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RESEARCH ARTICLE

Within-host and external environments differentially shape β-diversity across parasite life stages

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Abstract

1. Uncovering drivers of community assembly is a key aspect of learning how biological communities function. Drivers of community similarity can be especially useful in this task as they affect assemblage-level changes that lead to differences in species diversity between habitats. Concepts of β-diversity originally developed for use in free-living communities have been widely applied to parasite communities to gain insight into how infection risk changes with local conditions by comparing parasite communities across abiotic and biotic gradients.
2. Factors shaping β-diversity in communities of immature parasites, such as larvae, are largely unknown. This is a key knowledge gap as larvae are frequently the infective life-stage and understanding variation in these larval communities is thus key for disease prevention. Our goal was to uncover links between β-diversity of parasite communities at different life stages; therefore, we used gastrointestinal nematodes infecting African buffalo in Kruger National Park, South Africa, to investigate within-host and extra-host drivers of adult and larval parasite community similarity.
3. We employed a cross-sectional approach using PERMANOVA that examined each worm community at a single time point to assess independent drivers of β-diversity in larvae and adults as well as a longitudinal approach with path analysis where adult and larval communities from the same host were compared to better link drivers of β-diversity between these two life stages.
4. Using the cross-sectional approach, we generally found that intrinsic, within-host traits had significant effects on β-diversity of adult nematode communities, while extrinsic, extra-host variables had significant effects on β-diversity of larval nematode communities. However, the longitudinal approach provided evidence that intrinsic, within-host factors affected the larval community indirectly via the adult community.
5. Our results provide key data for the comparison of community-level processes where adult and immature stages inhabit vastly different habitats (i.e. within-host vs. abiotic environment). In the context of parasitism, this helps elucidate host

infection risk via larval stages and the drivers that shape persistence of adult parasite assemblages, both of which are useful for predicting and preventing infectious disease.

KEYWORDS
Nematoda, species replacement, *Syncerus caffer*, turnover

1 | INTRODUCTION

Understanding drivers of community composition remains a core subject of interest in community ecology (Buckley & Jetz, 2008; Shade, 2017) because uncovering how local communities are formed from larger species pools is key to understanding how biological communities function. Thus, β-diversity, or the ratio between regional and local species diversity (Whittaker, 1960), is useful as it can quantify the changes in species richness and species gain and loss that lead to differences in species diversity among habitats. For example, comparing patterns of β-diversity across various biotic and abiotic conditions, such as rainfall or habitat gradients has helped identify local environmental factors that determine community composition and structure across a variety of free-living taxa (e.g. Bishop et al., 2015; Fernandez-Going et al., 2013).

Similarly, insight into how parasite infection risk changes with local environmental conditions can be gained by comparing parasite communities across abiotic and biotic gradients. Indeed, concepts of β-diversity originally developed for use in free-living communities have been widely applied to parasite communities. For example, communities of adult parasites show varying patterns of β-diversity depending on geographic (Locke et al., 2012; Moss et al., 2020; Poulin, 2003) and environmental differences such as habitat structure and abiotic conditions (Blanar et al., 2016; Poulin et al., 2011; Warburton, Kohler, et al., 2016). Interestingly, immature stages of many of these same parasites are often the life stage responsible for transmission from one host to another; however, few studies have explicitly examined factors shaping β-diversity in this group.(see Thieltges et al., 2009 and Moss et al., 2020 for exceptions), despite the fact that understanding variation in immature parasite communities may be key for disease prevention (Murray et al., 2018).

Adult parasites are either totally (e.g. endoparasites) or partially (e.g. ectoparasites) reliant on their hosts. Consequently, this parasite life stage is subject to host immunological and physiological conditions, as well as host behaviours, like grooming, which can limit parasite survival and reproduction (Currie & Tahmasbi, 2008; Kemper et al., 2010). In contrast, the immature stages of key parasite groups, such as directly-transmitted nematodes and most ectoparasites, are not reliant on the host for habitat or other resources because they are free-living in the external environment. For example, the immature stages of many nematode species of medical and veterinary concern develop in faeces and soil (Larsen & Roepstorff, 1999). Likewise, the larvae of fleas feed on detritus in the host burrow while pupal stages are non-feeding (Krasnov et al., 2008). Thus, unlike the adults of these species, the immature parasites are often not dependent on close contact with a host. Instead, abiotic conditions, such as temperature and relative humidity, are the factors critical for their development and survival (Arene, 1986; Krasnov et al., 2001). This means that the adult and immature stages of these parasites, even though they are of the same species and in same locality, often experience very different local conditions, with adults more subject to the intrinsic factors of the within-host environment and immatures more subject to the extrinsic factors of the extra-host (i.e. abiotic) environment.

Although β-diversity of immature parasite stages is poorly understood, these parasite stages are functionally analogous to free-living species that have very different adult and larval stages, such as holometabolous insects or amphibians, two groups for which patterns of β-diversity in immatures have been examined. Among larval dragonflies and damselflies (i.e. odonates), for example, environmental conditions, such as water temperature, dissolved oxygen, altitude, and rainfall affect β-diversity (Mendes et al., 2021), while β-diversity of larvae amphibians is often related to habitat characteristics, such as pond size or vegetation structure, as well as climatic variables, such as seasonal temperature and precipitation (Knauth et al., 2019). Thus, these types of abiotic conditions are also likely to be important drivers of β-diversity in directly-transmitted immature parasites.

Adult parasites are intimately associated with the host; consequently, their survival and fecundity depend on host physiological and immune responses (Khokhlova et al., 2009; Tschirren et al., 2004). The shift in habitat and selective pressures experienced by adult parasites is once again analogous to the shift to terrestrial habitat experienced by adult amphibians and odonates. Adult amphibian β-diversity is strongly affected by dispersal and environmental filtering (e.g. da Silva et al., 2014), and habitat structure, particularly the presence of suitable vegetation, affects the β-diversity of adult odonates (Barzoki et al., 2021; Cleary et al., 2004). This type of filtering is parallel to a parasite finding a host (=habitat) with suitable physiological and immune responses. However, feedback between the adult and immature communities seems likely. The adult community and the habitat effects it experiences should shape the future immature community via differential reproduction, while the current immature community and environmental conditions it experiences should shape the future adult community via differential survival. However, the literature currently lacks any comprehensive comparison of the drivers of β-diversity in co-occurring communities of immature and adult life stages for any free-living or

parasitic species. Therefore, our understanding of the relative contributions of abiotic conditions or habitat suitability versus life-stage feedbacks toward shaping community structure of adult and immature organisms is limited.

In this study, our goal was to determine how the intrinsic, within-host and extrinsic extra-host factors influenced β-diversity at different parasite life history stages, adult and immature. Focusing on strongyle nematodes (Nematoda: Trichostronylidae) infecting the gastrointestinal (GI) tract of wild African buffalo (*Syncerus caffer*), we characterized β-diversity (Baselga & Orme, 2012), for both adult and immature communities, and investigated the links between host traits, abiotic conditions and β-diversity for each life stage. We predicted that (1) within-host factors would have a greater effect on adult nematode communities than extra-host ones because adult worms live within the host; (2) extra-host factors would have a greater effect on larval nematode communities because this life stage persists in host faeces and is directly subject to abiotic conditions and (3) there would be feedback effects of within-host factors on the larval community because the adult community begets the immature community.

2 | MATERIALS AND METHODS

2.1 | Study system

We used GI nematodes infecting African buffalo in Kruger National Park (KNP), South Africa to investigate drivers of adult and larval parasite community similarity. African buffalo are gregarious herbivores ubiquitous across sub-Saharan Africa. Buffalo also host a range of parasites and pathogens, including a variety of GI helminths (Ezenwa et al., 2019). Many of the helminths infecting African buffalo are strongyle nematodes that reside in the GI tract of their hosts where they mate and produce eggs. Eggs are released into the external environment with host faeces, where they hatch and develop into larvae. Egg hatching success is highly dependent on favourable temperatures and larval development rates depend on an appropriate combination of temperature and moisture conditions (O'Connor et al., 2006). Once larvae undergo several rounds of development and moulting, they become infective, third stage larvae (L3) that can be ingested by grazing hosts (O'Connor et al., 2006). After ingestion, L3s mature into adults in the host GI tract.

Given the life cycle of strongyle nematodes, adult and larval parasites may be subject to different selective pressures and there is potential for tight linkages between communities of different life stages. First, adult fecundity, as mediated by within-host factors, determines the egg community dispersed into the external environment. Second, egg and larval survival, as mediated by the extra-host factors pre-infection, coupled with post-infection larval survival mediated by within-host factors, together determine recruitment into the future adult community (Paterson & Viney, 2002). Thus, we tested our study predictions using two separate approaches: (1) a cross-sectional approach that examined each community at a single time point to assess independent drivers of β-diversity in infective larvae versus adults and (2) a longitudinal approach where adult communities, as well as progenitor and descendant infective larval communities, were compared to better link drivers of β-diversity between the two life stages. All animal protocols were approved by the University of Georgia (UGA) and Oregon State University (OSU) Institutional Animal Care and Use Committees (UGA AUP A2013 08-017-Y1-A0 and A2010 10-190-Y3-A5; OSU AUP 3822 and 4325).

2.2 | Host sampling

Female buffalo were sampled from June 2008 to August 2012 as part of a longitudinal anthelmintic treatment study (Ezenwa & Jolles, 2015). For the purposes of this work, only data from control (i.e. untreated) animals were used. Study individuals were captured, fitted with radio-collars to facilitate re-capture, and then sampled approximately every 6 months from two herds in the southern portion of KNP: Lower Sabie (LS) and Crocodile Bridge (CB). Data on herd (LS or CB), year, and season (i.e. wet versus dry) of capture provided information on abiotic conditions (e.g. temperature, humidity) experienced by the buffalo and larval nematodes. Body condition, age and nematode resistance genotype were host traits used to capture information on within-host factors affecting adult nematodes. Age was assessed using incisor eruption and wear patterns (Jolles, 2007), and body condition was measured via manual palpation and visual assessment of body fat using a scoring system ranging from 1 to 5 (Ezenwa et al., 2009). Nematode resistance genotype was identified and assigned to each animal based the presence or absence of the BL4-141 allele, an allele at the BL4 locus, a region near the interferon gamma gene, that is associated with phenotypic variation in the strongyle faecal egg count (FEC) of buffalo (Ezenwa et al., 2021). Crucially, phenotypic variation in FEC is associated anti-worm immunity in buffalo, thus the resistance genotype trait provides a reasonable proxy for the level of anti-worm defence mounted by different individuals (Ezenwa et al., 2021).

2.3 | Parasite sampling

To describe the infective larvae community hosted by individual buffalo, faecal samples collected from animals at capture were cultured and descendant larvae were identified using molecular barcoding as described in Budischak et al. (2015). Faeces collected from buffalo were cultured indoors at ambient temperature and humidity for approximately 10 days, and then L3 larvae were collected using a modified Baermann technique (Archie & Ezenwa, 2011). Since our indoor larval culturing conditions captured seasonal variation in ambient temperature and humidity in KNP, we used the larvae communities derived from our cultures as

a reasonable proxy for larvae derived from faecal pats on pasture. DNA was extracted from 5 to 45 individual larvae per faecal sample and sequenced at the ITS-2 region for species identification (Budischak et al., 2015), and only sequences with quality of 80% or greater were used for species identification (Ankola et al., 2021). Since individual buffalo were sampled longitudinally and each animal was sampled an average of 3.8 times (range 1–9), a single sampling point was chosen at random per host individual using a random number generator to obtain a cross-sectional sample of the larval community. This procedure resulted in a cross-sectional sample of 86 different immature worm communities comprised of nine different nematode taxa (Table S1) all collected from unique buffalo hosts.

To characterize the adult worm communities of each individual host, we used material from 33 female buffalo euthanized between 3 July and 18 August 2012 following South African National Parks (SANParks) Standard Operating Procedure for Lethal Population Control. These study animals originated from both the LS ($n = 16$) and CB ($n = 17$) herds. Data on individual host traits (e.g. age, condition) were collected post-mortem. Sampling of adult worms is described in Budischak et al. (2016). In short, the GI tract was removed from each carcass and the abomasum and small intestine were separated into sections. The contents of each section were collected following a standard rinsing protocol and a 2.5% aliquot of the resulting material was preserved in 5% phosphate-buffered formalin. Worms were isolated from GI tract contents and counted, then were morphologically identified at the USDA Agricultural Research Service, US National Parasite Collection (Budischak et al., 2015). Six different adult nematode taxa were isolated (Table S2). Because adult female *Haemonchus placei* are morphologically indistinguishable from *Haemonchus bedfordi* these two species were grouped together as *Haemonchus* sp. in all datasets. Likewise, undescribed *Parabronema* and *Trichostrongylus* species were classified according to their genus names. Overall, this resulted in a cross-sectional dataset of 33 distinct adult worm communities collected from individual buffalo.

Finally, 12 of the 33 buffalo with adult worm communities also had data on their progenitor and descendant larval communities, which was used to generate a longitudinal dataset for examining links between drivers of β-diversity across parasite life stages. Progenitor larvae refer to the community of worms hosted by members of the same herd at a time point prior to host necropsy and represent a proxy for the infective nematode community that each host was exposed to before the adult community was assessed via necropsy. Descendant larvae refer to L3s that were cultured directly from an individual host's faeces collected at necropsy and thus represent the offspring of the adult nematode community infecting that individual buffalo. A proxy for the progenitor larval community to which a focal host was exposed was derived from sampling a focal host's herd mates an average of 1.7 years (range: 0.6 to 2.8 years) prior to its necropsy date. Each progenitor larval community was generated by sampling 11–24 individuals (average 13.42) from the same herd as the focal host.

2.4 | Statistical analyses

2.4.1 | Cross-sectional approach

To test whether (1) within-host factors had a greater effect on adult nematode β-diversity than the extra-host factors and (2) extra-host factors had a greater effect on larval nematode β-diversity than the within-host factors, we used a cross-sectional approach focused on the presence-absence of larval and adult worms sampled from individual buffalo at a single time point. We calculated β-diversity based on the Sørenson index of similarity across sites (i.e. hosts), for 33 adult and 86 larval communities using the package *betapart* in R (R Core Team, 2020). Specifically, we used function *betapart.core*, to create the pairwise matrix necessary for computing multiple-site β-diversity measures, and function *betapart.multi*, to calculate β-diversity (Baselga & Orme, 2012). The pairwise matrix from *betapart.core* was then used in permutational multivariate analyses of variance (PERMANOVAs) to identify significant drivers of β-diversity for adult and larval nematode communities in individual hosts.

We included a series of predictor variables, some of which were previously found to be related to nematode communities in buffalo (host body condition, host age, host resistance genotype, host herd, season of host capture, year of host capture; Budischak et al., 2012, 2016, Ezenwa et al., 2021), as main effects in the PERMANOVAs, along with interactions between these variables. Predictor variables were of two different classes: those related to host traits (i.e. body condition, age, resistance genotype) and those related to extra-host abiotic factors (i.e. season, year, herd of origin). Herd roughly represents use of space by buffalo in our study population (Spaan et al., 2019), and given the lack of strong genetic sub-structuring between herds (Tavalire et al., 2019), we used herd as a proxy for the local environmental conditions and exposure to infective larvae that an individual buffalo experienced as it moved across the landscape. For adult worm communities, intrinsic host traits included resistance genotype, body condition, and age, while extrinsic, extra-host factors included herd and the month of capture since all hosts were captured and necropsied in the same season and year. For each host's larval community, host traits included resistance genotype, body condition, and age while extrinsic factors included herd, year and season. Models were implemented using *adonis* in the package VEGAN.

2.4.2 | Longitudinal approach

To test whether there were feedback effects of the intra-host environment on the larval community via the adult community, we used a longitudinal approach focused on presence-absence of larval and adult worm communities. First, β-diversity of a progenitor larval community that represented the potential pool of infective larvae an individual buffalo was exposed to via its herd mates was assessed. As in the cross-sectional approach, this was accomplished using *betapart*. The function *betapart.core* was used to create the

pairwise matrix necessary for computing multiple-site β-diversity measures and the function *betapart.multi* was used to calculate β-diversity of the progenitor larval community based on the Sørenson index of similarity (Baselga & Orme, 2012).

Then, in a step unique to the longitudinal approach, the β-diversity of two contemporaneous nematode communities were assessed for each individual buffalo that underwent necropsy: (1) an adult community obtained directly via necropsy and (2) a descendant larval community resulting from faeces collected at necropsy. We calculated point estimates (i.e. multivariate dispersion values) of β-diversity for both the adult and descendant worm communities for each host based on Sørenson similarity matrices using the function *betadisper* in package VEGAN, including bias adjustment for small sample sizes (McGoff et al., 2013). This function uses the similarity matrix to estimate a group centroid for each community and the dispersion value is assessed as the distance between each individual host's worm community and the group centroid (Anderson, 2006). This yielded a point estimate of β-diversity (i.e. dispersion value) that represents how similar the worm community of each individual buffalo was to the average worm community of all buffalo that underwent necropsy. This method is advantageous because it is based on relative, rather than absolute, differences thus allowing for a comparison between the adult and descendant worm communities of each host, even though the species richness of the communities might differ.

We then used path analysis to assess if the within-host and extra-host variables from the cross-sectional analyses affected adult and larval β-diversity in three possible ways: (1) predictor variables only affect adult β-diversity, (2) predictor variables simultaneously affect both larval and adult β-diversity or 3) predictor variables independently affect either larval or adult β-diversity. The path analysis approach also allowed us to test the effects of the worm communities on each other and infer the presence of feedbacks on β-diversity (e.g. progenitor larval community ➔ adult worm community ➔ descendant larval community). Thus, we were able to investigate the relative contributions of not only within-host and extra-host variables to β-diversity, but also the relative contribution of the β-diversity of the nematode community at one life history stage on another. Path analyses were implemented using the function *lavaan* in *vegan* and paths were specified from variables that were significant in the cross-sectional analyses to two dependent variables: adult parasite β-diversity and descendant larval parasite β-diversity. In addition, a path was specified from progenitor larval parasite β-diversity to adult parasite β-diversity to determine if adult parasite β-diversity was significantly affected by the level of progenitor larval parasite β-diversity it was potentially exposed to at a previous time point. Adult nematode communities can change significantly over time (Budischak et al., 2016), and the interval between our measure of potential exposure and isolation of the adult community at host necropsy ranged from 0.6 to 2.8 years. Thus, a path was also specified from the time elapsed since progenitor larvae sampling to host necropsy to determine whether the time between exposure sampling and adult parasite sampling impacted adult parasite β-diversity. Finally, a path was specified between adult parasite β-diversity and descendant larval parasite β-diversity to account for the possibility that the β-diversity of one life history stage could be a function of β-diversity at the prior stage. All possible combinations of a priori paths yielded 13 models reflecting three general structures: (1) descendant larval parasite β-diversity is shaped solely by adult parasite β-diversity (dependent model class [1 model]: Figure S1), (2) all or some combination of variables simultaneously affect adult and descendant larval parasite β-diversity (combination model class [8 models]: Figure S1), and (3) drivers of descendant larval parasite β-diversity are independent from drivers of adult parasite β-diversity (independent model class [3 models]: Figure S1). We ranked the 12 competing models using AIC and models were considered equivalent if ΔAIC ≤ 2. Model fit was evaluated using several metrics: (i) x^2 goodness-of-fit; (ii) Tucker–Lewis Index (TLI; and (iii) root mean square error of approximation (Kenny et al., 2015). As no other distribution improved model fit over a Gaussian distribution (Table S2) and partial residual plots showed no major deviations from normality (Figure S2), it was used for all structural models.

3 | RESULTS

3.1 | Within-host and extra-host drivers of β-diversity

The adult nematode community, which was comprised of six species (Table S3), was relatively similar among hosts (Sørensen index = 0.780) with the species replacement fraction (0.490) contributing nearly twice as much to community similarity than the richness difference fraction (0.290). This means that adult parasite β-diversity across host communities was driven more by species-for-species swapping, than by a gain or loss of species between host individuals. Overall, host traits influenced β-diversity of adult nematode communities. Both host age and resistance genotype had significant main effects on adult worm β-diversity, explaining approximately 13% and 10% of the variation between communities, respectively (Table 1), meaning that both hosts of similar age and genotype had significantly more similar adult worm communities. In addition, host age interacted with month and body condition to explain 12% and 8% of the variation in adult worm β-diversity (Table 1). Thus, hosts of the same age that were sampled in the same month, and hosts of the same age with similar body condition scores, had more similar adult worm communities.

The larval nematode community, which was comprised of seven species (Table S1), was highly similar among hosts (Sørensen index = 0.936). However, in contrast to the adult worm communities, larval communities were directly influenced by extra-host variables. The main effect of herd and year, and interactions between year and season and year and age all had significant effects on overall larval nematode β-diversity, although the percent of variation explained by each variable was 10% or less (Table 2). This means that hosts sampled from the same herd or during the same year had more similar

TABLE 1 Comparison of β-diversity and its components for adult helminth communities ($n = 33$) according to host-related (e.g. resistance genotype, age, body condition) and spatiotemporal variables (e.g. month of host capture, herd of host capture) using PERMANOVA. Asterisks and bold denote values and variables, respectively, that are significant.

Adult β-diversity	*df*	*F*	R^2	*p*
Month	1	−0.667	−0.008	0.941
Condition	1	−1.001	−0.012	0.963
Herd	1	4.067	0.048	0.091
Age	**1**	**11.080**	**0.129**	**0.013***
Resistance genotype	**1**	**8.378**	**0.098**	**0.014***
Month*condition	1	0.389	0.004	0.643
Month*herd	1	2.558	0.029	0.198
Condition*herd	1	4.912	0.057	0.061
Month*age	**1**	**10.277**	**0.120**	**0.016***
Age*condition	**1**	**7.004**	**0.082**	**0.028***
Herd*age	1	4.402	0.051	0.067
Month*resistance Genotype	1	−0.312	−0.004	0.892
Condition*resistance Genotype	1	1.710	0.020	0.267
Herd*resistance genotype	1	2.533	0.029	0.171
Age*resistance genotype	1	0.180	0.002	0.737

TABLE 2 Comparison of β-diversity components for larval (L_3) nematode communities ($n = 86$) to host-related (e.g. resistance genotype, age, body condition) and environment-related variables (e.g. year, season and herd) using PERMANOVA. Asterisks and bold denote values and variables, respectively, that are significant (*).

Larval β-diversity	*df*	*F*	R^2	*p*
Year	1	4.806	0.042	0.034*
Season	1	0.214	0.002	0.703
Condition	1	0.623	0.005	0.476
Herd	**1**	**10.581**	**0.092**	**0.001***
Age	1	0.732	0.006	0.435
Resistance genotype	1	1.742	0.015	0.205
Year*season	**1**	**4.807**	**0.052**	**0.042***
Year*condition	1	−0.152	−0.001	0.907
Season*condition	1	0.354	0.003	0.564
Year*herd	1	1.770	0.015	0.177
Season*herd	1	0.650	0.006	0.436
Condition*herd	1	0.324	0.003	0.581
Year*age	**1**	**12.513**	**0.109**	**0.003***
Season*age	1	1.506	0.013	0.229
Condition*age	1	0.298	0.003	0.660
Herd*age	1	2.046	0.018	0.168
Year*resistance genotype	1	1.373	0.012	0.249
Season*resistance genotype	1	0.810	0.007	0.387
Condition*resistance Genotype	1	1.556	0.017	0.205
Herd*resistance genotype	1	1.652	0.014	0.221
Age*resistance genotype	1	0.721	0.006	0.427

larval parasite communities. Similarly, hosts of the same age that were sampled in the same year and hosts that were sampled in the same year and same season had more similar larval parasite communities. Thus, although age, a host trait, interacted significantly with year, extra-host variables, including of herd, year, and the interaction between year and season were the variables that played a major role in shaping similarities between larval communities of individual hosts.

3.2 | Linkages between drivers of adult and larval β-diversity

Path analysis revealed evidence of feedback effects of adult parasite β-diversity on the larval parasite community. Of the 13 different models candidate models, seven emerged as top contenders all with a ΔAIC ≤2 (Figure 1; see Figure S3 for lower-ranked models). Although one model indicated that different variables were independently affecting adult and descendant larval parasite β-diversity (i.e. independent class, model 2) and one model indicated that different variables were entirely affecting descendant larval parasite β-diversity via the adult community (i.e. dependent class, model 3), the other top models were of the combination class of model showing a subset of variables simultaneously affecting adult and descendant larval parasite β-diversity. Nevertheless, some important commonalities emerged among the top models. First, the five combination models identified a mix of host and environmental variables simultaneously affecting adult and descendant larval worm communities (Table 3), suggesting that these variables were acting on both adult and descendant larval β-diversity.

Second, in all seven top models the host traits of body condition, resistance genotype, and age formed significant paths to adult β-diversity, while the extra-host variable of month frequently formed a significant path to both adult and descendant larval β-diversity. With respect to adult β-diversity, the models showed that communities were more similar with more time between sampling points and as hosts aged. However, hosts with the nematode resistance allele and hosts with poorer body condition had adult worm communities that were more dissimilar compared to non-resistant hosts or hosts with better body condition. Generally, descendant larval β-diversity was significantly lower, and larval communities were more similar, in July versus August and in older individuals. It is also important to note that the top models all explained a high degree of variation in adult (≥85.5%) and a lower, but still marked, degree of variation in descendant larval (~50%) community similarity (Table 3). In fact, the three models that had the highest R^2 values for the adult and larval

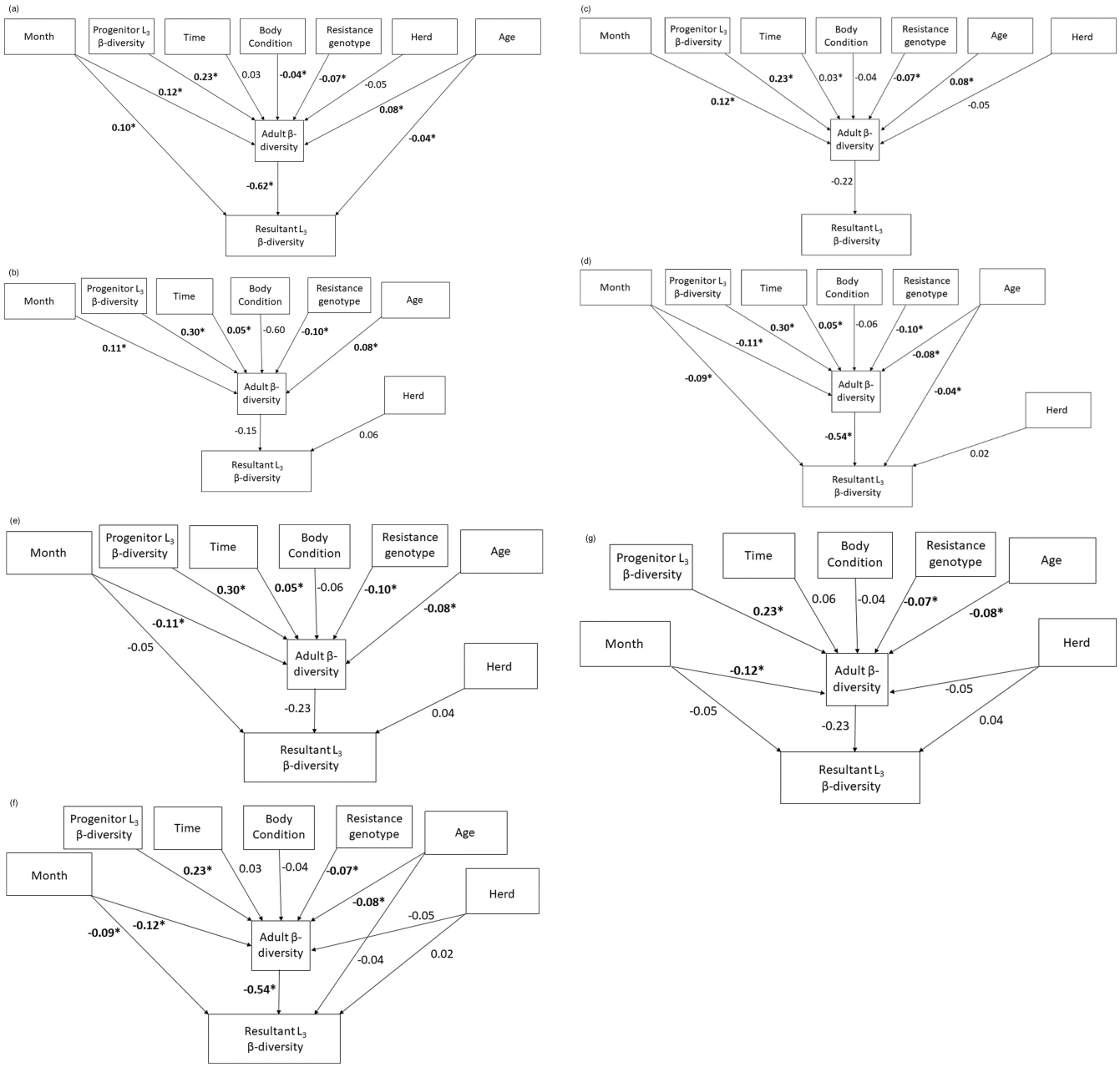

FIGURE 1 Best-fit path models of adult and larval community β-diversity. Exposure L3 indicates the larval community of all herdmates assessed at the time point prior to necrcopsy whereas resultant L3 indicates the larval community generated by a single host's adult community and assessed at host necropsy. Time indicates the length of time elapsed between the exposure L3 and adult communities in decimal years. Age indicates host age in decimal years. Values are analogous to beta weights and represent the standardized estimates of the variable to allow for comparison of strength and direction of path relationships among models. Significant ($p < 0.05$) paths are indicated with asterisks. Panels (a through g) correspond to models 1–7 in Table 3 and models with better fit are ranked first.

communities were all of the combination class, suggesting that these models more completely accounted for variation in these datasets (models 1, 4, and 6; Table 3).

Third, although β-diversity of the progenitor larval community was significant in all top models, the time elapsed between assessing progenitor larval and adult β-diversity did not always affect adult β-diversity in these models (Figure 1). This suggests that the progenitor larval community to which a buffalo is exposed contributes to adult community structure, but the effects of individual host traits on adult community assembly could be independent of time since infection. The effects of adult β-diversity on descendant larval β-diversity were also not consistent, but models that included a significant effect of the adult community on the larval community had higher R^2 values, suggesting some effect of adult β-diversity on larval β-diversity.

Fourth, both within-host and extra-host variables emerged as key predictors of adult and larval worm community similarity among the top models. Host age, body condition, resistance genotype, and

TABLE 3 Comparison of competing path models of overall β-diversity adult and resultant larval nematode infracommunities from the longitudinal approach. All models are ranked according to AIC and asterisks indicate the top competing models. Goodness-of-fit x^2 ($p \geq 0.05$), Tucker–Lewis Index (TLI ≥ 0.95), and root mean square error of approximation (RMSEA ≤ 0.08) values indicate overall measures of fit. The amount of variation explained in adult worm (R^2 adult) β-diversity and resultant larval β-diversity (R^2 L3) by each model and the class of model (i.e. larval variables dependent, in combination, or independent of adult variables) is also listed. Asterisks and bold denote top models.

Model	Model class	AIC	ΔAIC	x^2	TLI	RMSEA	R^2 adult	R^2 L3
1*	**Combination**	**92.386**	0	$p = 0.452$	**0.981**	**0.026**	**0.852**	**0.474**
2*	**Independent**	**93.149**	**0.763**	$p = 0.479$	**1.026**	**0.000**	**0.846**	**0.247**
3*	**Dependent**	**93.402**	**1.016**	$p = 0.464$	**1.003**	**0.000**	**0.852**	**0.166**
4*	**Combination**	**93.848**	**1.462**	$p = 0.417$	**0.923**	**0.052**	**0.846**	**0.591**
5*	**Combination**	**93.893**	**1.507**	$p = 0.468$	**1.008**	**0.000**	**0.846**	**0.295**
6*	**Combination**	**94.065**	**1.679**	$p = 0.244$	**0.597**	**0.119**	**0.852**	**0.461**
7*	**Combination**	**94.110**	**1.724**	$p = 0.274$	**0.655**	**0.110**	**0.852**	**0.303**
8	Combination	94.926	2.540	$p = 0.203$	0.497	0.133	0.852	0.456
9	Combination	95.185	2.799	$p = 0.197$	0.474	0.136	0.852	0.192
10	Combination	95.217	2.831	$p = 0.222$	0.536	0.128	0.852	0.293
11	Independent	97.779	5.393	$p = 0.192$	0.450	0.139	0.744	0.362
12	Independent	98.723	6.337	$p = 0.112$	0.211	0.167	0.731	0.408
13	Combination	99.658	7.272	$p = 0.112$	0.211	0.167	0.731	0.364

month of sampling were often strongly related to adult β-diversity, with these variables yielding significant paths in all models (Figure 1). Relationships suggesting a significant effect of host herd, age, or month of sampling on descendant larval β-diversity were slightly less consistent appearing in five of seven top models. Significant relationships between larval β-diversity, adult β-diversity and host age were also supported in three of severn top models (Figure 1). Thus, some variables that were significant in the PERMANOVAs for larvae, such as host age, were represented in the path analysis as having paths to both larval and adult communities. The longitudinal analyses therefore clarified the results of the cross-sectional analyses by providing a hypothesized structure for the models and identifying variables that likely affect both communities.

4 | DISCUSSION

In this study, we used a cross-sectional and a longitudinal approach to uncover extra- and intra-host drivers of community similarity in co-occurring adult and larval parasite communities in a host-nematode system. We also investigated possible links between adult and larval parasite communities due to shared drivers of community similarity for each life stage. Our results provide new insight for understanding community-level assembly processes for parasites with adult and immature life stages that inhabit vastly different habitats. Cross-sectional analyses revealed that host traits consistently affected adult nematode communities, while extrinsic, extra-host variables had a consistent effect on larval nematode communities. Longitudinal analyses showed that β-diversity of progenitor larval parasite communities significantly and consistently affected adult parasite β-diversity, while adult parasite β-diversity also affected the β-diversity of descendant larval parasite communities, although less consistently. This latter result suggests that host traits can influence the larval parasite communities via their effects on the adult parasite community. The longitudinal analyses also showed that some variables act independently on the larval parasite community and are not mediated by the adult parasite community. In combination, our cross-sectional and longitudinal approaches indicate that although adult parasite β-diversity community affects larval parasite β-diversity, there are also independent drivers of β-diversity that operate on each parasite life stage.

In general, we found that adult parasite β-diversity depended heavily on host traits. The cross-sectional and longitudinal analyses showed that the main effects of host age and resistance genotype, as well as the interaction between age and body condition, were key to predicting adult nematode β-diversity. Thus, the host plays a crucial role in shaping the adult parasite community. Host age often correlates with parasite diversity (e.g. Dugarov & Pronin, 2017; Timi et al., 2010) and previous findings in African buffalo indicate that younger individuals host more diverse nematode communities (Budischak et al., 2016), whereas older individuals host lower *Haemonchus* spp. burdens (Budischak et al., 2018). This suggests that some facet of host age can influence parasite assembly, including host-mediated processes, such as immunity or age-related host mortality, or parasite-mediated processes, such as interspecific competition between worms. Both low body condition (Gilot-Fromont et al., 2012; Martin et al., 2008; Warburton, Pearl, et al., 2016) and age (De Coster et al., 2010; Froy et al., 2019) can limit the strength and/or type of an individual's immune response. Therefore, the significant interaction of age and body condition on adult parasite β-diversity found here is consistent with previous work. Host resistance genotype also significantly contributed to adult parasite β-diversity. Buffalo with the nematode-resistant genotype reliably shed fewer worm eggs in their faeces and have higher levels of

anti-nematode immunity in their intestinal tract including eosinophils, mast cells and immunoglobulin A (Ezenwa et al., 2021). These immune mechanisms are known to be directly toxic and/or facilitate a type 2 immune response against worms (Linnemann et al., 2020; Obata-Ninomiya et al., 2020), potentially explaining why resistant hosts differ in nematode community similarity from non-resistant hosts. It is worth noting that the interaction between month of host capture and host age also had a significant effect on the adult nematode β diversity. This result could be linked to previous findings of seasonality of parasitism in buffalo. Buffalo experience higher parasite burdens in the dry season (Budischak et al., 2012, 2018; Gorsich et al., 2015), and in other African ungulates, external environmental conditions interact with parasitism such that individuals in the resource-poor dry season have higher nematode burdens (Ezenwa, 2004; Shearer & Ezenwa, 2020), likely due to some facet of condition-linked immunity (Beechler et al., 2012; Budischak et al., 2018).

Overall, extrinsic extra-host variables were key drivers of larval nematode β-diversity. In the cross-sectional analyses the main effects of herd and year and the interactions of year with season and host age were strongly associated with larval parasite β-diversity. In the longitudinal analyses, month and age appeared to be the most critical drivers of larval parasite community β-diversity. The effect of month in the longitudinal analysis is likely related to seasonality, and thus corresponds with results from the cross-sectional analysis. Season could affect larval nematode β-diversity via two different, but non-mutually exclusive mechanisms. First, seasonal differences in temperature could account for changes in the larval parasite community since larval nematode development is temperature-dependent (O'Connor et al., 2006). Although immature parasites in our study were cultured inside a building which could have dampened the seasonal variation in ambient environmental conditions experienced by larvae, thus not allowing for a one-to-one comparison with on-pasture conditions, general seasonal fluctuations in the abiotic environment were still likely key for larval parasite community. Second, seasonal changes in the quality of the larval habitat, in other words changes in the composition of host faeces, could also play a key role in larval parasite community structure. Faecal composition in African ungulates can vary due to the quality and type of forage available in different seasons (Tshabalala et al., 2010; Watermeyer et al., 2015). Given that larval development in strongyles is heavily impacted by faecal moisture content, the dry, low-quality forage available during the dry season could adversely affect the larval parasite community (O'Connor et al., 2006). Moreover, feeding stages of these larvae rely on bacteria and saprobiotic material in faeces and the African buffalo gut microbiome shifts significantly depending on year, season and forage quality (Sabey et al., 2021). Host age also significantly affects the gut microbiome of African buffalo (Sabey et al., 2021) creating a path for this host trait to affect larvae directly.

In total, our results suggest that some forms of extrinsic, abiotic variation takes the output (i.e. eggs) of the adult community and modify it, creating patterns of β-diversity in larvae that are not entirely dependent on the biotic, within-host environment, but also depends on the abiotic, extra-host environment. Indeed, parasites experience a dual environment that differs between adult and larval stages. For larvae, the extra-host environment affects both the development of off-host life stages and the presence or absence of suitable hosts for successful transmission (Maestri et al., 2020). Seasonality of infection occurs in nematodes hosted by African buffalo (Gorsich et al., 2014) and this pattern could be explained by seasonal changes in temperature and humidity on free-living parasite stages (e.g. Roberts & Grenfell, 1992). However, as noted above, the quality of forage also changes with season and this has been linked to changes in nematode burden (Ezenwa, 2004; Shearer & Ezenwa, 2020). Thus, season might impact both host- and abiotic-related variables with synergistic effects on the larval parasite community that are difficult to tease apart. Host herd, a variable that indicates distinct extra-host differences, like different local environmental conditions and variable exposure to infective larvae, was also strongly related to larval nematode β-diversity in our cross-sectional analysis. Herd roughly represents use of space by an individual as it moves with other buffalo across the landscape (Spaan et al., 2019). This should increase the likelihood that members of a herd are exposed to a similar suite of parasitic nematodes as they collectively move and forage. Thus, the extra-host effects of herd might also be difficult to separate from possible shared host traits among herd mates. Similarly, although no genetic structuring in KNP herds has been found (Lane-de Graaf et al., 2015; Tavalire et al., 2019) it could be possible that some other host traits might be linked to their herd identity.

Importantly, the shared influence of several host and abiotic predictors on adult and larval nematode communities suggests that some variables shape worm communities independently and via feedback effects from other life history stages. Our results indicate that host traits such as resistance genotype and body condition strongly influenced the adult nematode community, and adult nematode β-diversity in turn influenced the descendant larval nematode community. This suggests that adult parasite β-diversity depends heavily on key host traits that shape community assembly. Once these host traits act on the adult parasite community, then the eggs and subsequent early-stage larvae produced must pass through an abiotic filter in the external environmental to persist in the L3 community. In this way, adult nematode communities, and intrinsic host traits that shape it, echo through to larval assemblages, which are then further modified by extrinsic factors.

Little is known of both relative β-diversity of parasitic taxa at different life stages and that of free-living organisms that undergo similarly massive shifts in habitat and lifestyle between larval and adult forms (e.g. such as holometabolous insects and amphibians). For example, while the drivers of β-diversity in both adult and larval insect communities is an active area of research (e.g. Bishop et al., 2015; Simiao-Ferreira et al., 2018), there is little information to connect the community-level processes operating at each stage. Our results suggest that both adult communities and abiotic variables have strong effects on larval communities that could apply to free-living organisms. Thus, understanding that different processes

affect free-living communities of the same organisms at different life stages could inform which conservation and management techniques are most appropriate for each stage.

Overall, adult and larval nematode communities possessed relatively high levels of community similarity. Parasite β-diversity appeared to be generally affected by interactions that included intrinsic, host-related variables in adult nematode communities, whereas extrinstic extra-host variables appeared to be more critical drivers of larval parasite community similarity. However, the influence of host traits on the adult nematode community extended to the larval nematode community. Given that immature parasites are often the infective stage and adult parasites are often the agents of disease, understanding the drivers of variation in communities of both life stages becomes key for predicting and preventing diseases with this type of life history (Murray et al., 2018). Our results also provide essential data for comparing communities where adult and immature stages, either free-living or parasitic, inhabit vastly different habitats.

AUTHOR CONTRIBUTIONS

Elizabeth M. Warburton and Vanessa O. Ezenwa conceived of the study. Sarah A. Budischak, Anna E. Jolles and Vanessa O. Ezenwa collected data. Elizabeth M. Warburton performed the analyses and wrote the initial manuscript. All authors contributed critically to later drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.34tmpg4pt (Warburton et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1.

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