# Faecal microbiome varies with social group, age and bovine tuberculosis infection in the European Badger (*Meles meles*)

Natasha Meadows[1], Richard J Delahay[2], Robbie A. McDonald[3], Sian Powell[2], Kevin Hopkins[4], Laura Arnold[2], Xavier A. Harrison[1]*

[1] Centre for Ecology and Conservation, University of Exeter, Penryn TR10 9FE, UK

[2]Animal & Plant Health Agency, National Wildlife Management Centre, Sand Hutton, York, YO41 1LZ, UK

[3]Environment and Sustainability Institute, University of Exeter, Penryn TR10 9FE, UK

[4]Institute of Zoology, Zoological Society of London, London NW1 4RY

*Address for Correspondence x.harrison@exeter.ac.uk

KEYWORDS: Wildlife disease; host-microbe interactions*; Mycobacterium bovis*, microbial ecology.

## ABSTRACT

Host-associated microbes are key components of animal health and physiology, with particular importance for determining responses to pathogen infection. The gut microbiota is highly variable at the individual level, being shaped by a multitude of factors including diet, social behaviour, and age. Yet the relative influence of these traits on microbiota composition, and the consequences of this variation for host responses to pathogens remain unresolved. Here we investigate factors that shape the faecal microbiome in European badgers (*Meles meles*). Badgers act as a wildlife reservoir of *Mycobacterium bovis*, a zoonotic pathogen and the causative agent of bovine TB (bTB) in cattle, but the potential role of the microbiome in shaping patterns of infection and severity of disease are not known. Analysing 165 samples from 72 badgers over 3 years, we found that social group and age were key determinants of faecal microbiota composition, and identified several bacterial genera associated with bTB infection. Investigation of microbiome dynamics at the individual level using longitudinally sampled badgers revealed marked heterogeneity in age-dependent microbiome trajectories that were not detectable from population level trends in chronological age. These data provide novel insights into the factors associated with microbial community dynamics in complex wild systems and highlight the need for individual-level and longitudinal approaches to studying host-microbiome associations.

## INTRODUCTION

Host-associated microbial communities play an important role in biological processes including digestion and diet selection (Cholewińska et al., 2020; Trevelline & Kohl, 2022), social behaviour (Vernier et al., 2020), ageing (Wilmanski et al., 2021) and susceptibility to pathogens (King et al., 2016). Bacterial and fungal microbiotas vary widely among species, often with host phylogeny (Harrison et al., 2021; Youngblut et al., 2019), but also display substantial intraspecific variation within and among populations (Amato et al., 2013; Ingala et al., 2019; Kueneman et al., 2014; Liukkonen et al., 2024). Understanding the causes and consequences of variation in the microbiome at the individual level remains a major research goal (Worsley, Videvall, et al., 2024), especially for ecologically complex natural systems (reviewed in Marsh et al., 2024). Recent research focussed on wild animal microbiomes has revealed that individual traits such as age (Kohl et al., 2019; Rojas et al., 2023), diet (Jones et al., 2023), social status (Heitlinger et al., 2017) and social interactions (Pfau et al., 2023; Raulo et al., 2024) shape the structure and stability of individual symbiotic microbial communities, often in a sex-specific manner (Leech et al., 2021; Pafčo et al., 2019). However, we still lack a comprehensive understanding of the relative importance of these traits in determining microbiota dynamics, and of the potential impact of variation in host-microbe associations on key life history traits such as susceptibility to disease. Addressing these gaps in our knowledge is vital, both for understanding the importance of the microbiome in shaping host life history, and for predicting patterns of disease severity and transmission using metrics of host microbiota dynamics.

Here we investigate host-microbiome-pathogen associations in the European badger *(Meles meles)*, using samples from a large cohort of wild animals (n = 165 samples from 72 badgers over 3 years). The European badger has been the subject of considerable research, in part owing to its role in the epidemiology of *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB) in domestic cattle. Here, we explore the relationship between the faecal microbiome and host age, sex, body mass, social group membership, and *M. bovis* infection status. Across much of their geographic range badgers live in social groups where they engage in physical interactions, including playing and allogrooming (Fell et al., 2006), and sharing underground dens (setts) (Böhm et al., 2009). Recent work in wild mice has highlighted the relative importance of both direct (social) and indirect (space sharing)

interactions as transmission routes for anaerobic and aerotolerant suites of bacteria, respectively (Raulo et al., 2024), and increased rates of contact are thought to be a key mechanism driving the emergence of social group-level microbial signatures (Sarkar et al., 2020). Social group effects are common in wild systems, particularly in non-human primates (Bennett et al., 2016), though their effects can be diluted by other traits such as seasonal variation (Orkin et al., 2019) and potentially diet. Previous work on badgers has revealed significant within-group dietary specialisation, where individuals occupy unique foraging niches within social groups despite having access to similar resources (Robertson et al., 2014). Divergence in diet among members of the same group is expected to create heterogeneity in gut microbiome composition and dynamics (Jones et al., 2023) whilst higher rates of social interactions within groups will act simultaneously in the opposite direction by increasing the sharing of microbes (Raulo et al., 2024). Here we use the badger social system to quantify group and individual-level variation in the faecal microbiome, with a particular focus on understanding spatial and temporal dynamics linked to group affiliation and age.

Evidence from animal models has indicated that hosts can lose the ability to regulate their intestinal microbiota with age, leading to dysbiosis, which in turn accelerates senescence (Li et al., 2016), increases inflammation and compromises immunity (Thevaranjan et al., 2017). Previous work on badgers has identified age-related declines in immunity (Beirne et al., 2014), and the co-variation of rates of actuarial senescence with inbreeding and bTB infection (Hudson et al., 2023). Therefore, we expect that in badgers, advancing age should be associated with changes in overall microbiome composition linked to senescence, and that this effect might covary with infection status. Studies from other wild animal systems have found both a negative relationship between microbiota richness and chronological age (spotted hyenas, *Crocuta crocuta;* Rojas et al., 2023), or a relatively weak effect of age on declines in bacterial richness (Seychelles warblers, *Acrocephalus sechellensis*; Lee et al., 2025). Age-related declines in diversity at the *population level* may arise through selective disappearance of individuals with highly diverse microbiotas, or shifts in diet and behaviour at the individual level. However, few studies can disentangle these competing explanations due to the challenges of obtaining high-resolution longitudinal samples from wild hosts (Marsh et al., 2024).

Understanding the drivers of microbiome diversity is important in the study of infectious diseases because of the direct relevance of the microbiota to host immunity. Diverse microbiotas may be protective against infection by pathogens (Dillon et al., 2005; Knutie, 2020; Rebollar et al., 2016; Stecher et al., 2010), by acting either through direct mechanisms such as consuming nutrients that invading pathogens require (Spragge et al., 2023), or indirectly by priming the host immune system. Age-related declines in microbiome diversity (Rojas et al., 2023) may therefore be associated with increased pathogen susceptibility. Tuberculosis in badgers is typically a chronic infection primarily affecting the lungs and lymph nodes, with clinical presentations ranging from latent infection (with no apparent clinical signs) to systemic, disseminated disease affecting multiple organs (Gallagher & Clifton-Hadley, 2000). The primary route of transmission among badgers is believed to be inhalation of infectious respiratory droplets during close contact, but badgers with progressed disease may shed *M. bovis* in saliva, sputum, urine, faeces and pus from infected wounds (Clifton-Hadley et al., 1993). Given the most likely route of infection, we expect any association between the faecal bacterial microbiota (as a proxy for the gut microbiota) and bTB infection to be representative of indirect effects, where shifts in the gut microbiome may compromise systemic immunity (Thaiss et al., 2016) and make individuals more susceptible to both infection and the subsequent progression of disease. Previous work on humans has found clear differences in gut microbiome between healthy patients and those infected with *Mycobacterium tuberculosis* (Hu et al., 2019). Closely related *M. bovis* can also cause disease in humans and has a significant economic impact on livestock production in several parts of the world (Barnes et al., 2023). In some regions, including parts of the United Kingdom and Ireland, the infection circulates between badgers and cattle (Akhmetova et al., 2023; Crispell et al., 2019). Understanding the link between microbiome dynamics and bTB infection in badgers is important, as variation in the microbiome may be a driver of individual heterogeneity in infection status and severity.

Here we test the hypotheses that in European badgers i) social group membership determines gut microbiome composition, consistent with similar studies in other group-living animals (Sarkar et al., 2020), but that we will still observe substantial among-individual heterogeneity in gut microbiome within groups consistent with foraging niche partitioning; and ii) both the probability of infection with *M. bovis* and advances in host age will be

associated with shifts in microbiota composition. Specifically, we test the prediction that *M. bovis* infection will be associated with lower overall microbiome diversity. Collectively, our findings provide a novel assessment of the factors associated with microbiome variation in a wild social mammal.

## METHODS

*Sample Collection*

We use data from the long term Woodchester Park field study in Gloucestershire, UK, where the population of European badgers has been continuously monitored for almost 50 years. Boundaries of all social group territories are mapped every spring using bait marking (Delahay et al., 2000) and badgers are trapped at active setts (Cheeseman & Harris, 1982). Captured badgers are anaesthetised and identified with a unique tattoo given at first capture. Data on sex, mass and age class (juvenile < 1 year, adult >1 year) are recorded and each individual is assigned to a social group based on the territory in which they were captured. For those first caught as juveniles, we estimate known age to the nearest year from subsequent trapping data. During the routine examination of captured badgers from 2016-2018, a rectal swab was taken and stored dry at -20°C in the plastic swab tube prior to dispatch to the laboratory for characterisation of the faecal microbiome.

Mycobacterium bovis *(bTB) Infection Status*

The infection status of an individual is determined at every capture event using a combination of tests. These include *M. bovis* culture from tracheal and oesophageal aspirates, urine, faeces and swabs from any wounds, and testing of venal blood samples for antibodies (using the DPP® VetTB assay or the now discontinued Brock TB Stat-Pak test (Chembio Diagnostics Systems Inc., Medford, New York 11763, USA)) and a cellular immune response (the IFNγ-release assay;Ashford et al., 2020). Using a site-wide estimate of the prevalence of infection for each year, and the reported sensitivity and specificity of the assays, we calculated the cumulative contemporaneous posterior probability of infection for each individual badger, at each capture point during the study (Powell *et al*. in prep). This approach identified the first positive result in each individual's capture history and thereafter calculated the cumulative probability of infection at subsequent captures whilst accounting for potential false positive and false negative results, or disease resolution. In contrast to a binary classification system (i.e. infected or uninfected) this approach was deemed more likely to capture the impact of disease progression and time on the microbiome,

hypothesising that higher posterior probabilities of infection (either due to multiple positive assays, or longer infections) will be of greater impact.

*Sequencing and Bioinformatics*

We sent badger rectal swabs for DNA extraction at Fera, York, UK using Qiagen DNEasy kits followed by a heat inactivation step of 80°C for 20 minutes. This protocol inactivates *M. bovis,* rendering samples safe for processing in Biosafety Level 2 (BSL-2) facilities while preserving the integrity of DNA for subsequent molecular investigations (Doig, 2002; Mtafya et al., 2023; Sabiiti et al., 2018). We prepared sequencing libraries using a modified form of the protocol in Kozich et al., (2013), where we amplified a ~250bp section of the v4 region of the 16S rRNA gene (detailed protocol in Harrison et al., 2019). We used a Miseq nano cartridge to quantify inter-library variation in concentration, then pooled equimolar libraries for sequencing on a v2 chemistry 500bp PE MiSeq run.

We used the software R (R Core Team, 2023) for all downstream bioinformatics and statistical analysis. The package *dada2* (Callahan et al., 2016) was used to call Amplicon Sequence Variants (ASVs) and assign taxonomy using the SILVA v132 database (Quast et al., 2013; Yilmaz et al., 2014), using standard parameters. We used *phyloseq* (McMurdie & Holmes, 2013) to remove all ASVs with no Phylum level taxonomic annotation, as well as any assigned as Chloroplasts (Order), Mitochondria (Family) or Archea (Kingdom) (total = 2604 ASVs). We then used the R package *decontam* (Davis et al., 2018) to identify contaminants present in the negative sequencing controls. Using the 'prevalence' method and a threshold of 0.6, we identified and removed a further 722 ASVs. We then removed ASVs with 10 or fewer total reads, giving a final dataset of 844 ASVs across 165 samples, and a mean library size of 56,101 reads [range 13,569 – 267,515].

*Statistical Analysis*

We collected 165 rectal samples from 72 unique individuals (range 1-8 swabs per badger, where each swab represents a separate capture event). We were able to assign the age at capture for 62 individuals (145 samples) because they were first captured as cubs.

To examine variation in alpha diversity of microbiomes we rarefied libraries to the minimum per-sample read depth (13,569). Rarefaction curves indicated that the number of

species plateaued at about 10,000 reads, suggesting this cut-off sufficiently captures variation in diversity. We used a bivariate response model in the R package *brms* (Bürkner, 2018, 2021) to quantify predictors of bacterial microbiota richness. Here, badger mass and ASV richness were used as responses, with capture year, chronological age, *M. bovis* infection probability, and sex as fixed effect predictors (factors), and social group and badger ID as random intercept terms. This analysis allows us to control for other predictors of mass (e.g. sex and age) that may also influence ASV richness. A significant relationship between mass and richness manifests as a non-zero posterior correlation between these variables after controlling for other predictors. This dataset comprised 145 samples from 62 badgers allocated to 18 different social groups.

To investigate variation in patterns of beta diversity of microbiomes, we generated stacked bar plots of the relative abundance of the five most common bacterial Phyla across sampling years and social groups. We visualised differences in bacterial microbiota composition among social groups and among individuals using PCA ordinations on CLR-transformed community abundances, which allows us to use all sequencing data, even if per-sample sequencing depths differ, whilst also accounting for the compositionality of microbiome datasets (Gloor et al., 2017). Here we used only social groups with at least five samples (n = 131 observations of 54 badgers across 11 social groups) to ensure accurate estimation of group centroids. We used PERMANOVA on CLR-transformed community abundances to test the effects of social group, age, *M. bovis* infection probability and individual ID on microbiome composition (n = 145 samples from 62 known age badgers, age range 1-10).

We used the package *gllvm* (Niku et al., 2019) to fit Generalised Linear Latent Variable models to identify differentially abundant bacterial genera. We restricted the dataset to the top 50 most abundant genera and fitted a model including infection status, age and sex, and an infection x sex interaction, whilst controlling for variation among years (fixed effect) and repeated measures on the same individual (random intercept). We fitted the model to CLR-transformed abundances using a Gaussian distribution and specifying two latent variables.

To further investigate age-related shifts in the microbiome, we restricted the data to badgers of known age that had been sampled on at least three occasions (n = 6 badgers, range 3-8 samples per badger) and plotted microbiome trajectory by age for these

individuals. We also plotted temporal trends for 22 badgers (some of which were not of known chronological age) to investigate shifts in microbiome composition with changes in *M. bovis* infection probability.

## RESULTS

### Effects of Social Group and Age on Bacterial Microbiota Composition

Using data from 72 badgers sampled repeatedly over 3 years, we detected marked variation in bacterial community composition among social groups (Fig. 1A) and sampling years (Fig. 1B). The most abundant bacterial phyla detected in the badger gut microbiome were Firmicutes and Proteobacteria, followed by Fusobacteriota, Bacteroidota and Campylobacterota. Although the relative distribution of these phyla remained broadly stable over time, 2018 shows a large increase in the abundance of Proteobacteria compared to previous years (Fig 1B). PERMANOVA analysis indicated significant effects of social group, sex, chronological age and individual ID on bacterial microbiota composition (all $p < 0.003$; Table 1). Of these, individual ID ($r^2 = 32.5\%$) and social group ($r^2 = 16.5\%$) explained the most variation in microbiota composition. Conversely, there was no evidence for an effect of *M. bovis* infection probability on beta diversity at the population level ($p=0.6$; Table 1). Ordination of CLR-transformed bacterial communities supported these results, revealing marked variation both within- and among different social groups (Fig. 2).

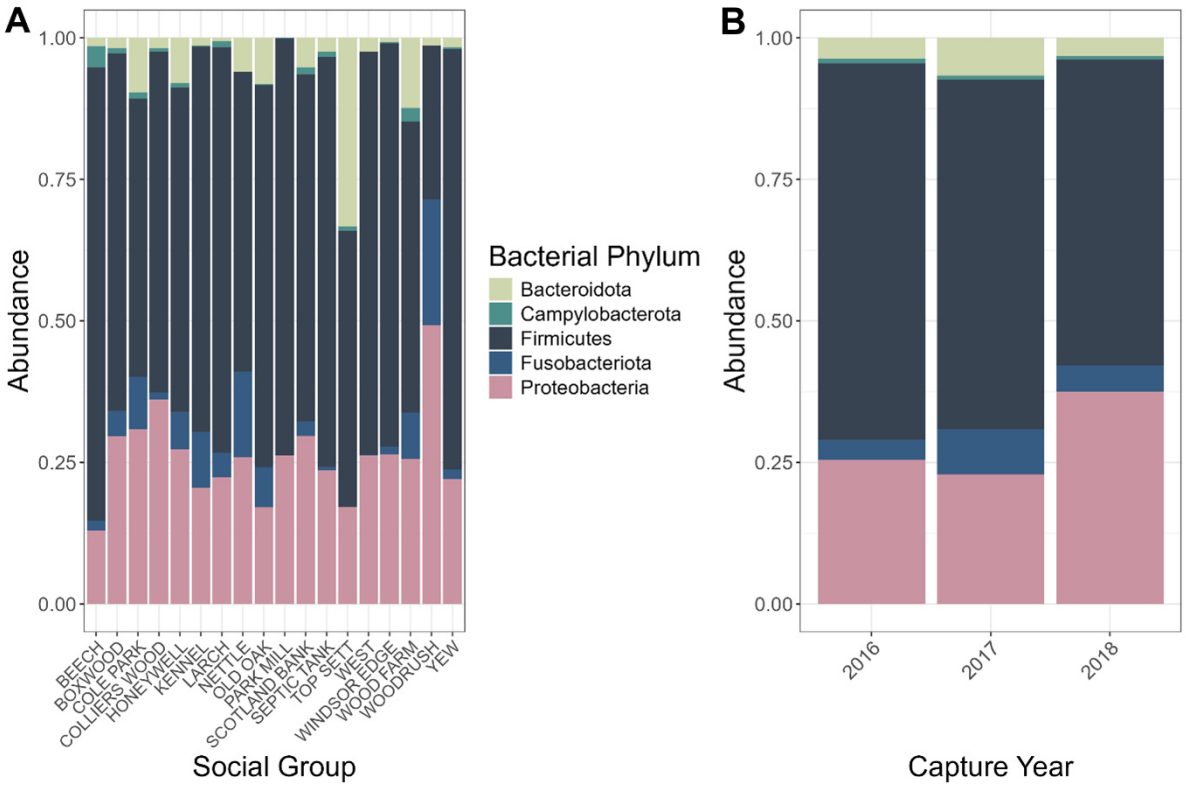


**Figure 1.** Compositional bar plots showing bacterial community structure across **(A)** 18 social groups and **(B)** 3 sampling years. Sample size was 165 badgers across all years and social groups.

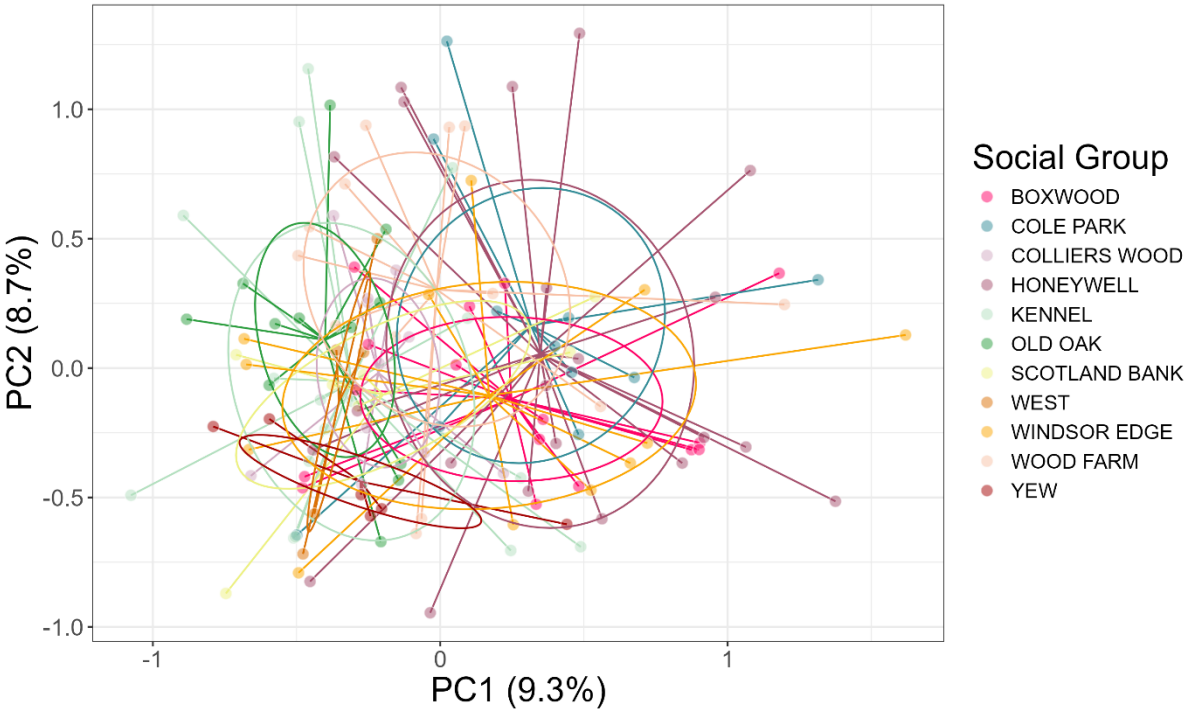


**Figure 2.** Principal Component Analysis (PCA) ordination of centre-log transformed community distances of badger faecal microbiotas. Each point is an individual sample from 54 badgers from 11 social groups, where each social group is represented by at least five sampled badgers. Lines connect individual samples to the centroid of the social group. Ellipses represent the 95% confidence interval of group centroids

| Term | DF | $R^2$ | F | P Value |
|---|---|---|---|---|
| Social Group | 17 | 0.16 | 1.57 | 0.001 |
| Chronological Age | 1 | 0.01 | 2.00 | 0.002 |
| Sex | 1 | 0.01 | 1.87 | 0.001 |
| *M. bovis* Infection Probability | 1 | 0.01 | 0.93 | 0.589 |
| Capture Year | 2 | 0.02 | 1.95 | 0.001 |
| Individual ID | 48 | 0.33 | 1.10 | 0.035 |
| Residual | 74 | 0.46 | | |

**Table 1.** PERMANOVA analysis investigating drivers of variation in beta diversity in badger faecal bacterial microbiotas. Significant terms shaded grey.

**Limited Variation Among Social Groups in Alpha Diversity**

Investigation of patterns of alpha diversity among social groups revealed limited evidence for differences in mean bacterial ASV richness (Fig. 3, Table 2). Instead, we detected significant within-group heterogeneity in bacterial richness consistent with the patterns observed in beta diversity (Fig. 3). This suggests that the observed variation in overall microbiota 'structure' *within* social groups (see Figs. 1A and 2) is driven partly by differences in bacterial community composition, where two badgers may have similar ASV richness but different species of bacteria present in the faecal microbiota.

A bivariate general linear mixed model (n = 145 measurements from 62 badgers of known age from 18 social groups) supported these patterns, indicating limited heterogeneity among groups in mean ASV richness (social group random effects SD = 0.04, 95% credible interval [0 - 0.11]; Table 2). There was no evidence for systematic variation in richness due to chronological age, sex, or bTB infection status (Table 2). We found no evidence of a correlation between microbiome richness and body mass at the individual level after controlling for the other variables (mean posterior correlation 0.26, 95% CI -0.56 – 0.93; Table 2). We also found no consistent variation in bacterial richness in relation to *M. bovis* infection probability within social groups (Fig. S1A) or sampling years (Fig. S1B).

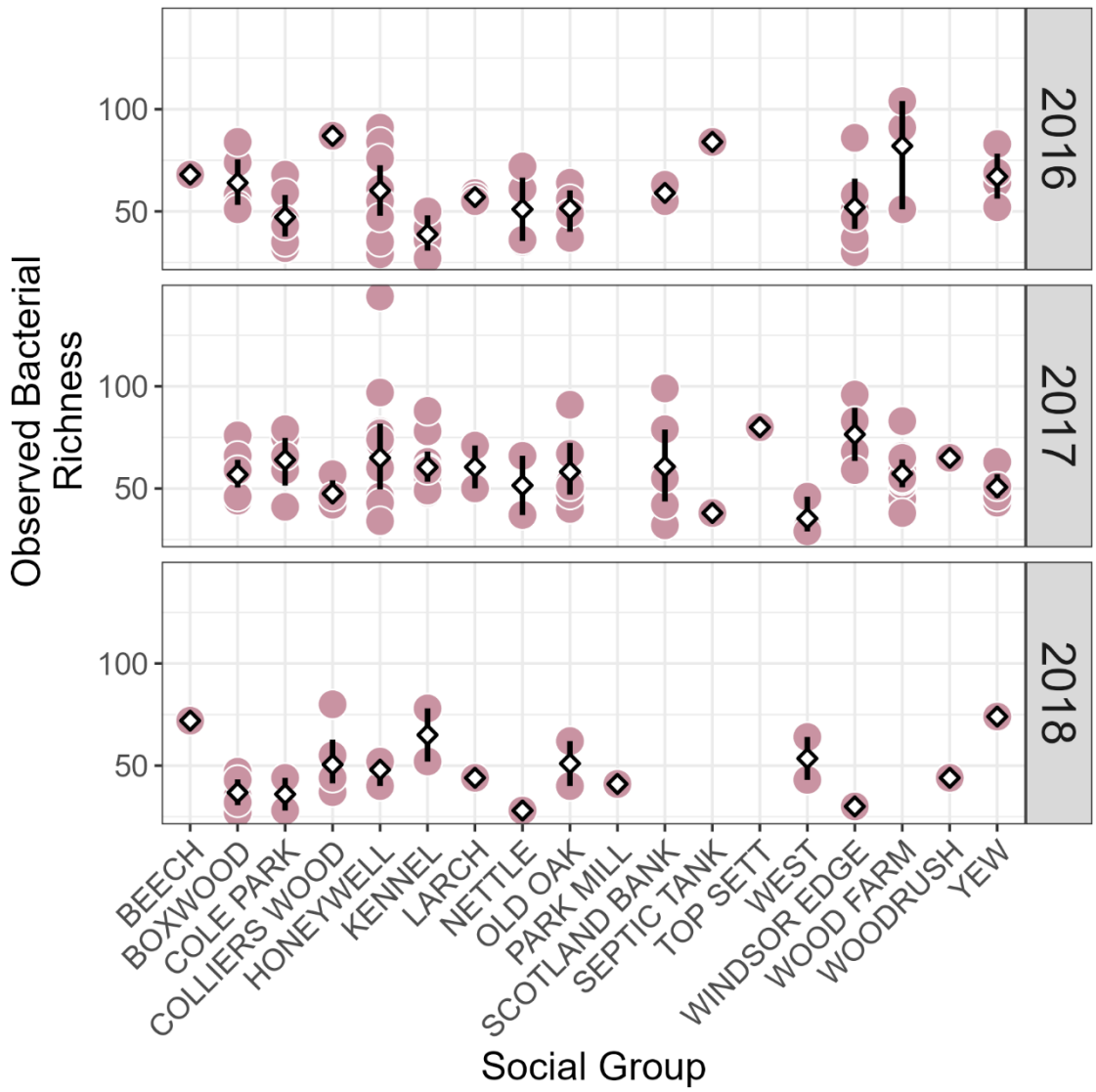


**Figure 3.** Bacterial richness for 18 social groups of badgers sampled between 2016 – 2018. Filled circles are raw data. White diamonds are group means and black bars represent standard errors.

| | Random Effects | | | |
|---|---|---|---|---|
| | Social Group (n=18) | **Mean** | **Lower95%** | **Upper95%** |
| | sd(Richness) | 0.05 | 0 | 0.13 |
| | sd(Weight) | 0.59 | 0.05 | 1.31 |
| | cor(Richness,Weight) | -0.08 | -0.95 | 0.94 |
| | Badger ID (n= 65) | **Mean** | **Lower95%** | **Upper95%** |
| | sd(Richness) | 0.13 | 0.03 | 0.22 |
| | sd(Weight) | 0.82 | 0.28 | 1.29 |
| | cor(Richness,Weight) | 0.26 | -0.52 | 0.93 |
| | **Fixed Effects** | | | |
| | | **Mean** | **Lower95%** | **Upper95%** |
| **RICHNESS** | Intercept (2016, Female) | 4.18 | 3.99 | 4.36 |
| | Capture Year 2017 | 0.04 | -0.08 | 0.15 |
| | Capture Year 2018 | -0.09 | -0.27 | 0.08 |
| | Chronological Age | -0.09 | -0.19 | 0.01 |
| | Chronological Age$^2$ | 0.01 | -0.002 | 0.02 |
| | Sex Male | -0.04 | -0.16 | 0.08 |
| | *M bovis* Infection Probability | 0.06 | -0.1 | 0.22 |
| **WEIGHT** | Intercept (2016, Female) | 6.31 | 5.2 | 7.44 |
| | Capture Year 2017 | 0.63 | 0.01 | 1.26 |
| | Capture Year 2018 | 0.26 | -0.66 | 1.17 |
| | Chronological Age | 0.65 | 0.08 | 1.23 |
| | Chronological Age$^2$ | -0.08 | -0.14 | -0.02 |
| | Sex Male | 1.54 | 0.8 | 2.29 |
| | *M bovis* Infection Probability | 0.34 | -0.58 | 1.28 |
| | **Family Specific Parameters** | | | |
| | | **Mean** | **Lower95%** | **Upper95%** |
| | shape_Observed | 16.41 | 11.17 | 23.96 |
| | sigma_weight | 1.51 | 1.3 | 1.76 |

**Table 2**. Model results from a bivariate mixed effects model examining the relative impacts of capture year, chronological age, sex and *M. bovis* infection probability on both microbiota richness and badger weight. Parameter estimates not crossing zero are shaded in grey.

**Associations With Individual Bacterial Genera**

Using generalised linear latent variable models (GLLVMs), we identified differences in microbiome composition linked to *M. bovis* infection probability, sex and age whilst controlling for individual ID (Fig. 4A). Males tend to possess greater relative abundance of the genera *Clostridium*, *Paeniclostridium* and *Streptococcus*, and lower abundances of *Porphyromonas* and *Fusobacterium*. Multiple ASVs appeared to increase with advancing age, including *Lachnoclostridium*, *Fusobacterium* and *Bacteroides*.

Individuals with higher infection probability had lower relative abundance of the genus *Lactobacillus*. We also detected a significant sex:infection interaction, where only females with higher infection probability had higher levels of *Plesiomonas*.

When examining residual correlations among genera after accounting for the effects of infection and individual ID, we identified several sets of genera that exhibited both positive and negative covariances. For example, several opportunistic pathogens including *Morganella*, *Citrobacter* and *Terrisporobacter* were positively correlated suggesting they may be more likely to co-occur in gut bacterial communities (Fig. 4B). We also observed negative residual correlations between *Lactobacillus* and opportunistic pathogens such as *Porphyromonas*.

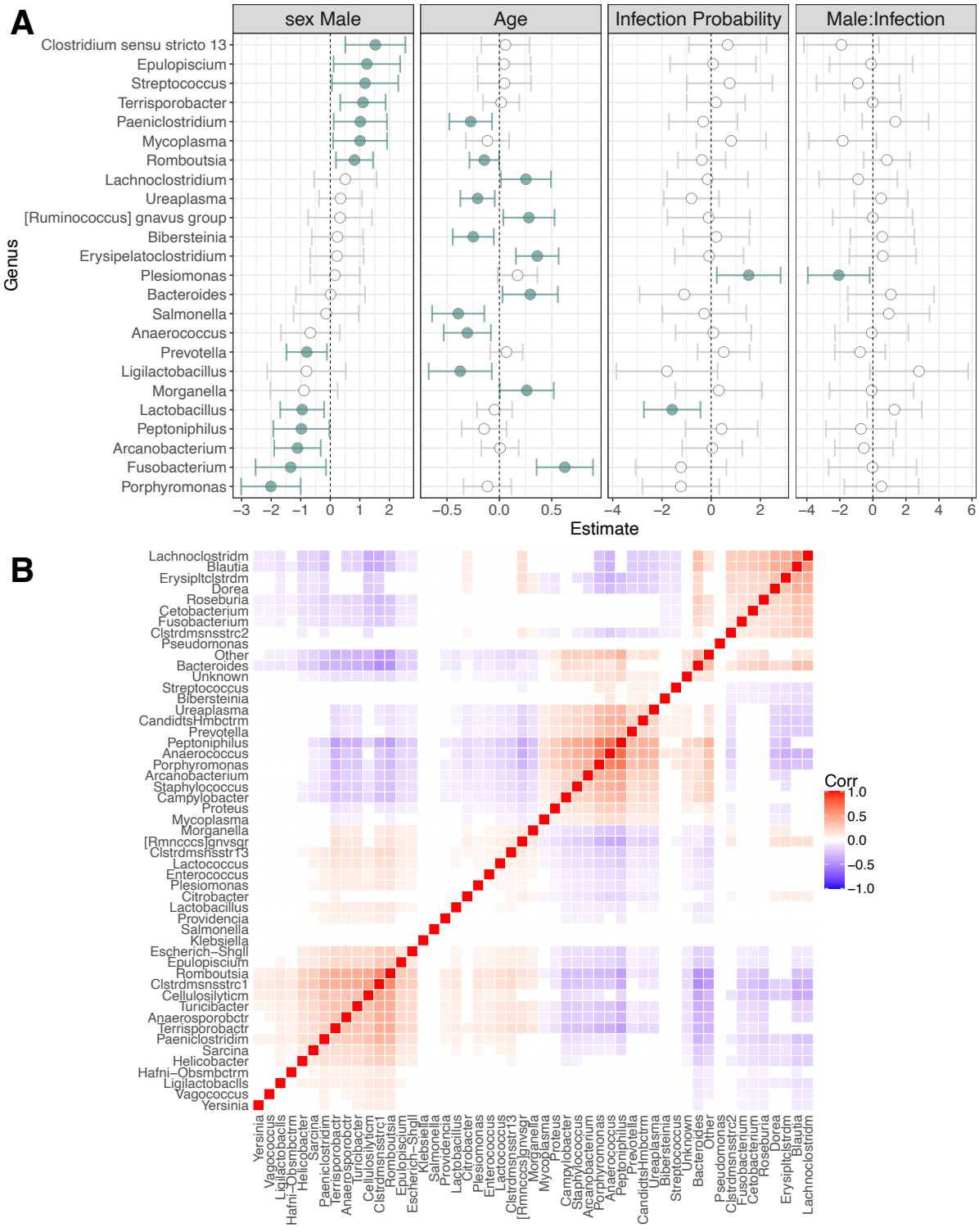


**Figure 4**. Output from a generalized linear latent variable model investigating differentially abundant bacterial genera by sex, age and *M. bovis* infection probability, whilst controlling for year and individual ID. **(A)** Model output showing significantly differentially abundant genera (green circles) for each predictor / interaction **(B)** Correlation plot identifying residual

covariation among bacterial genera in the sample after accounting for year, sex, age, infection and individual effects.

**Age-Dependent Dynamics**

We used data from six known-age badgers (ranging between 1 and 9 years old) that had been sampled three or more times throughout the study to identify marked variation among individuals in microbiome dynamics with advancing age for both alpha diversity (Fig. 5A) and beta diversity (Fig. 5B; Fig S2). Using data from 22 repeat-sampled badgers (some of which were of unknown chronological age), there was no indication that this direction or slope of shifts in microbiome was linked to *M. bovis* infection probability (Fig. S3). However, there was strong within-year variation in the bacterial microbiota in addition to among-year / age-dependent trends identified in Fig. 5A, B).

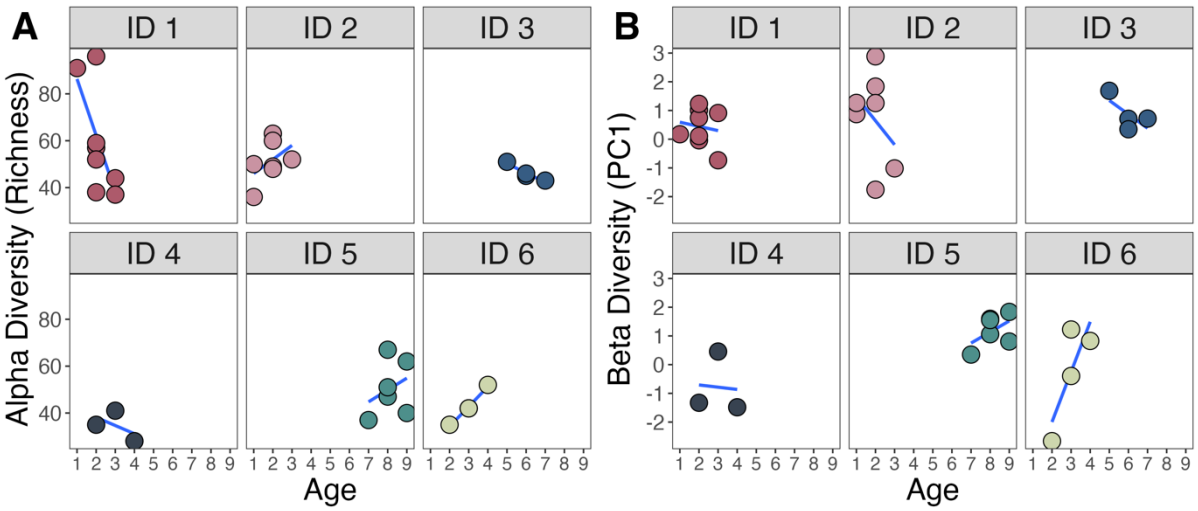


**Figure 5.** Dynamics of microbiome community composition with chronological age at the individual level for both alpha diversity **(A)** and beta diversity **(B)** for six badgers of known age with ≥3 samples (range 3-8 samples per badger)**.** Blue line indicates mean linear trend in trait indicative of shifts in microbiome over time.

## DISCUSSION

Our data indicate that individual identity, social group, sex and age all predict differences in the faecal microbiotas of group-living wild European badgers from a single population. Significant 'individuality' in the microbiome manifested via differences in both initial microbiome composition and microbiome dynamics within and across years, likely driven by individual dietary specialisation. We also found subtle effects of *M. bovis* infection probability on microbiome composition. Quantifying the drivers of variation in individual microbiota dynamics is a fundamental part of understanding temporal changes in both host health and immunity and patterns of social transmission of microbes (Marsh *et al* 2024).

### Microbiome Composition Varies With Social Group

Greater within-group similarity in microbiomes is predicted to arise through a range of potential factors including shared habitat and diet, as well as more frequent direct and indirect social contact and higher relatedness (reviewed in Sarkar et al., 2020). Several studies have identified social group membership as a strong predictor of microbiome composition in group-living species (Bennett et al., 2016; Moeller et al., 2016; Raulo et al., 2018; Tung et al., 2015; Vernier et al., 2020) though the precise drivers often vary. For example, a study of wild ring-tailed lemurs (*Lemur catta*) found that the social group microbiome remained distinct even when groups shared similar habitats (Bennett et al., 2016). Experimental work in Damaraland mole-rats (*Fukomys damarensis*) found that relatedness had no effect on the gut microbiome and that compositional similarity was driven primarily by a shared spatial and social environment (Bensch et al., 2023). As badgers from the same social group share discrete territories and setts, we hypothesised that animals from the same social group would share a unique microbial signature, but that the strength of this effect would be diluted by divergence in foraging niche among individuals. Our data were consistent with this hypothesis, revealing that social group membership accounted for about 16% of variation in the faecal microbiome. This effect operated principally through differences in overall composition (beta diversity) rather than systematic differences in overall richness (alpha diversity). Hence, different social groups tended to display similar mean bacterial richness but strong within-group variation in the number of ASVs detected. We also detected significant effects of time (year), albeit much weaker than the influence of social group. In contrast, research on white-faced capuchins (*Cebus*

*capucinus imitator*) revealed that temporal effects such as season can explain microbiome variation more than social group (Orkin et al., 2019), whilst in red squirrels (*Sciurus vulgaris*), population-level temporal traits like season and year can dominate individual level traits like sex and age (Ren et al., 2017a).

In this population of badgers, individual identity explained the greatest amount of variation (33%) in microbiota composition. Previous work using stable isotope analysis has revealed that badgers exhibit diverse and long-term dietary specialisation, with some animals preferring specific food types while others take a far more generalist approach, despite having access to the same resources (Robertson et al., 2014). Given the strong effect of diet on gut microbiota composition (Amato et al., 2015; Ren et al., 2017b; Wang et al., 2021) and stability (Jones et al., 2023), we expect that among-individual variation in foraging niche within a social group is an important driver of within-group microbiome heterogeneity. To test this hypothesis, future work could combine forensic metrics of diet from stable isotopes (e.g. Robertson et al., 2014) or metabarcoding with contemporaneous measures of gut or faecal microbiota. That such considerable within-group variation in the microbiota exists despite high within-group relatedness (Benton et al., 2016) suggests that host diet may outweigh any constraints on host microbiota due to higher genetic similarity. We did not measure relatedness in this study, but future work could attempt to quantify the relative importance of genetic versus environmental and behavioural drivers of variation in gut-associated microbes.

**Age, *M. bovis* Infection and the Importance of Individual Dynamics**

Both linear modelling and PERMANOVA analysis indicated that age is associated with shifts in the bacterial community composition of badger gut microbiomes. This result contrasts with previous work on European badgers that found no age-associated effects on alpha or beta diversity (Scott-Baumann et al., 2022), although that study was conducted on a small sample of carcasses (n = 12) and so may not be an accurate representation of the microbial landscape within live hosts. Host aging in badgers is frequently accompanied by corresponding changes in physiology and behaviour, including body condition, reproductive status, and social interactions (Beirne et al., 2014, 2015; Benton et al., 2018; Dugdale et al., 2011), which are expected to have knock-on effects on the microbiome. Age-associated

changes in the human microbiome have been well characterised (Yatsunenko et al., 2012), and have been implicated in the process of ageing and senescence (reviewed in Ghosh et al., 2022). We detected increases in the relative abundance of the genus *Bacteroides* with advancing age, which in humans is associated with senescence and lower survival (Wilmanski et al., 2021). We also found increases in the abundance of *Ruminococcus gnavus*, which is known to trigger host inflammatory responses and increase in relative abundance with age in humans (Crost et al., 2023). Age-related shifts in the microbiome have also been observed in non-human primates (Bennett et al., 2016; Pafčo et al., 2019), *Drosophila* (Leech et al., 2021), spotted hyenas (Rojas et al., 2023), meerkats (Risely et al., 2022) and birds (Lee et al., 2025), though several studies in other species have found no clear association between age and bacterial microbiota (Funosas et al., 2021; Lavrinienko et al., 2018; Maurice et al., 2015; Worsley et al., 2023). One potential explanation for these contrasting results is that population-level trends in age-microbiome relationships may be difficult to detect, even when using chronological age (e.g. Worsley et al., 2024, but see Rojas et al., 2023). Population-level data may mask important among-individual heterogeneity in ageing and microbiome trajectories, requiring that we investigate these patterns at the individual level (Risely et al., 2022; Björk et al., 2022). Recent work has identified fine-scale diurnal oscillations in gut microbiota composition in both meerkats (Risely et al., 2021) and spotted hyenas (Melville et al., 2024). These data from diverse mammalian systems highlight the importance of studying microbiome dynamics across a broad range of temporal resolutions to identify the causes and consequences of among-individual heterogeneity in microbiota composition.

Examining microbiome dynamics with chronological age at the individual level in our data revealed marked differences in individual-level microbial trajectories in European badgers. Age-dependent microbiome trajectories can arise in individuals because they occupy different ecological niches (e.g. foraging and climate), because the niches they occupy shift over time, or because they respond differently to similar ecological conditions with age. A key question is whether the direction and magnitude of an individual's age-dependent microbiome trajectory is linked to the pace of ageing. Previous work on this population has detected age and sex-dependent variation in disease progression (Benton et al., 2018), and variable rates of immune senescence and telomere shortening (Beirne et al.,

2014). Addressing this question requires that we integrate functional metrics of microbiome dynamics with long-term life history and physiological data to investigate the mechanisms by which the host microbiota may shape patterns of senescence. For example in *Drosophila*, age-related changes in gut microbiota composition have been linked to intestinal barrier dysfunction, altered immune gene activation and subsequently reduced lifespan (Clark et al., 2015).

We found no support for our prediction that bTB infection would be associated with lower microbiome richness, nor did we find differences in overall composition at the population-level. These findings provide no evidence of large-scale turnover in microbial profiles associated with infection, often used as evidence of 'dysbiosis' (Becker et al., 2015; Bowerman et al., 2020; DeCandia et al., 2020; Hassouneh et al., 2021; Macdonald et al., 2017; Worsley et al., 2021). The absence of any clear dysbiosis associated with *M. bovis* might be because it is only associated with advanced disease, whereas exposure to *M. bovis* and early stages of infection may have limited influence on overall microbiota composition. We detected subtle shifts in the relative abundance of key bacterial genera associated with *M. bovis* infection probability in badgers. A key finding is that animals with higher infection probabilities exhibited a lower relative abundance of the genus *Lactobacillus*, consistent with previous work on meerkats that identified decreases in the relative abundance of lactic acid bacteria (LAB) linked to TB exposure (Risely et al., 2023). LAB are important for modulating host immunity and preventing colonisation of pathogens (Bravo et al., 2022) which may explain the negative correlation between *Lactobacillus* and opportunistic pathogens like *Porphyromonas* in these data. That depleted abundances of LAB may indirectly determine susceptibility to respiratory pathogens such as *M. bovis* via effects on the host immune response remains a key hypothesis to be tested in future work.

We also detected a significant interaction between sex and infection, whereby female badgers with a higher probability of infection exhibited a higher relative abundance of the opportunistic pathogen *Plesiomonas*. In an Irish population of badgers, individuals infected with *M. bovis* were more likely to be coinfected with helminths (Kelly et al., 2022) though the effect was not sex-dependent. Male badgers possess different immune cell profiles to females (van Lieshout et al., 2020), are more likely to develop severe *M. bovis* infections (Graham et al., 2013) and show age-related shifts in immune profiles (van

Lieshout et al., 2020). These studies may help explain in part why we observed higher abundances of several opportunistic pathogens in males including *Mycoplasma*, *Clostridium* and *Paeniclostridium*. Future work should measure host cellular immunity in tandem with the absolute abundances of these microbes to investigate how co-infection dynamics may depend on host age, sex and commensal microbiome composition.

**Conclusion**

Here we have shown that social group, sex, sampling year and age shape the faecal microbiota of European badgers. We also detected subtle shifts in microbiota composition associated with *M. bovis* infection probability. Exploring these patterns at the individual level revealed marked variation in microbiome trajectory with time and age, suggestive of strong individuality in the microbiome likely linked to diet. Unravelling the importance of the microbiome for host traits like immunity and senescence requires that we adopt individual-level longitudinal approaches to these questions to account for marked variation in microbiome dynamics, and their subsequent impacts on host health.

## REFERENCES


Akhmetova, A., Guerrero, J., McAdam, P., Salvador, L. C. M., Crispell, J., Lavery, J., Presho, E., Kao, R. R., Biek, R., Menzies, F., Trimble, N., Harwood, R., Pepler, P. T., Oravcova, K., Graham, J., Skuce, R., du Plessis, L., Thompson, S., Wright, L., Byrne, A., & Allen, A. R. (2023). Genomic epidemiology of *Mycobacterium bovis* infection in sympatric badger and cattle populations in Northern Ireland. *Microbial Genomics*, *9*. https://doi.org/10.1099/mgen.0.001023

Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., Wilson, B. A., Nelson, K. E., White, B. A., & Garber, P. A. (2015). The Gut Microbiota Appears to Compensate for Seasonal Diet Variation in the Wild Black Howler Monkey (*Alouatta pigra*). *Microbial Ecology*, *69*, 434–443. https://doi.org/10.1007/s00248-014-0554-7

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Rex Gaskins, H., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A., & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra* ) gastrointestinal microbiomes. *The ISME Journal*, *7*, 1344–1353. https://doi.org/10.1038/ismej.2013.16

Ashford, R. T., Anderson, P., Waring, L., Davé, D., Smith, F., Delahay, R. J., Gormley, E., Chambers, M. A., Sawyer, J., & Lesellier, S. (2020). Evaluation of the Dual Path Platform (DPP) VetTB assay for the detection of *Mycobacterium bovis* infection in badgers. *Preventive Veterinary Medicine*, *180*, 105005. https://doi.org/10.1016/J.PREVETMED.2020.105005

Barnes, A. P., Moxey, A., Brocklehurst, S., Barratt, A., McKendrick, I. J., Innocent, G., & Ahmadi, B. V. (2023). The consequential costs of bovine tuberculosis (bTB) breakdowns in England and Wales. *Preventive Veterinary Medicine*, *211*, 105808. https://doi.org/10.1016/J.PREVETMED.2022.105808

Becker, M. H., Walke, J. B., Cikanek, S., Savage, A. E., Mattheus, N., Santiago, C. N., Minbiole, K. P. C., Harris, R. N., Belden, L. K., & Gratwicke, B. (2015). Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20142881. https://doi.org/10.1098/rspb.2014.2881

Beirne, C., Delahay, R., Hares, M., & Young, A. (2014). Age-Related Declines and Disease-Associated Variation in Immune Cell Telomere Length in a Wild Mammal. *PLOS ONE*, *9*, e108964. https://doi.org/10.1371/JOURNAL.PONE.0108964

Beirne, C., Delahay, R., & Young, A. (2015). Sex differences in senescence: the role of intra-sexual competition in early adulthood. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20151086. https://doi.org/10.1098/rspb.2015.1086

Bennett, G., Malone, M., Sauther, M. L., Cuozzo, F. P., White, B., Nelson, K. E., Stumpf, R. M., Knight, R., Leigh, S. R., & Amato, K. R. (2016). Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (*Lemur catta*). *American Journal of Primatology*, *78*, 883–892. https://doi.org/10.1002/AJP.22555

Bensch, H. M., Lundin, D., Tolf, C., Waldenström, J., & Zöttl, M. (2023). Environmental effects rather than relatedness determine gut microbiome similarity in a social mammal. *Journal of Evolutionary Biology*, *36*, 1753–1760. https://doi.org/10.1111/JEB.14208

Benton, C. H., Delahay, R. J., Robertson, A., McDonald, R. A., Wilson, A. J., Burke, T. A., & Hodgson, D. (2016). Blood thicker than water: kinship, disease prevalence and group size drive divergent patterns of infection risk in a social mammal. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20160798. https://doi.org/10.1098/rspb.2016.0798

Benton, C. H., Delahay, R. J., Smith, F. A. P., Robertson, A., McDonald, R. A., Young, A. J., Burke, T. A., & Hodgson, D. (2018). Inbreeding intensifies sex- and age-dependent disease in a wild mammal. *Journal of Animal Ecology*, *87*, 1500–1511. https://doi.org/10.1111/1365-2656.12878

Böhm, M., Hutchings, M. R., & White, P. C. L. (2009). Contact Networks in a Wildlife-Livestock Host Community: Identifying High-Risk Individuals in the Transmission of Bovine TB among Badgers and Cattle. *PLoS ONE*, *4*, e5016. https://doi.org/10.1371/journal.pone.0005016

Bowerman, K. L., Rehman, S. F., Vaughan, A., Lachner, N., Budden, K. F., Kim, R. Y., Wood, D. L. A., Gellatly, S. L., Shukla, S. D., Wood, L. G., Yang, I. A., Wark, P. A., Hugenholtz, P., & Hansbro, P. M. (2020). Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. *Nature Communications 2020 11:1*, *11*, 1–15. https://doi.org/10.1038/s41467-020-19701-0

Bravo, M., Combes, T., Martinez, F. O., Risco, D., Gonçalves, P., Garcia-Jimenez, W. L., Cerrato, R., Fernandez-Llario, P., & Gutierrez-Merino, J. (2022). Wildlife Symbiotic Bacteria Are Indicators of the Health Status of the Host and Its Ecosystem. *Applied and Environmental Microbiology*, *88*, e01385-21. https://doi.org/10.1128/AEM.01385-21

Bürkner, P. C. (2018). Advanced Bayesian multilevel modeling with the R package brms. *R Journal*, *10*, 395–411. https://doi.org/10.32614/RJ-2018-017/

Bürkner, P. C. (2021). Bayesian Item Response Modeling in R with brms and Stan. *Journal of Statistical Software*, *100*, 1–54. https://doi.org/10.18637/JSS.V100.I05

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583. https://doi.org/10.1038/nmeth.3869

Cheeseman, C., & Harris, S. (1982). Methods of marking badgers (*Meles meles*). *Journal of Zoology*, *197*, 289–292.

Cholewińska, P., Czyz, K., Nowakowski, P., & Wyrostek, A. (2020). The microbiome of the digestive system of ruminants – a review. *Animal Health Research Reviews*, *21*, 3–14. https://doi.org/10.1017/S1466252319000069

Clark, R. I., Salazar, A., Yamada, R., Pellegrini, M., Ja, W. W., & Correspondence, D. W. W. (2015). Distinct Shifts in Microbiota Composition during Drosophila Aging Impair Intestinal Function and Drive Mortality. *Cell Reports*, *12*. https://doi.org/10.1016/j.celrep.2015.08.004

Clifton-Hadley, R. S., Wilesmith, J. W., & Stuart, F. A. (1993). *Mycobacterium bovis* in the European Badger (*Meles meles*): Epidemiological Findings in Tuberculous Badgers from a Naturally Infected Population. *Epidemiology and Infection*, *111*, 9–19. https://www.jstor.org/stable/3863745

Crispell, J., Benton, C. H., Balaz, D., De Maio, N., Ahkmetova, A., Allen, A., Biek, R., Presho, E. L., Dale, J., Hewinson, G., Lycett, S. J., Nunez-Garcia, J., Skuce, R. A., Trewby, H., Wilson, D. J., Zadoks, R. N., Delahay, R. J., & Kao, R. R. (2019). Combining genomics and epidemiology to analyse bi-directional transmission of *Mycobacterium bovis* in a multi-host system. *ELife*, *8*, e45833. https://doi.org/10.7554/eLife.45833

Crost, E. H., Coletto, E., Bell, A., & Juge, N. (2023). *Ruminococcus gnavus* : friend or foe for human health. *FEMS Microbiology Reviews*, *47*. https://doi.org/10.1093/femsre/fuad014

Davis, N. M., Proctor, Di. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*, 1–14. https://doi.org/10.1186/S40168-018-0605-2/FIGURES/6

DeCandia, A. L., Brenner, L. J., King, J. L., & vonHoldt, B. M. (2020). Ear mite infection is associated with altered microbial communities in genetically depauperate Santa Catalina Island foxes (*Urocyon littoralis catalinae*). *Molecular Ecology*, *29*, 1463–1475. https://doi.org/10.1111/MEC.15325

Delahay, R. J., Brown, J. A., Mallinson, P. J., Spyvee, P. D., Handoll, D., Rogers, L. M., & Cheeseman, C. L. (2000). The use of marked bait in studies of the territorial organization of the European Badger (*Meles meles*). *Mammal Review*, *30*, 73–87. https://doi.org/10.1046/J.1365-2907.2000.00058.X

Dillon, R. J., Vennard, C. T., Buckling, A., & Charnley, A. K. (2005). Diversity of locust gut bacteria protects against pathogen invasion. *Ecology Letters*, *8*, 1291–1298. https://doi.org/10.1111/J.1461-0248.2005.00828.X

Doig, C. (2002). The efficacy of the heat killing of *Mycobacterium tuberculosis*. *Journal of Clinical Pathology*, *55*, 778–779. https://doi.org/10.1136/jcp.55.10.778

Dugdale, H. L., Pope, L. C., Newman, C., MacDonald, D. W., & Burke, T. (2011). Age-specific breeding success in a wild mammalian population: Selection, constraint, restraint and senescence. *Molecular Ecology*, *20*, 3261–3274. https://doi.org/10.1111/J.1365-294X.2011.05167.X

Fell, R. J., Buesching, C. D., & Macdonald, D. W. (2006). The Social Integration of European Badger (*Meles meles*) Cubs into Their Natal Group. *Behaviour*, *143*, 683–700. https://www.jstor.org/stable/4536370

Funosas, G., Triadó-Margarit, X., Castro, F., Villafuerte, R., Delibes-Mateos, M., Rouco, C., & Casamayor, E. O. (2021). Individual fate and gut microbiome composition in the European wild rabbit (*Oryctolagus cuniculus*). *Scientific Reports*, *11*, 766. https://doi.org/10.1038/s41598-020-80782-4

Gallagher, J., & Clifton-Hadley, R. S. (2000). Tuberculosis in badgers; a review of the disease and its significance for other animals. *Research in Veterinary Science*, *69*, 203–217. https://doi.org/10.1053/rvsc.2000.0422

Ghosh, T. S., Shanahan, F., & O'Toole, P. W. (2022). The gut microbiome as a modulator of healthy ageing. *Nature Reviews Gastroenterology & Hepatology*, *19*, 565–584. https://doi.org/10.1038/s41575-022-00605-x

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*, 294209. https://doi.org/10.3389/FMICB.2017.02224/BIBTEX

Graham, J., Smith, G. C., Delahay, R. J., Bailey, T., McDonald, R. A., & Hodgson, D. (2013). Multi-state modelling reveals sex-dependent transmission, progression and severity of tuberculosis in wild badgers. *Epidemiology and Infection*, *141*, 1429–1436. https://doi.org/10.1017/S0950268812003019

Harrison, X. A., McDevitt, A. D., Dunn, J. C., Griffiths, S. M., Benvenuto, C., Birtles, R., Boubli, J. P., Bown, K., Bridson, C., Brooks, D. R., Browett, S. S., Carden, R. F., Chantrey, J., Clever, F., Coscia, I., Edwards, K. L., Ferry, N., Goodhead, I., Highlands, A., … Antwis, R. E. (2021). Fungal microbiomes are determined by host phylogeny and exhibit widespread associations with the bacterial microbiome. *Proceedings Of The Royal Society B-Biological Sciences*, *288*, 20210552. https://doi.org/10.1098/RSPB.2021.0552

Harrison, X. A., Price, S. J., Hopkins, K., Leung, W. T. M., Sergeant, C., & Garner, T. W. J. (2019). Diversity-Stability Dynamics of the Amphibian Skin Microbiome and Susceptibility to a Lethal Viral Pathogen. *Frontiers in Microbiology*, *10*, 2883. https://doi.org/10.3389/FMICB.2019.02883/BIBTEX

Hassouneh, S. A. D., Loftus, M., & Yooseph, S. (2021). Linking Inflammatory Bowel Disease Symptoms to Changes in the Gut Microbiome Structure and Function. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.673632

Heitlinger, E., Ferreira, S. C. M., Thierer, D., Hofer, H., & East, M. L. (2017). The Intestinal Eukaryotic and Bacterial Biome of Spotted Hyenas: The Impact of Social Status and Age on Diversity and Composition. *Frontiers in Cellular and Infection Microbiology*, *7*, 262. https://doi.org/10.3389/fcimb.2017.00262

Hu, Y., Feng, Y., Wu, J., Liu, F., Zhang, Z., Hao, Y., Liang, S., Li, B., Li, J., Lv, N., Xu, Y., Zhu, B., & Sun, Z. (2019). The gut microbiome signatures discriminate healthy from pulmonary tuberculosis patients. *Frontiers in Cellular and Infection Microbiology*, *9*, 432616. https://doi.org/10.3389/FCIMB.2019.00090/BIBTEX

Hudson, D. W., McKinley, T. J., Benton, C. H., Delahay, R., McDonald, R. A., & Hodgson, D. J. (2023). Multi-locus homozygosity promotes actuarial senescence in a wild mammal. *Journal of Animal Ecology*, *92*, 1881–1892. https://doi.org/10.1111/1365-2656.13979

Ingala, M. R., Becker, D. J., Bak Holm, J., Kristiansen, K., & Simmons, N. B. (2019). Habitat fragmentation is associated with dietary shifts and microbiota variability in common vampire bats. *Ecology and Evolution*, *9*, 6508–6523. https://doi.org/10.1002/ECE3.5228

Jones, I., Marsh, K., Handby, T. M., Hopkins, K., Slezacek, J., Bearhop, S., & Harrison, X. A. (2023). The influence of diet on gut microbiome and body mass dynamics in a capital-breeding migratory bird. *PeerJ*, *11*, e16682. https://doi.org/10.7717/peerj.16682

Kelly, D. J., Marples, N. M., Byrne, R. L., Fogarty, U., Kenny, K., Cameron, H., Griffin, D., & Holland, C. V. (2022). An investigation of *Mycobacterium bovis* and helminth coinfection in the European badger *Meles meles*. *International Journal for Parasitology: Parasites and Wildlife*, *19*, 311–316. https://doi.org/10.1016/j.ijppaw.2022.11.001

King, K. C., Brockhurst, M. A., Vasieva, O., Paterson, S., Betts, A., Ford, S. A., Frost, C. L., Horsburgh, M. J., Haldenby, S., & Hurst, G. D. D. (2016). Rapid evolution of microbe-mediated protection against pathogens in a worm host. *The ISME Journal 2016 10:8*, *10*, 1915–1924. https://doi.org/10.1038/ismej.2015.259

Knutie, S. A. (2020). Food supplementation affects gut microbiota and immunological resistance to parasites in a wild bird species. *Journal of Applied Ecology*, *57*, 536–547. https://doi.org/10.1111/1365-2664.13567

Kohl, K. D., Brun, A., Caviedes-Vidal, E., & Karasov, W. H. (2019). Age-related changes in the gut microbiota of wild House Sparrow nestlings. *Ibis*, *161*, 184–191. https://doi.org/10.1111/IBI.12618

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, *79*, 5112–5120. https://doi.org/10.1128/AEM.01043-13/SUPPL_FILE/ZAM999104626SO1.PDF

Kueneman, J. G., Parfrey, L. W., Woodhams, D. C., Archer, H. M., Knight, R., & McKenzie, V. J. (2014). The amphibian skin-associated microbiome across species, space and life history stages. *Molecular Ecology*, *23*, 1238–1250. https://doi.org/10.1111/MEC.12510

Lavrinienko, A., Tukalenko, E., Mappes, T., & Watts, P. C. (2018). Skin and gut microbiomes of a wild mammal respond to different environmental cues. *Microbiome*, *6*, 1–16. https://doi.org/10.1186/S40168-018-0595-0/FIGURES/3

Lee, C. Z., Worsley, S. F., Davies, C. S., Silan, E., Burke, T., Komdeur, J., Hildebrand, F., Dugdale, H. L., & Richardson, D. S. (2025). Metagenomic analyses of gut microbiome composition and function with age in a wild bird; little change, except increased transposase gene abundance. *ISME Communications*, *5*, 8. https://doi.org/10.1093/ismeco/ycaf008

Leech, T., McDowall, L., Hopkins, K. P., Sait, S. M., Harrison, X. A., & Bretman, A. (2021). Social environment drives sex and age-specific variation in *Drosophila melanogaster* microbiome composition and predicted function. *Molecular Ecology*, *30*, 5831–5843. https://doi.org/10.1111/mec.16149

Li, H., Qi, Y., & Jasper, H. (2016). Preventing Age-Related Decline of Gut Compartmentalization Limits Microbiota Dysbiosis and Extends Lifespan. *Cell Host & Microbe*, *19*, 240–253. https://doi.org/10.1016/J.CHOM.2016.01.008

Liukkonen, M., Muriel, J., Martínez-Padilla, J., Nord, A., Pakanen, V., Rosivall, B., Tilgar, V., van Oers, K., Grond, K., & Ruuskanen, S. (2024). Seasonal and environmental factors contribute to the variation in the gut microbiome: A large-scale study of a small bird. *Journal of Animal Ecology*, *93*, 1475–1492. https://doi.org/10.1111/1365-2656.14153

Macdonald, S. E., Nolan, M. J., Harman, K., Boulton, K., Hume, D. A., Tomley, F. M., Stabler, R. A., & Blake, D. P. (2017). Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology. *PLOS ONE*, *12*, e0184890. https://doi.org/10.1371/journal.pone.0184890

Marsh, K. J., Bearhop, S., & Harrison, X. A. (2024). Linking microbiome temporal dynamics to host ecology in the wild. *Trends in Microbiology*, *32*, 1060–1071. https://doi.org/10.1016/J.TIM.2024.05.001

Maurice, C. F., Cl Knowles, S., Ladau, J., Pollard, K. S., Fenton, A., Pedersen, A. B., & Turnbaugh, P. J. (2015). Marked seasonal variation in the wild mouse gut microbiota. *ISME Journal*, *9*, 2423–2434. https://doi.org/10.1038/ismej.2015.53

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, *8*, e61217. https://doi.org/10.1371/JOURNAL.PONE.0061217

Melville, D. W., Rojas, C. A., Risely, A., & Theis, K. R. (2024). Subtle diurnal microbial rhythms in a large mammalian carnivore. *Journal of Mammalogy*, *XX*, 1–10. https://doi.org/10.1093/JMAMMAL/GYAE143

Moeller, A. H., Foerster, S., Wilson, M. L., Pusey, A. E., Hahn, B. H., & Ochman, H. (2016). Social behavior shapes the chimpanzee pan-microbiome. *Science Advances*, *2*. https://doi.org/10.1126/SCIADV.1500997

Mtafya, B., Qwaray, P., John, J., Sichone, E., Shoo, A., Gillespie, S. H., Ntinginya, N. E., & Sabiiti, W. (2023). A practical approach to render tuberculosis samples safe for application of tuberculosis molecular bacterial load assay in clinical settings without a biosafety level 3 laboratory. *Tuberculosis*, *138*, 102275. https://doi.org/10.1016/J.TUBE.2022.102275

Niku, J., Hui, F. K. C., Taskinen, S., & Warton, D. I. (2019). gllvm: Fast analysis of multivariate abundance data with generalized linear latent variable models in r. *Methods in Ecology and Evolution*, *10*, 2173–2182. https://doi.org/10.1111/2041-210X.13303

Orkin, J. D., Webb, S. E., Amanda, |, Melin, D., Webb, S. E., & Melin, A. D. (2019). Small to modest impact of social group on the gut microbiome of wild Costa Rican capuchins in a seasonal forest. *American Journal of Primatology*, *81*, e22985. https://doi.org/10.1002/AJP.22985

Pafčo, B., Sharma, A. K., Petrželková, K. J., Vlčková, K., Todd, A., Yeoman, C. J., Wilson, B. A., Stumpf, R., White, B. A., Nelson, K. E., Leigh, S., & Gomez, A. (2019). Gut microbiome composition of wild western lowland gorillas is associated with individual age and sex factors. *American Journal of Physical Anthropology*, *169*, 575–585. https://doi.org/10.1002/AJPA.23842

Pfau, M., Degregori, S., Johnson, G., Tennenbaum, S. R., Barber, P. H., Philson, C. S., & Blumstein, D. T. (2023). The social microbiome: gut microbiome diversity and abundance are negatively associated with sociality in a wild mammal. *Royal Society Open Science*, *10*. https://doi.org/10.1098/RSOS.231305

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*, D590. https://doi.org/10.1093/NAR/GKS1219

R Core Team. (2023). *R: The R Project for Statistical Computing*. https://www.r-project.org/

Raulo, A., Bürkner, P.-C., Finerty, G. E., Dale, J., Hanski, E., English, H. M., Lamberth, C., Firth, J. A., Coulson, T., & Knowles, S. C. L. (2024). Social and environmental transmission spread different sets of gut microbes in wild mice. *Nature Ecology & Evolution 2024 8:5*, *8*, 972–985. https://doi.org/10.1038/s41559-024-02381-0

Raulo, A., Ruokolainen, L., Lane, A., Amato, K., Knight, R., Leigh, S., Stumpf, R., White, B., Nelson, K. E., Baden, A. L., & Tecot, S. R. (2018). Social behaviour and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): In search of the role of immunity in the evolution of sociality. *Journal of Animal Ecology*, *87*, 388–399. https://doi.org/10.1111/1365-2656.12781

Rebollar, E. A., Hughey, M. C., Medina, D., Harris, R. N., Ibáñez, R., & Belden, L. K. (2016). Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of *Batrachochytrium dendrobatidis*. *The ISME Journal*, *10*, 1682. https://doi.org/10.1038/ISMEJ.2015.234

Ren, T., Boutin, S., Humphries, M. M., Dantzer, B., Gorrell, J. C., Coltman, D. W., McAdam, A. G., & Wu, M. (2017a). Seasonal, spatial, and maternal effects on gut microbiome in wild red squirrels. *Microbiome*, *5*, 163. https://doi.org/10.1186/s40168-017-0382-3

Ren, T., Boutin, S., Humphries, M. M., Dantzer, B., Gorrell, J. C., Coltman, D. W., McAdam, A. G., & Wu, M. (2017b). Seasonal, spatial, and maternal effects on gut microbiome in wild red squirrels. *Microbiome*, *5*, 163. https://doi.org/10.1186/S40168-017-0382-3/FIGURES/5

Risely, A., Müller-Klein, N., Schmid, D. W., Wilhelm, K., Clutton-Brock, T. H., Manser, M. B., & Sommer, S. (2023). Climate change drives loss of bacterial gut mutualists at the expense of host survival in wild meerkats. *Global Change Biology*, *29*, 5816–5828. https://doi.org/10.1111/GCB.16877

Risely, A., Schmid, D. W., Müller-Klein, N., Wilhelm, K., Clutton-Brock, T. H., Manser, M. B., & Sommer, S. (2022). Gut microbiota individuality is contingent on temporal scale and age in wild meerkats. *Proceedings of the Royal Society B: Biological Sciences*, *289*. https://doi.org/10.1098/rspb.2022.0609

Risely, A., Wilhelm, K., Clutton-Brock, T., Manser, M. B., & Sommer, S. (2021). Diurnal oscillations in gut bacterial load and composition eclipse seasonal and lifetime dynamics in wild meerkats. *Nature Communications 2021 12:1*, *12*, 1–12. https://doi.org/10.1038/s41467-021-26298-5

Robertson, A., McDonald, R. A., Delahay, R. J., Kelly, S. D., & Bearhop, S. (2014). Individual foraging specialisation in a social mammal: the European badger (*Meles meles*). *Oecologia*, *176*, 409–421. https://doi.org/10.1007/S00442-014-3019-2/FIGURES/5

Rojas, C. A., Holekamp, K. E., Viladomat Jasso, M., Souza, V., Eisen, J. A., & Theis, K. R. (2023). Taxonomic, Genomic, and Functional Variation in the Gut Microbiomes of Wild Spotted Hyenas Across 2 Decades of Study. *MSystems*, *8*, e0096522–e0096522. https://doi.org/10.1128/msystems.00965-22

Sabiiti, W., Azam, K., Esmeraldo, E., Bhatt, N., Rachow, A., & Gillespie, S. H. (2018). Heat inactivation renders sputum safe and preserves *Mycobacterium tuberculosis* RNA for downstream molecular tests. *Journal of Clinical Microbiology*, *57*. https://doi.org/10.1128/JCM.01778-18/ASSET/8B558040-470C-4D56-A1CA-A6F09FF072CE/ASSETS/GRAPHIC/JCM.01778-18-F0004.JPEG

Sarkar, A., Harty, S., Johnson, K. V. A., Moeller, A. H., Archie, E. A., Schell, L. D., Carmody, R. N., Clutton-Brock, T. H., Dunbar, R. I. M., & Burnet, P. W. J. (2020). Microbial transmission in animal social networks and the social microbiome. *Nature Ecology & Evolution 2020 4:8*, *4*, 1020–1035. https://doi.org/10.1038/s41559-020-1220-8

Scott-Baumann, J. F., Friedersdorff, J. C. A., Villarreal-Ramos, B., King, J., Hopkins, B., Pizzey, R., Rooke, D., Hewinson, G., & Mur, L. A. J. (2022). The Faecal Microbiome of the Wild European Badger *Meles meles*: A Comparison Against Other Wild Omnivorous Mammals from Across the Globe. *Current Microbiology*, *79*, 1–8. https://doi.org/10.1007/S00284-022-03064-4/FIGURES/5

Spragge, F., Bakkeren, E., Jahn, M. T., Araujo, E. B. N., Pearson, C. F., Wang, X., Pankhurst, L., Cunrath, O., & Foster, K. R. (2023). Microbiome diversity protects against pathogens by nutrient blocking. *Science*, *382*. https://doi.org/10.1126/SCIENCE.ADJ3502

Stecher, B., Chaffron, S., Käppeli, R., Hapfelmeier, S., Freedrich, S., Weber, T. C., Kirundi, J., Suar, M., McCoy, K. D., Von Mering, C., Macpherson, A. J., & Hardt, W. D. (2010). Like Will to Like: Abundances of Closely Related Species Can Predict Susceptibility to Intestinal Colonization by Pathogenic and Commensal Bacteria. *PLOS Pathogens*, *6*, e1000711. https://doi.org/10.1371/JOURNAL.PPAT.1000711

Thaiss, C. A., Zmora, N., Levy, M., & Elinav, E. (2016). The microbiome and innate immunity. *Nature 2016 535:7610*, *535*, 65–74. https://doi.org/10.1038/nature18847

Thevaranjan, N., Puchta, A., Schulz, C., Naidoo, A., Szamosi, J. C., Verschoor, C. P., Loukov, D., Schenck, L. P., Jury, J., Foley, K. P., Schertzer, J. D., Larché, M. J., Davidson, D. J., Verdú, E. F., Surette, M. G., & Bowdish, D. M. E. (2017). Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host & Microbe*, *21*, 455-466.e4. https://doi.org/10.1016/J.CHOM.2017.03.002

Trevelline, B. K., & Kohl, K. D. (2022). The gut microbiome influences host diet selection behavior. *PNAS*, *119*, 2117537119. https://doi.org/10.1073/pnas

Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J. C., Lynch, J., Grieneisen, L. E., Altmann, J., Alberts, S. C., Blekhman, R., & Archie, E. A. (2015). Social networks predict gut microbiome composition in wild baboons. *ELife*, *2015*. https://doi.org/10.7554/ELIFE.05224

van Lieshout, S. H. J., Badás, E. P., Mason, M. W. T., Newman, C., Buesching, C. D., Macdonald, D. W., & Dugdale, H. L. (2020). Social effects on age-related and sex-specific immune cell profiles in a wild mammal. *Biology Letters*, *16*, 20200234. https://doi.org/10.1098/rsbl.2020.0234

Vernier, C. L., Chin, I. M., Adu-Oppong, B., Krupp, J. J., Levine, J., Dantas, G., & Ben-Shahar, Y. (2020). The gut microbiome defines social group membership in honey bee colonies. *Science Advances*, *6*. https://doi.org/10.1126/SCIADV.ABD3431

Wang, Y., Smith, H. K., Goossens, E., Hertzog, L., Bletz, M. C., Bonte, D., Verheyen, K., Lens, L., Vences, M., Pasmans, F., & Martel, A. (2021). Diet diversity and environment determine the intestinal microbiome and bacterial pathogen load of fire salamanders. *Scientific Reports*, *11*, 20493. https://doi.org/10.1038/S41598-021-98995-6

Wilmanski, T., Diener, C., Rappaport, N., Patwardhan, S., Wiedrick, J., Lapidus, J., Earls, J. C., Zimmer, A., Glusman, G., Robinson, M., Yurkovich, J. T., Kado, D. M., Cauley, J. A., Zmuda, J., Lane, N. E., Magis, A. T., Lovejoy, J. C., Hood, L., Gibbons, S. M., … Price, N. D. (2021). Gut microbiome pattern reflects healthy aging and predicts survival in humans. *Nature Metabolism*, *3*, 274. https://doi.org/10.1038/S42255-021-00348-0

Worsley, S. F., Davies, C. S., Lee, C. Z., Mannarelli, M., Burke, T., Komdeur, J., Dugdale, H. L., & Richardson, D. S. (2024). Longitudinal gut microbiome dynamics in relation to age and senescence in a wild animal population. *Molecular Ecology*, *33*, e17477. https://doi.org/10.1111/mec.17477

Worsley, S. F., Davies, C. S., Lee, C. Z., Mannarelli, M.-E., Burke, T., Komdeur, J., Dugdale, H. L., & Richardson, D. S. (2023). *Assessing longitudinal gut microbiome dynamics in relation to age and senescence in a wild animal population*. https://doi.org/10.21203/rs.3.rs-3486843/v1

Worsley, S. F., Davies, C. S., Mannarelli, M.-E., Hutchings, M. I., Komdeur, J., Burke, T., Dugdale, H. L., & Richardson, D. S. (2021). Gut microbiome composition, not alpha diversity, is associated with survival in a natural vertebrate population. *Animal Microbiome 2021 3:1*, *3*, 1–18. https://doi.org/10.1186/S42523-021-00149-6

Worsley, S. F., Videvall, E., Harrison, X. A., Björk, J. R., Mazel, F., & Wanelik, K. M. (2024). Probing the functional significance of wild animal microbiomes using omics data. *Functional Ecology*, *38*, 2329–2349. https://doi.org/10.1111/1365-2435.14650

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*, 222–227. https://doi.org/10.1038/nature11053

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research*, *42*, D643. https://doi.org/10.1093/NAR/GKT1209

Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., Ley, R. E., & Farnleitner, A. H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications*, *10*, 2200. https://doi.org/10.1038/s41467-019-10191-3

## ACKNOWLEDGEMENTS

We thank the field and lab teams at Woodchester Park for trapping, sampling and testing badgers, and the landowners in the study area for permitting access.

## FUNDING STATEMENT

This work was funded by a BBSRC 'SWBIO' DTP studentship awarded to NM and XH, and CASE funding from the APHA. The long-term badger study at Woodchester Park is funded by Defra.

## COMPETING INTERESTS

The authors declare no competing interests.

## ETHICS

This research has been reviewed and approved by the University of Exeter ethics review committee (Project ID 5390712). All work on badgers was approved by the APHA Animal Welfare and Ethical Review Board and was carried out in accordance with the 1986 Animal (Scientific Procedures) Act under a Home Office project licence.

## DATA ACCESSIBILITY

All sequencing data are archived on the Sequence Read Archive (SRA) under project IDs PRJNA1269947 & PRJNA1273904. All code required to reproduce the analyses in this paper are available on GitHub at https://github.com/xavharrison/BadgerSpatialMicrobiomes2024