Fungal microbiomes are determined by host phylogeny and exhibit widespread associations with the bacterial microbiome.

Supplementary Material

METHODS

**ITS1F-2 and 16S rRNA amplicon sequencing**

To identify fungal communities, we amplified DNA for the ITS1F-2 rRNA gene using single index reverse primers and a modified protocol of Smith & Peay (1) and Nguyen et al. (2), as detailed in Griffiths et al. (3). We ran PCRs in duplicate using Solis BioDyne 5x HOT FIREPol® Blend Master Mix, 2μM primers and 1.5μl of sample DNA. Thermocycling conditions were 95 °C for 10 min, followed by 28 cycles of 95 °C for 30s, 52 °C for 20s and 72 °C for 30s, with a final extension of 72 °C for 8 minutes. We quality checked the PCR products using a 2200 TapeStation (Agilent, USA). We combined PCR replicates into a single PCR plate and cleaned products using HighPrep™ PCR clean up beads (MagBio, USA) according to the manufacturers’ instructions. To normalise the libraries, we combined 1ul of each sample and conducted a titration sequencing run with this pool using an Illumina v2 nano cartridge (paired end reads; 2 x 150bp) on the Illumina MiSeq at the University of Salford. Based on the percentage of reads sequenced per library, we calculated the volume required for the full sequencing run and pooled these accordingly. ITS rRNA amplicon sequencing was conducted using paired-end reads (2 x 250bp) using an Illumina v2 cartridge on the MiSeq platform at the University of Salford. We included negative controls (blank extractions) for six of the nine DNA extraction methods plus a blank consisting of PCR-grade water, as well as a fungal mock community as a positive control. We ran the same library twice to increase sequencing depth, and combined data within samples across these two runs in the data pre-processing stage.

To identify bacterial communities, we amplified DNA for the 16S rRNA V4 region using dual indexed forward and reverse primers according to Kozich et al. (4) and Griffiths et al. (5). We ran PCRs in duplicate as described above using thermocycling conditions of 95°C for 15 minutes, followed by 28 cycles of 95°C for 20s, 50°C for 60s and 72°C for 60s, and a final extension at 72°C for 10 minutes. After cleaning and quality checking (as above), we again sequenced an equivolume pool on an Illumina v2 nano cartridge as described above, then pooled samples according to read coverage and conducted a full paired-end sequencing run (2 x 250bp) using Illumina v2 chemistry. We included extraction blanks and a mock bacterial community as negative and positive controls, respectively.

**Pre-processing of amplicon sequence data**

We conducted all data processing and analysis in RStudio v1.2.1335 for R (6, 7). For 16S rRNA amplicon sequencing data, adapters and primers were automatically trimmed by the MiSeq BaseSpace software, but for ITS rRNA amplicon data we performed an additional trimming step in cutadapt (8) to remove these. We conducted amplicon sequence processing in DADA2 v1.5 (9) for both ITS rRNA and 16S rRNA amplicon data.

A total of 8,033,962 raw sequence reads from 934 samples (i.e. duplicate data for each sample from the two sequencing runs) were generated across the two ITS rRNA sequencing runs. Modal contig length was 225 bp (range 112-477 bp) once paired-end reads were merged. We did not conduct additional trimming based on sequence length as the ITS region is highly variable (10). We removed 29 amplicon sequence variants (ASVs) found in the negative controls and filtered out chimeras, and then assigned taxonomy using the UNITE v7.2 database (11). We combined sequence data for each sample across the two ITS rRNA
sequencing runs using the merge_samples function in phyloseq (12). After data processing, we obtained a median of 1425 reads per sample (range of 153 to 424,527). DADA2 identified 12 unique ASVs in the sequenced mock community sample comprising 12 fungal isolates.

A total of 6,657,351 raw sequence reads from 476 samples were generated during 16S rRNA sequencing. Modal contig length was 253 bp once paired-end reads were merged. We removed ASVs with length >260 bp (55 SVs; 0.004% of total sequences) along with chimeras and 17 SVs found in the negative controls. We assigned taxonomy using the SILVA v132 database (13, 14). We stripped out chloroplasts and mitochondria from samples, leaving a median of 3273 reads per sample (range of 153 to 425,179). DADA2 identified 20 unique ASVs in the sequenced mock community sample comprising 20 bacterial isolates.

**Alpha Diversity Models**

For model fitting, we filtered the data to only those samples with paired metrics of microbial richness for both kingdoms (201 observations from 42 species). For higher order taxonomic predictors and random effects, we binned all invertebrate classes into a single grouping to improve model performance, as otherwise invertebrate class and species were colinear. All vertebrate taxonomic groupings were equivalent to class (Mammalia, Aves etc). We fitted two models to these data. First, to quantify relative differences in richness between bacteria and fungi within a sample, we used GLMMs in the brms package, with i) Bernoulli errors and a logit link; ii) a binary response of ‘1’ if bacterial richness was higher than fungal richness, and ‘0’ otherwise; and iii) ‘Species’ nested within ‘Class’ as random intercepts. We did not include intermediate levels of taxonomy because replication at Order and Family levels was low relative to Class. We did not use a phylogenetic mixed model as not all species were represented in the TimeTree phylogeny. Second, to quantify absolute differences in microbial richness, we fitted a bivariate response LMM with both fungal and bacterial richness values as a two-column response with Class as a fixed effect, and Species as a random intercept. For all models, we used uninformative Cauchy priors for the random effects and Gaussian priors for fixed effects coefficients. We assessed model adequacy using visual inspection of chains to assess mixing and stationarity properties, as well as posterior predictive checks using the ‘pp_check’ function in brms.

**Beta Diversity Analysis**

To visualise differences in microbial community structure among samples, we i) plotted proportional abundance of microbial groups at the phylum level, aligned to the host phylogenetic tree, ii) agglomerated the data to class level and visualised the variation in CLR-transformed ratios for the five most abundant microbial classes in each kingdom for each species using jitter plots, and iii) conducted principal components analysis (PCA) using CLR-transformed abundance matrices for each kingdom.

**Network Statistics**

We calculated modularity of the class-level microbial networks comprising both positive and negative interactions using the modularity function after greedy clustering implemented in the igraph package and used bootstrap resampling to generate metrics of uncertainty around mean modularity measures. We used binomial GLM to test the hypothesis that the proportion of positive edges (correlations) varies by host class. We used permutations to randomise betweenness with
respect to microbial kingdom to test whether fungal nodes had higher or lower betweenness than expected by chance. We used permutation analysis to examine whether the frequency of the most abundant bacteria-fungal co-occurrences in each host class network were higher than expected by chance. Here, we shuffled the fungal phylum data for our class-specific data of positive co-occurrences to estimate a null distribution of the expected frequency of co-occurrences, and corrected p values for multiple testing using False Discovery Rate with the ‘p.adjust’ function.

**Host Diet**

To determine the effect of diet on bacterial and fungal community composition, we used only samples from the bird and mammal species and agglomerated the data for each host species using the merge_samples function in phyloseq. This gave us a representative microbiome for each host species, which we rarefied to the lowest number of reads for each combination of kingdom and host taxon (2,916 – 9,160 reads; bacterial read counts were low for lesser horseshoe bats and so this species was removed from this analysis) and extracted Euclidean distance matrices for each. We obtained dietary data for each host species from the EltonTraits database, which provides standardised and semi-quantitative diet data for host species based on descriptions from global handbooks and monographs. We extracted the Euclidean distances between host diets (birds and mammals separately) and correlated these with fungal and bacterial community distances using Mantel tests with Kendall rank correlations in the vegan package. We agglomerated the microbial data to class level and visualised the bacterial and fungal community compositions for mammals alongside pie charts displaying EltonTrait dietary data for each species. We also used a primary axis of the ordination of EltonTrait data to derive a 'dietary variation axis' which we used as a predictor for alpha diversity of birds and mammals.
**TABLE S1**: Details of host species and their origins, sex ratios, sample sizes and types, and storage and extraction methods for the study.

| Class                  | Common name            | Scientific name            | N   | Sex ratio (M: F: J: unknown: N/A) | Captive or Wild | Origin                        | Sample Type               | Collection Year | Tissue Storage                     | Extraction Kit                                                      |
|------------------------|------------------------|-----------------------------|-----|----------------------------------|------------------|-------------------------------|--------------------------|------------------|-------------------------------------|---------------------------------------------------------------------|
| Demospongia            | Vase sponge            | *Ircinia campana*           | 10  | 0: 0: 0: 10: 0                   | Wild             | Long Key, Florida, USA        | Tissue (choanosome)      | 2014             | 95% ethanol                         | Qiagen Blood and Tissue kit with proteinase K                        |
| Demospongia            | Golfball sponge        | *Cinachyrella sp.*          | 10  | 0: 0: 0: 10: 0                   | Wild             | Long Key, Florida, USA        | Tissue (choanosome)      | 2014             | 95% ethanol                         | Qiagen Blood and Tissue kit with proteinase K                        |
| Arachnida              | Hard tick              | *Amby loloma rotundatum*    | 10  | 0: 0: 10: 0: 0                   | Wild             | Montserrat, Caribbean         | Whole organism           | 2014             | 70% ethanol                         | Alkaline digest and ethanol precipitation                           |
| Malacostraca           | Blue swimming crab     | *Portunus segnis*           | 5   | 0: 0: 5: 0                       | Wild             | Malta                         | Gut                      | 2018             | 70% ethanol                         | Qiagen QIAmp Fast DNA Stool Mini kit                                |
| Malacostraca           | Brown shrimp            | *Cragon cragon*             | 10  | 0: 0: 10: 0: 0                   | Wild             | Liverpool, Lancashire, England | Gut                     | 2018             | Buffer AE and frozen at -20°C       | Qiagen Blood and Tissue kit with proteinase K                        |
| Insecta                | Cockroach              | *Diploptera punctata*       | 11  | 7: 1: 0: 3: 0                    | Captive          | Manchester Metropolitan University, Manchester, UK | Gut                      | 2018             | Liquid nitrogen and frozen at -80°C | Qiagen Blood and Tissue kit with proteinase K and lysozyme           |
| Insecta                | Honey bee              | *Apis mellifera*            | 10  | 0: 10: 0: 0: 0                   | Wild             | North West of England, UK     | Gut                      | 2016             | 100% ethanol and frozen at -20°C    | Qiagen Blood and Tissue kit with proteinase K and lysozyme           |
| Insecta                | Tsetse fly             | *Glossina tuscipes*         | 9   | 2: 7: 0: 0: 0                    | Wild             | Patira East, Uganda           | Whole organism           | 2019             | 70% ethanol                         | Qiagen Blood and Tissue kit with proteinase K                        |
| Insecta                | African palm weevil larvae | *Rhynchophorus phoenicis* | 6   | 0: 0: 6: 0                       | Wild             | Sapele Town, Delta State, Nigeria | Gut                      | 2019             | Frozen at -20°C                     | ZymoBIOMICS DNA mini kit                                              |
| Actinopterygii         | European eel           | *Anguilla anguilla*         | 10  | 0: 0: 10: 0: 0                   | Wild             | Cumbria, England              | Gut                      | 2009             | Frozen at -20°C                     | Qiagen PowerSoil kit                                                |
| Actinopterygii         | Foureye butterflyfish  | *Chaetodon capistratus*     | 10  | 0: 0: 10: 0: 0                   | Wild             | Bocas del Toro, Bahia Almirante, Panama | Gut                      | 2018             | 95% ethanol                         | Qiagen PowerSoil kit with proteinase K                               |
| Actinopterygii         | Yellowhead wrasse       | *Halichoeres garnot*        | 10  | 0: 0: 10: 0: 0                   | Wild             | Caye Caulker, Belize          | Gut                      | 2015             | 95% ethanol                         | Qiagen PowerSoil kit with proteinase K                               |
| Actinopterygii         | Barred hamlet          | *Hypoplectrus puella*       | 12  | 0: 0: 12: 0: 0                   | Wild             | Bocas del Toro, Bahia Almirante, Panama | Gut                      | 2018             | 95% ethanol                         | Qiagen PowerSoil kit with proteinase K                               |
| Amphibia               | Common midwife toad    | *Alytes obstetricans*       | 11  | 0: 0: 11: 0: 0                   | Captive          | London Zoo, London, UK        | Skin swab                | 2015             | Frozen at -20°C                     | Qiagen DNEasy kit                                                   |
| Amphibia               | Phofung river frog     | *Amieta hymenopus*          | 10  | 0: 0: 10: 0: 0                   | Wild             | Drakensberg National Park, South Africa | Tadpole mouthparts       | 2015             | 95% ethanol                         | Qiagen Blood and Tissue kit with proteinase K                        |
| Amphibia               | Common toad            | *Bufo bufo*                 | 10  | 0: 0: 10: 0: 0                   | Wild             | Norway                        | Whole organism           | 2009             | 70% ethanol                         | Phenol chlorophorm                                                  |
| Kingdom      | Scientific Name                      | Genus          | Species                        | Sample Type | Localised Site                      | Year     | Stabilisation | Franklin DNA Kit |
|-------------|--------------------------------------|----------------|--------------------------------|-------------|------------------------------------|----------|---------------|------------------|
| Mammalia    | Great-crested newt                    | Triturus       | cristatus                     | Wild        | Lancashire, England                | 2015     | 70% ethanol   | Phenol chlorophorm |
| Aves        | Reed warbler                         | Acrocephalus   | scirpaceus                    | Wild        | Lincolnshire, UK                  | 2018/19  | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Light-bellied brent goose             | Branta         | bernicla                      | Wild        | Iceland                            | 2017     | Frozen at -20°C | Qiagen PowerSoil kit |
| Aves        | Goldfinch                            | Carduelis      | carduelis                     | Wild        | Lincolnshire, UK                  | 2018/19  | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Stock dove                           | Columba        | oenas                         | Wild        | East Anglia, UK                   | 2014     | Frozen at -20°C | Qiagen QiAamp Fast DNA Stool Mini kit |
| Aves        | Woodpigeon                           | Columba        | palumbus                      | Wild        | East Anglia, UK                   | 2012     | Frozen at -20°C | Qiagen QiAamp Fast DNA Stool Mini kit |
| Aves        | Carrion crow                         | Corvus         | corone                        | Wild        | Cumbria, UK                       | 2019     | Frozen at -20°C | Qiagen Microbiome kit |
| Aves        | Blue tit                             | Cyanistes      | caeruleus                     | Wild        | Lincolnshire, UK                  | 2018     | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Yellowhammer                         | Emberiza       | citrinella                    | Wild        | Lincolnshire, UK                  | 2018     | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Reed bunting                         | Emberiza       | schoeniclus                   | Wild        | Lincolnshire, UK                  | 2018     | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Robin                                | Erithacus      | rubecula                      | Wild        | Lincolnshire, UK                  | 2018     | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Great tit                            | Parus          | major                         | Wild        | Lincolnshire, UK                  | 2018/19  | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Chiffchaff                           | Phylloscopus    | collybita                     | Wild        | Lincolnshire, UK                  | 2018/19  | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Collared dove                        | Streptopelia    | decacoto                      | Wild        | East Anglia, UK                   | 2014     | Frozen at -20°C | Qiagen QiAamp Fast DNA Stool Mini kit |
| Aves        | Turtle dove                          | Streptopelia    | turtur                        | Wild        | East Anglia, UK                   | 2014     | Frozen at -20°C | Quigen QiAamp Fast DNA Stool Mini kit |
| Aves        | Blackcap                             | Sylvia          | atricapilla                   | Wild        | Lincolnshire, UK                  | 2018     | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Aves        | Song thrush                          | Turdus         | philomelos                    | Wild        | Lincolnshire, UK                  | 2018     | Frozen at -20°C | Qiagen PowerSoil Pro kit |
| Mammalia    | Striped field mouse                  | Apodemus        | agrarius                      | Wild        | Chernobyl Exclusion Zone, Ukraine | 2017     | 100% ethanol and frozen at -20°C | Invitrogen Microbiome kit |
| Mammalia    | Yellow-necked mouse                  | Apodemus        | flavicolis                    | Wild        | Chernobyl Exclusion Zone, Ukraine | 2017     | 100% ethanol and frozen at -20°C | Invitrogen Microbiome kit |
| Mammalia    | Wood mouse                           | Apodemus        | sylvaticus                    | Wild        | Chernobyl Exclusion Zone, Ukraine | 2017     | 100% ethanol and frozen at -20°C | Invitrogen Microbiome kit |
| Mammalia    | Northern muriqui                      | Brachyteles     | hypoxanthus                   | Wild        | Caparao National Park, UK         | 2017/18  | RNA Later and frozen at -20°C | Qiagen QiAamp Fast DNA Stool Mini kit |
| Mammalia | Species                  | Genus          | Stella | Genus | Country                      | Location                           | Year | Preparation | Vendors           |
|----------|-------------------------|----------------|--------|-------|------------------------------|-----------------------------------|------|--------------|-------------------|
| Mammalia | Roe deer                | Capreolus capreolus | 7      |       | Wild                         | Faeces                            | 2019 | Frozen at -20°C | Qiagen Microbiome kit |
| Mammalia | Red deer                | Cervus elaphus  | 10     | 0: 0: 10: 0 | Wild                        | Faeces                            | 2018 | Frozen at -20°C | Qiagen QIAamp Fast DNA Stool Mini kit |
| Mammalia | Greater white-toothed shrew | Crocidura russula | 10     | 5: 5: 0: 0 | Wild                        | Faeces                            | 2018 | Frozen at -20°C | Qiagen PowerSoil kit |
| Mammalia | Eastern black rhino     | Diceros bicornis michaeli | 10     | 0: 10: 0: 0 | Captive                     | Faeces                            | 2011 | Frozen at -20°C | Qiagen QIAamp Fast DNA Stool Mini kit |
| Mammalia | Wild pony               | Equus ferus caballus | 10     | 5: 5: 0: 0 | Wild                        | Faeces                            | 2013 | Frozen at -20°C | Qiagen QIAamp Fast DNA Stool Mini kit |
| Mammalia | Hedgehog                | Erinaceus europaeus | 12     | 0: 0: 12: 0 | Wild                        | Faeces                            | 2019 | Frozen at -20°C | Qiagen Microbiome kit |
| Mammalia | Bank vole               | Myodes glareolus | 10     | 7: 3: 0: 0 | Wild                        | Faeces                            | 2017 | 100% ethanol and frozen at -20°C | Invitrogen Microbiome kit |
| Mammalia | Lesser horseshoe bat    | Rhinolophus hipposideros | 10     | 3: 5: 2: 0 | Wild                        | Faeces                            | 2016 | Frozen at -20°C | Zymo DNA Extraction kit |
| Mammalia | Capuchin monkey         | Sapajus libidinosus | 10     | 0: 0: 10: 0 | Wild                        | Faeces                            | 2017 | Frozen at -20°C | Qiagen QIAamp Fast DNA Stool Mini kit |
| Mammalia | Grey squirrel           | Sciurus carolinensis | 12     | 0: 0: 12: 0 | Wild                        | Faeces                            | 2019 | Frozen at -20°C | Qiagen Microbiome kit |
| Mammalia | Red squirrel            | Sciurus vulgaris  | 12     | 0: 0: 12: 0 | Wild                        | Faeces                            | 2019 | Frozen at -20°C | Qiagen Microbiome kit |
| Mammalia | Pygmy shrew             | Sorex minutus    | 10     | 5: 5: 0: 0 | Wild                        | Faeces                            | 2018 | 100% ethanol and frozen at -20°C | Qiagen PowerSoil kit |
RESULTS

Alpha-diversity measures remained relatively stable within a host species whether data were rarefied to 500, 1000, or 2500 reads (Figures 1 main text, Fig S1 below). Patterns between kingdoms were similar for each host species whether data were rarefied to 500 or 1000 reads, with the exception of slight increases in fungal diversity relative to bacterial diversity for two host species (blue tit, light-bellied brent goose) when data were rarefied to 1000 reads (Figure S1). Cross-kingdom patterns for each host species were also similar whether data were rarefied to 500 or 2500 reads (Figure S2), although four host species (chiffchaff, greater white-toothed shrew, light-bellied brent goose, reed warbler) showed greater differences between bacterial and fungal diversity when 2500 reads were used, and one (common midwife toad) had reduced differences (Figures 1 and S2).
TABLE S2

Proportion of variation in microbial community structure with (left) and without (right) accounting for sample metadata and wet lab preparation confounds.

| (a) FUNGI | Taxonomic Effects Only |
|-----------|------------------------|
| Predictor            | df | R²  | p value | df | R²  | p value |
| Sample Type          | 7  | 0.05| 0.001   |     |     |        |
| Tissue Storage       | 5  | 0.04| 0.001   |     |     |        |
| Extraction Kit       | 7  | 0.07| 0.001   |     |     |        |
| Class                | 2  | 0.02| 0.001   | 6  | 0.05| 0.001  |
| Order                | 6  | 0.05| 0.001   | 13 | 0.12| 0.001  |
| Species              | 18 | 0.09| 0.001   | 26 | 0.14| 0.001  |
| Residuals            | 303| 0.68|         | 303| 0.68|        |

| (b) BACTERIA | Taxonomic Effects Only |
|--------------|------------------------|
| Predictor            | df | R²  | p value | df | R²  | p value |
| Sample Type          | 6  | 0.06| 0.001   |     |     |        |
| Tissue Storage       | 6  | 0.16| 0.001   |     |     |        |
| Extraction Kit       | 7  | 0.12| 0.001   |     |     |        |
| Class                | 2  | 0.02| 0.001   | 6  | 0.09| 0.001  |
| Order                | 6  | 0.09| 0.001   | 12 | 0.21| 0.001  |
| Species              | 18 | 0.12| 0.001   | 27 | 0.27| 0.001  |
| Residuals            | 273| 0.42|         | 273| 0.42|        |
TABLE S3: Network statistics from class-specific microbial networks in Figure 3 in the main manuscript. ‘Modularity’ and ‘Groups’ statistics are derived from the cluster_fast_greedy function applied to *igraph* network objects. ‘Components’ data were extracted directly from the networks. Modularity was positively correlated with both number of groups (cor = 0.76) and number of components (cor = 0.86).

| Class       | Modularity | Groups | Components |
|-------------|------------|--------|------------|
| Mammalia    | 0.658      | 7      | 1          |
| Aves        | 0.719      | 23     | 14         |
| Insecta     | 0.781      | 10     | 6          |
| Actinopterygii | 0.806    | 16     | 11         |
| Amphibia    | 0.923      | 35     | 35         |
TABLE S4
PERMANOVA results of variation in microbial community structure calculated from Bray-Curtis distances among libraries rarefied to 500 reads (see Table 1 main manuscript).

(A) FUNGI

| Term       | Df | F.Model | R2   | Pr(>F)  | Sig. |
|------------|----|---------|------|---------|------|
| SampleType | 7  | 2.91    | 0.06 | 0.001   | ***  |
| TissueStorage | 4  | 3.48    | 0.04 | 0.001   | ***  |
| ExtractionKit | 7  | 3.82    | 0.07 | 0.001   | ***  |
| Class      | 2  | 3.51    | 0.02 | 0.001   | ***  |
| Order      | 6  | 3.39    | 0.06 | 0.001   | ***  |
| Species    | 18 | 1.90    | 0.10 | 0.001   | ***  |
| Residuals | 237 |     | 0.66 |         |      |
| Total      | 281 |     |      |         | 1    |

(B) BACTERIA

| Term       | Df | F.Model | R2   | Pr(>F)  | Sig. |
|------------|----|---------|------|---------|------|
| SampleType | 6  | 7.16    | 0.08 | 0.001   | ***  |
| TissueStorage | 6  | 10.57   | 0.12 | 0.001   | ***  |
| ExtractionKit | 7  | 9.05    | 0.12 | 0.001   | ***  |
| Class      | 1  | 10.33   | 0.02 | 0.001   | ***  |
| Order      | 6  | 7.69    | 0.09 | 0.001   | ***  |
| Species    | 18 | 3.44    | 0.12 | 0.001   | ***  |
| Residuals | 241 |     | 0.46 |         |      |
| Total      | 285 |     |      |         | 1    |
**FIGURE S1:** Shannon Diversity statistics for microbial data when libraries were rarefied to (A) 1000 reads per sample and (B) 2500 reads per sample. Horizontal bars show the median, boxes are 25th and 75th percentiles, and whiskers are the largest value or 1.5x the interquartile range if some values extend beyond that. Only species with both bacterial and fungal data are shown.
**FIGURE S2:** Posterior distributions from a Binomial mixed effects model examining probability of bacterial Shannon diversity being higher than fungal diversity when conditioned on Class. Estimates are in logits. White points are posterior means, and bars extend to 95% intervals. Shaded areas show full density of posterior samples.
FIGURE S3. Patterns of phylogenetic signal in alpha diversity for (A) bacteria and (B) fungi. Replication of species differs across microbial datasets, reflected by differing host phylogenies.
FIGURE S4: Effect of PC2 (secondary axis of a principal components analysis of host diet, on microbial richness for bacteria and fungi in mammals. Positive PC2 values indicate more fruits and seeds in the diet. Only the main effect of PC2 was retained in the top model, indicating no support for differing slopes dependent on whether fungal or bacterial communities are being modelled.
FIGURE S5: Bacterial community composition (agglomerated to class level) of 15 mammal species compared with crude foraging data for each host species (see main text for methods and sources). The five most abundant classes of fungi across all host species were Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Pezizomycetes and Sordariomycetes, for which Dothideomycetes and Eurotiomycetes showed the most variation between host species (Fig. S3). The five most abundant classes of bacteria were Actinobacteria, Alphaproteobacteria, Bacilli, Bacteroidia, and Gammaproteobacteria, which all varied considerably among host species.
FIGURE S6: Fungal community composition (agglomerated to class level) of 16 mammal species compared with crude foraging data for each host species (see main text for methods and sources).
FIGURE S7. Centred Log Ratio (CLR)-transformed abundance values from the five most abundant classes of (a) fungi and (b) bacteria identified across a range of host species. CLR-transformation is a normalisation method allowing comparison of abundance values across libraries of different sizes (read depths).
FIGURE S8

(A) Principal components analysis (PCA) of CLR-transformed microbial abundances for bacteria and fungi, with points coloured by host class. The first two axes of the ordination explained 19.4% and 8.84% of the variance in community structure for bacteria and fungi, respectively. PERMANOVA analysis revealed species ID to be the primary driver of variance in both taxa, accounting for 21.2% and 14.3% of the variance, respectively. However, there were also strong effects of sample handling and storage (see results). (B) PCA plots of bacterial (circles) and fungal (squares) community structure, faceted by host class, with points coloured by host order.
FIGURE S9: Microbial interaction networks for 40 species derived from cooccurrence analysis. Positive interactions (correlations) are shown in green, and negative interactions in red; blue nodes are bacteria and grey nodes and fungi. There was clear variation at the species level; for some host species, there were considerably more positive interactions (e.g., yellowhammers, pygmy shrews, greater white-toothed shrews, wood mouse, woodpigeon, yellow-necked mouse). In some species, there were slightly more negative interactions than positive (e.g., blackcap, goldfinch).
**FIGURE S10**: Number of positive (green) and negative (red) associations within bacterial-fungal co-occurrence networks for each host species.
FIGURE S1: Putative microbial interaction networks between bacterial (circles) and fungal (squares) taxa, coloured by microbial phylum. Networks were constructed using the R package SpiecEasi on CLR-transformed abundance values to detect non-random co-occurrence between groups of microbes.

FIGURE S11: Putative microbial interaction networks between bacterial (circles) and fungal (squares) taxa, coloured by microbial phylum. Networks were constructed using the R package SpiecEasi on CLR-transformed abundance values to detect non-random co-occurrence between groups of microbes.
**FIGURE S12**: Correlation between network modularity (A) and components (B) for each of 5 animal class networks. Both relationships are positive.
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