RESEARCH ARTICLE

Climate change drives loss of bacterial gut mutualists at the expense of host survival in wild meerkats

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Abstract

Climate change and climate-driven increases in infectious disease threaten wildlife populations globally. Gut microbial responses are predicted to either buffer or exacerbate the negative impacts of these twin pressures on host populations. However, examples that document how gut microbial communities respond to long-term shifts in climate and associated disease risk, and the consequences for host survival, are rare. Over the past two decades, wild meerkats inhabiting the Kalahari have experienced rapidly rising temperatures, which is linked to the spread of tuberculosis (TB). We show that over the same period, the faecal microbiota of this population has become enriched in Bacteroidia and impoverished in lactic acid bacteria (LAB), a group of bacteria including *Lactococcus* and *Lactobacillus* that are considered gut mutualists. These shifts occurred within individuals yet were compounded over generations, and were better explained by mean maximum temperatures than mean rainfall over the previous year. Enriched Bacteroidia were additionally associated with TB exposure and disease, the dry season and poorer body condition, factors that were all directly linked to reduced future survival. Lastly, abundances of LAB taxa were independently and positively linked to future survival, while enriched taxa did not predict survival. Together, these results point towards extreme temperatures driving an expansion of a disease-associated pathobiome and loss of beneficial taxa. Our study provides the first evidence from a longitudinally sampled population that climate change is restructuring wildlife gut microbiota, and that these changes may amplify the negative impacts of climate change through the loss of gut mutualists. While the plastic response of host-associated microbiotas is key for host adaptation under normal environmental fluctuations, extreme temperature increases might lead to a breakdown of coevolved host–mutualist relationships.

KEYWORDS
climate change, disease ecology, gut microbiome, host–microbe interactions, lactic acid bacteria, tuberculosis

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1 | INTRODUCTION

Wildlife populations across the globe are under increasing pressure from both climate change (Bellard et al., 2012) and infectious pathogens (Daszak et al., 2000; Jones et al., 2008; Tompkins et al., 2015). The link between climate change and infectious disease was recognized over two decades ago (Altizer et al., 2013; Baker et al., 2022; Harvell et al., 1999, 2002), with climate change altering disease dynamics through its impact on host susceptibility and demography, as well as parasite virulence and range shifts (Cohen et al., 2020; Kafle et al., 2020; Maynard et al., 2015; Paniw et al., 2022). However, little is known about how climate change and pathogen dynamics interact to shape the gut microbiota, a diverse bacterial community that is crucial for host physiological homoeostasis, pathogen defence, and thermal tolerance (Alavi et al., 2020; Honda & Littman, 2016; Jaramillo & Castañeda, 2021). Recent evidence suggests that the gut microbiota is affected directly and indirectly by climate change (Greenspan et al., 2020; Sepulveda & Moeller, 2020; Watson et al., 2019; Williams et al., 2022). However, whether such responses are adaptive or maladaptive for animal hosts remains unclear, because longitudinal time-series data that document gut microbial responses to climate change in natural populations, and the consequences for host fitness, are rare.

Gut microbial responses to climate change are proposed to be adaptive where microbiota plasticity in response to environmental cues allow hosts to rapidly adapt to ecological change (Alberdi et al., 2016). For instance, gut microbial commensals may increase host thermal tolerance (Jaramillo & Castañeda, 2021; Moeller et al., 2020) or confer protective effects from pathogens via modulation of host immunity or direct competition (Belkaid & Hand, 2014; Fleischer et al., 2022; Harris et al., 2019). Protective bacterial strains or groups of strains may therefore be expected to increase within the host population in response to a novel pressure, either through direct selection for beneficial bacterial strains, or through behavioural or developmental mechanisms that promote the colonization and transmission of beneficial strains (Moeller & Sanders, 2020).

Alternatively, changes to gut microbiotas may be maladaptive to the host when fitness outcomes for hosts and commensal microbes are mismatched (Carmody et al., 2021; Sommer et al., 2017). In such cases, strains that have either neutral or negative impacts on hosts may be able to increase in abundance, potentially outcompeting beneficial strains in the process. For example, experimentally warming temperatures during frog development leads to a breakdown in host-associated microbial homoeostasis and increases mortality later in life (Greenspan et al., 2020), suggesting that climate change might promote antagonistic interactions between hosts and gut microbes. However, distinguishing between adaptive and maladaptive microbial responses can be challenging for observational systems. Identifying whether climate-driven shifts in microbial communities are adaptive in natural populations requires long-term sampling of a population experiencing climate change, and where individual-level data on survival are available.

Meerkats (*Suricata suricatta*) inhabiting the South African Kalahari have experienced both climate change and an increase in tuberculosis (TB) prevalence over the past two decades (Müller-Klein et al., 2022; Paniw et al., 2019, 2022; Parsons et al., 2013; Van de Ven et al., 2020). Maximum temperatures have increased since the 1990s (Figure 1a) at a rate five times faster than the global average (Pattinson et al., 2022), which decreases pup survival and social group size (Paniw et al., 2022; Van de Ven et al., 2020). Mean seasonal rainfall, which is strongly linked to both fecundity and survival (Groenewoud & Clutton-Brock, 2021), is highly variable but has not changed over the past two decades (Figure 1b) (Pattinson et al., 2022). Nevertheless, drought years occur regularly and high maximum temperatures add to the negative fitness impacts of low rainfall, with interactions between temperature and rainfall strongly shaping meerkat survival and reproductive fitness (Paniw et al., 2019).

In conjunction with climate change, this population of Kalahari meerkats has also faced the rapid spread of TB, which is likely driven by the increasing frequency of extreme temperature events (Paniw et al., 2022). Meerkat TB is caused by the bacterial pathogen *Mycobacterium suricattae*, a member of the *M. tuberculosis* species complex, and is characterized by latent infections that progress to clinical signs in about 25% of cases (Donadio et al., 2022; Drewe et al., 2011; Müller-Klein et al., 2022). TB disease was first officially reported in this population in 1997, yet TB prevalence and social exposure, which can lead to latent infections, rapidly increased hence, with up to 40% of the population showing signs of disease in 2016 and 2017 (Figure 1c, as reported in Müller-Klein et al., 2022). Once clinical signs develop, individuals rapidly deteriorate and generally reach terminal stages in approximately 4–7 months (Müller-Klein et al., 2022; Patterson et al., 2021).

Here we link long-term changes in both climate and TB prevalence with shifts in the meerkat faecal microbiota from 1141 faecal samples collected from 235 individuals between 1997 and 2019 (Figure S1a). Faecal samples were collected from individually recognizable meerkats as part of a long-term research program established in 1993 (Clutton-Brock & Manser, 2016). Sampled meerkats were selected because they survived to at least 1 year of age and belonged to a social group that persisted at least 2 years. In total, our samples originate from 12% of the monitored population (Figure S1b). Previous studies on the gut microbiota of this population indicate that its ecology is rather unusual in that it undergoes strong shifts in composition across the day, probably in response to circadian patterns in foraging schedules (Risely et al., 2021a). These strong diurnal oscillations result in relatively low individuality in the gut microbiota, especially over long time periods (Risely et al., 2022). We applied 16S ribosomal RNA (rRNA) gene amplicon sequencing of the V4 region to generate amplicon sequence variants (ASVs; Risely et al., 2022). Faecal microbiota composition was quantified per sample (Figure 1d), and samples were paired with climate and biological metadata (Figure 1e–i), including season (Figure 1h), long-term maximum temperatures (Figure 1e) and rainfall (Figure 1f) over the previous year, and TB status at the time of sampling (Figure 1g).
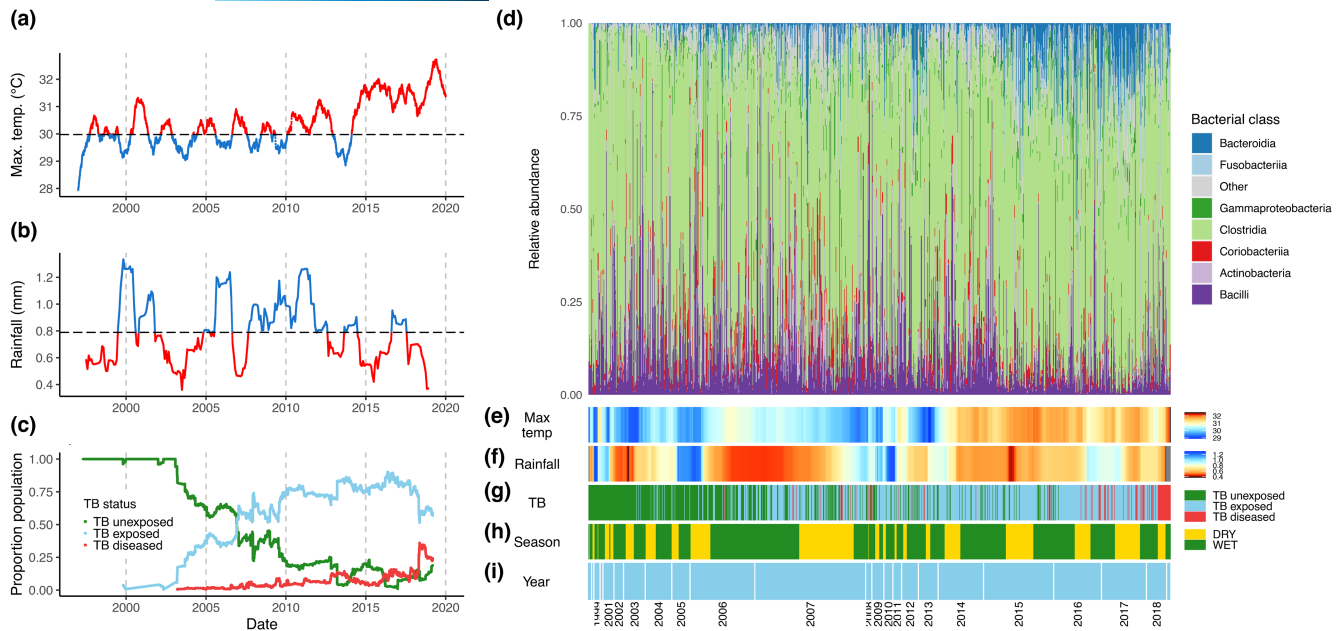
FIGURE 1 Trends in climate and TB prevalence across the study period (left panel) and composition of sample microbiota and associated metadata (right panel). Left panel: Rolling means (1 year window) in (a) maximum temperatures; (b) daily rainfall; and (c) prevalence in TB unexposed, exposed, and diseased individuals across the study period. Dashed vertical lines in (a, b) represent the means across the entire study period. Right panel: (d) faecal microbiota composition coloured by bacterial class; (e) mean daily maximum temperature in the year prior to sample collection; (f) mean daily rainfall in the year prior to sample collection; (g) TB stage at time of sample collection; (h) season the sample was collected (rainy and hot summer vs. dry and cool winter); (i) year of sample collection. TB, tuberculosis.

This study aims to (1) document shifts in the gut microbiota over the two-decade study period and test the extent to which observed changes reflect within-individual responses versus changes across generations; (2) identify climatic and biological predictors of groups of co-occurring genera that have increased or decreased over the study period; and (3) examine which groups of co-occurring taxa predict meerkat survival. We hypothesize that if observed changes are beneficial for meerkats, taxa that are associated with increased survival in the face of both TB and climate change should become more abundant over the study period. Yet, a loss of beneficial taxa or increase in taxa associated with higher mortality would rather indicate a breakdown in microbial homoeostasis.

2 | METHODS

2.1 | Study population and sample collection

The study population inhabits the Kalahari Desert region in South Africa (−26.96 S, 21.83 E). Individuals from this population are individually marked and have been monitored three to five times a week since 1993 by the Kalahari Meerkat Project (Clutton-Brock & Manser, 2016). A total of 1141 faecal samples from 235 individuals were analysed in this study, which were also analysed as part of Risely et al. (2021a). Seventy per cent of samples were collected from meerkats belonging to eight social groups, while the remaining 30% were collected from across 34 different social groups. Faecal

samples were collected from the ground immediately after a meerkat was observed defecating, and were stored next to an icepack and frozen within 8 h. For long-term storage, samples prior to 2008 were mostly frozen at −80°C (n = 461), or, after 2008, freeze-dried and kept at room temperature (n = 648). Freeze-drying is proposed to be preferable to freezing for biological samples where cold-chain transport and storage cannot be guaranteed, because freeze-dried DNA is robust to long-term storage and temperature changes (Bensch et al., 2022; Blekhman et al., 2016; Shen et al., 2021), even when stored at room temperature (Mareninov et al., 2013; Molnar et al., 2021; Wu et al., 2012).

We experimentally tested the effect of storage methods on quantified microbiota composition (see below for microbiota profiling methodology). Nine faecal samples from captive meerkats were either frozen or freeze-dried, storage method had negligible effects on measured faecal microbiota composition, with sample identity explaining 93% of variation in beta diversity (p < .001; Figure S6) (Risely et al., 2021a). In addition, we tested microbiota stability on 16 archived faecal samples originally collected from wild meerkats and freeze-dried between 2011 and 2013. The microbiota was quantified first in 2020 and again in 2022 and samples were kept at 4°C without buffer during this period. Microbial composition did not change over this 2-year period, with sample identity explaining 95% in beta diversity (Figure S7). These results indicate that the effect of sample identity overwhelms methodological effects. Moreover, reported trends are apparent in both frozen and freeze-dried samples (Figure S8).

2.2 | DNA extraction with internal standard, 16S rRNA amplification and sequencing

Before DNA extraction, NAP buffer was added to all faecal samples (Menke et al., 2017). A subsample of $0.6 \pm 0.05\,\mu g$ (wet) was taken, and $3\,\mu L$ of ZymoBIOMICS Spike-in Control I (High Microbial Load) was added to each subsample prior to DNA extraction. This internal standard consists of cells belonging to *Imtechella halotolerans* and *Allobacillus halotoleranss*, two species rarely found in faecal microbiota communities. An internal standard allows us to quantify ratios of absolute abundance by adding a known number of cells to each sample by which to normalize microbiota counts after sequencing. This method measures 16S copy number rather than absolute abundance, but has shown to accurately reflect variation in absolute abundances when care is taken to standardize faecal sample mass (Hardwick et al., 2017; Lin et al., 2019; Stämmler et al., 2016; Tourlousse et al., 2017). We have shown previously with this dataset that sample identity accounts for 90% of variation in estimated bacterial load, while 10% is technical variation (Risely et al., 2021a).

The bacterial genomic DNA was extracted using the NucleoSpin 96 Soil kit (Macherey-Nagel) following the manufacturer's instructions, and the hypervariable V4 region of the 16S rRNA gene was amplified using the primer pair 515 F (5′-GTGCCAGCMGCCGC GGTAA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′). We used the Fluidigm Access Array™ for Illumina Sequencing Systems for indexing and adding Illumina adaptor sequences. After purification (NucleoMag® NGS Clean-up and Size Select; Macherey-Nagel) and quantification (QuantiFlour® dsDNA Systemt; Promega) of barcoded samples, the normalized pooled sample library was sequenced as paired-end run on Illumina MiSeq platform at the Institute of Evolutionary Ecology and Conservation Genomics, Ulm University. Samples were sequenced across four Illumina runs (MiSeq Reagent Kit v2, 500-cycles). Extraction and PCR negative controls were included on all runs.

2.3 | Microbiota bioinformatics and normalization

All sequence reads were processed using QIIME2 version 2020.2 (Bolyen et al., 2018). Sequences were merged, quality filtered, and chimeras were removed using the DADA2 pipeline (Callahan et al., 2016) to generate ASVs (Callahan et al., 2016, 2017). Primers were trimmed and reads were truncated at 244 (forward) and 235 (reverse) base pairs. ASVs were assigned a taxonomy using SILVA version 132 (Pruesse et al., 2007). A tree was built using QIIME2's fragment insertion method (Janssen et al., 2018). ASVs were filtered if they were not bacteria, not assigned to a phylum (as these are assumed to be spurious), or if they were classified as mitochondria or chloroplasts. We used the function *decontam::isContaminant* (Davis et al., 2018) using the 'prevalence' method to identify sand microbes using 15 sand samples as a reference, and to remove them from the dataset. We then divided taxa counts per sample by *A. halotolerans*

abundance per sample to quantify ratios of absolute abundance across samples (described in Risely et al., 2021a). Both *Allobacillus* and *Imtechella* were then removed, and all further analyses were conducted on normalized reads. Because some samples had very high relative abundances of spike-in, we only retained samples for which read depth of the true microbiota (minus the internal reference) was over 5000. For downstream analyses, these counts were then CLR transformed to account for compositionality.

2.4 | Sample metadata

Most variables associated with each sample are described in detail in Risely et al. (2021a). Here, our aim was to quantify the contributions of date, TB, short- and long-term climate variables and body condition, to explaining any changes in microbiota composition across the study period, while controlling for important sources of variation identified in that study. The most important predictors of taxa abundances identified were time of day, meerkat age, season, as well as sequencing depth, sequencing run and sample storage. We therefore included these variables in all models (described below). In addition to variables already described, we quantified mean rainfall and maximum temperatures across the year prior to sample collection to capture long-term climate conditions. We chose a 1-year rolling window because shorter time periods (e.g. 6 months) captured seasonal dynamics that were already represented by season.

Tuberculosis stage (unexposed, exposed, diseased) was recorded for each sample, described in detail in Müller-Klein et al. (2022). In brief, individuals were considered diseased if they showed external clinical signs of TB such as characteristic submandibular lumps, and were considered exposed from the first day they were observed cohabiting with a diseased individual. Many individuals later in the study were born into groups affected by TB and therefore considered exposed at birth. Body condition was calculated as the residuals of a generalized additive mixed model (GAMM) predicting body weight against age, month and time of day (age being the strongest predictor of weight), applying all available weight data from the individuals included in this study. Weather data were sourced from the Van Zylsrus weather station, approximately 25 km away from the study area, and from long-term gauge and satellite data collected by the National Oceanic and Atmospheric Administration (NOAA) Climate Prediction Center (CPC) available at https://psl.noaa.gov/data/gridded. Daily maximum temperatures between the two sources were highly correlated ($r = .82$) yet daily rainfall was not correlated between the two datasets, reflecting the highly localized nature of rainfall (mainly thunderstorms) in the Kalahari. We therefore applied monthly mean values of rainfall to represent regional rainfall, which were highly correlated across the two datasets ($r = .76$). We measured time of day in reference to sunrise because this is more biologically meaningful than chronological time of day. We calculated daily sunrise times using *suncalc::getSunlightTimes* (Agafonkin & Thieurmel, 2017). We categorized season into wet (October to April) and dry (May to September).

2.5 | Statistical analysis of the faecal microbiota

To examine how overall composition (beta diversity) changed over the study period, we used a constrained ordination on a weighted Unifrac distance matrix and applying the function *vegan::capscale*. We excluded rare taxa represented by under 100 reads to allow model convergence. We included only time of day and year in the model, as we were specifically interested in quantifying variation attributed to these two variables. Statistical analysis of constrained ordinations was done using *vegan::anova.cca*. To examine the effect of meerkat identity on CAP scores, we modelled CAP2 values (which represented the effect of year) against meerkat identity as a random effect using a standard generalized linear mixed model and calculated the intraclass correlation coefficient (ICC) to quantify the contribution of meerkat identity. To examine average microbiotas per individual, we collapsed microbiota data by individual using *phyloseq::merge_samples*, which calculates the mean abundance per ASV per individual.

To visualize nonlinear trends in genus-level abundances across the study period, we fitted GAMMs to CLR transformed counts with a Gaussian distribution, with the function *mgcv::bam*. We limited analysis to 29 of the most abundance genera, which made up 77% of all reads, but also included *Lactobacillus*, a rarer taxon with only 15% prevalence, because we were particularly interested in lactic acid bacteria (LAB) taxa. We explicitly modelled date with a smoothing factor and included time of day, month, age, sequencing depth, storage, sequencing run, sequencing depth, meerkat ID (random effect) and social group (random effect) as covariables. We did not account for temporal autocorrelation (as we did in Risely et al., 2021a) because the relatively low-resolution sampling regime, combined with a highly dynamics community, meant there was very little temporal autocorrelation to account for.

While GAMs can account for nonlinear trends, they cannot account for covariation in taxa responses. To account for this, we applied generalized linear latent variable models (GLLVMs) using the function *gllvm::gllvm* (Niku et al., 2019), to CLR transformed counts, with two latent variables and applying a Gaussian distribution. GLLVMs are superior to methods such as differential abundance analysis because they model joint responses of species to explanatory variables and aim to tease apart the causes of species co-occurrence. We fitted two models: one that included only year of sample collection as the major variable of interest, controlling for time of day, sequencing depth and storage method, and including meerkat identity and social group as random effects. This model quantified overall trends in microbiota responses across the study period. To identify drivers of these trends, we fitted a second model that replaced date with a range of biological and climatic variables. Due to the number of potential predictor variables and some co-correlation between them (especially climatic variables) we conducted a model-selection process based on Akaike information criterion (AIC) to select the most parsimonious model. The best-fitting model included season as a binary factor (rather than short-term rainfall and temperature measures, which were highly correlated with each other), mean maximum temperature over the previous year, TB stage, body condition, age at sampling, as well as time of day, sequencing depth, storage and individual ID and social group as random effects. Only rainfall over the previous year was excluded, as it did not significantly add to model fit. The explanatory power of variables of interest was assessed by comparing variance explained to a model excluding season, mean maximum temperature over the previous year, TB stage, and body condition.

2.6 | Co-occurrence networks

To identify clusters of co-occurring genera, we examined co-occurrence associations between the 30 most common genera using the package NetCoMi (Peschel et al., 2021). Because network associations are strongly dependent on bacterial abundance or sequencing depth (e.g. if bacterial load is high, both taxa 1 and taxa 2 will have high abundances even though they are negatively associated, leading to false positive associations), we accounted for overall bacterial load by CLR transforming microbiota data for both co-occurrence networks and survival analyses. Only associations with a Spearman's correlation above 0.2 were retained. Co-occurring clusters were identified using the NetCoMi::netAnalyze function using the fast and greedy method. ASV counts were then collapsed by cluster membership for survival analysis.

2.7 | Survival analyses

To test the effects of faecal microbiota composition on future survival, we fitted mixed-effect Cox proportional hazard models using the function *coxme::coxme* (Therneau, 2022), with meerkat ID as a random effect. The model predicted whether a meerkat was alive or dead 6 months after each faecal sample was taken (dead $n = 316$, alive $n = 791$). We chose 6 months because over shorter periods sample sizes for dead versus alive were very unequal, while over longer periods the predictive power of models was lower (model AIC 6 months: 343; model AIC 1 year: 387). We based model section on AIC, using MuMIn::dredge (Barton, 2009) to compare the AIC of all potential candidate models, and selected final terms for inclusion by model averaging across top models ($\Delta AIC < 4$).

3 | RESULTS

3.1 | Faecal microbiota composition has shifted over two decades

We analysed 1141 faecal samples collected between 1997 and 2019 to examine trends in beta (between individual) diversity over the study period. We grouped samples into four study periods to account for unequal sampling across years (pre-2005, $n = 152$; 2005–2010, $n = 424$; 2010–2015, $n = 192$; 2015–2019, $n = 373$; Figure 2a), and fitted a constrained ordination (dbRDA) model on weighted
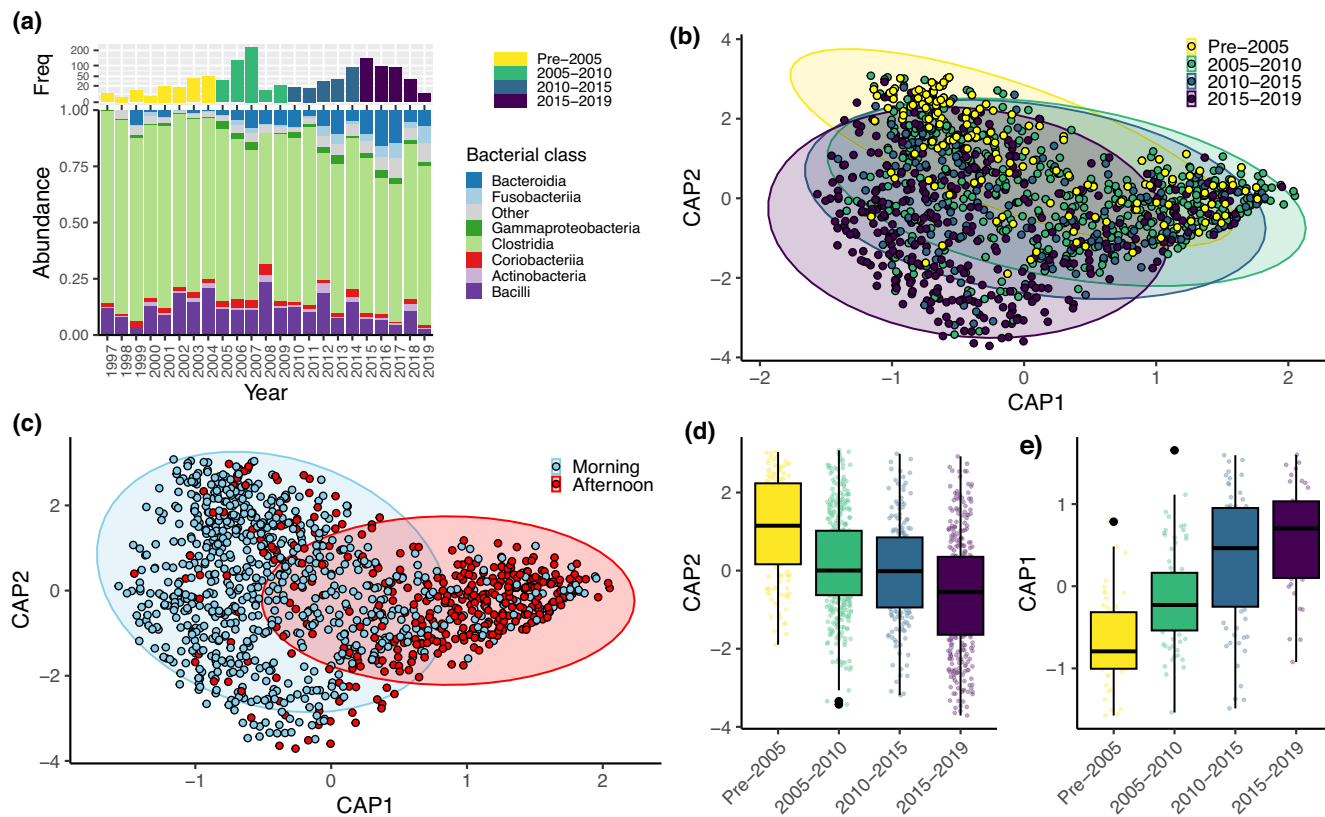
FIGURE 2 Faecal microbiota composition over the study period. (a) Microbiota composition by year, coloured by class, overlayed by a histogram of sample frequency per year, coloured by study period. (b) A constrained ordination (dbRDA) of faecal microbiota composition mapping variation to study period and (c) time of day. Ellipses show 90% confidence intervals around the group centroid. (b, c) Show the same ordination but coloured differentially; (d) CAP2 values from (b, c) grouped by study period; and (e) CAP1 scores from a separate constrained ordination where faecal microbiota is grouped by meerkat identity and modelled against study period (represented in this model by CAP1).

Unifrac distances. Beta diversity was associated with both study period (Figure 2b; $F = 27.4$, $p < .001$) and, as reported previously (Risely et al., 2021a), time of day (Figure 2c; $F = 78.8$, $p < .001$), with study period being largely captured by the second axis of variation (CAP2, Figure 2d).

We next examined the extent to which shifts in beta diversity reflect within-individual responses versus changes across generations. We tested the extent of within-individual responses by quantifying the contribution of individual identity towards explaining CAP2 scores presented above, whereby a contribution of zero would indicate that changes occurred within individuals, while a contribution of one indicates a highly individualized microbiota that does not shift over time. We examined generational responses by collapsing gut microbiota data by individual identity ($n = 235$ meerkats; an average of 4.8 samples per meerkat) to test whether the gut microbiota of individuals born earlier in the study period were, on average, different to those born later in the study period. We complemented this approach by examining shifts in the microbiotas of juveniles (<6months old) and adults (>6months old) separately, because changes to juvenile microbiotas may have long-lasting effects on microbiota development into adulthood.

Individual identity explained less than 1% variation in CAP scores (model ICC < 0.01), indicating that microbiota shifts represent

within-individual changes. Nevertheless, the average composition of the meerkat gut microbiota per individual also shifted over the study period (Figure 2e). Meerkats born later in the study period had, on average, higher abundances of Bacteroidia and lower abundances of Bacilli than meerkats born earlier in the study period (Figure S2). Moreover, the faecal microbiotas of both adults and juveniles changed similarly over the study period (Figure S3). These results indicate that shifts in gut microbiota over the study period reflect both within-individual changes over time as well as transgenerational changes to microbiotas of meerkats born later in the study period.

3.2 | Bacteroidia increased and lactic acid bacteria declined over the study period

To identify specific genera that increased or declined over the study period, we applied both nonlinear GAMMs and GLLVMs to the 30 most common genera. GAM modelling indicated most genera underwent nonlinear dynamics over the study period (Figure S4), with increases to *Bacteroides* (Figure 3a), *Fusobacterium* (Figure 3b) and *Rikenellaceae RC9 gut group* (Figure 3c), and declines in LAB, including *Lactococcus* (Figure 3d), *Pediococcus* (Figure 3e) and *Weissella* (Figure 3f).
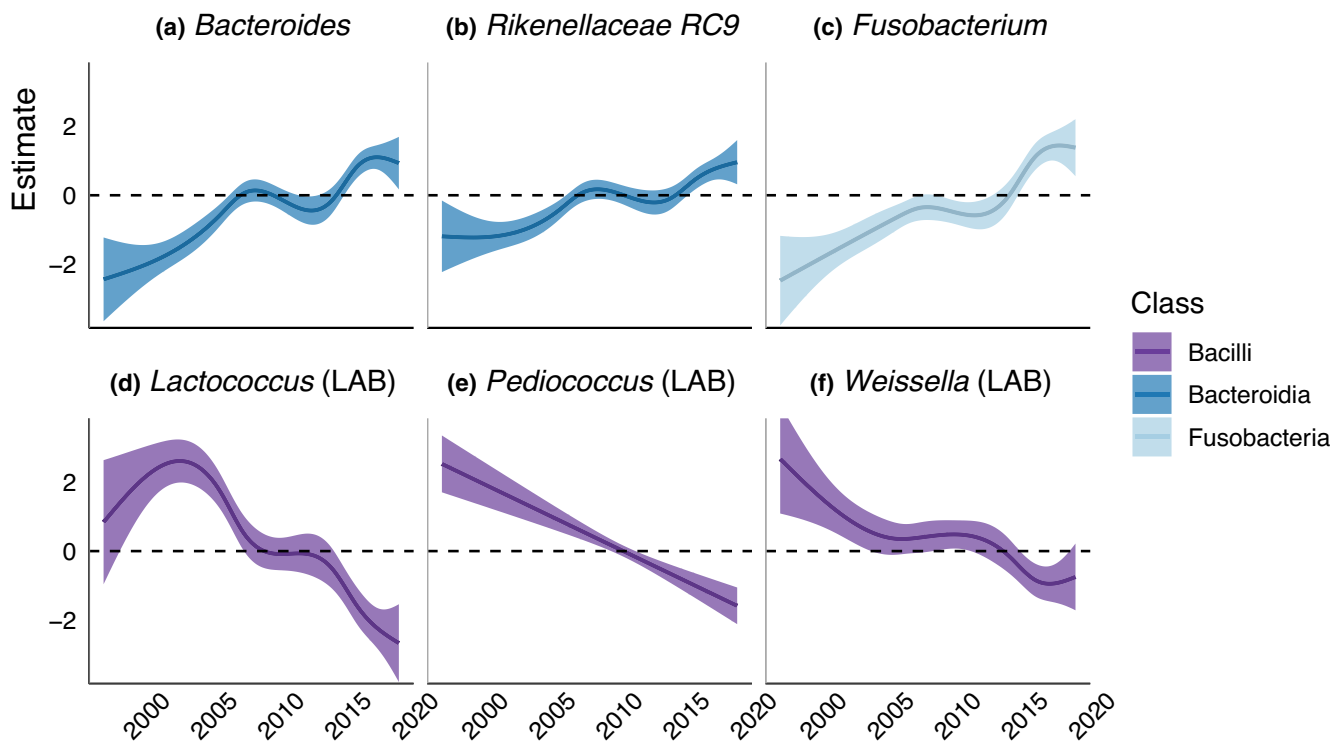
FIGURE 3 Nonlinear trends relative to the mean (dashed line) for six genera that either increased (a–c) or decreased (d–f) over the study period. See Figure S4 for all genera.

GLLVMs are a type of joint species distribution model and account for co-occurring groups of genera (Niku et al., 2019). We first fitted a simple model that included date as the predictor variable of interest, controlling for time of day, sequencing depth and storage method as fixed effects, and meerkat identity and social group as random effects to identify linear trends in genus-level abundances. Similar to the results from the GAMMs, genera from the class Bacteroidia (*Bacteroides*, *Parabacteroides* and *Rikenellaceae RC9 gut group*) and *Fusobacterium* increased (Figure 3a, top), whereas LAB (*Lactococcus*, *Pediococcus*, *Lactobacillus*, *Weisella*, and *Enterococcus*) declined over the study period (Figure 4a, bottom).

3.3 | Ecological correlates of increasing and decreasing genera

In order to identify ecological correlates of taxa that either increased or decreased over the study period, we fitted a second GLLV model that replaced sample date with a range of climatic and biological variables (Figure 4b–f). The most parsimonious model indicated that genera that increased over the study period tend to be more abundant in the dry season (Figure 4b), and when the mean maximum temperature over the previous year was high (Figure 4c). They were also more abundant in individuals that were TB exposed (Figure 4d) or had clinical signs of TB disease (Figure 4e), and, to some extent, in hosts with poorer body condition (Figure 4f). In contrast, genera that decreased over the study period tended to be more common

in the wet season, when long-term maximum temperatures are low, and in TB unexposed individuals (Figure 4a–e). They were largely not associated with body condition (Figure 4f). Average rainfall over the previous year did not improve model fit, but when included in the model was significantly associated with some genera, including a positive association with *Peptoclostridium* and *Clostridium sensu stricto 1*, and a negative association with *Christensenellaceae R7 group* and *Rikenellaceae R9 group* (Figure S5).

We examined residual correlations between genera after accounting for the effect of predictor variables, which highlighted strong positive correlations between increasing genera, with weaker positive correlations between decreasing taxa, and negative correlations between groups of increasing and decreasing clusters (Figure 4g). Climatic and biological variables explained 17% of covariation in genus abundances, and the model had a mean predictive power (R^2) of 38% across genera (max.=76% for Bacteroides, min.=6% for *Escherichia–Shigella*).

3.4 | Long-term maximum temperatures predict microbiota shifts better than long-term rainfall

To understand how interactions between long-term rainfall and maximum temperatures impact the faecal microbiota, we fitted a GLLV model that categorized faecal samples into whether they had been collected during a period with below or above average maximum temperatures and rainfall over the previous year ($n=281$),
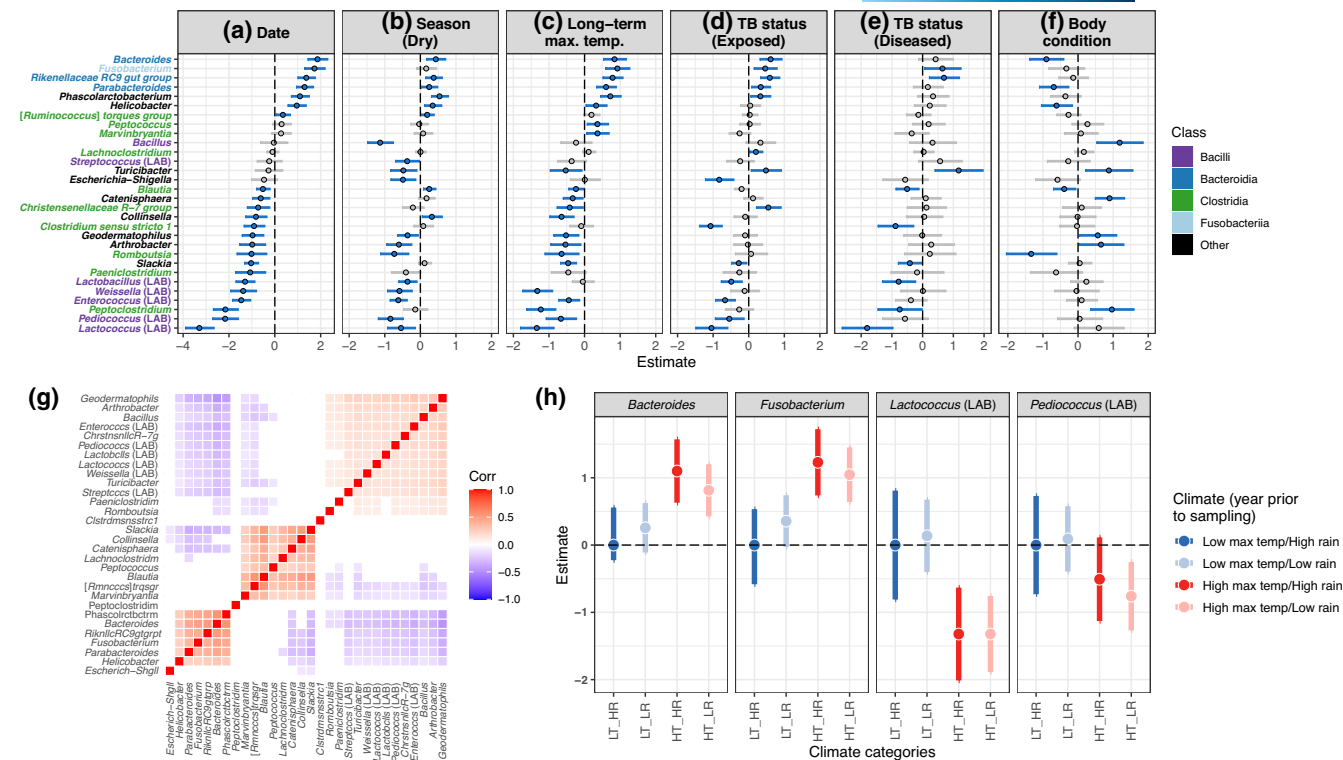
FIGURE 4 Genus-level changes to the faecal microbiota over the study period and associations with climate variables, TB infection status and body condition. (a–f) Estimates from two generalized latent variable models quantifying shifts in groups of co-occurring taxa by (a) date of sample collected; and, from a separate model, (b) season (dry vs. wet); (c) mean maximum temperature over the previous year; (d) TB status (exposed); (e) TB status (diseased); and (f) age-corrected body condition. Genus names are coloured by their taxonomic class and ordered by whether they have increased (top taxa) or decreased (bottom taxa) over the study period. (g) Residual correlations of taxa responses with red indicating positive correlations and blue negative correlations. Nonsignificant correlations are blank. (h) The effect of interactions between long-term maximum temperatures and rainfall on four key genera that increased or decreased over the study period. TB, tuberculosis.

and three other combinations (low temp./high rainfall, $n=281$; low temp./low rainfall, $n=323$; high temp./high rainfall, $n=175$; high temp./low rainfall, $n=351$). We expected that the impact of high maximum temperatures would depend on rainfall, yet this was not the case (Figure 4h). Abundances of genera exhibiting the strongest changes (*Bacteroides*, *Fusobacterium*, *Lactococcus* and *Pediococcus*) were shaped largely by maximum temperatures over the previous year, irrespective of long-term rainfall (Figure 4h).

3.5 | Co-occurring clusters of LAB are positively associated with survival

We tested whether changes to faecal microbiota composition conferred survival benefits using Cox proportional hazard models that predicted survival 6 months after each sample was taken. We included co-occurring clusters of genera as predictor variables, and controlled for known predictors of mortality including age, social status, TB disease status, season and mean rainfall and maximum temperature over the previous year, and included meerkat identity as a random effect. Due to the large number of possible predictors, the final model was built based on model averaging across optimum models (ΔAIC<4; Table S1).

Co-occurrence analysis identified five major groups of co-occurring clusters (Figure 5a), with *Bacteroides* and *Phascolarctobacterium* acting as hubs that were strongly associated with each other and negatively associated with other clusters. We collapsed faecal microbial counts based on cluster membership and modelled their association with future survival. We found that the cluster made up of LAB *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Lactobacillus* and *Weissella* had a small negative effect on meerkat mortality, that is, increased abundance of this cluster predicted higher meerkat survival (hazard ratio=0.6, $p=.04$; Figure 5b; Table S2). No other microbial cluster predicted survival, with nonmicrobial factors (TB status, social status, age, season and body condition) all having stronger associations with survival (Table S2). Effects of these nonmicrobial factors on survival are in line with findings from previous studies on this population (Groenewoud & Clutton-Brock, 2021; Paniw et al., 2019, 2022; Thorley et al., 2020).

4 | DISCUSSION

Meerkats inhabiting the Kalahari region in South Africa have experienced rapid climate change and increasing TB prevalence over
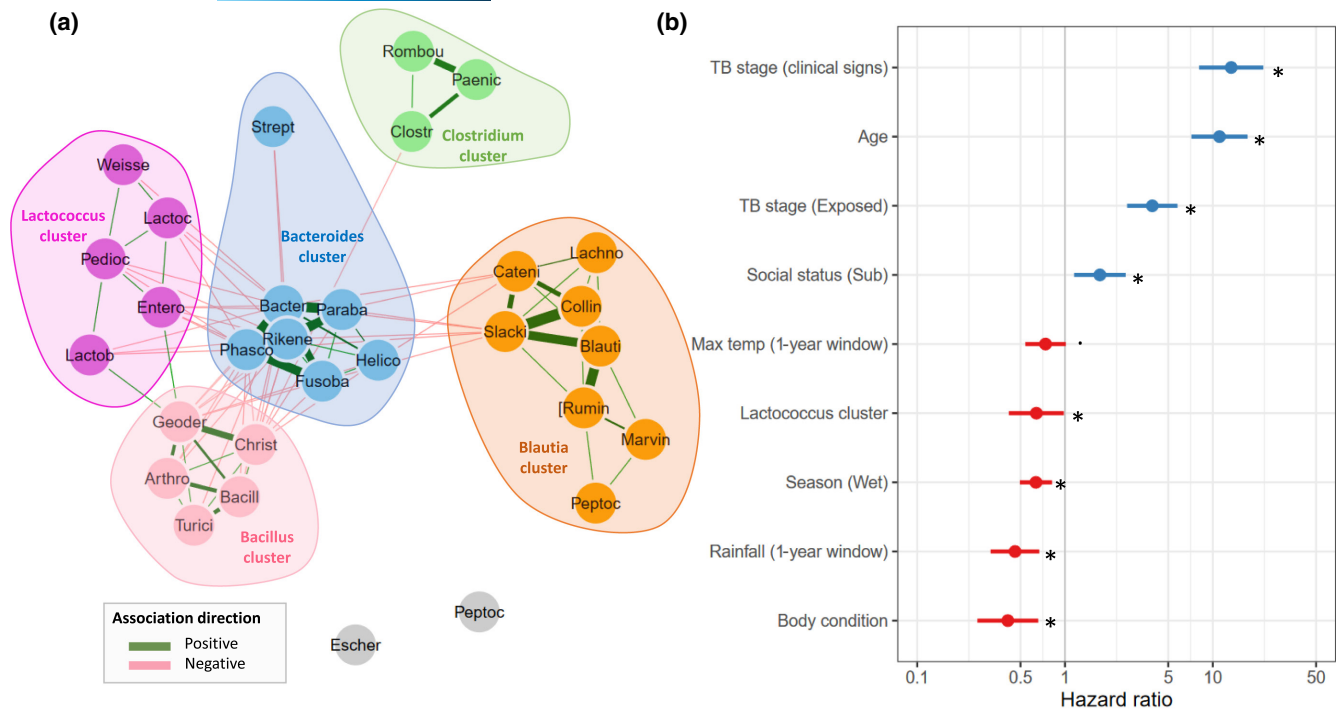
FIGURE 5 Associations of co-occurrence network clusters with mortality risk. (a) Co-occurrence network of the most abundant genera represented by the meerkat faecal microbiome, coloured and labelled by cluster membership. (b) Estimates from a Cox proportional hazards model predicting survival 6 months after each sample was taken. Positive estimates (blue) indicate a positive correlation with mortality (i.e. more likely to be dead), whilst negative values (red) indicate negative correlations (i.e. more likely to be alive). Stars indicate significant terms. See Table S2 for statistics.

the past two decades. We found a marked shift in microbiota composition during the same period, with increases in *Bacteroides* and *Fusobacterium*, and declines in lactic acid bacteria, such as *Lactococcus*, *Lactobacillus* and *Pediococcus*. Microbiota remodelling occurred within individuals over time, yet comparable shifts in juvenile microbiotas over the study period indicate transgenerational shifts may also play a role in maintaining a *Bacteroides*-rich microbiota. Taxa that increased were associated with the dry season, TB exposure and disease, and poor body condition, which were all linked to lower future survival, both in this study and others (Paniw et al., 2022; Van de Ven et al., 2020). We provide evidence that extreme temperatures over the previous year, rather than long-term rainfall, underpin the observed shifts, with further evidence that this relationship may be mediated by TB. The expansion of disease-associated pathobiome and simultaneous loss of mutualists LAB taxa, which were positively associated with future survival, indicate nonadaptive changes in the host-associated gut community.

This study provides the first evidence that climate change is altering gut microbiotas in a longitudinally monitored wildlife population. These findings are in line with cross-sectional studies that have also found support for climate-induced changes to gut microbiotas in wild polar bears and anoles (Watson et al., 2019; Williams et al., 2022), and experimental studies that demonstrate the direct link between temperature and the gut microbiota across taxonomically diverse hosts (Chevalier et al., 2015; Li et al., 2023; Moeller et al., 2020). A major goal for future research is to identify

the proximate mechanisms underpinning the link between climate change and changes in gut microbiotas. These are likely to encompass a combination of climate-induced changes in diet (e.g. in polar bears; Watson et al., 2019), host physiological stress and immunity (Stothart et al., 2019) and pathogen dynamics (Mori et al., 2021). In this population of Kalahari meerkats, our findings indicate that long-term maximum temperatures drive alterations to the gut microbiota via impacts on meerkat body condition and TB status. However, we cannot rule out a role for climate-induced diet changes, which was not measured in this study, nor completely disentangle the direct and indirect roles of temperature, TB and body condition, which requires higher resolution temporal sampling of individuals (e.g. weekly) as they progress across known disease states.

A major aim of our study was to examine whether shifts in the gut microbiota conferred survival benefits to hosts, which would be expected if host-associated microbiotas respond adaptively (for hosts) under novel selection pressures. We found that co-occurring groups of LAB were linked directly with higher survival likelihood, yet abundances of most LAB genera, with the exception of *Streptococcus*, declined over the study period. LAB taxa abundances were higher in the wet season, when long-term maximum temperatures were lower, and in TB unexposed individuals. Surprisingly, they were not linked to body condition, suggesting that their link to future survival is not mediated by condition. LAB taxa secrete lactic acid, which lowers gut pH, modulates immune responses and prohibits the colonization of inflammatory

and pathogenic microbes (Balcázar et al., 2007; Bravo et al., 2022; Campana et al., 2017), suggesting pathogen defence may be the primary evolutionary advantage of hosting LAB taxa. Many LAB strains are thought to be transmitted vertically via mammalian milk (Qi et al., 2022), and, in humans, their prevalence is thought to be supported by the consumption of fermented food such as yoghurts and dairy milk (Pasolli et al., 2020). While a decline in the sampling of milk-fed pups over the study period may provide one explanation for declines in LAB taxa, our results do not support this theory, as abundances of LABs in this population were not associated with age, and, moreover, the vast majority of samples were taken from meerkats post-weaning. An alternative explanation for their decline may include maternal stress, which has been linked to lower levels of LAB taxa in infants (Zijlmans et al., 2015), and which could reduce vertical transmission over generations.

The decline of LAB taxa was accompanied by a rise in co-occurring members of Bacteroidia and Fusobacteria. These taxa were associated with factors directly linked to higher mortality, including the dry season and TB exposure and disease, and therefore this emerging dominant group likely represents the pathobiome, defined as a group of microbes associated with a reduced health status and loss of host control over its microbiota (Bass et al., 2019). Our findings that extreme temperatures promote the expansion of the pathobiome support those from an experimental study on frogs, which showed that higher temperatures increase mortality by promoting gut microbial dysbiosis (Greenspan et al., 2020). Unexpectedly, the pathobiome was not directly associated with higher mortality, hinting that the negative effects of dysbiosis are specifically due to losses of health-associated taxa. Our findings indicate that novel selection pressures, such as those presented by climate change, promote the disruption of a eubiotic stable state, leading to the development of antagonism between hosts and their commensal microbiota (Johnson et al., 2021; Toby Kiers et al., 2010). The mechanisms by which hosts and microbiotas coevolve under such scenarios is a key area for future research and will be crucial for understanding the consequences of climate change on host and microbe evolution.

In conclusion, we present evidence for a distinct shift in faecal microbiota composition in a wild population of meerkats that has faced multiple stressors over the past two decades, most notably climate change and TB risk. These changes appear to be maladaptive for meerkats because LAB, which are associated with increased future survival, on average decreased over the study period. Our study represents the first evidence from a long-term study system that climate change is not only altering the demography of wild populations, but also impacts their microbial symbionts in a way that may exacerbate the negative impacts of global change on host fitness.

AUTHOR CONTRIBUTIONS

Alice Risely, Simone Sommer, Nadine Müller-Klein and Dominik W. Schmid were involved in conceptualization. Alice Risely and Nadine Müller-Klein were involved in formal analysis. Alice Risely and Kerstin Wilhelm were involved in investigation. Simone Sommer, Marta B. Manser and Tim H. Clutton-Brock were involved in resources. Marta B. Manser and Tim H. Clutton-Brock were involved in data curation. Alice Risely was involved in writing—original draft. Alice Risely, Nadine Müller-Klein, Dominik W. Schmid, Simone Sommer and Marta B. Manser were involved in writing—review and editing. Simone Sommer was involved in project administration. Simone Sommer, Alice Risely, Marta B. Manser and Tim H. Clutton-Brock were involved in funding acquisition.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All raw sequences are available at NCBI BioProject PRJNA764180. Processed data and R code to replicate analyses can be downloaded at https://zenodo.org/record/8102850 (Risely, 2023).

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SUPPORTING INFORMATION

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