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Experimental insight into the process of parasite community assembly

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Summary

1. Community assembly is a fundamental process that has long been a central focus in ecology. Extending community assembly theory to communities of co-infecting parasites, we used a gastrointestinal nematode removal experiment in free-ranging African buffalo to examine the community assembly patterns and processes.

2. We first asked whether reassembled communities differ from undisturbed communities by comparing anthelmintic-treated and control hosts. Next, we examined the temporal dynamics of assembly using a cross-section of communities that reassembled for different periods of time since last experimental removal. Next, we tested for evidence of assembly processes that might drive such reassembly patterns: environmental filtering based on host traits (i.e. habitat patches), interspecific interactions, priority effects and chance dispersal from the environmental pool of infective stages (i.e. the regional species pool).

3. On average, reassembled parasite communities had lower abundance, but were more diverse and even, and these patterns varied tightly with reassembly time. Over time, the communities within treated hosts progressively resembled controls as diversity and evenness decreased, while total abundance increased. Notably, experimental removal allowed us to attribute observed differences in abundance, diversity and evenness to the process of community assembly.

4. During early reassembly, parasite accumulation was biased towards a subordinate species and, by excluding stochastic assembly processes (i.e. chance dispersal and priority effects), we were able to determine that early assembly is deterministic. Later in the reassembly process, we established that host traits, as well as stochastic dispersal from the environmental pool of infective stages, can affect the community composition.

5. Overall, our results suggest that there is a high degree of resiliency and environmental dependence to the worm communities of buffalo. More generally, our data show that both deterministic and stochastic processes may play a role in the assembly of parasite communities of wild hosts, but their relative importance may vary temporally. Consequently, the best strategy for managing reassembling parasite communities may also need to shift over time.

Key-words: African buffalo, gastrointestinal nematode infection, host–parasite interactions, parasite community composition, reinfection dynamics

Introduction

Community assembly is a fundamental process that has long been a central focus in ecology (e.g. Gleason 1926; Clements 1938; Diamond 1975; Ricklefs 1987). Yet, the assembly processes governing the progression, resilience and stability of communities continue to be debated (reviewed in Weiher *et al.* 2011). We explored the community assembly patterns in a system that has received comparatively little attention, the parasites living within

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the hosts. Most organisms are infected with multiple parasite species (Petney & Andrews 1998), and both host fitness and parasite fitness are influenced by the presence of co-infecting parasites (Behnke *et al.* 2005; Telfer *et al.* 2010). Thus, in addition to informing our broader understanding of community assembly patterns, the processes shaping within-host parasite communities also have consequences for host population dynamics and disease transmission.

Understanding the factors underlying the community assembly remains a compelling issue in ecology because these processes shape the diversity and the stability of biological assemblages (Tilman 2004), and the predictability of community reestablishment after disruptions such as species invasions and environmental perturbations (Gleason 1926; Parker *et al.* 1999; Hubbell 2001; Tilman 2004). Community assembly processes can be deterministic, driven by environmental filtering and species interactions, or stochastic, resulting from chance colonization, priority effects or unpredictable disturbances (Chase & Myers 2011). In free-living communities, species are added to a habitat patch via dispersal from the regional species pool, but under deterministic assembly, some species will be filtered out because of incompatible abiotic (i.e. humidity or temperature) or biotic (i.e. competition) conditions (Clements 1938; Belyea & Lancaster 1999). In contrast, stochastic assembly processes include chance dispersal events without trait-based filtering and typically generate communities that are random subsets of the regional species pool. In some cases, chance dispersal is followed by priority effects where early arrivals gain a competitive advantage, and thus, not all stochastically assembled communities resemble the regional species pool (Gleason 1926; Diamond 1975). As a consequence of these distinct patterns, the structure of deterministic communities can often be predicted based on the knowledge of habitat traits and species needs, while the structure of stochastic communities cannot (Chase & Myers 2011).

For parasite communities, an individual host is a habitat patch and new infections represent successful dispersal events (Esch & Fernandez 1993). Parasite propagules harboured in or on other hosts or in the environment are analogous to the regional species pool. Since community assembly concepts are not taxon specific, these processes apply to all parasite types, although the notable differences in traits such as dispersal ability (e.g. ecto- vs. endoparasites) and capacity to reproduce within a habitat patch (e.g. micro- vs. microparasites) may create interesting differences in community assembly rates and structure. Just like free-living communities, parasite community assembly can be deterministic (i.e. shaped by host traits and/or species interactions) or stochastic (i.e. shaped by chance dispersal from the regional species pool and/or priority effects). For example, if parasite community assembly is deterministic and driven by environmental filtering, information about the habitat patch, in this case host traits such as age, sex or genotype, could be used to predict the susceptibility to new infections (i.e. community invisibility). Alternatively, if assembly is stochastic, susceptibility to new infections might be unpredictable because chance events dictate the order in which species establish, and one species might out-compete others simply due to priority effects (Johnson, de Roode & Fenton 2015). Furthermore, stochastic and deterministic processes can work in concert; a host's first infection may be a matter of chance, but the initial parasite may interact with subsequent species in a deterministic manner (e.g. trait-based competitive exclusion or facilitation via the modification of host immunity). Accordingly, understanding parasite community assembly can provide information on the predictability of re-infection following disturbance (e.g. clearance or treatment) and host susceptibility to invasion by novel parasites (Johnson, de Roode & Fenton 2015).

Despite the implications, knowledge of assembly processes in parasite communities lags far behind that of free-living species. Existing data suggest that parasite community structure can be highly variable, ranging from stochastic assemblages to strongly deterministic communities (Poulin 1996; Graham *et al.* 2009; Kennedy 2009; Krasnov *et al.* 2014). However, most parasite community assembly studies have examined the patterns of species co-occurrence using cross-sectional data sets, and whether it is possible to infer assembly processes from such observational data is controversial (Gotelli 2000; Timi & Poulin 2007; Fenton, Viney & Lello 2010; Dallas & Presley 2014). Because they provide empirical insight into assembly processes, experimental studies are frequently used for studying microbial, plant and animal communities (Segre *et al.* 2014; Dini-Andreote *et al.* 2015; Fayle *et al.* 2015) (Bender, Case & Gilpin 1984). Our best understanding of parasite communities comes from the combination of experimental, cross-sectional and longitudinal studies of trematode parasites in snail hosts; these communities are often deterministic assemblages structured by interspecific competition in predictable competitive hierarchies (Lie *et al.* 1968; Lim & Heyneman 1972; Kuris 1990; Sousa 1993). Whether this phenomenon is general across different host and parasite communities is unknown, in part, because experimental removal studies are rarely applied to parasite communities of wild hosts.

To advance our understanding of parasite community assembly in wild hosts, we used an experimental removal approach, supplemented with a longitudinal study, to test for evidence of specific community assembly processes. We took advantage of an anthelmintic treatment experiment that tracked free-ranging African buffalo (*Syncerus caffer*, Sparrman) in Kruger National Park, South Africa, over a 4-year period. Study animals were randomly assigned to a treated or control group at first capture, and treated animals were dosed with a slow-release fenbendazole bolus (Panacur, Intervet) at each recapture to eliminate gastrointestinal (GI) helminth ('worms' hereafter) infections. Study subjects were embedded within

much larger herds of non-study animals, representing between 4–9% of the total herd membership (Ezenwa & Jolles 2015). The GI worm community of buffalo in KNP is dominated by trichostrongyle nematodes (Budischak, Jolles & Ezenwa 2012; Budischak *et al.* 2015), which reproduce sexually inside the host and then pass eggs into the external environment via host faeces. The eggs hatch into larvae in the faeces and then disperse onto the surrounding vegetation where they get ingested by new hosts. Ingested larvae develop into adult worms in the host GI tract.

To address key questions about parasite community assembly, we tested whether reassembled and undisturbed communities differed by comparing parasite assemblages of anthelmintic-treated and control hosts. Next, we compared a cross-section of communities that reassembled for different periods of time to understand the temporal dynamics and resilience of GI worm communities. Finally, we tested for evidence of deterministic (environmental filtering, interspecific interactions) and stochastic (chance dispersal, priority effects) assembly processes that might underlie the differences between reassembled and control parasite communities. To evaluate the role of environmental filtering, we tested whether the differences among host traits (i.e. habitat patches) could explain the worm community composition. We searched for evidence of interactions among co-infecting parasite species by examining species co-occurrence patterns. Next, we determined whether chance dispersal could explain the parasite community composition by testing whether worm composition within host patches matched the availability of larvae on pasture (i.e. the regional species pool). Finally, we examined the temporal reassembly data for the patterns consistent with priority effects, specifically that early colonizers would become numerically dominant over time.

Materials and methods

COMPARING REASSEMBLED AND CONTROL COMMUNITIES

Over 200 female African buffalo were sampled from June 2008 to August 2012, and half were randomly assigned to receive treatment with a fenbendazole bolus delivered directly to the rumen. When administered at sufficient dosage, fenbendazole is 100% effective at killing adult and larval trichostrongyle nematodes (Williams 1991; Williams & Broussard 1995), and the slow-release bolus has an efficacy of approximately 160 days in African buffalo (Ezenwa *et al.* 2010). Study animals were re-captured at approximately 180-day intervals, and treated animals were re-dosed at each capture to clear any recolonizing worms. Each animal was captured 1–9 times throughout the study and animals lost to migration or mortality were replaced. At each capture, host traits including age, herd, condition, pregnancy status and aspects of immune function were assessed (see Ezenwa & Jolles 2015).

In addition, a subset of buffalo from the longitudinal study were euthanized and necropsied between 3rd July and 16th August 2012, and 33 individuals (9 treated and 24 control) were selected for cross-sectional parasite community analysis. To maximize the number of parasite communities sampled, we necropsied buffalo based on the presence of positive faecal egg counts, which screened for the following nematodes: trichostrongylids, *Trichuris* sp., *Strongyloides* sp. and cestodes: *Moniezia* sp. (Budischak *et al.* 2015). To sample adult worms, the abomasum and the small intestine of each infected animal were tied off post-mortem and removed from the carcass. GI tract contents were collected following the standard rinsing procedures and a 2·5% aliquot was preserved in 5% phosphate-buffered formalin. Formalin-fixed adult worm specimens were isolated from GI tract contents by rinsing the samples through 250- and 44-μm sieves. Specimens were counted and morphologically identified at the USDA Agricultural Research Service, US National Parasite Collection. Counts for each 2·5% sample were multiplied by 40 to estimate the total worm abundance (Budischak *et al.* 2015). No individuals were infected with *Trichuris* sp. or *Strongyloides* sp., and we excluded the cestode infection of a single host because these parasites could not be enumerated with the sampling methods used. Thus, our subsequent analyses were confined to trichostrongyle nematodes.

To determine whether reassembled communities differed from controls, we examined five measures of parasite community structure: total abundance, species-specific prevalence, Simpson's reciprocal diversity index ('diversity', hereafter), evenness and species richness (Simpson 1949; Magurran 2004; Oksanen *et al.* 2013) using the cross-sectional adult worm data set. Abundance is defined as the number of individuals of a given parasite species within a host, regardless of whether the host is infected, whereas prevalence refers to the proportion of infected hosts (Bush *et al.* 1997). Diversity and evenness are on scales of 0–1, where 1 represents high sample diversity or equal abundance of every species. Shapiro–Wilk tests on model residuals showed that abundance, diversity and evenness were not normally distributed, so Wilcoxon sign rank tests were used to compare the treated and control groups. We also determined whether the prevalence of each species differed between treated and control hosts using Z-tests. Since evenness cannot be calculated for communities with only 1 species, three host individuals (1 treated and 2 control) who had only 1 parasite species were dropped from evenness analyses. Richness was normally distributed, so analysis of variance was used to test for the differences in richness between groups. Finally, to account for sample size differences ($n = 9$ treated, 24 control) that might affect the species richness estimates, we used jackknife 1 rarefaction curves to examine the rates of species discovery and the effects of sample size on richness in treated and control hosts (Colwell 2013).

EXAMINING TEMPORAL REASSEMBLY PATTERNS

Using the cross-sectional data, we evaluated the relationship between time since most recent anthelmintic treatment (i.e. reassembly period) and four measures of community structure (total abundance, richness, diversity and evenness). To do so, we used linear regression analyses and excluded a single buffalo recaptured within a month of treatment since the interval was insufficient for drug efficacy. This point was also an extreme outlier for nematode diversity and evenness. In the remaining buffalo, reassembly periods ranged from 3 to 11 months. We ran identical linear regressions for control hosts to ensure that the interval between captures alone could not account for the

changes in parasite community composition. Finally, we visualized the resilience of treated communities by plotting the temporal trends in parasite community composition of treated hosts with the means from control hosts.

TESTING EFFECTS OF HOST TRAITS ON COMMUNITY ASSEMBLY

First, we tested whether deterministic assembly processes based on filtering by host traits (i.e. habitat patch differences) could explain the observed differences in community structure between treated and control communities. We considered only host traits that are not affected by the presence of worms (e.g. age, herd, sampling time [Julian date]), and excluded traits such as immune responses, host condition and pregnancy status because of the potential bidirectionality of interactions between these traits and worm infection. Using separate general linear models, we tested whether treated and control individuals in the cross-sectional data set differed with respect to age or sampling time, and chi-square tests were used to test for the differences in herd membership (Lower Sabie [LS] or Crocodile Bridge [CB] regions) between the treated and control groups.

Secondly, we used general linear models to test for the relationships between host traits (age, sampling time, herd) and the measures of parasite community composition (total abundance, *Haemonchus* sp. (*H*) abundance, *Cooperia fuelleborni* (Hung; *Cf*) abundance, diversity, evenness and richness) among the 24 control buffalo in the cross-sectional data set. Model residuals for diversity and total abundance and species-specific abundance were not normally distributed, so negative binomial generalized linear models were used in these cases. Due to the small sample size, we did not conduct similar analyses for treated buffalo.

Last, we examined the relationships between host traits and community structure among control buffalo sampled over the 4-year longitudinal study. These individuals were sublethally sampled for worm abundance and species composition at each capture. Faecal egg counts were used to quantify the total abundance of gastrointestinal tract worms. Species composition of worms shed in host faeces was determined by combining faecal egg count data with genetic identification of cultured larvae, a technique that provides accurate estimates of trichostrongyle abundance and species composition in African buffalo (Budischak *et al*. 2015). For each infected individual, we calculated five parasite community metrics, namely total abundance, species richness, diversity, evenness and species-specific abundance. We then examined the associations between these parasite community metrics and host traits, including age, herd (LS, CB or other [O]) and sampling time using general linear mixed models to account for repeated sampling of individuals. Sampling time was categorized by season (early dry: April–June; late dry: July–September; early wet: October–December; late wet: January–March).

EXAMINING THE ROLE OF SPECIES INTERACTIONS IN COMMUNITY ASSEMBLY

To test for the patterns consistent with the deterministic assembly due to interspecific interactions, in control communities, we examined the parasite abundance patterns using the cross-sectional adult worm data set and co-occurrence trends using the longitudinal larval data set. With the cross-sectional data set, we tested for a correlation between the abundance of *Cf* and *H* and then used general linear models to control for the effects of herd while alternately using each species as a response and predictor variable, a method shown to be an improvement over other co-occurrence tests for detecting species interactions (Fenton, Viney & Lello 2010). With the longitudinal data set, we used a repeated-measures chi-square test, the Cochran–Mantel–Haenszel test, to determine whether co-infection with the two most common nematodes occurred more or less likely than expected by chance. Less frequent co-occurrences than expected would provide preliminary evidence for competition, while more frequent co-infectious could indicate the facilitation, although other processes besides interspecific interactions could also create such patterns (Fenton, Viney & Lello 2010). We could not test for co-occurrence patterns using the cross-sectional (adult worm) data set because the low number of single infections (*H* only: $n = 1$; *Cf* only: $n = 1$) and the lack of uninfected individuals (a result of our sampling protocol) violated the minimum expected value assumption of the chi-square test. Likewise, we could not test for the abundance patterns with the longitudinal data set because species-specific abundance is a function of the total faecal egg count and is therefore not independent.

ENVIRONMENTAL AVAILABILITY AS A DRIVER OF COMMUNITY REASSEMBLY

To examine whether stochastic dispersal could explain the structure of reassembled communities, we first estimated the species composition of parasite propagules on pasture (i.e. the regional species pool). To estimate the environmental availability of infective larvae, we tracked the mean monthly parasite shedding in control buffalo using the longitudinal study population (104 individuals, 184 captures). Since buffalo are herd animals, and all study subjects resided in the same herds, we assumed that the 33 individuals sampled for the cross-sectional study were exposed to similar assemblages of parasites as were being released by the 104 control individuals tracked in the longitudinal study. Next, we compared the environmental larval exposure estimates of the two most prevalent nematode species to the adult worm community composition for the months corresponding to each host's reassembly period. For example, one cross-sectional buffalo was treated on 1 May 2012 and sampled for parasites on 8 August 2012, so we calculated the fraction of larvae of each species shed from May to July by control buffalo as an index of the regional species pool available to invade this open 'habitat patch'. Last, for each treated host, we used bootstrapping ($n = 1000$ replicates) to calculate the expected relative abundance of the two most common parasite species given the environmental availability of infective larvae. We ran separate simulations for each host to reflect its individual reassembly period and the total worm abundance. We compared the observed species composition data from each treated host to its bootstrap distribution to calculate the likelihood of observing such a composition given the estimated availability of infective stages in the environment.

Results

TREATED COMMUNITIES DIFFER FROM CONTROL COMMUNITIES

The cross-sectional adult worm data set showed that African buffalo were infected with seven species of

trichostrongyle nematodes. Parasite prevalence did not differ between control and treated hosts for any of these species (Table 1). Two species of *Haemonchus*: *H. placei* (Place) and *H. bedfordi* (Le Roux) were pooled because female specimens could not be reliably differentiated morphologically. *Cf* was the most prevalent and abundant species, infecting over 90% of individuals and comprising over 87% of all worms collected (Table 1). The two species of *Haemonchus* (*H*) infected almost 90% of buffalo, but at far lower abundance (Table 1). *Parabronema* sp. (*P*) was also common, infecting over 75% of hosts, and had relative abundance similar to *H* (Table 1). Relative abundance of *P* and *H* was higher in treated than in control buffalo. The other parasite species, *Trichostrongylus* sp. (*T*), *Africanastrongylus giganticus* (Hoberg, Abrams, and Pilitt; *Ag*) and *Africanastrongylus buceros* (Hoberg, Abrams and Ezenwa; *Ab*), were found at low prevalence and abundance in control communities (Table 1). *T* and *Ag* were absent from treated communities (Table 1). Species rarefaction curves suggest that at similar sample sizes, the same total species richness would be expected in the populations of treated and control hosts (Fig. 1). As such, the rare species missing from treated hosts (i.e. *T* and *Ag*) may simply be a result of the lower sample size of treated ($n = 9$) vs. control ($n = 24$) hosts.

Although group-level relative abundance and prevalence patterns were similar for treated and control hosts, there were some clear differences in parasite community composition between these groups at the individual host level. Total worm abundance was fourfold higher in control ($n = 24$) compared to treated buffalo ($n = 9$), and this difference was significant (Wilcoxon test: $W = 31{\cdot}5$, $P < 0{\cdot}001$; Fig. 2a). The difference was largely due to the higher abundance of *Cf* worms in control hosts ($W = 33$, $P = 0{\cdot}007$). No differences in the abundance of the other five parasite species were observed (*H*: $W = 98$, $P = 0{\cdot}95$; *P*: $W = 121$, $P = 0{\cdot}289$; *T*: $W = 88$, $P = 0{\cdot}44$; *Ab*: $W = 104$, $P = 0{\cdot}44$; *Ag*: $W = 92$, $P = 0{\cdot}61$). In terms of community structure, species richness ranged from 2 to 5 species for treated hosts and from 1 to 4 species for control hosts, but mean richness did not differ significantly between the two groups (ANOVA: $F_{1,31} = 0{\cdot}12$, $P = 0{\cdot}73$, Fig. 2b). However, on average, treated hosts had higher parasite diversity than control hosts (Wilcoxon test: $W = 119$, $P = 0{\cdot}032$; Fig. 2c). Similarly, evenness was higher in treated than in control hosts (treated: $n = 8$, control: $n = 22$, $W = 125$, $P = 0{\cdot}013$; Fig. 2d). Thus, although species richness was similar in both host groups, individual treated hosts had parasite communities that were less abundant, more diverse and more even than control hosts.

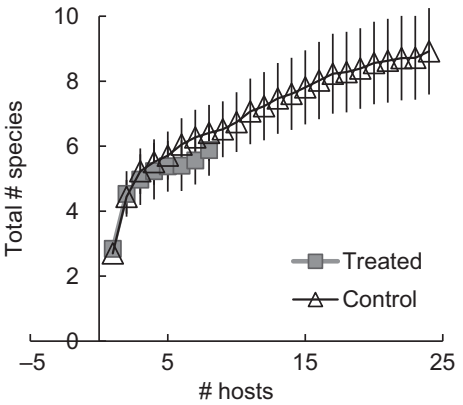

Fig. 1. Jackknife estimates (±1 SD) of species rarefaction curves for treated and control hosts by sample size.

REASSEMBLY PERIOD EXPLAINS THE DIFFERENCES BETWEEN TREATED AND CONTROL COMMUNITIES

With increasing time since the experimental removal, the parasite communities of treated hosts more closely resembled control communities. Among the cross-sectional hosts, worm abundance increased with time since last capture (linear regression, $n = 8$; $R^2 = 0{\cdot}45$, $t = 2{\cdot}61$, $P = 0{\cdot}04$), reflecting an estimated $35{\cdot}3 \pm 13{\cdot}5$ new infections per treated buffalo per day (Fig. 3a). Over time, the total abundance in treated hosts approached the levels in control hosts, but still fell short by over 3500 worms after 328 days (Fig 3a). There was no relationship between time since treatment and richness ($R^2 = 0{\cdot}09$, $t = -0{\cdot}78$, $P = 0{\cdot}47$; Fig. 3b), but the reassembly period was strongly and negatively correlated with diversity ($R^2 = 0{\cdot}80$, $t = -4{\cdot}43$, $P = 0{\cdot}007$; Fig. 3c). Reassembly period was

Table 1. Prevalence and relative abundance of nematodes for control ($n = 24$ buffalo, $n = 7295$ worms) and anthelmintic-treated ($n = 9$ buffalo, $n = 677$ worms) buffalo

	Prevalence (%)				Relative abundance (%)	
	Control	Treated	*Z* score	*P*-value	Control	Treated
Cooperia fuelleborni	96	89	−0·84	0·40	97	87
Haemonchus sp.	88	88	0	1	1·4	5·8
Parabronema sp.	88	75	0·74	0·46	1·1	5·3
Trichostrongylus sp.	8·3	0	−0·84	0·40	0·055	0
Africanastrongylus giganticus	4·2	0	−0·59	0·56	0·014	0
Africanastrongylus buceros	4·2	11	0·84	0·40	0·014	0·15

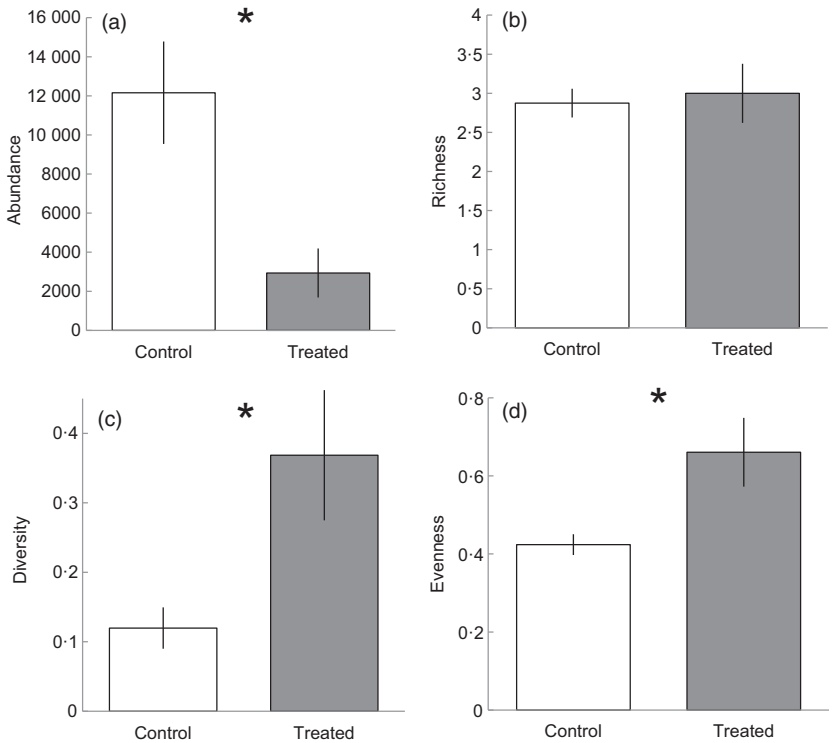

Fig. 2. Community composition metrics (±1 SE), for treated and control hosts, including (a) total abundance of adult worms, (b) species richness, (c) Simpson diversity and (d) evenness. Asterisk (*) indicates significant differences ($P < 0{\cdot}05$).

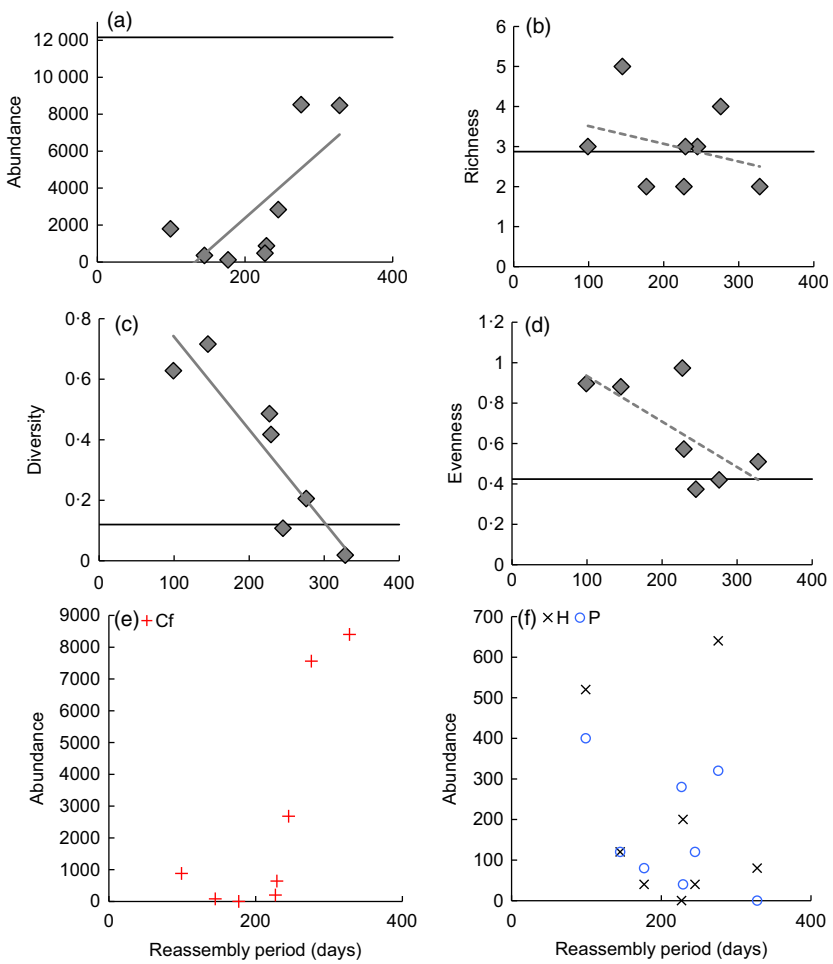

Fig. 3. Relationship between the reassembly period (days since last anthelmintic treatment) and (a) total abundance, (b) species richness, (c) Simpson diversity index, (d) evenness and species-specific abundance of (e) *Cooperia*, (f) *Haemonchus* and (f) *Parabronema*, adult worms in treated hosts. Solid lines indicate significant correlations and dashed lines indicate non-significant correlations. Horizontal black lines (a–d) indicate means for controls.

also negatively correlated with evenness, although this association was not statistically significant ($n = 7$; $R^2 = 0{\cdot}38$, $t = -2{\cdot}18$, $P = 0{\cdot}08$; Fig. 3d), suggesting that communities might become less even over time. In recolonized hosts, both richness and evenness reached average levels for control hosts by approximately 300 days post-treatment (Fig. 3). These patterns were driven by higher *Cf* abundance in hosts with a greater time interval since treatment, suggesting that hosts disproportionately accumulated *Cf* over time (Fig. 3e). Abundance of *H* and *P*, by contrast, did not vary with reassembly period (Fig. 3f). Importantly, among control hosts, capture interval was not related to any measure of parasite community composition ($n = 24$; abundance: $t = 0{\cdot}07$, $P = 0{\cdot}51$; richness: $t = -0{\cdot}05$, $P = 0{\cdot}96$; diversity: $t = -0{\cdot}73$, $P = 0{\cdot}47$; evenness: $n = 22$, $t = -0{\cdot}23$, $P = 0{\cdot}82$), suggesting that the patterns we observed in treated hosts were due to the process of reassembly and not to other factors associated with capture interval.

HOST TRAITS AS PREDICTORS OF PARASITE COMMUNITY STRUCTURE

In the cross-sectional study, treated ($n = 9$) and control ($n = 24$) hosts did not differ with respect to age (general linear model; Est = 10·3, t = 14·8, $P = 0{\cdot}49$), herd ($\chi^2 = 0{\cdot}034$, $P = 0{\cdot}85$) or sampling date (Est = 0·65, $t = 0{\cdot}11$, $P = 0{\cdot}91$), so these traits are insufficient to explain the differences observed between treated and control communities. However, when we looked at only controls, we found evidence that some host traits may affect the parasite community structure. Specifically, animals in the LS herd had lower *H* abundance and diversity than animals in the CB herd, and younger individuals had higher total and *Cf* abundance (Table 2); however, sampling time within the 5-week sampling window did not affect any aspect of parasite community structure (Table 2).

An effect of host traits on parasite community structure also emerged when we examined 104 control animals that were longitudinally sampled for larval worms. Trichostrongyle nematodes were the most common and abundant worms found, with all other worm species detected in <0·5% of faecal samples over the 4-year study period. Total abundance, *H* abundance and diversity decreased with age, while all the parasite community metrics, except for *Cf* abundance, varied among herds (Table 3). Similarly, all community metrics varied with sampling time except for *Cf* abundance and evenness (Table 3).

SPECIES INTERACTIONS AS DRIVERS OF PARASITE COMMUNITY STRUCTURE

Based on the temporal reassembly pattern whereby hosts preferentially accumulated *Cf* with increasing time since experimental clearance (Fig. 3e), we hypothesized that *Cf* might be a superior competitor and prevent additional *H* infections. However, for control animals from the cross-sectional study, the abundance of *Cf* and *H* adults was not correlated (Pearson's correlation: $n = 24$, $r = 0{\cdot}054$, $t = 0{\cdot}25$, d.f. = 22, $P = 0{\cdot}80$). This pattern did not change for either parasite species when herd was taken into account; *H* abundance was not a significant predictor of *Cf* abundance (general linear model, $n = 24$; *H*: Est = 5·87, $t = 0{\cdot}63$, $P = 0{\cdot}54$; herd: Est = 140, $t = 0{\cdot}87$, $P = 0{\cdot}39$), and vice versa (*Cf*: Est = 0·003, $t = 0{\cdot}63$, $P = 0{\cdot}54$; herd: Est = −8·36, $t = -2{\cdot}51$, $P = 0{\cdot}02$). Furthermore, contrary to our predictions, for animals from the longitudinal study, *Cf* and *H* co-occurred more often than expected by chance (Cochran–Mantel–Haenszel test, $n = 104$ individuals, 184 observations; odds ratio >44, $S = 206$, $P < 0{\cdot}0001$).

Table 2. Relationships between host traits and measures of parasite community composition among focal control buffalo sampled for adult worms. Analyses are negative binomial generalized linear models (abundance and diversity, d.f. = 3, 20) or general linear models (evenness: d.f. = 3,18; richness: d.f. = 3, 20)

	Total abundance			*Haemonchus* sp. abundance			*Cooperia fuelleborni* abundance		
	Estimate	*z* value	*P*-value	Estimate	*z* value	*P*-value	Estimate	*z* value	*P*-value
Intercept	−184	−1·05	0·30	−251	−1·33	0·18	−192	−0·89	0·37
Age	−0·012	−2·52	0·012*	0·0082	1·63	0·10	−0·013	−2·16	0·031*
Herd (LS)	0·31	0·78	0·44	−1·425	−3·51	0·0004***	0·33	0·68	0·50
Sampling time	0·016	1·08	0·28	0·021	1·34	0·18	0·016	0·92	0·36
	Diversity			Evenness			Richness		
	Estimate	*z* value	*P*-value	Estimate	*t* value	*P*-value	Estimate	*t* value	*P*-value
Intercept	87·3	0·42	0·68	18·0	0·69	0·50	−77·4	−0·41	0·69
Age	0·0095	1·74	0·082	0·0007	0·98	0·34	0·0023	0·45	0·66
Herd (LS)	−1·47	−3·20	0·0014**	−0·090	−1·49	0·15	−0·35	−0·82	0·42
Sampling time	−0·0070	−0·41	0·68	−0·0014	−0·68	0·51	0·0066	0·43	0·67

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3. Relationships between host traits and measures of parasite community composition among control, but infected, buffalo over the 4-year longitudinal study. Analyses are generalized linear mixed models with animal ID as a random effect. Sampling time is categorized by season: early wet = EW, late dry = LD, late wet = LW

	Total abundance				*Haemonchus* sp. abundance				*Cooperia fuelleborni* abundance			
	Estimate	d.f.	*t* value	*P*-value	Estimate	d.f.	*t* value	*P*-value	Estimate	d.f.	*t* value	*P*-value
Intercept	1·54	710	11·9	<0·0001***	37·7	243	5·22	<0·0001***	177	274	4·28	<0·0001***
Age	−0·006	620	−3·94	<0·0001***	−0·236	142	−2·68	0·0083**	0·075	205	0·15	0·88
Herd (LS)	−0·305	541	−3·14	0·002**	−24·4	143	−4·73	<0·0001***	56·1	205	1·91	0·058
Herd (O)	−0·013	825	−0·11	0·92	−10·5	168	−1·89	0·060	65·0	219	2·05	0·042*
Samp. time (EW)	0·279	923	3·44	0·0006***	20·1	260	3·91	0·0001***	−45·5	285	−1·54	0·13
Samp. time (LD)	0·132	883	1·67	0·096	3·05	258	0·57	0·57	22·0	285	0·71	0·48
Samp. time (LW)	0·047	884	0·52	0·60	3·10	272	0·49	0·63	61·9	292	1·68	0·093

	Diversity				Evenness				Richness[1]		
	Estimate	d.f.	*t* value	*P*-value	Estimate	d.f.	*t* value	*P*-value	Estimate	*z* value	*P*-value
Intercept	0·230	260	7·50	<0·0001***	0·637	195	18·4	<0·0001***	0·713	4·53	<0·0001***
Age	−0·001	174	−3·26	0·0014**	−0·0002	195	−0·65	0·52	−0·003	−1·43	0·15
Herd (LS)	−0·126	172	−5·84	<0·0001***	−0·062	195	−2·68	0·0080**	−0·237	−2·22	0·027*
Herd (O)	−0·090	179	−3·84	0·0002***	−0·016	195	−0·65	0·52	−0·206	−1·78	0·074
Samp. time (EW)	0·085	280	3·82	0·0002***	−0·005	195	−0·18	0·86	0·254	2·10	0·035*
Samp. time (LD)	0·040	280	1·74	0·083	−0·010	195	−0·35	0·72	0·145	1·13	0·26
Samp. time (LW)	0·012	291	0·43	0·67	−0·053	195	−1·59	0·11	0·139	0·93	0·35

[1]Poisson generalized linear mixed model.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

REASSEMBLED COMMUNITY RESEMBLANCE TO ENVIRONMENTAL EXPOSURE VARIES TEMPORALLY

Environmental parasite availability did not match the patterns of parasite community composition within hosts during the early stages of the reassembly process. Three of the four hosts sampled within 7 months (214 days) of treatment had significantly higher proportional abundance of *H* and lower proportional abundance of *Cf* than expected based on the availability of parasites in the environment (*P*'s < 0·014; Fig. 4). However, by approximately 8 months (244 days) after the treatment, observed *H* abundance in reassembled communities no longer exceeded expected values ($n = 3$ hosts, *P*'s > 0·34), and *H* abundance even fell below expectations in the host with the longest capture interval ($P < 0.001$). These patterns suggest that reassembled worm communities differed from expectations early during the reassembly process, and did not reflect the patterns of environmental availability until several months post-treatment.

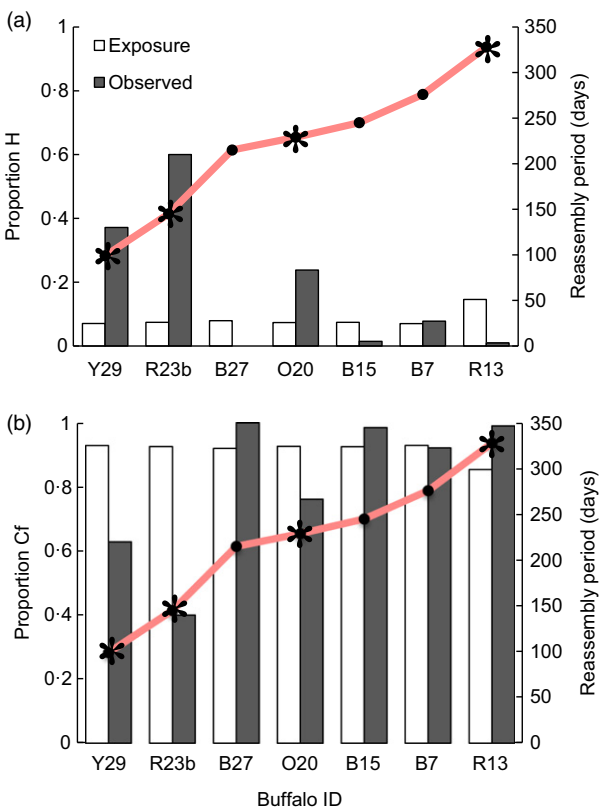

Fig. 4. Observed adult worm community composition in comparison with environmental availability of infective larvae. The line indicates reassembly period. Asterisk (*) indicates significant differences between observed and expected relative abundance estimates for each buffalo (one test for both species).

Discussion

We used an anthelmintic treatment to examine the patterns of parasite community assembly in free-ranging African buffalo, and then evaluated different assembly processes that could have generated these patterns. On average, reassembled parasite communities had lower abundance, but were more diverse and more even. Notably, our data suggest that the assembly processes shaping community composition may vary temporally. During

early reassembly, parasite accumulation was biased towards a rare species and we were able to exclude stochastic assembly processes (i.e. chance dispersal and priority effects). We also found no evidence of deterministic processes (i.e. host traits, species interactions) shaping early community assembly, but our ability to test for these deterministic processes was weak due to the low sample sizes. Later in the reassembly process, and in control communities, we established that host traits (i.e. habitat patch characteristics) can affect the community composition. Moreover, reassembled communities progressively resembled the environmental availability of infective stages over time (i.e. the regional species pool), suggesting that stochastic dispersal, in addition to filtering by host traits, may shape later parasite community assembly processes. This research highlights the complexity of parasite community assembly and the insights that can emerge from the integration of experimental, cross-sectional and longitudinal studies.

Since parasite community structure can vary spatially, temporally and among populations (Gotelli & Rohde 2002; Krasnov *et al.* 2011), it is not surprising that any two groups of hosts differ in parasite community structure. Notably, our experimental removal treatment allowed us to attribute observed differences in abundance, diversity and evenness to the process of community assembly. In our experiment, species richness was less sensitive to reassembly; average species richness of parasite communities did not differ between intact and experimentally perturbed communities, and rarefaction indicated that total species richness was similar between treated and control hosts after accounting for sample size. Over time, worm communities of treated hosts were increasingly comprised of one predominant species (*Cf*). However, early in the reassembly process, treated hosts had higher abundance of a subordinate species (*H*) than control hosts. The observed temporal change in infection could also arise if *H* is less sensitive to fenbendazole treatment than *Cf*, but livestock efficacy trials suggest that both species are equally susceptible to the drug (Williams 1991; Williams & Broussard 1995).

Despite the differences in mean community structure observed between treated and control hosts, our temporal data suggest that the parasite communities of buffalo were highly resilient to anthelmintic treatment; by 8 months post-treatment, treated hosts regained 75% of the mean abundance observed in controls and had nearly identical richness, diversity and evenness indices. Similar to our study, an anthelmintic treatment experiment in Soay sheep showed that hosts regained 87% of the mean GI nematode burden compared to control individuals at 6 months post-treatment (Craig *et al.* 2009). In contrast to our study, treated sheep also had less diverse nematode communities than controls after 6 months, while we found that treated buffalo had more diverse nematode communities up until approximately 7 months post-treatment. Despite our small sample size of treated hosts, the decline in community diversity with increasing reassembly period was very strong. These contrasting results are interesting because they demonstrate that the reassembly of similar parasite types (e.g. trichostrongyle nematodes) can vary greatly among host species, further emphasizing the need for additional experimental studies of parasite community assembly in free-ranging hosts.

Host traits, just like environmental conditions for free-living species, can affect the process of community assembly in a deterministic manner by filtering parasitic species based on their habitat requirements. Indeed, our data from control communities establish that each of the host traits we examined can affect the community composition. Younger buffalo had more abundant communities, a pattern commonly observed for nematode infections (Loukas, Constant & Bethony 2005; Verschave *et al.* 2014), including *Haemonchus* sp. and *Cooperia* sp. infections in livestock (Urquhart *et al.* 1966; Kloosterman, Ploeger & Frankena 1991). Likewise, seasonal variation in infection is common in wild ungulates (Turner & Getz 2010; Cizauskas *et al.* 2015), including buffalo (Gorsich, Ezenwa & Jolles 2014). The observed variation in worm community composition by herd was not surprising given that the focal herds differ in many factors, including body condition, survival, immunity and parasite prevalence and intensity (Ezenwa & Jolles 2015). Importantly, although host traits explained some variance in worm community composition among control animals, treated and control hosts did not differ significantly by age, herd membership or sampling period so these factors cannot explain the differences we observed between treated and control assemblages. This result does not exclude the possibility that host traits play a role early in community reassembly, but the direct examination of filtering by host traits would require a larger sample size of treated hosts. Moreover, the importance of filtering processes may vary with assembly time, so the patterns observed in control hosts may not reflect the processes occurring early in community reassembly.

The reassembly pattern where hosts preferentially accumulated *Cf* with increasing time since experimental clearance could potentially result from interspecific competition, another deterministic process. Competition-colonization theory predicts that dominant competitors will eventually out-compete other species in communities, erasing the differences in composition due to priority effects and the timing of disturbances (Tilman 2004). Thus, the pattern we observed could arise if the subordinate species (*H*) is a superior invader, but inferior competitor compared to the dominant species (*Cf*). In addition to having identical transmission modes, these two parasites species have similar development times in the environment and within hosts (Bowman 2009), so it seems unlikely that the pattern consistent with higher invasion potential of *H* is due to faster development. Furthermore, we found no evidence of competitive exclusion in the longitudinal study, but rather that the two species

co-occurred more often than expected by chance. Facilitation of one species by the other, potentially via host immune suppression, could explain this positive co-occurrence pattern (Lello *et al*. 2004; Maizels *et al*. 2012). However, *H* infections were generally of lower intensity and our sublethal sampling techniques may have failed to detect some single-species infections, artificially inflating the number of co-infections. Similar to interspecific competition, the temporal reassembly pattern whereby *H* abundance stagnated while *Cf* increased could have resulted from intraspecific competition among *H* adults and/or incoming larvae. Experimental data are needed to distinguish between the roles of inter- and intraspecific competition during community assembly. Overall, we detected no strong evidence of interspecific interactions that could explain the reassembly patterns. However, since we could only crudely test for species interactions in control communities, which our temporal assembly data suggest may have weaker species interactions than newly reassembled communities, a decline in interspecific competition over time is not improbable. For instance, immune stimulation during early reassembly could cause stronger immune-mediated interspecific interactions early in the reassembly process generating the observed temporal pattern.

Treated hosts were exposed to the regional pool of parasites in the environment, and if chance dispersal, a stochastic assembly process, drives community composition, we would have expected to see community compositions early in the reassembly process reflect the environmental availability. Furthermore, stochastic assembly followed by priority effects would lead to early colonizers dominating community composition. After accounting for temporal variation in larval exposure among hosts, we found evidence that communities in the early stages of reassembly differed from the environmental supply of parasites. Contrary to the predictions based on priority effects, the abundance of the subordinate species (*H*) was higher than expected based on the environmental availability of larvae during early reassembly, while over longer time-scales, treated hosts gained the dominant species (*Cf*), and their communities better reflected the estimated environmental availability of parasites. Thus, the observed temporal changes in community structure likely indicate the differences in infection processes within hosts (e.g. interspecific interactions, differential host susceptibility) soon after experimental removal, rather than the differences in exposure or priority effects. However, after longer time periods, stochastic dispersal from the regional pool of larvae may better explain the observed community composition patterns.

CONCLUSIONS

Our data suggest that both deterministic and stochastic processes may play a role in the assembly of parasite communities of African buffalo, but the relative importance of different processes may change through time. During early reassembly, we could exclude stochastic assembly processes but, due to the small sample size, our data were insufficient to identify the deterministic driver(s) of reassembly since we could not exclude filtering based on host traits or interspecific interactions. Later in reassembly and among control hosts, communities matched expectations of stochastic dispersal, but we also found evidence of deterministic, environmental filtering based on host age, herd and sampling time. Over time, reassembled communities began to closely resemble those of controls and the environmental availability of infective larvae, demonstrating their resilience following anthelmintic treatment. From a management perspective, it is important to understand the speed and resilience of parasite community reassembly since perturbations can potentially shift parasite communities towards more or less pathogenic combinations. In buffalo, for example, *H* is far more pathogenic than *Cf*, while high *Cf* abundance is associated with positive outcomes such as better condition, earlier onset of reproduction and higher survival (Budischak, Jolles & Ezenwa 2012; S. A. Budischak, A. E. Jolles & V. O. Ezenwa unpublished data). Therefore, our reassembly data suggest that parasite communities may potentially be more pathogenic during early recolonization. Importantly, our results suggest that since the drivers of assembly within a single system may not be static, the best strategy for managing parasite communities may also need to shift over time. Overall, this study highlights the dynamic nature of parasite community assembly and underscores the need for additional experimental studies in wild hosts to better understand parasite community assembly processes and the generality these patterns in natural populations.

Data accessibility

Data are available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.m00v5 (Budischak *et al*. 2016).

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