# Gut microbiota repeatability is contingent on temporal scale and age in wild meerkats

Alice Risely[1]*, Dominik W. Schmid[1], Nadine Müller-Klein[1], Kerstin Wilhelm[1] , Tim H. Clutton-Brock[2,3,4], Marta B. Manser[3,4,5], & Simone Sommer[1]

1. Institute for Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Germany
2. Large Animal Research Group, Department of Zoology, University of Cambridge, Cambridge, United Kingdom
3. University of Pretoria, Mammal Research Institute, Pretoria, South Africa
4. Kalahari Research Trust, Kuruman River Reserve, Northern Cape, South Africa
5. Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

Correspondence: risely@gmail.com

## Abstract

Inter-individual differences in gut microbiota composition are hypothesized to generate variation in host fitness – a premise for the evolution of host-gut microbe symbioses. However, recent evidence suggests that gut microbial communities are highly dynamic, challenging the notion that individuals harbour unique and stable gut microbial phenotypes. Leveraging a long-term dataset of wild meerkats, we reconcile these concepts by demonstrating that the relative importance of identity to shaping gut microbiota phenotypes compared to social group and annual variation depends on temporal scale. Across meerkat lifespan, annual variation overshadows the effects of identity and social group in predicting gut microbiota composition, with identity explaining on average less than 2% of variation across microbial phenotypes. However, identity is the strongest predictor of microbial phenotypes over shorter time periods, predicting on average 20% of variation before rapidly declining in explanatory power over longer periods. Decomposing drivers of variation highlight that identity, social group, and year are each associated with distinct phylogenetic groups of taxa. The effects of identity are also dependent on meerkat age, with the gut microbiota becoming more individualized and stable as meerkats get older. These findings illuminate the degree to which individualised gut microbial signatures can be expected, with important implications for the time frames over which gut microbial phenotypes may mediate host physiology, behaviour and fitness in natural populations.

## Introduction

Inter-individual differences in gut microbiota compositions can lead to variation in host health (Gupta et al. 2020), pathogen susceptibility (Rosshart et al. 2017, Leung et al. 2018, Alavi et al. 2020) and measures of fitness such as survival (Wilmanski et al. 2021, Worsley et al. 2021). Although the mechanisms underpinning these relationships remain poorly understood, one possibility is that hosts maintain individualized and stable microbial symbionts that are disproportionally important for mediating long-term physiological and behavioural phenotypes (Davidson et al. 2018). However, there is increasing evidence that gut microbial communities are highly dynamic (Grieneisen et al. 2021, Risely et al. 2021b, Vandeputte et al. 2021), and the role of individual identity

in shaping longitudinal dynamics remains puzzling (Gilbert et al. 2018). This uncertainty hinders efforts to unequivocally link gut microbiota communities to host phenotypes, to understand the temporal scales over which microbe-mediated selection may act, and to decipher how phylosymbiotic relationships between hosts and microbes evolve and persist (Groussin et al. 2020, Moeller and Sanders 2020, Mallott and Amato 2021).

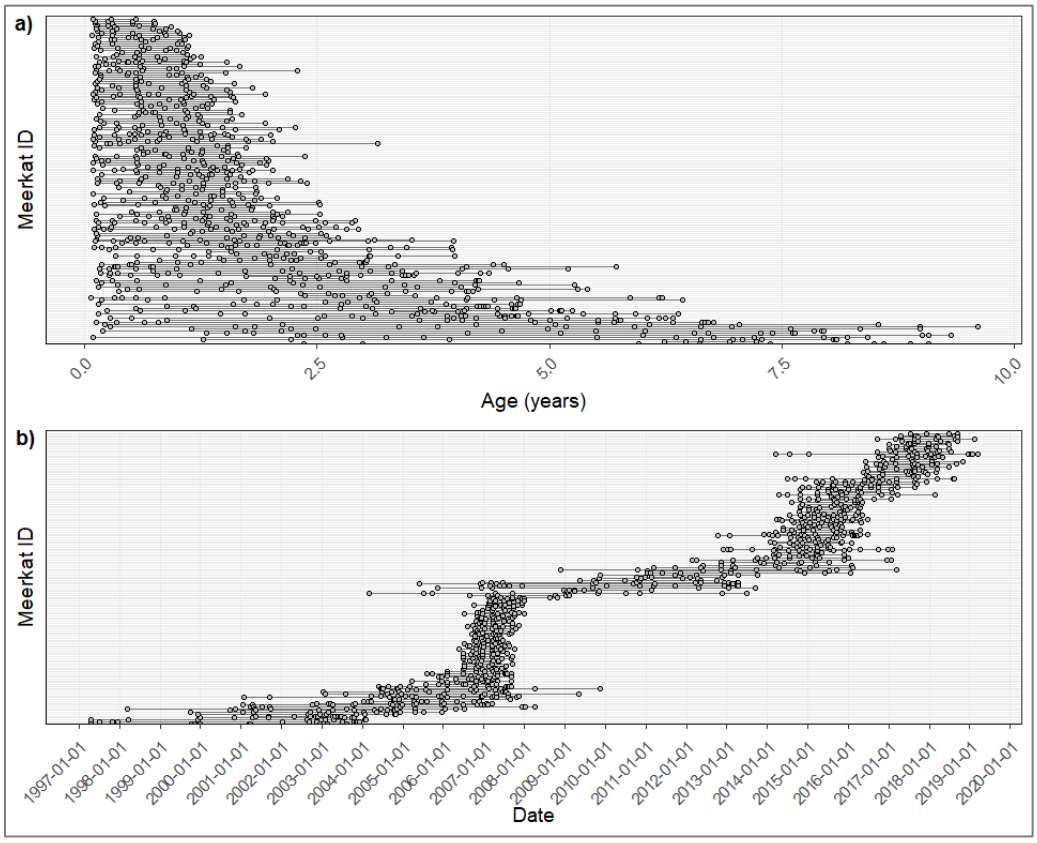
Similar to other labile phenotypes such as behaviour (Roche et al. 2016), the individuality of microbial phenotypes can be estimated via their repeatability through time. In humans from industrialized countries, gut microbiotas are characterised by high long-term repeatability across years (Franzosa et al. 2015, Lloyd-Price et al. 2017, Martinson et al. 2019), yet this long-term individuality conceals a highly dynamic community that is revealed by daily sampling (Vandeputte et al. 2021). Longitudinal studies of wild non-human primates also report highly dynamic gut microbiotas (Ren et al. 2015, Springer et al. 2017, Grieneisen et al. 2021), yet recent evidence suggests that individualised responses to changing environments limit the formation of individually unique microbial compositions over long time scales (Bjork et al. 2021). Whilst we have an emerging understanding of single factors underpinning the dynamic nature of overall community composition, including seasonality (Springer et al. 2017, Hicks et al. 2018, Baniel et al. 2021), age (Grieneisen et al. 2021), and social networks (Archie and Tung 2015, Moeller et al. 2016, Sarkar et al. 2020, Raulo et al. 2021), their relative contributions in shaping gut microbial phenotypes in natural populations remain obscure.

In this study we gathered longitudinal information on the repeatability and stability of the gut microbiota using 965 samples collected from 157 wild meerkats (*Suricata suricatta*) belonging to 22 social groups, sampled between 1997 and 2019 (Supplementary figure 1). Meerkats are small insectivorous mongooses living in social groups of two to fifty individuals in the arid regions of southern Africa. The population researched here is part of the Kalahari Research Project, which has monitored tagged individuals since 1993 (Clutton-Brock and Manser 2016). We analysed 16S gut microbiota data described previously (Risely et al. 2021b), and which was generated using an internal standard to quantify 16S copy number. Previous research demonstrated that the gut microbiota of

this population differs from that of primates in that it undergoes strong diurnal oscillations, yet weak seasonal changes (Risely et al. 2021b), generating particularly high microbial turnover rates on a daily basis.

We aimed to 1) quantify the relative contributions of meerkat identity, social group membership, and annual variation in explaining long-term gut microbiota composition; 2) identify microbial lineages that are most likely to vary across individuals, social groups, and year; 3) investigate whether the contribution from these three variables changes throughout time and with meerkat age; and 4) examine whether changes to individual repeatability are determined by shifts in overall community stability.

***Supplementary figure 1)*** *Sampling timelines ordered by a) meerkat age; and b) date the sample was taken.*

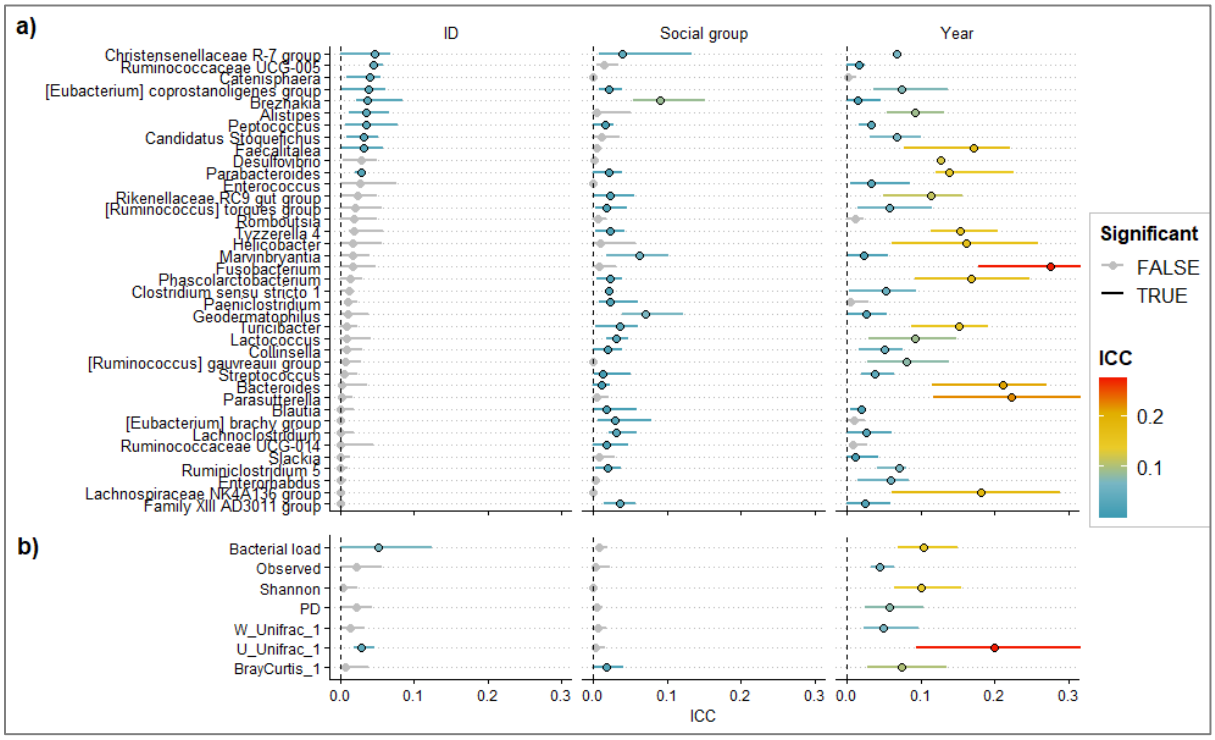


## Results

Strong annual variation across gut microbial phenotypes

Repeatability is defined as the proportion of total phenotypic variation that is attributed to individuals, and is also referred to as the Intraclass Correlation Coefficient (ICC) when applied other sources of variation such as social group or year (Nakagawa and Schielzeth 2010, Stoffel et al. 2017). We partitioned variation (ICC) attributed to either meerkat identity, social group membership or annual variation for 39 collapsed genera that were detected in 50% of samples, which together accounted for 82% relative abundance. Annual variation had the strongest effects across genera (87% significant; mean ICC = 0.081) with social group membership (62% significant; mean ICC = 0.02) and identity (26% significant; mean ICC = 0.016) having successively weaker effects (Fig. 1a). The genera *Christensenellaceae (R-7 group)* and *Ruminococcaceae (UCG-005)* were most likely to be characterised by inter-individual variation. Annual variation was also the most important predictor of most community diversity measures, and was particularly associated with suites of rarer, non-core taxa (captured by Unweighted Unifrac; Fig. 1b). Both individual identity and social group were largely unimportant for explaining gut microbiota diversity across the 20-year study period (Fig. 1b).

***Figure 1)*** *Contributions of meerkat identity, social group membership, and year on a) the abundances of 39 genera that were detected in at least 50% of samples, and b) community phenotypes, including bacterial load, three measures of alpha diversity, and the first axis of variation extracted from ordinations based on three beta diversity distances. Point colours are scaled by their relative effect size (ICC) and greyed out if they are not significant. PD = Faiths phylogenetic alpha diversity.*

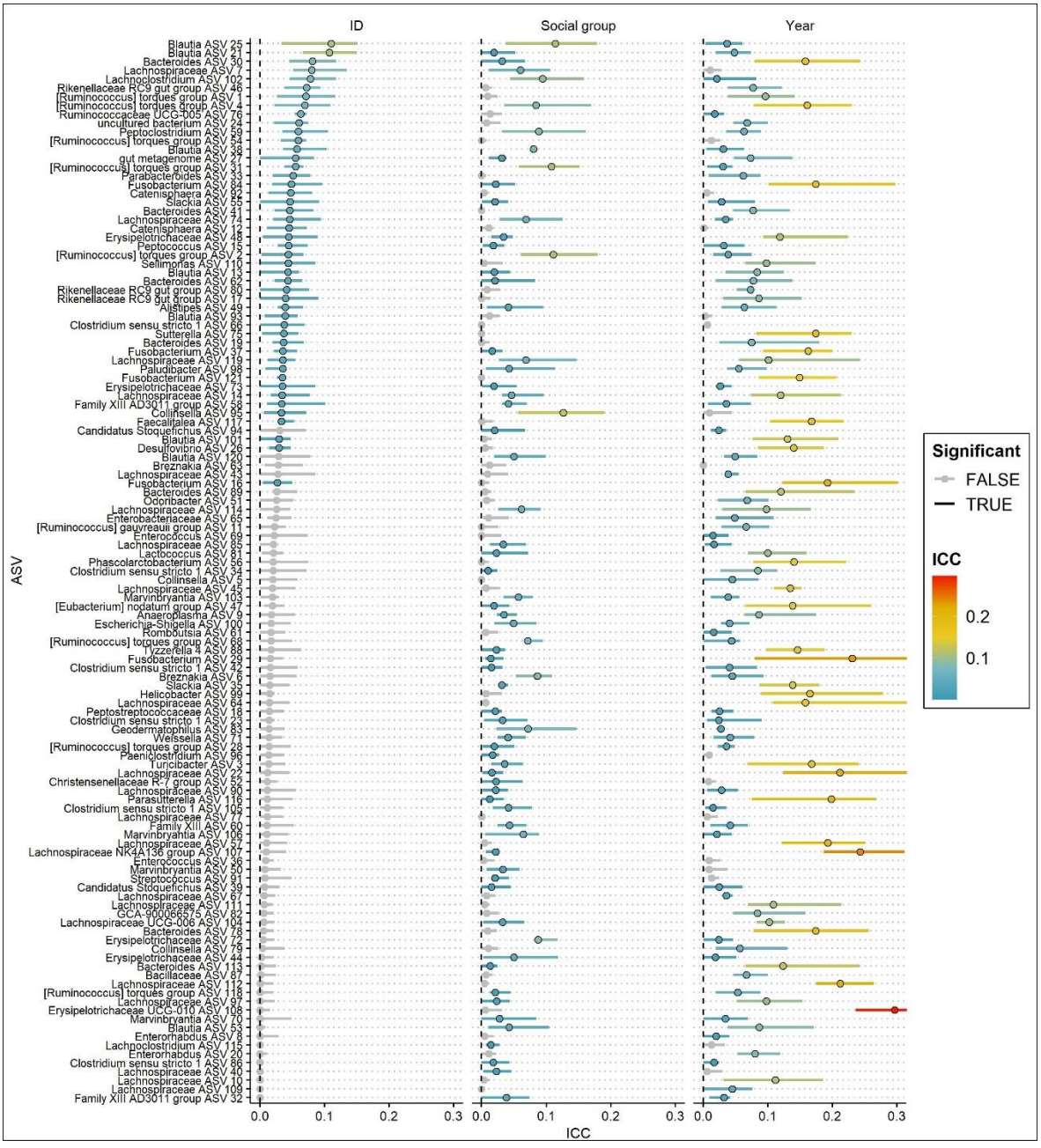



We next modelled taxa abundances at the amplicon sequence variant (ASV) level, including 121 ASVs that were detected in over 30% of samples and which together also accounted for 79% of relative abundance. Annual variation again had the strongest effects across ASVs (87% significant, mean ICC = 0.09±0.06 s.d), with social group membership (60% significant, mean ICC = 0.04±0.03 s.d.) and identity (39% significant, mean ICC = 0.05±0.02 s.d.) having weaker effects (Supplementary figure 2). Weak correlations existed between the effects of identity, year, and social group, with ASVs that tended to be characterised by identity, also tending to be characterised by social group membership (Pearson's r = 0.27, p = 0.003), whilst taxa characterised by inter-

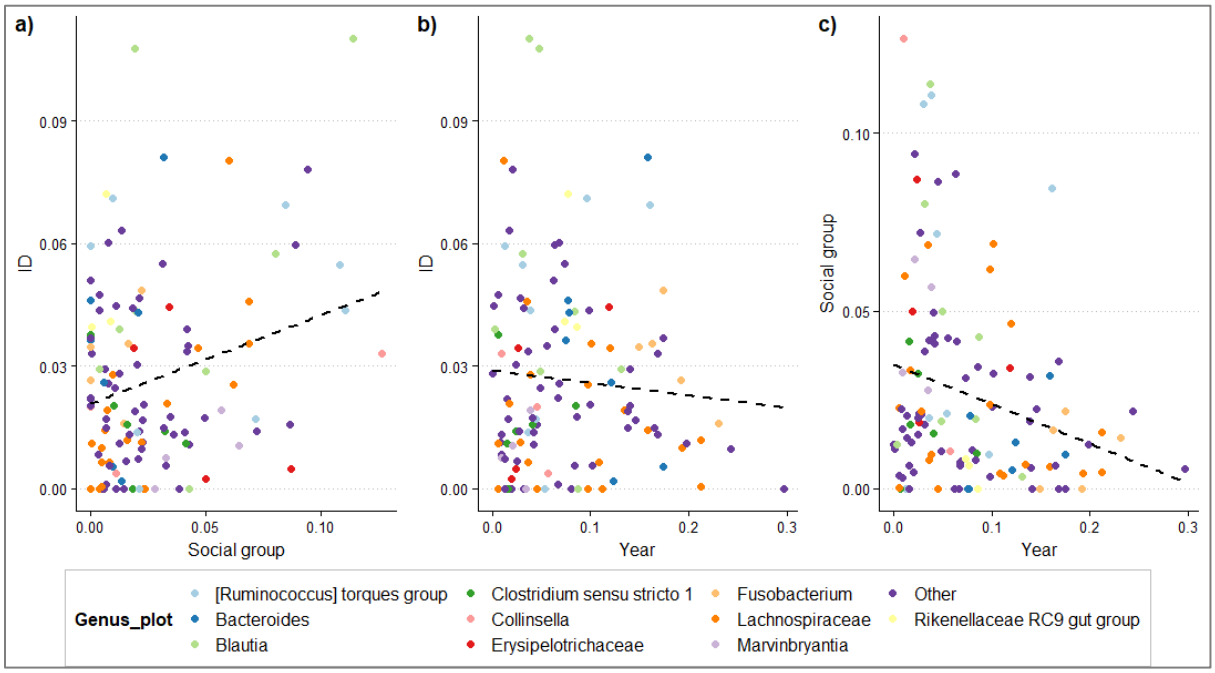
group variation tending to be buffered from annual effects (Pearson's r = -0.26, p = 0.006; Supplementary figure 3). The most individually repeatable ASVs belonged to the genera *Blautia* and *Bacteroides*. However, these genera were not significantly repeatable within individuals at the genus level, suggesting that identity effects often act at higher taxonomic resolutions than genus level.

We tested whether the weak influence of identity was dependent on model structure by excluding social group membership and year from models. Excluding social group and year considerably inflated the contributions of identity, with the majority of ASVs becoming significantly associated with identity (Supplementary figure 4).

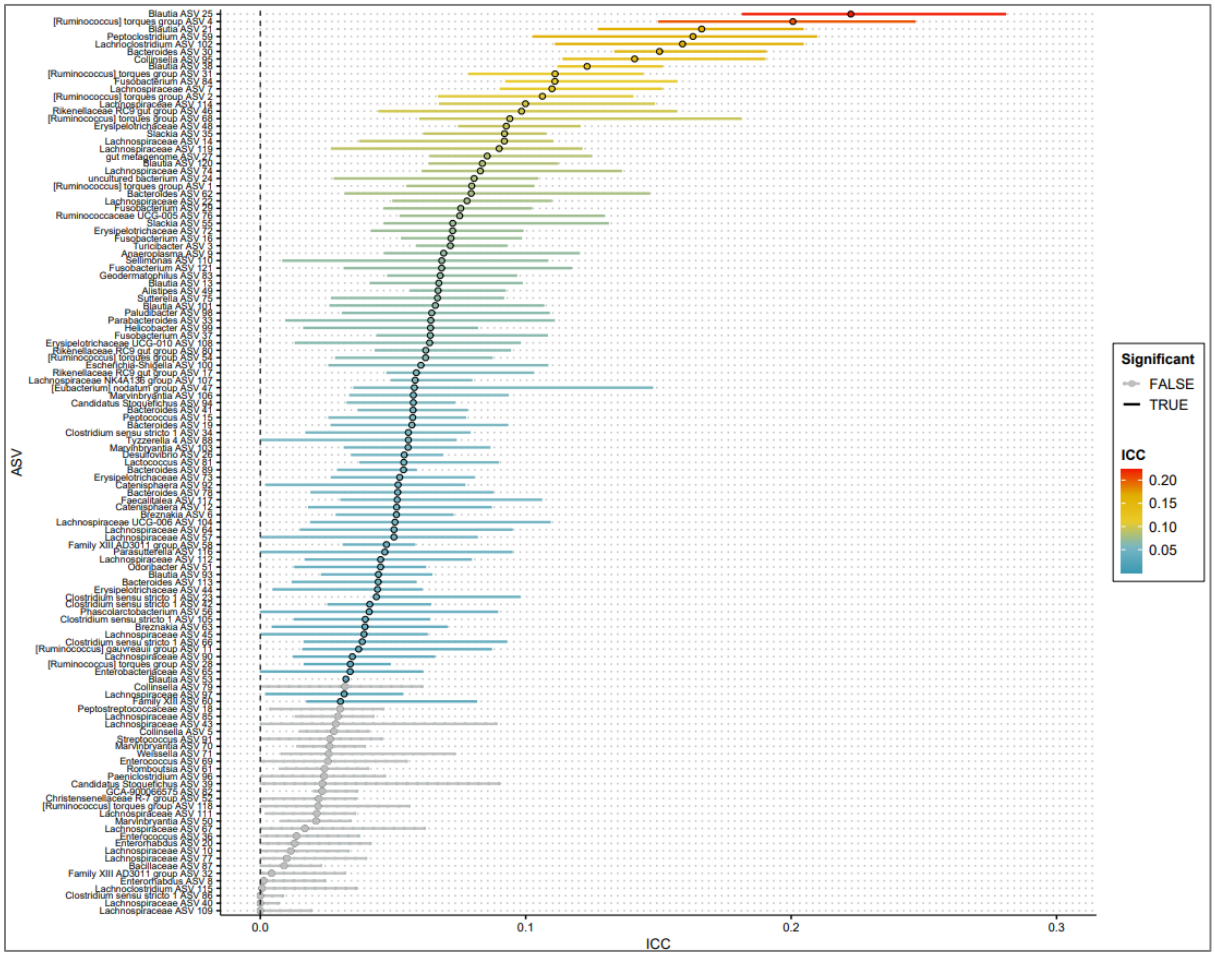
***Supplementary figure 2)*** *Contributions of meerkat identity, social group, and year on the abundances of 121 ASVs that were detected in at least 30% of samples. Point colours are scaled by their relative effect size (ICC), and greyed out if they are not significant. ASVs are ordered by contributions of meerkat identity.*



***Supplementary figure 3)*** *Correlations between ICC as a function of a) identity and social group; b) identity and year; and c) social group and year.*



***Supplementary figure 4)*** *Contributions of individual identity in explaining the abundances of 121 ASVs with prevalence over 30% when social group and year are excluded from models.*

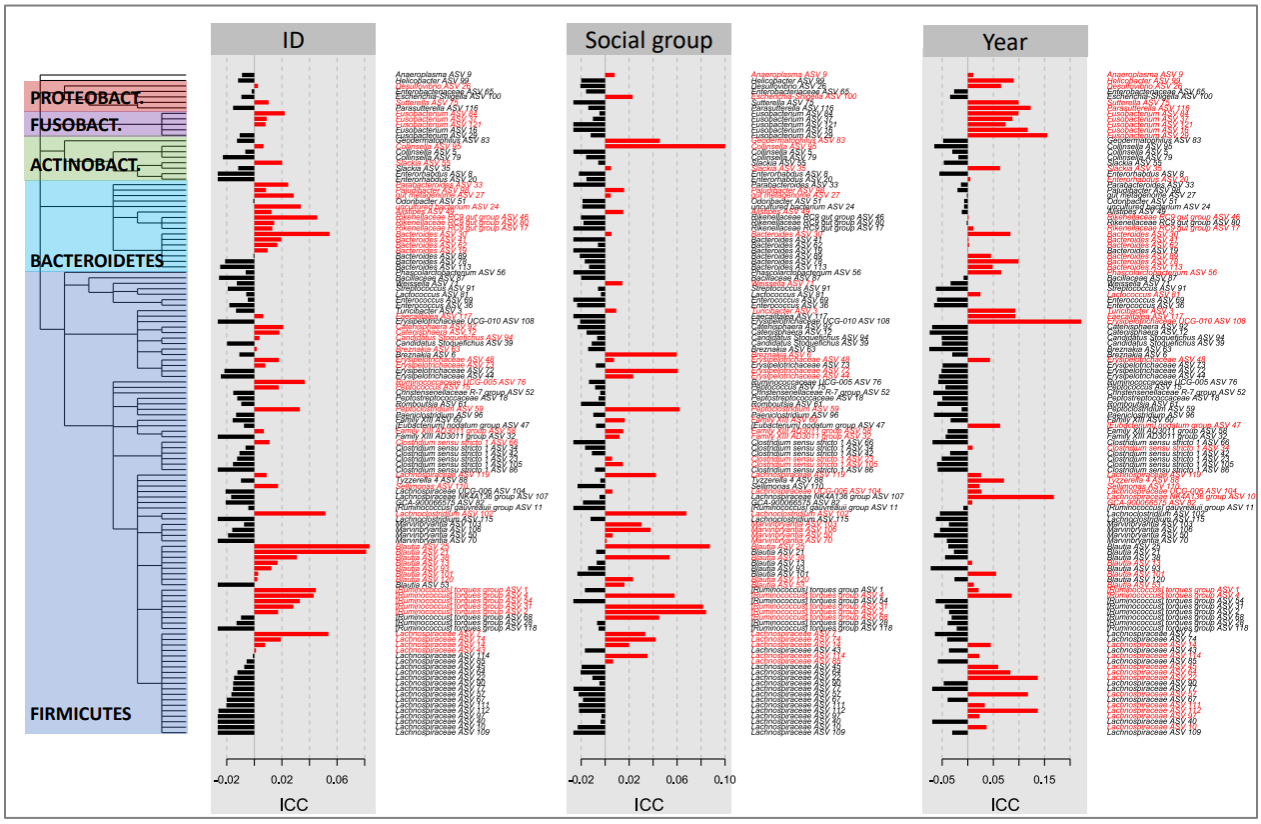



Localised phylogenetic signal in ICC

To test whether patterns in ICC were centred around particular phylogenetic branches, we estimated the phylogenetic signal in associations with identity, social group membership, and year. We found localised phylogenetic signals for individual identity (Moran's *I* = 0.00728, *P* = 0.014), social group (Moran's *I* = 0.00711, *P* = 0.018), and yearly variation (Moran's *I* = 0.02, *P* = 0.003; Fig. 2). Individual identity was predominantly associated with members of the Phylum Bacteroidetes, in particular *Rikenellaceae*, *Alistipes,* and some *Bacteroides* members, as well as some specific

Firmicutes genera, including *Blautia*, and *Ruminoccocus torques group*. In contrast, social group had wide-spread effects across members of Firmicutes, with particularly notable associations with cellulose-degrading *Marvinbryantia*, potentially reflecting different levels of plant consumption amongst social groups. Annual variation characterised members of the phyla Fusobacterium and Proteobacteria, some members of Bacteroides, and members of *Lachnospiraceae.* The most abundant genus, *Clostridium sensu stricto 1,* which undergoes strong diurnal oscillations (Risely et al. 2021b), demonstrated no phylogenetic signal in association with any variables.

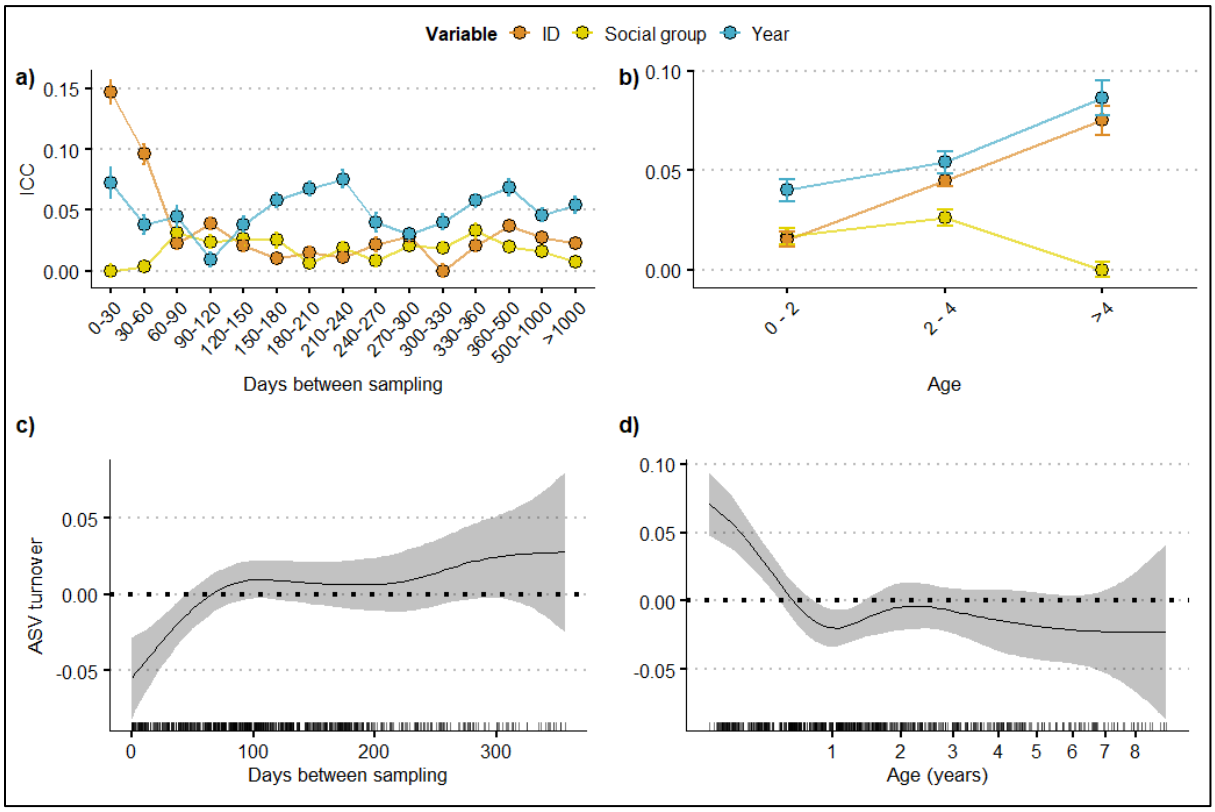
***Figure 2)*** *Phylogenetic signal in the contributions of individual identity, social group membership, and year across 121 ASVs with over 30% prevalence in the overall sample. ASVs for which ICC is higher than average are coloured in red. The major phyla are indicated.*



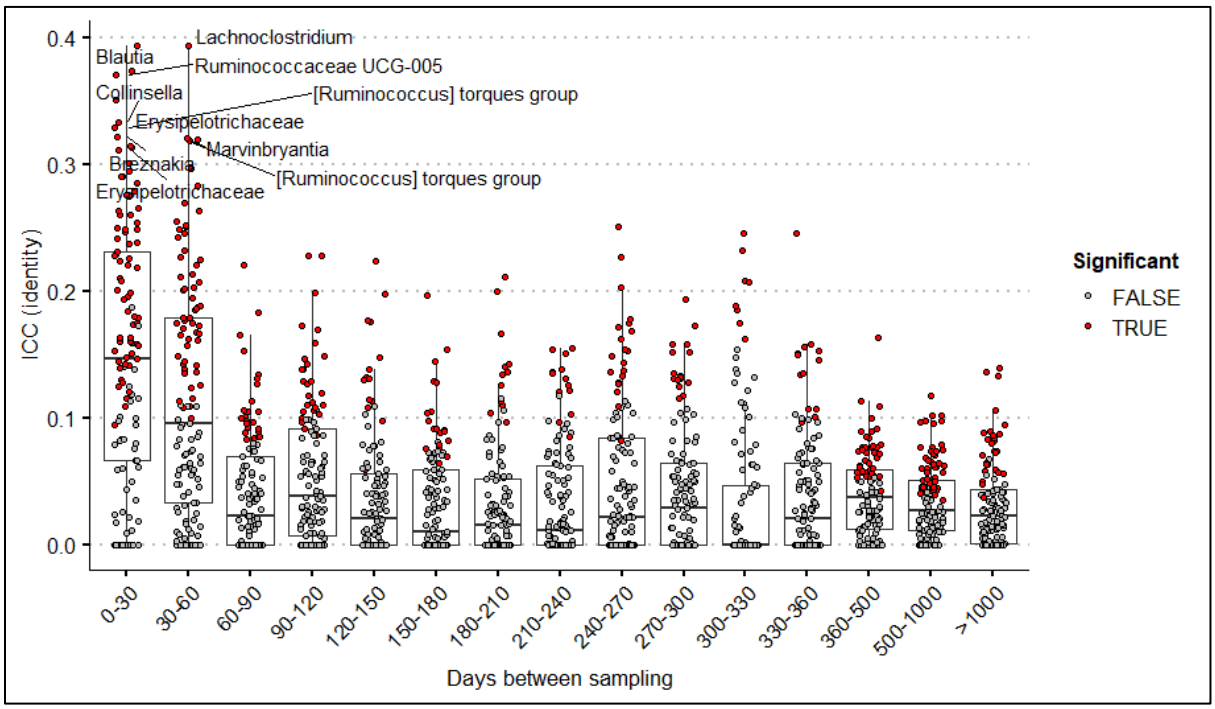
Effects of meerkat identity weaken over time

Whilst individual identity had weak effects over the whole study period, we hypothesised that it may be more important over shorter time frames. To test this, we compared contributions of identity, social group membership, and year to explain the abundances of the 121 ASVs analysed above. To do this, we selected two samples per individual that were collected either close together in time (within one month), or far apart (over one year), and repeating this process over a range of temporal time frames. As predicted, individual identity was more important than social group and year when longitudinal samples were taken within two months of each other, but the importance of identity decreased rapidly when individuals were sampled over long periods (Fig. 3a). When samples were longitudinally sampled within the same month, mean and median repeatability were 0.2 and 0.15, respectively, with some ASVs having a repeatability as high as 0.39 (Supplementary figure 5). When including only samples that were taken over two months apart, annual variation became the most important predictor of ASV abundances (Fig. 3a). Social group had, on average, weak effects (median ICC < 0.05) across all time frames.

***Figure 3)*** *Temporal trends in ICC (top panel) and ASV turnover between sampling events (lower panel) of meerkat gut microbiomes. Top panel: median ICC (and standard error) of individual identity, social group membership, and year from models predicting the abundances of 121 ASVs when samples are categorised by a) time intervals between sampling from the same individual; and b) different meerkat age categories. Bottom panel: Temporal predictors of ASV turnover between consecutive sampling events from the same individual, extracted from a GAMM, showing the association between ASV turnover and d) the number of days between samples; and e) the age of the meerkat at the point of the first sample.*



***Supplementary figure 5)*** *ICC estimates for individual identity for 121 ASVs with prevalence over 30%, when data are categorized based on longitudinal sampling period. ASVs which are significantly repeatable are indicated in red.*




Effects of meerkat identity are age dependent

There is evidence from wild baboons (*Papio cynocephalus*) that within-individual repeatability of many gut microbial taxa increases with age (Grieneisen et al. 2021), and we tested whether a similar pattern could be found in meerkats. Mean ASV repeatability did indeed increase with age, being lowest in young (< 2 years) meerkats (median = 0.015, 10% significantly repeatable), higher in adults (2 – 4 years; median = 0.05, 37% significantly repeatable), and highest in meerkats older than 4 years (median = 0.08, 35% significantly repeatable; Fig. 3b). Interestingly, the contribution of year also increased with age, possibly due to older meerkats being sampled over many years (Fig. 3b). In contrast, the effect of social group decreased in older meerkats (Fig. 3b), potentially suggesting that as gut microbial communities become more individualized

with age, microbial communities are buffered from group effects, such as horizontal transmission from group members.

Changes in repeatability are reflected by shifts in community stability

Low long-term repeatability is likely driven by a decline in community similarity over time (Springer et al. 2017, Gogarten et al. 2018), in which case we predict that the timeframe associated with low repeatability would be associated with high turnover, and vice versa. To test this association, we measured ASV turnover rate (the proportion of ASV appearing or disappearing) between consecutive samples taken from the same individual, and tested whether ASV turnover rate was predicted by the amount of time elapsed between samples and meerkat age.

Mean community turnover between sampling events was very high (~80% of ASVs appeared or disappeared between sampling events). Turnover increased with the amount of time elapsed between samples (Fig. 3c), yet also was dependent on meerkat age, with turnover being much higher in meerkats under one year of age (Fig. 3d; Supplementary table 1).

***Supplementary table 1)*** *Statistics from a GAMM predicting ASV turnover between consecutive samples.*

| Term | Estimate/edf | df | Statistic | p value |
|---|---|---|---|---|
| Intercept | 0.79 | | 55.6 | <0.0001 |
| Days between samples | 3.16 | 3.9 | 5.38 | 0.0005 |
| Age at first sample | 3.83 | 3.97 | 12.6 | <0.0001 |
| Year (random) | 15.7 | 22 | 11.9 | <0.0001 |
| ID (random) | 42.6 | 156 | 0.452 | 0.002 |
| Group (random) | 4.41 | 21 | 0.791 | 0.06 |

## Discussion

Longitudinal studies of wild populations are scarce but invaluable to dissect short- and long-term evolutionary and ecological dynamics. For gut microbiota, long-term data allows us to tease apart how much variation is explained by identity, social group membership, and yearly shifts in way that is not possible from cross-sectional studies conducted over short time scales. We leveraged an extensively sampled and well-studied wild meerkat population to show that, over long time periods, annual variation has stronger effects on the abundances of most common ASVs than identity or social group membership, and in addition is more influential in shaping overall alpha and beta diversity. However, the contribution of identity is considerably higher over shorter time periods, associated with specific phylogenetic groups of taxa, and increases with meerkat age. Increased repeatability with age is underpinned by an increase in overall microbial community stability, and not due to increased stability of particular taxa. These findings suggest that the downstream physiological effects of individualized gut microbiotas are likely to act over the scale of weeks or months, rather than years.

Our findings of weak long-term contributions of individual identity to gut microbial composition, an exponential decay in microbiota similarity within individuals over time, and increases in taxon repeatability with age, all align closely with those found in a decade long study of baboons (Bjork et al. 2021, Grieneisen et al. 2021), suggesting that such dynamics may be consistent across host species. These patterns in longitudinal dynamics may resolve the conflicting reports of the major drivers of gut microbiota dynamics across different species, with different findings being due to variable sampling periods and designs rather than inherent differences amongst species. Nevertheless, this does not preclude the possibility of differences in gut microbiota stability and associated drivers between host species. For example, individual host traits such as age, sex and social dominance rank generate individualized microbial signatures that are stable over short time frames in baboons (Bjork et al. 2021), yet these traits do not have a strong stabilizing effect in meerkats (Risely et al. 2021b).

We distinguished between taxa whose temporal variation is characterised by inter-individual, inter-group, or inter-annual variation. Pinpointing taxa associated with inter-individual variation is important for being able to link the gut microbiota to immutable host traits encoded by genetics and responsible for host fitness; yet, this is highly challenging when different sources of variation are nested in structure. In humans, members of the phylum Bacteroidetes tend to be characterised by strong inter-individual variation (Lloyd-Price et al. 2017), and this also appears to be the case in meerkats, with the Bacteroidetes genera *Alistipes, Rikenellaceae*, and *Bacteroides* all tending to be associated with meerkat identity. Some specific lineages of Firmicutes were also associated with identity, including *Blautia*, *Ruminoccocus torques group,* and *Christensenellaceae R-7 group*. Many of these genera have been found to be significantly heritable in mammals (Grieneisen et al. 2021), and therefore may be more likely to be associated with host traits such as genotype, physiology, or fitness. Together, these lines of evidence suggest that future studies of mammalian host-gut microbe interactions may benefit from focussing on these lineages as a potential mediator of host health and fitness.

We found that annual variation was mostly associated with members of the phyla Fusobacterium and Proteobacteria, as well *Bacteroides* (phylum Bacteroidetes) and *Lachnospiraceae* (Phylum Firmicutes). Fusobacterium and Proteobacteria tend to make up only a small component of mammalian gut microbial communities, with the exception of bats (Song et al. 2020), yet there is evidence that they are often highly abundant in diseased individuals suffering with a dysbiotic gut microbial community (Shin et al. 2015, Amitay et al. 2017, Rizzatti et al. 2017). The exact mechanism that causes this year-to-year variation remains unclear, given that the amount of rainfall is not an important predictor of gut microbiota composition in this population (Risely et al. 2021b). However, longer term climatic conditions, such as drought, are known to affect population health in this system. Climate extremes have long term effects on reproduction (Hodge et al. 2008, Bateman et al. 2013), mortality (Clutton-Brock et al. 1999), and tuberculosis prevalence (Patterson et al. 2017, Paniw et al. 2022), and together these may produce signals of dysbiosis in the gut microbiota.

Taken together, our findings call into question how close a match between host traits and the seemingly highly dynamic, and little individualised microbial phenotype can be expected. In contrast to stochastically-fluctuating taxa, individualized microbes are expected to be heritable (Grieneisen et al. 2021), and potentially associated with a host's genotype and evolutionary lineage (Moeller and Sanders 2020, Mallott and Amato 2021). As such, temporal stability and repeatability form the conceptual basis of linking commensal microbiota with, for instance, host immunogenetics and disease susceptibility (Montero et al. 2021). Low lifetime repeatability of most taxa may explain why reported associations between host genotype and gut microbiota composition are generally weak and rather specific (Rothschild et al. 2018, Suzuki et al. 2019, Davies et al. 2021). A focus on taxa that are moderately repeatable within individuals (e.g., members of *Christensenellaceae R-7 group*, *Alistipes, Rikenellaceae*, *Bacteroides, Blautia*, *Ruminoccocus torques group* in this study) will be important for understanding the genetic or ecological basis for why these taxa are relatively individualised compared to others.

Similarly, members of the same social group might be expected to share a substantial amount of microbial taxa (Sarkar et al. 2020), and this might translate into similar microbial responses to shared environments. Yet, group effects can be indistinguishable from environmental effects when social groups overlap in their territories, as is the case for baboons (Bjork et al. 2021). We found that social group membership played a secondary role to annual variation in explaining gut microbial variation, yet generally was associated with different suites of taxa, suggesting these associations are indeed shaped by social interactions between group members rather than underpinned by differences shared environments between groups. This underscores the importance of decomposing the often-nested effects of identity, social group membership, and long-term environmental conditions when synthesizing the relationship between gut microbial phenotype and both host genotype and fitness.

In conclusion, our findings demonstrate that the dynamics of specific lineages are differentially driven by either identity, social group membership, or annual variation, with implications for the mechanisms by which host-gut microbe symbioses function and

evolve. These results have important implications for how we study gut microbiota dynamics of natural populations in the future, and the time frames over which gut microbial phenotypes may mediate host physiology, behaviour and fitness.

## Material and Methods

### Study population and sample collection

The study population inhabits the Kalahari Desert region in South Africa (-26.96S, 21.83E). 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 and Manser 2016). Faecal samples have been collected across the entire study period from almost all monitored individuals. For this study, we analysed a subset of the samples included in (Risely et al. 2021b), excluding any individuals that had three samples or less. We therefore included a total of 965 samples collected from 157 wild meerkats (mean samples per ID = 7.5, min. = 4, max. = 14) belonging to 22 social groups, sampled between 1997 and 2019 (Supplementary figure 1). 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 hours. For long-term storage, samples were then either frozen at -80c (before 2008) or freeze-dried (after 2008). Effects of storage were minimal and are investigated in Risely et al. (2021b).

### 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 ±0.05 µg (wet) was taken, and 3µl 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 which are rarely found in gut 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 normalise 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 (Stämmler et al. 2016, Hardwick et al.

2017, Tourlousse et al. 2017, Lin et al. 2019). We have shown previously with this dataset that sample identity accounts for 90% of variation in estimated bacterial load, whilst 10% is technical variation (Risely et al. 2021b).

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-GTGCCAGCMGCCGCGGTAA-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.

Microbiome bioinformatics and normalisation

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 p. 2) to generate amplicon sequence variants (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 *Allobacillus halotolerans* abundance per sample to quantify ratios of absolute abundance across samples (described in Risely et al. 2021b). Both *Allobacillus* and *Imtechella* were then removed, and all further analysis were conducted

on normalised reads. Because some samples had very high relative abundances of spike-in, we only retained samples for which read depth of the true microbiome (minus the internal reference) was over 5,000.

Sample metadata

Detailed analysis of the biological and environmental factors that are associated with meerkat gut microbiotas was conducted in (Risely et al. 2021b). Here, our aim was to quantify the contributions of identity, social group, and year whilst 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 storage. We therefore included these variables in all models (described below). We measured time of day in reference to sunrise because this is more biologically meaningful than time of day. We calculated sunrise times per day using *suncalc::getSunlightTimes* (Agafonkin and Thieurmel 2017). We categorised season into wet (October to April) and dry (May to September).

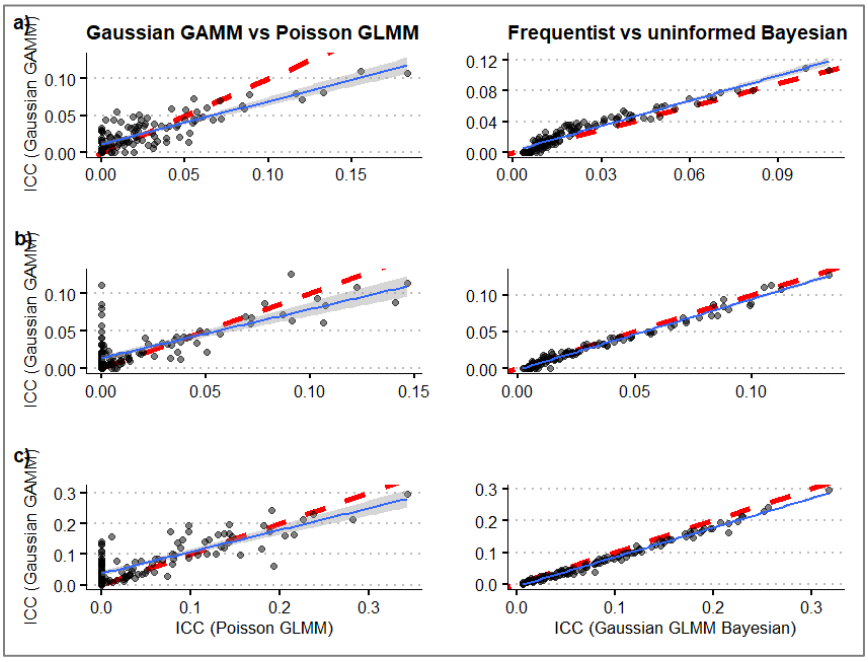
Statistical analysis

We quantified the contributions of individual identity, social group membership, and year for predicting the abundances of 39 genera that were detected in over 50% of samples, 121 single-taxon phenotypes at the ASV level that were detected in at least 30% of samples, and seven community phenotypes that represented measures of bacterial load, alpha diversity, and beta diversity. To do this, we estimated the adjusted Intraclass Correlation Coefficient (ICC) of all three variables when included as random effects in a Generalized Additive Mixed model (GAMMs), fitted using the *mgcv* package (Wood 2017), controlling for time of day, meerkat age, and sequencing depth as non-linear factors, and season (wet/dry), sample storage method, and sequencing run as fixed factors. We accounted for temporal autocorrelation by including an autocorrelation term in the model, nested by year.

ICC and 95% confidence intervals of the three random effects were calculated using the R function *rptGam::rptgam* (https://github.com/elipickh/rptGam). We chose a GAMM approach with a Gaussian distribution because both changes in microbiota abundances

across the day and with sequencing depth are non-linear, and ICC and 95% confidence intervals become increasingly challenging to estimate with other distributions such as Poisson. Reliable approaches for estimating ICC from models with negative binomial error distributions and/or zero inflation parameters are not yet available. GAMMs are also more likely to converge than linear models when data has high levels of nestedness, as it does here. Nevertheless, using a Gaussian approach may not always be appropriate if taxa counts are zero-inflated. We therefore also estimated ICC applying various modelling approaches, including linear models with square root transformed ASV counts modelled with a Poisson distribution, and applying both frequentist and Bayesian methods to linear models and comparing ICC estimates to GAMMs. All methods returned highly correlated estimates for ICC (Supplementary figure 6), suggesting that estimates are robust to different modelling approaches.

***Supplementary figure 6)*** *Comparison of ICC for a) individual identity, b) social group, and c) year for 121 ASVs when using the reported approach (GAMM with Gaussian distribution), versus ICC values from a Poisson GLM (left panel) and a Gaussian GLM using an uninformed Bayesian framework (right panel). The red dashed line indicates the 1:1 line.*



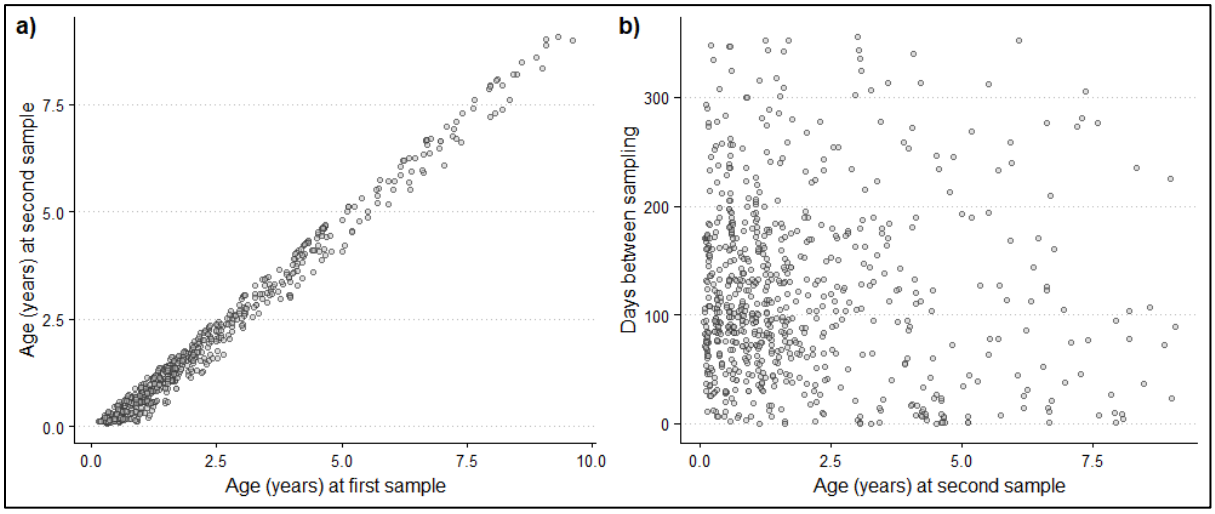
Phylogenetic signal

We tested for phylogenetic signal in ICC using the functions *phylosignal::lipamoran* and *phylosignal::phyloSignal* (Keck et al. 2016), applying Moran's I index as a measure of the correlation between ICC and bacterial phylogenetic structure.

ASV turnover

We estimates ASV turnover between consecutive samples collected from the same individual using the function *codyn::turnover* (Hallett et al. 2016). We then modelled changes to turnover using a GAMM with a Gaussian distribution and the number of days between samples and the age of the meerkat at the first sample as fixed effects, while accounting for meerkat ID, social group membership, and year as random effects. Note that because samples tended to be taken regularly from individuals across the sampling period, meerkat age at the first sample and meerkat age at the second sample were highly correlated (Pearson's $r = 0.97$, $p < 0.0001$; Supplementary figure 7a), therefore both variables had almost identical effects when included in the model. In addition, days between samples was not correlated with age at second sample (Pearson's $r = 0.0003$, $p = 0.9$; Supplementary figure 7b), therefore estimates were not bias by co-correlation between explanatory variables. Removing or adding variables had no effect on the model estimates, indicating that results are robust to changes in model structure.

***Supplementary figure 7)*** *Correlation between a) meerkat age when the first and second sample was taken for the analysis of ASV turnover between consecutive samples; and b) meerkat age (at second sample) and the number of days between samples.*



**Acknowledgments**

We are grateful to the Kalahari Research Trust and the Kalahari Meerkat Project for access to facilities and habituated animals in the Kuruman River Reserve, South Africa. This paper has relied on records of individual identities and/or life histories maintained by the Kalahari Meerkat Project and collected by scientists and volunteers. We thank the Northern Cape Conservation Service for permission to conduct fieldwork, and the South African Weather Service (SAWS) for providing weather data. We thank Ben Danzer for facilitating with sample collation and storage and Ulrike Stehle for contributing to lab work.

**Funding:**

German Research Foundation DFG SO 428/15-1 (SS); European Research Council 294494 (THCB); European Research Council 742808 (THCB); Human Frontier Science; Program RGP0051/2017 (THCB); University of Zurich (MBM); MAVA Foundation KRP 16026 (MBM, THCB).

**Author contributions:**

Conceptualization: AR, SS; Formal analysis: AR; Investigation: AR, KW; Resources: SS, MBM, THCB; Data Curation: MBM, THCB; Writing - Original Draft: AR, DWS; Writing - Review & Editing: AR, DWS, NMK, SS, MBM; Project administration: SS; Funding acquisition: SS, AR, MBM, THCB.

**Competing interests:**

Authors declare no competing interests.

**Data and materials availability:**

All sequences and processed data used in this study are available to download at (Risely et al. 2021a). Sequences are additionally stored under NCBI BioProject PRJNA764180. R code can be downloaded at https://github.com/Riselya/Microbiome-repeatability.

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