Effects of Land Use on Plague (*Yersinia pestis*) Activity in Rodents in Tanzania

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Abstract.

Understanding the effects of land-use change on zoonotic disease risk is a pressing global health concern. Here, we compare prevalence of *Yersinia pestis,* the etiologic agent of plague, in rodents across two land-use types—agricultural and conserved—in northern Tanzania. Estimated abundance of seropositive rodents nearly doubled in agricultural sites compared with conserved sites. This relationship between land-use type and abundance of seropositive rodents is likely mediated by changes in rodent and flea community composition, particularly via an increase in the abundance of the commensal species, *Mastomys natalensis,* in agricultural habitats. There was mixed support for rodent species diversity negatively impacting *Y. pestis* seroprevalence. Together, these results suggest that land-use change could affect the risk of local transmission of plague, and raise critical questions about transmission dynamics at the interface of conserved and agricultural habitats. These findings emphasize the importance of understanding disease ecology in the context of rapidly proceeding landscape change.

INTRODUCTION

Zoonotic pathogens, disease agents transmitted to humans from nonhuman animals, such as the Lyme disease spirochete (*Borrelia burgdorferi*) and the anthrax bacterium (*Bacillus anthracis*), are significant burdens on global public health.[1] In particular, bacterial zoonoses originating from wildlife constitute a growing problem, and their emergence and transmission into human populations are often correlated with poverty and rapid rates of environmental and ecological change.[1,2] Geographic hotspots of emerging and reemerging infectious diseases (EIDs) have been identified at a global scale, and are frequently located in the tropics and associated with high mammal biodiversity.[1,3] East Africa is one such identified EID hotspot, and harbors several emerging or re-emerging vector-borne pathogens, for example, Rift Valley fever virus, *Chikungunya* virus, and the plague bacterium, *Yersinia pestis*.[4–6] The region is also experiencing high rates of land-use change, making it a crucial and informative area to examine the effects of land-use change on zoonotic disease ecology.[7–9]

At a more local scale, relationships between land-use change and disease ecology have been suggested for a wide array of host–pathogen systems.[10–14] The "dilution effect" hypothesis, for example, suggests that the net effect of higher species diversity will be a reduction in disease risk at local scales[15]; therefore, conserved areas, which often host higher levels of biodiversity, may have reduced risk for human disease. The mechanisms accounting for such an effect include increases in relative abundance of high competence (amplifying hosts) and increases in total abundance of potentially susceptible hosts (susceptible host regulation).[15,16] However, relationships between biodiversity and disease risk are not uniform and appear to depend on the ecological context, the ecology of the host–pathogens involved,[17–20] and the metric of disease risk used.[21,22] Understanding these relationships is important; if we can understand and predict

changes in host and vector species assemblages as a function of land use, and the consequent risks of rodent-borne zoonotic diseases, then we can improve our ability to enact zoonotic disease control and possibly synergize both public health and conservation initiatives.

Y. pestis, the bacterium that causes plague, is an example of a vector-borne organism that constitutes an important public health concern in many parts of Africa.[4,23,24] Research on the eco-epidemiology of plague in East Africa has given relatively little direct attention to the role of land-use change in disease transmission, and has focused instead on the roles of factors such as climate, context, large mammal presence, and host abundance.[1,25–32]

One of the most common types of landscape change underway in East Africa, and elsewhere in the global tropics, is the conversion of wildlands to agricultural uses.[33–36] Cultivation in a variety of regions of East Africa has expanded by more than 70% in the last several decades.[37] Given that rodent abundance and community composition are known to be strongly impacted by land use,[16] we hypothesized that conversion of land to agriculture may affect landscape-level disease dynamics. To examine how this particular form of landscape change may influence the ecology of plague, we compared rodent communities and *Y. pestis* activity in conserved landscapes and in nearby converted agricultural fields, using a paired sampling design. Sampling was carried out in a region of northern Tanzania where plague is endemic and is a source of public health concern.[38] We compare metrics of rodent community assembly (i.e., abundance, richness, and diversity) and plague ecological risk (i.e., the presence/absence of plague activity and calculated abundance of seropositive rodents) in these two landscape types. Results from this work should contribute to our understanding of how landscape change influences wildlife communities and zoonotic disease risk on the local scale and can inform relevant intervention actions.

METHODS

Sampling locations.

Rodent trapping and pathogen sampling was carried out in three regions of north central Tanzania: the Tloma village vicinity (03°17'S, 35°40'E); the Kambi ya Nyoka village vicinity (03°18'S, 35°36'E); and the southern Manyara region (03°42'S, 35°43'E) (Figure 1). The sampling sites in the Tloma and Kambi ya Nyoka regions are situated at approximately 1,700-m elevation and located within and along the border of the Ngorongoro Conservation Area. The Manyara sites are situated at approximately 1,000-m elevation and located within and along the border of Lake Manyara National Park. This region of Tanzania is generally characterized as having a bimodal pattern of rainfall with peaks generally occurring in March–May and November–December.[39] Field sampling was carried out in this vicinity during June and July 2011, the onset of the dry season, shortly after the typical annual peak in human plague cases.[23]

We sampled rodents, as well as their fleas and pathogens, at six sites for this study. All sites were placed in a matched-pair design (across three site pairs, one in each of the three regions). Each site pair included two land-use treatment types: one site with "agricultural" use and one site with "conserved" use. Agricultural sites were actively under cultivation and in private ownership; they had little or no evidence of large wildlife presence (e.g., via dung or track). The dominant crop in agricultural sites was maize, but chickpeas, vegetables, tobacco, and millet were typically interspersed at lower densities between maize plantings. Conserved sites were at least 500 m inside the boundaries of conservation areas (Ngorongoro Conservation Area or Lake Manyara National Park) in which ecosystems were fully protected and typically consisted of closed-canopy evergreen forest. Tracks and signs of large wildlife species was frequently

observed in conserved sites. Agricultural sites were presumed to be formerly identical or similar to conserved sites prior to land conversion.

Rodent sampling.

At each site, we placed two sampling grids (< 1 km from each other) to encompass a wider range of environmental and agricultural (e.g., time of harvest, crops planted) variability. On each grid we placed 100 Sherman traps spaced 10 m apart. The three site pairs were separated from one another by approximately 15–50 km. Matched agricultural or conserved treatment sites within a pair were physically separated from one another by fences, fire trails, and patrolled/demarcated park boundaries. Agriculture/conserved site pairs were exposed to the same general climatic conditions and soil types. Sites within a given pair were sampled simultaneously. These a priori classifications of habitat type were confirmed by vegetation surveys at each site (see "Habitat descriptions").

Rodent traps were baited with a mix of maize meal, oats, and peanut butter. All traps were set in the late afternoon and checked at dawn. Captured rodents were identified to species in the field, sexed, weighed, marked with ear tags, sampled for blood under full anesthesia (using halothane), and released. After recovering the rodent from the trap, it was restrained over a container of ethanol while a technician collected fleas by passing a fine-toothed flea comb repeatedly over the animal from the base of the ears to the base of the tail. We then collected these fleas from the container. All fleas collected were identified to species morphologically; to confirm identities and eliminate possibility of cryptic species, a subset of rodents and fleas were also identified using DNA barcoding techniques with records deposited submitted to the Barcode of Life Project and made publicly available.[40] One group of morphologically and genetically distinct fleas (*Ctenopthalmus* sp.) could not be identified firmly to species level as all individuals

from this group were either female or were damaged in sampling, making species-level identification impossible. However, based on genetic clustering we included it in diversity analyses as a separate species. For site-level abundance analyses, we used the density of total fleas present on sampled hosts per hectare (one rodent sampling grid; species pooled) as a source of further information on plague transmission risk.[25]

At the conclusion of trapping at a given site, a subset of rodents was collected as voucher specimens (deposited both at the Sokoine University of Agriculture and the Smithsonian's National Museum of Natural History). Identities of voucher specimens were confirmed via cranial morphology; DNA barcodes of each species were then submitted to the Barcode of Life Project. For rodents, where species-level identification was difficult to determine on live animals, the identity of all individuals in question that were not lethally sampled was subsequently determined using a DNA barcoding approach. DNA taken from blood samples of these individuals was compared with reference DNA barcodes. To further ensure that cryptic species were not missed in the field, blood spots from a random subset of the animals not killed for voucher specimens (~10% of total catch per species) were used to confirm species identity using DNA barcodes. Our rodent identifications follow the taxonomy of Musser and Carleton,[41] except where updated by more recent research.[42,43]

Each site was trapped for three sequential nights (which previous work has shown captures the great majority of these species[44]), yielding approximately 3,600 trap nights (12 grids × 100 traps × 3 nights per trap). Because of low capture and recapture rates at some sites, we assessed abundance of rodents per site using the minimum number of rodents known alive, based on the number of unique individual rodents captured per site (i.e., rather than via mark recapture estimates). We reported and analyzed patterns of abundance between land-use types for all

rodents (species pooled). Rodent diversity at each trapping site was calculated using both

Shannon and Simpson diversity indices as well as species richness (Supplemental Table 1). All

rodent trapping and sampling was conducted in accordance with institutional animal care, and

use permits (Smithsonian Institution IACUC permit # 2009-04) and the guidelines of the

American Society of Mammalogists for the use of wild mammals in research.[45]

Habitat descriptions.

To describe the habitat quantitatively at each of our sampling areas, we overlaid a vegetation

sampling grid on each of our 12 rodent trapping grids, with 20×20 m spacing between each

point. At each point we placed 5 sample pins, with 1 m spacing between each pin (thus, there

were 250 pin drops per site). At each pin drop we identified the number of plants that touched

the 50-cm pin and the height at which they touched.[46] We recorded if the plant was an

agricultural species (identifying crop type), a grass, or a forb. To describe tree and shrub cover,

we recorded presence or absence of woody vegetation above 50 cm in height at each pin drop

point.

Detection of *Y. pestis* antibodies.

Y. pestis exposure was measured using an enzyme-linked immunosorbent assay (ELISA).[47,48]

Blood samples collected from field-sampled rodents were immediately separated using

centrifugation. The serum was then screened for the presence of antibodies against the specific

antigen (fraction 1 or F1 antigen) of *Y. pestis* using methods previously described.[49] In brief,

microtitre plates were coated with *Y. pestis* fraction 1 capsular antigen (F1) before adding test

serum samples. In this test, serum samples containing antibodies against F1 antigen (positive

serum) (Centers for Disease Control and Prevention, Fort Collins, CO) blocks the F1 antigen

from reacting with the enzyme labeled rabbit anti-F1 antibody (second antibody), which is not

species specific, hence enabling detection of plague antibodies in wild and domestic animals and

humans. In addition to positive and negative rabbit serum, positive control serum from rodents,

dogs, and humans were also used. The ELISA titers in these samples ($N = 107$) ranged from 1:16

to 1:256.

Assessing plague activity.

Given sampling constraints (e.g., some individuals were too small to provide the blood

volume needed for ELISA analyses), only a subset of the rodents captured was tested for plague

(Supplemental Table 2). All species were surveyed at each land-use type where they occurred.

To best assess plague activity at each site, we adapted the host community model of LoGiudice

and others[50] to calculate the expected density of seropositive animals at a site. The advantage of

using this approach is that it can make inferences that incorporate variation in host

seroconversion, without sampling all individuals at all sites, and has been broadly applied in

studies of the ecology of zoonotic disease.[21,50,51] In congruence with this method, the predicted

number of seropositive rodents for each species (plague activity index [PAI]) was calculated by

multiplying each species' abundance by its species-specific *Y. pestis* seroprevalence in each

land-use type (i.e., conserved versus agricultural) and summing the values for all species at each

site.

Statistical analysis.

To accurately calculate species richness and diversity given uneven capture success across

sites, we used rarefaction curves, calculated using individual-based abundance estimates, with

the analyses conducted in EstimateS 9.0. We used the Chao1 indicator for species richness, and

both Simpson and Shannon metrics of diversity. For all response variables (rodent abundance, diversity, and richness; flea abundance; PAI), we used a paired *t* test to compare differences among land use types with site pairs as the unit of replication. We also used a linear regression approach to test for correlations among response variables, here with site rather than site pair as the unit of replication. Comparison of community composition of fleas across treatments was also done with site rather than site pair as unit of replication. Statistics were run (except as indicated above) in R.[52] Results reported are mean ± SD.

RESULTS

Habitat descriptions.

A priori descriptions of habitats as agriculture or conserved forest were clearly supported by vegetation data, which varied strongly between sites. In agricultural sites, 50.0 ± 15.1% of all vegetation sampled consisted of agricultural crop species (the remainder was largely understory grasses and forbs growing between crops and crop rows). Agricultural plants were absent (< 0.01% plant cover) from conserved plots. Woody cover, largely absent in agricultural plots (4.9 ± 4.1%), was high (80.0 ± 13%) in conserved plots. Similarly, understory stratification (defined as the mean number of contacts of vegetation per pin) was also higher in conserved sites (3.7 ± 1.1) than in agricultural sites (2.2 ± 0.8).

Rodent abundance and diversity.

A total of 240 rodents were captured, representing nine rodent species (Supplemental Table 1). The two *Mus* spp. < 12 g in mass (members of the cryptic *Mus minutoides-musculoides* taxonomic complex[41]) were grouped together in all analyses given the difficulties with field identification and subsequent difficulties in DNA quality in genetic sequencing.

Rodent abundance (number of unique individuals; species pooled) tended to be higher in agricultural sites (156 agricultural animals versus 84 conserved animals), but the difference was not significant ($t = 2.26$, df $= 2$, $P = 0.15$) because of large variation among sites (Figure 2A). Both Shannon and Simpson measures of rodent diversity were significantly higher in conserved sites (Shannon $t = 18.8$, df $= 2$, $P = 0.003$; Simpson $t = 11.0$, df $= 2$, $P = 0.008$) (Figure 2B) than in paired agricultural plots. Rodent species richness was always greater in conserved sites (Supplemental Table 1), but this difference was only marginally significant ($t = 2.77$, df $= 2$, $P = 0.10$) (Figure 2B)

The numerically dominant rodent species in this study was the Natal Mastomys, *Mastomys natalensis*, which accounted for 56.7% of individual captures in this study. Abundance of *Mastomys natalensis* was highly variable between sites, but was consistently and significantly higher in agricultural sites (44.0 ± 2.0 individuals/ha) compared with conserved sites (2.3 ± 3.2 individuals/ha; $t = 47.2$, df $= 2$, $P = 0.0004$). Overall, 97% of all *Mastomys natalensis* captures were from agricultural sites (Supplemental Table 1). Most species in the study showed similar strong treatment preferences. *Mus* spp. were also much more common in agricultural plots (89% of all captures were in agriculture). All captures of *Grammomys dolichurus*, *Graphiurus murinus*, *Lophuromys makundii*, and *Praomys taitae* were in conserved plots. Of species captured more than five times, only *Lemniscomys striatus* was found relatively equally in both habitats (55% captures in conserved, 45% in agricultural habitats).

A total of nine flea species from five genera of rodents surveyed were identified (host–flea associations shown in Supplemental Table 3). Prevalence of flea infestation was moderate and not significantly different across site types, with 17% individuals in agricultural sites and 14% individuals in conserved sites having fleas. Intensity of infestation also did not differ across site

types (Supplemental Table 3). Site-level flea abundance (total number of fleas on host per hectare) was consistently higher in agricultural (17.0 ± 5.7 individuals/ha) as compared with conserved (5.7 ± 9.8 individuals/ha) sites, with this pattern approaching but not achieving significance ($t = 3.6$, df $= 2$, $P = 0.07$). In contrast, flea diversity (estimated via Shannon index) was consistently higher in conserved (2.0 ± 0.2) versus agricultural sites (1.2 ± 0.2), although this difference did not meet significance ($t = 3.9$, df $= 2$, $P = 0.06$). Community composition was, however significantly different across treatments (ANOSIM $R = 0.48$, $P = 0.03$). Notably, the most common flea, *Dinopsyllus lypusus* (33% of fleas identified), was only found in agricultural habitats. *Xenopsylla humilis* was also found only in agricultural habitats, but this species was represented by only a single individual flea. There were also four flea species that were found only in conserved habitats (*C. evidens, Ctenopthalmus* sp.*, D. longifrons,* and *Leptopsylla aethiopica*) although *Leptopsylla aethiopica* was also represented by only a single individual flea.

Y. pestis seroprevalence.

A total of 106 rodents representing eight species (43 in conserved and 63 in agricultural sites) were screened for *Y. pestis* antibodies (Supplemental Table 1). Three species (*Grammomys dolichurus*, *Lophuromys makundii*, *Mastomys natalensis*) tested seropositive for *Y. pestis* exposure. *Mastomys natalensis*, the most abundant species sampled and an avid human commensal, accounted for the majority of all seropositive rodents (75%, 9/12) (Supplemental Table 1; Figure 3). Seropositive *Mastomys natalensis* were only found in agricultural sites, and this was the only species that was seropositive in agricultural sites.

On the basis of the presence/absence of seropositive rodents at the site level only, plague had recently been active at five out of six of our study sites, suggesting that plague is broadly

endemic across the land-use mosaic sampled in this study. The predicted abundance of seropositive rodents (PAI) in our two land-use treatments was significantly higher in agricultural sites relative to conserved sites ($t = 3.6$, df $= 2$, $P = 0.04$) with roughly twice as many seropositive animals predicted in agricultural sites (1.08 ± 0.03 infected animals expected per hectare, compared with 0.51 ± 0.15 in conserved sites).

Rodent abundance (total number of individuals; species pooled) was not significantly related to calculated abundance of plague seropositives ($F_{1,4} = 3.7$, $r^2 = 0.48$, $P = 0.13$) (Supplemental Figure 1), although the trend was positive. There was a strong negative relationship between species diversity (as measured using the Simpson diversity index) and PAI at our sites ($F_{1,4} = 8.7$, $r^2 = 0.69$, $P = 0.04$) (Figure 4A). A similar pattern was observed using the Shannon Diversity Index, although this relationship was not quite significant ($F_{1,4} = 4.7$, $r^2 = 0.54$, $P = 0.09$) (Figure 4B). There was no significant relationship between Chao1 estimates of species richness and PAI ($F_{1,4} = 1.3$, $r^2 = 0.24$, $P = 0.31$) (Figure 4C). PAI was also strongly correlated with the relative abundance of the numerically dominant rodent *M. natalensis* ($F_{1,4} = 7.2$, $r^2 = 0.64$, $P = 0.05$) and with abundance of fleas per hectare ($F_{1,4} = 11.4$, $r^2 = 0.74$, $P = 0.03$).

DISCUSSION

In this study, land use was found to have an important influence on rodent communities. Two important measures of diversity (Shannon and Simpson) indicated that rodent communities were more diverse in conserved sites that had not been converted to agriculture (although species richness did not actually change significantly). On the whole, rodent abundance (species pooled) was not different between agricultural and conserved sites. *Mastomys natalensis*, however, was nearly 20 times more abundant in agricultural sites than conserved sites. These shifts in diversity and species composition are presumably the result of observed differences in vegetation structure

and composition associated with land-use conversion. Such patterns are consistent with observations made in other contexts.[26,53] Rodent species other than *Mastomys natalensis* also exhibited strong affinities for particular land-use types. However, all five rodent species captured in agricultural sites were also detected in conserved sites.

Flea community composition was also significantly affected by land use. Agricultural land use may favor species highly competent for transmitting *Y. pestis*. The two most common fleas observed in agricultural habitats, *D. lypusus* and *X. brasiliensis*, are both thought to be important and efficient vectors for plague transmission.[54–56] *D. lypusus* was completely absent from conserved sites and *X. brasiliensis* was five times more common in agricultural as conserved sites. The only other flea species in this study known to be competent at transmitting plague, *Ctenopthalmus calceatus*[57] was found at similarly low levels in both site types ($N = 3$ in agricultural sties, $N = 4$ in pastoral sites). Both species were specialists on *Mastomys natalensis.* However vector competency of many of the other flea species has not been studied.[26] Further research on vector competence of each species, as well as their propensity to bite humans, is necessary to fully evaluate the importance of the observed changes in flea community composition on transmission risk.[58]

These land-use-induced shifts in rodent community diversity and abundance had a strong effect on plague activity in our study region. The predicted abundance of seropositive rodents (PAI) was roughly twice as high in agricultural sites (1.08 ± 0.03 infected rodents expected per hectare, compared with 0.51 ± 0.15 in conserved sites) relative to conserved sites. This increase appears to be largely driven by the high numbers of *Mastomys natalensis* found in agricultural habitats, with the abundance of this species being very strongly correlated to plague seroprevalence. *Mastomys natalensis* is one of the most frequently seropositive species for *Y.*

pestis in Africa and is a widespread commensal species[38,59] (and an important host of various other human pathogens, including Lassa virus[60,61]). Analogous changes in community composition, with a common commensal species serving as a major reservoir and "key host" for a pathogen, have been reported elsewhere, e.g., in West Nile virus (American robin, *Turdus migratorius*),[62] Lyme disease (white-footed mouse, *Peromyscus leucopus*)[50] and other systems.[63] Given that globally community composition change appears to be more pervasive than net biodiversity change this is an important pattern to note.[64,65]

Aggregate rodent abundance was not related to predicted abundance of seropositive rodents, similar to other studies in East Africa that failed to detect differences in rodent abundance between villages with or without a history of plague.[26,66] On the basis of the biodiversity patterns exhibited in conserved versus agricultural lands, the dilution effect hypothesis would predict that there should be a reduced disease risk in conserved land. Our data indicate that the relationship between diversity (at the level of rodents) and plague risk (as measured using PAI) in this context depends entirely on the measure of biodiversity used: a strong negative relationship was observed using Simpson diversity index, the negative relationship approached significance but did not meet the threshold using Shannon diversity index, and no pattern observed when using Chao estimates of species richness. As these three indices respectively give increasing weight to rare species, our results suggest that differences in abundances of common species are more important in predicting the PAI. All of these metrics are routinely used in examining diversity-disease relationships, but multiple metrics are rarely reported.[10,18,22,67–69]

An important area for future research that will help extend the reach of these observations will be learning more about interactions between rodent communities in agricultural and conserved sites. All of the rodent species found in agricultural sites were also detected in paired

conserved sites. *Mastomys natalensis*, the dominant agricultural species, certainly demonstrates the capacity to move long distances between habitat types such as our paired treatment sites.[70] This creates the possibility for coupling of the pathogen transmission dynamics in these adjacent habitats. *Mastomys natalensis* populations in African agricultural fields exhibit extreme boom and bust cycles with densities of up to 1,000 rodents/ha reported during population explosions.[71–73] Rodent populations in African evergreen forests also cycled, but population peaks are rarely as high as those observed in *Mastomys natalensis*.[74,75] The juxtaposition of agricultural sites and conserved sites could influence disease dynamics if the rodent populations interact and allow source-sink dynamics for both host and pathogen population.

We were unable to test all captured rodents for *Y. pestis* presence, so our conclusions need to be interpreted in light of this caveat. Nevertheless, our host community model should accommodate incomplete sampling, and the species-specific seroprevalence patterns observed in the rodents sampled in this study are compatible with other surveys of rodent seroprevalence in the same region.[38] Also, although seroprevalence studies are widely adopted for plague surveillance, we note that plague seroprevalence studies offer insights into rates of *Y. pestis* resistance rather than infection or infectiousness, that is, animals that die of *Y. pestis* infection are not available to sample. Can *Mastomys natalensis* act as infectious plague hosts, or do they simply exhibit resistance to infection and not play a part in infecting fleas? Furthermore, the number of seropositive rodents per hectare does not translate directly to risk or occurrence of human disease because of many other factors that can affect exposure and infection, and progression from infection to overt disease.[63,76,77] Data on plague eco-epidemiology in East Africa are largely reliant on observations of disease in human populations combined with seroprevalence studies of rodents and other mammals. We currently lack a comprehensive

understanding of *Y. pestis* reservoir competence, transmission dynamics, and plague epidemiology in East Africa.[24,78]

Our results suggest that agricultural conversion can cause increases in plague activity. However, they also highlight many ongoing questions about the effects of land-use change on disease ecology and on the implications for disease control efforts. Understanding how landscape conversion and the interrelationships between different land-use types affect the ecology and epidemiology of plague in East Africa better positions us to design control measures for this disease in regions where it remains a real human health risk. Lessons learned from this case are also broadly important for understanding the dynamics of other zoonotic diseases in this era of rapid landscape change.

Synthesis.

Transmission risk for vector-borne diseases at the landscape level depends on multiple factors including: 1) abundance of animal hosts and vectors, 2) pathogen dynamics among those animal hosts and vectors, and 3) probability of contact between susceptible human hosts and infectious vectors. Conversion of wildlands for agriculture almost certainly increases the rate of encounter between humans and rodent hosts and vectors through an increase in human activity in converted landscapes. Our results show that, in the study area, the conversion of conservation areas to agriculture potentially increases the risk of plague transmission to humans via multiple pathways: not only are people more present in agricultural areas but the abundance of seropositive rodents also nearly doubled. The vector community may also be changed to favor species competent at transmitting plague, although more specific research on vector competence of rarer species would be needed to confirm this finding. In the context of sub-Saharan Africa, these observations are worth particular consideration given the rapid conversion of landscapes to

agriculture in this region. We suggest that future interdisciplinary work, exploring both ecological interactions between conserved to agricultural habitats, and the dynamics of realized human exposure, will be critical to understanding how land-use change affects plague risk in these areas.

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FIGURE 1. A map of the three site pairs in northern Tanzania at different scales, with nationally conserved areas designated in green. Insets of satellite images (from Google Earth) show that stark differences in land use occur directly outside the boundaries of these protected areas. These borders separate conserved and agricultural treatments used in this study at each of the three field sites: Kambi ya Nyoka (**A**), Tloma (**B**), and Manyara (**C**).

FIGURE 2. Mean difference (±SD) between land-use treatments (agricultural vs. conserved) of (**A**) rodent abundance, (**B**) rodent community diversity, and (**C**) plague activity index (PAI); positive values indicate increases in agricultural treatments. For rodent community diversity, the following metrics were used: Chao 1 species richness (CH), Shannon diversity (SH), and Simpson diversity (SI). Asterisk (*) indicates significant differences between agricultural and conserved sites for each response variable. Rodent abundance (**A**) was consistently but not significantly elevated in agricultural sites as compared with adjacent conserved sites. Diversity trended significantly lower in agricultural sites for all metrics. PAI (predicted plague seropositive rodents) was roughly twice as high in agricultural vs. conserved sites.

FIGURE 3. There were strong differences in the abundance (**A**) and plague seroprevalence (**B**) of rodent species and across the two land-use treatment types surveyed: agricultural (yellow) and conserved (green). Combining these two metrics provides an estimate of the likely number of plague seroprevalent rodents per species and treatment (**C**). Here we plot mean and SD (when appropriate). The eight species monitored in this study were *Gerbilliscus robustus* (GERO), *Grammomys dolichurus* (GRDO), *Graphiurus murinus* (GRMU), *Lemnisomys striatus* (LEST),

Lophuromys makundii (LOMA), *Mastomys natalensis* (MANA), *Mus minutoides* and *musculoides* (MUS), and *Praomys taitae* (PRTA).

FIGURE 4. A negative slope was observed for the relationship of plague activity index (PAI) and Simpson diversity (**A**), Shannon diversity (**B**), and species richness (**C**), with generally higher diversity and lower PAI in conserved sites. This relationship, however, was only significant for Simpson diversity.

TABLE 1

Flea species by habitat type (as percent of all fleas sampled). Host–flea associations are provided in Supplemental Table 3

Species	n	Agriculture (%)	Conserved (%)
Ctenophthalmus calceatus cabirus (Jordan and Rothschild, 1913)	7	6	8
Ctenophthalmus evidens (Jordan, 1929)	7	0	13
*Ctenophthalmu*s sp.	5	0	10
D. longifrons (Jordan and Rothschild, 1913)	6	0	12
D. lypusus (Jordan and Rothschild, 1913)	17	33	0
Leptopsylla aethiopica (Rothschild, 1909)	1	0	2
Listropsylla basilewskyi (Smit, 1960)	2	2	2
X. brasiliensis (Baker, 1904)	6	10	2
X. humilis (Jordan, 1925)	1	2	0

SUPPLEMENTAL FIGURE 1. No significant relationship was observed between plague activity index (PAI, expected number infected individuals) and rodent host abundance.

SUPPLEMENTAL TABLE 1

Rodent abundance, diversity, and plague risk indices (PAI, expected number of seropositive rodents) for agricultural (agr) and conserved (cons) sites in research sites in northern Tanzania

	Kambi ya Nyoka		Manyara		Tloma	
	cons	*agr*	*cons*	*agr*	*cons*	*agr*
Gerbilliscus robustus	0	0	1	2	0	0
Grammomys dolichurus	3	0	0	0	12	0
Graphiurus murinus	2	0	0	0	1	0
Lophuromys makundii	31	0	0	0	4	0
Lemniscomys striatus	6	3	1	0	0	2
Mastomys natalensis	1	44	6	46	0	42
Mus spp.	2	11	0	0	0	6
Praomys taitae	19	0	0	0	11	0
No. of individuals	64	58	8	48	28	50
Rodent richness (Chao1)	7.0	3.0	3.9	2.0	4.0	3.0
Simpson	1.54	0.82	0.93	0.55	1.49	0.7
Shannon	1.35	0.68	0.74	0.17	1.12	0.53
No. of plague positive	2	1	1	4	0	4
No. of tested	23	17	6	26	16	20
Plague activity index	2.73	4.31	2.5	4.5	0.9	4.11

SUPPLEMENTAL TABLE 2

Seroprevalence for *Yersinia pestis* antibodies (the number positive *versus* the number tested) in rodent species caught in trapping grids in conserved and agricultural areas

Species	Agricultural	Conserved	Total
Gerbilliscus robustus	0/1	–	0/1
Grammomys dolichurus	–	1/10	1/10
Graphiurus murinus	0/2	–	0/2
Lemniscomys striatus	0/2	0/2	0/4
Lophuromys makundii	–	2/13	2/13

Mastomys natalensis	9/60	0/5	9/65
Mus spp.	0/1	–	0/1
Praomys taitae	–	0/11	0/11

SUPPLEMENTAL TABLE 3

Host–flea associations (percent of all identified fleas), and overall intensity of infestation per habitat type per host species

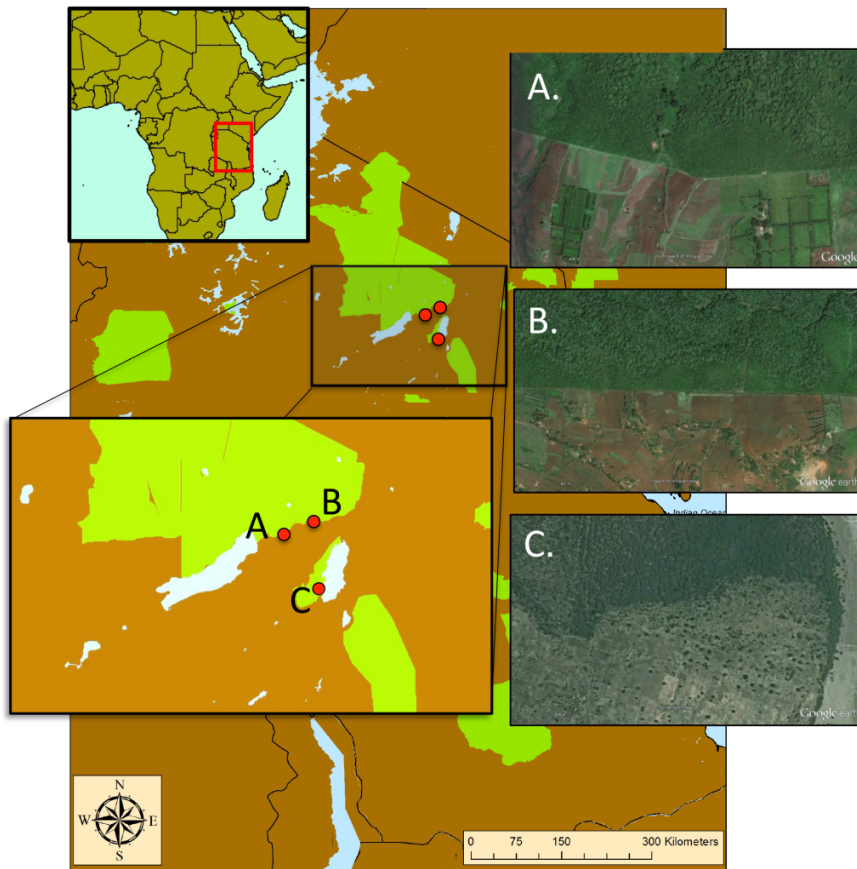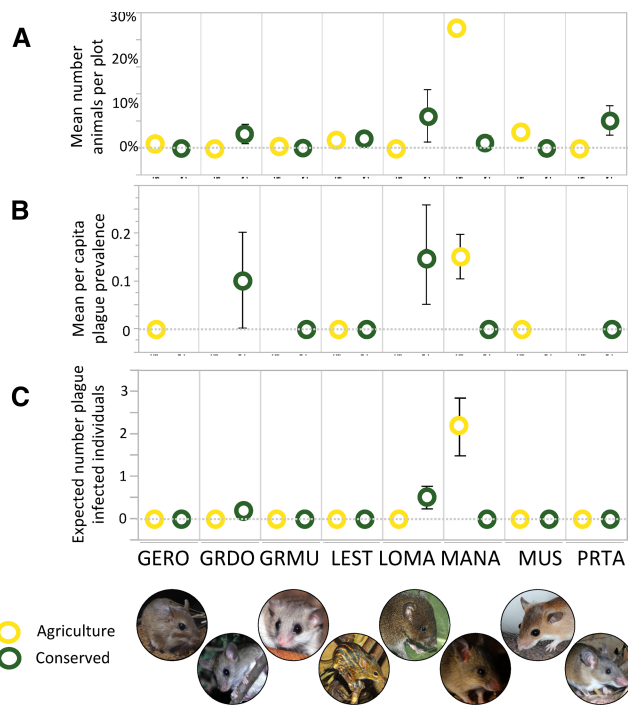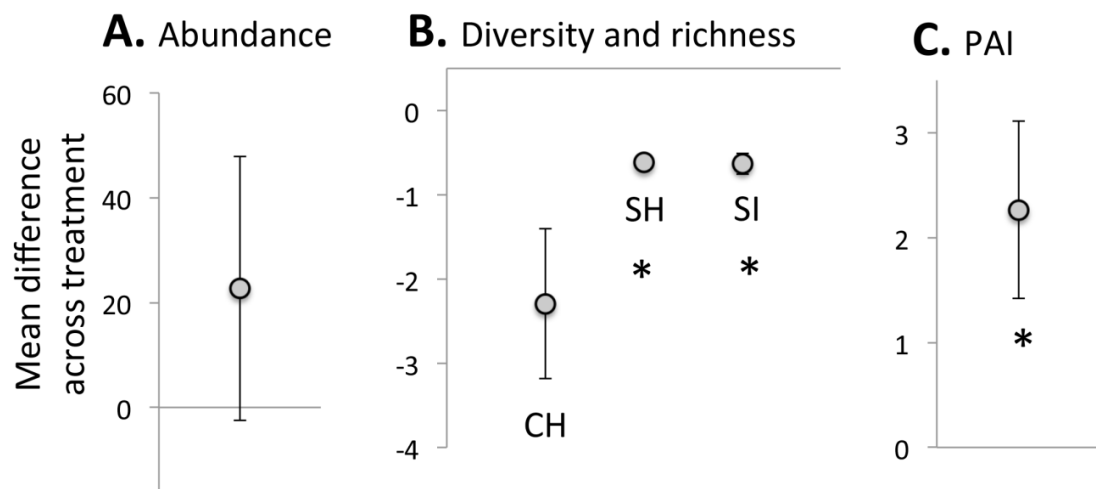| Species	*Lemniscomys striatus*	*Lophuromys makundii*	*Mastomys natalensis*	*Praomys taitae*
Ctenophthalmus calceatus cabirus	2%	4%	4%	2%
Ctenophthalmus evidens	–	12%	–	–
Ctenophthalmus sp.	–	8%	–	–
D. longifrons	2%	10%	–	–
D. lypusus	–	–	35%	–
Leptopsylla aethiopica	–	–	–	2%
Listropsylla basilewskyi	–	–	2%	2%
X. brasiliensis	–	–	12%	–
X.a humilis	–	–	2%	–
Intensity (agriculture)	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4	–
Intensity (conserved)	0.3 ± 0.5	–	0.1 ± 0.4	0.1 ± 0.3

Figure 1

Figure 2

A

Mean number animals per plot

30%

20%

10%

0%

B

Mean per capita plague prevalence

0.2

0.1

0

C

Expected number plague infected individuals

3

2

1

0

GERO   GRDO   GRMU   LEST   LOMA   MANA   MUS   PRTA

○ Agriculture
○ Conserved

Figure 3

A. Abundance

B. Diversity and richness

C. PAI

Figure 4

Figure S1