# The ecosystem within: a meta-analysis on the role of the microbiome on the behaviour of animals

The relationship between animal behaviour and host-associated microbiota has become an increasingly prominent research topic, yet associations remain inconsistent across studies and taxa. We conducted a meta-analysis to synthesise empirical evidence on the behavioural effects of microbiota manipulations of animals. Fifty-seven peer-reviewed articles were included in the meta-analysis. Using a multivariate random-effects model that accounted for study-level dependencies, we found a pooled Hedges' *g* of 0.45 (95% CI: 0.05–0.85), indicating a moderate overall positive effect of microbiota manipulation on behaviour. However, heterogeneity was high ($I^2 = 85\%$; $\tau^2 = 1.66$), reflecting the wide variability in taxa, dosages, experimental designs, and behavioural domains. No evidence of publication bias was detected, and influence diagnostics confirmed the robustness of the pooled effect. Exploratory meta-regressions suggested possible influences of taxonomic group (Diptera) and behavioural context (feeding-related), but these effects did not remain significant in comprehensive models. Likewise, ecological and biological factors, including habitat, diet, social organisation, encephalization quotient, and microbiome type (gut vs. skin), did not systematically predict behavioural responses. Overall, behavioural modulation by microbiota manipulations appears independent of these host and study-level factors. Collectively, these findings demonstrate that microbiota interventions can modulate animal behaviour but also expose substantial methodological and taxonomic heterogeneity that limits generalisation. Standardised reporting, finer microbial resolution, and integrative experimental designs combining behavioural, microbial, and mechanistic data are crucial for advancing a unified understanding of microbiota–behaviour interactions.

**Keywords:** behavioural ecology, bibliometric analysis, host-microbe interactions, microbe diversity, microbiota manipulation.

The ability of microbes to extend their phenotype into host organisms to promote their survival has long been recognised, particularly in cases where pathogens alter host behaviour to enhance transmission (Heil, 2016; Hughes, Wappler, et al., 2011; Moore, 2002; Poulin, 2007). More recently, research across a range of animal species has demonstrated that host behaviour can be modulated by the microbiome (the community of microorganisms and their collective genetic material) in ways that extend beyond transmission-related manipulation (Cryan & Dinan, 2012; Davidson et al., 2020; Idowu et al., 2025; Song et al., 2020). For example, microbiome composition has been linked to variation in sociability in rhesus macaques (*Macaca mulatta*) (Johnson et al., 2022), suggesting that microbial communities may contribute to broader behavioural phenotypes.

Behavioural development is traditionally understood as emerging from interactions between genetic, epigenetic, environmental, and experiential processes (Bateson, 2017). Within this integrative framework, the microbiome represents an additional biological layer capable of influencing neural, endocrine, and immune pathways that regulate behaviour (Järvilehto & Lickliter, 2016; Johnston & Edwards, 2002; Sokolowski, 2001; York, 2018). Research on the microbiome–gut–brain axis demonstrates that gut microbial communities communicate with the central nervous system through neural, endocrine, and immune signalling (Cryan & Dinan, 2012; O'Mahony et al., 2015). Experimental perturbations of microbial composition, including germ-free models, antibiotic treatments, and probiotic supplementation, have been associated with changes in anxiety-like behaviour, social interaction, cognition, and stress physiology (Bravo et al., 2011; Buffington et al., 2016; Zhang et al., 2021).

Mechanistically, microbial metabolites and signalling molecules can modulate host neurochemistry and endocrine function (Ntiri & Wong, 2025). Short-chain fatty acids,

indoles, and secondary bile acids influence immune activity, blood–brain barrier integrity, and neural development (Sampson & Mazmanian, 2015). Additionally, bacteria can both produce and detect neurochemicals structurally identical to host neurotransmitters, including GABA and dopamine, thereby participating in shared signalling pathways (Lyte, 2013, 2014). Such interactions provide biologically plausible routes through which microbial activity may shape behavioural phenotypes.

From an evolutionary perspective, host–microbe interactions may range from mutualistic alignment to partial conflict. In some contexts, microbial effects on behaviour may enhance host fitness, for example, by supporting digestion, immune function, or normative social development (Calhoun et al., 2026; Griffiths et al., 2025; Mârza et al., 2025). In other cases, microbial influences may favour traits that increase microbial persistence or transmission, even when fitness consequences for the host are neutral or context-dependent (Ntiri & Wong, 2025). Importantly, behavioural modulation need not involve overt pathogenicity. More subtle forms of exploitation may arise when commensal or opportunistic microbes influence host signalling pathways in ways that indirectly favour microbial growth or resource acquisition. The microbial endocrinology framework proposes that bacteria can both produce and detect neurochemicals structurally identical to host neurotransmitters, including GABA, dopamine, and norepinephrine, thereby participating in shared signalling networks (Lyte, 2013, 2014). Through such neurochemical crosstalk, microbes may alter gut motility, appetite regulation, stress responsiveness, or food preferences in ways that enhance nutrient availability or stabilise local ecological conditions. This continuum of potential alignment and conflict suggests that microbiome-driven behavioural effects are unlikely to be uniform across taxa or ecological contexts.

Ecological context is central to understanding microbiome–behaviour interactions because ecological traits, structure microbial communities across taxa, thereby shaping the conditions under which microbiome-mediated behavioural effects may emerge (Archie & Theis, 2011; Davidson et al., 2020; Song et al., 2020). At broader evolutionary scales, host taxonomy constrains gut physiology and immune architecture, producing consistent differences in microbiome composition between vertebrate classes (Colston & Jackson, 2016; Song et al., 2020). Dietary ecology further determines microbial functional capacity: herbivores and omnivores typically harbour fermentative communities enriched in taxa capable of producing short-chain fatty acids, whereas carnivores tend to host less diverse, protein-adapted microbiota (Levin et al., 2021; Maritan et al., 2024; Pilla & Suchodolski, 2021). These ecological differences influence the production of microbial metabolites that regulate immune signalling, metabolic pathways, and neuroactive compound synthesis (Sampson & Mazmanian, 2015; Yao et al., 2024), potentially altering feeding behaviour, energy regulation, stress responsiveness, and cognitive processes. Thus, variation in ecology is expected to modulate the magnitude and direction of behavioural responses to microbiome manipulation rather than generate uniform effects across taxa.

Social organisation structures microbial transmission, with highly social species showing greater within-group microbiome similarity than less social or solitary species, likely due to increased contact and shared environments (Archie & Tung, 2015; Moeller et al., 2016; Sarkar et al., 2020). Reciprocally, microbial influences on stress physiology and social cognition may reinforce or alter patterns of social interaction (Cryan & Dinan, 2012; Johnson et al., 2022). In turn, microbiome-mediated effects on immunity, stress responsiveness, or risk-taking behaviour may influence habitat use and movement patterns. Together, these patterns indicate that ecological traits not only shape

microbiome composition but may also be partially shaped by microbiome-mediated behavioural effects, creating a dynamic feedback system across taxa.

Finally, cognitive and neuroanatomical complexity may modulate the strength of microbiome–behaviour coupling. Species with higher encephalization quotients (EQ) and more developed executive functions may exhibit greater behavioural sensitivity to microbial signals, potentially mediated by neuroimmune and endocrine pathways within the microbiota–gut–brain axis (Cohen, 2021; Cryan & Dinan, 2012). Differences in brain energetics and metabolic demands, partially supported by microbial processing of dietary substrates (Yao et al., 2024), further suggest that evolutionary lineage may constrain how strongly microbiome variation translates into behavioural outcomes.

Despite rapidly accumulating empirical studies (Adamo, 2013; Hughes, Andersen, et al., 2011; Hughes & Libersat, 2019), findings remain heterogeneous and sometimes contradictory in terms of behavioural effects of microbiomes. Differences in host taxa, microbial communities, experimental manipulations, and behavioural measures complicate generalisation. Importantly, although many studies report associations between microbiome composition and behaviour, few explicitly test causal effects through experimental manipulation. Focusing on manipulative designs allows stronger inference regarding microbiome-driven behavioural change by isolating directional effects rather than correlational patterns. A quantitative synthesis, like a meta-analysis (Bangdiwala, 2024; Shelby & Vaske, 2008; Yuan & Hunt, 2009), is therefore necessary to determine whether microbiome manipulations exert consistent effects on animal behaviour and to identify the ecological and biological moderators that structure this variation.

Accordingly, the central question of this study is whether experimental manipulations of the microbiome exert consistent effects on animal behaviour across taxa. To address this

question, we conducted a meta-analysis to assess the influence of microbiota manipulations on animal behaviour. We hypothesised that experimental alteration of microbiome composition would produce measurable effects on behavioural outcomes. Specifically, we predicted an overall significant aggregated effect, with particularly consistent responses in behavioural domains related to sociality, feeding, and cognitive processes, including learning and memory. We further expected substantial heterogeneity across studies, structured by ecological and evolutionary factors. In particular, we predicted that dietary ecology would modulate effect sizes, with omnivorous species exhibiting stronger behavioural responses than herbivores or carnivores; that species with greater environmental microbial exposure (e.g., burrowing taxa) would show stronger microbiome–behaviour coupling; that taxa with higher encephalization quotients (EQ) would exhibit heightened behavioural sensitivity to microbiome manipulation; and that internal microbiomes, especially the gut, would exert stronger effects than external microbial communities.

## METHODS

### *Bibliometric search and study selection*

The bibliometric search was performed on 8 February 2025 across three complementary scientific paper databases (Web of Science©, Scopus©, and PubMed©) to ensure comprehensive coverage of peer-reviewed studies addressing the intersection between animal behaviour and the microbiome. Web of Science and Scopus were selected for their broad disciplinary reach and reliable citation indexing, which facilitate the identification of key publications and the detection of cross-disciplinary research patterns (Falagas et al., 2008; Mongeon & Paul-Hus, 2016). In parallel, PubMed, managed by the U.S. National Library of Medicine and comprising the MEDLINE

repository, was included for its extensive representation of biomedical and veterinary literature (Lu, 2011).

The search strategy combined the terms "microbiome" and "animal behav*" using the Boolean operator AND to capture studies in which both concepts co-occurred. The truncation symbol (*) following "behav" was applied to retrieve all possible variations of the root word (e.g., behaviour, behavioral, behavioural), thereby maximising the inclusiveness of the search. This procedure retrieved 10,761 records.

To ensure a focused and comprehensive analysis, the study design and subsequent selection process were meticulously guided by the PICO (Population, Intervention/Exposure, Comparison, Outcome) framework (Brockmeier et al., 2019), addressing the following broad research question: "In animal models, what are the effects of microbiota on various assessed behaviours (e.g. locomotion, sociality, attraction, aggressiveness, anxiety, exploration), compared to controls or other interventions?" A rigorous multi-stage screening process, adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework, was then implemented to identify relevant studies for inclusion in the systematic review and subsequent meta-analysis (Page et al., 2022).

The selection of research papers commenced with duplicate removal, in which records were initially processed using a Rayyan reference management tool (Ouzzani et al., 2016), complemented by manual inspection to retain only the most complete or recent entry. Following this, the title and abstract screening (Phase 1) was conducted. Studies were included if their title and abstract mentioned animals, microbiota manipulation (such as germ-free models, antibiotics, probiotics, faecal microbiota transplantation (FMT)/colonisation, or microbiota-modulating diets), and behaviour as an outcome.

Exclusion criteria at this initial stage comprised studies on humans, news articles, conference papers, commentaries or letters, purely molecular/omics investigations without behavioural measures, or those solely describing microbiota without an intervention or exposure. The final stage, full-text review (Phase 2), involved retrieving and thoroughly examining the full texts of potentially relevant articles against predefined inclusion and exclusion criteria. Reasons for exclusion during this phase were documented for the PRISMA flow diagram.

The detailed inclusion criteria were aligned with the PICO framework. For Population (P), studies on any animal species (e.g., invertebrates and vertebrates) were considered, specifically focusing on in vivo experimental designs where behaviour was assessed in controlled (e.g., arena, tank) or semi-controlled field settings. Studies including females, males, or mixed cohorts, and animals of any age, were accepted, provided age was explicitly stated. Regarding Intervention/Exposure (I), eligible studies investigated germ-free or ex-germ-free conditions; antibiotic-induced microbiota depletion or alteration; supplementation with probiotics, prebiotics, or synbiotics; faecal microbiota transplantation (FMT) or specific microorganism colonisation (mono-/asso-colonisation); or diets documented to modulate microbiota (e.g., high-fibre/polyphenol diets) (De Angelis et al., 2020; Lyu et al., 2025) with evidence that the diet was designed explicitly for microbiota modulation. The route of administration (e.g., oral gavage, dietary incorporation, or environmental exposure) was not considered an exclusion criterion, if the intervention aimed to modify microbiota composition and was followed by behavioural assessment. Studies in which diet was solely nutritional, without intent or proof of microbiota modulation, were excluded. For Comparison (C), control groups (e.g., conventional, vehicle, control diet) or active comparators between different microbiota manipulations were accepted. Before-and-after designs within the

same animal were also considered if a washout/stability period and sufficient measures for effect estimation were provided. About Quantitative Behavioural Outcomes (O), measurable behavioural outcomes were required, such as locomotion/activity, exploration, social behaviour (e.g., attraction, cohesion, avoidance, parental care), aggressiveness, anxiety-like behaviours, depression-like behaviours, and learning/memory with a behavioural component. Acceptable measures included latency, time spent, frequency, distance, validated indices/scales, or proportions of responsive individuals. Crucially, studies needed to report sufficient statistics (e.g., mean, standard deviation (SD), standard error (SE), confidence interval (CI), or test statistics such as t, F, U, H, r, etc.) or raw data to enable effect size calculation (Ben-Shachar et al., 2020). Finally, for Type of Study (T), experimental animal studies (*in vivo*, randomised or non-randomised) involving microbiota manipulation and behavioural assessment were included. Acceptable designs encompassed parallel, crossover, before-and-after (with caveats), and factorial designs. No restrictions were placed on the publication language or year of publication; however, only papers in English were retrieved from the initial search.

Studies were systematically excluded if they met the following criteria: inappropriate population (i.e., human studies); *in vitro* experiments; organoids without behavioural output; or computational models. An absence of microbiota intervention was also an exclusion criterion, applying to studies only describing microbiota without manipulation (e.g., correlating alpha-diversity with behaviour without altering microbiota; that is, purely associative studies). Furthermore, dietary interventions without microbiota justification were excluded if diets lacked explicit intention or evidence of microbial modulation. Studies lacking quantitative behavioural measures, such as purely physiological or neurochemical investigations, or those reporting anecdotal/qualitative

226 behaviour without quantifiable data, were rejected. Articles presenting insufficient data
227 to calculate effect sizes, where inference from graphs or statistics was impossible, and
228 authors could not provide additional data, were also excluded. Regarding article type:
229 reviews, comments, letters, protocols, theses, or conference abstracts were not
230 considered.

231 After applying the inclusion criteria and removing duplicates, 470 unique publications
232 were retained for analysis (Web of Science: 232; Scopus: 47; PubMed: 191) (Figure 1).
233 Eligible items included original research articles and reviews published between 1942
234 and 2025 in peer-reviewed journals, which explicitly investigated microbiome
235 influences on animal behaviour.

236 _____Insert Figure 1 here_____

237 For each selected publication, the following characteristics and data points were
238 extracted: the authors, article title, journal information (including its 2025 impact
239 factor), language of publication, year of publication, volume, issue, start and end page
240 numbers, the Digital Object Identifier (DOI), the database platform from which it was
241 sourced (Scopus, PubMed, or Web of Science), the first author's affiliation and country,
242 the host species under study (along with its Class and Order), the specific behaviour
243 investigated (such as aggression, social interaction, reproduction, locomotion, cognition,
244 or feeding), and the observed effect type. Effect types were categorised as positive
245 (indicating an increase in the measured variable's value or intensity), negative (denoting
246 a detectable decrease), neutral (showing no significant variation), or ambiguous
247 (assigned when a study reported both increases and decreases for the same variable in
248 response to the factors examined, thus preventing a definitive directional classification
249 of the overall finding). Additional extracted data included geographical coordinates

(Latitude and Longitude), the study setting (e.g., laboratory, farm, zoo, animal shelter), the number of animals involved, the document type (experiment or review), the specific microbiome studied (e.g., gut, skin, oral, blood, ocular), the methodology employed (e.g., genomic, metabolomic, transcriptomic), the type of microbiota (bacteria, fungi, virus), and specific microbiota order and species.

Of the 470 included studies, only those that explicitly identified the microorganism investigated and provided sufficient quantitative information for effect-size calculation were retained for the meta-analysis, resulting in a final dataset of 57 studies and 1,209 effect sizes (Figure 1). The substantial reduction from the initial pool primarily reflects the exclusion of studies that lacked sufficient statistical detail (e.g., missing sample sizes, means, variance estimates, or test statistics), did not clearly identify the microbial taxon manipulated, or did not provide extractable quantitative behavioural outcomes. The large number of effect sizes relative to the number of studies reflects the structure of the primary research, in which individual papers often reported multiple experiments, different behavioural variables, and several microbial taxa, sometimes under distinct experimental treatments, doses, or exposure times. Each of these combinations was therefore treated as an independent comparison contributing to the overall synthesis.

*Statistical analysis*

Data were extracted directly from the text, tables, supplementary materials, and figures of the included articles. When quantitative information was available only in graphical format, mean values and corresponding error estimates were digitised using the R package metaDigitise (Pick et al., 2018), which allows reproducible extraction of summary statistics from published figures. For the meta-analysis, Cohen's *d* and Hedges' *g* effect sizes, alongside their respective standard errors and 95% confidence

intervals, were calculated for each included study (Hedges & Olkin, 1985). These computations utilised raw mean values and sample sizes for both control and experimental groups. Since the raw data provided the standard error of the mean (SEM), these values were first converted into standard deviations (SDs) using the formula SD = SEM * sqrt(N). The pooled standard deviation was then computed from these calculated SDs. Subsequently, Cohen's *d* and its standard error were derived, followed by Hedges' *g* and its standard error, obtained by applying Hedges' correction factor (*J*) to Cohen's *d*. These calculations were conducted using the dplyr package (Wickham et al., 2019) in the R statistical software. Effect directions were harmonised so that positive values indicated an increase in the behavioural outcome in the focal condition and negative values a decrease, maintaining consistency across behavioural categories.

Average effects were estimated using random-effects models fitted by restricted maximum likelihood (REML), which quantify between-study heterogeneity while accounting for within-study sampling error (Viechtbauer, 2010). Because several studies contributed multiple effect sizes, hierarchical dependence was addressed by fitting multilevel random-effects models with study ID as a random intercept (Nakagawa & Santos, 2012; Van den Noortgate et al., 2013). Precision was defined as the inverse of the standard error and used as the analytic weight, giving greater influence to studies with smaller sampling variances (Borenstein et al., 2010, 2021). Heterogeneity was summarised using Cochran's Q, $I^2$, $H^2$, and $tau^2$, with 95% confidence and prediction intervals reported for global estimates.

To explore potential sources of heterogeneity in behavioural outcomes, we conducted subgroup analyses and meta-regressions using taxonomic, behavioural, and microbial moderators. For the meta-analysis, individual behaviours were grouped into broader categories: Anxiety-like (anxiety, anxiety-like), Depressive-like, Feeding-related

(feeding, attraction to food, foraging), Cognitive (cognition, memory), Social (social, maternal), Activity/Locomotion (locomotion, swimming), and Stress-related (stress, vigilance, abnormal behaviour). Subgroup analyses were performed when at least two independent comparisons ($k \geq 2$) were available per level, fitting separate random-effects models for each group. To better contextualize the results, we also report the number of independent comparisons per Order, highlighting which taxonomic categories had sufficient replication for stratified meta-analytic estimates. To avoid overfitting, categorical moderators with sparse categories were collapsed into an "Other" category using frequency-based thresholds. Meta-regressions were first run separately for each moderator and subsequently as multiple models that included all categorical predictors, with multicollinearity assessed using condition numbers derived from model matrices (Harrison et al., 2018). Predicted mean effects and 95% confidence intervals per moderator level were visualised using precision-weighted bubble plots.

To further explore ecological and biological sources of behavioural variation, we included meta-regression testing for effects of host-level traits and environmental context. Specifically, we modelled the influence of dietary ecology (herbivorous, omnivorous, insectivorous, etc), sociality (social, non-social), habitat type (burrower, non-burrower), and place (farm, laboratory, wild) as continuous or categorical moderators. These variables were hypothesised to modulate host–microbe–behaviour relationships by shaping microbial exposure, transmission routes, and selective pressures on neurobehavioural traits. Moderator analyses were conducted as exploratory tests of potential ecological drivers of heterogeneity. Because each moderator was evaluated in separate meta-regression models and the objective was to estimate effect sizes and associated uncertainty rather than to test a large set of simultaneous

hypotheses, we did not apply a formal False Discovery Rate (FDR) correction. Instead, effect estimates, standard errors, and confidence intervals are reported to allow transparent assessment of the magnitude and precision of moderator effects (Koricheva et al., 2013). In addition, the distribution of effect sizes across moderator categories was summarised to document potential imbalances in the underlying literature, which is dominated by a small number of well-studied taxa and ecological contexts. Sample sizes for each moderator category are reported in a Supplementary Table.

We also examined interactions between behavioural domain and microbiome type (gut, skin), assessing whether internal microbiomes exert stronger effects on behavioural outcomes than external microbiomes (Cryan et al., 2019; Sommer & Bäckhed, 2013). Each interaction was tested through mixed meta-regression models with multiplicative terms, and predictions were visualised as marginal effects using the *emmeans* package (Lenth et al., 2024).

Finally, we evaluated the role of encephalisation quotient (EQ) as a proxy for cognitive complexity and potential behavioural plasticity (Isler & van Schaik, 2009; Jerison, 1973). EQ values were compiled from published databases (Campi et al., 2011; Henriksen et al., 2016; Kohnken & Schwahn, 2016; Mayol-Cabré et al., 2020; Minervini et al., 2016; Murray, 2012) and modelled as a continuous moderator in multilevel meta-regressions. We predicted that species with higher EQ would display stronger microbiome–behaviour coupling, reflecting enhanced neurocognitive sensitivity to microbial modulation (Dunbar & Shultz, 2017; Herculano-Houzel, 2011).

Model diagnostics included forest plots, funnel plots, and contour-enhanced funnel plots (Peters et al., 2008) to evaluate asymmetry and potential publication bias. Egger's regression test (Egger et al., 1997) and Duval and Tweedie's trim-and-fill method

(Duval & Tweedie, 2000) were applied to estimate bias due to potentially missing studies. Robustness to study-level dependency was assessed using cluster-robust variance estimators (CR2) (Tipton, 2015) with clustering by study code. Leave-one-out analyses, Baujat influence diagnostics (Baujat et al., 2002), and cumulative meta-analyses were performed to identify influential cases and temporal trends. Heterogeneity sources were further examined through subgroup-specific $tau^2$ and $I^2$ statistics, and sensitivity analyses compared global versus multilevel model specifications (Fernández-Castilla et al., 2021).

In addition to behavioural outcomes, we evaluated whether experimental manipulation affected microbial diversity. For this purpose, paired data on microbial diversity in control and treatment conditions were extracted from the primary dataset (N = 18). Differences in diversity (ΔDiversity = Test − Control) were tested for normality (Shapiro–Wilk) and, because the distribution was non-normal, a Wilcoxon signed-rank test was used for comparison (Conover, 1999). A linear mixed-effects model (lme4) (Bates et al., 2015) with diversity as the dependent variable, Treatment (Control, Test) as a fixed factor, and random intercepts and slopes for Treatment nested within study ID [Diversity ~ Treatment + (1 + Treatment | Study ID)] was also fitted to account for study-level clustering. Degrees of freedom, 95% confidence intervals, and *P*-values for fixed effects were computed using Satterthwaite's approximation via the lmerTest package (Kuznetsova et al., 2017). Mean ΔDiversity values per microbial species were calculated together with standard errors, considering only taxa represented by at least two independent comparisons ($k \geq 2$), and the results were visualised using a horizontal bar chart (with error bars).

Data were screened for completeness, and rows with non-finite or zero variances were removed. Variables were standardised, and the directionality of effects was checked for

consistency across behavioural measures. Potential duplicate or redundant comparisons were aggregated, and hierarchical dependence was explicitly modelled to avoid pseudoreplication. Taxonomic information (Order) was merged from the raw dataset using unique study codes. All data processing and cleaning steps were scripted for full reproducibility.

To assess whether the primary studies employed sufficient sample sizes to detect the effects they reported, we conducted a formal power adequacy analysis based on each study's observed effect size. For every comparison, the required total sample size (N_required) to achieve 80% power at $\alpha = 0.05$ (two-tailed) was estimated using the closed-form solution of the two-sample t-test, assuming balanced group sizes (Serdar et al., 2021). Reported sample sizes (N_reported) were then compared against N_required to classify each study as adequately powered or underpowered. Achieved statistical power was also computed using the *pwr.t2n.test()* function based on the actual group sizes. To explore patterns in adequacy across biological, behavioural, and ecological moderators, we quantified the proportion of adequately powered studies within each categorical variable and tested deviations from chance at each category level using exact binomial tests. Independence between moderator categories and adequacy status (Yes/No) was examined using Pearson's chi-square tests applied to contingency tables. These analyses allowed us to evaluate whether taxonomic groups, behavioural domains, microbial categories, or ecological traits were systematically associated with higher or lower probability of adequate sample sizes, and to characterise overall patterns of statistical power within the evidence base. All power, adequacy, binomial, and chi-square analyses were implemented in R using *dplyr*, *purrr*, and *stats*.

All analyses were conducted in R (version 4.5.0) (R Core Team, 2025). The following packages were used: metafor for meta-analytic modelling (random-effects, multilevel,

and meta-regression analyses) (Viechtbauer, 2010), clubSandwich for robust standard errors (Pustejovsky, 2016), lme4 for mixed-effects modelling (Bates et al., 2015), dplyr, tidyr, stats, and purrr for data manipulation (Wickham et al., 2019), and broom for model tidying, ggplot2 for data visualisation (Wickham, 2016).

### *Ethical Note*

This study is a comprehensive meta-analysis based exclusively on data extracted from previously published scholarly articles. No primary experimental work involving live animals or human subjects was conducted. Therefore, formal ethical approval from an animal welfare committee was not required for this study under Brazilian legislation (CONCEA - Conselho Nacional de Controle de Experimentação Animal, 2023, 2024). Nonetheless, all analyses were conducted in accordance with the core ethical principles outlined in the ASAB/ABS Guidelines for the Treatment of Animals in Behavioural Research and Teaching (ASAB Ethical Committee/ABS Animal Care Committee, 2023) to ensure methodological integrity and best practices in the study of animal behaviour.

## RESULTS

### *Global effect and heterogeneity*

The final dataset comprised 57 independent studies contributing 1,209 effect sizes. A multivariate random-effects (REML) meta-analytic model, accounting for study-level dependencies, yielded a pooled Hedges' g of 0.45 (95% CI: 0.05 to 0.85), indicating a moderate, overall positive effect of microbiota manipulations on behavioural outcomes across studies (Supplementary Figure S1). Between-study heterogeneity was substantial ($I^2$ = 84.96%, $tau^2$= 1.658), suggesting that most of the variability in effect sizes

reflected actual differences between studies rather than sampling error. To address hierarchical dependence arising from multiple measures within studies, cluster-robust variance estimation (CR2) was applied. The robust model produced consistent results (β = 0.45, SE = 0.20, $t$ = 2.20, gl = 51.44, $P$ = 0.032), confirming the stability of the aggregate effect when adjusting for correlated data structures.

*Subgroup analyses - Host Order*

Effect sizes stratified by host Order were estimated for the three Orders represented by at least two independent comparisons in the dataset (Cypriniformes, Diptera, and Rodentia; Table 1; Supplementary Figure S2). Rodentia accounted for most comparisons (k = 46), whereas Diptera (k = 3) and Cypriniformes (k = 2) were represented by substantially fewer studies. Positive, non-significant effects were observed for Diptera and Rodentia, whereas Cypriniformes showed a negative effect size. Estimated variance within host orders (tau²) ranged from 0.67 to 7.15, and I² values ranged from 63% to 95%, indicating generally high heterogeneity.

**Table 1:** Effect sizes of behavioural modulation by microbiomes stratified by host Order. k = number of independent comparisons per host order; Estim. = estimated Hedges' g effect size; se = standard error of the estimate; Lower CI and Upper CI = lower and upper bounds of the 95% confidence interval; tau² = estimated between-study variance within each host Order; I² = percentage of total variation attributable to heterogeneity rather than sampling error.

| **Order** | **k** | **Estim.** | **se** | **Lower CI** | **Upper CI** | **z** | ***P*** | **tau²** | **I²** |
|---|---|---|---|---|---|---|---|---|---|
| Cypriniformes | 2 | -0.62 | 0.72 | -2.02 | 0.78 | -0.87 | 0.39 | 0.67 | 62.90 |
| Diptera | 3 | 2.44 | 1.61 | -0.72 | 5.60 | 1.51 | 0.13 | 7.15 | 94.57 |
| Rodentia | 46 | 0.38 | 0.20 | -0.02 | 0.78 | 1.86 | 0.06 | 1.27 | 78.11 |

442

443 *Subgroup analyses – Behavioural categories*

444 Effect sizes grouped by behavioural categories showed considerable variability (Table
445 2, Figure 2). Positive, non-significant effects were observed for Cognition and Feeding-
446 related behaviours, whereas Anxiety-like and Depressive-like behaviours were centred
447 near zero. Estimated variance within behavioural categories ($tau^2$) ranged from 0.22 to
448 7.46, and $I^2$ values ranged from 26% to 87%, indicating generally high heterogeneity.
449 The distribution of comparisons across behavioural categories is shown in Figure 3.

450 **Table 2:** Effect sizes of behavioural modulation by microbiomes stratified by
451 behavioural category. k = number of independent comparisons per behavioural
452 category; Estim. = estimated Hedges' g effect size; se = standard error of the estimate;
453 Lower CI and Upper CI = lower and upper bounds of the 95% confidence interval; $tau^2$
454 = estimated between-study variance within each behavioural category; $I^2$ = percentage
455 of total variation attributable to heterogeneity rather than sampling error.

| Group | k | Estim. | se | Lower CI | Upper CI | z | *P* | $tau^2$ | $I^2$ |
|---|---|---|---|---|---|---|---|---|---|
| Activity | 4 | 0.70 | 0.57 | -0.42 | 1.81 | 1.23 | 0.22 | 0.87 | 79.25 |
| Anxiety_like | 15 | -0.13 | 0.38 | -0.87 | 0.61 | -0.34 | 0.73 | 1.66 | 85.93 |
| Cognition | 10 | 0.86 | 0.53 | -0.18 | 1.90 | 1.62 | 0.11 | 2.22 | 86.46 |
| Depressive_like | 15 | -0.02 | 0.25 | -0.52 | 0.48 | -0.09 | 0.93 | 0.22 | 26.39 |
| Feeding_related | 4 | 1.98 | 1.51 | -0.99 | 4.94 | 1.31 | 0.19 | 7.46 | 87.61 |
| Social | 3 | 0.77 | 0.67 | -0.55 | 2.09 | 1.14 | 0.25 | 0.53 | 37.20 |
| Stress_related | 6 | 0.72 | 0.48 | -0.23 | 1.67 | 1.49 | 0.14 | 0.92 | 73.61 |

456

457 _____Insert Figure 2 here_____

458 _____Insert Figure 3 here_____

459

460 *Subgroup analyses - Microbial Order*

461 Grouping effect sizes by microbial Order revealed that manipulations involving
462 Lactobacillales yielded a positive, non-significant effect (g = 0.43, 95% CI: –0.14 to
463 0.99, $P = 0.14$) with high heterogeneity ($I^2 = 86.39\%$, $tau^2 = 2.01$). The combined group
464 Lactobacillales and Bifidobacteriales showed the largest effect size (g = 1.76, 95% CI: –
465 0.16 to 3.69, $P = 0.07$, $I^2 = 71.99\%$, $tau^2 = 2.58$), whereas Bifidobacteriales alone
466 exhibited a small negative, non-significant effect (g = –0.38, 95% CI: –1.21 to 0.44, $P =$
467 0.37, $I^2 = 65.66\%$, $tau^2 = 0.42$). Effects for Enterobacterales (g = 0.37, 95% CI: –1.96 to
468 2.70, $P = 0.76$) and Verrucomicrobiales (g = 0.06, 95% CI: –1.06 to 1.18, $P = 0.92$)
469 were also non-significant, with moderate to low heterogeneity.

470 *Subgroup analyses - Microbial species*

471 Species-level analyses (Table 3) further illustrated high variability. While most
472 estimates were non-significant, *Lactobacillus johnsonii* exhibited a significant negative
473 effect, indicating a potential reduction in behavioural outcomes under its influence.
474 Conversely, several other taxa, such as *Bifidobacterium longum* and *Lactiplantibacillus*
475 *plantarum*, showed large positive but imprecise effects, with confidence intervals
476 spanning zero and heterogeneity often exceeding 90%.

477

478 **Table 3:** Effect sizes of behavioural modulation by microbiomes stratified by microbial
479 species. k = number of independent comparisons per species; Estim. = estimated
480 Hedges' g effect size; se = standard error of the estimate; Lower CI and Upper CI =
481 lower and upper bounds of the 95% confidence interval; $tau^2$ = estimated between-study

variance within each species; $I^2$ = percentage of total variation attributable to heterogeneity rather than sampling error.

| Species | k | Estim. | se | Lower CI | Upper CI | z | *P* | $tau^2$ | $I^2$ |
|---|---|---|---|---|---|---|---|---|---|
| *Akkermansia muciniphila* | 2 | 0.06 | 0.57 | -1.06 | 1.18 | 0.10 | 0.92 | 0.00 | 0.00 |
| *Bifidobacterium breve* | 2 | 0.24 | 0.98 | -1.68 | 2.16 | 0.24 | 0.81 | 1.60 | 82.07 |
| *Bifidobacterium longum* | 2 | 1.68 | 2.30 | -2.83 | 6.19 | 0.73 | 0.47 | 9.96 | 94.12 |
| *Escherichia coli* | 3 | 0.42 | 1.29 | -2.10 | 2.95 | 0.33 | 0.74 | 3.231 | 65.334 |
| *Lacticaseibacillus casei* | 2 | 0.03 | 0.37 | -0.69 | 0.76 | 0.09 | 0.93 | 0.00 | 0.00 |
| *Lacticaseibacillus paracasei* | 4 | 0.30 | 0.69 | -1.05 | 1.64 | 0.43 | 0.67 | 1.17 | 72.84 |
| *Lacticaseibacillus rhamnosus* | 3 | 1.24 | 1.06 | -0.85 | 3.32 | 1.16 | 0.24 | 1.87 | 54.05 |
| *Lactiplantibacillus plantarum* | 5 | 1.10 | 1.21 | -1.26 | 3.47 | 0.92 | 0.36 | 6.50 | 90.36 |
| *Lactobacillus helveticus* | 3 | 0.21 | 0.51 | -0.78 | 1.21 | 0.42 | 0.67 | 0.35 | 45.15 |
| *Lactobacillus johnsonii* | 2 | -1.14 | 0.56 | -2.24 | -0.03 | -2.02 | 0.04 | 0.00 | 0.00 |
| *Lactococcus lactis* | 3 | -0.19 | 0.44 | -1.05 | 0.68 | -0.42 | 0.68 | 0.00 | 0.00 |
| *Limosilactobacillus reuteri* | 3 | -0.07 | 1.65 | -3.31 | 3.16 | -0.04 | 0.97 | 7.48 | 94.61 |

Our meta-analysis revealed significant associations between specific microorganisms and various behavioural categories. We observed that *E. coli*, *L. rhamnosus*, *L. plantarum*, and *B. longum* exerted a significant positive influence on behaviours grouped within the Cognition category (Figures 4, 5, and 6). This indicates that the

presence of these microorganisms is associated with increased cognitive-behavioural measures. Additionally, *L. plantarum* demonstrated a significant positive influence on behaviours within the Feeding-related category (Figure 4), suggesting an increase in these behaviours. In contrast, *L. rhamnosus* positively influenced behaviours in the Stress-related category (Figure 4), suggesting that its presence is associated with increased stress behaviours or indicators. Finally, *L. reuteri* exhibited a dual pattern of influence: it was associated with increases in behaviours within the Social category and decreases in behaviours within the Anxiety-like category (Figure 5).

_____Insert Figure 4 here_____

_____Insert Figure 5 here_____

_____Insert Figure 6 here_____

*Meta-regression of moderators*

To initially explore potential sources of heterogeneity, simple meta-regression models were conducted. These analyses revealed significant associations for two categorical predictors: Order Diptera ($\beta = 1.87 \pm 0.86$, $P = 0.030$) and behavioural category Feeding-related ($\beta = 2.34 \pm 0.92$, $P = 0.011$). These preliminary findings suggested that microbiota manipulations might exert greater behavioural effects in Diptera and in feeding-related contexts. However, when all moderators were included in a single multiple meta-regression model, none reached statistical significance.

To further explore potential sources of heterogeneity, we conducted additional meta-regression models including ecological and biological moderators (study setting, diet, habitat, and sociality). None of these variables reached statistical significance, although

some exhibited trends suggesting potential influences. Studies conducted under laboratory conditions ($\beta = 0.19 \pm 1.12$, $P = 0.87$) or in wild settings ($\beta = -0.01 \pm 1.58$, $P = 0.99$) did not differ significantly from those in farm environments. Similarly, diet type did not predict substantially behavioural outcomes, although saprophagous hosts showed a positive but non-significant trend ($\beta = 1.28 \pm 1.23$, $P = 0.30$). The comparison between burrowing and non-burrowing species revealed a marginally positive effect for non-burrowers ($\beta = 0.70 \pm 0.38$, $P = 0.066$), suggesting that species occupying open or surface habitats may exhibit slightly stronger behavioural responses to microbiota manipulations. Social organisation (social vs. solitary) did not influence behavioural modulation ($\beta = -0.14 \pm 0.55$, $P = 0.80$).

Finally, to assess whether behavioural outcomes varied according to the type of microbiome investigated, we conducted a meta-regression that included microbiome (gut, skin) as a moderator. The analysis revealed no significant differences among microbiome types ($QM_{(1)} = 0.03$, $P = 0.86$), indicating that the magnitude of behavioural effects was comparable across studies targeting distinct microbial communities. When controlling for other variables, the pattern remained unchanged ($\beta = -1.73 \pm 1.23$, $P = 0.16$), suggesting that the location of the microbiome did not systematically influence the behavioural responses observed. Additionally, we investigated whether species with higher encephalization quotients (EQ) exhibited stronger behavioural responses to microbiome manipulation. Across multiple models, including EQ as a continuous variable ($\beta = -0.07 \pm 0.10$, $P = 0.47$), as categorical terciles, and using non-linear splines, no significant relationship was detected.

*Microbial diversity*

Descriptive analysis of ΔDiversity revealed a range from a minimum decrease of –1.467 to a maximum increase of 5.455. The median ΔDiversity was 0.024 (interquartile range:

537 –0.380 to 0.250), with a mean of 0.107. A Shapiro–Wilk normality test (W = 0.602, $P <$
538 0.001) indicated that the distribution of ΔDiversity was significantly non-normal.
539 Consequently, a nonparametric Wilcoxon signed-rank test (W = 21914, N = 18, $P =$
540 0.660) was performed, which showed no statistically significant difference in median
541 diversity between the control and test conditions.

542 LMM analysis indicated that mean microbial diversity did not differ significantly
543 between experimental conditions. In the Control group, diversity averaged 4.27 ± 0.91
544 (mean ± SE; $t_{(17)} = 4.69$, $P = 0.00021$), whereas in the Treatment group it averaged 0.24
545 ± 0.23 ($t_{(17.14)} = 1.06$, $P = 0.302$), revealing no consistent overall effect of treatment.
546 However, random-effect estimates showed pronounced heterogeneity among studies
547 (intercept SD = 3.86; treatment SD = 0.93), with a strong positive correlation (r = 0.70)
548 between baseline diversity and treatment response. This pattern suggests that while
549 some studies reported marked shifts in microbial diversity following intervention, others
550 showed minimal or no change. Overall, there was no significant general effect of
551 treatment on microbial diversity, but responses were highly context-dependent across
552 taxa and experimental designs (Figure 7).

553 _____Insert Figure 7 here_____

554

555 *Publication bias, influence diagnostics and adequacy of sample sizes*

556 Funnel plot asymmetry tests did not indicate significant publication bias (Egger's
557 regression: z = 1.46, $P = 0.15$) (Figure 8). The Trim-and-Fill procedure did not impute
558 any missing studies (k filled = 0), and the adjusted pooled estimate remained unchanged
559 (g = 0.45). These results suggest that publication bias is unlikely to have significantly
560 influenced the overall conclusions. Furthermore, influence diagnostics identified one

study with a standardised residual exceeding 3.6 (Supplementary Material Table S1). Still, its exclusion did not substantially alter the pooled effect size or heterogeneity estimates, supporting the robustness of the findings.

_____Insert Figure 8 here_____

Across the studies included in the meta-analysis, the majority (≈96%) reported sample sizes sufficient to detect the effects observed (Figure 9). A binomial test confirmed that the proportion of adequately powered studies (884/1,186; 0.75) was significantly higher than expected by chance ($p < 0.001$), indicating a clear predominance of adequately powered studies overall. Only a small subset of studies was underpowered, predominantly those reporting small or very small effect sizes (Figure 9). The distribution of reported versus required sample sizes indicates that most studies met or exceeded the thresholds necessary for adequate statistical power. Analyses of the relationship between effect size and sample size show that studies with large and medium effects were almost universally adequately powered, whereas studies with smaller effects exhibited slightly lower adequacy, consistent with the higher sample sizes required to detect them (Figure 9). Calculations of achieved statistical power confirm that most studies reached the conventional 0.8 threshold, highlighting the robustness of the evidence base and suggesting that effect sizes reported are unlikely to be inflated due to low sample sizes (Figure 9).

_____Insert Figure 9 here_____

584 Analyses of sample size adequacy across study- and microbiome-related characteristics
585 revealed that most categories were dominated by adequately powered studies
586 (Supplementary Material Table S2; Supplementary Material Figures S3 and S4).
587 Specifically, for behavioural domains, studies examining anxiety-like, depressive-like,
588 social, cognition, and stress-related behaviours exhibited a significantly higher
589 proportion of adequately powered experiments, whereas activity- and feeding-related
590 behaviours did not differ significantly from chance. Across taxonomic classes,
591 mammalian studies were overwhelmingly adequately powered, whereas studies in other
592 classes showed non-significant deviations. Similarly, studies of omnivorous diets and
593 burrowing versus non-burrowing habitats were significantly enriched in adequately
594 powered experiments. Analyses by taxonomic order, sociality, and microbial
595 characteristics (including microbiome type, microbial taxa order, and specific microbial
596 species) also showed a clear predominance of adequately powered studies, with chi-
597 square tests confirming that adequacy was not uniformly distributed across categories
598 (all $p < 0.01$, except for microbiome type; Supplementary Material Table S2).

599

600 **DISCUSSION**

601 Our findings provide quantitative support for the hypothesis that microbiota
602 manipulations can influence animal behaviour. The meta-analysis revealed a moderate
603 overall positive effect (Hedges' $g = 0.45$), indicating that alterations in microbial
604 communities are associated with measurable behavioural changes across diverse animal
605 taxa. As in most ecological syntheses, this overall effect should be interpreted as an
606 average pattern across heterogeneous systems rather than a universal response across all
607 host species and experimental contexts. However, the absence of consistent changes in
608 microbial α-diversity suggests that behavioural responses are not necessarily driven by

609 broad shifts in community diversity, but rather by compositional or functional changes
610 within microbial assemblages.

611 Behavioural subgroup analyses showed positive trends for cognition, feeding-related,
612 stress-related, and social behaviours, consistent with the idea that host–microbe
613 interactions may influence behavioural domains linked to microbial acquisition,
614 maintenance, and transmission. Feeding and social behaviours are closely associated
615 with microbial exposure and exchange among hosts (Moeller et al., 2016; Sarkar et al.,
616 2020; Trevelline & Kohl, 2022). In contrast, anxiety-like and depressive-like behaviours
617 exhibited near-zero aggregated effects, suggesting that emotional regulation may be less
618 consistently influenced by microbiota manipulations or more dependent on specific host
619 taxa, microbial strains, or experimental contexts.

620 A considerable proportion of microbiome–behaviour research has focused on affective
621 states, particularly anxiety, depression, and stress-related behaviours, largely due to the
622 potential therapeutic applications of microbial interventions such as probiotics and
623 prebiotics in both humans and animals (Ji et al., 2023; Swanson et al., 2025). Rodent
624 models have been especially important for elucidating mechanisms linking microbial
625 communities to behaviour, including modulation of neurotransmitters such as serotonin,
626 dopamine, and γ-aminobutyric acid (GABA) (Cheng et al., 2022; Van Hul & Cani,
627 2023). These findings support the concept of a microbiota–gut–brain axis through
628 which microbial metabolites, immune signalling, and neuroendocrine pathways can
629 influence central nervous system function (Chakrabarti et al., 2022; Zhu, 2023).
630 Consistent with this broader literature, our dataset revealed that anxiety-like, stress-
631 related, and depressive-like behaviours were among the most frequently investigated
632 categories. Notably, the observation that some microbial taxa showed negative
633 associations with anxiety-like behaviours supports the possibility of strain- or

metabolite-specific effects on emotional regulation (Butler et al., 2025; Lalonde & Strazielle, 2022; Olavarría-Ramírez et al., 2023; Sacoor et al., 2024).

Despite these overall patterns, substantial heterogeneity was detected among studies ($I^2$ ≈ 85%). High heterogeneity is common in ecological and microbiome meta-analyses because biological responses often depend on multiple interacting factors, including host species, microbial composition, and experimental design. Moderator analyses revealed that several initially significant associations disappeared in multivariate models, suggesting that observed differences may reflect experimental or ecological variation rather than consistent biological effects. Ecological moderators such as diet, sociality, and habitat type did not show significant effects, although these factors remain plausible drivers of microbiome variation and behavioural responses in natural systems (Ley et al., 2008; Youngblut et al., 2019).

Overall, microbiota manipulations tended to increase the expression of normal behavioural patterns such as feeding, activity, cognition, and social behaviours, while abnormal or anxiety-like responses were generally reduced. These trends align with growing evidence that balanced microbial communities can contribute to neurochemical stability and emotional regulation through pathways including immune modulation, short-chain fatty acid production, vagal signalling, and neuroendocrine regulation (Bostick et al., 2022; Davidson et al., 2020; He et al., 2024; Kim, 2023; Merlo et al., 2024; O'Riordan et al., 2025; Ramadan et al., 2025; Singh et al., 2024). However, the high variability among studies indicates that microbiome-mediated behavioural effects depend heavily on host species, microbial composition, and experimental context, complicating the identification of consistent patterns across animal models.

Interpretation of taxonomic patterns must also consider strong biases in the available literature. The meta-analytic dataset revealed a pronounced concentration of studies in

659 model organisms, particularly rodents and *Drosophila*. In the broader concurrent
660 scientometric dataset, Rodentia accounted for the most studies (N = 200; 57.47%),
661 whereas Diptera comprised 31 studies (8.91%), all of which were exclusively
662 *Drosophila melanogaster*. The predominance of these tractable laboratory systems
663 likely reflects their experimental advantages, including standardised microbiome
664 manipulations, germ-free models, and controlled environmental conditions.
665 Consequently, the relatively stronger effect estimates observed in these groups may
666 partly reflect methodological factors rather than intrinsic biological differences among
667 taxa. The high heterogeneity detected within Rodentia ($I^2$ = 78%) and Diptera ($I^2$ =
668 95%) further reinforces that behavioural responses remain highly context-dependent
669 even within these model systems. Expanding research beyond model organisms will
670 therefore be essential to assess the generality of microbiome–behaviour relationships
671 across animal diversity.

672 The ecological context in which experiments are conducted may also influence
673 observed patterns. All studies included in the present synthesis were performed in
674 captive or semi-controlled environments. Captivity is known to alter microbial
675 communities relative to those of wild conspecifics (Clayton et al., 2016; McKenzie et
676 al., 2017; Schmidt et al., 2019), largely due to restricted diets, simplified environments,
677 and reduced opportunities for microbial transmission. These constraints may modify or
678 dampen naturally occurring microbiome–behaviour feedback, potentially limiting the
679 ecological generality of experimental results (Trevelline et al., 2019). Conversely,
680 controlled housing conditions, standardised diets, and the frequent use of germ-free or
681 antibiotic-treated models in microbiome research (Cryan & Dinan, 2012; Foster et al.,
682 2017), may amplify detectable microbial influences by reducing environmental noise
683 and increasing experimental precision. Thus, the moderate overall effect identified in

this meta-analysis likely reflects microbiome–behaviour dynamics under experimentally constrained ecological conditions. Comparative research in wild or semi-natural systems will be essential to determine whether these effects are weaker, stronger, or qualitatively different under natural ecological complexity.

The complementary analysis of statistical power and sample-size adequacy further strengthens the interpretation of the meta-analytic results. Most comparisons in the dataset were adequately powered to detect the reported effects, suggesting that the observed behavioural patterns are unlikely to be artefacts of systematically underpowered studies. However, smaller behavioural and taxonomic subgroups were represented by limited numbers of comparisons, highlighting the need for broader taxonomic and experimental coverage in future research.

Nevertheless, some species-level analyses included only a small number of independent comparisons ($k \leq 2$), and effect estimates for these microbial taxa should therefore be interpreted cautiously. Meta-regression and subgroup analyses based on limited datasets can be susceptible to spurious patterns (Higgins & Thompson, 2004). Despite this limitation, publication-bias and influence diagnostics did not indicate substantial distortions, and most studies were adequately powered to detect their reported effects.

Our analyses also indicated that microbial diversity did not change consistently following experimental interventions. Some studies reported increases in diversity after microbiome manipulations, whereas others observed reductions or no detectable changes (Chen et al., 2024; Gu et al., 2020; Hashikawa-Hobara et al., 2022; Ichikawa et al., 2023; Needham et al., 2022; Qiao et al., 2025; Tamayo et al., 2025). These contrasting outcomes likely reflect differences in host species, baseline microbial composition, intervention type, duration, and analytical approaches. Consequently, diversity metrics alone may not capture the functional microbial changes responsible for

behavioural modulation, reinforcing the need for standardised microbiome methodologies and more integrative analyses linking microbial composition, function, and host behaviour. Accordingly, species-level ΔDiversity estimates should be interpreted cautiously, as most microbial taxa were represented by only a small number of independent comparisons ($k \geq 2$), and these patterns are therefore exploratory rather than definitive.

Overall, this meta-analysis provides the first quantitative synthesis of experimental evidence linking microbiota manipulation to behavioural outcomes across animal taxa. While the results support the existence of microbiome–behaviour interactions, the high heterogeneity across studies and the taxonomic concentration in model organisms indicate that these effects are strongly context dependent. Future research should address these limitations through more standardised and integrative experimental designs, particularly by increasing consistency in microbiota manipulation protocols (e.g., antibiotics, probiotics, or faecal transplants), improving the standardisation of behavioural assays and their classification, and adopting more comparable approaches to microbiome characterisation and diversity estimation (a multi-omics approaches, including genomics, metabolomics, and transcriptomics). Such standardisation would reduce methodological variability and improve comparability across studies. Cross-species comparative and longitudinal studies will be particularly important for clarifying causal pathways and evolutionary patterns of microbiota–behaviour interactions. Expanding research to underrepresented taxa and ecological contexts will also improve the taxonomic and environmental scope of the field. Integrating behavioural ecology, microbiology, and neuroscience will be essential to develop a mechanistic understanding of how microbial communities influence animal behaviour

and how these interactions may be harnessed for applications in animal health and welfare.

Future research should address these gaps through more standardised and integrative experimental designs combining behavioural assessments with multi-omics approaches, including genomics, metabolomics, and transcriptomics.

## Data availability

All data is available at:

https://osf.io/enw5v/overview?view_only=5987bd6475774522b6ac2012664fda87

## REFERENCES


Adamo, S. A. (2013). Parasites: Evolution's neurobiologists. *Journal of Experimental Biology*, *216*(1), 3–10. https://doi.org/10.1242/jeb.073601

Archie, E. A., & Theis, K. R. (2011). Animal behaviour meets microbial ecology. *Animal Behaviour*, *82*(3), 425–436. https://doi.org/10.1016/j.anbehav.2011.05.029

Archie, E. A., & Tung, J. (2015). Social behavior and the microbiome. *Current Opinion in Behavioral Sciences*, *6*, 28–34. https://doi.org/10.1016/j.cobeha.2015.07.008

ASAB Ethical Committee/ABS Animal Care Committee. (2023). Guidelines for the ethical treatment of nonhuman animals in behavioural research and teaching. *Animal Behaviour*, *195*, I–XI. https://doi.org/10.1016/j.anbehav.2022.09.006

Bangdiwala, S. I. (2024). The importance of systematic reviews. *International Journal of Injury Control and Safety Promotion*, *31*(3), 347–349. https://doi.org/10.1080/17457300.2024.2388484

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bateson, P. (2017). *Behaviour, Development and Evolution*. OpenBook Publishers. https://www.openbookpublishers.com/product/490

Baujat, B., Mahé, C., Pignon, J. P., & Hill, C. (2002). A graphical method for exploring heterogeneity in meta-analyses: Application to a meta-analysis of 65 trials. *Statistics in Medicine*, *21*(18), 2641–2652. https://doi.org/10.1002/sim.1221

762 Ben-Shachar, M., Lüdecke, D., & Makowski, D. (2020). effectsize: Estimation of Effect Size
763 Indices and Standardized Parameters. *Journal of Open Source Software*, *5*(56), 2815.
764 https://doi.org/10.21105/joss.02815

765 Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2010). A basic introduction
766 to fixed-effect and random-effects models for meta-analysis. *Research Synthesis*
767 *Methods*, *1*(2), 97–111. https://doi.org/10.1002/jrsm.12

768 Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2021). *Introduction to*
769 *Meta-Analysis* (2nd ed.). Wiley. https://doi.org/10.1002/9780470743386

770 Bostick, J. W., Schonhoff, A. M., & Mazmanian, S. K. (2022). Gut microbiome-mediated
771 regulation of neuroinflammation. *Current Opinion in Immunology*, *76*, 102177.
772 https://doi.org/10.1016/j.coi.2022.102177

773 Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G.,
774 Bienenstock, J., & Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates
775 emotional behavior and central GABA receptor expression in a mouse via the vagus
776 nerve. *Proceedings of the National Academy of Sciences of the United States of*
777 *America*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

778 Brockmeier, A. J., Ju, M., Przybyła, P., & Ananiadou, S. (2019). Improving reference
779 prioritisation with PICO recognition. *BMC Medical Informatics and Decision Making*,
780 *19*(1), 256. https://doi.org/10.1186/s12911-019-0992-8

781 Buffington, S. A., Di Prisco, G. V., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., & Costa-
782 Mattioli, M. (2016). Microbial Reconstitution Reverses Maternal Diet-Induced Social
783 and Synaptic Deficits in Offspring. *Cell*, *165*(7), 1762–1775.
784 https://doi.org/10.1016/j.cell.2016.06.001

785 Butler, M. I., Kittel-Schneider, S., Wagner-Skacel, J., Mörkl, S., & Clarke, G. (2025). The Gut
786 Microbiome in Anxiety Disorders. *Current Psychiatry Reports*, *27*(5), 347–361.
787 https://doi.org/10.1007/s11920-025-01604-w

788 Calhoun, A. C., Shosanya, T., Long, B. K., Rehberger, J. K., & Sadd, B. M. (2026). Host-
789 associated beneficial gut microbiota boosts induced immunity and limits immune
790 deployment costs in bumblebees. *Journal of Animal Ecology*, *95*(1), 217–229.
791 https://doi.org/10.1111/1365-2656.70180

792 Campi, K. L., Collins, C. E., Todd, W. D., Kaas, J., & Krubitzer, L. (2011). Comparison of Area
793 17 Cellular Composition in Laboratory and Wild-Caught Rats Including Diurnal and
794 Nocturnal Species. *Brain, Behavior and Evolution*, *77*(2), 116–130.
795 https://doi.org/10.1159/000324862

796 Chakrabarti, A., Geurts, L., Hoyles, L., Iozzo, P., Kraneveld, A. D., La Fata, G., Miani, M.,
797 Patterson, E., Pot, B., Shortt, C., & Vauzour, D. (2022). The microbiota–gut–brain axis:
798 pathways to better brain health. Perspectives on what we know, what we need to
799 investigate and how to put knowledge into practice. *Cellular and Molecular Life*
800 *Sciences*, *79*(2), 80. https://doi.org/10.1007/s00018-021-04060-w

Chen, C.-M., Wu, C.-C., Kim, Y., Hsu, W.-Y., Tsai, Y.-C., & Chiu, S.-L. (2024). Enhancing social behavior in an autism spectrum disorder mouse model: investigating the underlying mechanisms of *Lactiplantibacillus plantarum* intervention. *Gut Microbes*, *16*(1), 2359501. https://doi.org/10.1080/19490976.2024.2359501

Cheng, Z., Zhang, L., Yang, L., & Chu, H. (2022). The critical role of gut microbiota in obesity. *Frontiers in Endocrinology*, *13*, 1025706. https://doi.org/10.3389/fendo.2022.1025706

Clayton, J. B., Vangay, P., Huang, H., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., Travis, D. A., Long, H. T., Van Tuan, B., Van Minh, V., Cabana, F., Nadler, T., Toddes, B., Murphy, T., Glander, K. E., Johnson, T. J., & Knights, D. (2016). Captivity humanizes the primate microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(37), 10376–10381. https://doi.org/10.1073/pnas.1521835113

Cohen, A. (2021). *Following the Gut: A Measurable Connection between the Gut Microbiome and Brain Size Thesis Advisor*. University of Colorado.

Colston, T. J., & Jackson, C. R. (2016). Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. *Molecular Ecology*, *25*(16), 3776–3800. https://doi.org/10.1111/mec.13730

CONCEA - Conselho Nacional de Controle de Experimentação Animal. (2023). *Guia Brasileiro de Produção, Manutenção ou Utilização de Animais em Atividades de Ensino ou Pesquisa Científica*.

CONCEA - Conselho Nacional de Controle de Experimentação Animal. (2024). *MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO*.

Conover, W. J. . (1999). *Practical nonparametric statistics* (3rd ed.). Wiley.

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–712. https://doi.org/10.1038/nrn3346

Cryan, J. F., O, K. J., M Cowan, C. S., Sandhu, K. V, S Bastiaanssen, T. F., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V, Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., … Dinan, T. G. (2019). The Microbiota-Gut-Brain Axis. *Physiological Reviews*, *99*, 1877–2013. https://doi.org/10.1152/physrev.00018.2018.-The

Davidson, G. L., Raulo, A., & Knowles, S. C. L. (2020). Identifying microbiome-mediated behaviour in wild vertebrates. *Trends in Ecology and Evolution*, *35*(11), 972–980. https://doi.org/10.1016/j.tree.2020.06.014

De Angelis, M., Ferrocino, I., Calabrese, F. M., De Filippis, F., Cavallo, N., Siragusa, S., Rampelli, S., Di Cagno, R., Rantsiou, K., Vannini, L., Pellegrini, N., Lazzi, C., Turroni, S., Lorusso, N., Ventura, M., Chieppa, M., Neviani, E., Brigidi, P., O'Toole, P. W., … Cocolin, L. (2020). Diet influences the functions of the human intestinal microbiome. *Scientific Reports*, *10*(1), 4247. https://doi.org/10.1038/s41598-020-61192-y

Dunbar, R. I. M., & Shultz, S. (2017). Why are there so many explanations for primate brain evolution? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727), 20160244. https://doi.org/10.1098/rstb.2016.0244

Duval, S., & Tweedie, R. (2000). Trim and Fill: A Simple Funnel-Plot-Based Method of Testing and Adjusting for Publication Bias in Meta-Analysis. *Biometrics*, *56*, 455–463. https://academic.oup.com/biometrics/article/56/2/455/7263515

Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, *315*(7109), 629–634. https://doi.org/10.1136/bmj.315.7109.629

Falagas, M. E., Pitsouni, E. I., Malietzis, G. A., & Pappas, G. (2008). Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. *FASEB J*, *22*(2), 338–342. https://doi.org/10.1096/fj.07-9492lsf

Fernández-Castilla, B., Declercq, L., Jamshidi, L., Beretvas, S. N., Onghena, P., & Van den Noortgate, W. (2021). Detecting Selection Bias in Meta-Analyses with Multiple Outcomes: A Simulation Study. *Journal of Experimental Education*, *89*(1), 125–144. https://doi.org/10.1080/00220973.2019.1582470

Foster, K. R., Schluter, J., Coyte, K. Z., & Rakoff-Nahoum, S. (2017). The evolution of the host microbiome as an ecosystem on a leash. *Nature*, *548*(7665), 43–51. https://doi.org/10.1038/nature23292

Griffiths, J. A., Nirmalkar, K., Wu, W. L., Krajmalnik-Brown, R., & Mazmanian, S. K. (2025). The gut microbiome shapes social behaviour across animal species. *Nature Reviews Microbiology*. https://doi.org/10.1038/s41579-025-01262-y

Gu, F., Wu, Y., Liu, Y., Dou, M., Jiang, Y., & Liang, H. (2020). *Lactobacillus casei* improves depression-like behavior in chronic unpredictable mild stress-induced rats by the BDNF-TrkB signal pathway and the intestinal microbiota. *Food & Function*, *11*(7), 6148–6157. https://doi.org/10.1039/D0FO00373E

Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., Robinson, B. S., Hodgson, D. J., & Inger, R. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ*, *2018*(5), e4794. https://doi.org/10.7717/peerj.4794

Hashikawa-Hobara, N., Otsuka, A., Okujima, C., & Hashikawa, N. (2022). Lactobacillus paragasseri OLL2809 Improves Depression-Like Behavior and Increases Beneficial Gut Microbes in Mice. *Frontiers in Neuroscience*, *16*, 18953. https://doi.org/10.3389/fnins.2022.918953

He, Y., Wang, K., Su, N., Yuan, C., Zhang, N., Hu, X., Fu, Y., & Zhao, F. (2024). Microbiota–gut–brain axis in health and neurological disease: Interactions between gut microbiota and the nervous system. *Journal of Cellular and Molecular Medicine*, *28*(18), e70099. https://doi.org/10.1111/jcmm.70099

877 Hedges, L. V. ., & Olkin, Ingram. (1985). *Statistical methods for meta-analysis* (I. Olkin, Ed.;
878 6th ed.). Academic Press.

879 Heil, M. (2016). Host Manipulation by Parasites: Cases, Patterns, and Remaining Doubts.
880 *Frontiers in Ecology and Evolution*, *4*, 80. https://doi.org/10.3389/fevo.2016.00080

881 Henriksen, R., Johnsson, M., Andersson, L., Jensen, P., & Wright, D. (2016). The
882 domesticated brain: genetics of brain mass and brain structure in an avian species.
883 *Scientific Reports*, *6*(1), 34031. https://doi.org/10.1038/srep34031

884 Herculano-Houzel, S. (2011). Brains matter, bodies maybe not: the case for examining
885 neuron numbers irrespective of body size. *Annals of the New York Academy of*
886 *Sciences*, *1225*(1), 191–199. https://doi.org/10.1111/j.1749-6632.2011.05976.x

887 Higgins, J. P. T., & Thompson, S. G. (2004). Controlling the risk of spurious findings from
888 meta-regression. *Statistics in Medicine*, *23*(11), 1663–1682.
889 https://doi.org/10.1002/sim.1752

890 Hughes, D. P., Andersen, S. B., Andersen, S. B., Hywel-Jones, N. L., Himaman, W., Billen, J., &
891 Boomsma, J. J. (2011). Behavioral mechanisms and morphological symptoms of zombie
892 ants dying from fungal infection. *BMC Ecology*, *11*, 13. https://doi.org/10.1186/1472-
893 6785-11-13

894 Hughes, D. P., & Libersat, F. (2019). Parasite manipulation of host behavior. *Current Biology*,
895 *29*(2), R45–R47. https://doi.org/10.1016/j.cub.2018.12.001

896 Hughes, D. P., Wappler, T., & Labandera, C. C. (2011). Ancient death-grip leaf scars reveal
897 ant–fungal parasitism. *Biology Letters*, *7*(1), 67–70.
898 https://doi.org/10.1098/rsbl.2010.0521

899 Ichikawa, S., Abe, R., Fujimoto, H., Higashi, K., Zang, L., Nakayama, H., Matsuoka, I., &
900 Shimada, Y. (2023). Paraburkholderia sabiae administration alters zebrafish anxiety-like
901 behavior via gut microbial taurine metabolism. *Frontiers in Microbiology*, *14*, 1079187.
902 https://doi.org/10.3389/fmicb.2023.1079187

903 Idowu, P. A., Mpofu, T. J., Magoro, A. M., Modiba, M. C., Nephawe, K. A., & Mtileni, B.
904 (2025). Impact of probiotics on chicken gut microbiota, immunity, behavior, and
905 productive performance—a systematic review. *Frontiers in Animal Science*, *6*, 1562527.
906 https://doi.org/10.3389/fanim.2025.1562527

907 Isler, K., & van Schaik, C. P. (2009). The Expensive Brain: A framework for explaining
908 evolutionary changes in brain size. *Journal of Human Evolution*, *57*(4), 392–400.
909 https://doi.org/10.1016/j.jhevol.2009.04.009

910 Järvilehto, T., & Lickliter, R. (2016). Behaviour Genetics: A Critical Perspective on the Role of
911 Genes in Behaviour. In *Encyclopedia of Life Sciences* (pp. 1–5). Wiley.
912 https://doi.org/10.1002/9780470015902.a0006181.pub3

913 Jerison, H. J. (1973). *Evolution of the Brain and Intelligence*. Elsevier.
914 https://doi.org/10.1016/B978-0-123-85250-2.X5001-9

915 Li, J., Jin, W., Liu, S. J., Jiao, Z., & Li, X. (2023). Probiotics, prebiotics, and postbiotics in health
916 and disease. *MedComm*, *4*(6), e420. https://doi.org/10.1002/mco2.420

917 Johnson, K. V.-A., Watson, K. K., Dunbar, R. I. M., & Burnet, P. W. J. (2022). Sociability in a
918 non-captive macaque population is associated with beneficial gut bacteria. *Frontiers in*
919 *Microbiology*, *13*, 1032495. https://doi.org/10.3389/fmicb.2022.1032495

920 Johnston, T. D., & Edwards, L. (2002). Genes, interactions, and the development of
921 behavior. *Psychological Review*, *109*(1), 26–34. https://doi.org/10.1037/0033-
922 295X.109.1.26

923 Kim, C. H. (2023). Complex regulatory effects of gut microbial short-chain fatty acids on
924 immune tolerance and autoimmunity. *Cellular and Molecular Immunology*, *20*(4), 341–
925 350. https://doi.org/10.1038/s41423-023-00987-1

926 Kohnken, R. A., & Schwahn, D. J. (2016). Lack of Chronic Histologic Lesions Supportive of
927 Sublethal Spontaneous Seizures in FVB/N Mice. *Comparative Medicine*, *66*(2), 105–
928 111. https://www.researchgate.net/publication/299901834

929 Koricheva, J., Gurevitch, J., & Mengersen, K. (2013). *Handbook of Meta-analysis in Ecology*
930 *and Evolution* (1st ed.). Princeton University Press.

931 Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in
932 Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13), 1–26.
933 https://doi.org/10.18637/JSS.V082.I13

934 Lalonde, R., & Strazielle, C. (2022). Probiotic effects on anxiety-like behavior in animal
935 models. *Reviews in the Neurosciences*, *33*(6), 691–701.
936 https://doi.org/10.1515/revneuro-2021-0173

937 Lenth, R. V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez,
938 F., Piaskowiski, J., Riebl, H., & Signmann, H. (2024). *emmeans: Estimated Marginal*
939 *Means, aka Least-Squares Means* (1.10.3; pp. 1–102).
940 https://github.com/rvlenth/emmeans.

941 Levin, D., Raab, N., Pinto, Y., Rothschild, D., Zanir, G., Godneva, A., Mellul, N., Futorian, D.,
942 Gal, D., Leviatan, S., Zeevi, D., Bachelet, I., & Segal, E. (2021). Diversity and functional
943 landscapes in the microbiota of animals in the wild. *Science*, *372*(6539), eabb5352.
944 https://doi.org/10.1126/science.abb5352

945 Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel,
946 M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of
947 Mammals and Their Gut Microbes. *Science*, *320*(5883), 1647–1651.
948 https://doi.org/10.1126/science.1155725

949 Lu, Z. (2011). PubMed and beyond: A survey of web tools for searching biomedical
950 literature. *Database*, *2011*, baq036. https://doi.org/10.1093/database/baq036

951 Lyte, M. (2013). Microbial Endocrinology in the Microbiome-Gut-Brain Axis: How Bacterial
952 Production and Utilization of Neurochemicals Influence Behavior. *PLoS Pathogens*,
953 *9*(11), e1003726. https://doi.org/10.1371/journal.ppat.1003726

954 Lyte, M. (2014). Host-microbiota neuroendocrine interactions influencing brain and
955 behavior. *Gut Microbes*, *5*(3), 381–389. https://doi.org/10.4161/gmic.28682

956 Lyu, Y., Pu, J., Deng, B., & Wu, C. (2025). Gut metabolome in companion animal nutrition—
957 linking diets to health. *Animals*, *15*(5), 651. https://doi.org/10.3390/ani15050651

958 Maritan, E., Quagliariello, A., Frago, E., Patarnello, T., & Martino, M. E. (2024). The role of
959 animal hosts in shaping gut microbiome variation. *Philosophical Transactions of the*
960 *Royal Society B: Biological Sciences*, *379*(1901), 20230071.
961 https://doi.org/10.1098/rstb.2023.0071

962 Mârza, S. M., Munteanu, C., Papuc, I., Radu, L., & Purdoiu, R. C. (2025). The Role of
963 Probiotics in Enhancing Animal Health: Mechanisms, Benefits, and Applications in
964 Livestock and Companion Animals. *Animals*, *15*(20), 2986.
965 https://doi.org/10.3390/ani15202986

966 Mayol-Cabré, M., Prats, E., Raldúa, D., & Gómez-Canela, C. (2020). Characterization of
967 monoaminergic neurochemicals in the different brain regions of adult zebrafish.
968 *Science of The Total Environment*, *745*, 141205.
969 https://doi.org/10.1016/j.scitotenv.2020.141205

970 McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., Alexiev, A.,
971 Amato, K. R., Metcalf, J. L., Kowalewski, M., Avenant, N. L., Link, A., Di Fiore, A., Seguin-
972 Orlando, A., Feh, C., Orlando, L., Mendelson, J. R., Sanders, J., & Knight, R. (2017). The
973 effects of captivity on the mammalian gut microbiome. *Integrative and Comparative*
974 *Biology*, *57*(4), 690–704. https://doi.org/10.1093/icb/icx090

975 Merlo, G., Bachtel, G., & Sugden, S. G. (2024). Gut microbiota, nutrition, and mental health.
976 *Frontiers in Nutrition*, *11*, 1337889. https://doi.org/10.3389/fnut.2024.1337889

977 Minervini, S., Accogli, G., Pirone, A., Graïc, J.-M., Cozzi, B., & Desantis, S. (2016). Brain Mass
978 and Encephalization Quotients in the Domestic Industrial Pig (Sus scrofa). *PLOS ONE*,
979 *11*(6), e0157378. https://doi.org/10.1371/journal.pone.0157378

980 Moeller, A. H., Foerster, S., Wilson, M. L., Pusey, A. E., Hahn, B. H., & Ochman, H. (2016).
981 Social behavior shapes the chimpanzee pan-microbiome. *Science Advances*, *2*(1),
982 e1500997. https://doi.org/10.1126/sciadv.1500997

983 Mongeon, P., & Paul-Hus, A. (2016). The journal coverage of Web of Science and Scopus: a
984 comparative analysis. *Scientometrics*, *106*(1), 213–228.
985 https://doi.org/10.1007/s11192-015-1765-5

986 Moore, J. (2002). *Parasites and the Behavior of Animals*. Oxford University Press.
987 https://doi.org/10.1093/oso/9780195084412.001.0001

Murray, K. A. (2012). Anatomy, Physiology, and Behavior. In M. A. Sukhow, K. A. Stevens, & R. P. Wilson (Eds.), *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* (pp. 753–763). Academic Press. https://doi.org/10.1016/B978-0-12-380920-9.00027-4

Nakagawa, S., & Santos, E. S. A. (2012). Methodological issues and advances in biological meta-analysis. *Evolutionary Ecology*, *26*(5), 1253–1274. https://doi.org/10.1007/s10682-012-9555-5

Needham, B. D., Funabashi, M., Adame, M. D., Wang, Z., Boktor, J. C., Haney, J., Wu, W.-L., Rabut, C., Ladinsky, M. S., Hwang, S.-J., Guo, Y., Zhu, Q., Griffiths, J. A., Knight, R., Bjorkman, P. J., Shapiro, M. G., Geschwind, D. H., Holschneider, D. P., Fischbach, M. A., & Mazmanian, S. K. (2022). A gut-derived metabolite alters brain activity and anxiety behaviour in mice. *Nature*, *602*(7898), 647–653. https://doi.org/10.1038/s41586-022-04396-8

Ntiri, E. S., & Wong, A. C. N. (2025). Microbial metabolites as engines of behavioral variation across animals. *Gut Microbes*, *17*(1), 2501191. https://doi.org/10.1080/19490976.2025.2501191

Olavarría-Ramírez, L., Cooney-Quane, J., Murphy, G., McCafferty, C. P., Cryan, J. F., & Dockray, S. (2023). A systematic review of the effects of gut microbiota depletion on social and anxiety-related behaviours in adult rodents: Implications for translational research. *Neuroscience and Biobehavioral Reviews*, *145*, 105013.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research*, *277*, 32–48. https://doi.org/10.1016/j.bbr.2014.07.027

O'Riordan, K. J., Moloney, G. M., Keane, L., Clarke, G., & Cryan, J. F. (2025). The gut microbiota-immune-brain axis: Therapeutic implications. *Cell Reports Medicine*, *6*(3), 101982. https://doi.org/10.1016/j.xcrm.2025.101982

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan - a web and mobile app for systematic reviews. *Systematic Reviews*, *5*(1), 210. https://doi.org/10.1186/s13643-016-0384-4

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2022). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Rev Panam Salud Publ*, *46*, e112. https://doi.org/10.26633/RPSP.2022.112

Peters, J. L., Sutton, A. J., Jones, D. R., Abrams, K. R., & Rushton, L. (2008). Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *Journal of Clinical Epidemiology*, *61*(10), 991–996. https://doi.org/10.1016/j.jclinepi.2007.11.010

Pick, J., Nakagawa, S., & Noble, D. (2018). metaDigitise: Extract and Summarise Data from Published Figures. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.metaDigitise

Pilla, R., & Suchodolski, J. S. (2021). The gut microbiome of dogs and cats, and the influence of diet. *Vet Clin N Am: Small Anim Pract*, *51*(3), 605–621. https://doi.org/10.1016/j.cvsm.2021.01.002

Poulin, Robert. (2007). *Evolutionary ecology of parasites* (2nd ed.). Princeton University Press.

Pustejovsky, J. E. (2016). clubSandwich: Cluster-Robust (Sandwich) Variance Estimators with Small-Sample Corrections. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.clubSandwich

Qiao, Y., Yu, J., Zhang, Z., Hou, Q., Guo, Z., & Wang, Y. (2025). Regulatory effects of *Lactobacillus zhachilii* HBUAS52074 $^{T}$ on depression-like behavior induced by chronic social defeat stress in mice: modulation of the gut microbiota. *Food & Function*, *16*(2), 691–706. https://doi.org/10.1039/D4FO04542D

R Core Team. (2025). *R: A Language and Environment for Statistical Computing* (4.5.0). R Foundation for Statistical Computing.

Ramadan, Y. N., Alqifari, S. F., Alshehri, K., Alhowiti, A., Mirghani, H., Alrasheed, T., Aljohani, F., Alghamdi, A., & Hetta, H. F. (2025). Microbiome Gut-Brain-Axis: Impact on Brain Development and Mental Health. *Molecular Neurobiology*, *62*(8), 10813–10833. https://doi.org/10.1007/s12035-025-04846-0

Sacoor, C., Marugg, J. D., Lima, N. R., Empadinhas, N., & Montezinho, L. (2024). Gut-Brain Axis Impact on Canine Anxiety Disorders: New Challenges for Behavioral Veterinary Medicine. *Veterinary Medicine International*, *2024*, 2856759. https://doi.org/10.1155/2024/2856759

Sampson, T. R., & Mazmanian, S. K. (2015). Control of Brain Development, Function, and Behavior by the Microbiome. *Cell Host & Microbe*, *17*(5), 565–576. https://doi.org/10.1016/j.chom.2015.04.011

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 and Evolution*, *4*(8), 1020–1035. https://doi.org/10.1038/s41559-020-1220-8

Schmidt, E., Mykytczuk, N., & Schulte-Hostedde, A. I. (2019). Effects of the captive and wild environment on diversity of the gut microbiome of deer mice (Peromyscus maniculatus). *ISME Journal*, *13*(5), 1293–1305. https://doi.org/10.1038/s41396-019-0345-8

Serdar, C. C., Cihan, M., Yücel, D., & Serdar, M. A. (2021). Sample size, power and effect size revisited: Simplified and practical approachin pre-clinical, clinical and laboratory studies. *Biochemia Medica*, *31*(1), 1–27. https://doi.org/10.11613/BM.2021.010502

Shelby, L. B., & Vaske, J. (2008). Understanding meta-analysis: A review of the methodological literature. *Leisure Sciences*, *30*(2), 96–110. https://doi.org/10.1080/01490400701881366

Singh, J., Vanlallawmzuali, Singh, A., Biswal, S., Zomuansangi, R., Lalbiaktluangi, C., Singh, B. P., Singh, P. K., Vellingiri, B., Iyer, M., Ram, H., Udey, B., & Yadav, M. K. (2024). Microbiota-brain axis: Exploring the role of gut microbiota in psychiatric disorders - A comprehensive review. *Asian Journal of Psychiatry*, *97*, 104068. https://doi.org/10.1016/j.ajp.2024.104068

Sokolowski, M. B. (2001). Drosophila: Genetics meets behaviour. *Nature Reviews*, *2*, 879–892.

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology*, *11*(4), 227–238. https://doi.org/10.1038/nrmicro2974

Song, S. J., Sanders, J. G., Delsuc, F., Metcalf, J., Amato, K., Taylor, M. W., Mazel, F., Lutz, H. L., Winker, K., Graves, G. R., Humphrey, G., Gilbert, J. A., Hackett, S. J., White, K. P., Skeen, H. R., Kurtis, S. M., Withrow, J., Braile, T., Miller, M., … McFall-Ngai, M. (2020). Comparative Analyses of Vertebrate Gut Microbiomes Reveal Convergence between Birds and Bats. *MBio*, *11*, e0290119. https://doi.org/10.1128/mBio

Swanson, K. S., Allenspach, K., Amos, G., Auchtung, T. A., Bassett, S. A., Bjørnvad, C. R., Everaert, N., Martín-Orúe, S. M., Ricke, S. C., Ryan, E. P., & Fahey, G. C. (2025). Use of biotics in animals: impact on nutrition, health, and food production. *Journal of Animal Science*, *103*, skaf061. https://doi.org/10.1093/jas/skaf061

Tamayo, M., Agusti, A., Molina-Mendoza, G. V., Rossini, V., Frances-Cuesta, C., Tolosa-Enguís, V., & Sanz, Y. (2025). Bifidobacterium longum CECT 30763 improves depressive- and anxiety-like behavior in a social defeat mouse model through the immune and dopaminergic systems. *Brain, Behavior, and Immunity*, *125*, 35–57. https://doi.org/10.1016/j.bbi.2024.12.028

Tipton, E. (2015). Small sample adjustments for robust variance estimation with meta-regression. *Psychological Methods*, *20*(3), 375–393. https://doi.org/10.1037/met0000011

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1895), 20182448. https://doi.org/10.1098/rspb.2018.2448

Trevelline, B. K., & Kohl, K. D. (2022). The gut microbiome influences host diet selection behavior. *Proceedings of the National Academy of Sciences*, *119*(17), e2117537119. https://doi.org/10.1073/pnas.2117537119

Van den Noortgate, W., López-López, J. A., Marín-Martínez, F., & Sánchez-Meca, J. (2013). Three-level meta-analysis of dependent effect sizes. *Behavior Research Methods*, *45*(2), 576–594. https://doi.org/10.3758/s13428-012-0261-6

Van Hul, M., & Cani, P. D. (2023). The gut microbiota in obesity and weight management: microbes as friends or foe? *Nature Reviews Endocrinology*, *19*(5), 258–271. https://doi.org/10.1038/s41574-022-00794-0

Viechtbauer, W. (2010). Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software*, *36*(3), 1–48. http://www.jstatsoft.org/

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (H. Wickham, Ed.; 2nd ed.). Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Yao, S., Han, J.-Z., Guo, J., Wang, X., Qian, L., Wu, H., Shi, W., Zhu, R.-J., Wang, J.-H., Dong, S.-S., Cui, L.-L., Wang, Y., Guo, Y., & Yang, T.-L. (2024). The Causal Relationships Between Gut Microbiota, Brain Volume, and Intelligence: A Two-Step Mendelian Randomization Analysis. *Biological Psychiatry*, *96*(6), 463–472. https://doi.org/10.1016/j.biopsych.2024.02.1012

York, R. A. (2018). Assessing the genetic landscape of animal behavior. *Genetics*, *209*(1), 223–232. https://doi.org/10.1534/genetics.118.300712

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*(1), 2200. https://doi.org/10.1038/s41467-019-10191-3

Yuan, Y., & Hunt, R. H. (2009). Systematic reviews: The good, the bad, and the ugly. *American Journal of Gastroenterology*, *104*(5), 1086–1092. https://doi.org/10.1038/ajg.2009.118

Zhang, Z., Xue, C., Ju, M., Guo, J., Wang, M., Yi, S., & Yi, X. (2021). Maternal gut dysbiosis alters offspring microbiota and social interactions. *Microorganisms*, *9*(8), 1742. https://doi.org/10.3390/microorganisms9081742

Zhu, L. (2023). Animal social behaviour and gut microbiome. *Frontiers in Microbiology*, *14*, 1210717. https://doi.org/10.3389/fmicb.2023.1210717

1137

1138

**Supplementary Material Captions**

**Figure S1:** Global forest plot showing the estimated effect sizes (Hedges' g) and 95% confidence intervals for all studies included in the meta-analysis. The multivariate random-effects model (REML), accounting for study-level dependencies, yielded a pooled Hedges' g of 0.45 (95% CI: 0.05–0.85), indicating a moderate, overall positive effect of microbiota manipulations on behavioural outcomes across studies. Each line corresponds to an individual research study, identified by its study number, behavioural category, microbial order, and species. The size of each square represents the inverse-variance weight assigned to the study, and the diamond denotes the overall pooled estimate. Details of each study (study ID) can be found in the dataset deposited in the Mendeley Data Repository, as indicated in the Declarations section.

**Figure S2:** Forest plots showing the estimated effect sizes (Hedges' g) and 95% confidence intervals for the three most frequently studied host Orders: Cypriniformes, Diptera, and Rodentia. Each line corresponds to an individual study, identified by its study number, behavioural category, and microbial species. Details of individual studies (study ID) are available in the dataset deposited in the Mendeley Data Repository, as referenced in the Declarations section.

**Figure S3.** Adequacy of sample sizes across behavioural and ecological variables. Each bar represents the proportion of studies classified as adequately powered ("Yes") versus underpowered ("No") within behavioural subcategories (e.g., activity, anxiety-like, cognition, depressive-like, feeding-related, social, stress-related) and across taxonomic, dietary, habitat, order, and sociality classifications. Binomial tests were used to evaluate significant deviations from equal proportions, highlighting the categories in which studies were predominantly adequately powered (Supplementary Material Table S2).

**Figure S4.** Adequacy of sample sizes across microbial classifications. The panel shows the proportion of studies classified as adequately powered ("Yes") versus underpowered ("No") across microbiome type (gut, skin), microbial orders, and specific microbial species. Statistical significance was evaluated using binomial tests to determine which microbial categories were consistently studied with sufficient sample sizes (Supplementary Material Table S2).

**Table S1:** Influence diagnostics for individual studies included in the meta-analysis. Each row corresponds to a single study, and the columns report several influence statistics used to assess the robustness of the overall model. rstudent = externally studentised residuals, measuring how much each study's effect size deviates from the model estimate; dffits = difference in fitted values when a study is omitted, indicating its influence on model predictions; cook.d = Cook's distance, reflecting the overall influence of each study on the fitted model; cov.r = covariance ratio, assessing the influence of a study on the precision of parameter estimates; tau2.del = between-study variance ($\tau^2$) when the study is deleted; QE.del = test of residual heterogeneity ($Q_e$) with the study removed; hat = diagonal elements of the hat matrix, quantifying study leverage; weight = inverse-variance weight assigned to each study; inf = overall influence index combining multiple diagnostic measures. Studies with extreme residuals or large influence values were inspected. Still, their exclusion did not materially alter the pooled effect size or heterogeneity estimates, supporting the robustness of the meta-analytic conclusions.

**Table S2:** Statistical summary of sample size adequacy across study characteristics and microbial variables. For each category, the number of studies classified as adequately powered ("Yes") versus underpowered ("No") is reported, along with the total proportion of adequately powered studies (prop_yes), binomial test p-values, and interpretation of significance. Chi-square tests of independence across categories are

also provided, indicating whether adequacy was significantly associated with the variable. Categories with $p < 0.05$ are highlighted as enriched considerably in adequately powered studies.