Bovine tuberculosis disturbs parasite functional trait composition in African buffalo

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Significance Statement:

Similar to abiotic disturbances like fires, floods, and droughts, emerging infectious diseases (EID's) 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 is most likely to affect. Yet, BTB is only one example of an EID and our novel framework can be applied to other EID's providing us with a novel method to evaluate their impacts and design mitigation strategies that acknowledge the complex parasite communities that exist worldwide.

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Abstract

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, however, no differences in overall parasite taxonomic composition between infected and uninfected individuals. The trait-based analysis revealed that direct-transmitted, quickly replicating parasites increased following BTB infection. This study demonstrates that trait-based approaches provide novel insight for understanding parasite community dynamics in the context of emerging infections.

Introduction

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 co-infection (reviewed in (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. (8)) and facilitative (e.g. (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 (11–16) although most studies still break existing networks of parasites into isolated pairwise comparisons (e.g. (17–23) that may fail to capture the true dynamics of co-infection.

Emerging infectious diseases act as ecological disturbances that can alter the structure of entire parasite communities (24), yet the impacts of emerging infections on the structure of the native parasite community are rarely explored (except see (18)). Disturbance ecology approaches that consider shifts in multivariate community composition have highlighted community responses to disturbance in terrestrial (e.g., (25)), marine (e.g., (26)), and freshwater (e.g., (27, 28)) communities of free-living organisms, and are increasingly used to understand the consequences of invasive species on native biodiversity (29, 30). Disturbance ecology may thus prove useful to predict the consequences of increasingly common emerging infections (11, 12) on native parasite communities.

Furthermore, disturbance ecology has the toolset to approach multi-parasite systems from not only taxonomic (species identity), but also functional (trait) perspectives by examining how functional traits of entire communities change with disturbance (31, 32). 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, 24, 33), and is necessary to understand how host communities (34) and vector communities (35) 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 (36–38) and track how these communities change with disturbance (31, 32) provide an intuitive, rigorous, and flexible approach that can advance our understanding of the consequences of novel parasite invasions. Applying such an approach to co-infection 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.

In this study, we apply the principles of trait-based disturbance ecology to understand how the arrival of a novel parasite affects 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) (18, 39–42), 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 (18, 43, 44) and body condition (i.e. wasting) (20, 42, 43); both are attributes that might permit the parasite to serve a "keystone" role, allowing us to evaluate how one parasite can restructure the rest of the parasite community. We developed a trait database for a diverse parasite community comprised 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 (31, 32).

We hypothesized that BTB infection would have contrasting effects on the parasite community. We predicted BTB to increase the occurrence of parasites when the dominant mechanism of interaction was enhanced susceptibility due to wasting and immune modulation (18) and decrease occurrence of parasites when the dominant mechanism of interaction was co-infected mortality due to wasting (20, 21). Because of 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 acquired BTB, to changes 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 & Parasite Diagnostics: Approximately 200 African buffalo were captured in Kruger National Park (KNP), South Africa, as part of a longitudinal study on gastrointestinal helminths and bovine tuberculosis which targeted young females (20). Individuals were followed for 4 years (or until they left the study due to death or emigration from the study area), and captured every 6 months, resulting in 1751 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 no-additive tubes. Feces was collected from the rectum using a gloved hand. Both blood and feces were placed on ice and transported back to the lab for

85 processing within 8 hours of collection. Once in the laboratory, serum was obtained by
86 centrifugation of the no-additive blood samples, and serum was then stored at -20°C. Whole
87 blood was frozen at -20°C until DNA was extracted for blood parasite detection (23). Feces was
88 processed on the same day of collection for gastrointestinal parasite detection (45).
89 Bovine tuberculosis (BTB) was diagnosed using a standard blood test (bovigam) that
90 evaluates the amount of interferon gamma produced in whole blood after stimulation with
91 tuberculosis antigens; this assay has been optimized for use in African buffalo (46). We
92 determined the date of conversion from BTB-negative to BTB-positive for all individuals in this
93 study using the protocol described in (20). We tested for the presence of 15 other parasites
94 including 5 viruses, 6 bacteria, 2 protozoa, 1 nematode and 1 trematode with diagnostics
95 available for African buffalo. There are numerous parasites in the system we are unable to detect,
96 but these 16 represent the most common parasites that have been described in buffalo and for
97 which detection is possible. The parasites are bovine herpes virus 1 (BHV), parainfluenza virus 3
98 (PI), adenvovirus 3 (Ad3), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial
99 virus (BRSV), *Brucella abortus* (Br), *Mannheimia haemolytica* (MH), Mycoplasma bovis (MB),
100 *Anaplasma centrale* (AC), *Anaplasma marginale* (AM), *Anaplasma omatajenne* (AO), *Theileria*
101 *parva* (TP), coccidia, *Schistosoma matthei* (SM) and strongyle nematodes (strongyles). While
102 the tick borne parasites (*Theileria parva*, *Anaplasma* spp.) (23), Coccidia, nematodes and flukes
103 (22, 47, 48) were diagnosed by presence of the parasite itself, the remainder of the parasites were
104 considered present when a buffalo's antibody status went from negative to positive between two
105 captures (49). Because buffalo are not known to clear *Brucella abortus* (50) or BVDV (51, 52),
106 once an animal seroconverted it was considered positive for the rest of the study. The viral

parasites are infections with shorter duration of clinical signs and buffalo are able to recover so multiple seroconversions were allowed per individual (PI3, Ad3, MH, BRSV, BHV).

Animal Selection for Inclusion:

We only included individuals that were captured at least two times prior to BTB conversion (Phase 1) and two times post BTB conversion (Phase 2), resulting in 29 individuals that were included as BTB converters. We then selected 29 'control' animals that did not acquire BTB during the study period that were matched with BTB animals for age (within 1 year), reproductive status (pregnant vs. not in the same phase) and capture date (+/- 2 months). Control animals (buffalo that never acquired BTB) were assigned the same "conversion" date as their paired BTB+ individual to divide captures into Phase 1 and Phase 2 – this kept the total samples the same for BTB+ and control animals in Phase 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, eight animals in each group (BTB+ and control) had an anthelmintic treatment (long-acting fenbendazole bolus) applied every six months for the duration of the study to reduce strongyle burdens (20).

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 prior to bolusing in June–July 2008 to the same 48 bolused animals measured 1 year later between June and 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 1 if they were positive for BTB and a 0 if negative at each capture. We then calculated the proportion of time each parasite species was present in the buffalo before and after BTB (Phase 1 and 2 respectively). If the parasite was tested for directly (such as *A. marginale* (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 *A. marginale* (AM) at time points 1,6 and 7, then the proportion of capture intervals it had AM for Phase 1 was 1/4 and Phase 2 was 2/4. If the parasite was detected with antibody seroconversion (such as PI3), then we calculated incidence of each parasite between successive captures, which was defined as a change in antibody titer from negative to positive in successive captures (BRSV, BVDV) or an increase in antibody titer greater than a certain percentage (as described by the manufacturer of the ELISA and in Glidden et al (49) (MH, MB, PI3, Ad3, BHV)). Incidence was then used to calculate the proportion of capture intervals during which an incident event occurred. More details on incidence calculation are described in Glidden et al (49).

Creation of the Trait Matrix

We created a categorical trait matrix based on nine traits of parasites that may influence transmission (e.g., (53, 54)) (Table 1, SI Appendix Table S1). Collectively, these traits represented basic aspects of parasite biology that are necessary to characterize the parasite community. We selected a broad suite of traits to understand which of the parasite traits likely to

be affected by the invasion of bovine tuberculosis; while also focusing on traits that may help us disentangle the effects that BTB may have due to wasting/co-mortality 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, Figure 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 two-dimensional solution (SI Appendix, Figure S1). Relationships among parasites matched expectations from 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 two univariate diversity metrics, functional richness (FRic; (38)) 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 with species richness. We used repeated measures ANOVAs with Bonferroni correction to compare richness between all groups (Phase 1 BTB vs Phase 1 control; Phase 2 BTB vs Phase 2 control; Phase 1 vs Phase 2 control; 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 indicator species analysis (ISA, multipatt in R package indicspecies) and examined statistical significance using a Monte Carlo randomization with 999 iterations (55). 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 four 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 Phases 1 and 2 using NMDS. We plotted each individual's taxonomic/functional parasite composition in Phase 1 and Phase 2 (as in (32)). 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 three-dimensional solutions. To aid in interpreting the ordinations, we examined parasite correlations with the first two axes ($r > 0.5$). We used permutation-based analysis of variance (PerMANOVA; (56)) to examine changes in the location of buffalo in parasite taxonomic ordination space between Phases 1 and 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(57). Dispersion is the average distance of each point from the multivariate group centroid and is a way to quantify the amount of multivariate space that is occupied by a given community.

For functional trait ordinations, we first converted the categorical trait matrix to a binary traits matrix (58) and then multiplied the control and BTB+ parasite matrices (individual*parasite) by the binary traits matrix (parasite*trait) to create individual*trait matrices

(58, 59), which we then ordinated using NMDS. Prior to ordination, we calculated Gower distances and applied log and Wisconsin transformations. Functional ordinations converged on stable two-dimensional solutions. We rotated each ordination to align with a vector of strongyle abundance to facilitate comparisons between ordinations (58), and because strongyles, a native parasite, are known to affect the survival of animals with BTB(20). 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 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 (57, 60). We conducted all analyses in R version 0.98.1062 using packages FD (25), vegan (61), and indicspecies (62).

Results

(1) *How did taxonomic and functional richness of parasite assemblages change over time in animals that acquired BTB versus those that did not?* Animals that acquired BTB experienced a greater increase in parasite assemblage richness than control animals. Taxonomic richness in BTB-infected animals increased by 3.3 species on average between Phase 1 and Phase 2, compared to an increase of 1.1 species in control animals (Figure 1; Table 2). Parasite functional richness (the number of unique trait combinations) was over three times greater in BTB-infected animals than in control animals (Figure 1; 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 in our BTB and control groups.

Animals that acquired BTB had slightly lower parasite richness prior to BTB conversion than control animals (Table 2; Figure 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, which is likely due to schistosome acquisition as buffalo age (48). BHV and BRSV were indicators of control buffalo in both phases and of BTB buffalo during Phase 2 (BHV: $p = 0.012$, BRSV: $p = 0.048$). However, these viral parasites were not indicators for BTB buffalo in Phase 1, which suggests that they may be associated with TB acquisition in this group.

(2) How did taxonomic and functional composition change over time in animals that acquired BTB versus 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: $df = 1$, $F = 3.83$, $p = 0.001$; Figure 2b; Table 2). These shifts represented changes in taxonomic composition that were associated with the loss of strongyle nematodes and *A. marginale* and the gain of *Brucella abortus* and schistosomes (Figure 2a; SI Appendix Table S2) for animals with BTB, and the loss of BHV and PI3 and a gain of *Brucella abortus* and nematodes for control animals (Figure 2b; SI Appendix Table S2). Despite these changes in parasite assemblage composition, the dispersion of parasite species did not differ between Phases 1 and 2 for either BTB+ or control animals (Control: $df = 1$, $F = 1.35$, $p = 0.28$; BTB: $df = 1$, $F = 1.05$, $p = 0.31$), 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 Phases 1 and 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 (Figure 2c, 2d). However, contrary 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 to 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+ 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 (Table SI Appendix 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 prior to infection, as evidenced by an increase in taxonomic richness and shifts in taxonomic composition. Furthermore, our analysis of functional traits highlighted 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

266 those already present in the parasite community were able to establish following BTB infection.
267 However, ordination and multivariate dispersion both showed that parasites occupied a smaller
268 region of trait space and had lower dispersion after the acquisition of BTB than before. This
269 pattern suggests that, while buffalo carried different parasite species post-BTB infection, the
270 traits that these species possessed were functionally similar to existing ones, which caused them
271 to cluster in trait space. This pattern is consistent with the idea that BTB alters host susceptibility
272 to parasites with particular suites of traits. Furthermore, our functional composition analysis
273 suggests that BTB shifted the parasite trait community towards contact-transmitted, simple life
274 cycle and fast replicating parasites, revealing a specific profile of pathogens that may be
275 facilitated by BTB.

276 Importantly, the changes to the parasite community in BTB-positive animals differed from
277 those seen in control animals. There were marginally significant increases in taxonomic and
278 functional richness through time in control animals, but the magnitudes of these increases were
279 2x less than in BTB-positive animals. Additionally, there were no differences in the dispersion of
280 Phase 1 and Phase 2 control buffalo in taxonomic space, suggesting that the parasite community
281 neither converged nor diverged over time. Control buffalo also shifted locations in the functional
282 space between Phase 1 and Phase 2, reflecting significant changes in parasite community
283 composition. Although we observed a contraction of functional space over time in all buffalo, in
284 control animals this contraction was only marginally significant and less than half the effect size
285 seen in BTB-positive animals. The pattern in control animals suggests that there are age- and/or
286 time-related shifts in the parasite community, but the magnitude of this shift differs when BTB is
287 present. Thus, the presence of certain parasites, like BTB, seems to catalyze extraordinary shifts
288 in community composition.

BTB has previously been described to alter the incidence and progression of individual microparasites in buffalo (Rift Valley fever: (18); *Brucella abortus*: (21)). 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. Additionally, our indicator species analysis suggested that two viral parasites, BHV and BRSV, were indicative of Phase 2 BTB buffalo, but not Phase 1 BTB animals, 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); this suggests 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 comprised of many parasites whose frequency of occurrence changed between Phases 1 and 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)* (63), as it suggests that one should not consider only the direct effects of TB in mitigation strategies but should also consider indirect effects via changing parasite communities. Beyond conservation, there are implications for public health and management as tuberculosis is a re-emerging disease worldwide (64–67). For instance, the prevention of co-infections may slow the progression of BTB infection, as has been discussed with helminths and BTB, where treatment of gastrointestinal parasites is known to

312 increase survival time for individuals infected with BTB (12, 20), and in brucella where the
313 presence of *Brucella abortus* slowed the invasion of BTB (21). A valuable next step would be to
314 evaluate whether the treatment of contact transmitted, quickly replicating parasites can slow the
315 progression of BTB infection, as has been found in humans (*Homo sapiens*) (68) and wild boar
316 (*Sus scrofa*) (69).

317 Interestingly, after BTB infection there was a small but significant decrease in two parasite
318 taxa: *Anaplasma marginale* and strongyles. Buffalo in this study that were infected with both
319 BTB and strongyles were much more likely to die (20) than those without strongyles, which
320 suggests that the decrease in strongyles may be due to coinfected mortality. However, previous
321 work by Gorsich et al (21) also demonstrated a co-infected mortality pattern between brucellosis
322 and BTB that we did not detect with this analysis. This is likely because that was a very small
323 effect, that is difficult to identify unless full longitudinal data are used – demonstrating the utility
324 of multiple types of analyses when evaluating the effect of an invading parasite on native
325 parasite communities.

326 We found some evidence that animals that acquired BTB began the study with different
327 parasite assemblages than those individuals that never acquired BTB. This may be due to the
328 non-random sample of animals we selected for inclusion in the study. Buffalo had to survive at
329 least 2 captures with BTB to be included in the study, and therefore we may only be assessing
330 the "healthiest" animals with BTB, rather than those that died quickly. Alternatively, there may
331 be a role for differences in susceptibility between BTB and control animals. Previous work has
332 suggested that susceptibility to BTB in buffalo may have a genetic basis, and while the
333 mechanism for susceptibility is unknown, it is possible that the genetic background of the
334 individuals that acquire BTB may also affect other diseases (43). Lastly, 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 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 novel 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 affected by the invasive disease, BTB. However, BTB is only one example of an emerging disease that may affect native parasite communities. As emerging diseases become more common (70) due to human activity (71, 72) and environmental changes (73–75), we must find new ways to evaluate their impacts and design mitigation strategies that acknowledge the complex parasite community that exists worldwide. We demonstrate 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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References

1. Steinmann P, Utzinger J, Du Z-W, Zhou X-N (2010) Multiparasitism a neglected reality on global, regional and local scale. *Adv Parasitol* 73:21–50.

2. Vaumourin E, Vourc'h G, Gasqui P, Vayssier-Taussat M (2015) The importance of multiparasitism: examining the consequences of co-infections for human and animal health. *Parasit Vectors* 8:545.

3. Cox FE (2001) Concomitant infections, parasites and immune responses. *Parasitology* 122 Suppl:S23–38.

4. Pedersen AB, Fenton A (2007) Emphasizing the ecology in parasite community ecology. *Trends Ecol Evol* 22(3):133–139.

5. Bordes F, Morand S (2015) Impacts of parasite diversity on wild vertebrates: limited knowledge but important perspectives. *Parasite Diversity and Diversification*, eds Morand S, et al. (Cambridge University Press, Cambridge), pp 77–90.

6. Bordes F, Morand S (2011) The impact of multiple infections on wild animal hosts: a review. *Infect Ecol Epidemiol* 1. doi:10.3402/iee.v1i0.7346.

7. Johnson PTJ, Buller ID (2011) Parasite competition hidden by correlated coinfection: using surveys and experiments to understand parasite interactions. *Ecology* 92(3):535–541.

8. Budischak SA, et al. (2018) Competing for blood: the ecology of parasite resource competition in human malaria-helminth co-infections. *Ecol Lett* 21(4):536–545.

9. Ezenwa VO, Jolles AE (2011) From host immunity to pathogen invasion: the effects of helminth coinfection on the dynamics of microparasites. *Integr Comp Biol* 51(4):540–551.

10. Lello J, McClure SJ, Tyrrell K, Viney ME (2018) Predicting the effects of parasite co-infection across species boundaries. *Proc Biol Sci* 285(1874). doi:10.1098/rspb.2017.2610.

11. Rynkiewicz EC, Pedersen AB, Fenton A (2015) An ecosystem approach to understanding and managing within-host parasite community dynamics. *Trends Parasitol* 31(5):212–221.

12. Johnson PTJ, de Roode JC, Fenton A (2015) Why infectious disease research needs community ecology. *Science* 349(6252):1259504.

13. Pedersen AB, Fenton A (2007) Emphasizing the ecology in parasite community ecology. *Trends Ecol Evol* 22(3):133–139.

14. Griffiths EC, Pedersen AB, Fenton A, Petchey OL (2014) Analysis of a summary network of co-infection in humans reveals that parasites interact most via shared resources. *Proc Biol*

Sci 281(1782):20132286.

15. Cariveau DP, Elijah Powell J, Koch H, Winfree R, Moran NA (2014) Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (Bombus). *ISME J* 8(12):2369–2379.

16. White LA, Forester JD, Craft ME (2017) Using contact networks to explore mechanisms of parasite transmission in wildlife: Contact networks: wildlife parasite transmission. *Biol Rev* 92(1):389–409.

17. Telfer S, et al. (2010) Species interactions in a parasite community drive infection risk in a wildlife population. *Science* 330(6001):243–246.

18. Beechler BR, et al. (2015) Enemies and turncoats: bovine tuberculosis exposes pathogenic potential of Rift Valley fever virus in a common host, African buffalo (Syncerus caffer). *Proceedings of the Royal Society of London B: Biological Sciences* 282(1805):20142942.

19. Ezenwa VO, Etienne RS, Luikart G, Beja-Pereira A, Jolles AE (2010) Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. *Am Nat* 176(5):613–624.

20. Ezenwa VO, Jolles AE (2015) Epidemiology. Opposite effects of anthelmintic treatment on microbial infection at individual versus population scales. *Science* 347(6218):175–177.

21. Gorsich EE, et al. (2018) Opposite outcomes of coinfection at individual and population scales. *Proc Natl Acad Sci U S A* 115(29):7545–7550.

22. Budischak SA, Hoberg EP, Abrams A, Jolles AE, Ezenwa VO (2016) Experimental insight into the process of parasite community assembly. *J Anim Ecol* 85(5):1222–1233.

23. Henrichs B, et al. (2016) Within guild co-infections influence parasite community membership: a longitudinal study in African Buffalo. *J Anim Ecol* 85(4):1025–1034.

24. Crowl TA, Crist TO, Parmenter RR, Belovsky G, Lugo AE (2008) The spread of invasive species and infectious disease as drivers of ecosystem change. *Front Ecol Environ* 6(5):238–246.

25. Laliberté E, Shipley B (2011) *FD: measuring functional diversity from multiple traits, and other tools for functional ecology* (R package version 1.0-11).

26. Graham NAJ, Jennings S, MacNeil MA, Mouillot D, Wilson SK (2015) Predicting climate-driven regime shifts versus rebound potential in coral reefs. *Nature* 518(7537):94–97.

27. Buisson L, Grenouillet G, Villéger S, Canal J, Laffaille P (2013) Toward a loss of functional diversity in stream fish assemblages under climate change. *Glob Chang Biol* 19(2):387–400.

28. Boersma KS, Bogan MT, Henrichs BA, Lytle DA (2014) Invertebrate assemblages of pools in arid-land streams have high functional redundancy and are resistant to severe drying. *Freshw Biol* 59(3):491–501.

29. Villéger S, Grenouillet G, Brosse S (2014) Functional homogenization exceeds taxonomic homogenization among E uropean fish assemblages. *Glob Ecol Biogeogr* 23(12):1450–1460.

30. Colin N, Villéger S, Wilkes M, de Sostoa A, Maceda-Veiga A (2018) Functional diversity measures revealed impacts of non-native species and habitat degradation on species-poor freshwater fish assemblages. *Sci Total Environ* 625:861–871.

31. Mouillot D, Graham NAJ, Villéger S, Mason NWH, Bellwood DR (2013) A functional approach reveals community responses to disturbances. *Trends Ecol Evol* 28:167–177.

32. Boersma KS, et al. (2016) Linking multidimensional functional diversity to quantitative methods: a graphical hypothesis-evaluation framework. *Ecology* 97:583–593.

33. Seabloom EW, et al. (2015) The community ecology of pathogens: coinfection, coexistence and community composition. *Ecol Lett*. doi:10.1111/ele.12418.

34. Han BA, Schmidt JP, Bowden SE, Drake JM (2015) Rodent reservoirs of future zoonotic diseases. *Proc Natl Acad Sci U S A* 112(22):7039–7044.

35. Evans MV, Dallas TA, Han BA, Murdock CC, Drake JM (2017) Data-driven identification of potential Zika virus vectors. *Elife* 6:e22053.

36. Mason NWH, Mouillot D, Lee WG, Wilson JB (2005) Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* 111(1):112–118.

37. Villéger S, Mason NWH, Mouillot D (2008) New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89(8):2290–2301.

38. Mouchet MA, Villéger S, Mason NWH, Mouillot D (2010) Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. *Funct Ecol* 24:867–876.

39. Laisse CJM, et al. (2011) Characterization of tuberculous lesions in naturally infected African buffalo (Syncerus caffer). *J Vet Diagn Invest* 23(5):1022–1027.

40. Cross PC, et al. (2009) Disease, predation and demography: assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels. *J Appl Ecol* 46(2):467–475.

41. Jolles AE, Cooper DV, Levin SA (2005) Hidden effects of chronic tuberculosis in African buffalo. *Ecology* 86(9):2358–2364.

42. Caron A, Cross PC, du Toit JT (2003) Ecological implications of bovine tuberculosis in African buffalo herds. *Ecol Appl* 13(5):1338–1345.

43. Tavalire HF, et al. (2018) Context-dependent costs and benefits of tuberculosis resistance traits in a wild mammalian host. *Ecol Evol* 181:674.

44. Beechler BR, Broughton H, Bell A, Ezenwa VO, Jolles AE (2012) Innate immunity in free-ranging African buffalo (Syncerus caffer): associations with parasite infection and white blood cell counts. *Physiol Biochem Zool* 85(3):255–264.

45. Budischak SA, Hoberg EP, Abrams A, Jolles AE, Ezenwa VO (2015) A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. *Mol Ecol Resour* 15(5):1112–1119.

46. Michel AL, Cooper D, Jooste J, de Klerk L-M, Jolles A (2011) Approaches towards optimising the gamma interferon assay for diagnosing Mycobacterium bovis infection in African buffalo (Syncerus caffer). *Prev Vet Med* 98(2-3):142–151.

47. Gorsich EE, Ezenwa VO, Jolles AE (2014) Nematode–coccidia parasite co-infections in African buffalo: Epidemiology and associations with host condition and pregnancy. *Int J Parasitol Parasites Wildl* 3(2):124–134.

48. Beechler BR, et al. (2017) Host immunity, nutrition and coinfection alter longitudinal infection patterns of schistosomes in a free ranging African buffalo population. *PLoS Negl Trop Dis* 11(12):e0006122.

49. Glidden CK, et al. (2018) Detection of Pathogen Exposure in African Buffalo Using Non-Specific Markers of Inflammation. *Front Immunol* 8:1944.

50. Gorsich EE, Ezenwa VO, Cross PC, Bengis RG, Jolles AE (2015) Context-dependent survival, fecundity and predicted population-level consequences of brucellosis in African buffalo. *J Anim Ecol* 84(4):999–1009.

51. Lanyon SR, Hill FI, Reichel MP, Brownlie J (2014) Bovine viral diarrhoea: pathogenesis and diagnosis. *Vet J* 199(2):201–209.

52. Coetzer JAW (ed)., Thomson GR (ed)., Tustin RC (ed). (1994) Infectious diseases of livestock with special reference to Southern Africa. Available at: http://agris.fao.org/agris-search/search.do?recordID=XF2015026354.

53. Lefevre T, et al. (2018) Transmission traits of malaria parasites within the mosquito: Genetic variation, phenotypic plasticity, and consequences for control. *Evol Appl* 11(4):456–469.

54. Ghosh S, Ferrari MJ, Pathak AK, Cattadori IM (2018) Changes in parasite traits, rather than intensity, affect the dynamics of infection under external perturbation. *PLoS Comput Biol* 14(6):e1006167.

55. Dufrene M, Legendre P (1997) Species Assemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. *Ecological Monographs* 67(3):345.

56. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46.

57. Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions.

Biometrics 62:245–253.

58. McCune B, Grace JB (2002) *Analysis of Ecological Communities* (MjM Software Design, Glenden Beach, Oregon).

59. Díaz S, Cabido M, Zak M, Martínez Carretero E, Araníbar J (1999) Plant functional traits, ecosystem structure and land-use history along a climatic gradient in central-western Argentina. *J Veg Sci* 10:651–660.

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

61. Oksanen J, et al. (2012) *vegan: Community Ecology Package* (R package version 2.0-3) Available at: http://CRAN.R-project.org/package=vegan.

62. De Cáceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. *Ecology* 90:3566–3574.

63. Aranaz A, et al. (2004) Bovine tuberculosis (Mycobacterium bovis) in wildlife in Spain. *J Clin Microbiol* 42(6):2602–2608.

64. Tom Navin, Scott McNabb, Jack T. Crawford (2002) The Continued Threat of Tuberculosis. *Emerging Infectious Disease journal* 8(11):1187.

65. Sohail M (2006) Tuberculosis: A re-emerging enemy. *J Mol Genet Med* 2(1):87–88.

66. De Lorenzo S, Tiberi S (2012) Tuberculosis a re-emerging disease. *Intern Emerg Med* 7(3):185–187.

67. Etter E, et al. (2006) Risk Analysis and Bovine Tuberculosis, a Re-emerging Zoonosis. *Ann N Y Acad Sci* 1081(1):61–73.

68. Li X-X, Zhou X-N (2013) Co-infection of tuberculosis and parasitic diseases in humans: a systematic review. *Parasit Vectors* 6:79.

69. Risco D, et al. (2014) Severity of bovine tuberculosis is associated with co-infection with common pathogens in wild boar. *PLoS One* 9(10):e110123.

70. Jones KE, et al. (2008) Global trends in emerging infectious diseases. *Nature* 451(7181):990–993.

71. Rogalski MA, Gowler CD, Shaw CL, Hufbauer RA, Duffy MA (2017) Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. *Philos Trans R Soc Lond B Biol Sci* 372(1712). doi:10.1098/rstb.2016.0043.

72. Cunningham AA, Daszak P, Wood JLN (2017) One Health, emerging infectious diseases and wildlife: two decades of progress? *Philos Trans R Soc Lond B Biol Sci* 372(1725). doi:10.1098/rstb.2016.0167.

73. Paull SH, et al. (2017) Drought and immunity determine the intensity of West Nile virus

epidemics and climate change impacts. *Proc Biol Sci* 284(1848). doi:10.1098/rspb.2016.2078.

74. Brenner F, Marwan N, Hoffmann P (2017) Climate impact on spreading of airborne infectious diseases. *Eur Phys J Spec Top* 226(9):1845–1856.

75. Machalaba C, et al. (2015) Climate Change and Health: Transcending Silos to Find Solutions. *Ann Glob Health* 81(3):445–458.

Table 1. Parasite trait definitions (Si Appendix, Table S1 has citations for each parasite). Traits that are 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 that are likely to play a major role in wasting or mortality are host compartment, site of replication and fitness effects.

Trait	Categories and definition
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 - primarily transmitted directly from one individual to another; environmental - primarily transmitted via contaminated fomites or ground; vector- transmitted by vectors (ticks, mosquitoes)
Life Cycle	simple - can complete a life cycle within one host; complex - parasite requires an intermediate host, vector, or environmental stage to complete 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

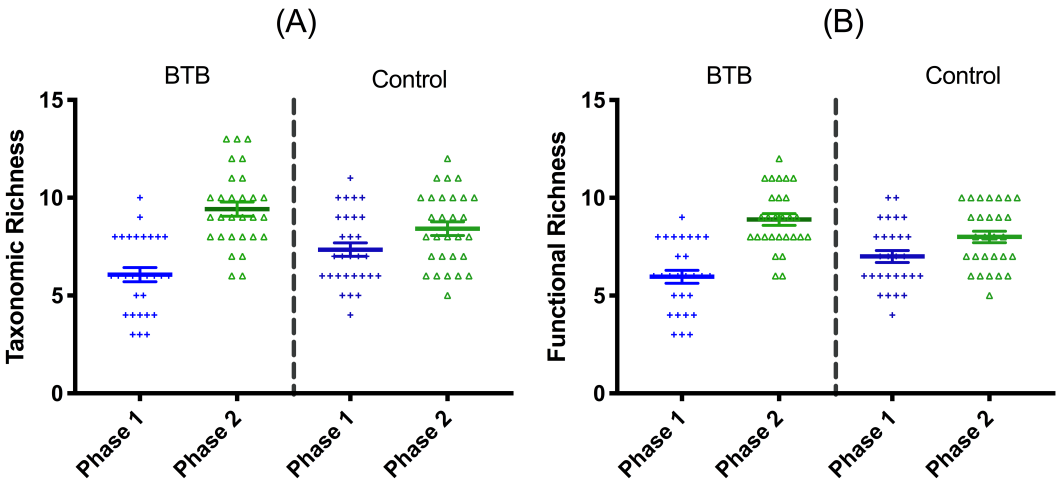
Primary body compartment	lung, GI tract, white blood cells, red blood cells, multi-site - site in the host of primary replication and/or the majority of the parasite life cycle
Site of replication	intra- or extracellular - whether the parasite replicates inside or outside host cells
Duplication time	the time it takes the parasite to duplicate its population; long- greater than one day; medium- between 5 & 24h; fast- <4h
Fitness effects	yes or no - Does the parasite reduce survival or fecundity in buffalo?

Table 2. RM-ANOVA (Taxonomic Richness $F=28.65$, $p<0.001$, Functional Richness $F=34.07$, $p<0.001$) with Bonferroni posthoc comparisons demonstrate that BTB-infected animals experienced an increase in parasite richness in Phase 2 to a greater degree than control animals. Significance: $p<0.01$***, $p<0.05$*

Comparison	Taxonomic Richness		Functional Richness	
	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

Figure 1. Phase 2 animals had higher parasite richness than Phase 1 animals, both taxonomically (panel A) and functionally (panel B). However BTB animals experienced a larger magnitude of increase in richness over time compared to control animals. Animals that acquired BTB had lower richness in Phase 1 than control animals and higher richness in Phase 2 than control animals. Statistics for between group comparisons are provided in Table 3. Lines represent means, bars are two standard error units, and each point is an individual buffalo.

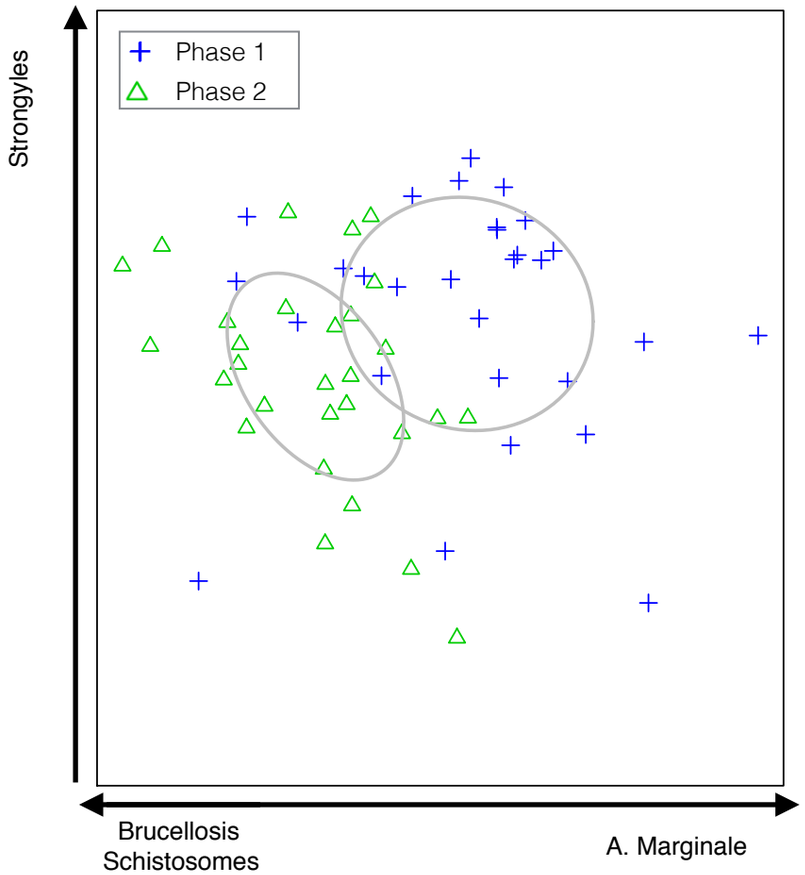
Figure 2. Nonmetric multidimensional scaling ordination of individual buffalo in parasite taxonomic space (panels A&B) and parasite trait space (panels C&D). Panel A is parasite taxonomic space for animals that acquired BTB (k = 3, Stress = 0.15), while Panel B is animals that did not acquire BTB (k = 3, Stress = 0.18). Panel C is parasite trait space for animals that acquired BTB (k = 2, Stress = 0.15) while panel D is animals that did not acquire BTB (k = 2, Stress = 0.18). The 95% confidence ellipses (gray) represent the standard deviation of the coordinates of Phase 1 and Phase 2 buffalo. Parasites that are correlated with the axes are listed alongside the ordinations A&B (Spearman correlation > 0.5; see supplementary material for details), while traits that correlate with the axes are listed alongside the ordinations C&D (Spearman correlation > 0.7, Tables S2 and S3 shows all associations greater than 0.5).


(A)
BTB
Control
Taxonomic Richness
15
10
5
0
Phase 1
Phase 2
Phase 1
Phase 2
(B)
BTB
Control
Functional Richness
15
10
5
0
Phase 1
Phase 2
Phase 1
Phase 2

Taxonomic Ordinations

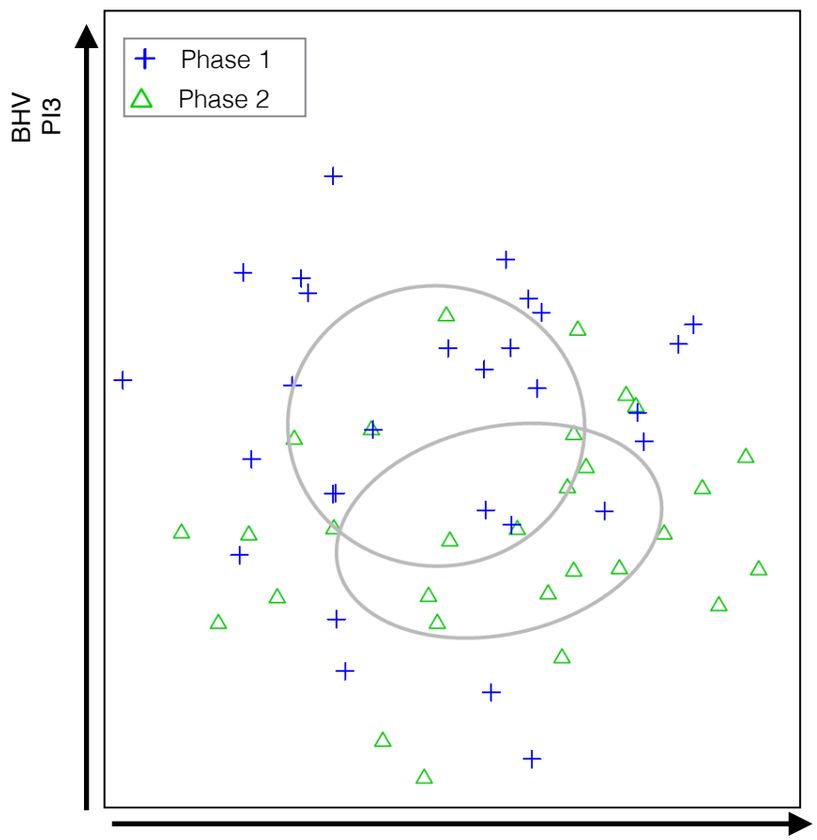
Animals that acquired BTB

(A)



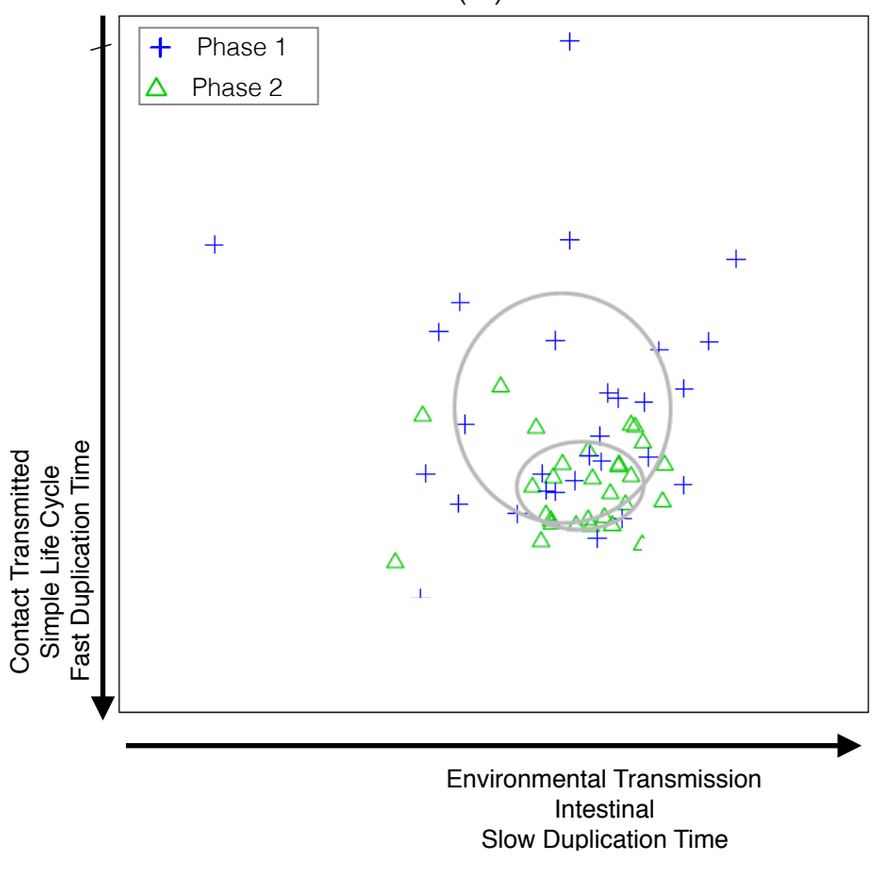
Animals that did not acquire BTB

(B)



Trait-based Ordinations

(C)



(D)