



# Spatiotemporal Dynamics of Early Life Microbiome Colonization in a Canine Model



Yehoon Jo<sup>2\*</sup>, Toby Branck<sup>1\*</sup>, Artemis S. Louyakis<sup>1</sup>, Jacob T. Nearing<sup>2-4</sup>, Amrisha Bhosle<sup>2-4</sup>, Matthew I. Jackson<sup>1</sup>, Christoph Brockel<sup>1</sup>, Curtis Huttenhower<sup>2-4\*</sup>, Kelsey N. Thompson<sup>2-4\*</sup>, Dayakar V. Badri<sup>1</sup>



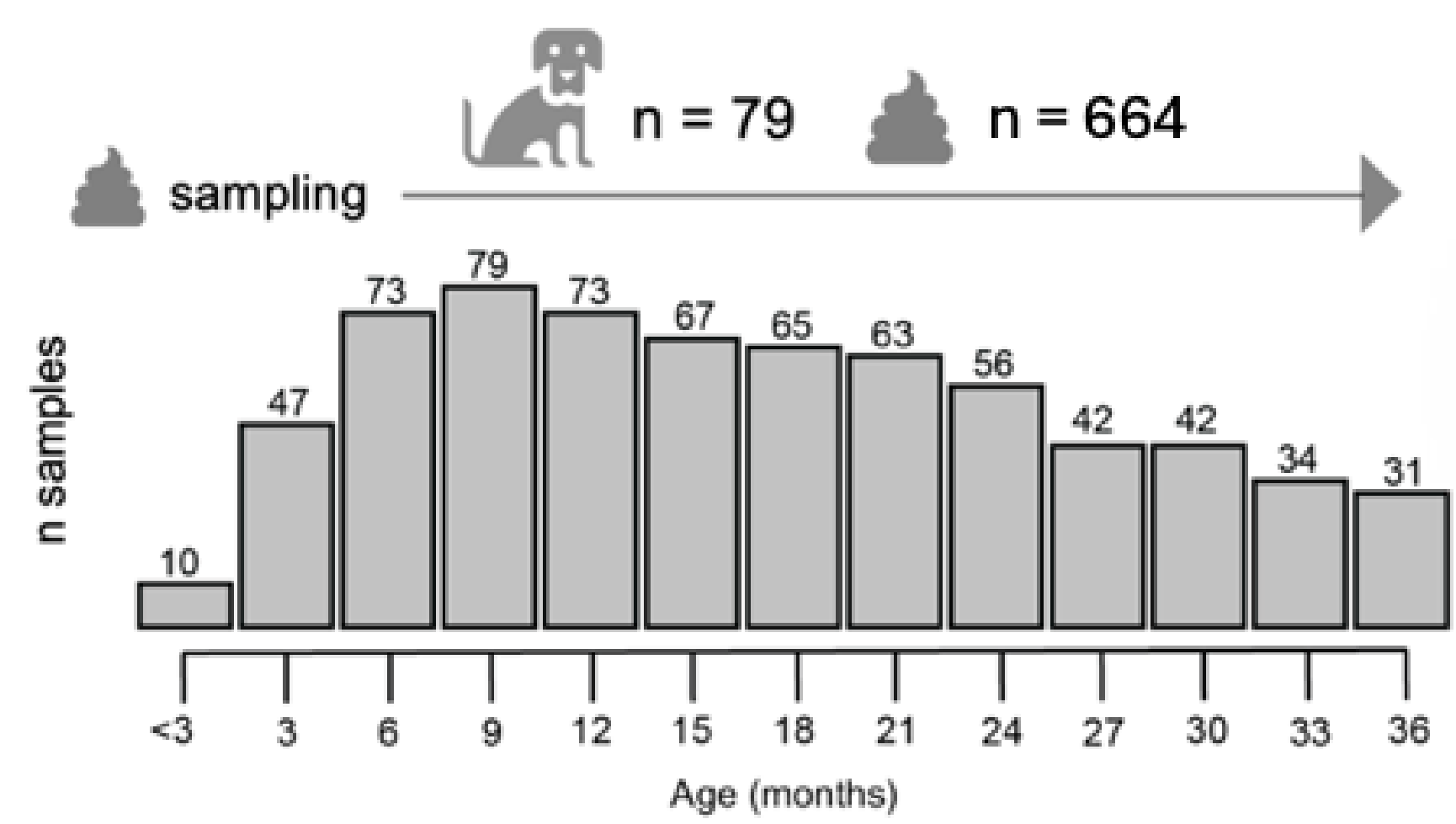
<sup>1</sup>Science and Technology Center, Hill's Pet Nutrition, Inc., Topeka, KS, USA, <sup>2</sup>Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA  
<sup>3</sup>Infectious Disease and Microbiome Program, Broad Institute of MIT and Harvard, Cambridge, MA, USA, <sup>4</sup>Harvard Chan Microbiome in Public Health Center, Harvard T.H. Chan School of Public Health, Boston, MA, USA



Early-life microbiome assembly represents a critical developmental window, yet the factors shaping colonization in companion animals remain poorly understood. Using 664 longitudinal stool metagenomes from 79 colony dogs sampled from 3 to 36 months of age, we characterized gut microbiome development and identified age-associated shifts in microbial composition, stability of colonizers, and within-species functional gene content.

## Longitudinal sampling from a canine model and study methodology

### Samples & Methods



Taxonomic, phylogenetic, and functional profiling

MetaPhlAn 4.0  
StrainPhlAn  
HUMAnN 4.0

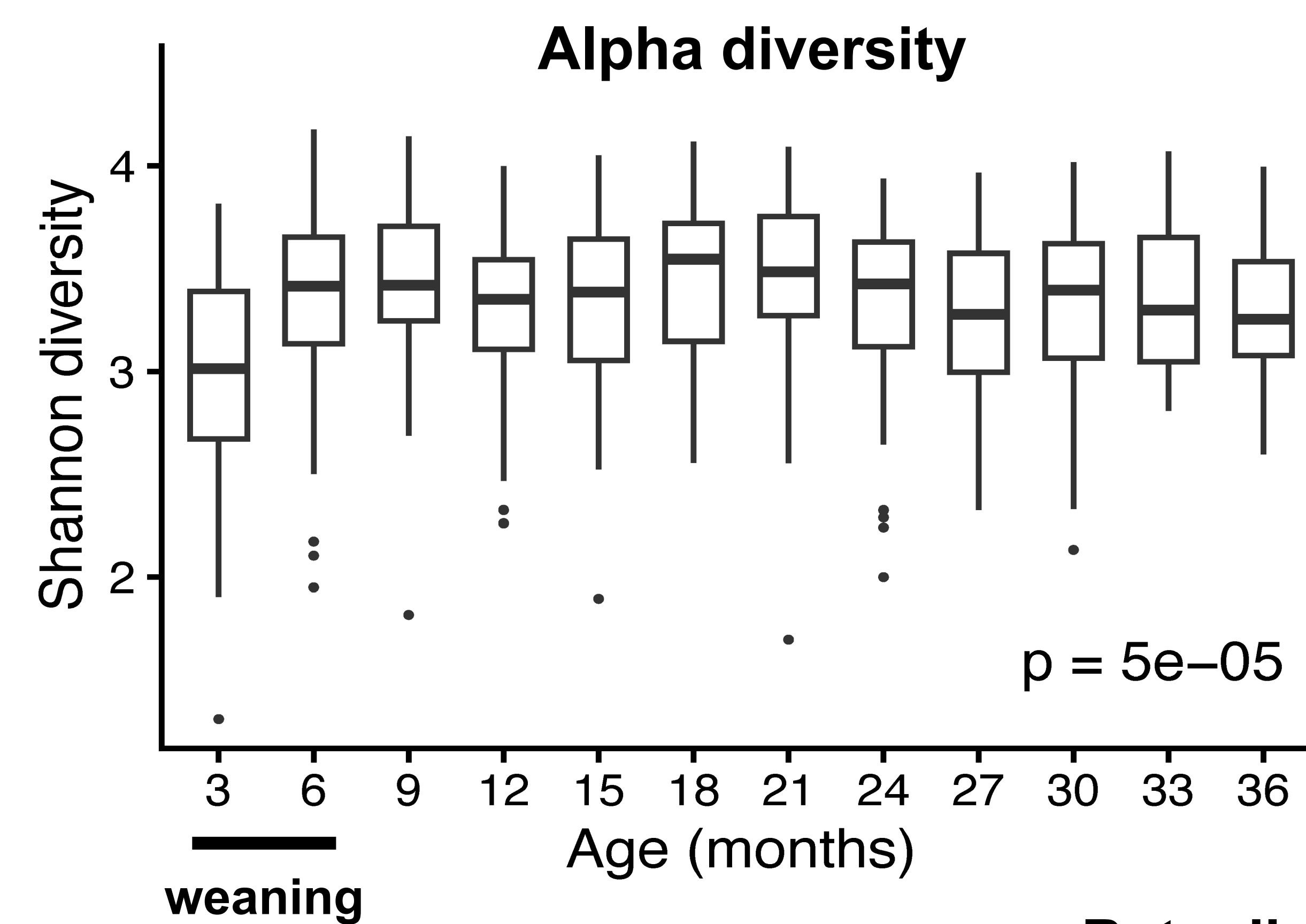
Statistical analysis

MaAsLin 3



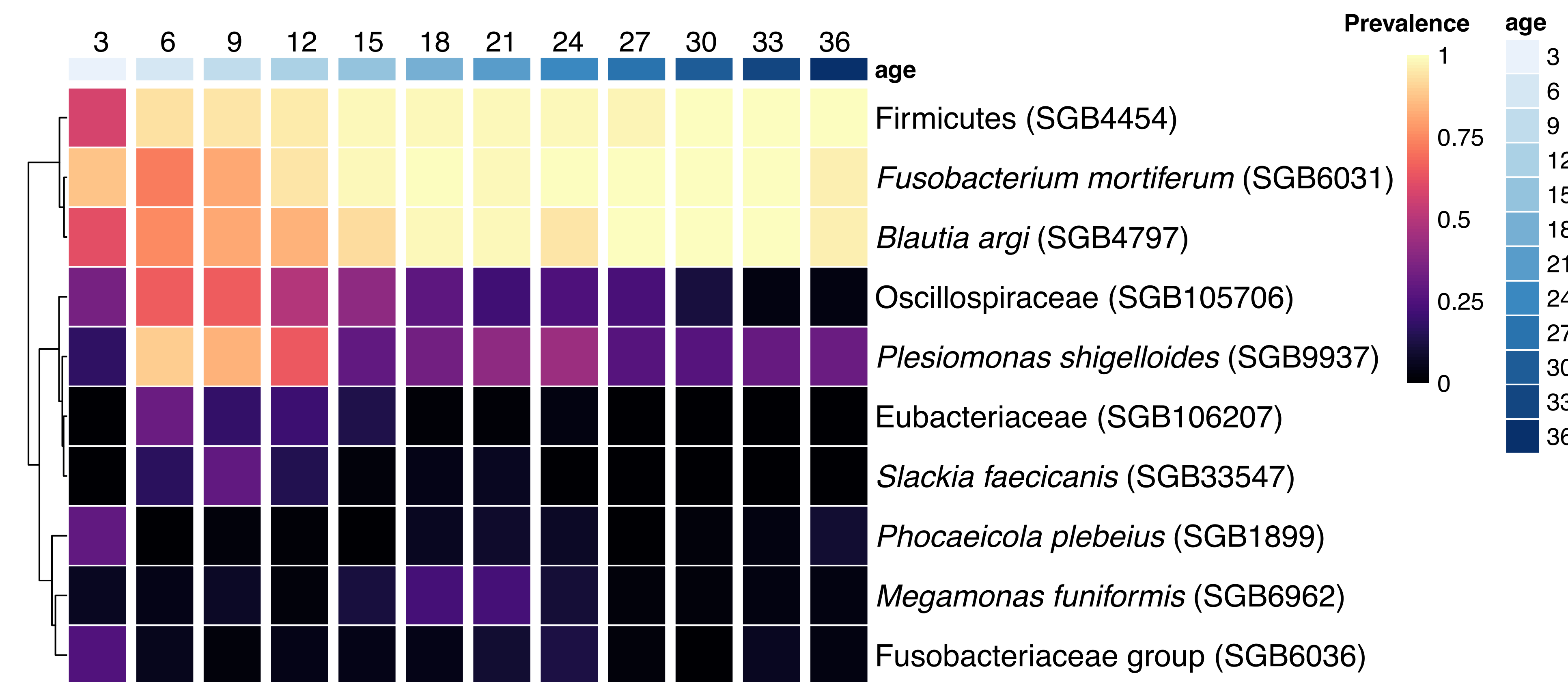
- Longitudinal canine cohort comprising 664 stool metagenomes collected approximately every 3 months from 79 colony dogs between 3 months and 3 years of age.
- Taxonomic and functional profiling using the bioBakery tool suite with rich metadata on diet, housing, breed, and lineage.

## Gut microbiome diversity increases after the introduction of solid food



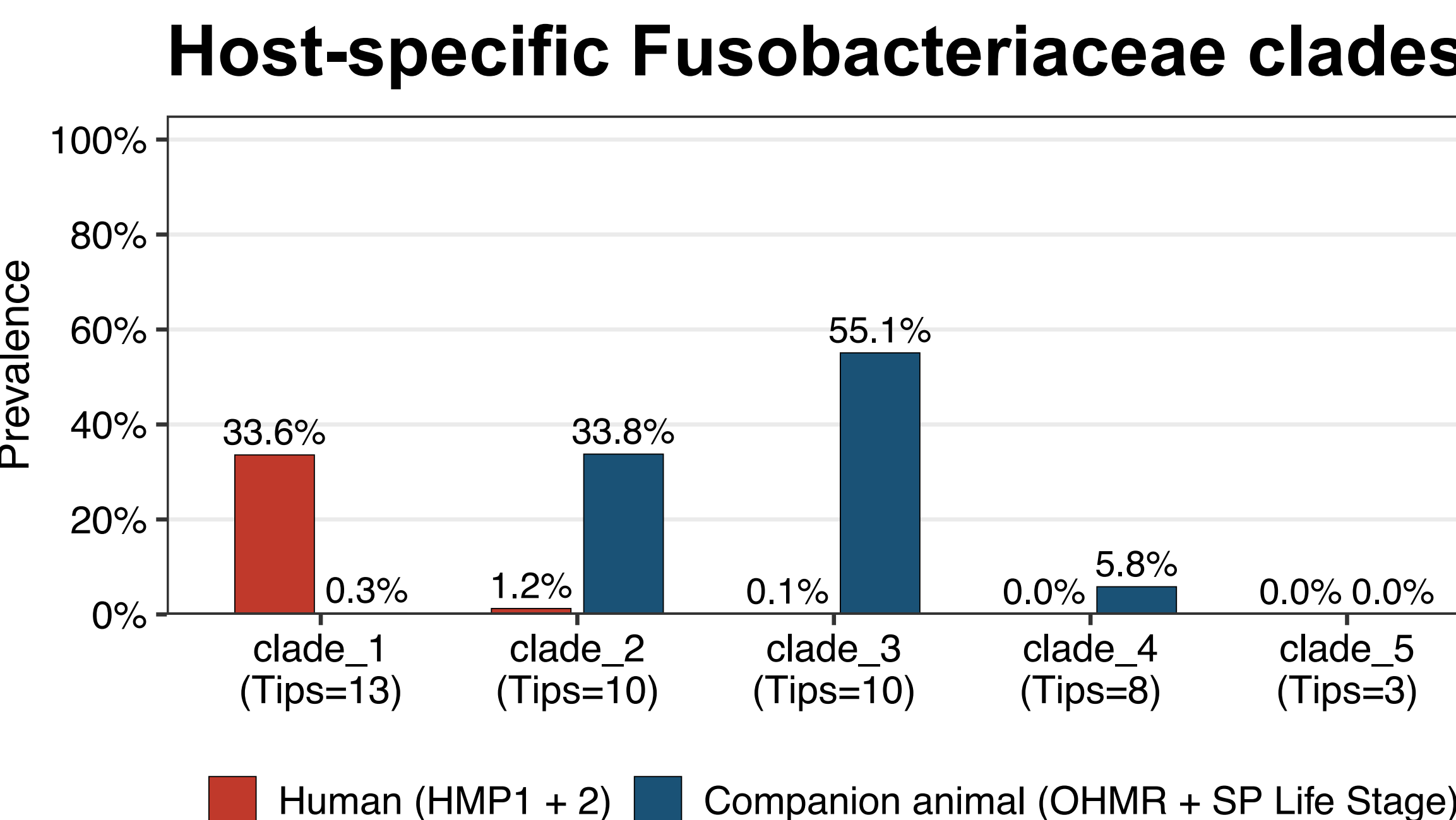
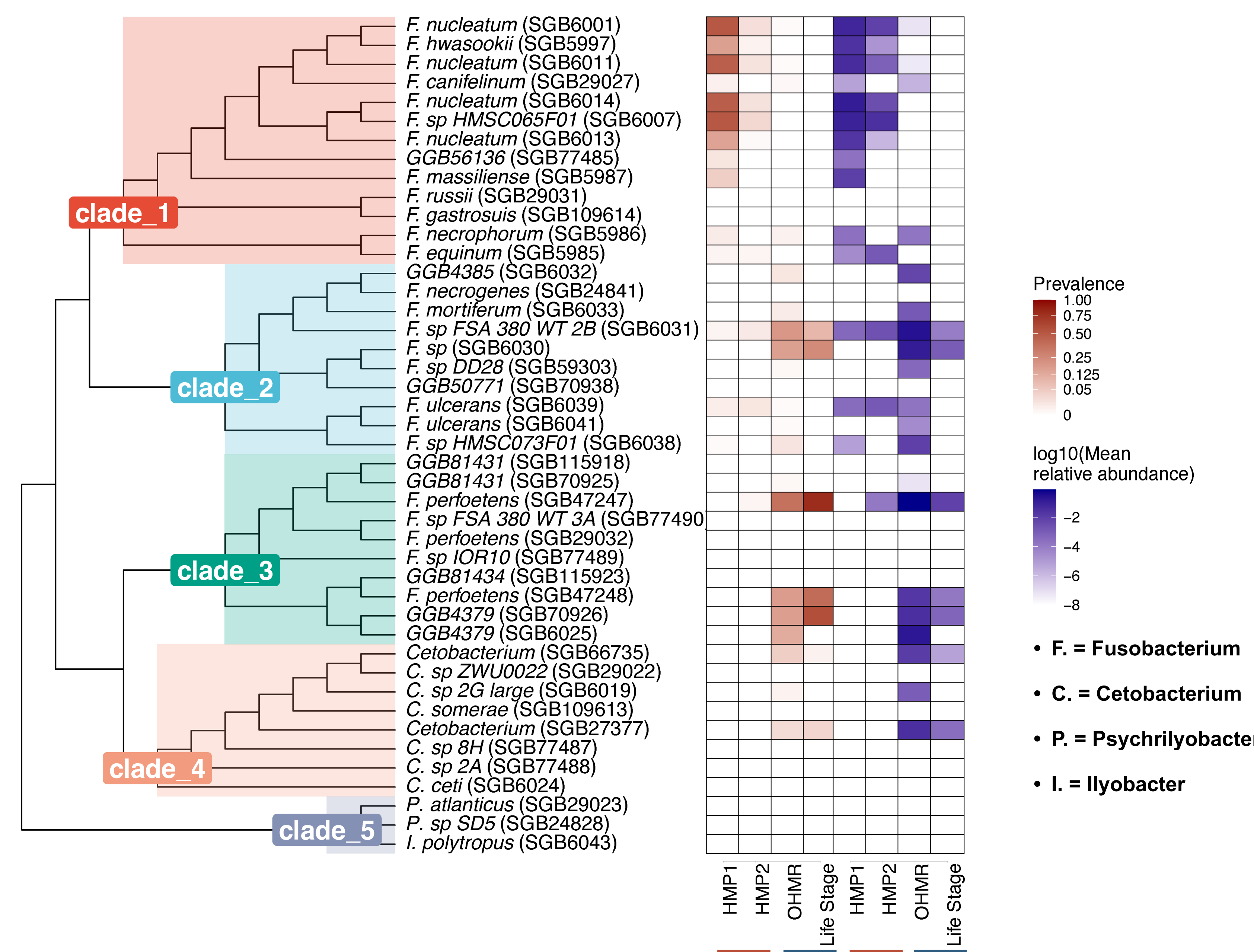
- Shannon diversity increased significantly with age following the introduction of solid food (Kruskal-Wallis  $p = 5e-05$ ).
- Beta diversity showed no clear global clustering by age.

## Individual taxa exhibit distinct age-associated trajectories



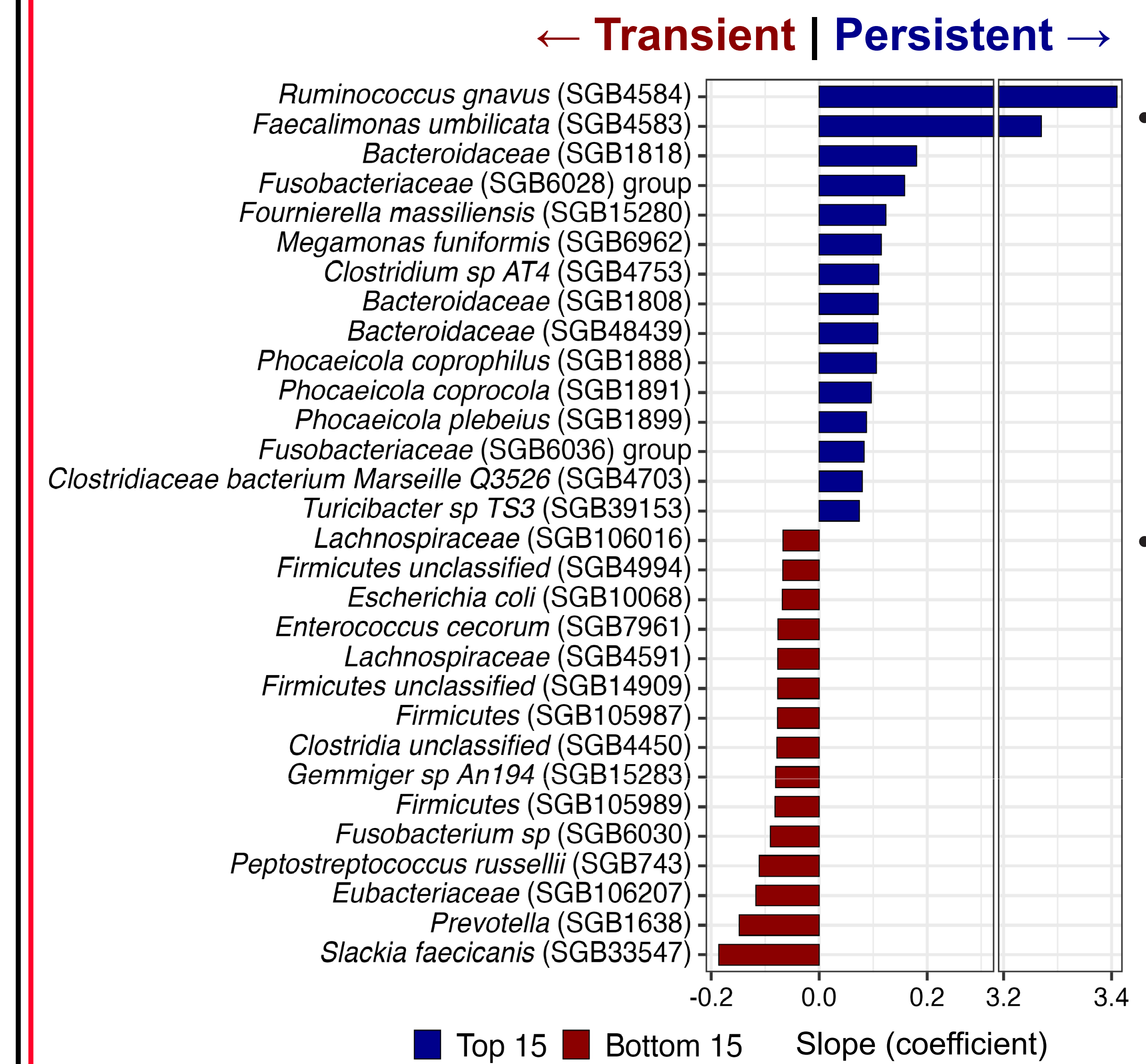
- MaAsLin 3 was used to identify taxa associated with age (fixed effects: age, total\_reads, sex, binary\_batch; random effect: dog; reference levels: male, Hills).
- Heatmap displays the top 10 taxa (FDR < 0.1) with prevalence  $\geq 20\%$  across samples.

## Fusobacteriaceae exhibit distinct host-associated phylogenetic clades



- Fusobacterium is a known bowel cancer-associated microbe in humans.
- Phylogenetic analysis identified distinct Fusobacteriaceae clades across humans and dogs.
- Humans and dogs typically harbored distinct microbial assemblages within Fusobacteriaceae.

## Some microbial taxa are retained more consistently over time



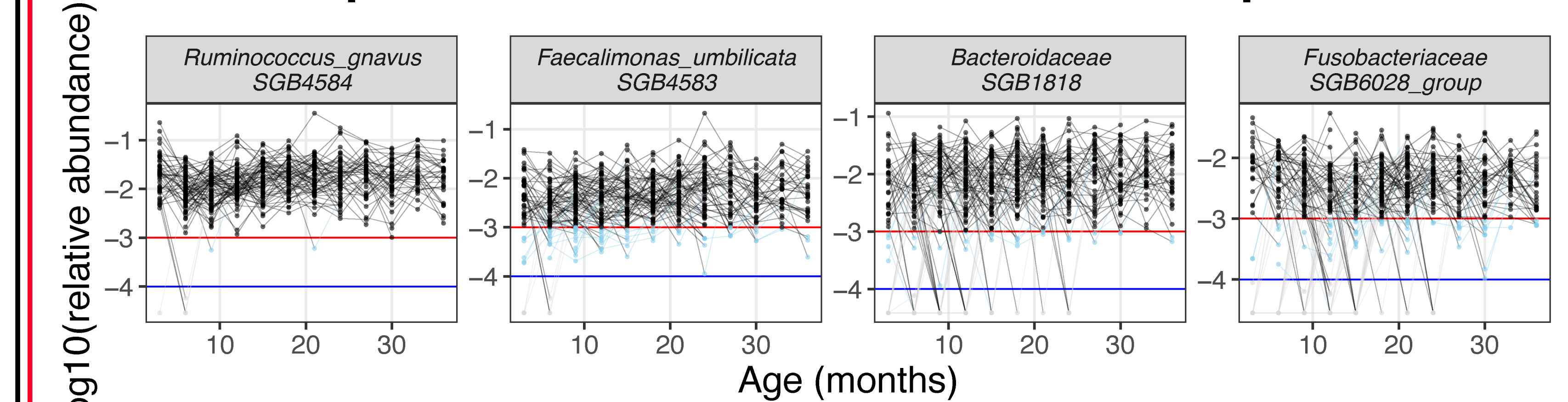
- Persistence across longitudinal intervals was modeled using generalized linear mixed models (GLMMs): persistent ~ interval months + (1|dog)
- Persistent taxa were more likely to remain above the presence threshold across time points, whereas transient taxa were more frequently lost over time.

## Persistence trends are consistent across dog cohorts

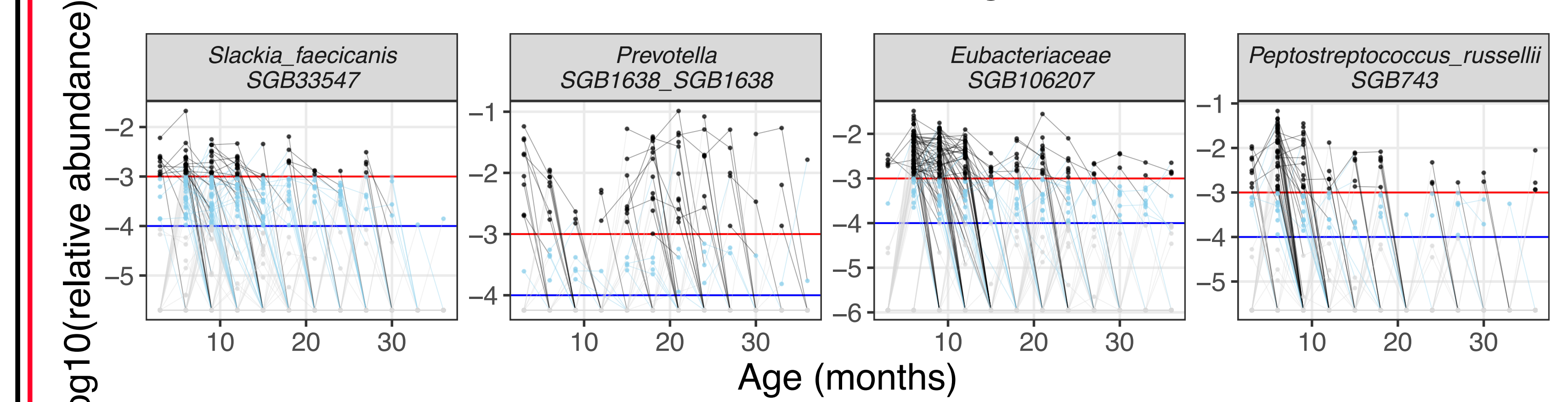


- Taxa with increasing persistence in SP Life Stage dogs also showed higher prevalence in an independent One Health Microbiome Resource (OHMR) dog cohort.

## Top 4 taxa remain stable across development



## Bottom 4 taxa are frequently lost over time



- present ( $\geq 1e-3$ )
- ambiguous ( $1e-4$ – $1e-3$ )
- absent ( $\leq 1e-4$ )

## Acknowledgments

We would like to thank Hill's Pet Nutrition and the National Institutes of Health for funding this work. We would also like to thank all of the pet partners and those that take care of them. For questions, please contact Yehoon Jo at [yehoon\\_jo@g.harvard.edu](mailto:yehoon_jo@g.harvard.edu) <http://huttenhower.sph.harvard.edu>

