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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.

\body

1 **Abstract**

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

15

16 **Introduction**

17 Wild hosts are infected with multiple parasites simultaneously(1–3). These species interact
18 directly and indirectly, and basic principles of community ecology apply to parasite assemblages
19 (4). Numerous studies have attempted to characterize the mechanisms and consequences of co-
20 infection (reviewed in (2, 5, 6)). However, it can be difficult to predict the direction and strength
21 of the outcomes (7) because parasite interactions can be both competitive (e.g. (8)) and
22 facilitative (e.g. (9, 10)) and the relative importance of these mechanisms varies. Investigators
23 have begun to apply community ecological principles to the field of disease ecology to
24 understand parasite interactions within a host (11–16) although most studies still break existing
25 networks of parasites into isolated pairwise comparisons (e.g. (17–23) that may fail to capture
26 the true dynamics of co-infection.

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

36 Furthermore, disturbance ecology has the toolset to approach multi-parasite systems from
37 not only taxonomic (species identity), but also functional (trait) perspectives by examining how
38 functional traits of entire communities change with disturbance (31, 32). When analyses are

39 limited to the taxonomic level, it is difficult to extrapolate beyond the specific parasite species
40 under study. Shifting the focus in disease ecology from taxon-based to trait-based approaches
41 can help us understand the mechanisms behind observed patterns in parasite community
42 composition and parasite transmission - a priority that has been emphasized in review papers (12,
43 24, 33), and is necessary to understand how host communities (34) and vector communities (35)
44 play a role in disease transmission.

45 Trait-based disturbance ecology thus has the potential to reveal the collective impacts of
46 the arrival of a novel parasite across entire parasite communities. Specifically, multivariate
47 ordination-based approaches that visualize the trait composition of communities (36–38) and
48 track how these communities change with disturbance (31, 32) provide an intuitive, rigorous, and
49 flexible approach that can advance our understanding of the consequences of novel parasite
50 invasions. Applying such an approach to co-infection questions may increase our capacity to
51 understand the community-wide impacts of invading parasites by identifying which native trait
52 combinations change with the arrival of the invaders.

53 In this study, we apply the principles of trait-based disturbance ecology to understand how
54 the arrival of a novel parasite affects taxonomic and functional community structure of a native
55 parasite community. We studied the effects of a well-characterized emerging, chronic parasitic
56 disease, bovine tuberculosis (BTB) (18, 39–42), on a community of 16 parasites in wild African
57 buffalo (*Syncerus caffer*). We focus on BTB because it is known to have dramatic effects on
58 immune function (18, 43, 44) and body condition (i.e. wasting) (20, 42, 43); both are attributes
59 that might permit the parasite to serve a “keystone” role, allowing us to evaluate how one
60 parasite can restructure the rest of the parasite community. We developed a trait database for a
61 diverse parasite community comprised of viruses, bacteria, protozoa, and helminths, and applied

62 taxonomic and trait-based approaches to analyze how parasite richness and community
63 composition changed in response to BTB infection. The unique longitudinal format of the data,
64 which involved sampling the parasite community in the same hosts over multiple years, allowed
65 us to implement a framework developed to understand the effects of disturbances on functional
66 trait diversity in multispecies communities (31, 32).

67 We hypothesized that BTB infection would have contrasting effects on the parasite
68 community. We predicted BTB to increase the occurrence of parasites when the dominant
69 mechanism of interaction was enhanced susceptibility due to wasting and immune modulation
70 (18) and decrease occurrence of parasites when the dominant mechanism of interaction was co-
71 infected mortality due to wasting (20, 21). Because of the opposing direction of these
72 hypotheses, predicting the overall effect of BTB on parasite community richness and structure is
73 challenging. Thus, we used a case-control design to compare changes in parasite community in a
74 group of buffalo that acquired BTB, to changes in a control group that was matched in terms of
75 age, herd, and time period but did not acquire BTB.

76 **Materials and Methods**

77 Study System & Parasite Diagnostics: Approximately 200 African buffalo were captured in
78 Kruger National Park (KNP), South Africa, as part of a longitudinal study on gastrointestinal
79 helminths and bovine tuberculosis which targeted young females (20). Individuals were followed
80 for 4 years (or until they left the study due to death or emigration from the study area), and
81 captured every 6 months, resulting in 1751 sample events. At each capture, blood and fecal
82 samples were obtained for parasite diagnostics. Blood was collected by jugular venipuncture into
83 lithium heparinized tubes and no-additive tubes. Feces was collected from the rectum using a
84 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), adenovirus 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 omatajense* (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

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

109 **Animal Selection for Inclusion:**

110 We only included individuals that were captured at least two times prior to BTB conversion
111 (Phase 1) and two times post BTB conversion (Phase 2), resulting in 29 individuals that were
112 included as BTB converters. We then selected 29 'control' animals that did not acquire BTB
113 during the study period that were matched with BTB animals for age (within 1 year),
114 reproductive status (pregnant vs. not in the same phase) and capture date (+/- 2 months). Control
115 animals (buffalo that never acquired BTB) were assigned the same "conversion" date as their
116 paired BTB+ individual to divide captures into Phase 1 and Phase 2 – this kept the total samples
117 the same for BTB+ and control animals in Phase 1 and 2 and facilitated comparison. This
118 allowed us to account for potential changes through time that were not associated with the
119 acquisition of BTB. In association with another study, eight animals in each group (BTB+ and
120 control) had an anthelmintic treatment (long-acting fenbendazole bolus) applied every six
121 months for the duration of the study to reduce strongyle burdens (20).

122 Importantly, we conducted a supplemental analysis and demonstrated that the bolus did
123 not affect parasite taxonomic or functional composition in our analysis. To verify that the bolus
124 (anthelmintic) did not change the parasite assemblage in our study, we compared 48 bolused
125 animals, measured prior to bolusing in June–July 2008 to the same 48 bolused animals measured
126 1 year later between June and August 2009. We found no differences in functional diversity
127 (Wilcoxon matched pairs signed rank, median difference 0.002, $p=0.253$), functional richness
128 (Wilcoxon matched pairs signed rank, median difference 0.008, $p=0.730$), or taxonomic diversity
129 (Wilcoxon matched pairs signed rank, median difference 0.05, $p=0.255$). This is likely because

130 the bolus reduces strongyle burden, but does not clear it entirely. Consequently, we included the
131 presence of strongyles as a parasite in our analyses.

132 **Creation of the Parasite Matrix**

133 The parasite matrix contained data on the parasites present in each buffalo at each capture. All
134 individuals were assigned a 1 if they were positive for BTB and a 0 if negative at each capture.
135 We then calculated the proportion of time each parasite species was present in the buffalo before
136 and after BTB (Phase 1 and 2 respectively). If the parasite was tested for directly (such as *A.*
137 *marginale* (SI Appendix), we determined the proportion of captures during which the parasite
138 was present in each phase. For instance, if Animal 1 was captured at 8 time periods, periods 1-4
139 in Phase 1 and periods 5-8 in Phase 2, and it had *A. marginale* (AM) at time points 1,6 and 7,
140 then the proportion of capture intervals it had AM for Phase 1 was 1/4 and Phase 2 was 2/4. If
141 the parasite was detected with antibody seroconversion (such as PI3), then we calculated
142 incidence of each parasite between successive captures, which was defined as a change in
143 antibody titer from negative to positive in successive captures (BRSV, BVDV) or an increase in
144 antibody titer greater than a certain percentage (as described by the manufacturer of the ELISA
145 and in Glidden et al (49) (MH, MB, PI3, Ad3, BHV)). Incidence was then used to calculate the
146 proportion of capture intervals during which an incident event occurred. More details on
147 incidence calculation are described in Glidden et al (49).

148 **Creation of the Trait Matrix**

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

153 be affected by the invasion of bovine tuberculosis; while also focusing on traits that may help us
154 disentangle the effects that BTB may have due to wasting/co-mortality and increased
155 susceptibility due to BTB infection.

156 **Statistical Analysis**

157 To evaluate whether our trait set appropriately captured representative aspects of parasite
158 biology, we first examined how parasites varied in their trait composition with a nonmetric
159 multidimensional scaling (NMDS) ordination of parasites in trait space (SI Appendix, Figure
160 S1). We calculated Gower dissimilarity from the categorical trait matrix and applied a Wisconsin
161 transformation to standardize before ordination. The ordination converged on a stable two-
162 dimensional solution (SI Appendix, Figure S1). Relationships among parasites matched
163 expectations from the literature. For instance, the intestinal parasites and tick-borne parasites
164 each clustered separately in multivariate space. The congruence between expectations and trait
165 space validated our trait selection and assignment.

166 To examine the effects of BTB on parasite taxonomic and functional richness, we
167 calculated two univariate diversity metrics, functional richness (FRic; (38)) and taxonomic
168 richness, for each buffalo in Phases 1 and 2. For categorical traits, FRic measures the number of
169 independent trait combinations and is directly comparable with species richness. We used
170 repeated measures ANOVAs with Bonferroni correction to compare richness between all groups
171 (Phase 1 BTB vs Phase 1 control; Phase 2 BTB vs Phase 2 control; Phase 1 vs Phase 2 control;
172 Phase 1 vs Phase 2 BTB). To assess which species of parasite changed with BTB infection, i.e.
173 were representative of each host group, we used an indicator species analysis (ISA, `multipatt` in
174 R package `indicspecies`) and examined statistical significance using a Monte Carlo
175 randomization with 999 iterations (55). ISA combines information on the relative abundances

176 and relative frequencies of species to determine an indicator value that represents the fidelity and
177 exclusivity of each parasite species to each of the four host groups: Phase 1 control, Phase 1
178 BTB, Phase 2 control, and Phase 2 BTB.

179 To examine the effects of BTB on parasite taxonomic and functional composition, we
180 visualized changes in the taxonomic and trait composition of each buffalo between Phases 1 and
181 2 using NMDS. We plotted each individual's taxonomic/functional parasite composition in
182 Phase 1 and Phase 2 (as in (32)). Examining shifts in the location of ordination space allowed us
183 to understand how taxonomic and trait composition of individual buffalo changed when they
184 acquired BTB. We then compared these changes with similar shifts in control buffalo during the
185 same time period (Phase 1 to Phase 2).

186 For taxonomic ordinations, we used Bray-Curtis distances and applied Wisconsin
187 transformations before ordination. We assessed ordination fit with overall stress; both taxonomic
188 ordinations converged on stable three-dimensional solutions. To aid in interpreting the
189 ordinations, we examined parasite correlations with the first two axes ($r > 0.5$). We used
190 permutation-based analysis of variance (PerMANOVA; (56)) to examine changes in the location
191 of buffalo in parasite taxonomic ordination space between Phases 1 and 2. We also compared the
192 multivariate dispersion of parasite associated with Phase 1 and Phase 2 buffalo using
193 homogeneity of group dispersions and permutation tests(57). Dispersion is the average distance
194 of each point from the multivariate group centroid and is a way to quantify the amount of
195 multivariate space that is occupied by a given community.

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

199 (58, 59), which we then ordinated using NMDS. Prior to ordination, we calculated Gower
200 distances and applied log and Wisconsin transformations. Functional ordinations converged on
201 stable two-dimensional solutions. We rotated each ordination to align with a vector of strongyle
202 abundance to facilitate comparisons between ordinations (58), and because strongyles, a native
203 parasite, are known to affect the survival of animals with BTB(20). We examined trait
204 correlations with the axes ($r > 0.5$). As with taxonomic composition, we tested for shifts in the
205 location of Phase 1 and Phase 2 animals in trait space with PerMANOVA, and homogeneity of
206 group dispersions of functional traits using permutation tests.

207 We also calculated multivariate dispersion (betadisper in R package vegan) to examine
208 differences in the dispersion of buffalo in taxonomic and functional space (57, 60). We
209 conducted all analyses in R version 0.98.1062 using packages FD (25), vegan (61), and
210 indicpecies (62).

211 **Results**

212 (1) *How did taxonomic and functional richness of parasite assemblages change over time in*
213 *animals that acquired BTB versus those that did not?* Animals that acquired BTB experienced a
214 greater increase in parasite assemblage richness than control animals. Taxonomic richness in
215 BTB-infected animals increased by 3.3 species on average between Phase 1 and Phase 2,
216 compared to an increase of 1.1 species in control animals (Figure 1; Table 2). Parasite functional
217 richness (the number of unique trait combinations) was over three times greater in BTB-infected
218 animals than in control animals (Figure 1; Table 2). Although we created our control group by
219 matching buffalo by age, herd, and observation period, we detected small differences in initial
220 taxonomic and functional richness of the parasite assemblages in our BTB and control groups.

221 Animals that acquired BTB had slightly lower parasite richness prior to BTB conversion than
222 control animals (Table 2; Figure 1).

223 We also found that indicator species differed by both BTB status and phase. Schistosomes
224 were a significant indicator of both control and BTB buffalo in Phase 2 ($p = 0.006$), suggesting
225 that buffalo acquired schistosomes regardless of BTB status, which is likely due to schistosome
226 acquisition as buffalo age (48). BHV and BRSV were indicators of control buffalo in both phases
227 and of BTB buffalo during Phase 2 (BHV: $p = 0.012$, BRSV: $p = 0.048$). However, these viral
228 parasites were not indicators for BTB buffalo in Phase 1, which suggests that they may be
229 associated with TB acquisition in this group.

230 *(2) How did taxonomic and functional composition change over time in animals that acquired*
231 *BTB versus those that did not?* BTB-infected animals occupied different locations in taxonomic
232 space after infection with BTB than before infection (PerMANOVA: $df = 1$, $F = 7.75$, $p = 0.001$),
233 and a similar change also occurred for control animals during the same time period
234 (PerMANOVA: $df = 1$, $F = 3.83$, $p = 0.001$; Figure 2b; Table 2). These shifts represented
235 changes in taxonomic composition that were associated with the loss of strongyle nematodes and
236 *A. marginale* and the gain of *Brucella abortus* and schistosomes (Figure 2a; SI Appendix Table
237 S2) for animals with BTB, and the loss of BHV and PI3 and a gain of *Brucella abortus* and
238 nematodes for control animals (Figure 2b; SI Appendix Table S2). Despite these changes in
239 parasite assemblage composition, the dispersion of parasite species did not differ between Phases
240 1 and 2 for either BTB+ or control animals (Control: $df = 1$, $F = 1.35$, $p = 0.28$; BTB: $df = 1$, $F =$
241 1.05 , $p = 0.31$), meaning that there was no contraction or expansion of multivariate taxonomic
242 space through time.

243 Both control and BTB-infected animals occupied different regions of trait ordination space
244 between Phases 1 and 2 (PerMANOVA: BTB: $df=1$, $F=5.69$, $P=0.001$; Control: $df=1$, $F=5.57$, p
245 $=0.001$), as in the taxonomic analysis, reflecting changes in functional trait composition for all
246 animals regardless of BTB status (Figure 2c, 2d). However, contrary to the taxonomic analysis,
247 the dispersion of functional traits contracted through time in both control and BTB-infected
248 buffalo (Control: $df=1$, $F=4.29$, $p=0.047$; BTB: $df=1$, $F=9.80$, $p=0.003$). Interestingly, the
249 magnitude of this contraction was almost double in BTB animals compared to control animals
250 (difference in distance to centroid between Phase 1 and Phase 2: Control = 0.027, BTB = 0.047).
251 The contraction in trait space for the BTB+ group was primarily associated with an increase in
252 contact-transmitted parasites with simple life cycles and fast replication times; the control group
253 contraction was not associated with any trait groups (Table SI Appendix S3; $r>0.7$). Notably, no
254 functional groups were lost entirely with the acquisition of BTB.

255 **Discussion**

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

264 When we evaluated how the trait assemblage changed with BTB infection, we found that
265 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.

289 BTB has previously been described to alter the incidence and progression of individual
290 microparasites in buffalo (Rift Valley fever: (18); *Brucella abortus*: (21)). However, our results
291 suggest that BTB may act as an ecological facilitator on a much larger scale than previously
292 suggested, affecting a range of contact-transmitted, fast replicating, and simple life cycle
293 parasites – traits typical of many viruses and bacteria. Additionally, our indicator species
294 analysis suggested that two viral parasites, BHV and BRSV, were indicative of Phase 2 BTB
295 buffalo, but not Phase 1 BTB animals, suggesting that BTB may increase the likelihood of
296 acquiring these parasites. This could be due to increased susceptibility or altered disease
297 progression - since both are diseases with a latent phase (BHV) or chronic carriers (BRSV); this
298 suggests that treatment and control efforts for these parasites may be warranted when BTB is
299 present in a host community. However, the taxonomic ordination space was comprised of many
300 parasites whose frequency of occurrence changed between Phases 1 and 2, and consequently it is
301 difficult to understand what other parasites may be affected, that are less well-described and
302 well-known. Our trait analysis was particularly valuable because it allowed us to identify traits of
303 parasites that may respond to the invasion of BTB.

304 Our finding that BTB alters the community of parasites has widespread implications for
305 managing health outcomes of BTB in wild animal populations, many of which are threatened or
306 endangered, such as Iberian Lynx (*Lynx pardinus*) (63), as it suggests that one should not
307 consider only the direct effects of TB in mitigation strategies but should also consider indirect
308 effects via changing parasite communities. Beyond conservation, there are implications for
309 public health and management as tuberculosis is a re-emerging disease worldwide (64–67). For
310 instance, the prevention of co-infections may slow the progression of BTB infection, as has been
311 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 coinfecting 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

335 are parasite assemblages that protect against the invasion of BTB within an individual; however,
336 our indicator species analysis revealed that none of the parasites we examined were strongly
337 associated with the BTB Phase 1 group. This suggests that a “protective” parasite community
338 was not evident in the buffalo in our study.

339 Our application of a novel functional diversity framework to examine trends in parasite
340 composition before and after acquisition of an emerging infectious disease allowed us to detect
341 patterns that were not apparent in previous studies and revealing the traits of parasites that may
342 be most likely affected by the invasive disease, BTB. However, BTB is only one example of an
343 emerging disease that may affect native parasite communities. As emerging diseases become
344 more common (70) due to human activity (71, 72) and environmental changes (73–75), we must
345 find new ways to evaluate their impacts and design mitigation strategies that acknowledge the
346 complex parasite community that exists worldwide. We demonstrate that incorporating
347 principles from community and functional ecology may allow researchers to understand the
348 community dynamics of pathogens and the consequences for host health in many contexts across
349 systems and scales.

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536

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 |

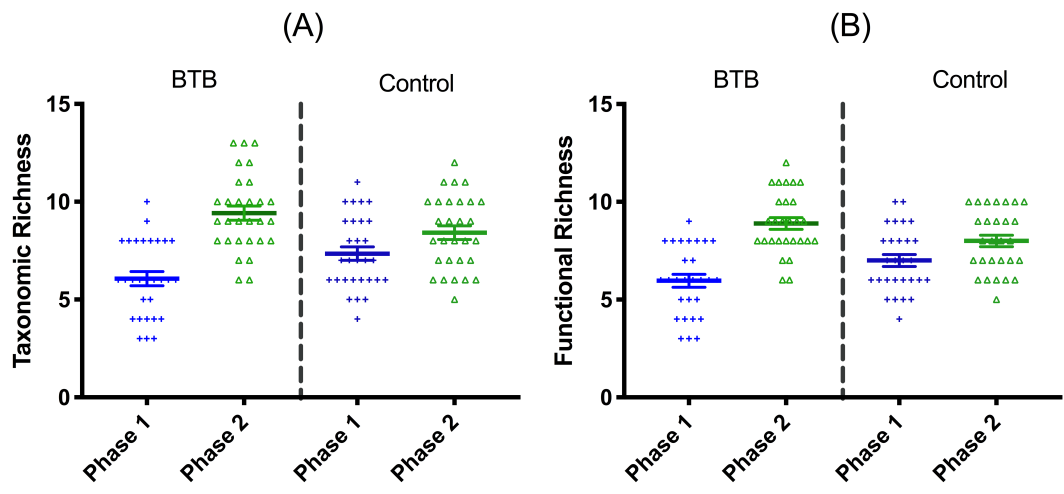
| | |
|--------------------------|---|
| Primary body compartment | lung, GI tract, white blood cells, red blood cells, multi-site - site in the host of primary replication and/or the majority of the parasite life cycle |
| Site of replication | intra- or extracellular - whether the parasite replicates 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.

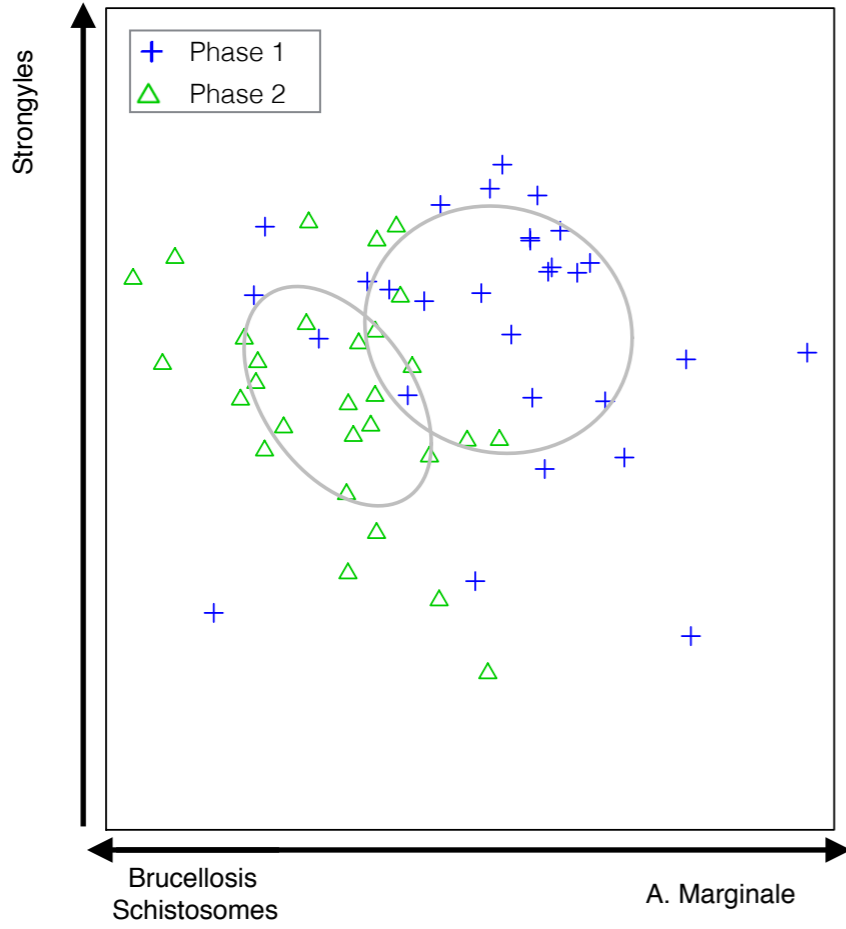
Figure 2. Nonmetric multidimensional scaling ordination of individual buffalo in parasite taxonomic space (panels A&B) and parasite trait space (panels C&D). Panel A is parasite taxonomic space for animals that acquired BTB ($k = 3$, Stress = 0.15), while Panel B is animals that did not acquire BTB ($k = 3$, 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).



Animals that acquired BTB

(A)

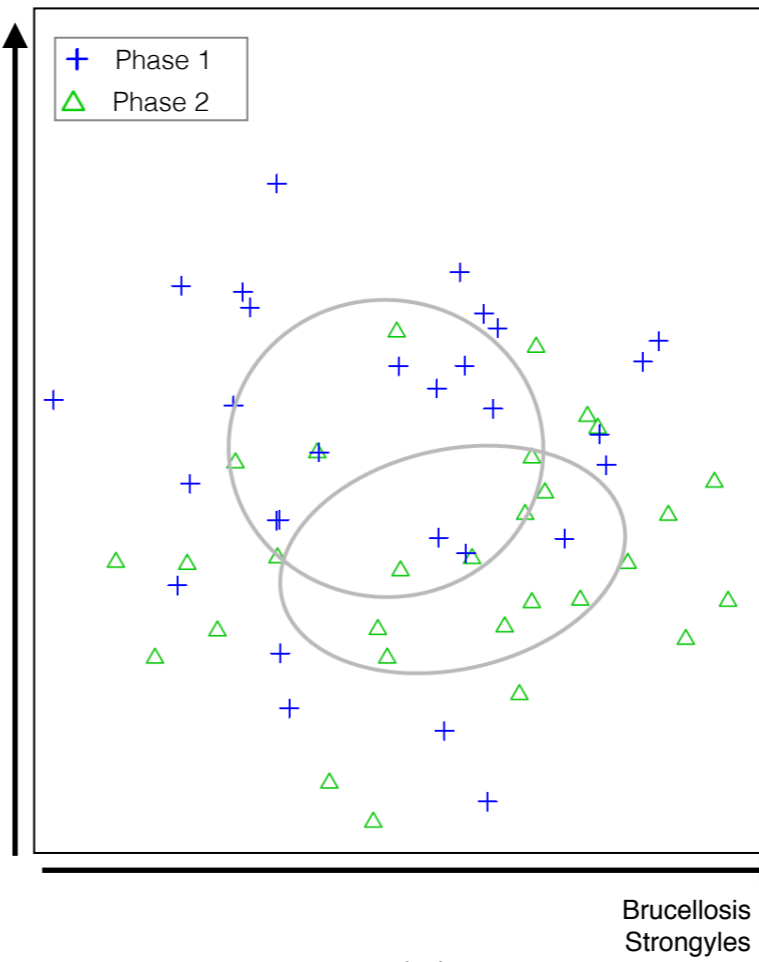
Taxonomic Ordinations



Animals that did not acquire BTB

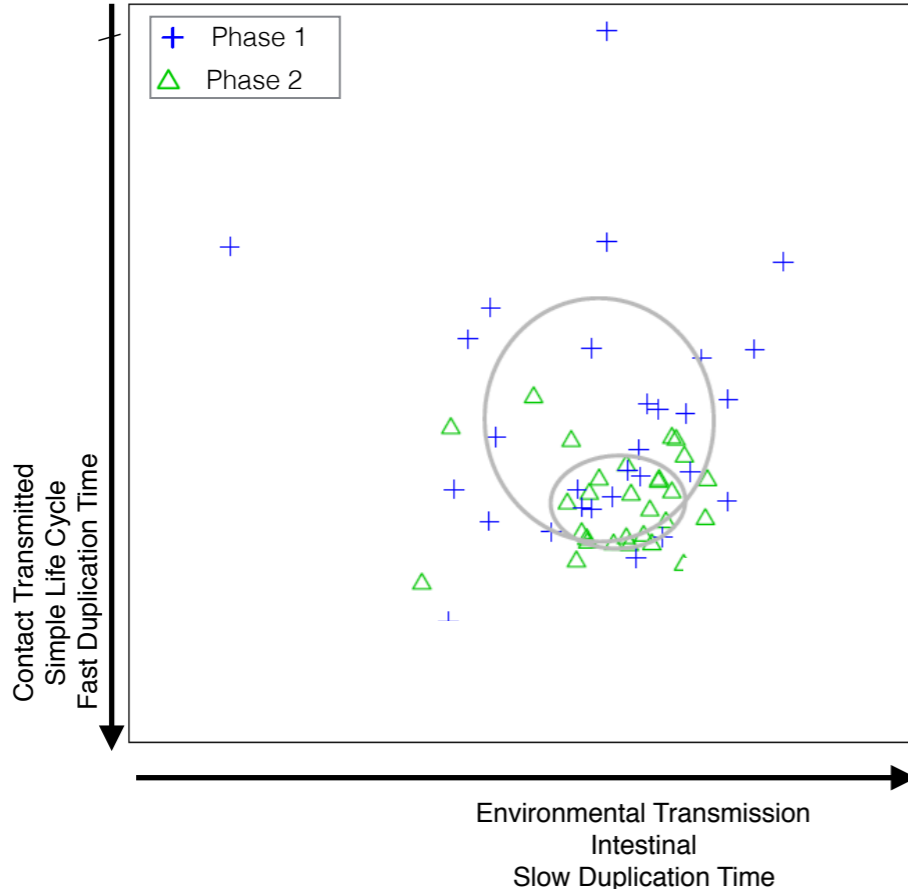
(B)

BHV
PI3



(C)

Trait-based Ordinations



(D)

Contact Transmitted Simple Life Cycle Fast Duplication Time

