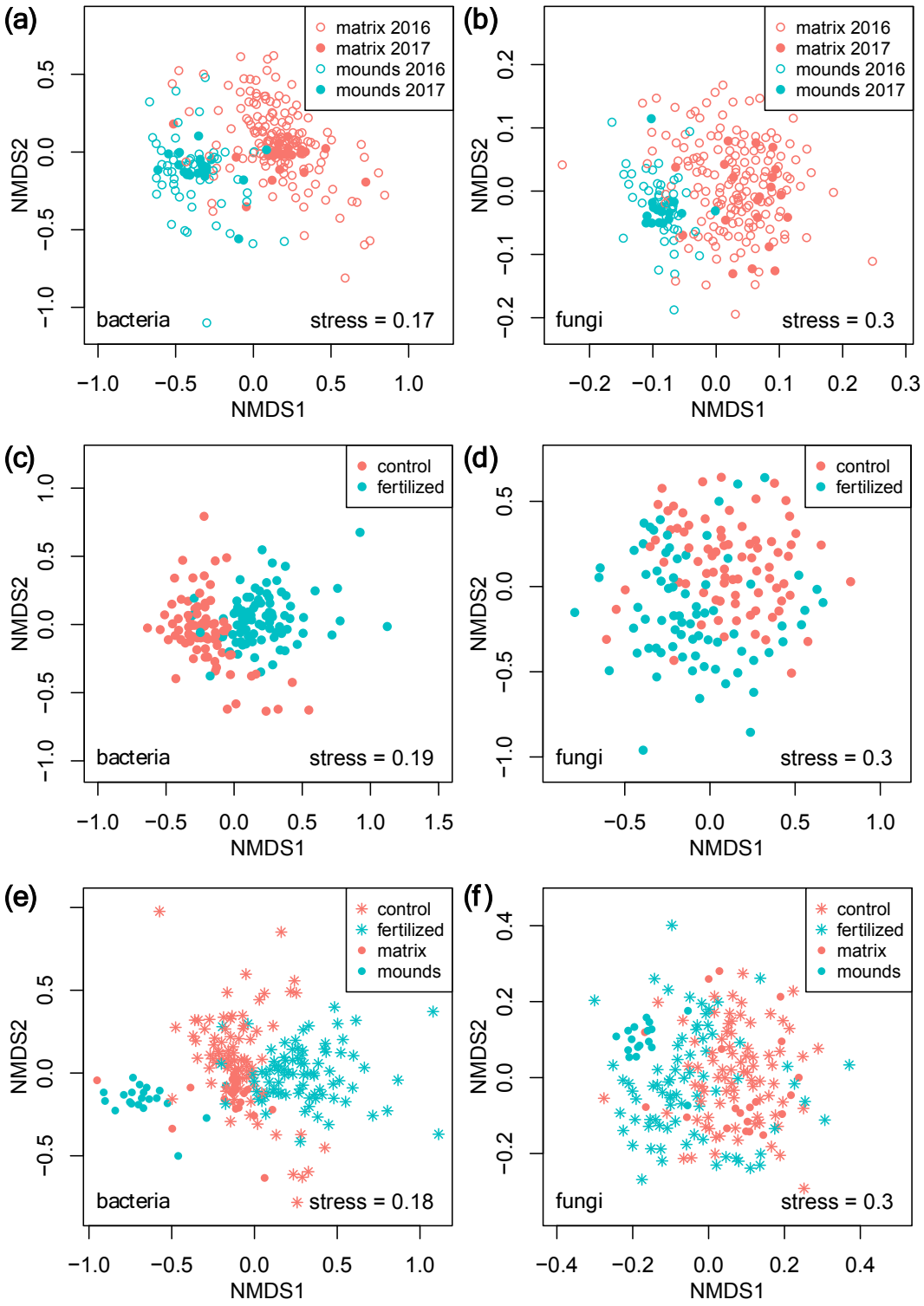

Figure 2. Compositional dissimilarity of microbial communities in mound, matrix, fertilized, and control soils. All plots show non-metric multidimensional scaling ordinations based on Bray-Curtis dissimilarities. **(a)** Bacterial and **(b)** fungal communities from transects in 2016 and 2017. Bacterial and fungal communities were compositionally distinct between mounds and matrix (bacteria: pseudo-F=37.2, df=(1, 221), p=0.001; fungi: pseudo-F=11.2, df=(1, 215), p=0.001). **(c)** Bacterial and **(d)** fungal communities were likewise compositionally distinct between fertilized and control patches in 2017 (bacteria: pseudo-F=20.2, df=(1, 159), p=0.001; fungi: pseudo-F=3.64, df=(1, 149), p=0.001). **(e)** Bacterial and **(f)** fungal communities from transects and fertilization experiment together in 2017, showing that mounds and fertilized patches were compositionally distinct (bacteria: pseudo-F=20.0, df=(1, 103), p=0.001; fungi: pseudo-F=4.11, df=(1, 90), p=0.001), as were matrix samples and control patches (bacteria: pseudo-F=6.30, df=(1, 102), p=0.001; fungi: pseudo-F=1.58, df=(1, 99), p=0.01). These plots additionally illustrate that fertilization made bacterial communities more dissimilar and fungal communities less dissimilar to those on termite mounds (see Figure S9). In all plots, each point represents the microbial community from a single sample, and the distances between points reflects degree of dissimilarity. Shaded ellipses show standard deviations of mound, matrix, fertilized patch, or control samples around their centroids. While some ordinations, especially for fungi, had high stress values, there was no clear breakpoint in screeplots for the transect, experimental, or combined datasets, and higher-dimension ordinations did not alter our interpretation of the data (see Figures S6, S7 and S8 for screeplots and ordinations in 3 dimensions).

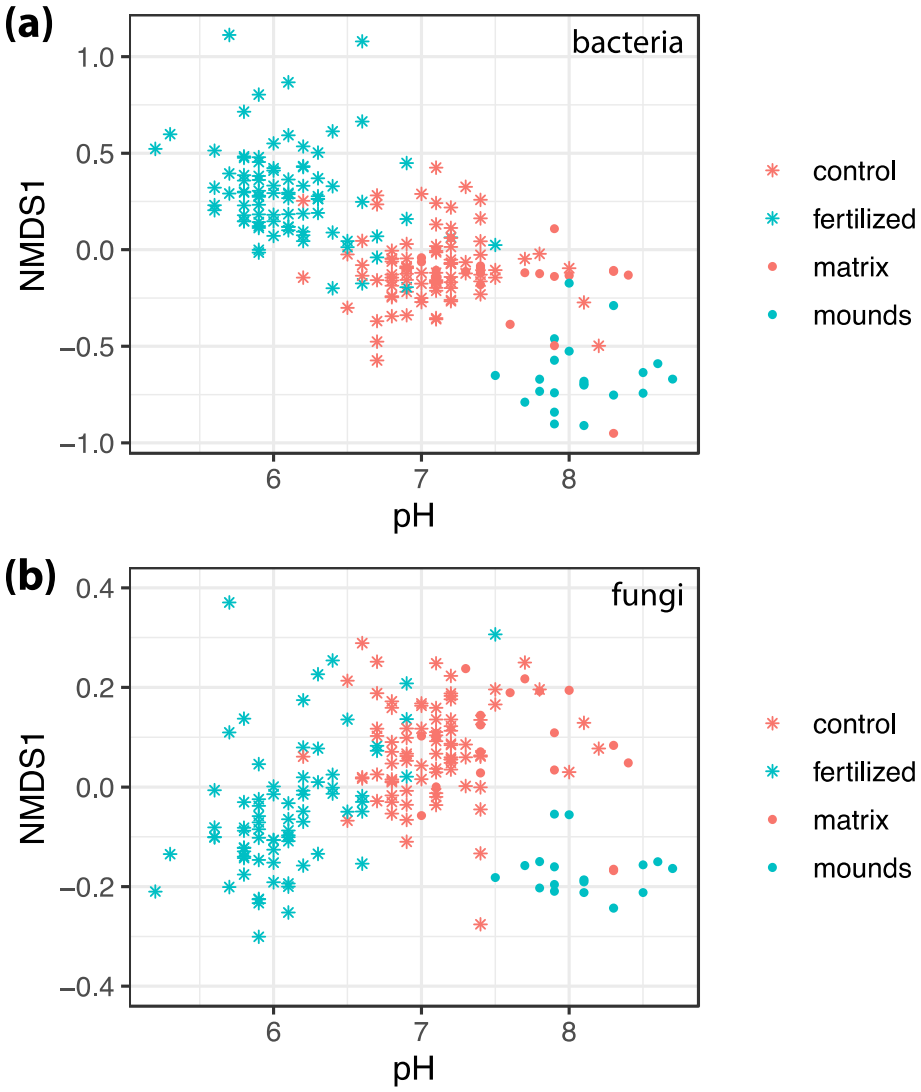

Figure 3. Relationship between microbial community composition and pH. Plots of scores on the first non-metric multidimensional scaling axis (NMDS1) for **(a)** bacterial communities and **(b)** fungal communities against pH for all samples collected in 2017. Each point represents a single sample, and NMDS1 functions here as one measure of variation in microbial community composition. Non-metric multidimensional scaling was performed in two dimensions using Bray-Curtis dissimilarities (see Figure 2e,f).

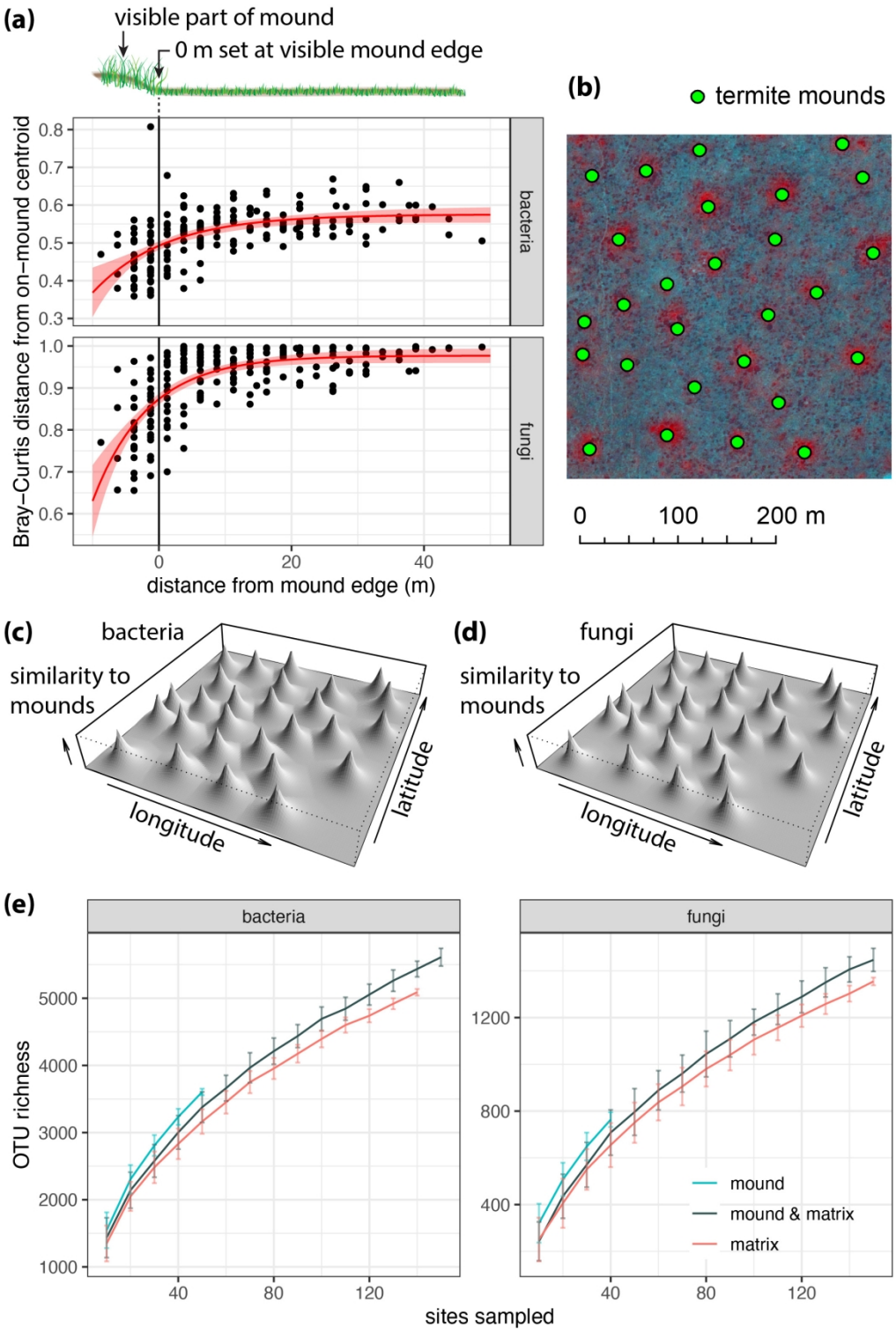

Figure 4. Spatial influences of termite mounds on microbial community composition and richness. Bray-Curtis dissimilarities from the centroid of the 2016 transect mound samples continue to increase for several meters beyond the visible mound edge. **(a)** Bacterial community dissimilarities with fitted curve $dissimilarity = 0.58 - 0.083e^{-0.091\ distance}$ (top panel), and fungal community dissimilarities with fitted curve $dissimilarity = 0.98 - 0.10e^{-0.12\ distance}$ (bottom panel). Shaded areas show 95% confidence intervals for fitted curves. Note that to facilitate comparisons between mounds of different sizes, the visible mound edge is set at 0 m for all transects, with mound centers located at negative distances; this labeling differs from other analyses in this study, in which 0 m refers to the mound center. **(b)** Locations of termite mounds (green dots) as inferred from a multispectral Quickbird satellite image (2.4-m resolution, here in false-color infrared); red patches in the image are areas of high primary productivity corresponding to termite mounds (Pringle *et al.* 2010). Given the inferred termite mounds locations in (b), extrapolating regression results from (a) across the landscape reveals spatial patterning in **(c)** bacterial and **(d)** fungal microbial communities, as indicated by Bray-Curtis dissimilarities (inverted here to reflect similarity for visual clarity) from the 2016 on-mound centroid. **(e)** Sample-wise rarefaction curves of OTU richness for bacterial communities (left panel) and fungal communities (right panel). Blue curves at top show the accumulation of richness for mound samples only; red curves at bottom show the richness of samples collected in the matrix only; and gray curves in the middle show a mix of mound and matrix samples in proportion to the areal coverage of mounds and matrix across the landscape. The higher richness of mixed mound/matrix samples relative to matrix-only samples shows that termite mounds increase microbial alpha diversity relative to landscapes without mounds. Error bars show standard deviations from 100 random rarefactions.

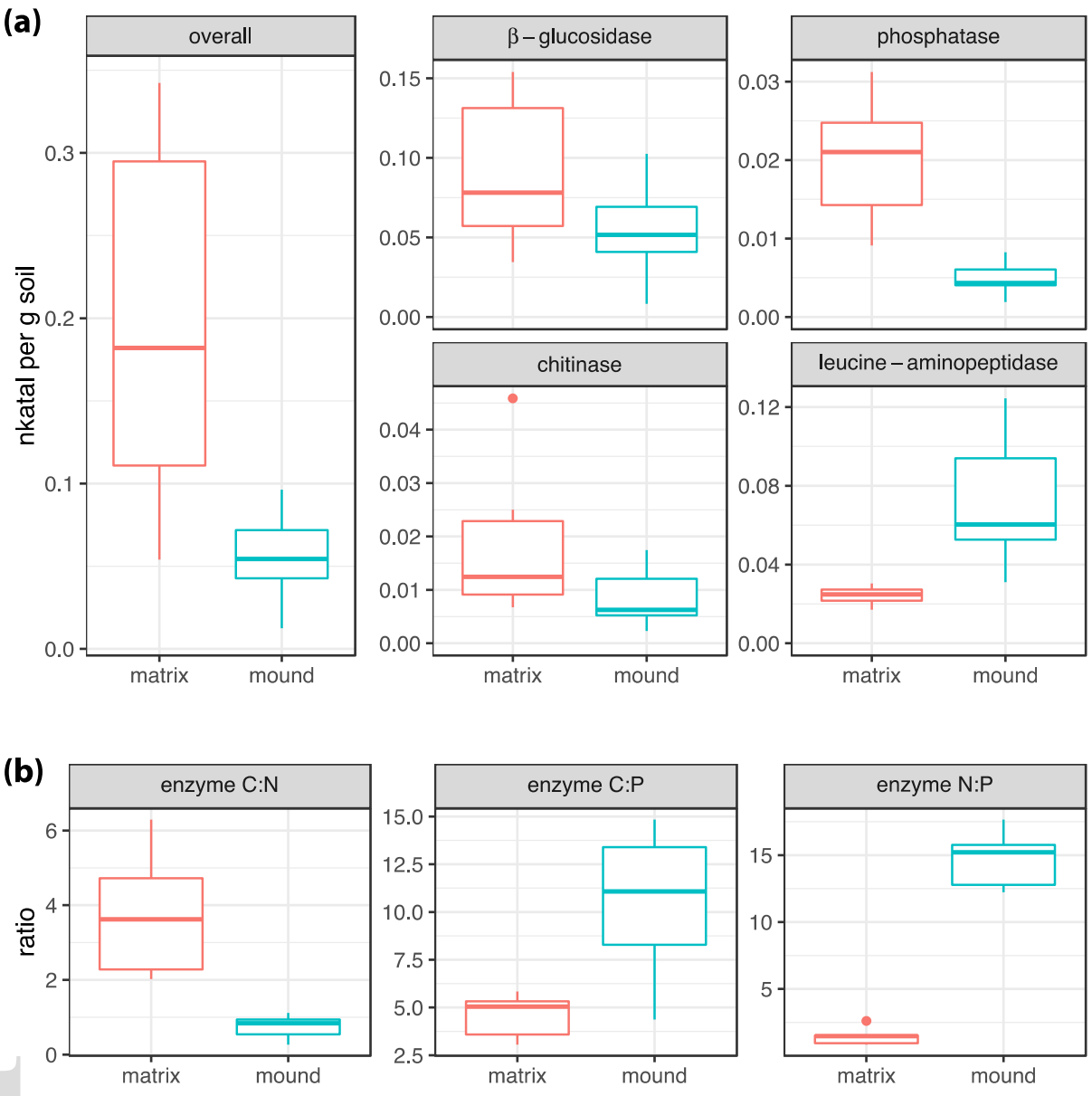

Figure 5. Effects of termite mounds on soil enzymatic activity. (a) Measured soil extracellular enzyme activities normalized by dry soil mass. Mound samples show lower overall hydrolytic enzyme activity compared to matrix samples (χ^2= 43.2, df=1, p<0.001; $n_{matrix} = n_{mound} = 7$ for all panels in this figure), as well as lower β-glucosidase, phosphatase, and chitinase activity (respectively: χ^2=36.0, df=1, p<0.001; χ^2=60.1, df=1, p<0.001; χ^2=19.3, df=1, p<0.001). Leucine aminopeptidase activity, however, was higher on mounds than in the matrix (χ^2=43.2, df=1, p=0.001). **(b)** Measured enzymatic activity ratios. Enzyme C:N (β-glucosidase:leucine-aminopeptidase) was lower on mounds than in matrix samples (χ^2=

51.3, df=1, $p<0.001$; n = 7 per sampling location), whereas both enzyme C:P (β-glucosidase:phosphatase) and enzyme N:P (leucine-aminopeptidase:phosphatase) were higher on mounds than in the matrix (C:P $\chi^2=46.3$, df=1, $p<0.001$; N:P $\chi^2=134.4$, df=1, $p<0.001$).