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Santa Barbara

The role of watering holes as hotspots of disease transmission in changing climates

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

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September 2020

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The role of watering holes as hotspots of disease transmission in changing climates

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Titcomb, G.T.; Hulke, J.; Mantas, J.N.; Young, H.S. *Using citizen science and metabarcoding to investigate herbivore parasite sharing at water sources in an East African savanna*. ESA Annual Meeting, Aug. 2019, Louisville, Kentucky, USA (Presentation).

Titcomb, G.T.; Hulke, J.; Orens, A; Mantas, J.N.; Gituki, B.C.; Young, H.S. *Parasite Safari: Using citizen science to understand herbivore parasite exposure risk at East African watering holes.* The Western Section of the Wildlife Society Meeting, Feb. 2019, Yosemite, California, USA. (Presentation)

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Titcomb, G.T.; Young, H.S. *Do watering holes concentrate parasites? Diving into the hotspots of savanna life across seasons and climates.* UCSB Graduate Student Symposium. Feb 2017. (Presentation)

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ABSTRACT

The role of watering holes as hotspots of disease transmission in changing climates

by

Georgia Titcomb

Humans impact the globe in numerous ways that have important yet variable effects on human and animal diseases. Anthropogenic changes may be particularly consequential where landscape resources increase transmission opportunities; however, these spatial hotspots of human and animal activity are a relatively understudied aspect of disease dynamics. Watering holes are an ideal system for studying such transmission hotspots amid accelerating global changes, as they draw together wildlife, domestic animals, and humans in arid climates that are increasingly impacted by climate change. In this dissertation, I used observational and experimental data to investigate plants, herbivores, and gastrointestinal parasites at these important ecological resources in a semi-arid savanna system in central Kenya.

I first examined how watering holes and associated herbivore aggregations shape plant communities that form the transmission substrate for many fecal-oral parasites. I found that herbivore aggregation near water was associated with decreased plant cover but opposing plant diversity patterns, depending on soil and rainfall. This was driven by changes in grass and tree cover and dominance shifts of two globally important grass species. I then used a two-year water manipulation experiment and observational study to examine the

extent to which herbivores and their gastrointestinal parasites aggregated near water sources under different gradients of water availability: aridity, recent rainfall, and distance from surface water. I found marked differences in dung and parasite aggregation at water by herbivore species, with elephants and cattle congregating strongly in arid conditions. However, all animals displayed some degree of increased watering hole use with at least one metric of decreased water availability, suggesting that drying environments may contribute to increased parasite concentration at these hotspots across species.

I then investigated gastrointestinal parasite communities in 18 sympatric and globally threatened herbivore species using DNA metabarcoding I found that host phylogeny and gut type were central in determining parasitic nematode sharing. I linked data on parasite spatial aggregation and sharing to data from an 8000-volunteer citizen science project measuring herbivore activity from camera traps to estimate parasite transmission near water relative to dry sites. I found that due to their abundance, degree of aggregation around water, and ability to share parasites, cattle were strong potential drivers of gastrointestinal parasite transmission for other herbivore species at watering holes. Together, these findings demonstrate predictable patterns of parasite transmission in resource-limited areas and have implications for understanding and predicting disease dynamics in humans, wildlife, and domesticated animals that live in increasingly dry landscapes.

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Chapter 1

1. Introduction

Background and Statement of the Problem

Humans are impacting the globe in myriad ways that also affect disease transmission. Climate changes can shift host and pathogen distributions, creating new outbreaks in some areas, and lowered risks in others; land use changes can drive both positive and negative changes in contact rates among humans and pathogens in the environment; and biodiversity loss can create opportunities for new diseases to emerge, while others may die out. Thus, the impacts of our anthropogenic activities on human and animal diseases remain highly uncertain, and they are likely to greatly depend on context, necessitating broader ecological research. Perhaps one of the few consistent characteristics of diseases across systems is stark aggregation and heterogeneity, such that certain individuals, species, or spaces may account for a disproportionate degree of transmission. However, despite obvious advantages for surveillance and research, there has been relatively little attention to the role of landscape features in acting as disease hotspots, nor their likely shifting roles amid environmental changes. Therefore, measuring and understanding the effects of human impacts on these hotspots will become increasingly important amid accelerating climate changes, a swelling

global population, and an unprecedent rate of biodiversity loss in both hosts and their

parasites.

1.1. Anthropogenic effects on diseases

1.1.1 Climate

Understanding the effects of climate changes on parasite transmission, prevalence, and

development are priorities for both conservation and public health (Patz 1996, Patz et al.

2005, Lafferty 2009). Thus, a substantial body of research has endeavored to monitor the

direct and indirect effects of climatic shifts on disease (Patz et al. 2000, Harvell et al. 2002,

Dobson et al. 2003, Brooks and Hoberg 2007). For example, increasing global temperatures

are likely to accelerate parasite development (McCue and Thorson 1964, Olwoch et al. 2008,

Weaver et al. 2010), but they may also increase parasite mortality (Pullan and Brooker 2012).

Temperature changes can also cause parasite range expansions and contractions (Lafferty

2009, Bebber et al. 2013); for example, in the spread of Bluetongue virus northward into

Europe via expansion of its midge vector (Purse et al. 2005), or in the potential spread of

Lyme disease in Canada via northward movement of its tick vector (Ogden et al. 2006).

Temperature can also alter host physiology, potentially eliciting immunosuppression under

stressful climatic conditions and increasing disease morbidity (Griffin 1989, Harvell et al.

2002). In addition to increased temperatures, climate change is likely to bring substantial

shifts in rainfall, but the direction of these effects will vary dramatically across the globe

(IPCC 2014). For example, increased rainfall stochasticity is likely to bring increased

instances of flooding, which are associated with cholera outbreaks (Reiner et al. 2012). For

many macroparasites, moister conditions can reduce mortality in the environment, especially

amid increasing temperatures (Weaver et al. 2010). In contrast, increased drying may promote other diseases, such as meningococcal meningitis, which has higher transmission rates in low humidity environments (Sultan et al. 2005). This, the complex, nonlinear effects of climate changes on disease have drawn attention to the need for further research that accounts for the ecology of the specific diseases in question (Lafferty 2009, Johnson et al. 2015).

1.1.2 Land use change and resource extraction

Throughout much of recent human history, humans have altered landscapes for development and extraction of resources. In some cases, these activities led to historical disease outbreaks: for example, deforestation during the Roman Empire led to increased standing water that promoted malaria spread to such an extent that humans avoided these infamous Pontine marshes for centuries (O'Sullivan et al. 2008). In the 1890s, construction of the Panama Canal brought many soldiers into contact with mosquitoes carrying yellow fever, leading to years of stalled development and then subsequent environmental destruction (Guzman et al. 2008). In more recent years, bushmeat hunting has been implicated in the emergence of Ebola (Leroy et al. 2004), and its sale in wet markets has been cited as the cause of the recent SARS-cov2 coronavirus (Andersen et al. 2020). Land use changes and resource extraction alter disease risks by either increasing or decreasing contact rates with infectious reservoirs in the environment (Gottdenker et al. 2014). However, patterns in changing contact rates may differ substantially among the different modes of disease transmission. In the case of vector-transmitted diseases, land-use changes may create new foci for vector reproduction, or they may alter host behavior to increase contact rates. For directly transmitted pathogens, changes that bring animals together (or humans and other animals together) can contribute to

increased transmission. Finally, for environmentally transmitted pathogens, any land use change that enables the survival of pathogenic stages or alters the spread of those stages can also influence disease risks.

1.1.3 Host Communities

Both climate and land use changes can also contribute to a third way in which humans affect disease transmission: by shifting the composition of animal communities. Changes in host community composition can have complex effects on disease transmission. For example, in the case of Lyme disease, the loss of 'incompetent' mammalian hosts that prevent the spread of *Borrelia burgdorferi*, the pathogen vectored by ticks, can lead to substantial increases in disease risk with declining biodiversity (Keesing et al. 2006). However, this is not universal, as additional hosts in a system can also have opposite amplification effects (Randolph and Dobson 2012). In an East African tropical savanna system, large wildlife exclusion led to a doubling in rodent populations and increases in the gastrointestinal parasite populations they sustained (Weinstein et al. 2017), and landscape-level increases in fleas that carry pathogens (Young et al. 2014). In contrast, in urban environments where defaunation is extreme, zoonotic diseases can be significantly reduced because contact rates with pathogens are substantially lowered (Kilpatrick et al. 2017, Wood et al. 2017). However, for the animals that thrive in urban environments, there are new potentials for contacts (Bradley and Altizer 2007, Gibb et al. 2020).

1.2 The importance of heterogeneity in disease transmission

Given the human and economic toll of disease spread in the Anthropocene and the uncertain and often contrasting effects of human activity on diseases as detailed above, disease

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prediction and surveillance has become an increasingly important focus (Morse et al. 2012).

However, elucidating contact networks among vectors, hosts, and the environment depending

on the relevant transmission mechanism is extremely complex. Researchers, therefore,

frequently rely on disease models to investigate patterns of spread, especially in wildlife

populations. From both empirical and modelling data, one essential aspect of many disease

systems is their aggregated nature; in other words, few individuals are responsible for most

infections (Woolhouse et al. 1997). Identifying these individuals or locations is thus a

priority for prediction, surveillance, and intervention (Morse et al. 2012, Holmes et al. 2018).

1.2.1 Hotspots and superspreaders

Across systems and transmission modes, diseases are often highly aggregated such that

relatively few hosts ('superspreaders') account for most parasites or subsequent exposures.

This can arise from numerous sources of heterogeneity that influence disease transmission

and progression, such as aggregated contact rates among individuals, vectors, or

environmental sources of infection and genetic factors that influence immunity (Anderson

and May 1991). Commonly referred to as the 20/80 rule, this pattern suggests that

approximately 20% of hosts may account for as much as 80% of disease transmissions

(Woolhouse et al. 1997). The ubiquity of this aggregation across systems, scales,

transmission modes, and life forms is also remarkable: for example, in the number of

individual contacts in transmission of STDs (Anderson and Garnett 2000), in the species that

tend to amplify and spread multi-host pathogens (Kilpatrick et al. 2006), in the number of

macroparasites found in mammalian hosts (Shaw et al. 1998), in plant diseases (Madden and

Hughes 1995), and in contact rates with water bodies that pose schistosomiasis risk

(Chandiwana and Woolhouse 1991). Importantly, this heterogeneity has critical implications

for the spread of disease throughout populations: it may dramatically increase R0 – the basic reproductive number that indicates the likelihood of disease spread in a susceptible population – (Woolhouse et al. 1997) and contribute to the explosiveness of pandemics (Lloyd-Smith et al. 2005). Thus, there have been calls for further work examining the sources of this variation and their impacts on disease spread using studies that consider community ecology (Johnson et al. 2015).

While there have been increasing efforts to quantify heterogeneity in contact rates among individuals that spread directly-transmitted pathogens (e.g. (Vanderwaal et al. 2017)), the roles of spatial heterogeneity and landscape features in explaining these patterns have been relatively underexplored since early efforts by pioneers of spatial epidemiology in the late 1800s (Ostfeld et al. 2005). Notably, landscape features and spatial heterogeneity can influence diseases of all types of transmission modes by altering host contact rates with infectious stages and/or pathogen persistence in the environment. For example, in the case of vector-transmitted pathogens, landscape patches can increase tick abundance (Allan et al. 2003), and for directly-transmitted tuberculosis, slums or city centers can act as hotspots of tuberculosis transmission in humans (Dowdy et al. 2012). However, despite the likely critical role of landscape heterogeneity in driving dynamics of environmentally-transmitted pathogens, there have been relatively few studies that quantify risk at disease hotspots relative to other areas (Paull et al. 2012). Perhaps one reason for this is that parasite stages in the environment have been historically challenging and time-consuming to quantify (Bass et al. 2015), and that host contacts with those stages are even more challenging to measure in humans (Smith 1998), let alone wild animals. Therefore, variation in transmission via environmental media has been modelled using spatially-explicit individual-based models,

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revealing important parameters such as density of resources in the environment (Bonnell et al. 2010) and seasonal use of those resources (Nunn et al. 2014). These recent efforts also underscore the need for further empirical data to determine whether landscapes can create the same 20/80 rule for those pathogens that are transmitted via the environment, and under what circumstances.

1.2.2 Multi-host and multi-parasite dynamics at hotspots

Given that individuals are often infected by more than one parasite species at any given moment (Bordes and Morand 2009), and landscape resources that increase animal contact rates can do so for many different host species, it is also important to consider multi-host and multi-parasite dynamics in these locations. For example, while increased contact rates around resource hotspots are likely to increase the per-capita infection risk for directly-transmitted pathogens and density-dependent environmentally-transmitted parasites, there can be opposing effects for parasites that have a limited number of infective stages that disperse widely over time and space. This 'safety in numbers' relationship (Buck et al. 2017) implies that while the overall parasite population increases with host aggregation, the burden experienced by individual hosts may be lowered; this phenomenon is likely to occur in many vector-transmitted parasites, sapronoses (free-living microbes that also cause infections (Kuris et al. 2014)), and parasitoids, among others (Buck et al. 2017). Therefore, it is important to consider the possibility that while increased host contacts around a shared resource cam increase parasite transmission for many diseases, the per-capita risk for hosts may contrast sharply.

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1.3 Anthropogenic changes and disease hotspots

Human activities can create disease hotspots if they attract hosts to new resources. For example, supplemental feeding of birds can promote the spread of avian conjunctivitis (Fischer et al. 1997), increased aggregation of food resources can increase infection and diversity of macroparasites (but not ectoparasites) in racoons (Wright and Gompper 2005b), and more recently, human aggregation in restaurants and bars likely promoted the spread of SARS-CoV-2 (Buonanno et al. 2020, Steinhauer 2020). Furthermore, anthropogenic drivers of resource hotspots can influence host behavior in such a way that transmission risk is further increased. For example, increased flying fox and human contact in peri-urban areas coupled with reduced fox migration is predicted to increase Hendra virus transmission (Plowright et al. 2011), and mongoose aggregation at garbage was associated with heightened aggression and tuberculosis prevalence transmitted via injuries (Flint et al. 2016). However, while there are examples of land use change in altering disease transmission, experimental studies are lacking (Gottdenker et al. 2014). Furthermore, the combined effects of land use change and climate are likely to be important at resource hotspots, especially if these stressors increase demand for the resource, but studies that explicitly consider this are rare.

1.4 Watering holes as ecological hotspots in a changing world

1.4.1 Global scale

Water is one of the most important resources for humans and animals worldwide, especially for the more than 2 billion people currently living in water-stressed systems (Oki and Kanae 2006). As climate changes are projected to expand dry land to more than 50% of the globe's

terrestrial surface by 2100 (Huang et al. 2016), this number will likely dramatically increase for humans, domestic animals, and wildlife. Thus, the potential role of water sources in aggregating animals and promoting disease transmission is likely to be important in many areas across the globe. For example, water sources have been implicated as avenues of disease transmission for paratuberculosis (Johne's disease), a major livestock pathogen worldwide (Sweeney et al. 2012), they have been associated with increased prevalence of tuberculosis-like lesions in deer and pigs in a Mediterranean ecosystem (Vicente et al. 2007), and stream sharing with livestock has been implicated in increased giardia prevalence in South American howler monkeys (Kowalewski et al. 2011). Indeed, human aggregation and contact with water has been implicated in the spread of diseases, including polio and many enteric viruses in swimming pools (Bonadonna and La Rosa 2019), and schistosomiasis across many regions of Africa (Steinmann et al. 2006).

1.4.2 East African savannas

Both natural and man-made watering holes found in East African savannas are widely recognized to be important sites for animal aggregation, particularly in drier climates (Valeix 2011). Given projected climate changes, altered host composition, and high parasite diversity, increased aggregations of a diverse array of animals and their parasites in these areas, these watering holes pose an ideal system in which to examine the anthropogenic drivers of disease transmission around shared resources.

1.4.3 Climate

In East African savannas, seasonal rainfall drives important cycles of productivity (Deshmukh 1984), and subsequent animal movement across scales (Holdo et al. 2009,

Goheen et al. 2013). For example, in the dry season, water may limit wildlife ranges, constraining them to areas with reliable access to water (e.g. Loarie, Aarde, & Pimm, 2009; Stears, Nuñez, Muse, Mutayoba, & McCauley, 2019; Western, 1975). In addition to affecting hosts, changes in precipitation and temperatures can also influence parasites in this system (Young et al. 2015, Titcomb et al. 2017). The projected temperature increases of 3°C by 2050 (Anyah and Qiu 2012) may influence development and mortality of many parasites in this system; for example, in accelerating the development of tick life stages (Randolph 1994) and hookworms, but also in increasing mortality at very high temperatures (Weaver et al. 2010). In addition to temperature changes, East Africa is projected to experience substantial shifts in rainfall. While global models predict an increase in total rainfall over East Africa (Niang et al. 2014), other studies have noted a decreasing trend in critically important 'long rains' (Camberlin and Philippon 2002, Funk et al. 2009). Importantly, independent of total rainfall, models project increased stochasticity, with more droughts and deluges (Williams and Funk 2011, Niang et al. 2014); both of which are extremes that may exacerbate diseases; for example, by immunosuppressing hosts during prolonged droughts and increasing parasite prevalence (Ezenwa 2004a), or by increasing waterborne diseases such as cholera during flooding events (Griffith et al. 2006). Finally, across parts of East Africa and much of Kenya, sand dams and other small reservoirs have become increasingly critical for buffering humans, their domestic animals, and wildlife against drought and unpredictable seasonality (Lasage et al. 2008, Ryan and Elsner 2016). These alterations signify yet another way in which humans shift landscapes in ways that can alter animal aggregations in response to climate change.

1.4.4 Wildlife

East Africa is a mammal biodiversity hotspot (Ceballos and Ehrlich 2006) that is also experiencing severe large mammal loss (Ceballos et al. 2005, Dirzo et al. 2014) due to poaching, land-use change, and climate change (Sala et al. 2000, Thuiller et al. 2006). As wildlife have been lost, cattle and other livestock have increasingly replaced wild mammal biomass (Ogutu et al. 2016, Hempson et al. 2017). These shifts in host communities are likely to have substantial impacts on disease transmission. For example, research in a Kenyan savanna system has documented extensive cascading effects of selective loss of large animals throughout the ecosystem (Goheen et al. 2018), with particular effects on parasite prevalence, diversity, and transmission dynamics (Keesing et al. 2013, Young et al. 2014, Weinstein et al. 2017, Titcomb et al. 2017)

1.4.5 Parasites

East Africa is affected by many important parasites for wildlife, domestic animals, and humans (Han et al. 2016). In addition to being an area of high parasite diversity (Pappalardo et al. 2020), it has also been identified as one of the regions most likely to be a source of future zoonotic disease emergence (Jones et al. 2008). While parasites greatly range in their effects on hosts (and recent calls for parasite conservation have noted that some can have indirect protective effects (Carlson et al. 2020)), many socially and economically important parasites cause serious morbidity and mortality among humans and animals, such as human and animal schistosomes, certain gastrointestinal helminths, and many tick-borne diseases (Wambwa 2005, Steinmann et al. 2006, Olwoch et al. 2008). For the numerous large mammalian herbivores that inhabit East African savannas, many of which are threatened or experiencing population declines (IUCN 2016), helminth parasites are diverse and abundant, and several of these parasites may be shared with closely related domestic animals (Round

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1968, Budischak et al. 2012, VanderWaal et al. 2014b), although the degree to which they are shared is uncertain (Walker et al. 2017). Although helminth many infections tend to be mild and sub-lethal, several species are known to pose significant health threats to wildlife, domestic animals, and humans. These include human and bovine schistosomes, fascioliasis in humans and herbivores, and certain trichostrongyle nematodes in wildlife and domestic animals that can also spillover to humans (Ashford and Crewe 2003). Furthermore, the effect of inter- and intra-species host aggregation on the prevalence and abundance of these parasites on the landscape level is likely to depend on the extent of host specificity and transmission via the environment.

1.5 Objective

In the context of changing climates, land use modifications, and shifting wildlife and livestock ratios, it is increasingly important to understand and identify the role of spatial hotspots of parasite transmission. However, the complexity of host-environment-parasite interactions that likely inform this relationship has often stymied empirical research. While disease models generally predict that resources that aggregate animals should promote disease transmission, we have little data on how this effect varies over climatic gradients, host species, and seasonal conditions. Furthermore, given the lack of data on the extent of parasite sharing among many hosts that share resources, we also have little perspective of the role of changing host composition on parasite transmission at these hotspots. My dissertation attempts to begin closing these knowledge gaps in linking host, environment, and parasite relationships at hotspots by investigating plant, herbivore, and gastrointestinal parasites at watering holes in a tropical savanna system in Kenya (Figure 1).

Figure 1. Schematic of dissertation chapters
In Chapter 2 I examine plant, climate, and herbivore interactions at watering holes; in Chapter 3, I examine herbivore, dung, and parasite aggregations; in Chapter 4 I quantify the degree to which gastrointestinal nematodes are shared among herbivores; and in Chapter 5 I link herbivores, parasites, and sharing to measure the degree to which watering holes may act as transmission hotspots. In the inset I illustrate the general mechanism by which these gastrointestinal nematodes are transmitted from adults in a host (square) that release many thousands of eggs in feces (circle) that develop into larvae that are consumed by a host. The dotted line signifies that certain parasites (e.g. *Trichuris*) can be transmitted as eggs.

Chapter 2 - *Savanna plant community responses to herbivore aggregation at water sources vary across abiotic gradients.*

In this chapter, I considered the ways in which herbivores and abiotic factors influence plant communities at water sources. I found that increasing herbivore aggregation near water was associated with opposite patterns in plant diversity depending on soil and rainfall context. This was predominantly driven by changes in tree cover and dominance shifts of two globally important grass species. These results emphasize the importance of context-dependent effects of large herbivores on plant diversity and apply them for the first time to plant dynamics at critical ecological hotspots where wild and domestic herbivores gather. In

the context of parasite transmission, given that many fecal-oral parasites for these animals are spread by consuming infectious stages in the environment, these findings have implications for host behavior and parasite survival and transmission at watering holes.

Chapter 3 - *Water sources aggregate parasites, with increasing effects in more arid conditions.*

In this chapter, I measured herbivore dung density and estimated parasitic nematode density in the environment, showing using experimental and observational datasets that water sources can aggregate herbivores and their gastrointestinal parasites by up to two orders of magnitude. Importantly, I found parasite aggregation was often strongest in arid areas and during dry periods. However, this effect was highly variable among herbivore species, with strongest effects observed for elephants and cattle. Thus, when water availability is reduced – a global pattern that is increasing amid climate changes and growing anthropogenic water use – risk of parasite exposure may increase substantially.

Chapter 4 - *The nemabiome in large mammalian herbivores: diet and gut morphology describe parasite richness and sharing*

In this chapter, I sought to better understand and quantify parasite sharing among wild herbivores and cattle. Using metabarcoding on fecal samples from 18 different herbivore species, I explored how parasite richness, phylogenetic diversity, and community composition varied with common predictors of parasite richness and sharing: host body size, range size, and group size, in addition to two less-commonly explored correlates: diet and gut morphology. I found that only these latter two metrics explained substantial variation in parasite richness and community composition, even after accounting for host phylogenetic

relationships. Together, these results demonstrate that gastrointestinal parasite sharing is common among large herbivores and is significantly predicted by diet and gut type.

Chapter 5 - *Cattle aggregations near water can create potential parasite transmission hotspots for other wildlife*

In my final chapter, I connected results from Chapters 3 and 4 to estimate the degree to which watering holes can increase parasite transmission, and how accounting for parasite sharing among sympatric hosts may change these estimates. Using a dataset generated by hundreds of thousands of camera trap identifications as part of an 8000-member citizen science project, I compared estimates of parasite transmissions in three contexts: at permanently filled water pans, experimentally drained water pans, and paired dry sites using data on animal activity in grazing and drinking, parasite density in the environment, and host density. I found that water sources can act as transmission hotspots for several species, most notably for elephants and cattle. Furthermore, I found that after accounting for parasite sharing, cattle had the potential to drive increased transmission gastrointestinal parasites among other bovids that shared the same water sources.

Cumulatively, the results from my dissertation will have implications for understanding and predicting disease dynamics in humans, wildlife and domesticated animals that live in dry landscapes. These areas are currently experiencing climatic shifts, land-use intensification, and rapid alterations to animal communities, further underscoring the importance of understanding disease transmission at water sources – resource hotspots at the nexus of these changes.

Chapter 2

2. Savanna plant community responses to herbivore aggregation at water sources vary across abiotic gradients

2.1 Abstract

Water sources support plants, humans, and animals, forming nodes of activity that can result in spatial heterogeneity across landscapes. However, global aridification and changing surface water supply threaten to change these ecosystems. Working in a semi-arid savanna in Kenya, we measured herbivore aggregation and plant height, cover (trees, grasses, and forbs), diversity, and composition at 17 paired water sources and dry sites. We analyzed differences in plant variables at water sources and dry sites across abiotic factors, examining effects of water proximity (tightly correlated to herbivore activity), soil type (nutrient-rich silt/clay vs. nutrient-poor sand), mean annual precipitation, and prior rainfall. The effect of surface water proximity and herbivore aggregation on plant communities varied substantially

depending on soil and rainfall. In arid areas with nutrient-poor sandy soils, forb and tree

cover were 50% lower at water sources compared to neighboring dry sites, species richness

was 15% lower, and a single species dominated 90% of transects. However, in mesic areas

with nutrient-rich finely textured soils, species richness was 25% *higher*, concurrent with the

decline of a dominant tall grass near water sources. Recent rainfall was important for grasses;

cover was higher relative to dry sites only during wet periods, a potential indication of

compensatory grazing. These findings suggest that divergent results of previous studies

examining water proximity and herbivore aggregation on vegetation diversity and

composition may depend on abiotic factors that can determine the degree and even direction

of effects. Where moisture and nutrient resources are high and promote the dominance of few

plant species, diversity can be elevated at water sources that aggregate herbivores as they

both promote grazing lawns and support trees; however, in arid conditions and sites with low

nutrient availability, diversity can be substantially reduced.

2.2 Introduction

Climate change and human development are rapidly altering the landscape of

terrestrial water sources and their associated ecological communities (de Wit and

Stankiewicz 2006, Vörösmarty et al. 2010), especially in dryland systems that cover 41% of

the globe (Millenium Ecosystem Assessment 2005). It is well-established that precipitation,

particularly in arid systems, causes dramatic differences in plant species composition,

biomass, and woody cover, and that changes to rainfall patterns will have substantial long-

term effects on plant communities (Deshmukh 1984, Sankaran et al. 2005). However, in

these arid systems, scarce surface waters can also aggregate domestic animals and wildlife,

causing extensive changes to surrounding plant communities (Landsberg et al. 2003, Hoshino

et al. 2009) due to herbivore effects on plant biomass, morphology, and community composition (Olff and Ritchie 1998, Jia et al. 2018).

The net effects of surface water sources—defined here as above-ground areas where freshwater collects—on plant communities likely depend on additional ecological stressors that constrain plant growth, notably including aridity, rainfall variability, and soil nutrient limitations. Together these factors also impact the magnitude of animal aggregation (e.g. low rainfall may cause stronger aggregations at water sources (Valeix 2011)), and the plant resilience to herbivory and trampling (e.g. (Louthan et al. 2013)). However, despite imminent changes in rainfall (IPCC 2014) and surface water supply (de Wit and Stankiewicz 2006), we have limited understanding of how abiotic factors modulate the effects of increased herbivore aggregation at water sources on plant communities. This is a significant knowledge gap given that these water sources provide critical resources to humans, their domestic animals, and wildlife, and will be increasingly important in the face of aridification; an accelerating process that will likely result in drylands covering more than half of Earth's surface within this century (Huang et al. 2016).

Animal aggregations at water sources impact vegetation via at least three major pathways: grazing, compaction/erosion, and nutrient addition. One of the most conspicuous effects of animal aggregation is radial vegetation patterning around water (termed 'piospheres' (Lange 1969)), in which many plants decline near water due to aggregating wildlife, resulting in landscape heterogeneity that characterizes savanna mosaics (Belsky 1995). This piosphere effect is usually attributed to grazing (Wesuls et al. 2012, Moreno García et al. 2014) or compaction (Andrew 1988, Thrash and Derry 1999), and most work on piosphere effects uses distance from water as a proxy for grazing gradients (e.g. Moreno

García et al., 2014). However, several studies have also found evidence for increased nutrient inputs near water (Tolsma et al. 1987, Perkins and Thomas 1993, Thrash and Derry 1999, Stumpp et al. 2005), especially for nitrogen and phosphorus that can be limiting in savanna systems (Pellegrini 2016).

Perhaps not surprisingly, given the multiple and sometimes contradictory effects that water sources can have on plants, a variety of plant community responses to provisional water sources have been observed. While plant height and cover generally decline (Thrash and Derry 1999), other studies note that nutrient-dense, fast-growing (Moreno García et al. 2014) and annual plants (Hoshino et al. 2009, Wesuls et al. 2012) tend to increase under heavy grazing near water. However, plant responses at water sources vary based on environmental variables such as habitat, year (Wesuls et al. 2012), distance to alternative water supply, soil, and prior rainfall (Thrash and Derry 1999). Responses in diversity metrics are more variable, with some studies reporting a steep decline in species richness (Landsberg et al. 2003) and diversity (Jawuoro et al. 2017) near water, while others find mixed or no measurable effects (Stumpp et al. 2005, Cheng et al. 2011). Authors of studies focusing on both specific plant traits and community diversity metrics have proposed that underlying site differences, such as mean annual precipitation, soil type, and grazing history could explain why specific plant responses are observed in one location, but not another (Stumpp et al. 2005, Wesuls et al. 2012). However, there remains no unifying explanation of these divergent results, and no extension of this question to a semi-arid environment where mean annual precipitation exceeds the extremely low rainfall levels ($< 200mm/year$) found in these studies, but which characterize much of the world's grazing lands.

Furthermore, despite increasing awareness of the importance of evolutionary history in eliciting community level responses (Cavender-Bares et al. 2009), relatively few studies have examined effects of water sources on plant diversity beyond measures of species richness, which does not account for similarities between closely-related species. Given that many water sources exhibit gradients of increased soil moisture and herbivore aggregation, we might detect signals of competition and environmental filtering, a process by which abiotic and biotic limitations impose selection on plant communities, reducing phylogenetic diversity (total length of all phylogenetic branches) and evolutionary divergence under stressful conditions (Tucker et al. 2017). One explanation for the apparently contrasting community-level effects observed across studies is site variation in aridity and productivity (Linstädter et al. 2014), as environmental gradients are well-established to mediate the effects of herbivores on a wide range of plant responses, and may amplify the effects of environmental filtering described above (Cavender-Bares et al. 2009). For example, when herbivory is strongest in low-productivity environments, vegetation communities may shift to those that can resist herbivory, especially grasses adapted to grazing, or to those that avoid herbivory via chemical and physical defenses or decreased accessibility (Mortensen 2013).

Exclosure experiment studies have explored grazing effects on plant communities across environmental contexts, and they may provide explanations for why plant responses to herbivore aggregations at water have been variable. A recent global meta-analysis showed herbivores to have strong negative effects on plant reproduction, biomass, abundance, and survival across ecosystems, and that changes in plant species richness and evenness are context-dependent (Jia et al. 2018). For grasslands in particular, exclosure studies have found that while herbivores can increase plant diversity, this varies by soil, rainfall, and herbivore

type (Olff and Ritchie 1998, Bakker et al. 2006, Young et al. 2013). In general, herbivores are thought to increase species richness by reducing dominant species cover (Koerner et al. 2018), thus allowing rarer species to persist, a relationship predicted by the Milchunas-Sala-Laurenth model when grazing is moderate and rainfall is high (Milchunas et al. 1988, Osem et al. 2002). In savannas, grazing can increase light resources and convert tall grass areas into productive grazing lawns (McNaughton 1984, Hempson et al. 2019), and their ability to do so is even more pronounced in absence of fire (Archibald and Hempson 2016). Thus, herbivores may increase plant diversity by promoting grazing lawns around water sources where they otherwise may not occur. Given that water can strongly aggregate herbivores, it is surprising that variation in grazing lawn responses at water sources are not well documented, and whether context-dependent patterns found from exclosure experiments and grazing lawn studies may explain contrasting results in the piosphere literature.

Exclosure studies have also found that soil type, aridity, and seasonality are three abiotic factors that can modulate herbivore effects on plants. Plant species richness tends to increase in the presence of herbivores on more nutrient-rich soils and decrease on nutrient poor soils (Olff and Ritchie 1998, Young et al. 2013). Aridity can also modulate the effects of herbivory: in arid areas, grazing often reduces species diversity, but has a unimodal effect in wetter grasslands, in which diversity increases with moderate grazing intensity (Milchunas et al. 1988, Bakker et al. 2006). Finally, seasonality can affect the degree to which certain plants compete for resources or facilitate growth, resulting in dominance shifts during wet and dry seasons (Veblen 2008). The role of these abiotic factors in modulating herbivore impacts could be magnified at water sources, given that aggregations can vary based on seasonality and aridity (Valeix 2011).

In this study we explored three outstanding questions about the net effects of water sources and herbivore aggregation on plants. We asked 1) How do water sources and their associated herbivore aggregations affect different plant groups, and how does this relationship vary based on water limitation (aridity, soil type, and season)? Given previous results suggesting reduced plant height and cover at water sources in arid areas (Thrash and Derry 1999), we expected that understory height and plant abundance (for trees, forbs, grasses) would decline near water, and that these effects would be starkest where water stress reduces grazing resilience. However, given that increased water and nutrients could potentially mitigate herbivore impacts on plants (Pringle et al. 2016), we expected smaller effects in wetter areas with higher nutrients. We extended this question to ask: 2) Does proximity to water sources affect plant diversity (as measured by species richness, Shannon Diversity, Faith's phylogenetic diversity, and mean pairwise distance), and does this vary by context? Considering that herbivores can strongly reduce total plant cover near water, we expected plant diversity to largely decline near water, where grazing and trampling would create strong environmental filters allowing few species to survive. We expected effects to be greatest in dry periods and arid areas, where abiotic conditions are a compounding filter. Finally, we investigated species-specific patterns to answer: 3) Which plants respond positively and negatively water sources, and is this context-dependent? Given previous results in very arid regions, we expected to find that species that are highly resistant to grazing and trampling would increase near water, but that others would decline. We expected this compositional change to be more pronounced where abiotic conditions are stressful to many plants (arid, nutrient-poor, sandy soil).

2.3 Methods

2.3.1 Site: Fieldwork was conducted at Mpala Research Centre in Laikipia County, Kenya (0º17' N, 37º52' E, 1600m elevation). Mpala is a mixed wildlife conservancy and cattle-ranch featuring, in addition to cows (*Bos taurus*) which account for approximately 30% of mammalian herbivore biomass (Augustine 2010), a diverse array of wild herbivores including elephants (*Loxodonta africana*), giraffe (*Giraffa camelopardalis*), zebra (*Equus quagga* and *Equus grevyi*), buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), and dik-dik (*Madoqua kirkii*) (see Augustine 2010 for relative densities of all herbivores) which are supported by man-made water sources created using small earth dams along seasonally-dry drainages (Figure 2B). Fieldwork was conducted at 17 water sources (average 400m in perimeter) and paired dry sites across a rainfall gradient (450-700 mm rainfall per year (Franz et al. 2010)); a range corresponding to transition from sub-desert scrub to grass-tree savanna (Shorrocks 2007) (Figure 2). Eight of the 17 water sources featured nutrient-rich, silt/clay soil with marked shrink-swell dynamics, while nine featured nutrient-poor high-drainage sandy soil (Figure 2; Appendix 1.1). Dry sites were selected by drawing a 1km line from each water source in a random direction within a predetermined range of degrees that controlled for elevation (± 25m) and soil type. To capture seasonal dryness (Figure 2E) we used a prior 30-day aggregate from daily rainfall data from Mpala (Caylor et al. 2017).

Figure 2. Mpala Water Sources

A) The 17 research sites across MRC spanned an aridity gradient ranging from 455 mm rainfall/yr (deep red sites) to 675 mm rainfall/yr (deep blue sites). Water sources B) were paired with dry sites randomly located 1km from any water supply. C) Transects extended 150m radially from each study site and were binned into three 50m distance intervals. D) Sites were spread across an annual rainfall gradient across two soil types. E) Sampling spanned four distinct periods (dark blue segments) that varied in prior rainfall totals (historical mean ± 95% CI for each month is shaded in light blue).

2.3.2 Vegetation surveys: Six 150m transects were surveyed at each water source and dry

site for each of four sampling seasons (Nov 2015, Feb 2016, Aug 2016, and Sept 2017)

selected to span a range of seasonal conditions. Transects extended radially from the water's

edge and were spaced at 60-degree intervals (Figure 2B). At dry sites, we began each transect

10 meters from the center to mimic the spatial sampling of watering sources (Figure 2C). At

each five-meter interval, we dropped a pin and recorded the species and maximum height of all vegetation touching the pin at any height. We did not measure the precise height of taller plants, but we recorded any individual above 500mm as ">500mm" for November 2015 and February 2016; and individuals above 1000mm were recorded as ">1m" for seasons August 2016 and September 2017. To ensure these sampling differences did not affect results, we truncated all measurements to 500mm and reran analyses. Results were almost identical (Appendix 1.2), and we thus presented full dataset results.

2.3.3 Soil: During August 2016, we collected and aggregated five topsoil (0-2cm) samples from three locations: the water's edge, 50m away from water, and at the dry site (1km away) for each of the 17 study sites. Aggregate samples were dried, sieved through 2mm mesh, and analyzed for total exchange capacity, pH, % organic matter, S, P (Bray II), Ca, Mg, K, Na, B, Fe, Mn, Cu, Zn, and Al at Brookside Laboratories (New Bremen, Ohio). Soils were classified as 'silt/clay' or 'sand' according to location and models published by Franz et al. 2010 (Figure 2D). The silt/clay soils (known as 'black cotton') are pellic vertisols characterized by shrink-swell dynamics, high productivity, and relatively low drainage, covering half of Laikipia and common across semi-arid Africa (Riginos 2009, IUSS Working Group WRB 2015). Sandy soils (commonly referred to as "red" soils) are ferric and chromic luvisols with lower productivity and better drainage than silt/clay soils, and also widespread throughout southern Africa (Augustine and McNaughton 2006, Pringle et al. 2007, Young et al. 2013). We performed linear discriminant analysis in JMP Pro 13 (SAS) to validate this grouping and to examine nutrient differences (Appendix 1.1). We found that Mn, % silt, Fe, and Cu sufficiently discriminated between soil types with 97% accuracy. We also used LMMs with post-hoc tests to compare both soils near water and at dry sites (Appendix 1.1). Results

showed that nutrient-rich silt/clay soils had significantly more Ca, Mg, K, Mn, Al, % silt, and % organic matter than nutrient-poor sandy soils. Sandy soils were higher in P, Fe, and % sand. Soils near water were higher in S, Na, B, P, and Fe; but they were lower in Al and % organic matter. There were no differences in pH, % clay, Zn, or Cu.

2.3.4 Herbivore Dung Surveys and Camera Trapping: Dung surveys were conducted concurrently to all vegetation surveys to measure animal aggregation near water. We counted fresh herbivore dung piles within a $1m^2$ quadrat every 10m along each transect at all water sources and dry sites. Dung was considered fresh if perceived to be less than 4 days old and was classified as either "grazer" (zebra, cow, buffalo), "mixed" (elephant, impala), or "browser" (gazelles, eland, giraffe). For each herbivore type, we calculated total dung pile count at water sources and dry sites across all sampling periods and sites (n=102 per herbivore group) To examine differences in herbivore dung counts by guild, we modeled dung counts (summed across each location) by herbivore type, site (water or dry), and soil type using a generalized linear mixed effects model (GLMM) with a Poisson distribution. We included location (n=17) as a random effect and tested significance of interactions using X^2 tests of nested models (Appendix S3). Given that herbivore dung density patterns at water sources compared to dry sites were similar across guilds, we then analyzed the sum of all counts as a function of distance to water (Appendix 1.4, section 1).

While dung counts have been shown to be a reliable metric of herbivore density on a broad scale (Barnes 2001), they do not necessarily indicate that herbivores spend more time trampling or foraging in locations where dung counts are higher. Therefore, we used camera trapping data to determine if broad scale dung patterns matched finer scale behavioral patterns. From April to August 2017, we placed one camera at each water source and dry

site. Of these deployments (n=34), 12 sites ran uninterrupted for a minimum of one week at both water sources and dry sites simultaneously (n=24 deployments; 387 trap nights total; Appendix 1.3). Images were classified by counting animals of a given species for each "trigger," which consisted of all images taken within a five minute interval (as in (Thorn et al. 2009)). We calculated the mean number of herbivores of each guild (grazer, mixed, and browser) per day for each deployment. We ran a GLMM with a negative binomial distribution using count as the response and site (water source/dry site), soil (silt/clay vs. sand), and herbivore (browser, mixed, or grazer) as fixed effects and location as a random effect, testing significance of all interactions using X^2 tests of nested models. Finally, to assess the degree to which our dung and camera trap data agreed with each other, we ran a Spearman's rank correlation test on dung and camera counts matched by herbivore, site (water vs dry), and location (n=72). Details are available in Appendix 1.3.

2.3.5 Height and cover analyses: To determine changes in plant height as a function of distance to water, we calculated the paired differences in mean maximum grass or forb height across all six transects at each 5m interval for each water source and dry site pair. For counts, we aggregated pin hits within three discrete distance bands from water (or the 0m mark at dry sites): 0-45m, 50-95m, 100-145m, calculating the percentage of pins that touched a "grass", "forb", "tree/shrub" or "bare ground" (out of a maximum 60 pin hits per distance band). Thus, for trees, percent cover refers to any cover above 1 meter. We modeled differences in height and percent cover of each vegetation type using linear mixed-effect models (LMMs): fixed effects included distance to water (a proxy for herbivore aggregation; Appendix 1.4) and the interaction with soil type (silt/clay vs. sand), in addition to mean annual precipitation (MAP), and prior rainfall (30-day aggregate). We modeled location (n=17) and sampling

period (n=4) as crossed random effects and ensured that variance was not inflated using the *car* package (Fox and Weisberg 2011). We performed regression analyses using the lme4 package (Bates et al. 2015) in R studio 3.5.1 (R Core Team 2016) and used the lmerTest package (Kuznetsova et al. 2017) to perform backwards stepwise selection using sequential F-tests. Summary tables of differenced and non-differenced data can be found in Appendix S5. Finally, we investigated the combined and separate effects of herbivore aggregation and distance from water on non-differenced plant cover using additional LMMs. Models were very similar to those fit with differenced data and show that distance to water and herbivore dung counts explain similar variation in plant cover (Appendix 1.4).

*2.3.6 Diversity analyses***:** We calculated species richness (SR) by summing the number of species across transects at each distance interval at watering sources and dry sites for each location and season. Because SR depends on abundance, we also calculated differences in rarified SR using the *vegan* package (Oksanen et al. 2016) for each experimental pair, using LMMs to explore variation over distance to water (a proxy for herbivore aggregation; Appendix S4), soil type, MAP, and prior 30 day rainfall, again checking for variance inflation. To ensure SR calculations were unaffected by greater spatial area covered in outer rings or at larger water sources, we verified that SR did not change among concentric rings at dry sites and used paired comparisons to the same distance interval at water (Appendix 1.6). We performed model selection using the same methods described above and repeated this procedure on Shannon diversity calculations.

      To explore other diversity metrics that account for evolutionary history, we created a phylogenetic tree using the Phylomatic tool, version 3 (http://phylodiversity.net/phylomatic/) (Webb and Donoghue 2005) and based on the APG III (2009) phylogeny. If species were not

available, we used genus-level classification. We then used Phylocom 4.2 to add branch lengths based on (Gastauer and Meira-Neto 2013). We chose two metrics to investigate different aspects of phylogenetic changes at water sources; Faith's PD (PD) and mean pairwise distance (MPD) (Tucker et al 2017; see Appendix 1.7 for details). We compared observed PD to a null model that randomized species abundances within sites, but we maintained sample richness ("richness" argument to ses.PD function in *picante*). We calculated standardized effect size differences for water sources and dry sites (ΔPD_{SES}), using distance from water and its interaction with soil type, plus prior rainfall and MAP as fixed effects, and location and season as random effects. We repeated this analysis for differences in standardized effect size for mean pairwise differences (MPD_{SES} and $MPD_{SES.AB}$), controlling for richness by comparison to null models (Webb et al. 2008). As with plant cover models, we examined the combined and separate effects of herbivore aggregation and distance from water on non-differenced diversity data using additional LMMs. Models were very similar to those fit with differenced data and show that distance to water and herbivore dung counts explain similar variation in plant diversity differences (Appendix 1.4).

2.3.7 Species-specific analyses: We used two approaches to investigate species-specific differences at water sources. First, we assessed dominant plant diversity at each site, following (van der Westhuizen et al. 2005). For each transect and distance bin, we identified the dominant plant species (greatest percent cover, excluding bare ground) and compared dominant species frequency across sites. Second, we used the most abundant 40 species that accounted for >90% of plant counts to construct LMMs that modeled change in percent cover by distance from water, soil type, and their interaction, using location and season as random

effects. We evaluated model parameters using Wald Chi-Squared tests via the lmerTest package (Kuznetsova et al. 2017).

For all analyses, given that soil and MAP covaried (i.e. silt/clay soil occurred only in high MAP areas), we compared models including either MAP or soil as a predictor. In all models, soil type explained more variation than MAP, but we noted that rainfall effects were likely to be important. Thus, we referred to silt/clay soils as "mesic" and sandy soils as "arid" in our results.

2.4 Results

2.4.1 Effect of water sources on herbivore aggregation

Both dung counts and camera trap data provided similar results, showing that grazers and mixed feeders were 1.5 to two times more abundant at water sources than dry sites (Figure 3). Meanwhile, browser dung and camera trap counts were only slightly elevated near water. Soil was significant in the model of dung counts for mixed feeders: counts were lower on mesic silt/clay than arid sand at both water and dry sites. However, there was no significant interaction between soil and site (water vs dry) for any group. Dung counts and camera trap sightings were significantly and moderately correlated (Spearman's $\rho = 0.52$. $p < 0.001$, n=72). This agreement suggests consistent broad patterns in herbivore aggregations around water. Finally, distance to water alone explained 50% of variation in total dung counts, and 62% when soil type and its interaction with water was included, indicating that water proximity describes most of the variation in herbivore aggregation, but with some differences by soil type. Specifically, total dung counts were ~1.3 times higher on sand compared to silt/clay at both dry sites and water sources. However, on both soils, dung counts at water

sources versus dry sites were 1.6 times higher (all distances) and 2.25 times higher in the 0-50m zone. Detailed results are provided in Appendix 1.3 and Appendix 1.4.

Figure 3. Herbivore activity at water sources

Measurements of herbivore activity at water sources and controls using both A) camera trapping and B) dung counts show that mixed feeders and grazers tend to be more strongly associated with water, while effects are smaller for browsers. There were no major differences depending on soil type, except that dung counts for mixed feeders were higher at both controls and water sources in high-stress contexts (drier sand) than lower-stress contexts (wetter silt/clay) (although the difference between control and water did not differ by soil type). Letters denote significantly different groups (across soil types) with Tukey's adjustment for multiple testing.

2.4.2 Effect of water sources and herbivore aggregation on understory height and tree, grass, forb, and bare ground cover

Distance from water was important in all models except for grass cover (Table 1; Figure 4). However, soil type strongly modulated the effect of distance on understory height and tree cover ($F_{1,2054} = 14.13$, $F_{2,177} = 8.82$ respectively; $p<0.001$ for both interactions; Figure 4A and C). Understory height was approximately 25% lower at the water's edge on mesic silt/clay but was reduced to a lesser extent on arid sand (-18% compared to dry sites). Tree cover did not differ between water sources and dry sites on mesic silt/clay (and even trended higher near water) but was reduced by one half in the sampling area closest to water on arid sandy soil. Meanwhile, forb cover was reduced by one half at the closest sampling area to water on both soil types ($F_{2,177} = 27.33$; $p<0.001$; Figure 4E); matching a pattern in which bare ground cover nearly doubled near water compared to dry sites ($F_{2,178} = 17.95$; $p<0.001$; Figure 4 F). Similarly, grass cover was 10% lower at water sources across all distances on silt/clay soil (but not sand; Figure 4D), with no effect of distance to water. Higher prior rainfall was associated with an increase in understory height and grass cover at water sources relative to dry sites (2mm increase per cm of rain, $F_{1,2054} = 14.13$; $p=0.01$ for Δ height, 1% increase per cm rain, $F_{1,190} = 18.81$; $p<0.001$ for Δ grass; Figure 4B and D), and a decrease in bare ground cover (-0.6% per cm rain, $F_{1,145} = 7.24$; $p=0.01$; Figure 4F). Mean annual precipitation was not an important factor in any model because soil type explained most of the variation in plant differences along the rainfall gradient. Location was a significant random effect in all models, but period was only important in models of understory height.

Figure 4. Differences in plant cover

Differences in understory height and percent cover (water sources minus controls) for different plant groups. Values above zero indicate higher levels at water sources. A) Understory height was reduced near water on both soil types, but this effect was more severe for wetter silt/clay soils than drier sandy soils. B) Understory height was more severely reduced at water sources during dry periods. C) Tree cover was reduced near water on drier sandy soil, but it was slightly elevated near water on wetter black silt/clay soil. D) Grass cover was lower near water on silt/clay soils, and this effect was strongest during periods of low rainfall. On sandy soils, grasses tended to be more abundant at water sources during wet periods. E) Forbs were reduced near water sources for both soil types. F) Bare ground counts showed the combined response of trees, forbs and grasses; bare ground was most frequent in the 0-45m zone close to water across all rainfall conditions, but it was reduced at further distances during wetter periods.

2.4.3 Effect of water sources and herbivore aggregation on plant diversity

Soil type interacted with distance in models of differences in rarefied species richness (SR), Shannon diversity (SD), and Faith's PD (PD) (Table 1). Remarkably, water source presence had opposing effects on all diversity metrics across the two soil types. On arid sand, SR, SD, and PD declined near water relative to dry sites (16%, 17%, and 15% decrease for each metric respectively in the 50m closest to water; p<0.001). However, on mesic silt/clay, all three metrics increased within the 100m closest to water compared to dry sites (13-22% increase in SR, 8-15% increase in SD (Figure 5), and 7-28% increase in PD). Higher prior rainfall also had a small positive effect on SD at water relative to dry sites (+0.2 units per 100 mm rain, $F_{1,183}$ = 4.26; p=0.04). When we controlled for SR, there were no differences in standardized effect size of PD or MPD between water sources and dry sites. However, $MPD_{SES.AB}$ was significantly lower near water on silt/clay soil ($F_{2,171}$ = 3.11; p=0.05; Table 1). However, the total explanatory power for the fixed effects in this model was low ($R^2_{Marginal}$ = 0.05), indicating that none of the parameters had a particularly strong effect compared to location and period variables (Table 1).

Figure 5. Water, herbivores, and plant diversity

Diversity metrics tended to increase with herbivore dung counts on wetter silt/clay soils but decreased on drier sandy soils (A). Since herbivore dung counts were elevated at water sources, this led to a pattern in which diversity was higher at silt/clay water sources compared to dry sites, and diversity was lower at sandy soil water sources compared to dry sites. B) A schematic of our results illustrates increased diversity and a transition from grass to grass/trees with increasing herbivore pressure at silt/clay sites, and decreased diversity and a transition from grass/trees to grass on sandy soils. Images show camera trap sightings at dry sites (bordered in red) and water sources (bordered in blue) on different soil types.

Table 1. Plant LMMs

Changes in vegetation height, cover, and diversity at water sources relative to dry sites. Parameters that increase vegetation or diversity near water are shaded blue, while those associated with declines are shaded orange. Intercepts for models of cover correspond to inner rings on sandy soils. Prior rainfall is scaled to cm for computational purposes.

Understory	Fixed						Random	Fit
	Intercept Sand	*Distance*		*Silt/clay*	*Distance: Silt/clay*		*Prior Rainfall*	$\sigma^2 / \tau_{Site} / \tau_{Season}$	R^2_M / R^2_C
ΔHeight (mm)	-57.94 -105, -11 -2.4 (0.03)	0.14 0.0, 0.3 2.2 (0.03)		-60.33 -110, -10 -2.4 (0.03)	0.36 0.55, 0.17 3.76 ***		2.01 0.5, 3.6 2.6 (0.01)	9506.5 2464.2 963.58	0.04 0.30

Cover

	Intercept Sand, Inner	*Middle*	*Outer*	*Silt/clay*	*Middle: Silt/clay*	*Outer: Silt/clay*	*Prior Rainfall*	$\sigma^2 / \tau_{Site} / \tau_{Season}$	R^2_M / R^2_C
ΔTrees	-12.10 -18.1, -6.2 -4.0 (.001)	8.48 5.1, 11.9 4.9 ***	10.14 6.8, 13.5 5.9 ***	15.83 7.2, 24.4 3.6 (.002)	-5.37 -10.4, -0.4 -2.1 (0.04)	-10.81 -15.8, -5.8 -4.3 ***		53.95 67.96 0.29	0.24 0.67
ΔForbs	-8.15 -11.7, -4.6 -4.5 ***	6.99 4.4, 9.6 5.4 ***	9.33 6.8, 11.9 7.1 ***					57.21 35.96 1.20	0.14 0.48
ΔGrass	-1.89 -8.9, 5.2 -0.5 (0.60)			-14.20 -23.9, -4.5 -2.9 (0.01)			0.95 0.5, 1.4 4.4 ***	88.41 95.42 0.0	0.25 0.64
ΔBare Ground	14.21 8.4, 20.0 4.8 ***	-7.92 -11.1,-4.7 -4.9 ***	-9.00 -12, -5.8 -5.5 ***				-0.06 -0.1, -0.02 -2.7 (.008)	88.74 86.62 2.56	0.11 0.55

Diversity

	Intercept Sand, Inner	*Middle*	*Outer*	*Silt/clay soil*	*Middle: Silt/clay*	*Outer: Silt/clay*	*Prior Rainfall*	$\sigma^2 / \tau_{Site} / \tau_{Season}$	R^2_M / R^2_C
ΔSR rarefied	-1.87 -3.1, -0.6 -3.0 (.006)	1.05 0.2, 1.9 2.4 (0.02)	1.89 1.0, 2.8 4.2 ***	3.28 1.7, 4.9 4.0 ***	0.01 -1.3, 1.3 0.0 (0.99)	-1.68 -3.0, -0.4 -2.6 (0.01)		3.43 1.87 0.33	0.29 0.57
ΔSD	-0.50 -0.7, -0.3 -4.6 ***	0.24 0.1, 0.4 3.3 ***	0.38 0.2, 0.5 5.4 ***	0.60 0.4, 0.9 14.6 ***	-0.08 -0.3, 0.1 -0.8 (0.42)	-0.33 -0.5, -0.1 -3.2 ***	0.02 0.0, 0.03 2.1 (0.04)	0.09 0.05 0.01	0.34 0.61
ΔPD	-143.80 -274,-13.9 -2.2 (0.04)	100.44 1.5,199.4 2.0 (0.05)	219.36 120, 318 4.4 ***	215.01 29.2, 401 2.3 (0.03)	129.72 -15.6, 275 1.8 (0.08)	-136.59 -281.9, 8.7 -1.8 (0.07)		43901 26092 797.34	0.22 0.52
ΔPD_{SES}	0.21 -0.2, 0.6 1.1 (0.30)							0.94 0.40 0.03	0.00 0.32
ΔMPD SES	0.27 -0.7, 1.2 0.6 (0.58)							6.61 2.03 0.28	0.00 0.26
ΔMPD SES.AB	0.10 -0.9, 1.1 0.2 (0.85)	0.04 -3.3, 0.4 0.1 (0.94)	0.47 -0.5, 1.4 1.0 (0.34)	-1.46 -3.3, 0.4 -1.6 (0.13)	1.79 0.4, 3.2 2.5 (0.01)	0.68 -0.7, 2.1 0.9 (0.35)		4.68 2.50 0.38	0.05 0.44

Legend:
Estimate
95% CI
T (P value)

2.4.4 Effect of water sources and herbivore aggregation on plant species responses

Dominant species composition near water also varied by soil type. On mesic silt/clay soil, there were fewer dominant species at dry sites than water sources, while the reverse was true on arid sandy soils (Figure 6). Notably, on silt/clay soils, the grass *Themeda triandra* was the most abundant species present for 42% of dry site transects, but only for 3% of transects near water. Meanwhile, on sandy soils, the grass *Cynodon dactylon* was the most abundant plant for 29% of dry site transects, but this shifted to 89% of transects close to water. More generally, the species-specific analyses revealed marked variation in plant performance around water sources (Figure 7).

Figure 6. Variation in plant dominance

A comparison of the relative frequency of the most abundant species for each transect at water sources and controls shows that few species dominate in a low stress context (wetter nutrient-rich silt/clay) controls (Themeda triandra and Pennisetum spp.), while only one species, Cynodon dactylon, typically dominates at high stress (dry, nutrient-poor sand) water sources. Distance from center (0-45m, 50-95m, 100-145m), which was closely associated with increased herbivore dung counts, is overlaid for each pie chart, demonstrating increasing effects with decreasing proximity to water. All grasses are shown in shades of green, except for stoloniferous Cynodon species in red hues, while trees and forbs are shown in blue and purple respectively.

Figure 7. Species-specific differences

Directional responses for each of the 40 plant species that comprise >90% of vegetation counts varied considerably in both magnitude and direction across distance and soil type (x-axis). Species that increased at water relative to controls are colored in green, while those that decreased are shaded gold. Significant differences from 0 (α=0.05) are outlined, and corresponding species are bolded. The average of the total percent cover for each species across sites is shown in grayscale to illustrate relative abundance of each species (ranging from 2-20% cover). Increasing outward distance correlates with decreasing herbivore density.

2.5 Discussion

Our results indicate that water source proximity, which was closely related to increased herbivore activity, has strong effects on plant communities, and that abiotic factors can influence the degree and direction of these effects. On mesic silt/clay soil, total grass cover declined, but the increase in grazing lawn species and trees resulted in a 25% increase in species richness, Shannon diversity, and phylogenetic diversity in just a 50-meter span where herbivores gathered. Meanwhile, on arid sandy soil, trees and forbs declined, resulting in a 15% decrease in diversity. Remarkably, a dominance change for two key grasses, *Themeda triandra*, a tussock-forming 'keystone' grass across savannas worldwide (Snyman et al. 2013), and *Cynodon dactylon,* a prostrate grass critical to grazing lawns (McNaughton 1984), provided a clear signal of differing plant communities near water on each soil type. Reduction of *T. triandra* near silt/clay water sources corresponded with nearly twice as many other dominant species, while *C. dactylon* dominated nearly every transect near sandy-soil water sources (90% of transects) (Figure 6). These results differed from our expectation that all but a few plant species would decline near water as a result of high herbivore pressure. Our observations indicate that increased water and nutrients can reduce elevated herbivore impacts in removing plant cover and reducing diversity around water sources that have been previously documented in piosphere research, suggesting that idiosyncratic previous results may have arisen partly due to site differences in primary plant stressors. While in some cases it is likely that previous work was unable to detect meaningful effects of grazing gradients at piospheres because conditions were already extreme (Stumpp et al. 2005), in another example, this may explain why (Landsberg et al. 2003) found that species richness was highest in piospheres with higher precipitation on nutrient-rich alluvial soils, and lowest on

piospheres with more nutrient-poor sandy soils and lower precipitation despite similar

grazing histories, another important co-factor that can modulate plant responses to herbivory.

2.5.1 How does water source proximity and herbivore aggregation affect different plant groups?

Our finding that mean understory height decreased at water sources corresponded

with our expectations and is supported by other studies (Andrew 1988, Landsberg et al. 2003,

Egeru et al. 2015). This is likely due to increased grazing and trampling due to herbivore

aggregation (Figure 3, Appendix 1.3 and Appendix 1.4). In addition to increasing bare

patches and reducing grass height (Graetz and Ludwig 1976), increased grazing pressure can

shift plant species composition to shorter species that spread laterally (Wesuls et al. 2012,

Hempson et al. 2015), as is typical of grazing laws (McNaughton 1984, Hempson et al.

2015). However, detailed tree, forb, and grass analyses indicated complex responses

dependent on soil and prior rainfall. For grasses, cover increased at water sources relative to

dry sites only during wet periods on both mesic silt/clay and arid sand contexts. This finding

may indicate 'compensatory growth,' when grazers can stimulate increased nitrogen uptake,

which, when coupled with increased moisture, results in positive vegetation responses

(McNaughton 1979). Indeed, elevated recent rainfall reduced the inverse relationship

between herbivore dung counts and grass cover near water on silt/clay soils and resulted in

increasing grass cover with increasing herbivore dung counts on sandy soils (Figure 4B, D,

F; Appendix 1.4).

While our results for sandy soils followed expectations, plant communities responded

to water sources and associated herbivore aggregations differently on mesic nutrient-rich,

silt/clay soils. Trees increased while grass cover decreased near water, suggesting that

grazing and abiotic factors can suppress tall grasses that exclude other species and improve tree persistence. Indeed, one study on black-cotton soils in this system found that grass competition could be just as important in limiting tree growth as herbivory and fire (Riginos 2009). Other studies have shown that herbivores can increase tree establishment by reducing competitively dominant grasses (Milchunas et al. 1988, Osem et al. 2002). Furthermore, our dung count results suggested that mixed feeders (including elephants that impose strong pressure on trees), were less common on silt/clay soil, possibly reducing tree herbivory relative to grazer pressure on grass. Other studies investigating tree-grass relationships in this system have found a negative relationship between tree density and grass biomass and that this may be modulated by soil texture for some dominant species (Riginos and Grace 2008, Riginos et al. 2009). Higher soil moisture near water sources can also facilitate tree growth on silt/clay soil. Since water is readily retained in fine soil particles compared to sand, deeper-rooted forms, which tend to be trees in this system (Holdo et al. 2018), may benefit from proximity to water sources. Indeed, other studies have noted denser tree cover near drainages on fine-textured soils, indicating competition between grasses and trees (Scholes and Archer 1997). Finally, increases in soil phosphorus, a possible limiting nutrient to savanna trees (Pellegrini 2016), near water (Appendix 1.1), coupled with potentially lower herbivory from mixed feeders (Appendix 1.3), and higher MAP and soil moisture, may explain why trees increased at water sources on silt/clay soils while other plant groups declined.

Cumulatively our results supported previous findings that water sources tend to reduce overall vegetation abundance, but only at sites with low water and soil nutrients. The most parsimonious explanation for patterns observed near water (e.g. decreased overall

cover, less forbs) is increased aggregation of herbivores near water (Figure 3; Figure 5; Appendix 1.4). One possible reason for divergent diversity patterns is that effects could depend on whether plants are limited by below-ground resources (nutrients and water) and disturbance (herbivory and trampling), or above-ground competition. This balance may be viewed as a transition from environmental filtering, in which low soil moisture and nutrients compounded with herbivore pressure can constrain many species (Poorter and Garnier 2007); to niche partitioning, in which herbivory coupled with increased soil moisture and nutrients can allow additional species, including highly productive and palatable grazing lawn grasses, to grow by dominant species removal (Osem et al. 2002). The balance of these above and below-ground variables can inevitably have strong effects on plant diversity and community composition (Maire et al. 2012).

2.5.2 Do water sources and herbivore aggregations affect plant diversity? Do abiotic factors mediate this relationship?

Given that plants were typically less abundant near sandy-soil water sources, it is unsurprising that species richness, Shannon diversity, and phylogenetic diversity were lower in these areas (Figure 5, Appendix 1.4). On arid sandy soil, diversity in the inner ring around water was very low, echoing previous findings in which the region closest to water had almost no vegetation (Perkins and Thomas 1993, Thrash and Derry 1999). However, we also found that richness and diversity increased up to 25% near water on silt/clay soil compared to dry sites. This is likely due to removal of dominant tall grass by trampling and grazing, facilitating growth of otherwise out-competed species (Scholes and Archer 1997, Osem et al. 2002). The positive relationship between Shannon diversity differences and prior rainfall also shows that seasonal rainfall promotes plant abundance and evenness near water. This

demonstrates that lower water stress and/or reduced herbivore activity during the wet season can promote growth of other species, indicating that water limitation and herbivory can act as environmental filters (Figure 5).

We also found that Faith's PD mirrored species richness patterns at water sources. This was as expected, given that these two metrics can be highly correlated (Cadotte et al. 2009, Tucker et al. 2017). When we used null models to control for species richness, we found no differences between water sources and dry sites in any context for PD and MPD, contrary to our expectations. This is likely because PD and SR were highly correlated for our phylogenetic tree (Pearson's r=0.92), which is not always the case, as this correlation can depend on tree shape, size, and spatial characteristics (Tucker and Cadotte 2013). After controlling for species richness, PD contributed little explanatory power for changes in plant diversity at water sources.

2.5.3 Which plants have positive and negative responses to water proximity and herbivore aggregation? Does this depend on abiotic context?

Our species-specific results provide key insights into potential mechanisms by which diversity metrics change depending on abiotic context. On mesic nutrient-rich silt/clay soils, the reduction of a dominant grass, *Themeda triandra*, at water sources resulted in a more diverse array of species comprising cover (Figure 6). Meanwhile, we found the opposite pattern on arid sandy soils; *Cynodon dactylon* dominated 89% of transects. We postulate that these opposite responses to herbivore aggregation can be attributed to variation in key plant characteristics whose tradeoffs become apparent across a gradient ranging from high competition (high abiotic resources and low herbivory) to elevated stress (low abiotic resources and high herbivory).

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Themeda triandra is considered one of the most important species in tropical grasslands worldwide (Snyman et al. 2013); while its presence can indicate healthy grassland, it can also decrease diversity by excluding other species (Fynn et al. 2004). *Themeda* often declines under heavy herbivory and drought, perhaps because it relies on above-ground seed reproduction, making it vulnerable to trampling and grazing (O'Connor 1994, Snyman et al. 2013). In contrast, *C. dactylon*, can proliferate with increased herbivory (McNaughton 1984, Egeru et al. 2015), and grow in bare patches where other species do not survive (Graetz and Ludwig 1976, Perkins and Thomas 1993). *Cynodon dactylon* can produce both stolons and rhizomes, even exhibiting plasticity depending on environmental pressures (Dong and de Kroon 1994), allowing it to propagate in heavily grazed areas, including water sources (van der Westhuizen et al. 2005, Jawuoro et al. 2017) and grazing lawns (McNaughton 1984, Porensky and Veblen 2015). Therefore, dominant plant reproductive and growth traits may be critical in determining community responses to herbivore aggregation across a range of systems and conditions.

This dominance change for only two species in opposing contexts is consistent with the framework outlined in Hempson et al. 2019 for grassland systems: with increasing herbivore pressure near water, plant communities were comprised by grazer resistors (*C. dactylon*), and in areas of lowered herbivore pressure and no fire, plant communities were dominated by generalist tolerators (*T. Triandra*). Our results also aligned with a broad, global pattern that large herbivores can have opposite effects on plant diversity depending on environmental context. Indeed, a meta-analysis of North American and European grasslands (Bakker et al. 2006) found that large herbivores increased plant diversity in high productivity areas, but decreased diversity in low-productivity sites, although (Koerner et al. 2018) found

that herbivore-induced change in dominance determined plant diversity responses to herbivory across rainfall contexts.

2.5.4 Additional Considerations: There are several aspects of this study to consider in interpreting results. First, in this system, MAP and soil type are collinear: nutrient-rich silt/clay soils occur in high MAP areas and sandy soils occur in drier areas; thus, results attributed to soil may have also been driven by rainfall (Fig. 1D). While a rainfall gradient exists within each of these soil types, we were unable to detect a significant signal of MAP. Studies incorporating a broader rainfall gradient across both soil types may reveal a stronger signal of MAP, an important gradient in this system (Goheen et al. 2013) and across grasslands globally (Rodríguez-Castañeda 2013). Second, in other soil contexts (high moisture/low nutrients or low moisture/high nutrients), we expect that herbivores and their physiological needs (which in turn are affected by these abiotic variables) will shape plant communities at water. If herbivores aggregate strongly on high moisture/low nutrient soils, we expect richness to increase if herbivores remove a dominant plant species. The extent to which this occurs may be lower if matrix richness is higher than that observed in our study. In low moisture/high nutrient contexts, we expect herbivore aggregation to reduce richness, but this effect may be lower if increased nutrients can promote greater herbivory tolerance or avoidance. Third, our measurement of seasonality as the accumulated 30-day rainfall total likely varies in relevance to different plants, as growth and uptake vary among species and functional groups (Breshears and Barnes 1999, Ogle and Reynolds 2004). Different species will likely display different responses to both rainfall totals and variability. Fourth, in many grassland ecosystems, fire and herbivory interact to shape plant communities (Archibald and Hempson 2016, Donaldson et al. 2018, Hempson et al. 2019). Fire has not been a major

consumer in the study area for over 60 years (Okello et al. 2008), due to fragmentation by roads and suppression by humans (Pringle et al. 2015), an increasingly common pattern (Andela et al. 2017). Therefore, our results are likely largely driven by herbivores that interact with soil and rainfall factors to shape plant communities.

While our study suggests that the net effects of herbivores and soil moisture at water sources on plant communities are mediated by soil type and rainfall, it is also likely that the degree to which herbivores impact vegetation at water varies seasonally and across a rainfall gradient, as aridity may promote animals, especially those that are heavily water dependent, to more strongly congregate near water. Additional experimental studies could also assess the impacts of adding and removing water sources on herbivore behavior, plant responses, diversity, and ecosystem functioning. While our results are likely to be broadly applicable to naturally-occurring or dammed water sources, it is likely that plant cover around boreholes or water troughs (the focus of most previous literature on the topic, e.g. (Thrash and Derry 1999)) is more likely to be reduced, as these water sources provide little additional moisture to plants to compensate for trampling and grazing, and which concentrate herbivores in very small areas (Stumpp et al. 2005). Finally, while we did not quantify drainage depth or water source hydrology, they likely describe variation in plant responses that we were unable to capture in our analyses. Given the global importance of natural and dammed water sources similar to those used in our study (e.g. Lasage, Aerts, Mutiso, & de Vries, 2008; O'Connor, 2001), our results provide valuable insight into vegetation responses at these key savanna resources.

2.6 Conclusion

Humans and their domestic animals increasingly rely on water sources to mitigate effects of increased rainfall stochasticity and drought susceptibility (Lasage et al. 2008), and the same is likely to be true for wildlife. Water sources in arid environments function as ecological hotspots that may also contribute to landscape-level diversity by imposing spatial heterogeneity in grazing (Augustine 2003), giving rise to vegetation mosaics that are likely to be essential in structuring these ecosystems (Swanson et al. 1988). However, amid rapid climatic changes and development, humans are dramatically modifying surface water supply and distribution (de Wit and Stankiewicz 2006, Gosling and Arnell 2016), requiring a clearer understanding of plant community responses at these water sources. Our findings suggest that where nutrients and moisture are plentiful, water proximity and herbivore aggregation may be associated with increased plant diversity as the relative abundance of a dominant species declines, but in arid low-nutrient conditions and very high herbivore pressure, diversity may decline. It is likely that higher herbivore densities suppress tall grasses that dominate in high productivity sites, allowing rarer species and grazing lawn grasses to flourish. In low productivity sites, high herbivore densities act as a compounding environmental filter such that fewer species survive and reproduce. Our findings imply that abiotic factors can explain the direction and extent of long-term effects of water sources and herbivore aggregations on plant composition and diversity, an increasingly important topic amid continually changing water supply across arid landscapes.

Laikipia was converted to commercial and group ranches, communal lands, and conservation areas. Mpala's land and resources are managed by a board of trustees including international and Kenyan institutions focused on a mission of science, education, conservation, and community outreach. We thank Mpala Research Centre and Kenya Wildlife Service for facilitating this work. Fieldwork for this project is permitted under the Kenyan National Commission for Science, Technology, and Innovation (NACOSTI/P/16/0782/10585) and Kenya Wildlife Service (KWS/BRM/5001). We are grateful to Jenna Hulke, Michelle Long, and Douglas Branch for additional field assistance. We thank Grace Charles for helpful insight that contributed to this project.

Coauthors and contributions:

Godfrey Amooni[1] was pivotal in assisting in plant identifications and conducting surveys, along with John Mantas[1], who assisted with dung surveys and camera trap monitoring. Hillary Young[1,2] collaborated on study design, interpretation of results, and manuscript preparation for publication.

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Chapter 3

3. Water sources aggregate parasites, with increasing effects in more arid conditions

3.1 Abstract

Landscape heterogeneity and climate can influence animal behavior and movement in ways that profoundly alter disease transmission. In the case of fecal-oral parasites, water resources may aggregate large groups of many different host species in small areas, concentrate infectious material, and function as disease hotspots. This may be exacerbated where water is scarce and for species that require frequent access to standing water. However, while many studies have documented the role of water in drawing animals together, there has been little work, and no experimental study, on the effects of water

sources on disease transmission, much less any comparison across a range of hosts and climatic contexts.

Working in an East African savanna system, we show via experimental and observational methods that the presence of standing water (whether natural or artificial) can increase the density of both wild and domestic herbivore feces, and thus, the concentration of fecal-oral parasites in the environment, by up to two orders of magnitude, creating landscape hotspots of disease transmission. Furthermore, our results show that this effect is amplified in drier areas and following periods of low rainfall, creating dynamic and heterogeneous disease landscapes across space and time. However, this effect varies markedly by herbivore species, with strongest effects observed for two large, water-dependent animals that are of critical concern for conservation and development: elephants and cattle. Thus, for these animals, even under regular climatic conditions, water resources may entail a complex trade-off between a critical resource (water) with an important risk (parasite exposure). When water availability is reduced – a global pattern that is increasing amid climate changes and growing anthropogenic water use – risk of parasite exposure may increase substantially, posing multiple threats to these critical taxa.

3.2 Introduction

For many environmentally-transmitted parasites, landscape heterogeneity can create localized transmission hotspots that have the potential to markedly affect overall parasite exposure risk (Paull et al. 2012, Leach et al. 2016, Dougherty et al. 2018). Water sources may serve as important transmission foci in a landscape, as they can concentrate a wide range of hosts in a small area where parasite exposure may be increased via drinking or nearby foraging (Vicente et al. 2006, Nunn et al. 2011). Furthermore, water sources are particularly

important in arid climates, where the impacts of climate change are likely to be especially pronounced (IPCC 2014). Climate change is predicted to lead to half of the globe's surface being covered by dry land by the end of this century (Huang et al. 2016), underscoring the growing importance of stable access to water in these regions for the two billion people currently living in water-stressed areas (Oki and Kanae 2006).

While more than 60,000 large dams have been built across the globe in the past 60 years, their density is lowest in Africa, emphasizing the importance of smaller water sources that are not well recorded. Across Kenya, sand dams and other small reservoirs are critical for buffering humans, their domestic animals, and wildlife against drought and unpredictable seasonality (Lasage et al. 2008, Ryan and Elsner 2016). The shifting landscape of water availability (de Wit and Stankiewicz 2006) can subsequently influence herbivore behavior (Redfern et al. 2003, Ogutu et al. 2014), and potentially result in increased aggregations and parasite transmission where water is relatively scarce. Although the potential for water sources to function as disease hotspots has been previously noted (Paull et al. 2012, Nunn et al. 2014), there is very little quantitative work on their role in influencing transmission, and no studies have focused on a broad range of hosts and parasites across a rainfall gradient.

Water sources are recognized to aggregate animals, particularly in drier climates (Western 1975, Valeix et al. 2008a). For example, water distribution can control the movements of the largest population of elephants in the world, with aggregations rising at the limited number of water sources remaining during the dry season (Chamaillé-Jammes et al. 2007a). However, the degree to which different herbivores gather at water depends on each species' diet and physiology. Grazers, for example, tend to associate with water more commonly than browsers (Western 1975, Valeix et al. 2008b, Hayward and Hayward 2012),

and this is likely to be especially true for man-made water sources, as opposed to rivers (Smit et al. 2007). However, even for animals that acquire water from rivers, increasing regional fluctuations in river flow (Snoussi et al. 2007) are likely to contribute to surface-water scarcity that may drive animals to share standing water sources. Furthermore, rainfall seasonality influences the degree to which animals concentrate at water (Western 1975) with heightened aggregations during dry seasons relative to rainy periods (Chamaillé-Jammes et al. 2007b, Sutherland et al. 2018). However, despite widespread acknowledgment that water aggregates animals, there is scarce quantitative data on the degree to which animals congregate relative to their background density, or how this varies across regional climatic gradients. Understanding these patterns will provide critical new information about relative parasite risk at the spatial scale at which transmission is most likely to be relevant (Morgan et al. 2004).

Animal aggregations in general are well-established to drive increased risk of disease transmission (McCue and Thorson 1964, Chamaillé-Jammes et al. 2008), particularly for density-dependent parasites (Anderson and May 1978). Fecal-oral transmitted parasites, including many helminths that inflict serious morbidity on domestic and wild herbivores, release many thousands of parasitic ova into the environment upon host defecation. Many of these parasites commonly infect herbivorous mammalian hosts when they drink water or consume forage contaminated with infective parasite stages from feces (e.g. Strongylida, R. C. Anderson, 2000). Increased time spent at water should, in theory, lead to both increased density of dung (and thus parasites), and increased risk of exposure of infective stages via drinking and eating. This link has been supported by modeling work (Nunn et al. 2014) and by one observational study on red deer noting that deer aggregation at water sources was

positively associated with prevalence of *Elaphostrongylosis cervi* (Vicente et al. 2006). In other systems, food resources have been manipulated to study corresponding increases in directly transmitted pathogens in racoons (Wright and Gompper 2005b), and carcass sharing among carnivores has been thought to increase potential for pathogen transmission (Ogada et al. 2012). However, there has been no large scale or experimental work designed to test the role of water sources in increasing potential for parasite sharing.

While water-driven aggregation of parasites likely occurs across a variety of landscapes where water is scarce and highly concentrated, East African tropical savannas provide an ideal place to investigate this phenomenon as they are home to a diverse array of herbivores in a largely water limited landscape. Common wild African ungulates include many locally or globally declining species such as zebra (*Equus burchellii* and *Equus Grevyi*), giraffe (*Giraffa camelopardalis)*, elephant (*Loxodonta* africana), buffalo (*Syncerus caffer*), and impala (*Aepyceros melampus*), all of which are infected by a diverse and abundant community of helminths (Round 1968). While many parasites are host-specific, substantial parasite sharing occurs even among taxonomically divergent species (Wells et al. 2018) when those species overlap spatially (VanderWaal et al. 2014b). Notably, several important parasites (e.g. trichostrongyle nematodes) are shared with closely-related domestic animals or with humans (Round 1968, Walker et al. 2017). While many parasite sharing links remain uncertain (Walker et al. 2017), it is likely that several pose significant health threats (Bull 1994, Ashford and Crewe 2003).

In this study, we asked two specific questions: **1) To what extent do water sources concentrate herbivores, their feces, and thus, fecal-oral parasites, across multiple herbivore species?** We addressed this question using a two-year water removal and

replenishment experiment in an East African tropical savanna system, We hypothesized that the presence of a water source would increase the density of herbivores and their feces; and, based on density of parasite eggs in dung, the density of parasites deposited in the environment. Based on prior work documenting herbivore water dependence, we expected this effect to be most apparent for large herbivores and grazers; specifically, elephants, cattle, buffalo, and zebra. We then extended our first question to ask: **2) How do herbivore dung and parasite density at watering holes vary across different rainfall contexts?** We addressed this question using observational data gathered from water sources spread across a broad rainfall gradient and over three years of sampling in central Kenya. Given that surface water is a vital resource for many animals in this region, we hypothesized that all herbivores would exhibit a pattern in which dung and parasite density concentrated close to water following periods of low rainfall and in more arid areas, with stronger results for more water-dependent animals. These findings will be important for understanding shifting risk of parasite exposure for several threatened wildlife species in response to changing water supply due to climate changes.

3.3 Methods

3.3.1 Experimental system: Research was conducted at two mixed wildlife and cattle ranching properties in tropical savanna ecosystems of Laikipia county, in central Kenya: Ol Pejeta Conservancy and Mpala Research Centre (Figure 8). At Ol Pejeta Conservancy (0.0043° S, 36.9637° E), we established five sites each consisting of one pair of water pans (10 pans total) and 1 'dry' (no-pan) site (Figure 8A). Dry sites were determined by randomly selecting a coordinate from a range of locations 1km from the experimental water pan and at least 1km from any other water source. The two water pans were located 400m to 1km apart,

and during the experiment one of these pans was drained (experimental pan) for one year and then refilled, while one was left filled throughout the experiment (filled pan) (Figure 8B and C). To measure herbivore activity at water sources, we conducted camera trapping throughout the experiment at each water pan (Appendix 2.1). We performed dung surveys at each site once before draining water from the experimental pan in each pair in October 2016. We repeated the dung surveys at each of the water pans plus dry sites (every 3 months, n=5 resurveys during the experiment) before refilling in January 2018 and resurveying (n=3 surveys post refilling). Dung surveys were performed along six 150m transects that extended radially outward from the water source (or center of the dry site). A 1m^2 quadrat was placed every 10m (n=16 quadrats per transect), and the volume of all large mammalian herbivore dung was estimated and classified as 'fresh' ($\leq$ 3 days) or 'old' (> 3 days) (See Appendix 2.2 for detailed description of dung volume measurement methods). Dung was crushed to avoid counts in subsequent surveys. After a drought in June 2017, quadrats were laid down on both sides of the transect to increase sampling area and density per square meter was calculated by averaging across the two quadrats.

3.3.2 Observational site: To investigate patterns in herbivore abundance, dung density, and parasite density across multiple climatic conditions, we extended these protocols at 20 man-made dams at Mpala Research Centre (0.283° N, 37.867° E) (n=17, described in Titcomb *in review*) and Ol Pejeta Conservancy (n=3). While our experimental pans were confined to one rainfall zone (~700mm/yr), these dams span a steep rainfall gradient ranging from 460 mm/year to 760 mm/year (Figure 8D), marking a transition from sub-desert scrub to grass/tree savanna (Shorrocks 2007). We again included paired dry sites located 1km away

Figure 8. Experimental and observational field sites
Experimental and observational study sites are located in central Kenya. A) We used five pairs of water pans (blue dots) with matched dry sites (white dots) at Ol Pejeta Conservancy. B and C) Experimental pans were filled and surveyed at the beginning of the study ("Pre", n=1) before being drained ("During", n=5) and refilled ("Post", n=3). D) Distribution of observational sites located across Mpala Research Centre's rainfall gradient. E) Schema of sampling transects that radiated outwardly from both water pans and dams. F) Sites were consistently utilized by both wildlife and domestic animals as measured by camera traps.

from any water source. Dry sites were determined by randomly selecting a coordinate from a range of locations 1km from the dam and at least 1km from any other water source. From April-September 2017, one camera was placed at each dam and dry site for one month (Appendix 1.3). Dung surveys were conducted using the same methods as in the experimental system, with the exception that quadrats were laid on only one side of the transect. Five surveys were conducted from November 2015 – October 2017 at all sites at

Mpala; two surveys were repeated at the Ol Pejeta Dams during November 2015 and September 2016.

3.3.3 Photograph analysis*: We uploaded photographs from all cameras to a citizen science website (https://www.zooniverse.org/projects/gtitcomb/parasite-safari) where volunteers assisted in classifying photographs by counting animals and assessing how many were drinking and/or grazing. Image sets were retired after 5 classifications. An animal was determined to be present if at least 3 of the 5 classifications stated its presence and counts for each activity were averaged. The final dataset was created by calculating independent triggers: sequences of classifications that occurred within uninterrupted five-minute periods. We assumed that single-photo triggers corresponded to five seconds of animal presence. We then integrated animal counts within these triggers by multiplying the average count (present, drinking, and grazing) by the duration of the trigger. We then calculated daily individual*seconds at each site for each animal by summing within each day that the camera was running. We analyzed data from a total of 666 trap nights across water pans from the three stages of the experiment, focusing our analyses on the dry season (June – October; Appendix 2.1).

3.3.4 Parasite detection in feces and soil*: We estimated parasite eggs in the environment (eggs/m^2) as the product of median fecal egg counts (eggs/g) by the physical density of fresh dung for each species (g feces /cm^3) and the density of fresh dung in the environment (cm^3/m^2). Quantification methods for dung density in environment are detailed in Appendix 2.2, and physical density of dung was estimated based on published and field measurements of herbivore feces (Chame 2003, Appendix 2.2). To quantify parasite density, we conducted fecal egg counts on fresh herbivore dung samples (n=131) collected across multiple years

and locations at Mpala Research Centre (Table 2). We quantified the number of eggs per

gram of feces from each of the focal herbivores using the mini-FLOTAC protocol (Barda et

al. 2013). Fecal egg counts are reported in Table 2. To place our FEC measurements in

context with values from other studies, we also conducted a literature search of reported FEC

values for the six focal herbivores in this study. Our values fell within ranges found in studies

conducted throughout East and Southern Africa (Appendix 2.3).

Table 2. Fecal egg counts from Mpala herbivores
Fecal egg counts for focal animals sampled during the study used to estimate total parasite density. Individual
animals were sampled across Mpala Research Centre over multiple seasons and years (2015-2017). Values are
qualitatively similar to egg counts found in these species from a range of studies across Africa (Appendix 2.3).

Species	N	Median EPG	Mean EPG	SD	SE
Cow	16	255	292.19	301.08	75.27
Elephant	26	540	733.69	875.27	143.89
Zebra	22	820	1108.41	939.83	236.31
Impala	26	125	162.77	171.77	31.92
Buffalo	20	20	55.15	79.03	17.67
Giraffe	21	0	26.43	90.79	5.77

We also measured parasite eggs present in soils at all sites by subsampling surface

soil (<1cm depth) from damp soil near the water's edge ("0m Wet"), dry soil next to the

water ("0m Dry"), and 50m from the center of the control site ("Control") for each of five

transects. We first filtered soils using a 2mm sieve and combined 4g filtered soil from each

of the five transects to create a homogenized 20g composite sample. For wet soils, we

measured 25g total and calculated the wet and dry weights using a replicate composite

sample. To measure parasite eggs in each soil sample, we followed a sedimentation-

floatation protocol (Azian et al. 2008), using 0.1% Tween 80 to wash soil, and Sheather's

sugar as a floatation solution. We counted all unhatched and intact strongyle-type eggs that

rose to the cover slip following fifteen minutes of centrifugation at 500g. Finally, for wet soil samples, we used dry soil weight to calculate eggs per gram of dry soil.

3.3.5 Statistical analyses

3.3.5.1 Question 1: To what extent do water sources concentrate herbivores, their dung and fecal-oral parasites, for multiple herbivore species?

Herbivore activity: We compared herbivore activity (daily individual*seconds) at filled and experimental pans recorded from camera traps using generalized linear mixed models with a negative binomial distribution. We tested for the significance of the interaction between experiment status (pre, during, or post – draining) and treatment (filled or drained) for each of our six focal species using X^2 tests. Site (n=5) and month (n=7) were included as random effects.

Dung and parasite density: We compared parasite and dung density (eggs/m^2 and cm^3/m^2 respectively) at filled and experimental pans using generalized linear mixed hurdle models with a zero-inflation component and Gaussian conditional component. Density was cube-root transformed to meet residual normality assumptions for the Gaussian component for all models (elephants, cattle, zebra, giraffe, and buffalo), except for impala, which was log-transformed. Note that zebra dung densities reflect both *Equus grevyi* and *Equus burchellii*, as the dung of these two species are indistinguishable.

We tested the effect of experiment status (pre-draining, during the experiment, and post-refill) on differences between dung density at filled and experimental pans, assuming that a significant interaction between status and treatment in either the conditional or zero-inflated components of the model signified changes due to water manipulation. We also included outward distance from water (log-transformed) as a fixed effect, while period

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(n=10) and site (n=5) were included as random effects. For interpretability, we also analyzed the log-ratio of dung density for all dung and parasites summed together. Exponentiating the log-ratio provides an intuitive estimate of relative dung and parasite density. Models follow a similar structure as negative binomial GLMMs and are presented in Appendix 2.4.

We also created GLMMs for dry sites and filled pans to test the relationship between dung density at water and dry sites throughout the experiment. While dung density for most species differed at water sources compared to dry sites, and was more than eight times higher (at the 0m mark) for all animals together, only impala dung density changed at filled pans compared to dry sites throughout the experimental period, indicating that in almost all cases, significant results were likely a result of changes to experimental pans only (Appendix 2.5).

Parasites in soil: We used a zero-inflated negative binomial generalized linear mixed model to test whether soil parasite egg densities in dry soil next to drained and filled water pans differed as a function of experiment status. We included site (n=5) and period (n=10) as random effects.

3.3.5.2 Question 2: How do herbivores, their dung, and parasite density at watering holes vary across different rainfall contexts?

To understand how rainfall impacts herbivore activity and dung and parasite density across contexts we used camera trap, dung count and parasite data collected at watering holes and at dry sites for the same species as in our experimental analyses.

Herbivore activity: We compared daily individual*seconds of herbivore activity at water sources and dry sites using generalized linear mixed models with a negative binomial distribution. We also tested for interactions between site type (water or dry) and mean annual precipitation and prior 30-day rainfall, including random effects for site ID (n=17). Mean

annual precipitation values were derived from (Franz et al. 2010), and prior rainfall data were available from Mpala's long term rainfall monitoring project (Caylor et al. 2017).

Dung and parasite density: To understand differences in dung and parasite density at water sources compared to dry sites, we again used zero-inflated hurdle models with a cube-root transformation of positive data to test the interactions between water presence and cumulative prior 30-day rainfall, mean annual precipitation, and outward distance, including random effects for site (n=20) and period (n=5).

Parasites in soil: Finally, we used a zero-inflated negative binomial generalized linear mixed model to test whether soil parasite egg densities differed among sample type (wet soil, dry soil next to the water's edge, and dry soil 1km from water). We included location (n=20) and period (n=5) as random effects. All analyses were performed in R 3.5.1 (R Core Team 2016).

3.4 Results

Together, our combined results from both the experimental and observational systems showed that parasite density was elevated at water sources, but that this varied considerably by herbivore species (Figure 9).

3.4.1 Question 1: To what extent do water sources concentrate dung and thus, fecal-oral parasites, for multiple herbivore species?

Water removal resulted in significantly reduced herbivore activity at experimental water sources relative to filled water sources for all animals together, elephants, and both zebra species combined. For all animals, activity at experimental pans was 75% of that at filled pans at the beginning of the experiment, but this dropped to 20% when water was removed, and rose to 60% after water was replenished. While the interaction between

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experimental status and treatment was not significant for cattle, buffalo, giraffe, and impala,

herbivore activity for each of these animals trended lower at drained experimental water

sources relative to filled water sources at the beginning of the experiment (Appendix 2.1).

Figure 9. Parasite density at water and dry sites
Comparisons of estimated parasite eggs contributed by each species at constant water sources and dry sites (calculated from both experimental and observational datasets) show considerable consistency across species. Estimates from the experimental system compare filled pans only to dry sites. Note that both graphs are visualized on the $\log_{10}$ scale.

Dung density at filled water sources relative to experimental water sources increased

when water was drained for all animals together (Figure 10), and herded cattle and elephants

separately (Appendix 2.6). The magnitude of this effect was largest for elephants: when

experimental pans were drained, dung density was estimated to be more than six times higher

at filled pans (at the 0m mark), while it was no different between experimental and control

pans pre-draining or post-refilling (Table 3; Appendix 2.6). We found a similar pattern for

cattle, as dung aggregation at filled pans was more than three times higher during the drained

period (at the 0m mark), but at no other phase of the experiment. Since cattle and elephants

accounted for the largest proportion of total dung density (approximately 75%, Appendix

2.6), this drove a similar pattern for all dung summed together. However, we detected no effect of experimental water draining for zebra, impala, or giraffe considered separately and buffalo dung density was slightly higher at experimental sites after refilling.

The model of parasite density using fecal egg count data (pooled across species) showed substantially elevated parasite density at filled pans relative to drained pans (Table 3). Parasite density at filled pans compared to experimental pans was estimated to be three times higher during the experiment, but no different before or after draining (Figure 10).

Finally, we found that parasite density in dry soil at the edge of the water pan was consistent across treatments throughout the experiment (X^2_2 for the interaction between experiment status and treatment = 0.14, p = 0.93), suggesting that water removal did not substantially affect the density of parasites found in dry soil.

3.4.2 Question 2: How does herbivore dung and parasite density at watering holes vary across different rainfall contexts?

While herbivore activity measured from camera traps was approximately 3.5 times higher at water sources relative to controls for all herbivores together, and was significantly elevated for elephants, giraffe, buffalo, and zebra specifically, we found no significant interaction between mean annual precipitation and water presence. This was likely due to the short duration of deployments and low statistical power, as in general, herbivore activity declined with increasing annual precipitation at both control sites and water sources for all species summed together (Appendix 2.1).

Figure 10. Changes in dung and parasite density following water manipulation
Visualized log ratios of dung and parasite density at filled water pans relative to drained water pans throughout the experiment (pre-draining, during experiment, and post-refilling). Points and lines that lie above 0 indicate increased density at filled pans relative to drained pans. Larger points designate averages at each 10m outward distance interval. Species-specific figures illustrating hurdle models for zero-inflated data are available in Appendix 6.

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Table 3. GLMM hurdle models comparing filled and experimental water pans

Coefficients are presented for both the conditional and zero-inflation components of the models ("Cond", and "Zero"). Parameters signifying a decline in density at experimental pans "During" or "Post" experiment are bordered by a solid line. When dung density increased with outward distance (a pattern contrary to our expectations), parameters are bordered by a dotted line. The intercept corresponds to 0m from water prior to conducting the experiment ("Pre").

Estimate ±SE t (P-value)	All		Elephant		Cow		Zebra	
	Cond	Zero	Cond	Zero	Cond	Zero	Cond	Zero
Intercept Pre, Filled, 0m	5.77 ± 0.87 6.65 (<0.001)	-2.92 ± 0.67 -4.34 (<0.001)	5.41 ± 0.91 5.97 (<0.001)	0.01 ± 0.59 0.01 (0.99)	5.19 ± 0.63 8.24 (<0.001)	-0.88 ± 0.63 -1.39 (0.16)	3.80 ± 0.79 4.78 (<0.001)	3.00 ± 0.79 3.80 (<0.001)
During	-0.83 ± 0.93 -0.90 (0.37)	0.36 ± 0.66 0.54 (0.59)	-0.54 ± 0.97 -0.56 (0.58)	0.35 ± 0.60 0.58 (0.56)	-1.08 ± 0.66 -1.62 (0.10)	-0.81 ± 0.60 -1.34 (0.18)	-0.86 ± 0.66 -1.30 (0.19)	0.30 ± 0.69 0.44 (0.66)
Post	-0.82 ± 0.98 -0.84 (0.40)	-0.07 ± 0.71 -0.10 (0.92)	-1.63 ± 1.00 -1.62 (0.11)	-0.66 ± 0.63 -1.05 (0.29)	-1.23 ± 0.70 -1.77 (0.08)	-1.68 ± 0.64 -2.64 (0.01)	-1.17 ± 0.71 -1.64 (0.10)	1.04 ± 0.73 1.42 (0.16)
Drained	0.27 ± 0.29 0.94 (0.35)	0.52 ± 0.46 1.13 (0.26)	-0.56 ± 0.57 -0.99 (0.32)	-0.38 ± 0.39 -0.98 (0.33)	-0.38 ± 0.33 -1.14 (0.25)	-0.06 ± 0.35 -0.18 (0.86)	0.63 ± 0.37 1.72 (0.09)	**0.85 ± 0.36 2.34 (0.02)**
Outward Distance	**-0.30 ± 0.04 -7.55 (<0.001)**	**0.22 ± 0.07 3.31 (0.001)**	0.01 ± 0.07 0.08 (0.94)	**0.37 ± 0.05 7.17 (<0.001)**	**-0.18 ± 0.04 -5.13 (<0.001)**	**0.41 ± 0.05 7.76 (<0.001)**	-0.06 ± 0.12 -0.50 (0.62)	**-0.73 ± 0.09 -8.05 (<0.001)**
During: Drained	**-1.01 ± 0.33 -3.09 (0.002)**	0.62 ± 0.50 1.23 (0.22)	0.17 ± 0.69 0.25 (0.81)	**1.33 ± 0.46 2.88 (0.004)**	-0.05 ± 0.36 -0.14 (0.89)	**1.20 ± 0.39 3.09 (0.002)**	-0.41 ± 0.42 -0.97 (0.33)	0.58 ± 0.41 1.41 (0.16)
Post: Drained	-0.66 ± 0.34 -1.91 (0.06)	0.28 ± 0.54 0.52 (0.60)	1.13 ± 0.65 1.74 (0.08)	**0.94 ± 0.45 2.09 (0.04)**	-0.03 ± 0.36 -0.10 (0.92)	**0.90 ± 0.41 2.17 (0.03)**	-0.57 ± 0.48 -1.19 (0.23)	0.33 ± 0.46 0.73 (0.47)
σ^2_{Site}	0.20	0.20 0.49	0.00	0.28	0.24	0.52	0.12	0.68
σ^2_{Period}	0.82	1.67	0.77	0.47	0.56	0.49	0.55	0.57
$\sigma^2_{Residual}$	1.67		1.69	1.69	1.22	1.22	1.37	1.37
R^2_M	0.102				0.111		0.084	
R^2_C	0.286				0.289		0.218	
N = 1440								

(continued)	Buffalo		Impala (log)		Giraffe		Parasites	
	Cond	Zero	Cond	Zero	Cond	Zero	Cond	Zero
Intercept Pre, Filled, 0m	4.20 ± 0.57 7.36 (<0.001)	0.83 ± 0.79 1.05 (0.29)	0.50 ± 0.49 1.02 (0.31)	1.63 ± 0.72 2.25 (0.02)	0.92 ± 0.47 1.95 (0.05)	2.91 ± 0.78 3.72 (<0.001)	36.49 ± 5.56 6.56 (<0.001)	-2.97 ± 0.67 -4.44 (<0.001)
During	-0.06 ± 0.60 -0.10 (0.92)	1.14 ± 0.79 1.44 (0.15)	**-0.81 ± 0.37 -2.20 (0.03)**	0.44 ± 0.69 0.64 (0.52)	0.08 ± 0.35 0.23 (0.82)	-0.04 ± 0.68 -0.05 (0.96)	-5.63 ± 5.89 -0.96 (0.34)	0.48 ± 0.67 0.73 (0.47)
Post	-0.54 ± 0.69 -0.79 (0.43)	1.93 ± 0.88 2.20 (0.03)	**-0.87 ± 0.39 -2.22 (0.03)**	0.51 ± 0.73 0.69 (0.49)	0.06 ± 0.37 0.16 (0.87)	-0.15 ± 0.71 -0.21 (0.84)	-6.37 ± 6.21 -1.03 (0.31)	0.03 ± 0.71 0.04 (0.97)
Drained	-0.02 ± 0.38 -0.06 (0.95)	-0.67 ± 0.41 -1.61 (0.11)	-0.13 ± 0.55 -0.25 (0.81)	**1.44 ± 0.58 2.47 (0.01)**	-0.09 ± 0.37 -0.24 (0.81)	0.57 ± 0.57 0.99 (0.32)	0.81 ± 2.17 0.37 (0.71)	0.49 ± 0.45 1.10 (0.27)
Outward Distance	-0.07 ± 0.06 -1.16 (0.25)	**0.25 ± 0.06 3.93 (<0.001)**	-0.03 ± 0.09 -0.37 (0.71)	**-0.17 ± 0.08 -2.12 (0.03)**	0.09 ± 0.07 1.19 (0.23)	**-0.23 ± 0.10 -2.41 (0.02)**	**-1.28 ± 0.30 -4.34 (<0.001)**	**0.24 ± 0.06 3.92 (<0.001)**
During: Drained	-0.52 ± 0.47 -1.10 (0.27)	0.19 ± 0.49 0.39 (0.70)	0.37 ± 0.59 0.62 (0.53)	-0.85 ± 0.63 -1.36 (0.17)	0.08 ± 0.39 0.21 (0.83)	-0.14 ± 0.62 -0.23 (0.82)	**-7.09 ± 2.42 -2.93 (0.003)**	0.64 ± 0.48 1.32 (0.19)
Post: Drained	-0.91 ± 0.59 -1.52 (0.13)	**-1.40 ± 0.58 -2.40 (0.017)**	-0.06 ± 0.62 -0.09 (0.92)	-1.00 ± 0.66 -1.52 (0.13)	-0.10 ± 0.40 -0.26 (0.80)	-0.45 ± 0.64 -0.71 (0.48)	-4.53 ± 2.53 -1.79 (0.07)	0.29 ± 0.52 0.56 (0.57)
σ^2_{Site}	0.29	0.63	0.00	0.32	0.13	0.51	3.69	3.71
σ^2_{Period}	0.40	0.63	0.19	0.54	0.22	0.51	26.60	26.63
$\sigma^2_{Residual}$	1.05	1.05	1.26	1.26	0.58	0.58	156.85	157.48
R^2_M	0.155				0.045		0.084	
R^2_C	0.309				0.201		0.232	
N = 1440								

Mean annual precipitation and prior rainfall were important parameters in models of dung density for cattle, elephants, zebra, and all animals combined (Table 4, Figure 11, Appendix 2.6). In the driest locations (~460mm/year) closest to water and following no rainfall, cattle dung density was three orders of magnitude higher at water relative to dry sites 1km from any water source, but this elevated density decreased as mean annual precipitation and outward distance increased. This pattern was also strong for elephants: in dry areas following periods of no rainfall, elephant dung was predicted to be ten times higher close to water, but this effect weakened as MAP and outward distance increased (Table 4, Appendix 2.6). Zebra dung density was no different between water and dry sites when there was little prior rainfall or low MAP, and we even observed potential aversion to water during the wettest periods in areas of high MAP. Similarly, impala dung density was slightly elevated near water in low-rainfall locations but was depressed near water in wetter conditions. We also observed slightly higher dung density levels at watering holes relative to dry sites for buffalo and giraffe in low-rainfall conditions, and there was a significant interaction between MAP and prior rainfall for giraffe (Table 4, Appendix 2.6).

Critically, outward distance from water, mean annual precipitation, and prior rainfall all modulated parasite density in the environment at water sources compared to dry sites. In areas were MAP was lowest (450mm/year) and prior 30-day rainfall was 0mm, parasite density was estimated to be more than 150 times higher than dry sites in the closest area to water. This effect decreased sharply as MAP, prior rainfall and outward distance increased (Table 4; Figure 11).

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Figure 11. Dung and parasite density ratios across water gradients
Visualized log ratios of dung and parasite density at watering holes relative to non-water control sites across differing levels of mean annual precipitation, prior rainfall, and outward distance. Points and lines that lie above 0 indicate increased density at water relative to the rest of the environment. Larger points represent averages for each value of MAP, prior rainfall, or outward distance. Species-specific figures illustrating zero-inflated data are available in Appendix 2.6.

Table 4. GLMM hurdle models comparing water sources and dry sites

Coefficients are presented for both the conditional and zero-inflation components of the hurdle models ("Cond", and "Zero"). Parameters signifying a negative relationship between density and each covariate are shaded in blue (as hypothesized), while a positive relationship is shaded in orange (contrary to expectations). The intercept corresponds to dung and parasite density at dry sites when distance and prior rainfall are zero and MAP is the lowest level observed (450 mm/yr).

Estimate ±SE t (P-value)	All		Elephant		Cow		Zebra	
	Cond	Zero	Cond	Zero	Cond	Zero	Cond	Zero
(Intercept) MAP: 4.5 Dist: 0, Rain: 0	3.86 ± 0.49 7.85 (<0.001)	-0.88 ± 0.40 -2.22 (0.027)	4.59 ± 0.60 7.62 (<0.001)	0.63 ± 0.58 1.09 (0.28)	3.32 ± 0.37 8.86 (<0.001)	1.89 ± 0.58 3.23 (<0.001)	3.51 ± 0.38 9.20 (<0.001)	1.52 ± 0.53 2.90 (<0.001)
MAP	-0.33 ± 0.20 -1.68 (0.092)	**0.45 ± 0.12 3.83 (<0.001)**	-0.22 ± 0.29 -0.74 (0.46)	**0.95 ± 0.31 3.08 (0.002)**	0.20 ± 0.14 1.42 (0.16)	**0.72 ± 0.24 2.98 (0.003)**	-0.01 ± 0.15 -0.05 (0.96)	0.32 ± 0.25 1.27 (0.20)
Water	**3.14 ± 0.43 7.38 (<0.001)**	**-2.43 ± 0.37 -6.61 (<0.001)**	**2.33 ± 0.67 3.48 (0.001)**	**-2.70 ± 0.44 -6.19 (<0.001)**	**1.03 ± 0.40 2.60 (0.009)**	**-3.03 ± 0.48 -6.31 (<0.001)**	-0.01 ± 0.56 -0.03 (0.98)	-0.05 ± 0.47 -0.11 (0.91)
Distance	0.06 ± 0.07 0.91 (0.36)	0.01 ± 0.05 0.12 (0.91)	0.24 ± 0.12 1.93 (0.053)	0.01 ± 0.07 0.11 (0.91)	-0.03 ± 0.07 -0.37 (0.71)	**-0.18 ± 0.08 -2.15 (0.032)**	-0.09 ± 0.07 -1.23 (0.22)	-0.03 ± 0.06 -0.39 (0.70)
Rain	**-1.41 ± 0.27 -5.21 (<0.001)**	**0.74 ± 0.21 3.54 (<0.001)**	0.38 ± 0.44 0.86 (0.39)	**1.59 ± 0.28 5.63 (<0.001)**	0.00 ± 0.27 0.01 (0.99)	**1.09 ± 0.31 3.48 (0.001)**	-0.10 ± 0.29 -0.36 (0.72)	**0.60 ± 0.27 2.25 (0.02)**
MAP: Water	**-0.38 ± 0.19 -2.00 (0.045)**	**0.45 ± 0.15 3.04 (0.002)**	0.10 ± 0.36 0.29 (0.77)	0.41 ± 0.22 1.84 (0.065)	**-0.36 ± 0.17 -2.13 (0.034)**	0.16 ± 0.21 0.76 (0.45)	-0.11 ± 0.22 -0.52 (0.61)	0.21 ± 0.19 1.12 (0.26)
Distance: Water	**-0.49 ± 0.09 -5.68 (<0.001)**	**0.18 ± 0.07 2.48 (0.013)**	**-0.50 ± 0.14 -3.55 (<0.001)**	**0.31 ± 0.09 3.56 (<0.001)**	-0.12 ± 0.08 -1.52 (0.13)	**0.44 ± 0.10 4.58 (<0.001)**	0.01 ± 0.11 0.05 (0.96)	-0.17 ± 0.10 -1.70 (0.09)
Rain: Water	-0.05 ± 0.32 -0.16 (0.87)	**1.23 ± 0.25 4.99 (<0.001)**	-0.72 ± 0.52 -1.37 (0.17)	0.15 ± 0.32 0.46 (0.64)	-0.33 ± 0.31 -1.07 (0.28)	0.27 ± 0.36 0.76 (0.45)	0.15 ± 0.38 0.39 (0.69)	**0.95 ± 0.33 2.86 (0.004)**
σ Site	0.43	0.19	0.09	0.74	0.10	0.50	0.12	0.65
σ Period	0.69	0.67	0.33	0.73	0.25	0.80	0.13	0.69
σ Residual	2.08	2.08	1.91	1.91	0.91	0.91	1.18	1.18
R^2_M	0.128		0.032		0.042		0.010	
R^2_C	0.244		0.062		0.119		0.031	
N = 2816								

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(continued)	Buffalo		Impala		Giraffe		Parasites	
	Cond	Zero	Cond	Zero	Cond	Zero	Cond	Zero
(Intercept) MAP: 4.5 Dist: 0, Rain: 0	4.17 ± 0.50 8.31 (<0.001)	4.22 ± 0.93 4.55 (<0.001)	1.18 ± 0.26 4.59 (0.00)	2.22 ± 0.51 4.36 (<0.001)	1.51 ± 0.19 8.09 (<0.001)	1.85 ± 0.42 4.42 (<0.001)	46.09 ± 8.03 5.74 (<0.001)	-2.72 ± 0.74 -3.69 (<0.001)
MAP	-0.04 ± 0.18 -0.20 (0.84)	**-1.19 ± 0.28 -4.24 (<0.001)**	-0.17 ± 0.10 -1.67 (0.10)	0.00 ± 0.23 0.02 (0.98)	-0.17 ± 0.10 -1.61 (0.11)	0.36 ± 0.23 1.54 (0.12)	**-4.04 ± 1.31 -3.09 (0.002)**	**0.43 ± 0.11 3.78 (<0.001)**
Water	0.11 ± 0.54 0.20 (0.84)	**-1.73 ± 0.62 -2.80 (0.005)**	-0.46 ± 0.37 -1.23 (0.22)	**-2.15 ± 0.74 -2.92 (0.003)**	-0.29 ± 0.27 -1.06 (0.29)	**-0.99 ± 0.47 -2.09 (0.036)**	**34.12 ± 8.06 4.23 (<0.001)**	**-4.73 ± 0.85 -5.58 (<0.001)**
Distance	0.08 ± 0.10 0.86 (0.39)	0.16 ± 0.11 1.38 (0.17)	-0.06 ± 0.05 -1.18 (0.24)	-0.09 ± 0.10 -0.92 (0.36)	0.04 ± 0.04 1.04 (0.30)	0.01 ± 0.07 0.20 (0.84)	0.53 ± 0.48 1.10 (0.27)	0.00 ± 0.05 -0.05 (0.96)
Rain	-0.52 ± 0.27 -1.93 (0.054)	**1.64 ± 0.35 4.73 (<0.001)**	**-0.35 ± 0.16 -2.24 (0.025)**	-0.55 ± 0.40 -1.37 (0.17)	0.00 ± 0.13 -0.01 (0.99)	-0.23 ± 0.27 -0.87 (0.38)	**-9.70 ± 1.93 -5.04 (<0.001)**	**0.80 ± 0.20 4.12 (<0.001)**
MAP: Water	-0.23 ± 0.20 -1.15 (0.25)	0.41 ± 0.24 1.74 (0.08)	0.18 ± 0.18 0.98 (0.33)	0.45 ± 0.37 1.21 (0.23)	0.22 ± 0.13 1.70 (0.09)	**0.56 ± 0.22 2.52 (0.012)**	**-3.01 ± 1.36 -2.20 (0.028)**	**0.47 ± 0.14 3.40 (0.001)**
Distance: Water	-0.06 ± 0.12 -0.52 (0.60)	0.10 ± 0.14 0.70 (0.48)	0.01 ± 0.07 0.14 (0.89)	0.12 ± 0.14 0.89 (0.38)	0.04 ± 0.06 0.80 (0.42)	-0.05 ± 0.10 -0.56 (0.58)	**-3.15 ± 0.62 -5.08 (<0.001)**	**0.21 ± 0.07 3.01 (0.003)**
Rain: Water			0.41 ± 0.22 1.88 (0.060)	**1.58 ± 0.52 3.07 (0.002)**	-0.19 ± 0.18 -1.04 (0.30)	**0.87 ± 0.33 2.63 (0.012)**	-0.37 ± 2.32 -0.16 (0.87)	**1.18 ± 0.23 5.06 (<0.001)**
σ Site	0.00	0.64	0.20	0.35	0.00	0.48	2.63	0.20
σ Period	0.30	1.49	0.00	0.26	0.00	0.25	4.79	0.68
σ Residual	0.95	0.95	0.56	0.56	0.55	0.55	15.43	15.43
R^2_M							0.128	
R^2_C							0.226	
N = 2816								

In our negative binomial model of eggs found in soil, parasite densities differed significantly based on soil sampling location ($X^2_3 = 135.7$, p <0.001). Parasites (eggs per gram of soil) were more than two orders of magnitude higher in wet soils (mean ± SE = 31.1±8.0), and four times higher in dry soils (1.02±0.35), near the water's edge compared to dry soils 1km from water (0.26±0.13) (Figure 12).

Figure 12. Parasite egg density in soils
Parasite density was substantially elevated in wet soils compared to dry sites. Density in dry soils was also higher, but to a much lesser extent. Letters denote statistically significant groups after false-discovery-rate correction for multiple testing. Error bars show mean ± SE.

3.5 Discussion

Utilizing both experimental and observational datasets we show that water sources strongly concentrate herbivores, herbivore dung and parasite density, with the extent of concentration typically negatively related to prior rainfall and mean annual precipitation. The effects were greatest for cattle and elephants, with these animals driving patterns in total dung and estimated parasite density given the high relative abundance of their dung and

relatively high average parasite fecal egg count. However, all species showed at least some negative interaction between dung density at water sources and annual or recent rainfall, suggesting that risk of fecal-oral parasite exposure at water sources will typically be elevated in drier conditions.

3.5.1 Question 1: Water sources strongly increase dung and parasite density for some herbivores

Our experimental results provide causal evidence that availability of surface water increases herbivore activity and concentrates herbivore dung and fecal-oral parasites in the environment. Specifically, we show that water removal reduced both herbivore activity and total dung density by more than 50% and total estimated parasite density by up to two-thirds relative to sites with water, which remained constant relative to dry sites 1km from any water source throughout the experiment. Importantly, we found that this pattern was largely driven by two globally important species: cattle and elephants, but that responses were substantially weaker for other herbivore species in the study, demonstrating differing balances of resource requirements and parasite exposure risk.

Rapid decreases in elephant activity and dung aggregation following water removal corresponds with previous studies that found that elephants are strongly dependent on water distribution (Loarie et al. 2009), and can quickly alter their movements in response to water availability (Chamaillé-Jammes et al. 2013, Bohrer et al. 2014). Given that elephants are mixed feeders that consume both grasses and trees (Merwe et al. 1988), and often forage across large areas that likely have low parasite contamination, it is likely that drinking and ingestion of contaminated forage near watering holes contributes a substantial source of intraspecific fecal-oral parasite infections. While nematode infections in elephants, a species

classified as vulnerable by the IUCN, are common (Appendix 4.3), at least one study identified parasitism and starvation as likely causes of death for 38 young elephants that died during a period of severe drought in Kenya (Obanda et al. 2011), underscoring the importance of monitoring parasite infections as part of conservation efforts.

Our similar finding that dung density substantially increased near water for cattle is also intuitive. Daily cattle water requirements are relatively high compared to other savanna herbivores (Kay 1997), necessitating their need for frequent drinking. High dung concentration at water sources results from large herd sizes and heavy congregations around a small water source. Given that cow movements are typically dictated by humans, the clear change in dung density during the experiment reflects the rapid ability for humans and their livestock to adapt to changes in water distribution. While water sources do increase exposure to fecal-oral parasites for cattle, other human management practices that concentrate individuals -- such as use of cattle corrals (bomas), salt licks, or simply by maintaining high herd densities -- likely function similarly. The relative importance of water aggregation on cattle parasite risk and sharing with wildlife may thus depend greatly on other regional management practices.

Interestingly, we did not find that all animal species responded substantially to water manipulation. This is likely to be partially explained by the fact that herbivores must partition their time and energy between foraging and seeking water. When forage quantity or quality is reduced near water, some species may choose to drink and depart, rather than stay and forage near water (Redfern et al. 2003). Indeed, in Chapter 2, we found that understory height, grass and forb cover were all reduced near water; this is consistent with well-documented plant loss near water sources in other systems (Thrash and Derry 1999, Smit et al. 2007). Given

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that elephants and cattle are strongly water dependent, water requirements may override the need for superior forage. For other large and water-dependent grazers, such as zebra and buffalo, foraging requirements might limit their ability to consume sufficient material in proximity to water (Redfern et al. 2003). In particular, (Redfern et al. 2003) noted that buffalo and zebra may experience a strong and moderate tradeoff, respectively, between water and forage quantity and quality. Similarly, (Groom and Harris 2010) found that grass biomass, rather than water, was a limiting resource for zebra in areas of high livestock density and frequent droughts. Browsers, such as giraffe and impala are less water dependent as their digestive systems allow them to better retain water (Estes 2012). Lack of suitable browse near heavily impacted water likely explains why giraffe dung is not substantially elevated at water. Finally, species-level differences in defecation behavior may also explain some of the variation observed. Male zebra and impala are territorial, and often demarcate territory with middens. If midden location is independent of water location, then dung density may not correlate tightly with proximity to water.

Finally, risk of predation at water sources (Hayward and Hayward 2012) may also explain why certain herbivores were less likely to have higher average dung density near water than others, as predation risk is often higher near water (Valeix et al. 2010). This explanation is also parsimonious with our results given that the large body size of elephants affords some protection from predation (Sinclair et al. 2003) and in this system cattle move exclusively with human protection, reducing risk of increased predation. By contrast, other species may experience a heightened risk of predation (Crosmary et al. 2012), particularly those that are smaller in size (Sinclair et al. 2003). In particular, this may explain why impala, which are strongly constrained by predation (Ford et al. 2014), had little dung

accumulation at water sources, despite having moderate water requirements (Redfern et al. 2003). Indeed, our camera trapping data showed more than twice as much carnivore activity at water sources at both Mpala Research Centre and Ol Pejeta Conservancy than at dry sites 1km from water (Appendix 4.2). Further research may thus show that predators indirectly influence herbivore exposure to fecal-oral parasites via a landscape of fear.

3.5.2 Question 2: Reduced mean annual precipitation and prior rainfall concentrate dung and parasites around watering holes for most species

The results from our observational dataset expand upon our experimental findings, showing that lower recent rainfall and mean annual precipitation often exacerbate the extent to which animal dung is concentrated around water. While these effects varied by species, elephant, cattle, giraffe, and buffalo dung were all concentrated more strongly at water sources in areas of low mean annual precipitation, suggesting that the density of their fecal-oral parasites is also substantially increased when water is limiting. Furthermore, several species demonstrated a dependence on short-term rainfall – when prior rainfall was low (<50 mm over 30 days), giraffe, buffalo, zebra, and impala dung was found at a higher density than at control sites, suggesting a shifting tradeoff between water requirements and forage and/or predation risk. Together, these results suggest that climatic context has the potential to substantially alter exposure to fecal-oral parasites for these species. While projections for future annual rainfall patterns in our study region are mixed (Adhikari et al. 2015), local rainfall prediction models point to reductions in important seasonal long rains (Funk et al. 2008, Williams and Funk 2011). Indeed, increased global temperature projections (IPCC 2014), broadscale aridification (Huang et al. 2016), and increased competition for water with

humans (de Wit and Stankiewicz 2006) may drive certain wildlife to congregate more strongly at water, likely increasing their exposure to fecal-oral parasites.

While the results of this study have clear implications for parasitism within the impacted species, the implications of total increase in parasite density on other species will depend on the extent of cross species parasite sharing that occurs. While species-level identification of parasites was not within the scope of this study (and is not possible from morphological analysis alone, although many linkages remain uncertain due to uneven sampling and heavy focus on domestic species (Walker et al. 2017)), literature reviews indicate that while hosts tend to have specific parasites, all species share parasites with at least one other host species (Round 1968). However, elephants rarely share strongyle parasites with other species, indicating that the strong implications of water in concentrating elephant parasites are likely to be largely confined to elephants. However, cattle appear to share multiple gastrointestinal helminths with a wide range of species (Walker et al. 2017), and their strong aggregations at water may function to increase exposure to these parasites for other animals when they drink water or forage nearby. Given that cattle comprise approximately one half of all herbivore biomass in the broader region (Ogutu et al. 2016), even a small degree of overlap in parasite sharing may have substantial effects on parasitism in other wildlife (Morgan et al. 2004). This may be another way in which human domination of landscapes increases threats to wildlife. Alternatively, parasite treatment in cattle may also reduce transmission of shared helminths, although increasing anthelminthic resistance is a growing concern (Kaplan and Vidyashankar 2012).

In addition, parasite aggregation may also have substantial indirect (nonconsumptive) impacts on animal behavior and fitness, much as predators may impose a 'landscape of fear'

in prey. Recent work has begun to focus on the role of disgust in facilitating host avoidance of parasites, thereby imposing 'landscapes of disgust' in hosts (Weinstein et al. 2018, Buck et al. 2018). Therefore, animal behavior may also complicate our findings if animals are able to detect parasites in water or the environment. For example, (Ndlovu et al. 2018) found that elephants avoided water with heavy *E. coli* contamination (which is likely to be correlated with other fecal-oral parasites), (Ezenwa 2004b) found that dik-dik avoided feeding near feces, and (Amoroso et al. 2019) found that red-fronted lemurs strongly avoided waterholes contaminated with feces. These studies suggest that in certain cases, the costs of parasite exposure may significantly alter animal behavior and foraging.

Notably, many conclusions of this study are based on using fecal egg counts as a proxy for risk of fecal-oral parasite exposure. One potential shortcoming of this approach is that we use average parasite density per species to extrapolate to larger scales, including sampling periods and seasons. While this assumption allows us to ask questions at a larger scale, it is clear that seasonality can affect fecal egg counts (FECs) for different host and parasite species in different directions, and many studies have shown variation in herbivore FEC based on seasonality (Ezenwa 2004a, Thurber et al. 2011, Cizauskas et al. 2015). Any consistent deviation in infection intensity across seasons could thus either dampen the effect of water or heighten it. Studies have found both increases and decreases in fecal egg counts over rainfall seasons and periodic droughts for the herbivore species examined in this study. However, previous work from this study site found that drought was associated with increased fecal egg counts for six of nine bovid species (Ezenwa 2004a), with no species showing decreased fecal egg counts during drought. Thus, our results are likely to be a conservative estimate of the impacts of drought on aggregating parasites. A second important

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consideration is that fecal egg count is only a coarse proxy of risk of exposure as parasites must develop and survive in the environment before ingestion by a new host. If climatic conditions are unfavorable, many parasites may die before reaching their host. While helminth development tends to accelerate in warmer temperatures, it can arrest when exceedingly hot (Anderson 2000). The degree to which climate conditions affect parasite survival in the environment will depend on the parasite in question and its resilience to changing temperatures and ability to adapt (Rohr et al. 2011). However, moist conditions near water may also mitigate parasite desiccation. Unfortunately, the specific parameters are unknown for most of the wildlife parasites in this system, and so we are unable to incorporate those impacts on risk. Future modelling work may be able to explore the extent to which variation in these parameters might alter the basic findings of this study.

3.6 Conclusion

This study shows that water sources cause large scale – up to 150-fold – increases in strongyle parasites of large herbivores compared to dry sites during the driest conditions, although the effects vary strongly across herbivore species. Critically, we show that climatic context greatly modifies these patterns, with much stronger levels of parasite aggregation in rainfall-limited times and sites. Given increasing global aridification, these findings suggest that parasite exposure may increase dramatically for both domestic species and wildlife in many regions. This also suggests that human management of surface water - for their use and that of their domestic species - is an important but hitherto unexplored way in which humans influence wildlife parasite exposure. This influence will likely only increase as water becomes increasingly scarce and livestock biomass continues to increase regionally (Ogutu et al. 2016) and globally (Pelletier and Tyedmers 2010). Cumulatively, these findings highlight

the multiple potential pathways in which humans can affect wildlife parasitism and behavior via climate change and domestic animal management.

Coauthors and contributions:

Jenna Hulke[1,2] assisted greatly in coordinating and executing all aspects of fieldwork and lab work, in addition to John Mantas[2], who performed countless dung identifications and surveys. Ivan Rodriguez[3] assisted in soil collection and sampling for nematodes, while Hillary Young[2,3] collaborated on experimental design and interpretation of findings.

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Chapter 4

4. The nemabiome in large mammalian herbivores: diet and gut morphology describe parasite richness and sharing

4.1 Abstract

Patterns in wildlife parasite diversity and sharing can dramatically impact health, abundance, and behavior of wildlife, livestock, and people. However, despite the ubiquity of parasites, we have a rudimentary understanding of parasite communities in wild taxa. Metabarcoding methods hold the potential to vastly improve our understanding of parasite community ecology, but this potential is only just starting to be realized. In this study, we used metabarcoding to explore gastrointestinal nematode communities of 18 different species of large mammalian herbivores from Kenya. We explored three broad questions using this dataset: 1) What explains variation in parasite MOTU richness and phylogenetic diversity

among hosts? 2) How do parasite communities vary among hosts? and 3) Which host species are central to a parasite sharing network?

We found that host species identity explained 50% and 55% of variation in an individual's parasite richness and diversity, respectively, and 74% of variation in parasite community composition. Despite previous results suggesting that host body size, range size, and group size tend to be positively correlated with parasite richness, we found that none of these hypothesized correlates were significant in our dataset. Rather, we found that a mixed feeder diet was linked to increased richness, and that host gut morphology was central to parasite community. Our investigation of parasite sharing revealed that hosts occupying central positions in the host phylogeny were central in the host-parasite sharing network and that geographic range size was surprisingly not a significant predictor.

Together our findings emphasize the close co-evolutionary history between large mammalian herbivores and gastrointestinal nematodes, and they highlight stark differences in parasite communities among herbivores with different gut morphologies. Our results underscore the power of molecular methods in enhancing our understanding of parasite richness, community, and sharing for a broad range of globally threatened hosts.

4.2 Introduction

Parasites account for approximately 40% of animal diversity (Dobson et al. 2008), play roles in structuring host communities (Poulin 1999), and dominate food webs (Thompson et al. 2005, Lafferty et al. 2006). However, our understanding of parasite diversity remains greatly underexplored. Despite scientific focus on single host-parasite interactions, the majority of parasites infect multiple hosts (Woolhouse et al. 2001), and the majority of hosts are infected by an array of parasites at any given time (Bordes and Morand

2009). Studies that do focus on multiple hosts and multiple parasites find significant changes in conclusions about transmission (Pilosof et al. 2015), and note that parasite diversity may impose selection pressure (Bordes and Morand 2009), demonstrating that the breadth of parasite communities can sculpt a host's evolution. These findings have ushered in calls for more focus on parasite community ecology and consideration of both host and environmental factors in structuring parasite communities on both an individual and host species level (Guernier et al. 2004)˙ (Poulin 2014). This is particularly true given climate-induced global changes in host species distributions (Parmesan and Yohe 2003, Root et al. 2003, Thomas et al. 2004), biodiversity declines (Dirzo et al. 2014, Ceballos et al. 2015), and increasing human-wildlife overlap (Cardillo et al. 2004, Karanth et al. 2010), which threaten to dramatically change multi-host-multi-parasite relationships across a wide array of species, with large potential downstream effects on hosts, parasites, and ecosystem function. Historically such comprehensive multi-host multi-parasite analyses have often been prohibitive due to sampling limitations. For example, gastrointestinal nematodes parasitize a wide range of large mammalian herbivore species, yet accurate identifications often require adult specimens that are often only retrieved by opportunistic sampling of deceased hosts or by culturing larvae. However, metabarcoding and other high-throughput sequencing methodologies, while still in their nascent stages for this use (Selbach et al. 2019), hold enormous promise to shed light on questions related to multiple parasite infections (Bass et al. 2015, Titcomb et al. 2019).

Parasite species richness is an important first metric in thinking about multiple parasite infections. Several studies have suggested that parasite species richness is a trait shared among individuals of different host species (Krasnov et al. 2008, Bordes and Morand

2008). The role of host phylogeny in explaining variation in parasite richness has also been considered, as hosts and parasites may retain deep evolutionary linkages throughout their lineages (Poulin 1995, 2007, Morand and Poulin 2003). However, factors that predict parasite richness beyond host identity have been challenging to disentangle (Kamiya et al. 2014, Morand 2015). Three host-specific predictors have been widely proposed as correlates of parasite species richness across systems: host body size, geographical range, and population density (Lindenfors et al. 2007, Bordes and Morand 2009). A recent meta-analysis (Kamiya et al. 2014) tested the effect of these correlates, finding evidence that host body size, geographical range size, and population density tend to be positively correlated with parasite species richness across parasite groups, but that this could depend on spatial scale. While herbivore feeding strategies and gut morphologies have not been commonly tested as predictors of parasite richness, given that transmission in many ungulates occurs via feeding and that many gastrointestinal parasites occupy specific niches within the intestinal tract (Rohde 1994), it would follow that variation in parasite richness may also reflect these differences.

In many cases, these questions have only been applied to parasite species richness on the population or species level, and not at the scale of individuals (i.e. the infracommunity as opposed to the component community (Bush et al. 1997)), an important distinction whose relationship remains underexplored (Poulin 2007), and still remain limited in number due to limitations in large datasets containing data on multi-host, multi-parasite species interactions. Furthermore, parasite phylogenetic diversity beyond species richness is rarely investigated given relatively poor understanding of phylogenetic distances between parasites of wild

hosts. Thus, molecular methods provide unprecedented potential to efficiently answer these fundamental questions across a broad array of systems and scales.

Beyond the search for consistent patterns in parasite species richness, questions relating to community composition expose major knowledge gaps. Are the same signals of host species identity detectable when applied to parasite communities? Previous work has explored this question in small mammal and ectoparasite (Krasnov et al. 2008) and endoparasite (Dallas et al. 2019b) communities, finding that parasite communities tend to be similar among host individuals of the same species. However, little is known about the extent to which parasite relatedness changes our understanding of community composition, or which host characteristics, aside from host species identity, are related to parasite communities. Furthermore, these questions have not been extensively explored for gastrointestinal helminths, nor have they been applied to a diverse array of large herbivores. Thus, we still have poor understanding of the generality of these patterns and lack a high-resolution snapshot of a sympatric and diverse host community.

Finally, given that these sympatric herbivores share food and water resources that may serve as transmission foci for gastrointestinal helminths, there have been attempts to construct potential parasite sharing networks using literature records or morphological identifications (VanderWaal et al. 2014b, Walker et al. 2017). Constructing these networks is useful because several node-specific metrics can be used to identify hosts that are central to a network in four particular ways: a) by having a high number of total parasite links (high degree), b) by sharing parasites with distinct groups, acting as a bridge (high betweenness), c) by sharing parasites with many other hosts (high closeness), and d) by sharing parasites with other hosts that also share many parasites, thereby occupying central positions in the

overall network (high eigenvector centrality). Together, central hosts may affect parasite

transmission dynamics for many other species. Conversely, examining species with a high

number of unique links can also illuminate potential parasite extinctions when hosts are lost

(Lafferty 2012), a growing threat given global declines in wildlife (Dirzo et al. 2014).

While several databases archive much-needed information on host-species parasite

relationships (Gibson et al. 2005, Nunn and Altizer 2005), both the value and the limitation

of these datasets is that they aggregate data from many times and places, potentially

obscuring patterns in host-parasite sharing among individuals at a given time or location.

Understanding parasite communities within hosts, therefore, is likely to be sample-size

limited, potentially biased or harmful to hosts, time-consuming, and difficult to replicate

without harnessing environmental samples (e.g. fecal samples, swabs, urine, etc.). Molecular

methods offer a potential solution to this problem by cheaply and efficiently screening

hundreds of samples for parasitic genes of interest (Bass et al. 2015). Metabarcoding, the

process of selectively amplifying and sequencing a gene of interest across many different

samples, has been used increasingly in disease ecology studies (Titcomb et al. 2019). One

important feature of this method is that resulting sequences are clustered by their genetic

similarity and matched to species names documented in genetic databases. Often, when

species names are lacking, Molecular Operational Taxonomic Units (MOTUs) are used as a

proxy for parasite species. Thus, metabarcoding methodology can be used to investigate host-

parasite communities and to explore relationships between host phylogeny and parasite

diversity. For large mammalian herbivores that are declining worldwide, metabarcoding

could begin to fill major knowledge gaps in patterns of parasite diversity and sharing (Walker

et al. 2017).

Here, we use metabarcoding to analyze parasitic nematode DNA in dung samples from 18 sympatric large mammalian herbivore species. Using molecular operational taxonomic units (MOTUs) as a proxy for parasite species, we ask several questions to investigate parasite patterns in this important and threatened group of hosts.

1) What explains parasite MOTU richness and phylogenetic diversity among large herbivores?

We expected that metabarcoding results would correspond to past morphological work in that host species identity would explain a large proportion of variation in parasite richness and diversity within individuals (i.e. infra-community). We also suspected that after accounting for variation due to species identity, infra-community richness and diversity would increase with host body size, range size, and social group size. Furthermore, given that mixed feeders likely sample vegetation from a broader range of landscape features, and ruminants likely have more complex gut morphologies that provide a range of parasite habitats, we hypothesized that they would have higher parasite diversity. We also expected to find these patterns at the host species level (component community richness), after controlling for sampling effort.

2) What explains parasite MOTU community composition among large herbivores?

We expected to find that host species identity would explain substantial variation in parasite communities due to strong phylogenetic linkages between hosts and parasites. We also anticipated that hosts with different feeding strategies would have different parasite communities because their parasite exposure would vary depending on plant material consumed (i.e. browse may be less contaminated with feces than grass). Furthermore, if parasite communities are determined by availability of habitat in host digestive tracts, we

hypothesized that parasite communities among ruminants, pseudoruminants, and hindgut fermenters would differ. Finally, we surmised that communities may differ as a function of host body size, home range size, and social group size, as these variables are linked to parasite habitat, host immunity, and breadth of parasite transmission.

3) To what extent are parasite MOTUs shared among hosts, and which hosts species play pivotal roles in a network of parasite sharing?

We expected that host-parasite linkages would be highly aggregated: the majority of parasite MOTUs would be found in one or two host species, but that parasite MOTU sharing among a large proportion of hosts would be rare, as is typical of host-parasite networks(Shaw et al. 1998). Furthermore, given results from literature analyses (Dallas et al. 2019a), we expected to find that hosts with large home ranges would occupy central positions in the host-parasite network.

4.3 Methods

4.3.1 Sample collection and sequencing

Fecal samples were collected during three sampling seasons (June-July 2014, March 2015, and August 2016) from 18 large herbivore species at Mpala Research Centre (Table 5). Samples were collected from fresh herbivore dung (avoiding contact with the ground or vegetation), and stored at 4°C for 1-4 hours before extraction (Kartzinel et al. 2015). We used the ITS-2 ribosomal DNA region to differentiate among nematode taxa (Hung et al. 2000). Nematode DNA was amplified using the NC1 forward and NC2 reverse primers (Avramenko et al. 2017). We performed two rounds of qPCR on all 527 samples plus positive, negative, and extraction controls to screen for nematode DNA presence before sequencing. Reaction mixtures for qPCR were comprised of 5uL fast SYBR green mastermix (Applied

Biosystems), 0.2uL of NC1 and 0.2uL of NC2 forward and reverse primers (concentration =

10uM), 3.6uL of water, and 1uL DNA extract for 10uL reaction mixtures. Fast qPCR

conditions included an initial holding stage of 95°C for 20 seconds followed by 40 cycles of

95°C for 3 seconds and 60°C for 30 seconds, before a final melting curve of 95°C for 15

seconds, 68°C for 1 minute, and 88°C for 15 seconds. Samples for which the mean CT value

< 35 (n=321) were selected for metabarcoding using NC1/NC2 primer pairs with unique tag

combinations. PCR reaction mixtures for metabarcoding included 2.5 mM MgCl2, 200 µM

each dNTP, 0.1 mg/mL BSA, 4% DMSO, 0.2 µM each primer, Amplitaq Gold polymerase,

and 2 µL DNA extract in 15 µL reactions. Thermocycling involved an initial denaturing at

95°C for 10 min, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1

min, and ending with a 5 min extension at 72°C. Sample concentrations were standardized

and pooled before sequencing with negative, positive, and extraction controls on an Illumina

MiSeq (2x300bp with 24 million reads).

Sequence demultiplexing, identifications, and quality controls were performed using

OBITOOLS (Boyer et al. 2016) software. Specifically, we assembled pair-end reads,

assigned sequences to their original samples, removed low quality sequences and those

which may have arisen due to sequencing errors, discarded single sequences, and assigned

sequences to parasite taxa documented in GenBank. We did not discard sequences with a low

match (all were >70% similarity) to those available in GenBank because existing records are

depauperate and low matches were disproportionately high in understudied and threatened

host species (Appendix 3.2).

Table 5. Summary of samples screened using qPCR and metabarcoding

Species	qPCR N	qPCR Prevalence	Metabarcoding N	Metabarcoding Positive	Metabarcoding Prevalence	Total Prevalence
Buffalo	47	0.20	16	12	0.75	0.26
Camel	9	0.89	8	5	0.63	0.56
Cattle	49	0.55	27	20	0.74	0.41
Dik-dik	50	0.62	22	15	0.68	0.30
Donkey	14	0.86	13	9	0.69	0.64
Eland	32	0.63	18	15	0.83	0.47
Elephant	40	1.00	39	25	0.64	0.63
Giraffe	38	0.42	18	8	0.44	0.21
Goat	17	0.00	0	NA	NA	0.00
G. gazelle	31	0.87	25	20	0.80	0.65
G. zebra	33	0.94	27	20	0.74	0.61
Hartebeest	28	0.82	20	12	0.60	0.43
Hippo	18	0.61	10	9	0.90	0.50
Hybrid zebra	4	0.75	2	1	0.50	0.25
Impala	41	0.83	22	17	0.77	0.41
Kudu	12	0.17	3	3	1.00	0.25
Oryx	13	1.00	13	6	0.46	0.46
Plains zebra	35	0.94	23	18	0.78	0.51
Sheep	10	0.00	0	NA	NA	0.00
Warthog	22	0.59	13	8	0.62	0.36
Waterbuck	10	0.20	2	1	0.50	0.10
Total	553	0.61	321	224	0.70	0.41

Sequences were then clustered based on a 98% similarity threshold and the most abundant sequence within each cluster (MOTU) was taken to be representative of that cluster. We experimented with different clustering thresholds to determine if conclusions changed based on this threshold. For example, while 98% is generally considered to be a reasonable standard threshold, (Avramenko et al. 2018) found that intraspecies similarity in cattle nematodes at this locus ranged from 99.2% to 100%, while interspecies similarity ranged from 49.0% to 98.8%, providing a second logical potential cutoff of 99%. We performed all analyses using both the 98% and 99% datasets. Results are qualitatively similar, but we chose to present data from the 98% dataset in the main text in keeping with

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prior work (Srivathsan et al. 2015), while providing results from the 99% dataset in Appendix 3.3.

We used two additional methods to control for potentially erroneous reads. First, we investigated the distribution of reads generated from negative controls (n=120) and subtracted the maximum number of negative control reads for each MOTU from all other samples, as recommended in (Elbrecht and Steinke 2018). This process eliminated all reads from 90 samples. We then rarified all samples to 1000 reads using *vegan* (Oksanen et al. 2016) to form a matrix of relative read abundance (RRA) for all parasite MOTUs and samples (Deagle et al. 2019). We then excluded all host/MOTU combinations with RRA less than 1% to control for any remaining low-abundance sequencing errors. The final MOTU table consisted of 224 samples with at least one positive record of any of the 112 MOTUs. Our second method was to investigate the distribution of read counts in negative controls and samples. We then imposed a cutoff threshold based on negative control read counts (3060). We rarified all sample reads to 3060 reads and excluded all host/MOTU combinations with RRA less than 1%. We found that the first analysis was more conservative in that it reduced low-level MOTU reads in a wide array of samples; thus, we present these results in the main text.

4.3.2 Fecal egg count comparison

To evaluate the sensitivity of the molecular methodology for nematode infections, we also conducted fecal egg counts using the modified McMaster method on a subset (n=37) of samples from impala and warthogs collected from a different site (Gorongosa National Park), but analyzed concurrently to all Mpala samples. Almost all individuals were positive for nematode eggs (35/37) using the McMaster fecal egg float method. Prevalence using

metabarcoding methods was 60% and there were no false positives. These results suggest

that metabarcoding prevalence estimates are likely to be conservative.

4.3.3 Statistical Analyses

4.3.3.1 What explains parasite MOTU richness and phylogenetic diversity among large

herbivores?

Infra-community richness: We calculated within-host richness by counting the number

of MOTUs for each individual sampled. We included all samples with zero reads (including

those that were not sequenced due to negative qPCR readings) for richness comparisons. We

used a zero-inflated generalized linear model with a Poisson error structure to examine the

relationship between parasite richness and host species identity. We performed post-hoc

comparisons using the false-discovery rate correction. After examining variation among

species, we then constructed new Poisson GLMMs of MOTU richness while accounting for

host phylogeny using MCMCglmm. We included log-transformed home-range size, body

mass, and social group size, diet (browser, mixed, or grazer) and gut morphology (ruminant,

pseudo-ruminant, or hind-gut fermenter) as fixed effects. Measurements of average body

size, home range size, and group size were obtained from the PanTHERIA database (Jones et

al. 2009) for all host species in our study. When information was not available in the

database or varied from local patterns (e.g. group size for eland at Mpala is not >500),

estimates from our study region were used (Appendix 3.4).

Host species level richness: Due to uneven host species sampling, total parasite MOTU

richness for each host species (including all zeros) was estimated using the specpool function

in *vegan* (Oksanen et al. 2016). Total parasite richness was compared across species using

the first-order jack-knife estimate because it is less prone to bias than other methods (Walther

and Morand 1998, Walther and Moore 2005). We then used a phylogenetic least-squares (PGLS) model to regress estimated component-community richness by log-transformed home-range size, body mass, and social group size, diet, and gut morphology as explanatory variables, while accounting for host phylogeny. We compared all submodels using delta AICc values.

Phylogenetic diversity: Since species richness and phylogenetic diversity are usually tightly correlated, we used the ses.pd function in *picante* (Kembel et al. 2010) to investigate differences in parasite diversity beyond MOTU richness (using 'richness' as our null model). We then regressed resulting z-values by 1) host species using linear models, and 2) by host characteristics after accounting for host phylogeny using an MCMCglmm, just as in our models of parasite richness. Best models were again selected based on AICc values.

4.3.3.2 What explains parasite MOTU community composition among large herbivores?

We first ordinated the data using non-metric multidimensional scaling to collapse parasite community data to two dimensions based on a binary Jaccard distance matrix. We compared patterns in parasite communities among host species using the *adonis2* function in *vegan* to perform a perMANOVA on the Jaccard distance matrix constructed from the individual by parasite MOTU matrix using host species as an explanatory variable. To account for parasite phylogeny, we then calculated the unweighted UniFrac distances of all samples with at least one positive MOTU using the *phyloseq* package (McMurdie and Holmes 2013) and repeated the perMANOVA analysis using this distance matrix. To then examine potential explanatory power of our hypothesized covariates (body size, range size, group size, diet, and gut morphology), we then calculated 1) a Jaccard distance matrix based on prevalence of each MOTU with each host 2) a UniFrac distance matrix based on average individual UniFrac

distances within each species. We used perMANOVA to examine additional effects of explanatory variables beyond host taxonomic order and family.

Finally, to determine whether host and parasite phylogenies showed signals of coevolution, we performed a Procrustes cophylogenetic analysis (Balbuena et al. 2013) using the *paco* package (Hutchinson et al. 2017). We calculated a goodness of fit statistic (m^2) from 1000 permutations to assess whether the multivariate matrices showed phylogenetic congruence.

4.3.3.3 To what extent are parasite MOTUs shared among hosts, and which hosts species play pivotal roles in a network of parasite sharing?

We built a bipartite species-level network for all host species with at least 7 positive individual samples (n=14 host species). We then projected the network to a unipartite network of hosts and calculated node metrics using the *igraph* package (Csardi and Nepusz 2006). We then examined potential relationships between host node metrics and home range size, body mass, social group size, diet, and gut morphology using linear models that accounted for host phylogeny (*pgls* package in R). Given that host and parasite phylogenies showed a strong signal of co-evolution, we also included the mean patristic distance of each host to all other species as a covariate. To determine best models of each node centrality metric, we compared AICc values. Finally, to investigate the effect of removing certain host species from the network on parasite extinctions, we used the *NetworkExtinction* package (Corcoran et al. 2019) to calculate resulting parasite extinctions.

4.4 Results

4.4.1 Parasite MOTU richness and diversity is explained largely by host species identity, with highest richness and diversity in mixed feeders.

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4.4.1.1 Infra-community richness: We found that host species identity accounted for approximately 50% of the variation in parasite MOTU richness within all samples (n=527, X^2_{18}=214.89, p < 0.001), with Grevy's zebra, Plains zebra, Impala, Hartebeest, Elephant, and Grant's gazelle having high richness, and Cattle and Warthog having relatively low richness (Table 6; Figure 13). After accounting for host phylogeny, only herbivore diet significantly accounted for variation in parasite richness. Mixed feeders had higher parasite MOTU richness, and browsers had lower richness (Table 7; Figure 13A).

Table 6. Parasite richness and diversity contrasts among hosts
Contrasts for each species from both the zero-inflated GLM investigating the relationship between infracommunity MOTU richness and host species identity (following a false discovery rate correction for multiple testing) and the linear model testing for differences in deviations from expected phylogenetic diversity. Animals that tended to have greater richness or diversity are shaded in green, while those with lower relative richness or diversity are shaded blue. Positive values in the zero-inflation component indicate increased probability of individuals with zero parasite MOTUs.

| Host Species | MOTU Richness | | | | | | | | Phylogenetic Diversity | | | |
| | *Conditional Component* | | | | *Zero-inflation Component* | | | | | | | |
Contrast	Est.	SE	t ratio	p	Est.	SE	t ratio	p	Est.	SE	t ratio	p
G. gazelle	0.76	0.09	8.47	<0.001	-0.91	0.38	-2.4	0.11	-0.699	0.208	-3.354	0.002
G. zebra	0.61	0.09	6.49	<0.001	-0.74	0.36	-2.05	0.16	-0.932	0.208	-4.474	<0.001
Elephant	0.61	0.09	7.01	<0.001	-0.82	0.34	-2.44	0.11	-1.055	0.191	-5.529	<0.001
P. zebra	0.71	0.09	7.54	<0.001	-0.36	0.35	-1.06	0.61	-0.932	0.217	-4.287	<0.001
Donkey	0.04	0.16	0.25	0.84	-0.92	0.55	-1.67	0.26	-1.378	0.309	-4.457	<0.001
Oryx	0.33	0.16	2	0.09	-0.16	0.54	-0.29	0.92	-0.115	0.353	-0.327	0.744
Hartebeest	0.34	0.12	2.78	0.01	-0.02	0.38	-0.06	0.95	0.483	0.258	1.871	0.088
Camel	-0.05	0.22	-0.21	0.84	-0.56	0.66	-0.85	0.72	-0.548	0.384	-1.426	0.185
Impala	0.36	0.11	3.37	<0.001	-0.02	0.32	-0.07	0.95	-0.563	0.217	-2.59	0.018
Eland	-0.22	0.15	-1.54	0.18	-0.23	0.37	-0.63	0.72	0.769	0.234	3.281	0.003
H. zebra	0.43	0.36	1.18	0.3	0.79	1.1	0.72	0.72	-1.306	0.839	-1.556	0.154
Hippo	-0.31	0.19	-1.65	0.17	-0.38	0.48	-0.79	0.72	-0.856	0.309	-2.768	0.012
Warthog	-0.95	0.29	-3.23	<0.001	-0.06	0.53	-0.12	0.95	1.581	0.309	5.116	<0.001
Dik-dik	-0.22	0.15	-1.54	0.18	0.5	0.32	1.55	0.29	0.939	0.28	3.36	0.002
Cattle	-0.69	0.16	-4.17	<0.001	-0.12	0.33	-0.37	0.9	1.466	0.213	6.894	<0.001
Giraffe	0.03	0.17	0.2	0.84	1	0.4	2.51	0.11	0.762	0.309	2.464	0.023
Kudu	-1.05	0.5	-2.08	0.08	0.48	0.76	0.62	0.72	0.622	0.597	1.042	0.334
Buffalo	-0.24	0.16	-1.51	0.18	0.72	0.35	2.09	0.16	0.204	0.258	0.79	0.455
Waterbuck	-0.48	0.61	-0.79	0.51	1.82	1.02	1.79	0.24	1.558	0.839	1.856	0.088

Table 7. MOTU richness model accounting for host phylogeny and characteristics
Summary tables for the full and reduced models of MOTU richness among individuals of different herbivore species after accounting for phylogenetic relationships using MCMCglmm models.

Full Model

Niterations = 100,000 Burnin = 5000 Thin = 50	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	0.0008	-2.904	3.313	1900	0.999
Diet [Grazer]	0.341	-0.928	1.620	1900	0.553
Diet [Mixed Feeder]	**1.478**	**0.335**	**2.752**	**1754**	**0.025 ***
Gut [Psuedoruminant]	0.060	-2.527	2.291	1900	0.975
Gut [Ruminant]	-1.213	-3.693	1.081	1900	0.280
ln(Body Mass)	-0.165	-0.681	0.311	1900	0.499
ln(Social Group Size)	0.299	-0.381	0.883	1920	0.311
ln(Home Range Size)	0.003	-0.243	0.280	2186	0.993
Phylogeny	1.84	0.00343	5.059	1900	
Units	3.404	2.526	4.333	1306	
DIC	1542.912				
Lambda (mode)	0.235	0.0008	0.600		

Reduced Model

Iterations = 100,000 Burnin = 5000 Thin = 50	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	-0.803	-2.763	1.023	1900	0.354
Diet [Grazer]	0.538	-0.291	1.501	1900	0.222
Diet [Mixed Feeder]	**1.578**	**0.680**	**2.474**	**1988**	**0.0011**
Phylogeny	1.248	0.146	2.897	1900	
Units	3.323	2.470	4.225	1807	
DIC	1547.833				
Lambda (mode)	0.217	0.059	0.478		

4.4.1.2 Component community richness: After accounting for sample size, total parasite richness pooled across all individuals of a host species varied considerably. Again, wild equids (plains zebra, Grevy's zebra), Grant's gazelle, and hartebeest had high estimated richness. Interestingly, buffalo were estimated to have substantially more parasites on the species-level than the individual level while cattle and warthog were also estimated to have

low relatively low species-level richness (Figure 13B). However, we found that none of our hypothesized predictors showed a significant relationship with component-community richness, as our best model included only the intercept (Appendix 3.1).

Figure 13. Parasite MOTU richness across host species
MOTU richness including and excluding qPCR zeros following rarefaction to 1000 reads (A). (B) Estimated component community (species-level) richness.

4.4.1.3 Infra-community diversity: Phylogenetic diversity (independent of species richness) was also host dependent (R^2=0.55, $F_{18,197} = 13.31$, p<0.001), with hosts that had high MOTU richness exhibiting lower-than-expected phylogenetic diversity (Figure 14). Notably, there was a significant negative relationship between MOTU richness and phylogenetic diversity ($X_1^2 = 74.31$, p<0.001), except for cattle and kudu, which showed increasing diversity with increasing richness. The best model of variation in phylogenetic diversity included only MOTU richness as a predictor (Table 8).

Table 8. Phylogenetic diversity MCMCglmm model

Summary tables for the full and reduced MCMCglmm models of individual Z-values derived from comparing observed parasite phylogenetic diversity to null models. Models account for phylogenetic similarity among herbivore species.

Full Model

	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	-1.300	-4.125	1.108	1900	0.282
Diet [Mixed]	-0.217	-0.906	0.544	1900	0.535
Diet [Browser]	0.324	-0.701	1.314	1900	0.471
Gut [Pseudoruminant]	-1.643	-3.389	0.150	1900	0.076
Gut [Ruminant]	-0.096	-2.021	1.746	1900	0.886
ln(Body Mass)	0.086	-0.343	0.461	1900	0.633
ln(Social Group Size)	0.208	-0.282	0.674	1900	0.365
ln(Home Range Size)	-0.109	-0.304	0.102	1900	0.267
MOTU richness	**-0.178**	**-0.219**	**-0.135**	**1900**	**<0.01 ***
Phylogeny	1.382	0.145	3.378	1900	
Units	0.608	0.492	0.729	1900	
DIC	519.77				
Lambda	0.725	0.330	0.889		

Reduced Model

	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	-1.129	-2.907	0.627	2146	0.193
MOTU richness	**-0.178**	**-0.218**	**-0.135**	**1900**	**<0.01 ***
Phylogeny	1.517	0.430	2.931	1772	
Units	0.606	0.485	0.727	1900	
DIC	516.83				
Lambda	0.706	0.510	0.873		

Figure 14. Relationship between MOTU richness and phylogenetic diversity
Phylogenetic diversity was significantly lower than expected after accounting for MOTU richness, and it trended lower in hindgut fermenters (HGF) compared to ruminants (R) or pseudoruminants (PR).

4.4.2 Parasite MOTU composition among hosts depends on host species identity and gut morphology.

Host species identity was also highly significant in describing parasite community composition in individuals. Specifically, 54% of variation in parasite community composition was described by host species identity at Mpala (n=224, $F_{17,205}$=13.431, p < 0.001) (Figure 15). When we accounted for parasite phylogenetic relatedness, we found that host species identity accounted for an even greater fraction of variation among parasite communities in individuals (71%; $F_{18,205}$=28.257, p < 0.001). Indeed, host and parasite phylogenies showed a significant signal of phylogenetic congruence (m^2 =, p<0.001, n=1000

permutations; see figures in Appendix 3.2). At the host species level, parasite communities

were significantly related to their taxonomic order and family. Although host families were

nested within gut morphology levels, we found that gut morphology described a substantial

degree of variation in parasite communities (Figure 16; Sequential R^2 for MOTU table =

0.16, $F_{2,15}$= 2.53, p=0.001; sequential R^2 for UniFrac distances: = 0.175, $F_{2,15}$= 3.466,

p=0.002; Table 9).

Table 9. PerMANOVA results for parasite community data in host species
PerMANOVAs are based on distance matrices of a) MOTU prevalence and b) mean UniFrac distances. Terms
are listed by sequential sum of squares.

Parameter	Df	*Seq. Sum Squares*	R^2	*F Ratio*	*Pr(>F)*	*Seq. Sum Squares*	R^2	*F Ratio*	*Pr(>F)*
		MOTU Prevalence				**UniFrac Distances**			
Taxonomic Order	2	1.895	0.271	4.418	0.001 ***	1.535	0.380	7.523	0.001 ***
Gut Morphology	2	1.086	0.156	2.533	0.001 ***	0.730	0.181	3.578	0.001 **
Taxonomic Family	2	0.694	0.099	1.618	0.008 *	0.552	0.136	2.706	0.003 **
Diet	2	0.895	0.128	2.087	0.011 **	0.155	0.038	0.759	0.706
ln(Group Size)	1	0.482	0.069	2.245	0.007 **	0.223	0.055	2.191	0.059
ln(Body Mass)	1	0.481	0.069	2.243	0.01 *	0.188	0.046	1.838	0.103
ln(Home Range)	1	0.164	0.023	0.764	0.759	0.049	0.012	0.478	0.851
Residual	6	1.287	0.184			0.612	0.151		
Total	17	6.983	1.000			4.043	1.000		

Figure 15. NMDS plots of parasite communities among individual hosts

Non-metric multidimensional scaling based on Jaccard distances (A) and UniFrac distances (B) demonstrates species-level partitioning in parasite communities.

Figure 16. NMDS plots of species-level differences in parasite communities
NMDS plots of species-level data demonstrate clear partitions based on gut morphology using both Jaccard distances for a host-parasite prevalence matrix (A) and a distance matrix using average UniFrac distances for each host species (B).

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4.4.3 Most parasite MOTUs are specific to a single host species, but cattle and eland show elevated degree, betweenness, and closeness centrality.

We found that 52% of all MOTUs were found in only one host species (Figure 17). The maximum number of hosts was 10; this MOTU had a best identity of *Cooperia sp.* (95%) and was found in all ruminants except cattle and waterbuck. After calculating node metrics, we identified cattle and eland as being important species in a network of parasite sharing, as they exhibited relatively high degree, betweenness, and closeness centrality across all parasites (Figure 18). While dik-dik and giraffe had high eigenvector centrality, values for other metrics were relatively low. Furthermore, we found that a host's mean phylogenetic distance to all other hosts in the network was negatively associated with degree centrality ($F_{1,12}$= 8.49, p=0.01), but not for other metrics (Figure 18; Appendix 3.1). While this distance was negatively correlated with eigenvector centrality, gut morphology was a more important predictor. Specifically, ruminants had higher eigenvector centrality than herbivores with other gut types ($F_{2,11}$= 13.29, p=0.001). Finally, our analyses highlighted the important role of elephants in maintaining parasite diversity: loss of elephants as hosts will likely lead to substantial subsequent losses in parasite species (Figure 19).

Figure 17. Aggregated distribution of host range among parasite MOTUs
The distribution of host species that each parasite MOTU infected was highly aggregated: more than half infected only one host species, while <5% infected 8 or more host species.

Figure 18. Node metrics for hosts connected in a network of parasite sharing
The unipartite projection of host and parasite sharing (A) shows the relationships between large mammalian herbivores based on their nematode parasites. Node level metrics showed few relationships to host characteristics, except that mean phylogenetic distance to all other hosts was negatively associated with eigenvector centrality and significantly covaried with degree centrality (p=0.01) (B). Ruminants had higher eigenvector centrality than other gut types (p=0.001).

Figure 19. Parasite losses with host extinctions
Experimental single host extinctions revealed that elephants accounted for a disproportionate number of unique links, demonstrating that elephant extinctions would likely lead to substantial loss in parasite diversity. Bars are shaded by each species' endangerment status as assigned by the IUCN red list (IUCN 2016), with species population trends (+ increasing, - decreasing, . stable) shown in parentheses.

4.5 Discussion

Our metabarcoding analysis of parasites infecting a diverse array of herbivores revealed robust signals of host species identity in determining parasite MOTU richness and phylogenetic diversity, with strong evidence of host and parasite cophylogeny. While we found no relationship between parasite richness or diversity and host home range, body mass, or social group size, we did find evidence that richness is higher in mixed feeders and hindgut fermenters, and that host gut morphology is closely linked to nematode communities. Finally, hosts occupying central positions in a host phylogeny are central nodes in a network of parasite sharing, with evolutionarily distinct species, such as elephants, accounting for a large proportion of single-host parasites.

4.5.1 What explains parasite MOTU richness and phylogenetic diversity among large herbivores?

Our finding that host species identity explained a significant proportion of variation in individual (infracommunity) richness agreed with our hypothesis and prior findings in other systems; however, the degree to which this was the case was somewhat surprising given that all hosts were found within a 200 km^2 area and exhibited a high degree of spatial overlap (Ezenwa 2003). When we examined MOTU richness without accounting for parasite phylogeny, richness was particularly high in equids, a finding that has been noted previously (Walker et al. 2017). One potential explanation for this draws upon the hypothesis that total parasite *biomass*, rather than number of species, is constrained by host attributes (Poulin 2007). By this hypothesis, higher parasite richness should be associated with higher relative abundance of smaller-bodied parasites. Indeed, equids are known for being infected by an array of 'small strongyles' (Corning 2009), and our results also signify this. While there is no immediate explanation for lower infracommunity parasite richness in cows and buffalo, we hypothesize that these animals may be parasitized by relatively larger nematode species.

Beyond host species identity, we found little evidence that body size, home-range size, or social group size is related to parasite MOTU richness on either the individual or species level. This was somewhat surprising given prior results (Dallas et al. 2019a). Our finding that richness was highest in mixed feeders was interesting because it may indicate that more diverse food sources, and thus more diverse transmission sources, may be related to increased parasite richness. While this has not been documented in large mammalian herbivores, it has been noted in trophically-transmitted fish parasites (Locke et al. 2014). Accounting for parasite phylogeny substantially augmented our results. Interestingly, we

found a clear negative relationship between MOTU richness and phylogenetic diversity across most hosts, an indication of phylogenetic conservatism, in which closely related parasites are more ecologically similar in that they infect the same host species (Poulin et al. 2011). We found that this signal extended to gut morphology: phylogenetic diversity was consistently lower than expected in hindgut fermenters than ruminants, even after controlling for MOTU richness.

Together these results showed little support for other host characteristics in predicting parasite richness and diversity. These findings differ from our expectations and previous findings in which home range size may be an important predictor of infracommunity parasite species richness (Nunn et al. 2003, Lindenfors et al. 2007), but align with a synthesis finding no relationship between home range size and component community richness for ungulates, and a negative relationship for other mammalian taxa (Bordes et al. 2009). When examining parasite richness on the host species-level, total helminth richness has been shown to be positively correlated with geographic range size in rodents (Feliu et al. 1997), and others have found the same patterns in carnivores (Torres et al. 2006, Lindenfors et al. 2007).

Our finding that individual MOTU richness was unrelated to body size is similar to analyses across mammal taxa (Morand and Poulin 1998), but differs from findings for a range of ungulate parasites in which host body size was positively correlated with total parasite species richness (Ezenwa et al. 2006). Like one comparative study across primates (Nunn et al. 2003), we found no significant relationship between social group size and parasite diversity, and similarly to a comparative study in ungulates (Ezenwa et al. 2006), the trend was negative. Our lack of significant results indicates that if there is a relationship between these variables and individual-level MOTU richness, it may be relatively weak,

although our power was low given 18 total species investigated (power = 0.5 to detect an R^2 of 0.2). Another possible reason for a lack in significant relationships is that parasite sharing among sympatric species may complicate estimates of group size, especially when those species are commonly found in close proximity to each other (VanderWaal et al. 2014b). Indeed, parasite overlap in a group of sympatric bovids has been shown in this system (Ezenwa 2003).

4.5.2 What explains parasite MOTU community composition among large herbivores?

High parasite community fidelity within host species mirrors our findings for parasite richness; this is the first use of molecular methods to reveal this pattern for gastrointestinal parasites among a diverse array of large mammalian herbivores. These results correspond to similar results for ectoparasite communities in rodents (Krasnov et al. 2005, 2006) and carnivores (Huang et al. 2014) that also emphasize the importance of host phylogeny. The stronger signal of host species identity in UniFrac results, which accounts for phylogenetic relationships among parasites, (71% vs 54%) and highly significant results from our cophylogeny analysis further demonstrate the strong evolutionary relationship between these nematode parasites and their large herbivore hosts. While the importance of accounting for this phylogeny has been highlighted (Poulin 1995), studies testing its significance have found inconsistent results among host and parasite taxa. For example, while Poulin (2010) found a weak exponential decay in the similarity of parasite faunas with increasing host phylogenetic distance in Canadian freshwater fishes, patterns varied substantially by parasite type. Seifertová et al. (2008) found that phylogenetic distance was more predictive than spatial distance in metazoan fish parasite communities, but at the scale of habitat realms, Bush et al. (1990) found that host phylogeny was less important than host habitat. While the strong

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patterns in our work demonstrate the tight cophylogenetic relationship between large herbivores and nematode parasites, it remains to be seen if this is broadly applicable to other groups.

Other work has shown how the 'host environment' (host mass, reproductive rate, longevity, and trophic status) explains variation in parasite community composition in rodents (Dallas and Presley 2014). In our large mammalian herbivore study, we found evidence that gut morphology, a key aspect of host environment, explained a large degree of parasite community composition. Surprisingly, we saw comparatively little evidence that other covariates explained significant variation in parasite communities after controlling for host taxonomy. Increasing the number of host species in a variety of regions will increase power to detect meaningful differences and to see if they are indeed replicated over space. We hypothesize that host traits that are conserved across phylogeny and related strongly to parasite habitat are important predictors of parasite communities. Together, our findings may be helpful in predicting host-parasite links, a growing focus of parasite ecology (Dallas et al. 2017).

4.5.3 To what extent are parasite MOTUs shared among hosts, and which hosts species play pivotal roles in a network of parasite sharing?

Our finding that host-parasite links were highly aggregated aligns with our expectation and previous findings in the literature (Walker et al. 2017). By examining specific nodes, we found that eland and cattle were more central in the host-parasite network than other host species. Our finding that a host species' mean phylogenetic distance to all others was predictive of degree centrality is also a signal of host-parasite cophylogenetic signal, and has also been suggested by models based on herbivore parasite records from

Botswana (Walker et al. 2017). If hosts that are more closely related to each other are more likely to share parasites, then it follows that they will have central positions in this web. Interestingly, some species with high phylogenetic distinctiveness still shared some parasites with core animals (Figure 18), demonstrating the potential for a small number of parasites to have unexpectedly high host ranges (Figure 17). When we investigated this further, the majority of parasite MOTUs found in more than one type of gut morphology, were in the superfamily Strongyloidea, whose parasites have been recorded in a wide range of animals (Round 1968, Anderson 2000). The correlation between degree centrality and host phylogeny is useful because it could be used to predict parasite sharing networks when sympatric hosts are known. Unexpectedly, we found that gut morphology was a better predictor of eigenvector centrality than any other metric, even after accounting for host phylogeny. High ruminant centrality indicates that there are likely to be several parasite species that are frequently shared among herbivores in this group compared to herbivores with other gut types. Finally, our analysis investigating parasite losses with single host species extinctions demonstrated that loss of elephants would have disproportionately large negative effects on parasite diversity. Elephant extinction in our network of 14 different host species led to a loss of nearly 25% of parasite MOTUs included in the network, underscoring the importance of this threatened species for parasite biodiversity.

4.5.4 Additional considerations

There are several additional aspects to consider in this analysis. First, our estimates of parasite prevalence using molecular methods were lower than prevalence recorded from fecal egg counts, suggesting that our results may be conservative with respect to estimates of parasite species richness. However, richness analyses partitioned by zero and positive data

revealed similar patterns, suggesting that these results are robust to low prevalence. Second, metabarcoding has additional technical limitations that could potentially bias results. One important example is primer specificity: while the primer and target gene sequenced here has been shown to effectively differentiate among parasitic nematode species (Avramenko et al. 2017), it is likely that certain parasite species were more efficiently amplified than others. While we controlled for this by analyzing our data as binomial host/parasite associations, (rather than relative read abundance), it is possible that differential amplification may have affected our results. The qualitative correspondence of our data to both parasite ecological theory and the literature indicate that metabarcoding has strong potential to both confirm and extend ecological theories (e.g. as has been shown in (Kartzinel et al. 2015)).

Finally, and perhaps most glaringly, the fact that we were unable to reliably identify parasite MOTUs illustrates the dearth of genetic identifications currently available in Genbank. Aside from missing records, at least one study has found large inaccuracies in species identifications (Valkiunas et al. 2008), although this is constantly improving over time (Leray et al. 2019). Indeed, some best identifications for elephant parasites in this study matched most closely (but only at ~75% similarity) with a parasite family known to only infect marsupials (Cloacinidae). There still remain large gaps in genetic knowledge for a broad array of parasites, especially for those not known to infect humans or their domestic animals (Selbach et al. 2019), but which may be critically important for conservation of understudied wildlife species (Walker et al. 2017). Only with parasite species identifications, and some understanding of the ecology of those species, will scientists will be able to better link parasite life history traits and morphologies to broad ecological patterns and theories (Valkiunas et al. 2008). For example, exploring the hypothesized link between parasite

biomass and host species attributes (Poulin 2007) may be possible without extensive lethal sampling, as parasite-specific body size measurements can be linked to their detection in host feces. Knowledge of parasite virulence for each host species could be used to weight edges in host-parasite sharing networks to identify hosts that are central to costly interactions. Although metabarcoding is not a panacea to dwindling parasitological expertise, our results provide compelling evidence for its potential to greatly augment our understanding of broad patterns in parasite ecology. Future efforts to connect decades of intricate descriptions and natural history from parasites preserved in collections with their genetic sequences will further our understanding of parasite ecology in a changing world.

4.6 Conclusions

Our finding that parasite MOTU richness and community composition exhibited high host fidelity and traced host phylogeny mirrors findings from the literature, but they also highlight the importance of the host gut as a habitat. The clear partitioning in parasite communities among herbivores with different gut morphologies demonstrates that phylogenetically conserved host traits that directly constrain parasite habitats account for a large proportion of variation in nematode communities. This contrasts with findings that patterns in host body size, group size, and range size are predictive, and indicate that consideration of variation in parasite resources within the host might explain a substantial degree of the variation in parasite sharing outcomes. Furthermore, our findings suggest that hosts that are centrally located in the host phylogeny may be important for parasite sharing networks. While much work remains to be done in connecting parasite natural history to genetic sequences, these efforts hold enormous promise to shed light on important hidden facets of the biodiversity iceberg.

Coauthors and contributions:

Johan Pansu[1,3] assisted in establishing the pipeline for molecular work and performed bioinformatics. Kaia Tombak[2,3] performed fieldwork and assisted in study design and interpretation of results. Matt Hutchinson[3] and Tina Hansen[3] assisted with lab work and provided feedback on analytical approaches and interpretations. Tyler Kartzinel[4] collected samples and performed extractions. Hillary Young[5] assisted in study design and interpretation of results. Rob Pringle[3] facilitated lab work and provided feedback on study design and interpretation.

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Chapter 5

5. Cattle aggregations near water can create potential parasite transmission hotspots for other wildlife

5.1 Abstract

Water sources provide critical resources to wildlife, livestock, and humans, and they are likely to be especially important in the face of increasing global aridification and rainfall uncertainty. However, heightened animal aggregations near water may also promote the transmission of parasites, creating stark transmission heterogeneity across space. While this hotspot effect has often been assumed to occur around water sources, it has been infrequently tested or quantified. Furthermore, despite the high degree of overlap in wildlife and livestock resource use and parasite sharing, interspecific parasite transmission dynamics are rarely considered. In this study, we used observational and experimental data to compare estimates of parasite transmission at permanent water sources, dry sites, and experimentally drained

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water pans using host activity, parasite density, and parasite sharing data collected from an East African tropical savanna where cattle and several wildlife species of conservation concern overlap. We found that per unit area, water sources markedly increased parasite transmission estimates, but that this hotspot effect varied strongly by host species. When we considered interspecific parasite sharing, estimated parasite transmissions per unit area were up to 10,000 times higher than dry sites for elephants, but they were not significantly higher in giraffe. Results were similar, but more muted, from the experimental data: transmissions per unit area were up to 200 times higher near permanent water than drained water pans for elephants and three times as high for giraffe. Notably, cattle aggregation at water drove greatly increased risk for bovids and giraffe given their higher degree of parasite sharing with these animals. These findings underscore the substantial degree of heterogeneity in parasite transmissions across space, and they illustrate the effect of accounting for interspecific sharing at resources were diverse host assemblages concentrate.

5.2 Introduction

Humans are increasingly modifying natural landscapes, including surface water availability and distribution, via climate change, land use change, and livestock husbandry; all of which, in turn, have stark implications for disease transmission in humans, domestic animals, and wildlife (Patz et al. 2008, Van Campen and Rhyan 2010). Surface water is an increasingly critical landscape feature for these groups in the face of widespread global aridification (Huang et al. 2016) and increasingly unpredictable rainfall (IPCC 2014). However, surface water also has the potential to concentrate individuals and their parasites (Chapter 3), potentially increasing disease transmission risk within species (Paull et al. 2012, Zvidzai et al. 2013). However, as humans and their livestock increasingly overlap with and

replace wildlife (Hempson et al. 2017), it is also critical to account for parasite sharing across species at these hotspots. However, the effects of water sources on parasite sharing is rarely tested using empirical data due to complexities in estimating both exposure risk and parasite sharing across species, space, and time.

Water sources are well-known to aggregate animals in a relatively small space (Valeix 2011), and this may lead to increased risk of directly-transmitted diseases by elevating contact rates among infectious individuals (Vicente et al. 2007) or infectious material in the environment (Nunn et al. 2014). While spaces that substantially increase contact rates (hotspots) have been considered analogous to superspreading individuals that disproportionately account for new cases (Paull et al. 2012), there have been few attempts to quantify the degree to which landscape features can elevate disease transmission risk. While recent advances in contact modelling and GPS tracking for diseases (Dougherty et al. 2018) have enabled better understanding of the role of hotspots in increasing risks for directly transmitted pathogens (VanderWaal et al. 2014a, Vanderwaal et al. 2017), it remains extremely challenging to collect empirical data on contact rates with infectious material in the environment to explicitly inform models that can account for hotspot effects.

Because true disease transmission is challenging to quantify (Craft 2015), mathematical models provide critical insight into broader scale dynamics. These disease models often rely on a central parameter that modulates transmission of an infectious agent. In the simplest epidemiological models, the transmission rate is usually the product of the average number of contacts (per unit time) and the probability of transmission given contact. For macroparasites that have infectious stages in the environment, total transmissions per unit time and space can be thought of as the product of the density of parasites in the

environment, the rate at which each host consumes parasites, and the density of hosts in the environment (Anderson and May 1991) (Figure 20). However, most epidemiological models (whether for directly transmitted microparasites or environmentally transmitted macroparasites) do not account for variation in infectiousness or contacts over space or time, which can lead to inaccuracies in predicting disease outbreaks and extinctions (Lloyd-Smith et al. 2005). While previous studies have more thoroughly examined the effect of varying individual infectiousness in altering directly transmitted infections, variation in contact rates for environmentally transmitted infections across space may also be important (Paull et al. 2012, Leach et al. 2016). Identifying locations of increased transmission risk among animals that also share parasites to varying degrees will better inform a landscape of risk for different host species.

Figure 20. Macroparasite compartment model
Schematic illustrating a compartment model for macroparasite infections (Anderson and May 1991, Dobson and Hudson 1992). For this study, we compared total parasite transmissions, the movement of infectious stages in the environment to viable parasites within hosts (shown in red), in three different contexts: permanent water sources, where parasites and hosts are often concentrated, drained water sources, and dry sites. Transmissions (T) are the product of ingestion rate (approximated by contact time) (β), parasites (W), and hosts (H) per unit area and time.

Given that many mammal species that share a common resource (e.g. water and vegetation surrounding water) also share parasites (Round 1968, Vicente et al. 2007), it is especially important to consider the ways in which parasite sharing may alter understanding of exposure and transmission in different environmental contexts. Perhaps the most famous examples have been in the field of vector-borne diseases, where host community has been shown to either amplify or 'dilute' risk of Lyme disease (Keesing et al. 2006). For directly-transmitted diseases, recent work has further emphasized the need to consider a full host community in understanding transmission; for example, in the ability of dogs to drive infection dynamics of canine parvovirus in lions (Behdenna et al. 2019), and in circulation of bovine tuberculosis among possums, ferrets, deer, and pigs in New Zealand (Nugent 2011). For environmentally-transmitted parasites, the effect of parasite sharing is often challenging to quantify directly, but has been shown to drive increased strongyle nematode abundance among several bovid species in areas where their habitats overlapped (Ezenwa 2003), and modeling work has implied substantial differences in parasite dynamics between multi- and single-species networks (Pilosof et al. 2015). While few studies have attempted to quantify elevated disease risks at shared resources (e.g. (Wright and Gompper 2005a, Vicente et al. 2006)), even fewer have quantified the effect of multi-species contacts at hotspots that promote parasite transmission.

Accounting for parasite sharing among multiple host species may be particularly important for diseases that are density dependent – i.e. greater host populations lead to increased transmission, as is the case for many fecal-oral parasites that are transmitted via the environment (May and Anderson 1979). Fecal-oral transmitted parasites, including gastrointestinal helminths that cause substantial morbidity on domestic and wild herbivores,

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and massive economic losses on a global scale (Charlier et al. 2014), release many thousands of eggs into the landscape upon host defecation. These parasites then infect herbivorous mammals when they ingest infective parasite stages that disperse from feces via drinking or grazing (e.g. Strongylid nematodes (Anderson 2000)) (Figure 20). Notably, several important gastrointestinal parasites (e.g. trichostrongyle nematodes) are shared with closely-related domestic animals or with humans (Round 1968, Walker et al. 2017), and it is likely that several pose significant health threats (Bull 1994, Ashford and Crewe 2003).

Given that cattle are increasingly dominating many landscapes (Hempson et al. 2017), it is important to account for their density and parasite sharing with wildlife. While cattle and wildlife overlaps occur globally (Hempson et al. 2017), their co-occurrence is common in East Africa, where many wildlife species of conservation concern overlap with larger ranching operations or smaller scale community grazing (Keesing et al. 2018). Indeed, aerial wildlife counts have shown that livestock biomass in Kenya grew to 8.1 times that of wildlife in 2011-2013 compared to 3.5 times wildlife biomass in 1977-1980 (Ogutu et al. 2016). Substantial research has investigated the compatibility of wildlife and cattle ranching operations, especially if tourism and ranching provide economically beneficial outcomes for communities (Homewood et al. 2012). While parasite sharing at water points may occur in many arid locations with mixed herbivore assemblages, East African tropical savannas provide an ideal context to investigate differing transmission risks across space. High cattle and herbivore densities across this area are also often supported by provisional water sources that concentrate many water-dependent animals (Chapter 3). Furthermore, many wildlife species in these landscapes are either threatened or experiencing population declines (IUCN 2016), such as zebra (*Equus burchellii* and *Equus Grevyi*), giraffe (*Giraffa camelopardalis)*,

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elephant (*Loxodonta* africana), buffalo (*Syncerus caffer*), and impala (*Aepyceros melampus*), and which are often infected by a diverse array of gastrointestinal parasites (Round 1968).

In this study, we connected two years of empirical data on herbivore behavior and parasite density at water sources, nearby dry locations, and experimentally-drained water pans to answer two questions: **1) Are intraspecific parasite transmissions elevated near permanent water sources compared to dry sites and drained pans? How does this vary across herbivore species?** We expected that single-species transmissions would be elevated in highly water-dependent animals (elephants and cattle), but that other herbivores would experience similar transmission risk at water and dry sites or drained pans. We then extended these questions to incorporate shared parasites: **2) After accounting for parasite sharing among species, how does this relationship change?** We expected that any animal with significant parasite overlap with either cattle or elephants would have an altered perspective of transmission risk at water. Given that elephants share relatively few parasites with other species (Chapter 4), we expected to find that cattle would be the primary drivers of this pattern.

5.3 Methods

5.3.1 Study Site*:* Fieldwork was performed at Ol Pejeta Conservancy (0.0043° S, 36.9637° E), where we established five experimental sites (described in Chapter 3), each with one pair of water pans (10 pans total) and 1 'dry' (no-pan) site. Dry site coordinates were randomly selected from a range of locations 1km from the experimental water pan and at least 1km from any other water source. One of the two water pans (located 0.4 - 1km apart at each site) was drained for one year and then refilled, while one remained filled throughout the experiment. For the observational component of this study, we used data from the dry site

and water pan that remained filled for the duration of the experiment. For the experimental

component, we compared drained to filled pans for the period from Jan 2017 – Feb 2018,

excluding March and April 2017, as animals were observed drinking rainwater from drained

pans during these wet-season months. We also repeated analyses for pre- or post- water

manipulation when both pans were filled to ensure that there were no differences in

aggregation around water (Appendix 4.1).

5.3.2 Field Data for Parameters:

5.3.2.1 Herbivore activity (β): We measured herbivore activity (Figure 21A) using camera

traps deployed at each water pan and dry site (n=15). We positioned cameras to capture

animal movements at each water source or center of a dry site and performed walk tests to

determine detection distances prior to deployment. All cameras had maximum detection

distances between 12 and 15 meters across sites. Cameras were set to take 3 image bursts if

movement or heat was sensed with minimal delay (1-5 seconds). We maintained cameras for

a two-year period from August 2016 – August 2018, servicing on a monthly basis. Excluding

failed or shortened deployments, the dataset comparing dry sites to filled pans was comprised

of 5888 trap nights, and the experimental comparison was comprised of 2392 trap nights.

Images from all camera deployments were uploaded to our citizen science project in

Zooniverse, the world's largest online citizen science platform

(https://www.zooniverse.org/projects/gtitcomb/parasite-safari). Nearly 8000 volunteers

assisted in classifying photographs by counting animals and assessing how many were

drinking and/or grazing in each image set. Image sets were retired after 5 classifications. We

determined consensus identifications by comparing all classifications for each image set; an

animal was considered present if at least 3 of the 5 classifications said so, and then counts for

each activity (present/drinking/grazing) were averaged for each species. We then determined

the mean daily per-capita duration of animals seen drinking or grazing per m^2 (β) by 1)

Calculating the elapsed time within uninterrupted image sequences (10s) for each species, 2)

summing total duration within a day, and 3) dividing by the area covered by each camera

(with a 50 degree angle and 15m detection distance, this was 98m^2). We assumed that single-

photo triggers corresponded to five seconds of animal presence.

5.3.2.2 Parasite Density (W): We used parasite density (eggs per m^2) estimates (Figure 21B)

from dung surveys and fecal egg floats described in Chapter 2. Because parasite density

tended to be especially elevated in the 50m near water (the same area captured by camera

traps), we used average parasite density per m^2 within the inner 50m radius of water to

represent areas near water. In summary, we calculated parasite density (eggs/m^2) by

multiplying dung volume from field surveys (cm^3/m^2) by physical dung density (g feces

/cm^3) and fecal egg counts (eggs/g) performed at a nearby location and cross-referenced with

fecal egg counts reported in the literature from focal species across Africa (Chapter 3).

5.3.2.3 Parasite Sharing (Λ): We used a distance matrix (Figure 21C) calculated from

gastrointestinal parasite community similarities from metabarcoding data (full details

available in Chapter 4). Specifically, we calculated the Jaccard distance of parasite

communities at the host species level. We chose Jaccard distances because this metric

quantifies the degree of overlap based on species occurrences, and thus provides a

conservative estimate of parasite sharing (i.e. there are likely to be more false negatives than

false positives using this method). We also conducted a literature search of host-parasite

records to compare metabarcoding Jaccard matrices with records found in the literature.

While published accounts are biased toward domestic species, we found similar general patterns in parasite sharing among our focal hosts (Appendix 4.3).

5.3.2.4 Herbivore Density (H): We again used our camera trapping results to estimate the total number of herbivores found drinking or grazing per m^2 (Figure 21D). We calculated the daily total density of individuals of each species by summing the mean count for each image set within each day and dividing by the area encompassed by the camera.

5.3.3 Equations:

We combined the above parameters to estimate the degree to which estimated transmissions (T) were elevated at filled water pans compared to dry sites or drained pans (a 'hotspot effect') when 1) intraspecific transmissions only were considered, and 2) when interspecific transmissions were considered. We then quantified the effect of considering parasite sharing on changing this hotspot effect at water sources.

5.3.3.1 Question 1: Are intraspecific parasite transmissions elevated near permanent water sources compared to dry sites or drained pans?

Models of helminth transmission are more complex than the more straightforward SI, SIS, and SIR models for directly-transmitted microparasites (Kilpatrick and Altizer 2010), as they account for worm survival and mortality outside the host (Figure 20). We focused on one critical element of this model that we expected to differ significantly between water sources and dry sites: the transmission of parasites from the environment to hosts. According to the model (Anderson and May 1991, Dobson and Hudson 1992), transmission occurs via the product of W (infective parasites), β (the rate of parasite ingestion), and H (the number of hosts).

Figure 21. Estimation of parasite transmissions using field data
Transmissions (T) were estimated by combining numerous types of data: A) The daily per-capita time spent drinking or grazing per m^2 based on camera trapping data showed that most animals tended to spend more time in areas near water compared to dry sites, but this was especially elevated for elephants and zebra. B) The average parasite density in the environment was estimated from dung and fecal egg counts, and it was notably higher for cattle and elephants compared to dry sites. C) Parasites were more closely shared among bovids (cattle, impala, giraffe, and buffalo) than other species. D) The total number of individuals grazing or drinking was considerably higher for cattle and zebra at water compared to dry sites. (Means ± SEs). Filled pan and dry site values in A and D are shown from the full camera trap dataset; drained values are shown for the subset of months when pans were empty.

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For each herbivore (i) at each of the five water and dry sites (j), we estimated

transmission T, (per unit area) in each context using:

$$T_{ij} = Transmission_{ij} = \beta_{ij} * W_{ij} * H_{ij}$$

Where β is the average daily individual time spent drinking or grazing (per m^2) and W is

the density of parasites (eggs per m^2), and H is the average daily number of hosts drinking or

grazing (per m^2). We made several assumptions by simplifying this equation: 1) daily time

spent drinking or grazing was proportional to the number of bites per day, 2) parasite

development and survival was consistent across sites, 3) the same proportion of susceptible

individuals were found at water and dry sites, and 4) transmission dynamics were fully

density-dependent.

We then used the log ratio of transmission at sites with and without water (either dry site

or drained pan) for each host species (i) and location (j), to determine a 'hotspot effect'

describing the magnitude to which transmission was increased near water:

$$\text{Hotspot Effect}_T = dT_{intraspecific_{ij}} = \ln\left(\frac{T_{water_{ij}}}{T_{dry_{ij}}}\right)$$

5.3.3.2 Question 2: After accounting for parasite sharing among species, how does this

hotspot effect change?

The above equations only compared transmission events within a single host species.

However, many gastrointestinal parasites are shared among species, as shown in Chapter 4.

Therefore, we extended the equation above to incorporate parasite sharing by summing the

contacts that each host species made with potentially infectious parasites of other species

(Webster et al. 2017), and weighting by parasite sharing:

$$T_{interspecific_{ij}} = \sum_{k=1}^{n} H_{ij} * \beta_{ij} * W_{ijk} * \Lambda_{jk}$$

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Where transmissibility is modified by parasite sharing Λ, and where i is the focal host, j is each site, and k is each additional host in the system. By multiplying the sharing matrix by parasite density, we made a further assumption that sharing distances incorporated variation in parasite fecundity from fecal egg counts. In other words, if host A shared a third of its parasites with host B, we assumed that on average, host B would be exposed to a third of host A's parasites. Because different parasite species may exhibit different fecundity levels, we also repeated our analyses using dung volume as the source of exposure, assuming that transmission risk was proportional to dung volume (Appendix 4.1). We found that results were qualitatively similar.

After calculating estimated transmissions using data collected at both filled water sources and dry sites or drained pans, we again compared the change in the transmissions in each of these contexts to find a hotspot effect:

$$\text{Hotspot Effect}_\text{T} = dT_{interspecific_{ij}} = \ln\left(\frac{T_{water_{ij}}}{T_{dry_{ij}}}\right)$$

Finally, we calculated the difference in this hotspot effect to determine the degree to which accounting for multiparasite sharing changed results:

$$\Delta \text{Hotspot Effect} = dT_{interspecific_{ij}} - dT_{intraspecific_{ij}}$$

5.3.4 Statistical Analyses:

5.3.4.1 Question 1: Are intraspecific parasite transmissions elevated near water sources compared to dry sites?

We created a linear mixed-effects model (LMM) of the log ratio of transmissions for water and dry sites or drained pans (hotspot effect), using location (n=5) as a random effect and host species as a main effect. We then performed post-hoc comparisons for the different

species, testing whether their means significantly differed from zero and adjusting for multiple comparisons using false discovery rate (FDR) estimation.

5.3.4.2 Question 2: After accounting for parasite sharing among species, how does this hotspot effect change?

We created an identical LMM to model the hotspot effect across species after accounting for interspecific parasite contacts. We again performed FDR-adjusted post-hoc tests of means that differed significantly from zero. To investigate the effect of incorporating parasite sharing on the change in transmissions at water relative to dry sites, we created another LMM of the pairwise differences in hotspot effect, using host species as a main effect and location (n=5) as a random effect. We performed FDR-adjusted post-hoc tests to determine whether these pairwise differences significantly differed from zero for each species.

All models were fit using the *lmerTest* package (Kuznetsova et al. 2017) and post-hoc tests were performed using *emmeans* (Lenth 2020). Analyses were performed in R version 4.0.1 (R Core Team 2016).

5.4 Results

5.4.1 Question 1: *Are water sources hotspots of parasite transmission rates?*

5.4.1.1 Filled pans vs. dry sites: We found that cattle and elephants had significantly elevated (~700 and 10,000 times higher respectively) estimated parasite transmissions per m^2 at water relative to dry sites (p=0.04 and p=0.008 respectively) (Table 10). Water sources were not significant parasite transmission hotspots for buffalo, giraffe, zebra, and impala when these hosts were considered alone, although rates trended higher near water for all species except giraffe. Given that all animals tended to spend a greater amount of time either

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drinking or grazing near water relative to dry sites (Figure 21A), this was likely to be driven by differences in dung and parasite density in these locations.

Table 10. Post-hoc tests of hotspot effects across species
Tests on the intra- and inter- specific LMMs for the effect of water sources in increasing parasite transmissions (hotspot effect).

Species	Test	Hotspot Effect$_T$	SE	df	Lower CI	Upper CI	T-ratio	P$_{adj}$	
Observational Comparison (Dry Site vs. Filled)									
Buffalo	Intra	3.422	2.071	20	-0.897	7.741	1.653	0.137	
	Inter	5.300	1.820	20	1.490	9.110	2.904	**0.013**	*
Cow	Intra	6.530	2.071	20	2.211	10.850	3.154	**0.015**	*
	Inter	6.330	1.820	20	2.530	10.140	3.470	**0.007**	*
Elephant	Intra	9.174	2.071	20	4.854	13.493	4.430	**0.002**	*
	Inter	9.190	1.820	20	5.390	13.000	5.039	**<0.001**	**
Giraffe	Intra	-1.087	2.413	20	-6.122	3.947	-0.450	0.657	
	Inter	3.040	2.090	20	-1.330	7.400	1.452	0.162	
Impala	Intra	3.504	2.071	20	-0.815	7.824	1.692	0.137	
	Inter	4.840	1.820	20	1.030	8.640	2.651	**0.018**	*
Zebra	Intra	5.489	2.071	20	1.169	9.808	2.651	**0.031**	*
	Inter	5.590	1.820	20	1.790	9.400	3.066	**0.012**	*
Experimental Comparison (Filled vs. Drained)									
Buffalo	Intra	2.028	1.259	14	-0.672	4.727	1.611	0.194	
	Inter	2.494	1.070	14	0.208	4.780	2.340	0.052	.
Cow	Intra	3.452	0.898	14	1.525	5.379	3.842	**0.011**	*
	Inter	3.411	0.900	14	1.481	5.340	3.791	**0.012**	*
Elephant	Intra	3.535	1.133	14	1.104	5.965	3.119	**0.023**	*
	Inter	3.632	1.100	14	1.276	5.990	3.307	**0.016**	*
Giraffe	Intra	-0.518	1.770	14	-4.315	3.279	-0.293	0.774	
	Inter	0.773	1.330	14	-2.080	3.630	0.581	0.570	
Impala	Intra	-0.734	1.963	14	-4.944	3.476	-0.374	0.774	
	Inter	1.039	1.440	14	-2.058	4.140	0.720	0.570	
Zebra	Intra	3.320	1.219	14	0.705	5.935	2.723	**0.033**	*
	Inter	3.224	1.160	14	0.736	5.710	2.779	**0.030**	*

5.4.1.2 Filled pans vs. drained pans: Our experimental results showed similar patterns to filled pans and dry site comparisons, but to a smaller extent. Cattle, elephants, and zebra had

elevated (~30, 45, and 35 times higher respectively) estimated daily parasite transmissions per m^2 at filled pans relative to drained pans, but this was only marginally significant after adjusting for multiple comparisons (p=0.065). There were no significant differences in risk for other animals during the experiment, nor for any animal either pre- or post- experiment (See Appendix 4.1 for pre- and post- results).

Figure 22. Hotspot effect of water sources after accounting for parasite sharing
The estimated degree to which parasite transmissions were elevated at water relative to dry sites per unit time and area was substantially higher for all species except giraffe (mean effect ± 95% CI plus FDR-adjusted p-value for difference from 0).

5.4.2 Question 2: *After accounting for parasite sharing among species, how does this hotspot effect change?*

5.4.2.1 Filled pans vs. dry sites: After accounting for parasite sharing among host species, all animals except giraffes had significantly heighted estimates of parasite transmissions near water sources relative to dry sites (Figure 22). Elephants again had the greatest hotspot effect, and after considering parasite sharing, water sources became a newly significant transmission hotspot for buffalo, zebra, and impala, with transmissions per unit area near water averaging ~200, 250, and 125-time that of dry sites respectively (p=0.03, p=0.03, and p=0.04). The effect of considering additional species in changing the degree to which transmission

estimates were increased near water was significantly elevated for buffalo, giraffe, and impala (Figure 23, Table 11); this hotspot effect was ~4 times greater for impala (p=0.01) and ~6.5 times greater for buffalo (p<0.001) when accounting for parasite sharing with other species compared to intraspecific transmissions only. While the hotspot effect was more than 60 times greater for giraffe after accounting for sharing (p<0.001), it was still comparable between water and dry sites because intraspecific giraffe transmission estimates trended lower at water relative to dry sites. Cattle were the greatest contributors to these changes because they had the most parasite community overlaps with these other animals (Figure 24), and they had very high relative parasite density near water compared to dry sites (Figure 21B).

5.4.2.2 Filled pans vs. drained pans: Accounting for parasite sharing significantly increased estimates of parasite transmissions near water sources relative to drained pans for giraffe and impala by approximately 3.5 and 6-fold, respectively (p=0.02, p=0.001). Despite these changes, total transmissions were not significantly higher at filled pans relative to drained pans for these animals, although estimates were centered 2-3 times greater with high variance (Table 11). After accounting for parasite sharing transmissions for all animals were estimated to be higher near filled pans relative to drained pans, but this was only significant for cows, elephants, and zebra.

Table 11. Post-hoc tests of the change in hotspot effects among species
Tests on the LMM describing the change in estimated hotspot effect after considering interspecific parasite sharing.

Species	Δ Hotspot EffectT	SE	df	Lower CI	Upper CI	T-ratio	Padj	
Observational Comparison (Dry Site vs. Filled)								
Buffalo	1.877	0.374	20	1.097	2.658	5.018	**<0.001**	***
Cow	-0.199	0.374	20	-0.979	0.581	-0.532	0.901	
Elephant	0.020	0.374	20	-0.760	0.800	0.054	0.958	
Giraffe	4.123	0.479	20	3.124	5.121	8.613	**<0.001**	***
Impala	1.333	0.374	20	0.553	2.114	3.563	**0.004**	**
Zebra	0.105	0.374	20	-0.675	0.886	0.282	0.937	
Experimental Comparison (Filled vs. Drained)								
Buffalo	0.459	0.425	19	-0.431	1.349	1.079	0.588	
Cow	-0.041	0.380	19	-0.837	0.756	-0.107	0.916	
Elephant	0.081	0.425	19	-0.809	0.972	0.191	0.916	
Giraffe	1.294	0.425	19	0.403	2.184	3.041	**0.020**	*
Impala	1.774	0.380	19	0.977	2.570	4.661	**0.001**	**
Zebra	-0.096	0.380	19	-0.892	0.701	-0.251	0.916	

Figure 23. Hotspot changes after accounting for interspecific parasite sharing
Visualization of the change in hotspot effect (the degree to which transmission estimates were elevated at water relative to dry sites) after accounting for interspecific parasite sharing across each of our sites. Positive effects are shown in blue, and negative effects are shown in red. Accounting for interspecific parasite sharing significantly increased the hotspot effect for buffalo, giraffe, and impala, while other species remained unchanged.

Figure 24. Parasite transmissions contributed by each focal host species
The estimated maximum number of transmissions (assuming density-dependent transmission and no parasite mortality in the environment) contributed by each host species (95% CIs) to each focal species (panels) included in the study. For non-bovids (elephants and zebra) that shared fewer parasites with other animals, most transmissions were intraspecific. For bovids, most transmissions were estimated to arise from cattle. The sum of all contacts in each location provided an estimate of total transmission (T).

5.5 Discussion

Together, our results highlight the degree to which water sources may increase parasite transmission risk and emphasize the importance of considering the broader host community in assessing disease risk across space. Comparisons between permanent water sources and dry sites showed substantial increases in transmission risk at water for most species, and our experimental results also supported this, albeit with weaker results. However, both sets of findings illustrate that accounting for parasite sharing among hosts is especially important when a highly abundant animal that heavily aggregates near shared resources also shares its parasites with other animals in the system. In our study, cattle drove changes in parasite transmission risk for other wild bovids around water sources. Given that cattle are

increasingly replacing wildlife in many systems (Hempson et al. 2017), it is important to consider ways in which their management can affect parasitism in wildlife.

Other studies have also underscored the importance of cattle management for managing disease risk for wildlife that share parasites. For example, by spraying cattle with acaricides humans reduced landscape-level tick abundance (Keesing et al. 2013, 2018), lowering disease risk for a wide range of wildlife that were parasitized by the same ticks and their pathogens. This outcome is particularly promising for efforts to promote cattle and wildlife co-existence, especially amid growing human wildlife conflicts and the need to both conserve wildlife and maintain economically feasible solutions for human communities (Homewood et al. 2012, Keesing et al. 2018). While our findings show that in the case of cattle with untreated gastrointestinal helminth infections, wildlife transmissions likely increase, clearing these infections would likely render this effect negligible. However, rising concern that the of broadscale use of antihelminthics has led to drug-resistant parasites cautions against 'global worming'(Kaplan and Vidyashankar 2012), and new studies highlighting the unexpected benefits that parasites provide to hosts have prompted a global conservation plan for wildlife parasites (Carlson et al. 2020). While reducing parasite infections in cattle are unlikely to alter transmission risk near water relative to background areas, altering their movement and behavior around water likely will. For example, grazing livestock in areas that are further from water may reduce dung and parasite density at these sites.

Our findings also highlight the marked species-specific differences in host behavior and parasite density that contribute to parasite transmission. One of the most obvious driving forces for animal aggregations at water is host-specific water requirements. Indeed, we found

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that per-capita time spent at water was especially elevated relative to background areas for elephants, a highly water-dependent mammal (Chamaillé-Jammes et al. 2013). Giraffe and impala, on the other hand, showed little difference in time spent at water relative to dry sites; one reason for this may be that they require relatively less water than other species in our study (Estes 2012). Several additional external factors likely drive variation in host behavior around water, such as foraging requirements, fear of predators, and parasite avoidance. In particular, browsing species like giraffe likely have very few overall contacts with parasites because they consume forage high in trees, which are often reduced in the inner area closest to water (Thrash and Derry 1999) (Chapter 2). However, ingesting water contaminated with feces may increase risk if infectious larvae, eggs, oocysts etc. are able to survive at the water's surface. In contrast, grazing species such as zebra, buffalo, and cattle may spend more time near water if animal activity promotes highly attractive grazing lawns near water, but this can depend on herbivore density and soil and precipitation gradients (Chapter 2). Predators can also influence host behavior at water sources via a landscape of fear. Smaller animals such as impala may avoid water if it elevates risk of predation (Crosmary et al. 2012). Indeed, our camera traps indicated substantially elevated carnivore detections near water relative to dry sites (Appendix 4.2). Finally, recent studies have shown that hosts may successfully avoid parasite infection if they can detect parasites in the environment (Weinstein et al. 2018, Buck et al. 2018). Examples of this "landscape of disgust" have been noted in dik-dik that avoid feces when feeding (Ezenwa 2004b), and in elephants and lemurs that avoid heavily-contaminated water sources (Ndlovu et al. 2018, Amoroso et al. 2019).

This study is one of the first attempts to quantify the effect of water sources as transmission hotspots relative to background areas, as measuring variation in contact rates

with parasites in the environment is extremely challenging (Paull et al. 2012, Craft 2015). Therefore, disease models are critically important for understanding the dynamics of environmentally transmitted parasites. While constructing entire host and parasite population models was outside the scope of this study, our findings that 1) water sources contribute substantial heterogeneity to parasite transmissions and 2) incorporating interspecific parasite sharing can modify the hotspot effect of water sources, will be useful for informing models that more explicitly account for variation in risk across space, just as variation in host-host contact rates can change modelling outcomes for directly-transmitted pathogens (Lloyd-Smith et al. 2005, Paull et al. 2012).

There are several important additional considerations for this study. Firstly, we made the important assumption that the degree to which parasites are shared among hosts is proportional to the transmissibility of those parasites among hosts. While we believe that this is a reasonable assumption in the absence of additional data, there is much individual variability in transmission probability which can have substantial implications for disease outcomes (Lloyd-Smith et al. 2005). Additionally, we did not consider the effect of parasite removal from the environment in this study. Because gastrointestinal worms release many thousands of eggs that then produce infective larvae, we assumed that parasite removal via grazing would not substantially reduce the number of infective parasites remaining in the environment. However, additional models may show that accounting for parasite removal is important, especially if animal foraging densities are exceptionally high. For many parasites that have a limited number of infectious stages in the environment, the per-capita infection risk may be decreased when hosts aggregate, despite overall increases in transmission success (Buck et al. 2017). This may indeed be the case for tick-borne diseases, as high host

density near water may reduce risk as fewer infectious stages are left to infect additional hosts. Indeed, supplementary tick drags performed concurrent to this study indicated reduced numbers of questing ticks around water sources (Appendix 4.4).

In addition to host immunological constraints on parasite infection, parasite-parasite interactions within hosts may also be important determinants of transmission success. While we did not consider these dynamics in this study, they may be important in parasite epidemiology (Karvonen et al. 2019). Finally, we did not account for seasonal dynamics in host or parasite behavior and survival. Seasonality can alter host use of water (Valeix 2011) (Chapter 3), parasite burdens and output in hosts (Ezenwa 2004a, Ndlovu et al. 2009), and parasite survival in the environment (Pullan and Brooker 2012). Consideration of these parameters is an important next step in understanding the role of environmental hotspots in elevating parasite transmission, especially amid global aridification and climate changes.

5.6 Conclusions

Our findings illustrate that after accounting for animal activity, parasite density in the environment, and parasite sharing among different host species, water sources may markedly increase parasite transmission relative to background areas. Our results further emphasize that parasite sharing among sympatric hosts can be critical for understanding transmission risk within a heterogeneous landscape. Our finding that cattle were highly abundant, aggregated strongly at water, and shared parasites with other herbivores in the system suggests that they can act as important drivers of parasite infections in other wild bovids and giraffe, depending on the parasite in question. While additional modelling and consideration of the seasonality and parasite mortality is needed to understand overall infection dynamics,

these findings have important implications for livestock management in areas where resources are shared with wildlife.

Coauthors and contributions:

Jenna Hulke[1,2] assisted in coordinating and executing all aspects of fieldwork and lab work, in addition to John Mantas[2], who performed countless dung identifications and surveys. Benard Gituku[3] facilitated all work and contributed to experimental design at Ol Pejeta Conservancy, while Hillary Young[2,4] collaborated on experimental design and interpretation of findings.

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Appendices

Appendix 1. Chapter 2

Appendix 1.1. Soil analyses

Section 1.1.1 Soil classification using linear discriminant analysis (LDA)

Given the large number of differences in soil variables, and the uncertainty as to which variables might best moderate the effects of water on vegetation properties, we classified soils *apriori* into two groups – red sand and black silt/clay soils using a soil map from Franz 2010. We used linear discriminant analysis (LDA) to explore variables that most clearly differentiated between these soil types and to examine support for this grouping. Because soils in the immediate vicinity of water tended to have different chemical properties than dry sites or 50m from water (Table A1.1.2), we excluded the 17 soil samples collected 0m from water to build the model (therefore, total n=34). First, we checked for univariate normality and equal variance among groups for each soil type. We applied transformations to ensure these assumptions were met (noted in Table A1.1.2). We performed LDA using JMP Pro 13.1 (SAS, Cary, NC), followed by forward stepwise variable selection using sequential F-tests (additional terms that improved the model with $p<0.05$ were included). While the model including all soil variables successfully discriminated between the groups (with no misclassifications; Figure A1.1.1, Table A1.1.2), we found that a combination of only four variables were needed to discriminate between red sand and black silt/clay soils with 97%

accuracy (one misclassification). These were: manganese, % silt, iron, and copper (Figure
A1.1.1).

In the reduced model, one sample was misclassified. Upon further investigation, this
sample was found to lie very close to the geographic boundary between these soil types (less
than 1km). Given that the LDA showed a reasonably discrete grouping of these two soil
types, we chose not to define a third 'transition' group, as it would introduce additional
complexity with limited explanatory improvement. We also noted model performance in
classifying the 17 samples collected near water sources that were not used to build the model;
all but one sample was classified correctly, while three were misclassified using the full
model (Table A1.1.1).

Figure A1.1.1 Canonical plot of sample scores within each soil type grouping for full (A) and
reduced (B) models.

Table A1.1.1 Classification results for full and reduced models from LDA

Model	Number of terms	Data Subset	Count	Number misclassified	Percent misclassified
Full	11	Dry site & 50m	34	0	0
		Water 0 m	17	3	17.6
Reduced	4	Dry site & 50m	34	1	2.9
		Water 0 m	17	1	5.9

Section A1.1.2 Differences in soil properties at water sources across soil types

To compare differences in soil properties at water sources versus dry sites, we constructed linear mixed-effect models (LMMs) and performed post-hoc comparisons of group means for each variable. We used soil type (black silt/clay or red sand), distance (0m from water, 50m from water, or control), and their interaction as fixed effects and location (n=17) as random effects. We checked for normality of residuals following regression and performed appropriate transformations in ensure equal variance among groups. We determined parameter significance using stepwise selection using the lmerTest package in R

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to identify the top model for each measurement. When effects were significant, we performed post-hoc comparisons with Tukey's method of adjustment for multiple comparisons (Table A1.1.2).

Table A1.1.2 Summary of least-square means and 95% confidence intervals for soil properties. Results are grouped by the combination of fixed-effect terms included in the best model. Significantly different means are denoted by letters where relevant (lowest value corresponds to "a") and higher means are shaded darker. Transformations applied prior to analyses are indicated in parentheses below each variable heading.

Soil Type	Location	Fixed Effects = Soil Type + Distance + Soil Type:Distance			
		Organic Matter (none)	Bray II P (log)	Fe (inverse)	Al (none)
Silt/clay	Water 0	4.13 ab (3.35-4.92)	16.79 bd (11.11-25.40)	86.9 b (74.3-104.6)	547.1 ab (489.2-605.0)
	Water 50	4.78 b (3.99-5.56)	16.33 abc (10.80-24.70)	62.5 a (55.8-71.3)	602.7 bc (544.8-660.6)
	Dry Site	4.90 b (4.12-5.69)	7.94 a (5.25-12.00)	72.8 ab (63.7-84.8)	678.2 c (620.3-736.1)
Sand	Water 0	2.70 a (1.96-3.44)	38.33 ce (25.68-57.20)	153.6 c (119.8-213.9)	472.4 a (417.0-527.7)
	Water 50	3.43 ab (2.69-4.17)	37.28 de (24.98-55.63)	79.6 ab (69.4-93.2)	527.9 ab (472.6-583.3)
	Dry Site	2.71 a (1.97-3.45)	18.11 bd (12.14-27.03)	71.1 ab (55.8-71.3)	603.5 bc (548.1-658.9)

Soil Type	Fixed Effects = Soil Type						
	Total Exchange (none)	Ca (log)	Mg (none)	K (none)	Mn (none)	% Silt (none)	% Sand (none)
Silt/clay	14.99 a (11.81-18.16)	2549 b (2077-3126)	507.2 b (435.9-578.5)	808.0 b (711.9-904.1)	275.5 b (242.6-308.4)	43.0 b (38.1-47.8)	49.5 a (43.9-55.2)
Sand	21.39 b (18.02-24.76)	1592 a (1313-1931)	393.2 a (326.0-460.5)	648.2 a (557.6-738.9)	162.7 a (131.6-193.8)	26.0 a (21.4-30.6)	63.8 b (58.5-69.2)

Location	Fixed Effects = Distance		
	S (inverse)	Na (inverse)	B (log)
Water 0	15.71 c (12.92-20.03)	40.7 b (32.4-54.6)	0.52 b (0.44-0.63)
Water 50	10.60 b (9.25-12.41)	27.6 a (23.5-33.4)	0.48 ab (0.40-0.58)
Dry Site	8.55 a (7.65-9.68)	26.0 a (22.4-31.0)	0.41 a (0.34-0.49)

Fixed Effects= none			
Zn (log)	Cu (log)	pH (none)	% Clay (none)
2.57 (2.28-2.86)	1.65 (1.55-1.74)	6.85 (6.73-6.98)	8.9 (7.3-10.5)

Appendix 1.2. LMM for truncated understory height data

Since maximum understory height was truncated at 500mm for the first two sampling seasons, and not the latter two seasons, we truncated all data at 500mm and reran the analysis to ensure that sampling differences did not affect model outcomes. Table A1.2.1 shows a side-by-side comparison of models built using the original data (left) and truncated data (right). Results are very similar.

Table A1.2.1 Comparison models for differences in understory height using either original data (left), or data truncated to 500mm (right).

Predictors	Original Data: Δ Height (mm)				Truncated Data: Δ Height (mm)			
	Est.	*CI*	*Statistic*	*p*	*Est.*	*CI*	*Statistic*	*p*
(Intercept)	-57.94	-104.57 – -11.30	-2.44	**0.031**	-50.68	-88.64 – -12.72	-2.62	**0.018**
Outward Distance	0.14	0.01 – 0.27	2.18	**0.030**	0.14	0.03 – 0.25	2.43	**0.015**
Silt/clay Soil	-60.33	-110.38 – -10.28	-2.36	**0.030**	-53.66	-99.08 – -8.24	-2.32	**0.033**
30-day Prior Rain	2.01	0.46 – 3.56	2.55	**0.011**	1.73	0.37 – 3.08	2.50	**0.013**
Distance: Silt/clay Soil	0.36	0.17 – 0.55	3.76	**<0.001**	0.30	0.14 – 0.47	3.61	**<0.001**
Random Effects								
σ^2	9506.53				7314.48			
$\tau_{00\ site}$	2464.17				2045.19			
$\tau_{00\ season}$	963.58				433.47			
Observations	2077				2077			
Marginal R^2	0.043				0.046			
Conditional R^2	0.296				0.287			

Appendix 1.3. Measurements of herbivore abundance

Section A1.3.1 Dung Surveys

For each herbivore type, we calculated total dung pile count at water sources and dry sites across all sampling periods and locations (n=102 per herbivore group) We modeled dung counts (summed across each site) by herbivore type, water status, and soil type using a generalized linear mixed effects model with a Poisson distribution. We included location (n=17) as a random effect and tested significance of interactions using X^2 tests of nested models.

Table A1.3.1 Herbivore dung counts at water sources and dry sites for each guild and soil type.

Herbivore	Site Type	Soil	N	Mean Count	SD	SE	95% CI
Browser	Dry	mesic silt/clay	8	11.63	2.97	1.05	2.49
		arid sand	12	11.50	9.10	2.63	5.78
	Water	mesic silt/clay	8	12.00	5.61	1.98	4.69
		arid sand	12	14.75	11.01	3.18	7.00
Mixed	Dry	mesic silt/clay	8	10.50	3.30	1.16	2.75
		arid sand	12	20.92	13.74	3.97	8.73
	Water	mesic silt/clay	8	22.38	6.46	2.28	5.40
		arid sand	12	39.58	26.74	7.72	16.99
Grazer	Dry	mesic silt/clay	8	25.38	8.90	3.14	7.44
		arid sand	12	19.92	10.97	3.17	6.97
	Water	mesic silt/clay	8	39.63	13.92	4.92	11.64
		arid sand	12	37.75	21.21	6.12	13.47

Table A1.3.2 Coefficients and estimates for the best Poisson GLMM of herbivore dung counts. The intercept corresponds with browser dung counts at dry sites with mesic silt/clay soils.

Predictors	Estimate	SE	95% CI	Statistic	p
			Dung Counts (96m^2)		
(Intercept)	2.37	0.24	1.90 – 2.85	9.77	**<0.001**
Site Type [Water]	0.17	0.09	-0.01 – 0.34	1.87	0.061
Herbivore [Mixed]	0.03	0.12	-0.20 – 0.25	0.24	0.809
Herbivore [Grazer]	0.78	0.1	0.58 – 0.99	7.49	**<0.001**
Soil [Arid sand]	-0.12	0.31	-0.73 – 0.48	-0.4	0.689
Site Type [Water]: Herbivore [Mixed]	0.5	0.11	0.28 – 0.72	4.5	**<0.001**
Site Type [Water]: Herbivore [Grazer]	0.39	0.11	0.18 – 0.60	3.62	**<0.001**
Herbivore [Mixed]:Soil [Arid sand]	0.5	0.12	0.28 – 0.73	4.33	**<0.001**
Herbivore [Grazer]:Soil [Arid sand]	-0.23	0.11	-0.44 – -0.01	-2.07	**0.038**
Random Effects					
σ^2	0.05				
$\tau_{00 \ Site}$	0.41				
ICC $_{Site}$	0.89				
Observations	120				
Marginal R^2 / Conditional R^2	0.317 / 0.926				

Table A1.3.3 Type II ANOVA table for best model of dung counts. The three-way interaction was not significant ($\chi_2^2 = 3.04$; p=0.21), and the interaction between Soil and Water/Dry site was not significant ($\chi_1^2 = 1.52$, p=0.22).

Source	X^2	Df	Pr(>Chisq)	
Site Type	168.90	1	< 0.001	***
Herbivore	272.39	2	< 0.001	***
Soil	0.06	1	0.8062	
Site Type:Herbivore	20.92	2	< 0.001	***
Herbivore:Soil	62.88	2	< 0.001	***

Table A1.3.4 Post-hoc tests of dung counts averaged over levels of soil type (Tukey adjustment for comparison of 6 estimates).

Herbivore	Site Type	Count	SE	df	Lower CI	Upper CI	Group
Browser	Dry	10.15	1.64	Inf	7.40	13.93	A
Browser	Water	11.85	1.89	Inf	8.67	16.19	AB
Mixed	Dry	13.56	2.14	Inf	9.95	18.47	B
Grazer	Dry	19.81	3.06	Inf	14.64	26.81	C
Mixed	Water	25.84	3.95	Inf	19.15	34.88	D
Grazer	Water	34.28	5.18	Inf	25.48	46.10	E

Section A1.3.2 Camera Traps

During the interval from April to August 2017, we placed one camera trap at each water source and paired dry site for at least one week (n=34 deployments total). Of these deployments, 12 sites ran uninterrupted for a minimum of one week at both water and dry sites simultaneously (n=24 deployments; 387 trap nights total). Images were classified by counting the number of animals of a given species for each "trigger," which consisted of all images taken within a five minute interval (as in (Thorn et al. 2009)). We calculated the mean total number of herbivores of each guild (grazer, mixed, and browser) per day for each deployment. We ran a generalized linear mixed effects model with a negative binomial distribution using count as the response and type (water/dry site), soil (mesic silt/clay vs. arid sand), and herbivore (browser, mixed, or grazer) as fixed effects and site as a random effect, testing significance of all interactions using X^2 tests of nested models.

Table A1.3.5 Summary of camera trap deployments across sites at Mpala.

Water/ Dry	Site	Camera Model	Begin Date & Time	End Date & Time	Trap Nights
Water	1	Reconyx HC500 Hyperfire	4/27/2016 9:20	5/18/2016 9:39	21.01
Dry	1	Reconyx HC500 Hyperfire	4/27/2016 12:16	5/18/2016 10:02	20.91
Water	2	Reconyx HC500 Hyperfire	6/30/2016 16:10	7/19/2016	18.72
Dry	2	Reconyx HC500 Hyperfire	6/30/2016 16:27	7/19/2016	18.72
Water	5	Reconyx HC500 Hyperfire	8/9/2016	8/22/2016	12.90
Dry	5	Reconyx HC500 Hyperfire	8/9/2016 15:27	8/22/2016 12:52	12.89
Water	6	Reconyx HC500 Hyperfire	8/22/2016	8/30/2016 17:05	8.15
Dry	6	Reconyx HC500 Hyperfire	8/9/2016	8/22/2016 13:18	12.93
Water	7	Reconyx HC500 Hyperfire	6/7/2016 16:07	6/30/2016 15:05	22.96
Dry	7	Reconyx HC500 Hyperfire	6/7/2016 15:43	6/30/2016 14:56	22.97
Water	10	Reconyx RM45 Rapidfire	6/6/2016 11:40	6/16/2016 13:27	10.07
Dry	10	Reconyx RM45 Rapidfire	6/6/2016 12:00	6/27/2016 11:12	20.97

Water	12	Moultrie MCG-M880	7/2/2016 9:56	7/19/2016 11:34	17.07
Dry	12	ScoutGuard 860C	4/4/2016 12:22	4/22/2016 8:27	17.84
Water	13	Reconyx HC500 Hyperfire	6/7/2016 12:48	6/30/2016 12:32	22.99
Dry	13	Reconyx HC500 Hyperfire	6/7/2016 12:27	6/30/2016 12:44	23.01
Water	14	Moultrie MCG-M880	8/22/2016	8/30/2016 14:50	8.18
Dry	14	Reconyx HC500 Hyperfire	8/22/2016	8/30/2016 14:51	8.20
Water	15	Reconyx HC500 Hyperfire	4/26/2016	5/18/2016 11:49	21.90
Dry	15	Reconyx HC500 Hyperfire	4/26/2016	5/7/2016 14:36	11.03
Water	16	Reconyx HC500 Hyperfire	7/19/2016 13:10	8/9/2016 12:41	20.98
Dry	16	Reconyx HC500 Hyperfire	7/19/2016 13:37	8/9/2016 12:43	20.96
Water	17	Reconyx HC500 Hyperfire	6/30/2016 13:58	7/11/2016 23:01	11.38
Dry	17	Reconyx HC500 Hyperfire	7/1/2016 2:47	7/19/2016 12:14	18.39

Table A1.3.6 Summary of average daily camera trap sightings of individuals of each herbivore type at water sources and dry sites for each soil type.

Herbivore	Site Type	Soil	N	Daily sightings	SD	SE	95% CI
Browser	Dry	Mesic silt/clay	5	0.04	0.04	0.02	0.05
		Arid sand	7	0.08	0.09	0.03	0.08
	Water	Mesic silt/clay	5	0.10	0.14	0.06	0.18
		Arid sand	7	0.21	0.28	0.11	0.26
Mixed	Dry	Mesic silt/clay	5	0.21	0.21	0.09	0.26
		Arid sand	7	0.67	1.11	0.42	1.02
	Water	Mesic silt/clay	5	0.64	0.24	0.11	0.30
		Arid sand	7	2.20	2.85	1.08	2.64
Grazer	Dry	Mesic silt/clay	5	0.29	0.27	0.12	0.33
		Arid sand	7	0.58	0.64	0.24	0.60
	Water	Mesic silt/clay	5	1.26	1.03	0.46	1.28
		Arid sand	7	1.12	0.69	0.26	0.64

Table A1.3.7 Coefficients and estimates for the best Negative-Binomial GLMM of herbivore camera trap sightings. The intercept corresponds to browser sightings at dry sites with mesic silt/clay soils.

Predictors	Log Mean	std. Error	95% CI	Statistic	p
			Daily Camera Sightings		
(Intercept)	-3.30	0.73	-4.72 – -1.87	-4.54	**<0.001**
Site Type [Water]	1.06	0.35	0.37 – 1.74	3.02	**0.003**
Herbivore [Grazer]	1.99	0.66	0.70 – 3.27	3.02	**0.003**
Herbivore [Mixed]	2.17	0.65	0.90 – 3.44	3.34	**0.001**
Soil [Arid sand]	0.63	0.40	-0.15 – 1.40	1.56	0.11
Random Effects					
σ^2	1.03				
$\tau_{00 \; Site}$	0.10				
ICC_{Site}	0.09				
Observations	72				
Marginal R^2 / Conditional R^2	0.55 / 0.59				

Table A1.3.8 Type II ANOVA table for best model of camera trap sightings. No interactions were significant.

Source	X^2	Df	Pr(>Chisq)	
Site Type	9.12	1	0.003	**
Herbivore	11.21	2	0.004	**
Soil	2.53	1	0.11	

Table A1.3.9 Post-hoc tests of camera trap sightings averaged over levels of soil type (Tukey adjustment for comparison of 6 estimates).

Site Type	Herbivore	Mean Daily Sightings	SE	df	Lower CI	Upper CI	Group
Dry	Browser	0.05	0.03	65	0.01	0.20	A
Water	Browser	0.15	0.09	65	0.04	0.52	B
Dry	Mixed	0.44	0.16	65	0.21	0.92	B D
Water	Mixed	1.28	0.36	65	0.73	2.22	C E
Dry	Grazer	0.37	0.14	65	0.17	0.78	BC
Water	Grazer	1.06	0.31	65	0.59	1.91	DE

Finally, to assess the degree to which our dung and camera trap data agreed with each other, we ran a Spearman's rank correlation test on dung and camera counts matched by herbivore type, site, and treatment (n=72).

Figure A1.3.1 Dung counts and camera trap sightings at the site level were correlated across all soil and herbivore types (Spearman's rho = 0.52, p<0.001).

Appendix 1.4. Herbivore dung density as a covariate

Models from the main text use distance from water as a proxy for herbivore aggregation at water sources. To examine the relationship between herbivore dung density, distance from water, and plant cover, we provide two sets of analyses. First, we report the correlation of total dung density (counts from all herbivores, see Appendix 1.3 for a breakdown by herbivore guild) with outward distance for water sources and dry sites (Section 1). Second, after aggregating total herbivore dung density across periods as a measure of long-term herbivore impacts, we reanalyzed non-differenced plant cover and diversity data using herbivore dung counts as a covariate in linear mixed models (Section 2).

Section A1.4.1 The relationship between total dung density and distance from water

Figure A1.4.1: Total herbivore dung counts were highly dependent on distance from water on both soil types.

Table A1.4.1 LMM estimates for a model of total herbivore dung counts (per 6m^2) (averaged over periods and within distance bins). While dung counts were slightly elevated on arid sandy soils compared to mesic silt/clay soils, this difference was less significant than parameters describing distance from water.

Predictors	Estimates	std. Error	CI	T-value	p
(Intercept)	0.12	0.02	0.09 – 0.15	7.04	**<0.001**
Site Type [Water]	0.15	0.02	0.11 – 0.20	7.27	**<0.001**
Distance [50-95]	0.01	0.02	-0.03 – 0.04	0.44	0.661
Distance [100-145]	0.00	0.02	-0.03 – 0.04	0.26	0.793
Soil [Sand]	0.04	0.02	0.00 – 0.07	2.02	**0.044**
Site Type [Water] * Distance [50-95]	-0.14	0.03	-0.18 – -0.09	-5.37	**<0.001**
Site Type [Water] * Distance [100-145]	-0.13	0.03	-0.18 – -0.08	-5.04	**<0.001**
Site Type [Water] * Soil [Sand]	0.04	0.02	0.00 – 0.09	2.16	**0.031**
Random Effects					
σ^2	0.00				
$\tau_{00\ \text{Site}}$	0.00				
Observations	96				
Marginal R^2 / Conditional R^2	0.623 / 0.678				
Marginal R^2 / Conditional R^2 for model fit with only Site Type x Distance	0.500 / 0.661				

Section A1.4.2 Plant analyses using herbivore dung density as a covariate

We refit all models using non-differenced plant cover and diversity data with herbivore dung density as a covariate. Parameters that significantly interacted with Site Type [Water] largely aligned with parameters that were significant using differenced data. Models were largely similar to those reported in the main text. Grass cover was lower at water sources compared to dry sites on mesic silt/clay soils, but the opposite effect was true for trees (Figure A1.4.2). Forb cover was strongly influenced by rainfall, and bare ground cover increased with increasing herbivore dung density on mesic silt/clay soils, but levelled-out on arid sand, where grass cover tended to increase. In several instances, herbivore dung density explained much of the same variation as outward distance from water, with model R^2 values changing minimally when outward distance was replaced by only herbivore dung density.

Table A1.4.2 LMM estimates for grass, forb, tree, and bare ground cover. Parameters positively associated with vegetation near water have solid borders, while those associated with declines have dotted borders. Parameters that agree with those in the main text are shaded in green, while those that are newly significant (and not in differenced data) are shaded in yellow. Significant parameters (not tested in the main text) are shaded in blue, and baseline and insignificant parameters remain unshaded. Intercepts for models correspond to inner rings on sandy soils. We also provide R^2 values models fit with only herbivore dung density or outward distance from water.

Predictors	Grass		Forb		Tree		Bare Ground		Height	
(Intercept)	0.66	14.2	0.09	3.69	0.29	6.93	0.31	5.64	212.50	5.39
	0.05	***	0.02	***	0.04	***	0.05	***	39.45	***
Site [Water]	-0.02	-1.03	-0.08	-6.1	-0.11	-4.48	0.07	1.13	-80.06	-11.22
	0.02	0.31	0.01	***	-0.03	***	0.06	0.26	7.13	***
Rain	-0.01	-0.67	0.18	13.14	0.00	0.22	-0.04	-1.73	-5.98	-0.96
	0.02	0.501	0.01	***	0.02	0.82	0.02	0.08	6.25	0.338
Rain * Site [Water]	0.08	3.06			0.05	2.54	-0.05	-2.06	37.65	4.88
	0.03	**			0.02	**0.01**	0.03	**0.04**	7.72	***
Dist [Mid]			0.00	0.00	-0.01	-0.47	-0.01	-0.34		
			0.01	1	0.01	0.64	0.02	0.733		
Dist [Outer]			0.00	0.00	0.00	0.04	0.00	0.18	-0.08	-1.85
			0.01	1	0.01	0.96	0.02	0.86	0.04	0.065
Dist [Middle] * Site [Water]			0.07	3.7	0.08	3.81	-0.09	-3.45	0.31	5.21
			0.02	***	0.02	***	0.03	***	0.06	***
Dist [Outer] * Site [Water]			0.09	4.94	0.06	3.1	-0.11	-4.22		
			0.02	***	0.02	**	0.03	***		
Soil [Silt/Clay]	0.22	5.41			-0.2	-7.43	-0.23	-3.95	42.64	2.01
	0.04	***			0.03	***	0.06	***	21.20	0.044
Soil [Silt/Clay] * Site [Water]	-0.11	-4.86			0.05	2.97	0.18	2.61	-35.63	-6.71
	0.02	***			0.02	**	0.07	**	5.31	***
Herbivores	0.11	1.09			-0.19	-2.14	-0.51	-2.10		
	0.10	0.28			0.09	**0.03**	0.24	**0.04**		
Herbivores * Site [Water]							0.37	1.32		
							0.28	0.19		
Soil [Silt/Clay] * **Herbivores**	-0.41	-2.61			0.64	5.28	0.83	2.23		
	0.16	**			0.12	***	0.37	**0.03**		
Soil [Silt/Clay] * Herbivores * Site [Water]							-0.84	-2.00		
							0.42	**0.05**		

					Legend	
					Est.	T-val
					SE	p-val

	Grass	Forb	Tree	Bare Ground	Height
σ^2	0.01	0.01	0.01	0.01	7285.96
$\tau_{00\ \text{Location}}$	0.00	0.00	0.00	0.00	1843.77
$\tau_{00\ \text{Season}}$	0.01	0.00	0.01	0.00	5292.59
Marginal R^2 / Conditional R^2	0.183 / 0.611	0.346 / 0.582	0.178 / 0.638	0.219 / 0.550	0.067 / 0.529
R^2 Herbivore model	0.005 / 0.520	0.024 / 0.407	0.021 / 0.573	0.046 / 0.447	0.028 / 0.495
R^2 Site * Distance model	0.015 / 0.535	0.063 / 0.447	0.026 / 0.567	0.083 / 0.494	0.049 / 0.516
	dAICc = 1.3	dAICc = 22.3	dAICc = 0.3	dAICc = 26.3	dAICc = 216.7

Table A1.4.3 Type III ANOVA tables for each of the models listed in Table A1.4.2.

Model	Parameters	Sum Sq	Mean Sq	Num DF	Den DF	F value	Pr(>F)
Grass	Rainfall	0.022	0.022	1	399.52	2.58	0.11
	Site Type	0.138	0.138	1	384.68	16.41	***
	Soil	0.117	0.117	1	49.6	13.90	***
	Herbivores	0.013	0.013	1	390.38	1.51	0.22
	Rainfall:Site Type	0.079	0.079	1	382.26	9.38	0.002
	Site Type:Soil	0.199	0.199	1	386.11	23.64	***
	Soil:Herbivores	0.057	0.057	1	390.25	6.82	0.009
Forbs	Rainfall	1.033	1.033	1	401.97	172.73	***
	Site Type	0.073	0.073	1	381.94	12.28	***
	Distance	0.158	0.079	2	381.94	13.21	***
	Site Type:Distance	0.158	0.079	2	381.94	13.21	***
Tree	Rainfall	0.028	0.028	1	398.42	5.60	0.02
	Site Type	0.032	0.032	1	391.43	6.34	0.01
	Distance	0.069	0.035	2	385.51	6.84	0.001
	Soil	0.171	0.171	1	77.92	33.87	***
	Herbivores	0.014	0.014	1	398.1	2.70	0.1
	Rainfall:Site Type	0.033	0.033	1	383.17	6.44	0.01
	Site Type:Distance	0.078	0.039	2	385.97	7.72	***
	Site Type:Soil	0.045	0.045	1	389.48	8.83	0.003
	Soil:Herbivores	0.141	0.141	1	393.73	27.88	***
Bare	Rainfall	0.123	0.123	1	400.3	15.36	***
	Site Type	0.039	0.039	1	395.45	4.88	0.03
	Distance	0.150	0.075	2	388.16	9.32	***
	Soil	0.100	0.100	1	93.7	12.49	***
	Herbivores	0.011	0.011	1	398.19	1.32	0.25
	Rainfall:Site Type	0.034	0.034	1	382.59	4.26	0.04
	Site Type:Distance	0.151	0.075	2	387.58	9.38	***
	Site Type:Soil	0.054	0.054	1	397.24	6.79	0.01
	Site Type:Herbivores	0.000	0.000	1	392.28	0.05	0.83
	Soil:Herbivores	0.033	0.033	1	398.2	4.15	0.04
	Site Type:Soil:Herbivores	0.032	0.032	1	395.67	4.02	0.05
Height	Site Type	1544583	1544583	1	4134	211.99	***
	Distance	49154	49154	1	4134	6.75	0.009
	Rainfall	49821	49821	1	4146.7	6.84	0.009
	Soil	10154	10154	1	16.2	1.39	0.25
	Site Type:Distance	197697	197697	1	4134	27.13	***
	Site Type:Rainfall	173354	173354	1	4134	23.79	***
	Site Type:Soil	327606	327606	1	4134	44.96	***

Table A1.4.4 LMM estimates for plant diversity metrics. Parameters positively associated with diversity near water have solid borders, while those associated with declines have dotted borders. Parameters that agree with those in the main text are shaded in green, while those that are newly significant (and not in differenced data) are shaded in yellow. Significant parameters (not tested in the main text) are shaded in blue, and baseline and insignificant parameters remain unshaded. Intercepts for models correspond to inner rings on sandy soils. We also provide R^2 values models fit with only herbivore dung density or outward distance from water.

Each cell shows: Est (top-left), T-val (top-right) / SE (bottom-left), p-val (bottom-right).

Predictors	SD	Richness	PD	ses.PD	ses.MPD	ses.MPDab
(Intercept)	2.3 27.3 / 0.08 ***	11.86 17.2 / 0.69 ***	1011.5 11.7 / 86.3 ***	-2.68 -7.1 / 0.38 ***	-2.8 -3.1 / 0.9 **	-5.02 -4.87 / 1.03 ***
Site [Water]	-0.48 -4.9 / 0.1 ***	-3.25 -3.1 / 1.04 **	-250.5 -3.4 / 73.9 ***	1.1 3.9 / 0.28 ***		3.28 4.01 / 0.82 ***
Rain	0.04 5.67 / 0.01 ***	0.27 9.16 / 0.03 ***	27.9 5.8 / 4.8 ***	0.06 3.74 / 0.01 ***	0.1 2.9 / 0.04 **	
Rain * Site [Water]	0.02 2.56 / 0.01 0.01		16.6 2.7 / 6.2 **			
Dist [Mid]	0.05 1.12 / 0.05 0.27	0.27 0.9 / 0.3 0.37	-6.0 -0.2 / 36.1 0.87			-0.56 -2.34 / 0.24 0.02
Dist [Outer]	0.06 1.34 / 0.05 0.18	0.38 1.27 / 0.3 0.20	-5.89 -0.2 / 36.0 0.87			-0.81 -3.41 / 0.24 ***
Dist [Mid] * Site [Water]	0.27 3.48 / 0.08 ***	1.55 3.05 / 0.51 **	199.3 3.4 / 59.4 ***			
Dist [Out] * Site [Water]	0.28 3.62 / 0.08 ***	1.48 2.98 / 0.49 **	180.8 3.1 / 58.1 **			
Soil [S/C]	-0.43 -4.9 / 0.09 ***	-2.65 -4.4 / 0.6 ***	-247.3 -3.4 / 73.0 ***	-0.58 -3.3 / 0.18 ***	-1.4 -4.2 / 0.3 ***	-1.08 -2.26 / 0.48 0.02
Soil [S/C] * Site [Water]	0.32 4.66 / 0.07 ***	2.36 5.08 / 0.47 ***	158.6 3.0 / 52.1 **			-1.33 -3.31 / 0.4 ***
Herbivores	-0.39 -1.2 / 0.34 0.25	-9.32 -2.6 / 3.66 0.01	-79.4 -0.3 / 258.7 0.76	3.65 2.35 / 1.55 0.02		10.08 2.92 / 3.45 **
Herbivores * Site [Water]		8.59 2.03 / 4.22 0.04		-5.3 -3.19 / 1.66 ***		-14.6 -3.59 / 4.07 ***
Soil [S/C] * **Herbivores**	2.09 4.43 / 0.47 ***	11.07 3.74 / 2.96 ***	832.9 2.3 / 358.0 0.02			
Soil [S/C] * Hbvs * **Site [Water]**						
σ^2	0.08	2.94	43334.8	0.69	4.15	2.96
$\tau_{00 \text{ Location}}$	0.01	0.47	8312.0	0.1	0.3	0.66
$\tau_{00 \text{ Season}}$	0	0.06	14051.6	0.26	2.97	2.63
Marginal R^2 / Cond. R^2	0.392 / 0.471	0.342 / 0.442	0.264 / 0.514	0.131 / 0.428	0.077 / 0.484	0.133 / 0.589
R^2 Herbivore	0.031 / 0.227	0.012 / 0.231	0.003 / 0.435	0.001 / 0.401	0.001 / 0.497	0.002 / 0.567
R^2 Site Type * Distance	0.062 / 0.268 dAICc = 10.4	0.046 / 0.273 dAICc = 10.8	0.033 / 0.462 dAICc = 47.0	0.015 / 0.420 dAICc = 1.2	0.006 / 0.507 dAICc = 3.7	0.011 / 0.582 dAICc = 0.9

Legend: Est. T-val / SE p-val

Table A1.4.5 Type III ANOVA tables for each of the models listed in Table S4.

Model	Parameters	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
	Site Type	0.335	0.335	1	390.97	4.34	0.04
	Distance	2.658	1.329	2	381.63	17.24	***
	Herbivores	0.361	0.361	1	375.71	4.68	0.03
	Rainfall	7.117	7.117	1	345.84	92.32	***
SD	Soil	0.552	0.552	1	151.48	7.16	0.008
	Site Type : Distance	1.205	0.602	2	382.21	7.81	***
	Site Type : Rainfall	0.504	0.504	1	378.58	6.54	0.01
	Site Type : Soil	1.673	1.673	1	388.36	21.70	***
	Soil : Herbivores	1.511	1.511	1	392.4	19.60	***
	Site Type	5.660	5.660	1	369.21	1.93	0.17
	Distance	66.768	33.384	2	382.8	11.37	***
	Herbivores	0.192	0.192	1	390.67	0.07	0.8
	Rainfall	246.643	246.643	1	282.66	83.97	***
SR	Soil	14.831	14.831	1	114.12	5.05	0.03
	Site Type : Distance	32.494	16.247	2	382.12	5.53	0.004
	Site Type : Herbivores	12.158	12.158	1	366.5	4.14	0.04
	Site Type : Soil	75.784	75.784	1	391.96	25.80	***
	Soil : Herbivores	41.179	41.179	1	390.1	14.02	***
	Site Type	37853	37853	1	386.94	0.87	0.35
	Distance	534705	267353	2	379.65	6.17	0.002
	Herbivores	91278	91278	1	391.43	2.11	0.15
	Rainfall	4051249	4051249	1	390.26	93.49	***
PD	Soil	183765	183765	1	96.41	4.24	0.04
	Site Type : Distance	562019	281010	2	380.17	6.48	0.002
	Site Type : Rainfall	304923	304923	1	377.32	7.04	0.008
	Site Type : Soil	401516	401516	1	384.26	9.27	0.002
	Soil : Herbivores	234651	234651	1	389.74	5.41	0.02
	Site Type	12.613	12.613	1	386.52	17.38	***
	Herbivores	1.258	1.258	1	384.53	1.73	0.19
ses.PD	Rainfall	10.125	10.125	1	378.03	13.95	***
	Soil	7.274	7.274	1	17.81	10.02	0.005
	Site Type : Herbivores	8.658	8.658	1	379.38	11.93	***
ses.MPD	Soil	74.120	74.120	1	15.31	17.85	***
	Rainfall	34.714	34.714	1	332.79	8.36	0.004
	Site Type	40.649	40.649	1	386.54	13.73	***
	Distance	34.684	17.342	2	380.4	5.86	0.003
	Herbivores	6.487	6.487	1	391.75	2.19	0.14
ses.MPDab	Soil	46.395	46.395	1	17.72	15.67	***
	Site Type : Herbivores	38.152	38.152	1	383.76	12.89	***
	Site Type : Soil	32.364	32.364	1	386.03	10.93	0.001

Figure A1.4.2 Percent cover for grasses (A), trees (B), forbs (C), and bare ground (D) are visualized along a continuum of herbivore dung density across soil types (facets), and at both water sources (blue) and dry sites (red).

Figure A1.4.3 Grass cover declined with increasing herbivore dung density on mesic silt/clay soils, and this effect was especially apparent during low-rainfall periods (first panel). On arid sandy soils, herbivores interacted with rainfall such that grass cover increased with increasing herbivores and rainfall.

Figure A1.4.4 Different diversity metrics for plant communities on different soil types (facets) at water sources (blue) and dry sites (red). Unadjusted diversity metrics are shown in the left column, while resulting Z-values after comparison to null models are shown in the right column. A horizontal line is drawn to demonstrate the threshold at which diversity is significantly reduced compared to null models.

Appendix 1.5. Plant cover and diversity summaries

Table A1.5.1 Summary means and standard errors for tree, forb, grass, and bare ground cover totals by soil type and water presence (water source vs dry site).

Soil Type	Water Presence	Distance (m)	Tree	Forb	Grass	Bare
Silt/clay	Dry	0-145	14.98 ± 1.29	20.84 ± 2.27	83.76 ± 1.79	8.96 ± 1.37
	Water source	0-45	18.68 ± 1.72	11.11 ± 1.45	70.38 ± 2.75	23.46 ± 2.46
		50-95	21.78 ± 2.17	20.54 ± 2.34	74.63 ± 2.47	14.46 ± 1.93
		100-145	18.01 ± 1.95	21.4 ± 2.6	73.81 ± 2.08	15.00 ± 2.02
Sand	Dry	0-145	26.14 ± 2.1	18.05 ± 1.98	67.35 ± 2.54	20.28 ± 2.2
	Water source	0-45	14.04 ± 1.64	11.27 ± 1.58	69.68 ± 2.31	27.7 ± 2.64
		50-95	22.51 ± 2.33	16.17 ± 1.91	70.79 ± 2.29	20.71 ± 1.96
		100-145	24.18 ± 2.23	19.79 ± 2.13	71.65 ± 2.3	18.24 ± 2.16

Table A1.5.2 Summary means and standard errors of paired differences (grouped by site; n=17 over 4 sampling periods) in tree, forb, grass, and bare ground cover between water sources and dry sites for different soil types and outward sampling distance.

Soil Type	Distance (m)	Δ Tree	Δ Forb	Δ Grass	Δ Bare
Silt/clay	0-45	3.69 ± 1.72	-9.73 ± 1.69	-13.38 ± 2.81	14.5 ± 2.26
	50-95	6.79 ± 1.57	-0.31 ± 1.91	-9.14 ± 2.67	5.5 ± 1.78
	100-145	3.03 ± 1.43	0.55 ± 1.61	-9.95 ± 2.07	6.04 ± 1.73
Sand	0-45	-12.1 ± 2.23	-6.78 ± 1.59	2.33 ± 2.42	7.42 ± 3.12
	50-95	-3.63 ± 2.33	-1.88 ± 1.67	3.44 ± 2.29	0.44 ± 1.97
	100-145	-1.97 ± 1.92	1.74 ± 1.68	4.3 ± 2.25	-2.04 ± 2.22

Table A1.5.3 Summary means and standard errors for species richness, Shannon Diversity, and Phylogenetic Diversity totals by soil type and water presence (water source vs dry site).

Soil Type	Water presence	Distance	SR	SD	PD
Silt/clay	Dry	0-45	10.88 ± 0.44	2.27 ± 0.08	1015 ± 43
		50-95	11.12 ± 0.41	2.33 ± 0.07	991 ± 46
		100-145	11.42 ± 0.45	2.36 ± 0.07	1014 ± 48
	Water source	0-45	12.34 ± 0.41	2.46 ± 0.06	1082 ± 61
		50-95	13.6 ± 0.51	2.67 ± 0.07	1290 ± 67
		100-145	13.04 ± 0.46	2.6 ± 0.07	1166 ± 56
Sand	Dry	0-45	11.89 ± 0.26	2.42 ± 0.04	1142 ± 51
		50-95	12.29 ± 0.24	2.52 ± 0.04	1169 ± 46
		100-145	12.05 ± 0.3	2.47 ± 0.05	1134 ± 45
	Water source	0-45	9.92 ± 0.41	1.98 ± 0.08	977 ± 61
		50-95	11.38 ± 0.36	2.32 ± 0.06	1113 ± 54
		100-145	11.96 ± 0.32	2.41 ± 0.06	1193 ± 54

Table A1.5.4 Summary means and standard errors of paired differences (grouped by site; n=17 over 4 sampling periods) in diversity metrics between water sources and dry sites for different soil types and outward sampling distance.

Soil Type	Distance	ΔSR	ΔSD	ΔPD
Silt/clay	0-45	1.45 ± 0.51	0.19 ± 0.09	69 ± 51
	50-95	2.47 ± 0.51	0.34 ± 0.08	299 ± 49
	100-145	1.62 ± 0.48	0.24 ± 0.07	151 ± 51
Sand	0-45	-1.88 ± 0.41	-0.42 ± 0.07	-139 ± 50
	50-95	-0.84 ± 0.32	-0.19 ± 0.05	-45 ± 44
	100-145	-0.01 ± 0.32	-0.04 ± 0.05	74 ± 41

Appendix 1.6. Radial sampling and species richness

A radial sampling design was selected to allow us to identify distance effects of water sources. However, this method has the potential to influence diversity results, which we explore and address here. One potential tradeoff of the fixed radial sampling design is that summed species counts across transects might result in higher species richness in outer rings than inner rings, as the total area covered is larger (despite equal sampling effort). In other words, increased species richness in outer rings might be an artefact of scale. To verify that analyses of changes in all metrics of diversity are not affected by the sampling methodology, we first asked whether species richness (SR), Shannon diversity (SD), and phylogenetic diversity (PD) changed with outward distance at dry sites only. We built LMMs for dry site data only, regressing SR, SD, and PD by outward distance, using sampling period and site as random effects. We then compared this model to one containing only random effects using F-tests. For no model was distance a significant parameter (Figure A1.6.1; Table A1.6.1).

Second, because the perimeter of water sources varied among sites (due to differing size of water bodies) but remained constant at dry sites (set at a predefined distance of 10m radius from center point), we investigated whether the perimeter of each water source had any explanatory power for differences in diversity. We calculated differences between water sources and dry sites for each of these diversity metrics, and we constructed models that included the interaction of soil type, outward distance, and perimeter (with site and sampling period as random effects) and performed backwards selection using lmerTest. We found that the perimeter was not an important factor in any model (Figure A1.6.2, Table A1.6.2).

Figure A1.6.1 None of the diversity metrics examined increased with increasing outward distance at dry sites, despite an increased sampling area. In contrast, there were differences by distance at water sources.

Table A1.6.1 F-values for the effect of distance in each model of species richness (SR), Shannon diversity (SD), and phylogenetic diversity (PD) at dry sites only.

Effect of Distance on:	SS	MS	$F_{2,179.03}$	Pr(>F)
SR	4.948	2.474	0.8777	0.42
SD	0.229	0.115	1.6117	0.20
PD	2304.3	1152.1	0.0255	0.97

Figure A1.6.2 Water source perimeter did not affect differences in measurements of species richness (SR), Shannon diversity (SD), and phylogenetic diversity (PD) compared to dry sites.

Table A1.6.2 F-values for the combined effects of perimeter and distance from water for each model of differences in species richness, Shannon Diversity, and Phylogenetic Diversity show that only distance and soil type are important parameters; water source perimeter has very little explanatory power.

Response	Parameter	SS	MS	$F_{num,den}$	Pr(>F)
ΔSR	Dists:Perim:Soil	53.01	26.50	$F_{2,167.6}=1.71$	0.18
	Dists:Perim	9.998	4.999	$F_{2,169.4}=0.32$	0.73
	Perim:Soil	7.228	7.228	$F_{1,13.5}=0.466$	0.51
	Perim	4.754	4.754	$F_{1,14.6}=0.306$	0.59
	Dists:Soil	**184.15**	**92.07**	$\mathbf{F_{2,171.39}=5.93}$	**0.003**
ΔSD	Dists:Perim:Soil	0.209	0.105	$F_{2,167.4}=1.19$	0.31
	Perim:Soil	0.0577	0.0577	$F_{1,13.3}=0.65$	0.43
	Dists:Perim	0.2659	0.1329	$F_{2,169.3}=1.50$	0.23
	Perim	0.0055	0.0055	$F_{1,14.4}=0.062$	0.81
	Dists:Soil	**0.9710**	**0.4855**	$\mathbf{F_{2,171.3}=5.46}$	**0.005**
ΔPD	Dists:Perim:Soil	134866	67433	$F_{2,170.3}=1.48$	0.23
	Perim:Soil	4477	4477	$F_{1,13.3}=0.098$	0.76
	Dists:Perim	62478	31239	$F_{2,172.3}=0.68$	0.51
	Perim	1443	1443	$F_{1,14.4}=0.032$	0.86
	Dists:Soil	**583024**	**291512**	$\mathbf{F_{2,174.2}=6.38}$	**0.002**

Appendix 1.7. Phylogenetic Metrics Justification

First, we used Faith's PD as a measure of the total evolutionary history at water sources compared to dry sites. Aside from being a frequently-used metric across a broad range of ecological studies (Tucker et al. 2017), changes in PD can provide insight into significant changes in community composition; higher PD has been associated with communities with higher productivity (Cadotte 2013) and stability (Cadotte et al. 2012) and may be a potential proxy for functional diversity (Srivastava 2012), but see (Mazel et al. 2018). We calculated PD for each community using the picante package in R (Kembel et al. 2010). However, because PD is the sum of branch lengths represented by a community, and is correlated with the number of branch tips (species richness), several studies have highlighted that many patterns in community composition are primarily explained by species richness, rather than PD (Venail et al. 2015). After controlling for richness, higher PD at certain sites might reflect a greater breadth of diversity across the phylogenetic tree.

We also asked if communities were more divergent at water sources compared to dry sites using mean pairwise distance (MPD) and abundance weighted MPD (MPD$_{AB}$) as metrics. MPD has been identified as an 'anchor' metric of α-diversity for ecological studies, as it can measure the similarity among species in a community (Tucker et al. 2017). Abundance-weighted measures also account for the relative abundance of different species (Cadotte et al. 2010). At water sources, high levels of herbivore pressure might prevent the growth of many species, resulting in communities comprised of fewer species that share similar lineages (i.e. low MPD). However, MPD may increase at water sources if distinct lineages can thrive where they otherwise would not.

Appendix 2. Chapter 3

Appendix 2.1. Camera trap data and herbivore activity analyses

Table A2.1.1 Summary of experimental system camera trap deployments at Ol Pejeta Conservancy used to assess herbivore activity during dry season months. A summary of observational deployments at Mpala Research Centre is provided in Table A1.3.5.

Status	Site Name	Treatment	Start	End	Trap Nights	Camera Model
Pre	Jericho	Filled	10/18/2016	10/31/2016	13	Moultrie M-880
		Drained	8/13/2016	8/27/2016	14	Moultrie M-880
	Kambi	Filled	9/30/2016	10/14/2016	14	Moultrie M-880
		Drained	9/24/2016	10/14/2016	20	Moultrie M-880
	Oscar	Filled	10/18/2016	11/2/2016	15	Moultrie M-880
		Drained	9/30/2016	10/18/2016	18	Moultrie M-880
	Sidai	Filled	9/30/2016	10/18/2016	18	Moultrie M-880
		Drained	9/30/2016	10/18/2016	18	Moultrie M-880
	Tangi	Filled	11/25/2016	11/30/2016	6	Moultrie M-880
		Drained	10/18/2016	11/2/2016	15	Moultrie M-880
During	Jericho	Filled	7/10/2017	7/25/2017	15	Moultrie M-880
		Drained	7/10/2017	7/25/2017	15	Moultrie M-880
	Kambi	Filled	9/22/2017	10/25/2017	33	Moultrie M-880
		Drained	7/11/2017	7/18/2017	7	Moultrie M-880
			9/6/2017	9/16/2017	10	Moultrie M-880
	Oscar	Filled	7/25/2017	8/26/2017	32	Moultrie M-880
		Drained	7/25/2017	9/6/2017	43	Moultrie M-880
	Sidai	Filled	5/30/2017	6/30/2017	31	Moultrie M-880
		Drained	6/15/2017	7/14/2017	29	Moultrie M-880
	Tangi	Filled	6/15/2017	7/12/2017	27	Moultrie M-880
		Drained	6/15/2017	6/24/2017	9	Moultrie M-880
			7/25/2017	7/30/2017	5	Moultrie M-880
Post	Jericho	Filled	6/18/2018	7/5/2018	17	Moultrie M-880
		Drained	5/31/2018	6/12/2018	12	Moultrie M-880
	Kambi	Filled	6/29/2018	7/5/2018	6	Moultrie M-880
		Drained	5/15/2018	6/15/2018	31	Moultrie M-880
	Oscar	Filled	8/23/2018	9/14/2018	22	Moultrie M-880
		Drained	8/5/2018	8/24/2018	19	Moultrie A-30
	Sidai	Filled	5/30/2018	7/4/2018	35	Moultrie A-30
		Drained	5/15/2018	5/18/2018	3	Moultrie A-30
			5/30/2018	6/3/2018	4	Moultrie A-30
			6/30/2018	7/9/2018	9	Moultrie M-880
	Tangi	Filled	7/4/2018	8/20/2018	47	Moultrie M-880
		Drained	5/14/2018	5/29/2018	15	Moultrie A-30

Table A2.1.2 Coefficients (log-means), standard errors, and statistical results for best negative binomial GLMMs of herbivore activity (daily individual*seconds) for the experimental system at Ol Pejeta Conservancy. Significant negative interactions between experiment status (pre, during, post) and treatment (filled, drained) indicate substantial decreases in activity at experimental pans relative to filled pans after the first period of the experiment.

Key:	Est. ±SE Z p	All	Elephant	Cattle	Zebras	Buffalo	Impala	Giraffe
Intercept (Filled, Pre)		8.40 ±0.49 17.02 ***	6.03 ±0.65 9.32 ***	8.68 ±0.59 14.63 ***	6.9 ±0.87 7.9 ***	6.17 ±1.55 3.97 ***	5.91 ±1.07 5.52 ***	5.79 ±1.07 5.39 ***
Drained		0.56 ±0.41 1.35 0.177	-0.26 ±0.62 -0.43 0.669	-1.72 ±0.56 -3.07 **0.002**	-2.08 ±0.6 -3.46 **0.001**	-2.17 ±1.25 -1.74 0.082	-2.38 ±0.91 -2.61 **0.009**	-2.86 ±0.71 -4.04 ***
During		-0.86 ±0.36 -2.41 **0.016**	0.31 ±0.61 0.51 0.613	-0.47 ±0.62 -0.76 0.45	-2.59 ±0.62 -4.2 ***	-0.72 ±1.17 -0.61 0.539	-1.86 ±0.99 -1.88 0.059	-3.11 ±0.79 -3.95 ***
Post		0.33 ±0.37 0.9 0.367	0.61 ±0.68 0.9 0.368	-1.26 ±0.44 -2.88 **0.004**	0.06 ±0.67 0.09 0.925	0.68 ±0.44 1.53 0.125	-0.43 ±0.5 -0.86 0.391	-0.63 ±0.55 -1.16 0.246
Filled:During		-1.73 ±0.51 -3.38 **0.001**	-2.15 ±0.84 -2.56 **0.011**		-1.76 ±0.82 -2.15 **0.031**			
Filled:Post		-1.48 ±0.53 -2.76 **0.006**	-0.87 ±0.85 -1.02 0.307		-1.41 ±0.85 -1.66 0.098			
σ^2		1.88	2.82	3.33	2.74	3.15	3.3	3.75
$\tau_{00\ Location}$		0.79	0.78	0.31	2.64	7.59	1.4	3.62
$\tau_{00\ Month}$		0.00	0.00	0.00	0.00	0.58	1.22	0.00
Marginal R^2 / Conditional R^2		0.23 0.46	0.15 0.34	0.20 0.26	0.29 0.64	0.08 0.74	0.13 0.52	0.18 0.59

Figure A2.1.1 Total daily animal activity measured by camera traps at each location throughout the experiment. Individual sites are shown in color, while the average across sites is shown in gray.

Table A2.1.3 Coefficients (log-means), standard errors, and statistical results for best negative binomial GLMMs of herbivore activity (daily individual*seconds) at observational water sources and dry sites at Mpala Research Centre. No interactions between water presence and mean annual precipitation were significant.

Key: Est. SE Z p	All	Elephant	Impala	Cattle	Giraffe	Buffalo	Zebra
Intercept (Dry site, MAP = 4.6)	11.00 ±1.98 5.55 ***	7.65 ±7.09 1.08 0.28	9.95 ±7.24 1.37 0.17	21.56 ±10.95 1.97 **0.05**	8.77 ±4.47 1.96 **0.05**	-6.02 ±12.01 -0.5 0.62	8.01 ±4.94 1.62 0.11
Water	1.44 ±0.40 3.57 ***	3.80 ±0.74 5.10 ***	0.62 ±0.86 0.72 0.47	1.59 ±1.03 1.54 0.12	1.86 ±0.93 2.00 **0.05**	3.21 ±1.26 2.55 **0.01**	2.69 ±0.82 3.30 **0.001**
MAP (÷100)	-0.96 ±0.37 -2.58 **0.01**	-1.30 ±1.30 -1.00 0.32	-1.21 ±1.33 -0.91 0.36	-3.13 ±2.03 -1.55 0.12	-1.27 ±0.79 -1.59 0.11	0.93 ±2.18 0.43 0.67	-0.96 ±0.90 -1.07 0.29
σ^2	2.5	3.32	3.51	4.65	4.51	4.22	3.25
$\tau_{00 \ Month}$	0.00	4.02	4.36	0.00	0.00	11.58	1.84
$\tau_{00 \ Number}$	0.11	2.29	0.00	0.00	0.00	0.00	0.00

Figure A2.1.2 Mean daily activity for all herbivores together across 12 sites with camera trapping data at Mpala Research Centre. While there was no significant interaction for any species, activity was substantially higher at water sources relative to dry sites.

Appendix 2.2. Quantifying herbivore dung

A2.2.1 *Determination of 'fresh' vs 'old' dung:*

Before the experiment began, we collected fresh dung from herbivore species included in the study

(defecation was directly observed). Dung was weighed and dimensions of pellets were taken. For

elephant and buffalo, a 30cm^3 sample was used. We placed dung in the field for two weeks and

reweighed at several intervals. We found that most dung samples were very dry by day three (Figure

A2.2.1); therefore, we used visible internal and external moisture content, presence of arthropods, and

odor to gauge whether dung was fresh or old during our subsequent surveys. Dung quantification was

applied consistently across all surveys and treatment locations by the same surveyor (J. Mantas).

Figure A2.2.1 Weight of dung pellets or 30mL dung samples over time

A2.2.2 *Dung volume calculation:*

To expedite field measurements, we devised a methodology to calculate the approximate volume of

herbivore dung in each quadrat. For species that did not have easily countable pellets (elephants,

buffalo, cattle), we counted the number of 64cm^2 units of dung for each species found in each

quadrat. We then multiplied this by 4cm for elephants and 2cm for buffalo and cattle to account for

differing average depths. For all other species, we counted the number of pellets and multiplied by standard measurements from (http://www.scielo.br/pdf/mioc/v98s1/v98s1a14.pdf) to obtain the final volume.

A2.2.3 *Physical dung density:*

We used the physical density of dung to convert parasite eggs per gram of feces to eggs per cm^3. We used conversions listed in A2.2.1 based on field measurements from above.

Table A2.2.1 Species-specific dung properties used to calculate parasite density

	Elephant	Cow	Zebra	Buffalo	Impala	Giraffe
Density (g/cm^3)	0.58	0.95[†]	0.79	0.95	1.66	1.66[‡]
Pellet size (cm^3)	8x8x4 = 256	8x8x2 = 128	6x4x1.5 = 36	8x8x2 = 128	1.1x0.6x0.6 = 0.396	2.5x2.5x1.5 = 9.375

[†]Density was assumed to be similar to buffalo measurements
[‡]Density was assumed to be similar to impala measurements

Appendix 2.3. Fecal egg counts literature search

To compare fecal egg counts from the focal species in our study, we conducted a Web of Science literature search using the search criteria TS=((fecal AND egg AND count) AND (cow OR cattle OR elephant OR zebra OR giraffe OR buffalo OR impala)). Of the 299 results, 193 studies were selected based on the relevance of their abstracts, but only 7 contained specific FEC data from any of the focal species located in Africa. We therefore supplemented this search by investigating citations from these studies and by additional Google Scholar searches. All references are provided in Table A2.3.1.

Figure A2.3.1 Mean fecal egg counts reported in studies from our literature search of reports across Africa. Values observed from sampling at our study site (green triangles) generally follow similar patterns to other studies but fall slightly below weighted and overall study means for zebra, elephant, impala, and buffalo, although our observed values fall within the range of prior study values.

Table A2.3.1 Fecal egg count data and references for focal species included in our study

Species	N	Mean	Med.	Error	Prev.	Location	Note	Method	Ref
Buffalo	100	2.07			0.3	S. Africa	Wet	MM	(Beechler et al. 2012)
Buffalo	100	4.44			0.69	S. Africa	Dry	MM	(Beechler et al. 2012)
Buffalo	375	298		23 SE		S. Africa	Early dry	MM	(Caron et al. 2003)
Buffalo	375	409		29 SE		S. Africa	Late dry	MM	(Caron et al. 2003)
Buffalo	60	349		54 SE	0.85	Mpala		MM	(Ezenwa 2003)
Buffalo	40	274				Kenya	Dry	MM	(Ezenwa 2004a)
Buffalo	11	294				Kenya	Normal	MM	(Ezenwa 2004a)
Buffalo	167	251				S. Africa	M; Fig	MM	(Ezenwa and Jolles 2008)
Buffalo	226	251				S. Africa	F; Fig	MM	(Ezenwa and Jolles 2008)
Buffalo	78	1000				S. Africa	Fig	MM	(Gorsich et al. 2014)
Buffalo	448	800				S. Africa	Fig	MM	(Gorsich et al. 2014)
Buffalo	129	400				S. Africa	Fig	MM	(Gorsich et al. 2014)
Buffalo	208	300				S. Africa	Fig	MM	(Gorsich et al. 2014)
Buffalo	100	300				S. Africa	Fig	MM	(Gorsich et al. 2014)
Buffalo	103	94.5		173 SD		S. Africa	Y, Positives	MM	(Penzhorn 2000)
Buffalo	283	120.6		143 SD		S. Africa	A, Positives	MM	(Penzhorn 2000)
Cattle	18	246				Ghana	F	MM	(Agyei 1991)
Cattle	6	22		58 SD		Tanzania		NA	(Brito et al. 2013)
Cattle	6	90		305 SD		Tanzania		NA	(Brito et al. 2013)
Cattle	8	85		152 SD		Tanzania		NA	(Brito et al. 2013)
Cattle	210	319		62 SE		Ethiopia		MM	(Degefu et al. 2011)
Cattle	98	48			0.14	Kenya	Y	MM	(Kabaka et al. 2014)
Cattle	321	18.4			0.14	Kenya	A	MM	(Kabaka et al. 2014)
Cattle	349	296		37.3 SE	0.51	Kenya		MM	(Kanyari et al. 2010)
Cattle	46	80				Tanzania	A; Fig	MM	(Keyyu et al. 2005)
Cattle	46	150				Tanzania	Y; Fig	MM	(Keyyu et al. 2005)
Cattle	46	300				Tanzania	J; Fig	MM	(Keyyu et al. 2005)
Cattle	23	100				Tanzania	A; Fig	MM	(Keyyu et al. 2005)
Cattle	23	150				Tanzania	Y; Fig	MM	(Keyyu et al. 2005)
Cattle	23	200				Tanzania	J; Fig	MM	(Keyyu et al. 2005)
Cattle	32	125				Tanzania	A; Fig	MM	(Keyyu et al. 2005)
Cattle	31	200				Tanzania	Y; Fig	MM	(Keyyu et al. 2005)
Cattle	31	300				Tanzania	J; Fig	MM	(Keyyu et al. 2005)
Cattle	31	245	200	31 SE	0.9	Kenya	T	NaCl Float	(Knafo 2008)
Cattle	423	180.4			0.55	S. Africa		MM	(Ndlovu et al. 2009)
Cattle	600	291	272	183 SD	0.7	Rwanda	Dry (15)	MM	(Sun et al. 2018)
Cattle	600	246	248	178 SD	0.63	Rwanda	Wet (15)	MM	(Sun et al. 2018)
Cattle	57	229			0.84	Kenya	C; Fig	MM	(Waruiru et al. 2000)
Cattle	56	325			0.93	Kenya	Y; Fig	MM	(Waruiru et al. 2000)
Cattle	52	159			0.75	Kenya	A; Fig	MM	(Waruiru et al. 2000)
Cattle	64	150				Kenya	C	MM	(Waruiru et al. 2000)
Elephant	187	1100		500 SD		Botswana		MM	(Baines et al. 2015)
Elephant	241	500		600 SD		Botswana		MM	(Baines et al. 2015)

Species	N	Mean		Error	Prev	Country	Group	Method	Citation
Elephant	63	1409	1375			Namibia		MM	(Brumfitt 2005)
Elephant	63	2204	2138			Namibia		MM	(Brumfitt 2005)
Elephant	19	202	50	319 SD	0.93	Kenya	F	MM	(King'ori et al. 2020)
Elephant	7	121	50	236 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	16	106	75	125 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	35	320	200	419 SD	0.93	Kenya	F	MM	(King'ori et al. 2020)
Elephant	4	275	175	333 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	14	171	50	272 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	46	146	100	205 SD	0.93	Kenya	F	MM	(King'ori et al. 2020)
Elephant	8	200	100	276 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	19	89	50	133 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	25	36	0	67 SD	0.93	Kenya	F	MM	(King'ori et al. 2020)
Elephant	10	0	0	0 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	22	23	0	46 SD	0.93	Kenya	M	MM	(King'ori et al. 2020)
Elephant	578	1694		61 SE	0.96	Kenya		MM	(Parker et al. 2020)
Elephant	119	736		84 SE		Namibia	M	MM	(Thurber et al. 2011)
Elephant	70	976		134 SE		Namibia	F/J	MM	(Thurber et al. 2011)
Giraffe	21	77			0.33	UK	Captive	MM	(Melbourne 1978)
Giraffe	14	0	0		0.06	Kenya	Est mean	MM	(VanderWaal et al. 2014b)
Impala	692	963		39 SE	0.96	Mpala		MM	(Ezenwa 2003)
Impala	442	660				Kenya	Dry	MM	(Ezenwa 2004a)
Impala	225	467				Kenya	Normal	MM	(Ezenwa 2004a)
Impala	112	55				Zambia	Cool dry	MM	(Nalubamba et al. 2012)
Impala	112	39				Zambia	Hot dry	MM	(Nalubamba et al. 2012)
Impala	112	264				Zambia	Wet	MM	(Nalubamba et al. 2012)
Impala	102	216		18 SE		Uganda	Nat. Park	CF	(Ocaido et al. 1999)
Impala	76	247		31 SE		Uganda	Ranched	CF	(Ocaido et al. 1999)
G. Zebra	39	569	500	60 SE	0.82	Kenya	T	NaCl Float	(Knafo 2008)
G. Zebra	15	1306				Kenya	J	MM	(Mwatenga 2017)
G. Zebra	47	1187				Kenya	J	MM	(Mwatenga 2017)
G. Zebra	145	1635				Kenya	A	MM	(Mwatenga 2017)
G. Zebra	15	1100		300 SE		Kenya			(Rubenstein 2010)
P. Zebra	76	317.9				Uganda	F	MM	(Fugazzola and
P. Zebra	65	241.5				Uganda	M	MM	(Fugazzola and
P. Zebra	31	473	350	67 SE	0.61	Kenya	T	NaCl Float	(Knafo 2008)
P. Zebra	5	2500	2150	628 SE	1	Namibia		Krecek 1983	(Krecek et al. 1987)
P. Zebra	15	2100		300 SE		Kenya			(Rubenstein 2010)
P. Zebra	247	1600		250 SE		Namibia	J; Fig	MM	(Turner 2009)
P. Zebra	247	2600		200 SE		Namibia	Y; Fig	MM	(Turner 2009)
P. Zebra	247	2500		100 SE		Namibia	A; Fig	MM	(Turner 2009)
P. Zebra	10	1225		104 SE	0.8	Kenya	Ranched	MM	(Wambwa et al. 2004)
P. Zebra	10	1620		204 SE	1	Kenya	Free Ranging	MM	(Wambwa et al. 2004)

Notes: J = Juvenile, Y = Yearling, A = Adult, F = Female, M = Male
Fig: Values estimated from figures, Tab: Values calculated from table
MM = McMaster (or modified) egg float method

Appendix 2.4. Best models of dung density log-ratios

To enable direct comparisons between filled and experimental pans, we calculated the log ratio for dung density (cm^3/m^2) at filled and experimental water pans for all parasites, all dung together, and for dung of each of the six most common species (elephants, cattle, zebra, impala, giraffe, and buffalo). Note that zebra dung densities reflect both *Equus grevyi* and *Equus burchellii*, as the dung of these two species are indistinguishable.

$$y = \log\left(\frac{Density_{filled} + 1}{Density_{experimental} + 1}\right)$$

We used linear mixed-effect models to test the effect of experiment status (pre-draining, during the experiment, and post-refill) on the log ratio of dung density. We also included outward distance and its interaction with status as fixed effects, while period (n=10) and site (n=5) were included as random effects. The best model of dung density log ratio was determined using backwards stepwise selection using the lmerTest package (Kuznetsova et al. 2017), and 95% confidence intervals of model coefficients were determined by bootstrapping the final model 10000 times and calculating the 95% bias-corrected confidence interval, as this method is considered robust to deviations from normal data in mixed effect models (Thai et al. 2013). If bootstrap intervals found coefficient estimates that overlapped with 0, they were dropped from the final model.

Results are qualitatively similar to results presented in the main text; however, adding a nominal value (+1) to all data resulted in biased estimates for herbivore species with low dung density. Log-ratio models are therefore presented here for ease of interpreting significant effects.

Table A2.4.1 Best models of dung and parasite log ratios at filled vs. experimental pans. Parameters that increased and decreased dung at filled pans relative to drained pans are shaded green and orange respectively. Both 95% profile and bias-corrected bootstrap CIs (from 10,000 bootstraps) are provided for each coefficient.

Species	Intercept (0m, Pre)	During	Post	Distance (1=100m)	Distance: During	Distance: Post	σ^2 σ^2_{site} σ^2_{period}
All	-0.15 -0.27 -1.23, 0.93 -1.21, 0.96 0.786	1.97 3.31 0.80, 3.14 0.74, 3.12 **0.002**	0.73 1.16 -0.50, 1.96 -0.44, 1.89 0.25	0.24 0.41 -0.89, 1.36 -0.92, 1.35 0.682	-1.18 -1.87 -2.41, 0.06 -2.41, 0.088 0.062	-0.19 -0.28 -1.49, 1.11 -1.44, 1.11 0.777	5.62 0.03 0.04
Cow	-0.03 -0.06 -0.99 – 0.93 -1.14, 1.01 0.95	1.87 3.83 0.91, 2.84 0.76, 3.04 ***	0.96 0.52 -0.06, 1.97 -0.19, 2.21 0.064	0.046 0.09 -0.95, 1.04 -1.14, 1.29 0.93	-1.27 -2.28 -2.36, -0.17 -2.59, -0.001 **0.023**	-0.30 -0.52 -1.46, 0.85 -1.71, 1.00 0.60	4.45 0.17 0.00
Elephant	-0.035 -0.086 -0.89, 0.82 -0.86, 0.35 0.932	0.66 2.01 -0.066, 1.38 **0.045, 1.31** 0.078	0.51 1.47 -0.26, 1.27 -0.17, 1.19 0.18				5.50 0.28 0.02
Zebra	0.53 7.72 0.38, 0.68 0.39, 0.67 ***						3.39 0.00 0.00
Impala	0.19 3.47 0.072, 0.311 0.062, 0.346 **0.006**	-0.16 -2.87 -0.284, -0.038 -0.315, -0.026 **0.02**	-0.15 -2.61 -0.283, -0.024 -0.313, -0.015 **0.03**				0.18 0.00 0.00
Buffalo	-0.22 -1.32 -0.60, 0.16 **-0.33, -0.12** 0.23						2.20 0.10 0.05
Giraffe	-0.028 -0.50 -0.14, 0.085 -0.11, 0.052 0.62			0.11 2.00 0.002, 0.218 0.018, 0.207 **0.046**			0.46 0.00 0.00
All Parasites	0.50 0.80 -0.73, 1.72 -0.66, 1.69 0.45	2.12 3.10 0.78, 3.46 0.82, 3.38 **0.01**	0.99 1.37 -0.42, 2.40 -0.33, 2.30 0.20				27.60 0.00 0.05

Legend: Estimate
t-value
95% Profile interval
95% Boot interval
Pr(>|t|)

Table A2.4.2 Post-hoc tests using Tukey correction for multiple comparisons. For best models that included an interaction between distance and experiment status, estimates reflect ratios at 0m outward distance. The converted ratios are shown in the far-right column of the table.

Species	Null Hypothesis	Estimate	SE	Z	P	95% CI: Log Ratio		Ratio Estimate
						Lower	Upper	
All	During - Pre == 0	1.973	0.597	3.307	0.003	0.584	3.361	**7.189**
	Post - Pre == 0	0.730	0.629	1.161	0.469	-0.734	2.193	2.075
	Post - During == 0	-1.243	0.398	-3.125	0.005	-2.168	-0.317	**0.289**
Cow	During - Pre == 0	0.924	0.258	3.578	<0.001	0.323	1.525	**2.519**
	Post - Pre == 0	0.730	0.272	2.682	0.02	0.097	1.364	**2.075**
	Post - During == 0	-0.194	0.172	-1.127	0.490	-0.595	0.217	0.824
Elephant	During - Pre == 0	0.659	0.328	2.01	0.106	-0.104	1.423	1.933
	Post - Pre == 0	0.509	0.346	1.47	0.298	-0.296	1.314	1.664
	Post - During == 0	-0.150	0.219	-0.69	0.766	-0.659	0.359	0.861
Impala	During - Pre == 0	-0.161	0.056	-2.873	0.011	-0.291	-0.030	**0.851**
	Post - Pre == 0	-0.153	0.059	-2.605	0.024	-0.291	-0.016	**0.858**
	Post - During == 0	0.007	0.037	0.190	0.980	-0.080	0.094	1.007
All Parasites	During - Pre == 0	2.121	0.685	3.099	0.005	0.528	3.714	**8.339**
	Post - Pre == 0	0.9898	0.722	1.372	0.348	-0.689	2.669	2.691
	Post - During == 0	-1.131	0.456	-2.480	0.034	-2.193	-0.0694	**0.323**

For our observational dataset, we also calculated the log ratios of dung and parasite density at watering holes and at controls using the formula below.

$$y = \log\left(\frac{Density_{waterhole} + 1}{Density_{control} + 1}\right)$$

Log ratios of density at waterholes relative to controls were analyzed using linear mixed-effect models testing the interactions between cumulative prior 30-day rainfall, mean annual precipitation, and outward distance, including random effects for site (n=20) and period (n=5). Best models were again determined using backwards stepwise selection using the lmerTest package (Kuznetsova et al. 2017). Final confidence intervals were again determined by bootstrapping the final model 10000 times and calculating the 95% bias-corrected percentile interval. Any non-significant interactions were dropped from the model and re-run.

Table A2.4.3 Best models of the log ratio of dung and parasite density at watering holes versus dry sites. Coefficients indicating increased dung and parasite density at water are shaded green, while those indicating decreased relative density are shaded orange.

Species	Intercept	Distance	MAP	Rain	Distance:MAP	Rain:MAP	$\sigma^2 / \sigma^2_{site} / \sigma^2_{period}$
All	6.86	-3.65	-0.88	-0.92	0.51		7.36
	5.40	-2.56	-3.90	-4.55	2.01		
	4.36, 9.36	-6.45, -0.85	-1.32, -0.44	-1.33, -0.52	0.011, 1.00		0.01
	4.49, 9.03	-6.15, -1.05	-1.27, -0.46	-1.32, -0.51	0.049, 0.94		0.00
	***	**0.01**	***	***	**0.045**		
Cow	2.42	-0.63	-0.23	-0.33			3.28
	4.35	-6.01	-2.40	-2.17			
	1.31, 3.59	-0.83, -0.42	-0.43, -0.04	-0.66, -0.033			0.03
	1.74, 3.34	-0.83, -0.43	-0.39, -0.11	-0.67, -0.068			0.02
	***	***	**0.02**	**0.03**			
Elephant	3.91	-0.718	-0.477				6.42
	4.92	-4.90	-3.46				
	2.27, 5.51	-1.01, -0.43	-0.75, -0.19				0.07
	2.82, 4.85	-1.01, -0.43	-0.627, -0.300				0.01
	***	***	**0.002**				
Zebra	2.16		-0.36	-3.01		0.48	2.59
	2.66		-2.49	-2.66		2.44	
	0.56, 3.82		-0.65, -0.075	-5.25, -0.78		0.092, 0.88	0.03
	0.85, 3.86		-0.67, -0.13	-5.13, -1.36		0.20, 0.87	0.00
	0.009		**0.014**	**0.008**		**0.015**	
Impala	-0.56	0.83	0.11		-0.15		0.21
	-2.45	3.44	2.62		-3.48		
	-1.01, -0.11	0.36, 1.31	0.026, 0.19		-0.23, -0.065		0.00
	-1.06, -0.08	0.28, 1.44	0.024, 0.19		-0.25, -0.056		0.00
	0.015	***	**0.009**		***		
Buffalo	0.28			-0.30			1.38
	3.21			-2.88			
	0.092, 0.48			-0.50, -0.095			0.00
	0.15, 0.45			-0.55, -0.093			0.02
	0.008			**0.004**			
Giraffe	0.14			-0.18			0.61
	2.84			-2.82			
	0.04, 0.24			-0.32, -0.054			0.01
	0.063, 0.22			-0.32, -0.055			0.00
	0.01			**0.007**			
All Parasites	9.42	-1.48	-0.99	-2.11			35.07
	5.94	-4.33	-3.58	-4.74			
	6.31, 12.53	**-2.15, -0.81**	-1.54, -0.45	-2.98, -1.24			0.11
	6.69, 11.79	**-2.13, -0.83**	-1.45, -0.52	-2.92, -1.06			0.00
	***	***	**0.001**	***			

Legend:
Estimate
t-value
95% Profile interval
95% Bootstrap interval
Pr(>|t|)

Appendix 2.5. Comparisons between filled water pans and dry sites

Table A2.5.1 GLMM Hurdle model coefficients for comparisons between dry sites and filled water pans throughout the duration of the experimental study at Ol Pejeta Conservancy. There were no significant interactions between filled pans and experimental status for any species except impala.

	All	Elephant	Cow	Zebra	Buffalo	Impala	Giraffe	Parasites
Conditional Model								
(Int.)	3.86 2.08, 5.65 ***	4.22 1.80, 6.65 **0.001**	3.16 1.58, 4.75 ***	3.8 2.59, 5.01 ***	4.28 2.84, 5.73 ***	1.05 0.20, 1.90 **0.016**	1.44 0.92, 1.96 ***	24.95 13.3, 36.56 ***
During	-0.94 -2.8, 0.92 0.323	-0.42 -2.46, 1.6 0.686	-0.59 -2.1, 0.96 0.457	-0.79 -1.98, 0.4 0.197	-0.77 -2.16, 0.6 0.278	-0.51 -1.44, 0.42 0.281	-0.06 -0.44, 0.3 0.742	-5.42 -17.45, 6.6 0.377
Post	-0.92 -2.9, 1.05 0.36	-0.32 -2.4, 1.77 0.767	-1.15 -2.8, 0.45 0.16	-0.63 -1.9, 0.67 0.345	-1.36 -2.85, 0.1 0.074	-0.62 -1.60, 0.35 0.211	-0.03 -0.4, 0.36 0.884	-4.76 -17.45, 7.9 0.462
Filled	2.35 1.47, 3.24 ***	1.17 -1.0, 3.37 0.298	2.29 1.02, 3.56 ***	0.23 -1.2, 1.66 0.755	0.32 -0.97, 1.6 0.622	0.25 -0.01, 0.50 0.062	-0.61 -1.84, 0.6 0.331	14.15 7.66, 20.64 ***
ln(Dist+1)	-0.01 -0.15, 0.1 0.847	0.18 -0.2, 0.56 0.333	0.03 -0.14, 0.2 0.752	-0.12 -0.3, 0.04 0.144	0.02 -0.16, 0.2 0.814	-0.02 -0.04, 0.01 0.133	0.02 -0.07, 0.1 0.662	-0.44 -1.40, 0.52 0.37
During: Filled	0.1 -0.6, 0.77 0.773	-0.12 -1.7, 1.45 0.877	-0.53 -1.65, 0.6 0.351	-0.04 -0.78, 0.7 0.919	0.43 -0.47, 1.3 0.353	-0.38 -0.61, -0.15 **0.001**	0.25 -0.38, 0.9 0.436	-0.24 -5.12, 4.64 0.924
Post: Filled	0.12 -0.6, 0.83 0.739	-1.3 -2.9, 0.26 0.102	-0.1 -1.22, 1.0 0.867	-0.54 -1.39, 0.3 0.215	0.85 -0.3, 1.95 0.133	-0.29 -0.52, -0.06 **0.012**	0.21 -0.4, 0.86 0.524	-1.37 -6.52, 3.78 0.602
Filled: ln(Dist+1)	-0.41 -0.58, -0.2 ***	-0.18 -0.6, 0.24 0.404	-0.28 -0.47, -0.1 **0.005**	0.01 -0.3, 0.33 0.937	-0.22 -0.5, 0.06 0.122	0.03 -0.00, 0.06 0.096	0.07 -0.17, 0.3 0.569	-1.5 -2.74, -0.25 **0.018**
Zero-Inflated Model								
(Int.)	-1.18 -2.7, 0.35 0.131	2.04 0.61, 3.46 **0.005**	3.16 1.61, 4.72 ***	0.15 -1.3, 1.57 0.838	1.39 -0.23, 3.0 0.092	1.5 -15.2, 18.2 0.86	1.37 0.01, 2.72 **0.048**	-1.14 -2.55, 0.26 0.11
During	0.82 -0.7, 2.37 0.297	0.52 -0.75, 1.8 0.422	-1 -2.4, 0.42 0.167	1.06 -0.3, 2.44 0.131	1.11 -0.37, 2.6 0.141	-10.09 -28.4, 8.23 0.28	-0.08 -1.37, 1.2 0.905	0.93 -0.49, 2.36 0.199
Post	0.5 -1.1, 2.14 0.547	-0.27 -1.6, 1.04 0.686	-1.58 -3.06, -0.1 **0.035**	1.51 0.03, 2.98 **0.045**	1.05 -0.5, 2.63 0.19	-11.16 -29.63, 7.3 0.236	-0.1 -1.5, 1.26 0.881	0.61 -0.90, 2.11 0.43
WPC	-1.9 -3.3, -0.48 **0.009**	-2.31 -3.6 – -1.1 ***	-4.52 -5.9 – -3.1 ***	2.84 1.6 – 4.09 ***	-0.36 -1.7 – 1.0 0.596	1.35 -0.42 – 3.1 0.135	2.07 0.45, 3.69 **0.012**	-2.02 -3.36, -0.69 **0.003**

(cont.)	All	Elephant	Cow	Zebra	Buffalo	Impala	Giraffe	Parasites
ln(Dist+1)	-0.05	-0.03	-0.14	0.09	-0.03	0.02	0.05	-0.04
	-0.2, 0.09	-0.2, 0.16	-0.3, 0.04	-0.06, 0.2	-0.2, 0.16	-0.23, 0.27	-0.1, 0.2	-0.16, 0.09
	0.51	0.734	0.121	0.223	0.721	0.889	0.522	0.589
During: Filled	-0.5	-0.15	0.18	-0.75	-0.03	-0.79	0.11	-0.45
	-1.5, 0.49	-1.1, 0.83	-0.86, 1.2	-1.5, 0.01	-0.98, 0.9	-1.82, 0.23	-0.9, 1.12	-1.38, 0.48
	0.325	0.766	0.737	0.052	0.944	0.128	0.827	0.342
Post: Filled	-0.78	-0.44	-0.14	-0.51	0.86	0.24	0.02	-0.71
	-1.87, 0.3	-1.4, 0.54	-1.2, 0.94	-1.35, 0.3	-0.29, 2.0	-0.89, 1.37	-1.0, 1.06	-1.71, 0.30
	0.163	0.378	0.805	0.231	0.141	0.676	0.976	0.168
Filled: ln(Dist+1)	0.3	0.49	0.67	-0.81	0.22	-0.37	-0.43	0.33
	0.03, 0.57	0.26, 0.73	0.43, 0.92	-1.1, -0.55	-0.06, 0.5	-0.76, 0.02	-0.74, -0.1	0.08, 0.58
	0.028	*******	*******	*******	0.12	0.06	**0.007**	**0.01**
Random Effects								
σ^2	2.9	3.16	1.37	1.6	1.01	0.09	0.36	161.88
$\tau_{00\ Site}$	0.06	0	0.06	0.01	0.02	0	0	1.71
$\tau_{00\ Period}$	0.7	0.56	0.3	0.24	0.35	0.18	0	28.58
N	1440	1440	1440	1440	1440	1440	1440	1440
M. R^2 / C. R^2	0.097 / 0.285		0.178 / 0.347	0.055 / 0.182	0.085 / 0.329	0.174 / 0.724		0.098 / 0.240

Appendix 2.6. Dung and parasite composition by host

Figure A2.6.1 A) Dung composition across all sampling sites for Ol Pejeta and Mpala. The rank abundance for herbivore species was the same in both locations, with elephants comprising a greater proportion of total dung density at Mpala. B) Visualization of parasite density as a function of outward distance at water sources and controls at Mpala. Elephants contribute the vast majority of total parasites.

Figure A2.6.2 Dung/parasite density and probabilities at filled pans, experimental pans, and dry sites throughout the experiment (pre-draining, during experiment, and post-refilling). When there was a significant interaction between status and treatment, best fit lines are shaded in color. When filled water sources differed from dry sites, lines are shaded in black/orange.

Figure A2.6.3 Dung/parasite density and probabilities at water sources and dry sites across different rainfall contexts and outward distance from water. When there was a significant interaction between site type (water/dry) and each covariate, best fit lines are shaded in color.

Appendix 3. Chapter 4

Appendix 3.1. Supplementary model selection and linear model tables

Table A3.1.1 PGLS Model selection table for component community MOTU richness. No model performed significantly better than the model containing only the intercept.

(Int.)	Diet	Gut Morph.	log(Body Mass)	log(Home Range)	log(Social Group)	df	logLik	AICc	Delta	Weight
21.76						1	-66.245	134.7	0	0.321
19.44				0.9582		2	-65.765	136.3	1.59	0.145
33.72			-3.261	2.505		3	-64.694	137.1	2.36	0.099
23.45			-0.3056			2	-66.225	137.3	2.51	0.091
22.02					-0.1067	2	-66.244	137.3	2.55	0.09
16.82	+					3	-64.952	137.6	2.88	0.076
20.2				0.97	-0.3283	3	-65.756	139.2	4.49	0.034

Table A3.1.2 Best linear pgls models for node-specific metrics.

Parameters	Betweenness Est. $\pm$ SE T (P)	Closeness Est. $\pm$ SE T (P)	Degree Est. $\pm$ SE T (P)	Eigenvector Est. $\pm$ SE T (P)
(Intercept)	5.819 $\pm$ 2.711 2.146 (0.05)	0.012 $\pm$ 0.006 1.921 (0.08)	16.980 $\pm$ 4.510 3.766 (0.003)	0.115 $\pm$ 0.113 1.021 (0.329)
ln(Grp Size)		0.002 $\pm$ 0.001 1.601 (0.14)		
Gut Type [PR]				-0.093 $\pm$ 0.276 -0.338 (0.742)
Gut Type [R]				0.684 $\pm$ 0.143 4.764 (0.001)
Distance			-0.085 $\pm$ 0.029 -2.914 (0.013)	

	Est.	CI	Est.	CI	Est.	CI	Est.	CI
κ	1.000	(fixed)	1.000	(fixed)	1.000	(fixed)	1.000	(fixed)
λ	0.000	(NA, 0.988)	0.010	(NA, 0.813)	1.000	(NA, NA)	0.000	(NA, 0.979)
δ	2.425	(0.039, NA)	0.033	(0.002, 0.254)	1.814	(0.159, NA)	2.496	(0.044, NA)
Multiple F	0.000		0.136		0.013		0.001	
Adjusted R^2	0.000		0.107		0.370		0.654	

Appendix 3.2. Phylogenetic tree construction and visualization

To account for nematode phylogeny in our diversity and parasite community analyses, we constructed a phylogenetic tree with each tip corresponding to the dominant sequence for each MOTU. First, we created a subset of 164 sequences (from all 64291 sequences) based on the MOTU table generated from metabarcoding. Because multiple sequences were clustered together for each MOTU based on their similarity, we selected the most abundant sequence for each MOTU as the basis of phylogeny construction.

We aligned sequences using the ClustalOmega algorithm from the *msa* package (Bodenhofer et al. 2015). Output sequences were 497 base pairs in length. We then compared candidate phylogenetic models using the *phangorn* package (Schliep 2011), with the best model (lowest BIC) being the Kimura (1980) model (Kimura 1980) with gamma correction (K80+G). We then used this model to produce phylogenetic distances between the top sequences for each MOTU using the dist.dna function in *ape* (Paradis and Schliep 2019). Finally, we constructed trees from the resulting distance matrix using the neighbor-joining method and unweighted pair group method with arithmetic mean (UPGMA). The neighbor-joining tree was more parsimonious and was thus used for all further analyses.

We visualized trees by first plotting tips with the corresponding best parasite identity obtained from Genbank (Figure A3.2.1). We then determined the 'core host' for each parasite by simply extracting the corresponding host with the highest average relative read abundance (RRA) for the given MOTU (Figure A3.2.2). Note that by comparing Figures A3.2.1 and A3.2.2, it is evident that many elephant parasites have extremely low matches to current parasite records.

Figure A3.2.1 Resulting Neighbor-Joining tree constructed from filtered nematode sequences. Leaves are labeled with the best Genbank identity and are colored by their best identity score.

Figure A3.2.2 Resulting Neighbor Joining tree constructed from filtered nematode sequences. Tips are labeled with the 'core' host species, which we assigned based on host with the highest average relative read abundance for the given MOTU. Tips are colored by the number of host species in which the MOTU was detected with RRA > 0.01.

Figure A3.3.3 Tanglegram connecting hosts (left) with their nematode parasites (right), organized by phylogeny. Host species are colored by their gut morphology, with ruminants in blue, hindgut fermenters in red, and pseudoruminants in green.

Figure A3.3.4: Phylogenetic tree estimated from MOTUs with tips overlaid with the 'dominant' host species. Darker host shading indicates increased sharing across host species. Hosts are colored by family.

Appendix 3.3. Analysis using a 99% MOTU clustering threshold

To ensure that our results were robust to the similarity threshold for sequence clustering, we conducted all analyses using a 99% threshold. Here we recreate all figures and statistical tables in the main text using the 99% threshold. Importantly, none of the major conclusions of the study are changed by increasing the clustering threshold. Throughout this appendix, any p-values that are significant using this dataset only (and not the 98% threshold) are shown in bold, red text. Values that are not significant using this dataset, but which are significant using the 98% dataset are shown in bold, blue text.

A3.3.1 Data properties:

Following clustering and filtering we detected sufficient parasite DNA in 230 samples (compared to 224), and the final dataset contained 779 MOTUs (compared to 112).

A3.3.2 Richness results:

Table A3.3.1: Contrasts for each species from the zero-inflated GLMM investigating the relationship between infracommunity MOTU richness and host species identity (following a false discovery rate correction for multiple testing). Animals that tended to have greater richness or phylogenetic diversity are shaded in green, which those with lower richness or lower-than-expected phylogenetic diversity are shaded in blue. This table mirrors Table 2 from the main text.

| | MOTU Richness | | | | | | | | Phylogenetic Diversity | | | |
| **Host** | *Conditional Component* | | | | *Zero-inflation Component* | | | | | | | |
Contrast	Est.	SE	T	P	Est.	SE	T	P	Est.	SE	T	P
G. gazelle	0.63	0.09	7.25	***	-1.05	0.38	-2.73	0.1	-0.64	0.26	-2.47	0.05
G. zebra	0.51	0.09	5.67	***	-0.87	0.36	-2.38	0.1	-1.44	0.26	-5.59	***
Elephant	0.47	0.09	5.43	***	-0.82	0.33	-2.45	0.1	-1.38	0.24	-5.73	***
Oryx	**0.36**	**0.15**	**2.4**	**0.04**	-0.46	0.54	-0.85	0.61	0.34	0.42	0.83	0.52
Donkey	0.02	0.16	0.16	0.93	-0.9	0.54	-1.65	0.24	-0.73	0.37	-1.96	0.14
P. zebra	0.44	0.1	4.51	***	-0.36	0.34	-1.06	0.55	-1.07	0.28	-3.89	0.00
Hartebeest	**0.09**	**0.13**	**0.72**	**0.69**	-0.16	0.38	-0.43	0.74	0.40	0.32	1.28	0.35
Impala	0.52	0.09	5.44	***	-0.02	0.32	-0.06	0.95	-0.73	0.28	-2.64	0.03
Camel	-0.24	0.23	-1.06	0.5	-0.54	0.65	-0.83	0.61	0.65	0.49	1.33	0.35
Eland	-0.11	0.13	-0.88	0.6	-0.18	0.36	-0.52	0.72	**0.44**	**0.30**	**1.48**	**0.30**
Warthog	-0.54	0.21	-2.55	0.03	0.23	0.44	0.51	0.72	**-0.02**	**0.39**	**-0.04**	**0.97**

Dik-dik	-0.27	0.14	-1.91	0.11	0.53	0.32	1.68	0.24	**0.54**	**0.31**	**1.76**	**0.19**
Hippo	**-0.82**	**0.24**	**-3.39**	*******	-0.4	0.49	-0.82	0.61	**0.44**	**0.37**	**1.19**	**0.38**
Giraffe	-0.02	0.16	-0.14	0.93	0.86	0.38	2.25	0.1	**0.28**	**0.37**	**0.76**	**0.53**
H. zebra	-0.04	0.45	-0.09	0.93	0.79	1.1	0.72	0.64	-0.55	1.07	-0.52	0.68
Cattle	-0.8	0.16	-5.06	***	-0.1	0.31	-0.3	0.8	1.66	0.26	6.30	***
Buffalo	-0.07	0.14	-0.53	0.75	0.76	0.34	2.24	0.1	0.10	0.33	0.31	0.80
Kudu	-0.15	0.28	-0.54	0.75	0.79	0.64	1.23	0.47	0.53	0.62	0.85	0.52
Waterbuck	0.04	0.43	0.1	0.93	1.89	1.01	1.88	0.19	1.17	1.07	1.09	0.40

Table A3.3.2 MOTU richness model accounting for host phylogeny and characteristics
Summary tables for the full and reduced models of MOTU richness among individuals of different herbivore species after accounting for phylogenetic relationships using MCMCglmm models (compare to Table 7 in the main text).

Full Model

Niterations = 100,000 Burnin = 5000 Thin = 50	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	0.228	-4.139	3.774	1765	0.915
Diet [Grazer]	0.348	-1.235	1.841	1900	0.641
Diet [Mixed Feeder]	**1.792**	**0.228**	**3.251**	**1900**	**0.032 ***
Gut [Psuedoruminant]	-0.356	-3.108	2.593	1900	0.755
Gut [Ruminant]	-1.702	-4.256	1.363	1900	0.206
ln(Body Mass)	-0.195	-0.808	0.461	1816	0.519
ln(Social Group Size)	0.397	-0.356	1.194	1900	0.286
ln(Home range Size)	-0.006	-0.343	0.367	1900	0.973
Phylogeny	2.656	0.0028	8.411	1900	
Units	8.058	6.217	10.039	1493	
DIC	1717.885				
Lambda (mode)	0.0043	0.0004	0.515		

Reduced Model

Iterations = 100,000 Burnin = 5000 Thin = 50	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	-0.980	-3.220	1.213	1893	0.342
Diet [Grazer]	0.726	-0.423	1.860	1751	0.221
Diet [Mixed Feeder]	**2.033**	**0.818**	**3.133**	**1900**	**0.002 ****
Phylogeny	1.717	0.086	4.343	1900	
Units	7.789	6.101	9.810	1619	
DIC	1722.317				
Lambda (mode)	0.107	0.021	0.372		

Table A3.3.3 PGLS Model selection table for component community MOTU richness. No model performed significantly better than the model containing only the intercept. Compare to Table A3.1.1 in the Appendix.

(Int.)	Diet	Gut Morph.	ln(Body Mass)	ln(Home Range)	ln(Social Group)	df	logLik	AICc	Delta	Weight
150						1	-83.335	169	0	0.28
173.4				-6.616		2	-82.705	170.4	1.43	0.137
213.8			-10.13			2	-82.786	170.6	1.6	0.126
175.5					-13.52	2	-82.915	170.8	1.85	0.111
149.3	+					3	-81.359	170.9	1.92	0.107
162.2	+			-7.307		4	-80.49	173	4	0.038
150						1	-83.335	169	0	0.28

A3.3.3 Phylogenetic Diversity Results

Table A3.3.4 Summary tables for the full and reduced MCMCglmm models of individual Z-values derived from comparing observed parasite phylogenetic diversity to null models. Models account for phylogenetic similarity among herbivore species. Compare to Table 8 in the main text.

Full Model

	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	-2.378	-4.994	0.484	1900	0.098
Diet [Mixed]	-0.173	-1.057	0.716	1900	0.679
Diet [Browser]	0.298	-0.830	1.485	2055	0.553
Gut [Pseudoruminant]	0.349	-1.587	2.487	1900	0.727
Gut [Ruminant]	0.429	-1.639	2.572	1900	0.654
ln(Body Mass)	0.543	-0.008	1.129	1900	0.056 .
ln(Social Group Size)	0.005	-0.221	0.261	1900	0.961
ln(Home Range Size)	-0.243	-0.686	0.215	1900	0.257
MOTU richness	**-0.078**	**-0.102**	**-0.055**	**1900**	**<0.001 *****
Phylogeny	1.571	0.153	3.799	1686	
Units	1.423	1.167	1.711	1900	
DIC	742.592				
Lambda	0.454	0.181	0.771		

Reduced Model

	Posterior Mean	l-95% CI	u-95% CI	Effective Sample Size	pMCMC
(Intercept)	-2.594	-4.362	-0.645	1900	0.011 *
MOTU richness	**-0.081**	**-0.104**	**-0.059**	**2054**	**<0.001 *****
Phylogeny	1.669	0.372	3.361	1900	
Units	1.427	1.157	1.700	1900	
DIC	740.962				
Lambda	0.494	0.290	0.739		

Table A3.3.5 PerMANOVA results for parasite community data in host species
PerMANOVAs are based on distance matrices of a) MOTU prevalence and b) mean UniFrac distances. Terms are listed by sequential sum of squares. Compare to Table 9 in the main text.

		MOTU Prevalence				UniFrac Distances			
Parameter	Df	Seq. Sum Squares	R^2	F Ratio	Pr(>F)	Seq. Sum Squares	R^2	F Ratio	Pr(>F)
Taxonomic Order	**2**	**1.690**	**0.234**	**3.274**	**0.001 *****	**0.965**	**0.274**	**3.332**	**0.001 *****
Gut Morphology	**2**	**1.085**	**0.150**	**2.103**	**0.003 ****	**0.682**	**0.194**	**2.356**	**0.008 ****
Taxonomic Family	**2**	**0.716**	**0.099**	**1.388**	**0.038 ***	0.254	0.072	0.876	0.575
Diet	**2**	**0.876**	**0.121**	**1.697**	**0.012 ***	0.283	0.080	0.976	0.466
log(Group Size)	**1**	**0.546**	**0.076**	**2.115**	**0.003 ****	0.163	0.046	1.129	0.318
log(Body Mass)	**1**	**0.503**	**0.070**	**1.949**	**0.016 ***	0.198	0.056	1.370	0.180
log(Home Range)	1	0.247	0.034	0.957	0.517	0.107	0.030	0.736	0.672
Residual	6	1.548	0.215			0.868	0.247		
Total	17	7.211	1.000			3.519	1.000		

Table A3.3.6 Best linear pgls models for node-specific metrics. Compare to table A3.1.2.

Parameters	Betweenness Est. ± SE T (P)	Closeness Est. ± SE T (P)	Degree Est. ± SE T (P)	Eigenvector Est. ± SE T (P)
(Intercept)	5.881 ± 2.485 2.367 (0.034)	-0.003 ± 0.010 -0.342 (0.740)	15.955 ± 4.165 3.830 (0.002)	0.301 ± 0.133 2.250 (0.048)
ln(Grp Size)				-0.163 ± 0.067 -2.438 (0.035)
ln(Body Mass)		0.001 ± 0.0005 2.900 (0.016)		
Diet [Grazer]		0.004 ± 0.002 2.074 (0.064)		
Diet [Mixed]		0.008 ± 0.002 3.884 (0.003)		
Gut Type [PR]				0.201 ± 0.236 0.848 (0.416)
Gut Type [R]				0.893 ± 0.142 6.286 (<0.001)
Distance			-0.077 ± 0.028 -2.768 (0.017)	

	Est.	CI	Est.	CI	Est.	CI	Est.	CI
κ	1.000	(fixed)	1.000	(fixed)	1.000	(fixed)	1.000	(fixed)
λ	0.000	(NA, NA)	0.261	(0.00, NA)	1.000	(NA, NA)	0.000	(NA, NA)
δ	2.331	(0.035, NA)	0.018	(0.001, 0.12)	1.814	(0.159, NA)	2.362	(0.037, NA)
Multiple F	0.00		0.003		0.013		0.001	
Adjusted R^2	0.00		0.659		0.370		0.776	

Figures

Figure A3.3.1 MOTU richness including and excluding qPCR zeros following rarefaction (A). B. Estimated component community (species-level) richness.

Figure A3.3.2 Phylogenetic diversity was significantly lower than expected, given MOTU richness, across almost all host species; however, it was significantly lower on average in hindgut fermenters compared to ruminants.

Figure A3.3.3 Non-metric multidimensional scaling based on Jaccard distances (A) and UniFrac distances (B) demonstrates strong species-level partitioning in parasite communities. Note that warthogs were substantially different using the Jaccard distances and are shown in panel A inset.

Figure A3.3.4 NMDS plots of species-level data (averaged across individuals of each host species) demonstrates clear partitions based on gut morphology using both Jaccard (A) and UniFrac (B) distance matrices.

Figure A3.3.5 The distribution of host species that each parasite MOTU infected was highly aggregated: more than half infected only one host, while a very small number infected more than 8 different host species.

Figure A3.3.6 The unipartite projection of host and parasite sharing (A) shows the relationships between large mammalian herbivores based on their nematode parasites. Node level metrics showed few relationships to host characteristics, except that mean phylogenetic distance to all other hosts was negatively associated with eigenvector centrality and significantly covaried with degree centrality (p=0.01) (B). Ruminants had higher eigenvector centrality than other gut types (p=0.001) and mixed feeders had higher closeness centrality (for 99% dataset only). Compare to Figure 18 in the main text.

Figure A3.3.7 Experimental single host extinctions revealed that elephants accounted for a disproportionate number of unique links, demonstrating that elephant extinctions would likely lead to substantial loss in parasite diversity. Colors correspond to IUCN red list categories and population trends: D – Domestic, LC – Least Concern, NT – Near Threatened, VU – Vulnerable, and EN – Endangered, with (+) indicating increasing populations, (.) indicating stable populations, and (-) showing declining populations.

Appendix 3.4. Host species data

Table A3.4.1 Host characteristics used as predictors in models of MOTU richness, diversity, and community composition. The majority of data is supplied by the PanTHERIA database(Jones et al. 2009). Where modifications were made (due to missing data or values that were not relevant to our study site), we reference new values.

Species	Order	Family	Binomial	Mass kg	Range km^2	Grp Size	Diet	Gut
Elephant	Proboscidea	Elephantidae	*Loxodonta africana*	3940	594	3.4	M	HGF
Warthog	Artiodactyla	Suidae	*Phacochoerus africanus*	82.5	1.58	3	G	HGF
Hippo	Artiodactyla	Hippopotamidae	*Hippopotamus amphibius*	1520	8[1]	20	G	PR
Donkey	Perissodactyla	Equidae	*Equus asinus*	180	142	4.7	G	HGF
P. zebra	Perissodactyla	Equidae	*Equus burchellii*	277	128	10	G	HGF
Hartebeest	Artiodactyla	Bovidae	*Alcelaphus buselaphus*	164	2.75	20	G	R
Buffalo	Artiodactyla	Bovidae	*Syncerus caffer*	593	63.8	12	G	R
Giraffe	Artiodactyla	Giraffidae	*Giraffa camelopardalis*	955	82.5	15	B	R
Camel	Artiodactyla	Camelidae	*Camelus dromedarius*	488	10.2†	10	B	PR
Waterbuck	Artiodactyla	Bovidae	*Kobus ellipsiprymnus*	202	1.3	12	G	R
Oryx	Artiodactyla	Bovidae	*Oryx gazella*	186	61.5[2]	20	G	R
G. gazelle	Artiodactyla	Bovidae	*Gazella granti*	55	0.71[3]	20	M	R
G. zebra	Perissodactyla	Equidae	*Equus grevyi*	403	6.3[3]	4	G	HGF
Dik-dik	Artiodactyla	Bovidae	*Madoqua kirkii*	4.77	0.077[3]	2.5	B	R
Impala	Artiodactyla	Bovidae	*Aepyceros melampus*	52.3	3.93[4]	15.25	M	R
Eland	Artiodactyla	Bovidae	*Taurotragus oryx*	561	197	20	M	R
Kudu	Artiodactyla	Bovidae	*Tragelaphus strepsiceros*	205	2[5]	12	B	R
Cattle	Artiodactyla	Bovidae	*Bos taurus*	613	10.2	120	G	R

† Since camels at our study site are herded in much the same way as cattle, we assigned the same estimated home range.

Table References:

1. (Stears et al. 2019)
2. (Lehmann 2015)
3. (Grant et al. 1992)
4. (Ford et al. 2014)
5. (Tilahun 2019)

Appendix 4. Chapter 5

Appendix 4.1. Model results using alternate data

To test the sensitivity of our results to certain assumptions, we repeated our analyses using different data sources. First, we eliminated parasite fecal egg counts from the analysis, instead assuming that dung volume was proportional to risk for each host species. Second, we restricted our calculation of contacts to time spent grazing only (i.e. we assumed no parasite transmission from drinking water). Third, we combined these two data types (dung volume and grazing data only). Results were similar: all significant coefficients that matched results in the text are shaded in green. Coefficients that were significant in the text but were not in the modified analyses are shaded in blue. Insignificant coefficients that match results in the main text remain unshaded. Finally, we also tested whether comparisons between filled and experimental pans differed in hotspot effects before or after water was drained and refilled. Results showed that there were no significant differences during these periods.

A4.1.1 Dung volume only

Table A4.1.1 Post-hoc comparisons of the intra- and inter- specific LMMs for the effect of water sources in increasing parasite transmissions relative to dry sites using only dung data (i.e. no assumptions about parasite fecundity were made).

Species	Test	Hotspot Effect	SE	df	Lower CI	Upper CI	T-ratio	P_{adj}	
Buffalo	Intra	3.42	2.33	14.8	-1.54	8.39	1.47	0.19	
Buffalo	Inter	4.32	2.14	12.8	-0.31	8.94	2.02	0.10	.
Cow	Intra	6.53	2.33	14.8	1.57	11.49	2.81	**0.04**	*
Cow	Inter	6.23	2.14	12.8	1.60	10.85	2.92	**0.04**	*
Elephant	Intra	9.17	2.33	14.8	4.21	14.14	3.94	**0.01**	***
Elephant	Inter	9.20	2.14	12.8	4.58	13.82	4.31	**0.01**	***
Giraffe	Intra	-1.09	2.72	22.3	-6.73	4.55	-0.40	0.69	
Giraffe	Inter	1.60	2.45	19.1	-3.53	6.72	0.65	0.52	
Impala	Intra	3.50	2.33	14.8	-1.46	8.47	1.51	0.19	
Impala	Inter	3.96	2.14	12.8	-0.67	8.58	1.85	0.10	.
Zebra	Intra	5.49	2.33	14.8	0.52	10.45	2.36	0.07	.
Zebra	Inter	5.72	2.14	12.8	1.10	10.34	2.68	**0.04**	*

Table A4.1.2 Post-hoc comparisons of the LMM describing the change in estimated hotspot effect after considering intraspecific sharing for dung data only (no parasite counts included).

Species	Δ Hotspot Effect	SE	df	Lower CI	Upper CI	T-ratio	P_{adj}	
Buffalo	0.90	0.33	22	0.22	1.57	2.76	**0.03**	*
Cow	-0.30	0.33	22	-0.98	0.37	-0.93	0.54	
Elephant	0.03	0.33	22	-0.65	0.70	0.08	0.94	
Giraffe	2.75	0.44	22	1.84	3.65	6.30	**<0.001**	***
Impala	0.45	0.33	22	-0.22	1.13	1.40	0.35	
Zebra	0.23	0.33	22	-0.44	0.91	0.71	0.58	

4.1.2 Grazing Activity Only

Table A4.1.3 Post-hoc comparisons of the interspecific LMM for the effect of water sources in increasing parasite transmission relative to dry sites using only grazing data (i.e. no instances of drinking were considered as additional avenues of parasite transmission).

Species	Test	Hotspot Effect	SE	df	Lower CI	Upper CI	P_{adj}	
Buffalo	3.96	1.83	19.6	0.14	7.77	2.16	**0.05**	*
Cow	5.18	1.83	19.6	1.36	9.00	2.83	**0.03**	*
Elephant	6.28	1.83	19.6	2.46	10.10	3.44	**0.02**	*
Giraffe	1.34	2.64	32.8	-4.04	6.72	0.51	0.61	
Impala	4.43	1.83	19.6	0.61	8.25	2.42	**0.04**	*
Zebra	4.62	1.83	19.6	0.80	8.43	2.52	**0.04**	*

Note: The change in the hotspot effect is identical to the one reported in the main text (Table 11).

Table A4.1.4 Post-hoc comparisons of the interspecific LMM for the hotspot effect of water sources in increasing parasite transmission relative to dry sites using only grazing data and dung data (i.e. no drinking data or parasite egg counts included).

Species	Test	Hotspot Effect	SE	df	Lower CI	Upper CI	P_{adj}	
Buffalo	2.97	1.91	18.9	-1.02	6.97	1.56	0.16	
Cow	5.08	1.91	18.9	1.08	9.08	2.66	**0.05**	*
Elephant	6.29	1.91	18.9	2.29	10.29	3.29	**0.02**	*
Giraffe	-0.09	2.74	32.4	-5.67	5.49	-0.03	0.98	
Impala	3.55	1.91	18.9	-0.45	7.55	1.86	0.12	
Zebra	4.74	1.91	18.9	0.74	8.74	2.48	**0.05**	*

Note: The change in the hotspot effect is identical to Table A4.1.2 in this appendix.

4.1.3 Grazing Activity Only for Filled vs. Drained Pans

Table A4.1.5 Post-hoc comparisons for the intra- and interspecific LMMs describing the effect of water sources in increasing parasite transmission at drained and filled pans using only grazing data (no drinking).

Species	Test	Hotspot Effect	SE	df	Lower CI	Upper CI	T-ratio	P_adj
Buffalo	Intra	1.31	1.09	12	-1.08	3.69	1.20	0.38
	Inter	1.48	0.83	14	-0.29	3.26	1.79	0.14
Cow	Intra	2.63	0.62	12	1.29	3.97	4.28	**0.01**
	Inter	2.59	0.61	14	1.27	3.91	4.22	**0.01**
Elephant	Intra	1.73	1.09	12	-0.65	4.11	1.58	0.28
	Inter	3.33	1.62	14	-0.15	6.81	2.05	0.12
Giraffe	Intra	0.40	1.60	12	-3.08	3.88	0.25	0.81
	Inter	1.20	0.84	14	-0.60	3.00	1.43	0.21
Impala	Intra	-1.29	2.10	12	-5.86	3.28	-0.61	0.66
	Inter	0.48	1.55	14	-2.85	3.82	0.31	0.76
Zebra	Intra	2.40	1.08	12	0.05	4.74	2.23	0.14
	Inter	2.30	1.00	14	0.16	4.45	2.30	0.11

Table A4.1.6 Post-hoc comparisons of the LMM describing the change in estimated hotspot effect for filled vs. drained pans using only grazing data (no drinking time was included).

Species	Hotspot Effect	SE	df	Lower CI	Upper CI	T-ratio	P_adj
Buffalo	0.46	0.40	17	-0.39	1.30	1.15	0.53
Cow	-0.04	0.36	17	-0.80	0.71	-0.11	0.91
Elephant	0.08	0.40	17	-0.76	0.93	0.20	0.91
Giraffe	0.77	0.57	17	-0.42	1.96	1.36	0.53
Impala	1.77	0.36	17	1.02	2.53	4.96	**<0.001**
Zebra	-0.10	0.36	17	-0.85	0.66	-0.27	0.91

4.1.4 Pre and post draining comparisons

Table A4.1.7 Post-hoc comparisons of the interspecific LMMs for the hotspot effect of filled pans in increasing parasite transmission relative to drained pans using only grazing data (i.e. no drinking data included).

Species	Period	Hotspot Effect	SE	df	Lower CI	Upper CI	T-ratio	P_{adj}	
Buffalo	Pre	0.542	1.393	45	-2.264	3.347	0.389	0.831	
	Post	-2.622	1.093	45	-4.823	-0.421	-2.399	0.062	.
Cow	Pre	0.548	0.990	45	-1.446	2.543	0.554	0.831	
	Post	0.930	0.934	45	-0.950	2.810	0.996	0.487	
Elephant	Pre	1.429	1.214	45	-1.016	3.874	1.177	0.736	
	Post	-2.796	1.072	45	-4.955	-0.638	-2.609	0.062	.
Giraffe	Pre	0.365	1.488	45	-2.631	3.362	0.246	0.831	
	Post	-2.643	1.654	45	-5.974	0.688	-1.598	0.234	
Impala	Pre	2.386	1.493	45	-0.622	5.393	1.598	0.703	
	Post	-1.014	1.378	45	-3.789	1.762	-0.736	0.559	
Zebra	Pre	-0.229	1.066	45	-2.376	1.918	-0.215	0.831	
	Post	-0.142	1.252	45	-2.663	2.379	-0.113	0.910	

Appendix 4.2. Camera trapping details

Camera traps were set at filled water pans and paired dry sites located at least 1km from any water. The trapping period spanned August 2016-August 2018, and cameras were monitored on a monthly basis. Animal interference, dead batteries, or camera malfunctions reduced the set of usable trap nights; however, each site had at least 200 (Table 4.2.1).

Table 4.2.1 Total trap nights for each water source and paired dry site at each study location.

Location	Observational Data			Experimental Data		
	Dry Site	**Filled**	**Total**	**Drained**	**Filled**	**Total**
Jericho	557	402	959	314	223	537
Kambi	380	204	584	291	100	391
Oscar	227	278	505	262	136	398
Sidai	437	376	813	157	165	322
Tangi	513	480	993	256	289	545
Total	2114	1740	3854	1280	913	2193

The more than 600,000 classifications performed by ~8000 volunteers were aggregated to determine consensus IDs for downstream analyses. To ensure that public IDs were robust, we compared them to IDs by our research team for 2195 image sets. Identifications were similar and highly correlated; our IDs matched 99% of public IDs, and public IDs matched 91% of expert IDs (some missed a second species). Counts were also very similar (Figure A4.2.1).

Figure A4.2.1 1:1 Correlation of public and expert counts demonstrates that on average, members of the public counted the same number of animals in each image as did our team (experts).

To examine our wildlife activity results over time, we averaged the product of total

individuals and duration of each visitation (individuals x seconds) at water sources and dry

sites for each hour of the day. We found substantially higher activity for both herbivores and

carnivores (Figure A4.2.2, Table A4.2.2).

Figure A4.2.2 Herbivore and carnivore activity over time at water sources and dry sites. Herbivores tended to be more active during daylight hours, while carnivores exhibited crepuscular and nocturnal activity.

Table A4.2.2 Post-hoc comparisons of the log-ratio of carnivore activity (individual * seconds) at water sources compared to dry sites. Spotted hyena, jackals, and lions had ~25 times more activity near water than away from it, while other carnivores showed no difference.

Species	Hotspot Effect	Hotspot Effect′	SE	df	Lower CI	Upper CI	T-ratio	P_{adj}	
Spotted Hyena	3.20	24.59	1.17	11.1	0.62	5.78	2.73	**0.046**	*
Striped Hyena	-0.01	0.99	1.17	11.1	-2.59	2.57	-0.01	0.995	
Aardwolf	0.23	1.26	1.17	11.1	-2.35	2.81	0.20	0.991	
Jackal	3.28	26.64	1.17	11.1	0.70	5.86	2.80	**0.046**	*
Cheetah	0.55	1.74	1.17	11.1	-2.03	3.13	0.47	0.991	
Leopard	0.37	1.44	1.17	11.1	-2.22	2.95	0.31	0.991	
Lion	3.30	27.16	1.17	11.1	0.72	5.88	2.81	**0.046**	*

Appendix 4.3. Database search on host-parasite records

To compare metabarcoding results to host-parasite records in the literature, we compiled data from three major sources: 1) The London Natural History Museum's host-parasite database (Gibson et al. 2005), 2) The Global Mammal Parasite Database (Nunn and Altizer 2005), and 3) The checklist of helminth parasites of African mammals (Round 1968).

We restricted our search to parasite species expected to be amplified using the Nem1 and Nem2 primers for the ITS2 region. We counted each record of a host-parasite interaction as a single reference; therefore, references that contained information about multiple hosts and parasites accounted for numerous reference counts. A summary of reference counts for each host species and parasite family is shown in Figure A4.3.1. Together, we collected data on 297 different parasite groups, of which 257 were parasites identified to genus and species. We excluded entries that were listed to genus-level, as parasite sharing could not be determined (e.g. *Trichostrongylus sp.* may refer to many parasites; leaving such entries in the dataset may lead to networks that indicate a higher degree of sharing than in reality).

Figure A4.3.1 Total number of references for each parasite family included in our search.

Figure A4.3.2 Comparison of unipartite projections of hosts connected by shared parasites for both metabarcoding and literature data.

Figure A4.3.3 A) Comparison of Jaccard similarity values for each focal host included in our analyses. B) Metabarcoding and literature Jaccard distances were strongly correlated (ρ=0.73). Metabarcoding suggested a higher degree of sharing in points shown below the black 1:1 line, while literature suggested higher sharing in points above the line.

Appendix 4.4. Questing tick abundance at water sources and dry sites

We performed tick drags on addition to dung surveys along radial transects that extended outward from water sources and paired dry sites. A 1m x 1m drag cloth was towed behind a researcher for each of three 50m intervals extending from water (or center of each dry site). We also repeated drags in the opposite direction to increase sampling area. Larvae, nymphs and adult ticks were collected and counted at each 50m interval.

We aggregated tick counts by summing across the six outward transects within each 50m distance interval before conducting analyses (total drag length per observation = 600m). We used negative binomial GLMMs to model questing tick abundance for larvae, nymphs, and adults, including site (water vs dry) as a fixed effect, and period (n=7) and location (n=5) as random effects.

Table A4.4.1 Coefficients, standard errors, and statistics for each negative binomial GLMM of questing tick abundance.

Predictors	Larvae				Nymph				Adult			
	Log-Mean	SE	Z value	p	Log-Mean	SE	Z value	p	Log-Mean	SE	Z value	p
Intercept (Dry Site)	5.21	0.32	16.17	**<0.001**	1.72	0.5	3.79	**<0.001**	-0.96	0.4	-2.31	**0.02**
Water Source	-1.73	0.21	-8.41	**<0.001**	-0.41	0.1	-2.98	**0.003**	-0.26	0.2	-1.17	0.24
Random Effects												
σ^2/ σ^2_{Site}/ σ^2_{Period}	0.97 / 0.25 / 0.24				0.61 / 0.82 / 0.23				1.41 / 0.51 / 0.27			
Observations	198				198				198			
R^2_M / R^2_C	0.340 / 0.562				0.025 / 0.642				0.008 / 0.361			

Figure A4.4.1 Comparisons of ticks at filled water pans and paired dry sites for each tick life stage. Asterisks denote significant paired differences in tick abundance between the two site types. Violins illustrate the highly aggregated distribution of tick counts, overlaid by group means and standard errors.