Bovine tuberculosis disturbs parasite functional trait composition in African buffalo

Brianna R. Beechler[a,1,2], Kate S. Boersma[b,1], Peter E. Buss[c], Courtney A. C. Coon[a,d], Erin E. Gorsich[e,f,g], Brian S. Henrichs[e], Adam M. Siepielski[h], Johannie M. Spaan[e], Robert S. Spaan[i], Vanessa O. Ezenwa[j], and Anna E. Jolles[a,e]

[a]Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331; [b]Department of Biology, University of San Diego, San Diego, CA 92110; [c]Veterinary Wildlife Services, South African National Parks, Kruger National Park, Skukuza 1350, South Africa; [d]Felidae Conservation Fund, Mill Valley, CA 94941; [e]Department of Integrative Biology, Oregon State University, Corvallis, OR 97331; [f]Zeeman Institute: Systems Biology and Infectious Disease Epidemiology Research, University of Warwick, Coventry CV4 7AL, United Kingdom; [g]School of Life Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom; [h]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701; [i]Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331; and [j]Odum School of Ecology & Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

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Novel parasites can have wide-ranging impacts, not only on host populations, but also on the resident parasite community. Historically, impacts of novel parasites have been assessed by examining pairwise interactions between parasite species. However, parasite communities are complex networks of interacting species. Here we used multivariate taxonomic and trait-based approaches to determine how parasite community composition changed when African buffalo (*Syncerus caffer*) acquired an emerging disease, bovine tuberculosis (BTB). Both taxonomic and functional parasite richness increased significantly in animals that acquired BTB than in those that did not. Thus, the presence of BTB seems to catalyze extraordinary shifts in community composition. There were no differences in overall parasite taxonomic composition between infected and uninfected individuals, however. The trait-based analysis revealed an increase in direct-transmitted, quickly replicating parasites following BTB infection. This study demonstrates that trait-based approaches provide insight into parasite community dynamics in the context of emerging infections.

emerging disease | invasion biology | disease community

Wild hosts are infected with multiple parasites simultaneously (1–3). These species interact directly and indirectly, and basic principles of community ecology apply to parasite assemblages (4). Numerous studies have attempted to characterize the mechanisms and consequences of coinfection (reviewed in refs. 2, 5, 6). However, it can be difficult to predict the direction and strength of the outcomes (7), because parasite interactions can be both competitive (e.g., ref. 8) and facilitative (e.g., refs. 9, 10), and the relative importance of these mechanisms varies. Investigators have begun to apply community ecological principles to the field of disease ecology to understand parasite interactions within a host (4, 11–15), although most studies still break existing networks of parasites into isolated pairwise comparisons (e.g., refs. 16–22) that may fail to capture the true dynamics of coinfection.

Emerging infectious diseases act as ecological disturbances that can alter the structure of entire parasite communities (23), yet the impacts of emerging infections on the structure of the native parasite community are rarely explored (except see ref. 17). Disturbance ecology approaches that consider shifts in multivariate community composition have highlighted community responses to disturbance in terrestrial (e.g., ref. 24), marine (e.g., ref. 25), and freshwater (e.g., refs. 26, 27) communities of free-living organisms and are increasingly used to explore the consequences of invasive species on native biodiversity (28, 29). Thus, disturbance ecology may prove useful for predicting the consequences of increasingly common emerging infections (11, 12) on native parasite communities.

Furthermore, disturbance ecology has the toolset needed to approach multiparasite systems from both taxonomic (species identity) and functional (trait) perspectives by examining how functional traits of entire communities change with disturbance (30, 31). When analyses are limited to the taxonomic level, it is difficult to extrapolate beyond the specific parasite species under study. Shifting the focus in disease ecology from taxon-based to trait-based approaches can help us understand the mechanisms behind observed patterns in parasite community composition and parasite transmission—a priority that has been emphasized in review papers (12, 23, 32)—and is necessary to understand how host communities (33) and vector communities (34) play a role in disease transmission.

Trait-based disturbance ecology thus has the potential to reveal the collective impacts of the arrival of a novel parasite across entire parasite communities. Specifically, multivariate ordination-based approaches that visualize the trait composition of communities (35–37) and track how these communities change with disturbance (30, 31) provide an intuitive, rigorous, and flexible approach that can advance our understanding of the consequences of novel parasite invasions. Applying such an approach to coinfection questions may increase our capacity to understand the community-wide impacts of invading parasites by identifying which native trait combinations change with the arrival of the invaders.

Significance

Similar to abiotic disturbances like fires, floods, and droughts, emerging infectious diseases (EIDs) act as key disturbances that can have cascading effects on native parasite communities within hosts. Here we investigate an EID of great concern for wildlife and human health: bovine tuberculosis (BTB) in African buffalo. Our application of a functional diversity framework to examine trends in parasite composition before and after acquisition of BTB revealed traits of parasites that BTB are most likely to affect. Yet BTB is only 1 example of an EID, and our framework can be applied to other EIDs, providing a way to evaluate their impacts and design mitigation strategies that acknowledge the complex parasite communities existing worldwide.

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[1]B.R.B. and K.S.B. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: brianna.beechler@oregonstate.edu.

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In this study, we applied the principles of trait-based disturbance ecology to understand how the arrival of a novel parasite affects the taxonomic and functional community structure of a native parasite community. We studied the effects of a well-characterized emerging, chronic parasitic disease, bovine tuberculosis (BTB) (17, 38–41), on a community of 16 parasites in wild African buffalo (*Syncerus caffer*). We focus on BTB because is known to have dramatic effects on immune function (17, 42, 43) and body condition (i.e., wasting) (19, 41, 42); both are attributes that might permit the parasite to serve a "keystone" role, allowing us to evaluate how 1 parasite can restructure the rest of the parasite community. We developed a trait database for a diverse parasite community composed of viruses, bacteria, protozoa, and helminths and applied taxonomic- and trait-based approaches to analyze how parasite richness and community composition changed in response to BTB infection. The unique longitudinal format of the data, which involved sampling the parasite community in the same hosts over multiple years, allowed us to implement a framework developed to understand the effects of disturbances on functional trait diversity in multispecies communities (30, 31).

We hypothesized that BTB infection would have contrasting effects on the parasite community. We predicted that BTB would increase the occurrence of parasites when the dominant mechanism of interaction was enhanced susceptibility due to wasting and immune modulation (17) and would decrease the occurrence of parasites when the dominant mechanism of interaction was coinfected mortality due to wasting (19, 20). Given the opposing direction of these hypotheses, predicting the overall effect of BTB on parasite community richness and structure is challenging. Thus, we used a case-control design to compare changes in parasite community in a group of buffalo that had acquired BTB and in a control group that was matched in terms of age, herd, and time period but did not acquire BTB.

Materials and Methods

Study System and Parasite Diagnostics. Approximately 200 African buffalo were captured in Kruger National Park, South Africa, as part of a longitudinal study on gastrointestinal helminths and BTB that targeted young females (19). Individuals were followed for 4 y (or until they left the study due to death or emigration from the study area) and were captured every 6 mo, resulting in 1,751 sample events. At each capture, blood and fecal samples were obtained for parasite diagnostics. Blood was collected by jugular venipuncture into lithium heparinized tubes and tubes without additive. Feces was collected from the rectum using a gloved hand. Both blood and feces were placed on ice and transported back to the laboratory for processing within 8 h of collection. Once in the laboratory, serum was obtained by centrifugation of the no-additive blood samples and then stored at −20 °C. Whole blood was frozen at −20 °C until DNA extraction for blood parasite detection (22). Feces was processed on the day of collection for gastrointestinal parasite detection (44).

BTB was diagnosed using a standard blood test (bovigam) that evaluates the amount of IFN-γ produced in whole blood after stimulation with tuberculosis antigens, optimized for use in African buffalo (45). We determined the date of conversion from BTB-negative to BTB-positive for all individuals in this study using a previously described protocol (19). We tested for the presence of 15 other parasites, including 5 viruses, 6 bacteria, 2 protozoa, 1 nematode, and 1 trematode, with diagnostics available for African buffalo. There are numerous parasites in the system that we are unable to detect, but these 16 are the most common parasites described in buffalo and for which detection is possible: bovine herpes virus 1 (BHV), parainfluenza virus 3 (PI), adenovirus 3 (Ad3), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), *Brucella abortus* (Br), *Mannheimia haemolytica* (MH), *Mycoplasma* bovis (MB), *Anaplasma centrale* (AC), *Anaplasma marginale* (AM), *Anaplasma omatajenne* (AO), *Theileria parva* (TP), coccidia, *Schistosoma matthei* (SM), and strongyle nematodes (strongyles). While the tick-borne parasites (e.g., TP, *Anaplasma* spp.) (22), Coccidia, nematodes, and flukes (21, 46, 47) were diagnosed by the presence of the parasite itself, the remaining parasites were considered present when a buffalo's antibody status went from negative to positive between 2 captures (48). Because buffalo are not known to clear Br (49) or BVDV (50, 51), once an animal seroconverted, it was considered positive for the remainder of the study. The viral parasites cause infections with a shorter duration of clinical signs and buffalo are able to recover, so multiple seroconversions were allowed per individual (PI3, Ad3, MH, BRSV, and BHV).

Animal Selection for Inclusion. We only included individuals that were captured at least twice before BTB conversion (phase 1) and twice after BTB conversion (phase 2), resulting in 29 individuals included as BTB converters. We then selected 29 control animals that did not acquire BTB during the study period, which were matched with BTB animals for age (within 1 y), reproductive status (pregnant vs. not pregnant in the same phase), and capture date (±2 mo). Control animals (buffalo that never acquired BTB) were assigned the same "conversion" date as their paired BTB-positive individual to divide captures into phase 1 and phase 2; this kept the total samples the same for BTB-positive and control animals in phases 1 and 2 and facilitated comparison. This allowed us to account for potential changes through time that were not associated with the acquisition of BTB. In association with another study, 8 animals in each group (BTB-positive and control) had an anthelmintic treatment (long-acting fenbendazole bolus) applied every 6 mo for the duration of the study to reduce strongyle burdens (19).

Importantly, we conducted a supplemental analysis and demonstrated that the bolus did not affect parasite taxonomic or functional composition in our analysis. To verify that the bolus (anthelmintic) did not change the parasite assemblage in our study, we compared 48 bolused animals, measured before bolusing in June–July 2008, with the same 48 bolused animals measured 1 y later June–August 2009. We found no differences in functional diversity (Wilcoxon matched-pairs signed-rank, median difference, 0.002; $P = 0.253$), functional richness (Wilcoxon matched-pairs signed-rank, median difference, 0.008; $P = 0.730$), or taxonomic diversity (Wilcoxon matched-pairs signed-rank, median difference, 0.05; $P = 0.255$). This is likely because the bolus reduces strongyle burden but does not clear it entirely. Consequently, we included the presence of strongyles as a parasite in our analyses.

Creation of the Parasite Matrix. The parasite matrix contained data on the parasites present in each buffalo at each capture. All individuals were assigned a score of 1 if they were positive for BTB and 0 if negative at each capture. We then calculated the proportion of time that each parasite species was present in the buffalo before BTB (phase 1) and after BTB (phase 2). If the parasite was tested for directly (e.g., AM; *SI Appendix*), we determined the proportion of captures during which the parasite was present in each phase. For instance, if animal 1 was captured at 8 time periods, periods 1–4 in phase 1 and periods 5–8 in phase 2, and it had AM at time points 1, 6, and 7, then the proportion of capture intervals in which it had AM was 1/4 for phase 1 and 2/4 for phase 2. If the parasite was detected with antibody seroconversion (e.g., PI3), then we calculated the incidence of each parasite between successive captures, defined as a change in antibody titer from negative to positive in successive captures (e.g., BRSV, BVDV) or an increase in antibody titer greater than a certain percentage, as described by the manufacturer of the ELISA device and by Glidden et al. (48) (e.g., MH, MB, PI3, Ad3, BHV). This incidence was then used to calculate the proportion of capture intervals during which an incident event occurred. Details on incidence calculation are available elsewhere (48).

Creation of the Trait Matrix. We created a categorical trait matrix based on 9 traits of parasites that may influence transmission (e.g., refs. 52, 53) (Table 1 and *SI Appendix*, Table S1). Collectively, these traits represent basic aspects of parasite biology necessary to characterize the parasite community. We selected a broad suite of traits to understand which parasite traits are likely to be affected by the invasion of BTB, while also focusing on traits that may help us disentangle the possible effects of BTB due to wasting/comortality and increased susceptibility due to BTB infection.

Statistical Analysis. To evaluate whether our trait set appropriately captured representative aspects of parasite biology, we first examined how parasites varied in their trait composition with a nonmetric multidimensional scaling (NMDS) ordination of parasites in trait space (*SI Appendix*, Fig. S1). We calculated Gower dissimilarity from the categorical trait matrix and applied a Wisconsin transformation to standardize before ordination. The ordination converged on a stable 2D solution (*SI Appendix*, Fig. S1). Relationships among parasites matched expectations based on the literature; for instance, the intestinal parasites and tick-borne parasites each clustered separately in multivariate space. The congruence between expectations and trait space validated our trait selection and assignment.

To examine the effects of BTB on parasite taxonomic and functional richness, we calculated 2 univariate diversity metrics, functional richness (FRic) (37) and taxonomic richness, for each buffalo in phases 1 and 2. For categorical traits, FRic measures the number of independent trait combinations and is directly comparable to species richness. We used repeated-measures ANOVA with Bonferroni correction to compare richness among all groups (phase 1 BTB vs. phase 1 control, phase 2 BTB vs. phase 2 control, phase 1 vs. phase 2 control, and phase 1 vs. phase 2 BTB). To assess which species of parasite changed with BTB infection (i.e., were representative of each host group), we used an

Table 1. Parasite traits

Trait	Categories and definitions
Size of parasite	Macro: large enough to be visible with the naked eye; micro: not large enough to be visible with the naked eye
Cellularity	Acellular (e.g., viruses); single (e.g., most bacteria and protozoa); multi-trematode, nematodes
Primary transmission mode	Contact: transmitted primarily directly from 1 individual to another; environmental: transmitted primarily via contaminated fomites or ground; vector: transmitted by vectors (e.g., ticks, mosquitoes)
Life cycle	Simple: can complete a life cycle within 1 host; complex: parasite requires an intermediate host, vector, or environmental stage to complete the life cycle
Length of infection	Chronic: parasite with a "carrier or latent stage" in buffalo or that animals do not clear with an immune response; acute: parasite that animals typically clear with an immune response
Primary body compartment	Lung, gastrointestinal tract, white blood cells, red blood cells; multisite: site in the host of primary replication and/or the majority of the parasite life cycle
Site of replication	Intracellular or extracellular: whether the parasite replicates inside or outside host cells
Duplication time	The time it takes the parasite to duplicate its population; long: >1 d; medium: 5–24 h; fast: <4 h
Fitness effects	Yes or no: Does the parasite reduce survival or fecundity in buffalo?

Traits likely to play a major role in changing susceptibility are size of parasite, cellularity, primary transmission mode, life cycle, duplication time, and length of infection. Traits likely to play a major role in wasting or mortality are host compartment, site of replication, and fitness effects. Details of each parasite are provided in *SI Appendix*, Table S1.

indicator species analysis (ISA; multipatt in the R package indicspecies) and examined statistical significance using a Monte Carlo randomization with 999 iterations (54). ISA combines information on the relative abundances and relative frequencies of species to determine an indicator value that represents the fidelity and exclusivity of each parasite species to each of the 4 host groups: phase 1 control, phase 1 BTB, phase 2 control, and phase 2 BTB.

To examine the effects of BTB on parasite taxonomic and functional composition, we visualized changes in the taxonomic and trait composition of each buffalo between phase 1 and phase 2 using NMDS. We plotted each individual's taxonomic/functional parasite composition in phase 1 and phase 2(as in ref. 31). Examining shifts in the location of ordination space allowed us to understand how taxonomic and trait composition of individual buffalo changed when they acquired BTB. We then compared these changes with similar shifts in control buffalo during the same time period (phase 1 to phase 2).

For taxonomic ordinations, we used Bray–Curtis distances and applied Wisconsin transformations before ordination. We assessed ordination fit with overall stress; both taxonomic ordinations converged on stable 3D solutions. To aid interpretation of the ordinations, we examined parasite correlations with the first 2 axes ($r > 0.5$). We used permutation-based analysis of variance (PerMANOVA) (55) to examine changes in the location of buffalo in parasite taxonomic ordination space between phase 1 and phase 2. We also compared the multivariate dispersion of parasite associated with phase 1 and phase 2 buffalo using homogeneity of group dispersions and permutation tests (56). Dispersion, the average distance of each point from the multivariate group centroid, is a way to quantify the amount of multivariate space occupied by a given community.

For functional trait ordinations, we first converted the categorical trait matrix to a binary traits matrix (57) and then multiplied the control and BTB-positive parasite matrices (individual*parasite) by the binary traits matrix (parasite*trait) to create individual*trait matrices (57, 58), which we then ordinated using NMDS. Before ordination, we calculated Gower distances and applied log and Wisconsin transformations. Functional ordinations converged on stable 2D solutions. We rotated each ordination to align with a vector of strongyle abundance to facilitate comparisons between ordinations (57) and because strongyles, a native parasite, are known to affect the survival of animals with BTB (19). We examined trait correlations with the axes ($r > 0.5$). As with taxonomic composition, we tested for shifts in the location of phase 1 and phase 2 animals in trait space with PerMANOVA and the homogeneity of group dispersions of functional traits using permutation tests.

We also calculated multivariate dispersion (betadisper in R package vegan) to examine differences in the dispersion of buffalo in taxonomic and functional space (56, 59). We conducted all analyses in R version 0.98.1062 using packages FD (24), vegan (60), and indicspecies (61).

Results

How Did Taxonomic and Functional Richness of Parasite Assemblages Change over Time in Animals That Acquired BTB and Those That Did Not? Animals that acquired BTB experienced a greater increase in parasite assemblage richness compared with control animals. Taxonomic richness in BTB-infected animals increased by 3.3 species on average between phase 1 and phase 2, compared with an increase of 1.1 species in control animals (Fig. 1 and Table 2). Parasite functional richness (i.e., number of unique trait combinations) was over 3 times greater in BTB-infected animals than in control animals (Fig. 1 and Table 2). Although we created our control group by matching buffalo by age, herd, and observation period, we detected small differences in initial taxonomic and functional richness of the parasite assemblages between our BTB and control groups. The animals that acquired BTB had slightly lower parasite richness before BTB conversion compared with controls (Table 2 and Fig. 1).

We also found that indicator species differed by both BTB status and phase. Schistosomes were a significant indicator of both control and BTB buffalo in phase 2 ($P = 0.006$), suggesting that buffalo acquired schistosomes regardless of BTB status, likely due to schistosome acquisition as buffalo age (47). BHV and BRSV were indicators of control buffalo in both phases and of BTB buffalo in phase 2 (BHV, $P = 0.012$; BRSV, $P = 0.048$). However, these viral parasites were not indicators for BTB buffalo in phase 1, suggesting that they may be associated with BTB acquisition in this group.

How Did Taxonomic and Functional Composition Change over Time in Animals That Acquired BTB and Those That Did Not? BTB-infected animals occupied different locations in taxonomic space after infection with BTB than before infection (PerMANOVA; df = 1, F = 7.75, $P = 0.001$), and a similar change also occurred for control animals during the same time period (PerMANOVA;

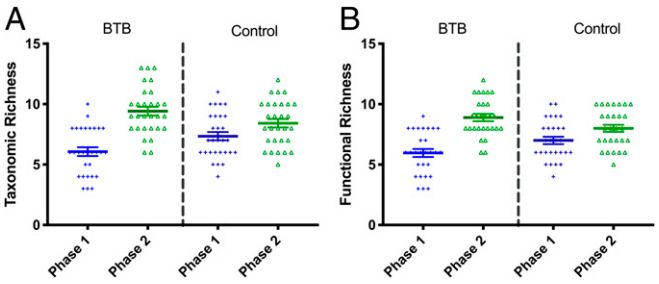

Fig. 1. Phase 2 animals had higher parasite richness than phase 1 animals, both taxonomically (*A*) and functionally (*B*). However, BTB animals experienced a larger magnitude of increase in richness over time compared with control animals. Animals that acquired BTB had lower richness than control animals in phase 1 and higher richness than control animals in Phase 2. Statistics for between-group comparisons are provided in Table 2. Lines represent means, bars are 2 SE units, and each point is an individual buffalo.

Table 2. Parasite richness in BTB-infected and control animals in phase 1 and phase 2

	Taxonomic richness		Functional richness	
Comparison	Mean difference	*P* value	Mean difference	*P* value
Phase 1 vs. phase 2 (BTB)	3.345	<0.001**	2.929	<0.001**
Phase 1 vs. phase 2 (control)	1.069	0.051	1	0.067
Phase 1 BTB vs. phase 1 control	1.276	0.031*	1.036	0.067
Phase 2 BTB vs. phase 2 control	1	0.099	0.8929	0.067

Repeated-measures ANOVA (taxonomic richness: F = 28.65, $P < 0.001$; functional richness: F = 34.07, $P < 0.001$) with Bonferroni post hoc comparisons.
*$P < 0.05$.
**$P < 0.01$.

df = 1, F = 3.83, $P = 0.001$) (Fig. 2*B* and Table 2). These shifts represent changes in taxonomic composition associated with the loss of strongyle nematodes and AM and the gain of Br and schistosomes (Fig. 2*A* and *SI Appendix*, Table S2) for animals with BTB, and the loss of BHV and PI3 and a gain of Br and nematodes for control animals (Fig. 2*B* and *SI Appendix*, Table S2). Despite these changes in parasite assemblage composition, the dispersion of parasite species did not differ between phase 1 and phase 2 for either BTB-positive or control animals (control: df = 1, F = 1.35, $P = 0.28$; BTB-positive: df = 1, F = 1.05, $P = 0.31$), meaning that there was no contraction or expansion of multivariate taxonomic space through time.

Both control and BTB-infected animals occupied different regions of trait ordination space between phase 1 and phase 2 (PerMANOVA; BTB: df = 1, F = 5.69, $P = 0.001$; control: df = 1, F = 5.57, $P = 0.001$), as in the taxonomic analysis, reflecting changes in functional trait composition for all animals regardless of BTB status (Fig. 2 *C* and *D*). However, in contrast to the taxonomic analysis, the dispersion of functional traits contracted through time in both control and BTB-infected buffalo (control: df = 1, F = 4.29, $P = 0.047$; BTB: df = 1, F = 9.80, $P = 0.003$). Interestingly, the magnitude of this contraction was almost double in BTB animals compared with control animals (difference in distance to centroid between phase 1 and phase 2: control, 0.027; BTB, 0.047). The contraction in trait space for the BTB-positive group was primarily associated with an increase in contact-transmitted parasites with simple life cycles and fast replication times; the control group contraction was not associated with any trait groups (*SI Appendix*, Table S3; $r > 0.7$). Notably, no functional groups were lost entirely with the acquisition of BTB.

Discussion

BTB infection changed the taxonomic and trait composition of parasites in African buffalo. Individual buffalo harbored different parasites after BTB infection than they did before infection, as evidenced by an increase in taxonomic richness and shifts in taxonomic composition. Furthermore, our analysis of functional traits highlights that BTB fosters an increase in parasites with specific trait patterns (i.e., fast replication, contact-transmitted) after BTB infection. Understanding changes in this context may allow us to predict how an invasive disease like BTB may alter native parasite communities and better create disease control programs that consider the context of the parasites into which the emerging disease enters.

When we evaluated how the trait assemblage changed with BTB infection, we found that functional richness increased, indicating that parasites with trait combinations different from those already present in the parasite community were able to establish themselves following BTB infection. However, ordination and multivariate dispersion both showed that parasites occupied a smaller region of trait space and had lower dispersion after the acquisition of BTB than before. This pattern suggests that while buffalo carried different parasite species after BTB infection, the traits that these species possessed were functionally similar to existing ones, which caused them to cluster in trait space. This pattern is consistent with the idea that BTB alters host susceptibility to parasites with particular suites of traits. Furthermore, our functional composition analysis suggests that BTB shifted the parasite trait community toward contact-transmitted, simple life cycle, and fast-replicating parasites, revealing a specific profile of pathogens that may be facilitated by BTB.

Importantly, the changes to the parasite community in BTB-positive animals differed from those seen in control animals. There were marginally significant increases in taxonomic and functional richness through time in control animals, but the magnitudes of these increases were 2-fold lower compared with those in BTB-positive animals. In addition, there were no

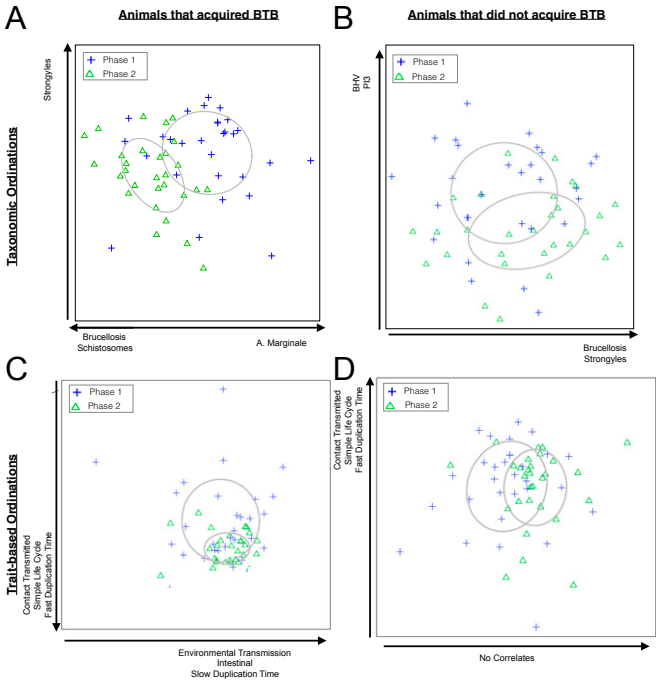

Fig. 2. Nonmetric multidimensional scaling ordination of individual buffalo in parasite taxonomic space (*A* and *B*) and parasite trait space (*C* and *D*). (*A*) Parasite taxonomic space for animals that acquired BTB ($k = 3$; stress = 0.15). (*B*) Animals that did not acquire BTB ($k = 3$; stress = 0.18). (*C*) Parasite trait space for animals that acquired BTB ($k = 2$; stress = 0.15). (*D*) Animals that did not acquire BTB ($k = 2$; stress = 0.18). The 95% confidence ellipses (gray) represent the SD of the coordinates of phase 1 and phase 2 buffalo. Parasites correlated with the axes are listed alongside the ordinations in *A* and *B* (Spearman correlation >0.5; *SI Appendix*), while traits that correlate with the axes are listed alongside the ordinations in *C* and *D* (Spearman correlation >0.7; *SI Appendix*, Tables S2 and S3 show all associations >0.5).

differences in the dispersion of phase 1 and phase 2 control buffalo in taxonomic space, suggesting that the parasite community neither converged nor diverged over time. Control buffalo also shifted locations in the functional space between phase 1 and phase 2, reflecting significant changes in parasite community composition. Although we observed a contraction of functional space over time in all buffalo, in control animals this contraction was only marginally significant and less than half the effect size seen in BTB-positive animals. The pattern in control animals suggests that there are age- and/or time-related shifts in the parasite community, but the magnitude of this shift differs when BTB is present. Thus, the presence of certain parasites, like BTB, seems to catalyze extraordinary shifts in community composition.

BTB has previously been described to alter the incidence and progression of individual microparasites in buffalo, such as Rift Valley fever (19) and Br (20). However, our results suggest that BTB may act as an ecological facilitator on a much larger scale than previously suggested, affecting a range of contact-transmitted, fast-replicating, and simple life cycle parasites—traits typical of many viruses and bacteria. In addition, our indicator species analysis suggests that 2 viral parasites, BHV and BRSV, were indicative of phase 2 BTB buffalo but not of phase 1 BTB buffalo, suggesting that BTB may increase the likelihood of acquiring these parasites. This could be due to increased susceptibility or altered disease progression, since both are diseases with a latent phase (BHV) or chronic carriers (BRSV), suggesting that treatment and control efforts for these parasites may be warranted when BTB is present in a host community. However, the taxonomic ordination space was composed of many parasites whose frequency of occurrence changed between phase 1 and phase 2, and, consequently, it is difficult to understand what other parasites may be affected that are less well described and well known. Our trait analysis was particularly valuable because it allowed us to identify traits of parasites that may respond to the invasion of BTB.

Our finding that BTB alters the community of parasites has widespread implications for managing health outcomes of BTB in wild animal populations, many of which are threatened or endangered, such as Iberian lynx (*Lynx pardinus)* (62), suggesting that we should consider not only the direct effects of TB in mitigation strategies, but also indirect effects via changing parasite communities. Beyond conservation, there are implications for public health and management, as tuberculosis is a reemerging disease worldwide (63–66). For instance, the prevention of coinfections may slow the progression of BTB infection, as has been discussed with helminths and BTB, where treatment of gastrointestinal parasites is known to increase survival time in animals infected with BTB (12, 19), and in *Brucella*, where the presence of Br slowed the invasion of BTB (20). A valuable next step would be to evaluate whether the treatment of contact-transmitted, fast-replicating parasites can slow the progression of BTB infection, as has been seen in humans (*Homo sapiens*) (67) and wild boar (*Sus scrofa*) (68).

Interestingly, after BTB infection, there was a small but significant decrease in 2 parasite taxa: AM and strongyles. Buffalo in this study that were infected with both BTB and strongyles were much more likely to die (19) than those without strongyles, suggesting that the decrease in strongyles may be due to coinfected mortality. However, previous work by Gorsich et al. (20) also demonstrated a coinfected mortality pattern between brucellosis and BTB that we did not detect with this analysis. This is likely because it was a very small effect that is difficult to identify unless full longitudinal data are used—demonstrating the utility of multiple types of analyses when evaluating the effect of an invading parasite on native parasite communities.

We found some evidence that animals that acquired BTB began the study with different parasite assemblages than those individuals that never acquired BTB. This may be due to the nonrandom sample of animals that we selected for inclusion in the study. Buffalo had to survive at least 2 captures with BTB to be included in the study, and thus we may be assessing only the "healthiest" animals with BTB, rather than those that died quickly. Alternatively, there may be a role for differences in susceptibility between BTB and control animals. Previous work has suggested that susceptibility to BTB in buffalo may have a genetic basis, and while the mechanism for susceptibility is unknown, it is possible that the genetic background of the individuals that acquire BTB may affect other diseases as well (42). Finally, it is possible that there are parasite assemblages that protect against the invasion of BTB within an individual; however, our indicator species analysis revealed that none of the parasites that we examined were strongly associated with the BTB phase 1 group. This suggests that a "protective" parasite community was not evident in the buffalo in our study.

Our application of a functional diversity framework to examine trends in parasite composition before and after acquisition of an emerging infectious disease allowed us to detect patterns that were not apparent in previous studies and revealing the traits of parasites that may be most likely to be affected by the invasive disease BTB. However, BTB is only 1 example of an emerging disease that may affect native parasite communities. As emerging diseases become more common (69) due to human activity (70, 71) and environmental changes (72–74), we must find new ways to evaluate their impacts and design mitigation strategies that acknowledge the complex parasite community that exists worldwide. We have demonstrated that incorporating principles from community and functional ecology may allow researchers to understand the community dynamics of pathogens and the consequences for host health in many contexts across systems and scales.

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