# Additional file

## Contents

# Supplementary Methods

**Table S1** PRISMA checklist 2020

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review. | Title page |
| **ABSTRACT** | | | |
| Abstract | 2 | See the PRISMA 2020 for Abstracts checklist. | Page 3 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of existing knowledge. | Page 4-5 |
| Objectives | 4 | Provide an explicit statement of the objective(s) or question(s) the review addresses. | Page 5 |
| **METHODS** | | | |
| Eligibility criteria | 5 | Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses. | Page 5-6 |
| Information sources | 6 | Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted. | Page 6 |
| Search strategy | 7 | Present the full search strategies for all databases, registers, and websites, including any filters and limits used. | Page 5-6 and S2 Table |
| Selection process | 8 | Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process. | Page 5-8 and Page 9 (Figure 1) |
| Data collection process | 9 | Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process. | Page 6-7 |
| Data items | 10a | List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect. | Page 5-7 |
| | 10b | List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information. | Page 6-7 and Page 10-13 (Table 1) |
| Study risk of bias assessment | 11 | Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process. | Page 7 and Supplementary Methods |
| Effect measures | 12 | Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results. | Page 7 and |

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| | | | Supplementary Methods |
| Synthesis methods | 13a | Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)). | Page 7 and Supplementary Methods |
| | 13b | Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions. | Page 7 and Supplementary Methods |
| | 13c | Describe any methods used to tabulate or visually display results of individual studies and syntheses. | Page 7 and Supplementary Methods |
| | 13d | Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. | Page 7 and Supplementary Methods |
| | 13e | Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression). | Page 7 and Supplementary Methods |
| | 13f | Describe any sensitivity analyses conducted to assess robustness of the synthesized results. | Page 7 and Supplementary Methods |
| Reporting bias assessment | 14 | Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases). | Page 7 and Supplementary Methods |
| Certainty assessment | 15 | Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome. | Page 7 |
| **RESULTS** | | | |
| Study selection | 16a | Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram. | Page 8-9, (Figure 1) |
| | 16b | Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded. | Page 8-9, (Figure 1) and Supplementary Methods |
| Study characteristics | 17 | Cite each included study and present its characteristics. | Page 10-13 (Table 1) |

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| Risk of bias in studies | 18 | Present assessments of risk of bias for each included study. | Page 20 and S5-7 Tables and S16-18 Figure |
| Results of individual studies | 19 | For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. | Page 14-18 Figure 2-7 and S1-9 Figure and S3 Table |
| Results of syntheses | 20a | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. | Page 20 and S5-7 Tables and S16-18 Figure |
| | 20b | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. | Page 14-18 Figure 2-7 and S1-9 Figure |
| | 20c | Present results of all investigations of possible causes of heterogeneity among study results. | Page 18 and S10-15 Figure |
| | 20d | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. | Page 18 and S1-9 Figure |
| Reporting biases | 21 | Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed. | Page 20 and S5-7 Tables and S16-18 Figure |
| Certainty of evidence | 22 | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. | Page 20 and S8 Table |
| **DISCUSSION** | | | |
| Discussion | 23a | Provide a general interpretation of the results in the context of other evidence. | Page 20-22 |
| | 23b | Discuss any limitations of the evidence included in the review. | Page 23 |
| | 23c | Discuss any limitations of the review processes used. | Page 23 |
| | 23d | Discuss implications of the results for practice, policy, and future research. | Page 23 |
| **OTHER INFORMATION** | | | |
| Registration and protocol | 24a | Provide registration information for the review, including register name and registration number, or state that the review was not registered. | Page 5 |
| | 24b | Indicate where the review protocol can be accessed, or state that a protocol was not prepared. | Page 5 |

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| | 24c | Describe and explain any amendments to information provided at registration or in the protocol. | Page 5 |
| Support | 25 | Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review. | Title Page (Page 2) |
| Competing interests | 26 | Declare any competing interests of review authors. | Title Page (Page 2) |
| Availability of data, code and other materials | 27 | Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. | Title Page (Page 2) |

**Table S2** Search key

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| **Search key** | (normal OR general OR healthy) **AND** (population OR participant OR participants OR volunteer OR volunteers OR subject OR subjects OR adult OR adults OR adolescent OR adolescents OR child OR children OR infant OR infants OR newborn OR newborns OR birth cohort OR pediatric* OR elderly OR elders) **AND** (probiotic OR probiotic* OR bifidobac* OR lactobac* OR escherichia OR streptococcus OR saccharomyces OR bacillus OR pediococc* OR leuconostoc) **AND** random* |
| **MEDLINE (Expanded)** | ("normal"[All Fields] OR "normalisation"[All Fields] OR "normalisations"[All Fields] OR "normalise"[All Fields] OR "normalised"[All Fields] OR "normalises"[All Fields] OR "normalising"[All Fields] OR "normalization"[All Fields] OR "normalizations"[All Fields] OR "normalize"[All Fields] OR "normalized"[All Fields] OR "normalizer"[All Fields] OR "normalizers"[All Fields] OR "normalizes"[All Fields] OR "normalizing"[All Fields] OR "normally"[All Fields] OR "normals"[All Fields] OR ("drugs, generic"[MeSH Terms] OR ("drugs"[All Fields] AND "generic"[All Fields]) OR "generic drugs"[All Fields] OR "generic"[All Fields] OR "family characteristics"[MeSH Terms] OR ("family"[All Fields] AND "characteristics"[All Fields]) OR "family characteristics"[All Fields] OR "generation"[All Fields] OR "generations"[All Fields] OR "general"[All Fields] OR "general s"[All Fields] OR "generalisability"[All Fields] OR "generalisable"[All Fields] OR "generalisation"[All Fields] OR "generalization, psychological"[MeSH Terms] OR ("generalization"[All Fields] AND "psychological"[All Fields]) OR "psychological generalization"[All Fields] OR "generalization"[All Fields] OR "generalisations"[All Fields] OR "generalise"[All Fields] OR "generalised"[All Fields] OR "generalises"[All Fields] OR "generalisibility"[All Fields] OR "generalising"[All Fields] OR "generalities"[All Fields] OR "generality"[All Fields] OR "generalizability"[All Fields] OR "generalizable"[All Fields] OR "generalizations"[All Fields] OR "generalize"[All Fields] OR "generalized"[All Fields] OR "generalizes"[All Fields] OR "generalizing"[All Fields] OR "generally"[All Fields] OR "generals"[All Fields] OR "generate"[All Fields] OR |

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| | "generated"[All Fields] OR "generates"[All Fields] OR "generating"[All Fields] OR "generation s"[All Fields] OR "generational"[All Fields] OR "generative"[All Fields] OR "generatively"[All Fields] OR "generativity"[All Fields] OR "generator"[All Fields] OR "generator s"[All Fields] OR "generators"[All Fields] OR "generically"[All Fields] OR "genericity"[All Fields] OR "generics"[All Fields]) OR ("healthies"[All Fields] OR "healthy"[All Fields])) AND ("populate"[All Fields] OR "populated"[All Fields] OR "populates"[All Fields] OR "populating"[All Fields] OR "population"[MeSH Terms] OR "population"[All Fields] OR "population groups"[MeSH Terms] OR ("population"[All Fields] AND "groups"[All Fields]) OR "population groups"[All Fields] OR "populations"[All Fields] OR "population s"[All Fields] OR "populational"[All Fields] OR "populous"[All Fields] OR ("participant"[All Fields] OR "participant s"[All Fields] OR "participants"[All Fields] OR "participate"[All Fields] OR "participated"[All Fields] OR "participates"[All Fields] OR "participating"[All Fields] OR "participation"[All Fields] OR "participations"[All Fields] OR "participative"[All Fields] OR "participator"[All Fields] OR "participators"[All Fields]) OR ("participant"[All Fields] OR "participant s"[All Fields] OR "participants"[All Fields] OR "participate"[All Fields] OR "participated"[All Fields] OR "participates"[All Fields] OR "participating"[All Fields] OR "participation"[All Fields] OR "participations"[All Fields] OR "participative"[All Fields] OR "participator"[All Fields] OR "participators"[All Fields]) OR ("volunteer s"[All Fields] OR "volunteered"[All Fields] OR "volunteers"[MeSH Terms] OR "volunteers"[All Fields] OR "volunteer"[All Fields] OR "volunteering"[All Fields]) OR ("volunteer s"[All Fields] OR "volunteered"[All Fields] OR "volunteers"[MeSH Terms] OR "volunteers"[All Fields] OR "volunteer"[All Fields] OR "volunteering"[All Fields]) OR ("subject"[All Fields] OR "subject s"[All Fields] OR "subjects"[All Fields] OR "subjects s"[All Fields]) OR ("subject"[All Fields] OR "subject s"[All Fields] OR "subjects"[All Fields] OR "subjects s"[All Fields]) OR ("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) OR ("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) OR ("adolescences"[All Fields] OR |

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| | "adolescency"[All Fields] OR "adolescent"[MeSH Terms] OR "adolescent"[All Fields] OR "adolescence"[All Fields] OR "adolescents"[All Fields] OR "adolescent s"[All Fields]) OR ("adolescences"[All Fields] OR "adolescency"[All Fields] OR "adolescent"[MeSH Terms] OR "adolescent"[All Fields] OR "adolescence"[All Fields] OR "adolescents"[All Fields] OR "adolescent s"[All Fields]) OR ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields] OR "child s"[All Fields] OR "children s"[All Fields] OR "childrens"[All Fields] OR "childs"[All Fields]) OR ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields] OR "child s"[All Fields] OR "children s"[All Fields] OR "childrens"[All Fields] OR "childs"[All Fields]) OR ("infant"[MeSH Terms] OR "infant"[All Fields] OR "infants"[All Fields] OR "infant s"[All Fields]) OR ("infant"[MeSH Terms] OR "infant"[All Fields] OR "infants"[All Fields] OR "infant s"[All Fields]) OR ("infant, newborn"[MeSH Terms] OR ("infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "newborn"[All Fields] OR "newborns"[All Fields] OR "newborn s"[All Fields]) OR ("infant, newborn"[MeSH Terms] OR ("infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "newborn"[All Fields] OR "newborns"[All Fields] OR "newborn s"[All Fields]) OR ("birth cohort"[MeSH Terms] OR ("birth"[All Fields] AND "cohort"[All Fields]) OR "birth cohort"[All Fields]) OR "pediatric*"[All Fields] OR ("aged"[MeSH Terms] OR "aged"[All Fields] OR "elderly"[All Fields] OR "elderlies"[All Fields] OR "elderly s"[All Fields] OR "elderlys"[All Fields]) OR ("elder s"[All Fields] OR "elders"[All Fields] OR "sambucus"[MeSH Terms] OR "sambucus"[All Fields] OR "elder"[All Fields])) AND ("probiotic s"[All Fields] OR "probiotical"[All Fields] OR "probiotics"[MeSH Terms] OR "probiotics"[All Fields] OR "probiotic"[All Fields] OR "probiotic*"[All Fields] OR "bifidobac*"[All Fields] OR "lactobac*"[All Fields] OR ("escherichia"[MeSH Terms] OR "escherichia"[All Fields] OR "escherichiae"[All Fields]) OR ("streptococcus"[MeSH Terms] OR "streptococcus"[All Fields]) OR ("saccharomyces"[MeSH Terms] OR "saccharomyces"[All Fields] OR "saccharomyce"[All Fields]) OR ("bacillu"[All Fields] OR "bacillus"[MeSH Terms] |

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| | OR "bacillus"[All Fields]) OR "pediococc*"[All Fields] OR ("leuconostoc"[MeSH Terms] OR "leuconostoc"[All Fields] OR "leuconostocs"[All Fields])) AND "random*"[All Fields] |
| **EMBASE (Expanded)** | (normal OR general OR healthy) AND ('population'/exp OR population OR participant OR participants OR 'volunteer'/exp OR volunteer OR 'volunteers'/exp OR volunteers OR subject OR subjects OR 'adult'/exp OR adult OR 'adults'/exp OR adults OR 'adolescent'/exp OR adolescent OR 'adolescents'/exp OR adolescents OR 'child'/exp OR child OR 'children'/exp OR children OR 'infant'/exp OR infant OR 'infants'/exp OR infants OR 'newborn'/exp OR newborn OR newborns OR 'birth cohort'/exp OR 'birth cohort' OR (('birth'/exp OR birth) AND ('cohort'/exp OR cohort)) OR pediatric* OR 'elderly'/exp OR elderly OR elders) AND ('probiotic'/exp OR probiotic OR probiotic* OR bifidobac* OR lactobac* OR 'escherichia'/exp OR escherichia OR 'streptococcus'/exp OR streptococcus OR 'saccharomyces'/exp OR saccharomyces OR 'bacillus'/exp OR bacillus OR pediococc* OR 'leuconostoc'/exp OR leuconostoc) AND random* |
| **Cochrane Register of Controlled Trials (CENTRAL) (Expanded)** | (normal OR general OR healthy) AND (population OR participant OR participants OR volunteer OR volunteers OR subject OR subjects OR adult OR adults OR adolescent OR adolescents OR child OR children OR infant OR infants OR newborn OR newborns OR birth cohort OR pediatric* OR elderly OR elders) AND (probiotic OR probiotic* OR bifidobac* OR lactobac* OR escherichia OR streptococcus OR saccharomyces OR bacillus OR pediococc* OR leuconostoc) AND random* |

**Table S3** Definitions of gut microbiota diversity outcomes reported in the included studies.

| α-diversity indices | Definition | Reference |
|---|---|---|
| Shannon diversity index | The Shannon diversity index shows how diverse the species in a given community are. It rises with the number of species and the evenness of their abundance. The higher the index is, the more diverse the species are in the habitat. If the index equals 0, only one species is present in the community. The index has no upper limit. According to the current data, there are no clearly defined reference values for the "ideal" Shannon diversity of the microbiome. For the definition of low diversity, the cut-off points in the literature range from 2.0 to 4.0. | [1–4] |
| Observed OTUs | An OTU table contains the number of sequences that are observed for each operational taxonomic unit (OTUs) in each sample. An OTU can be defined as a collection of 16S rRNA sequences that have a certain percentage of sequence divergence. Columns usually represent samples, and rows represent genera or species-specific taxonomic units (OTUs). | [4, 5] |
| Chao1 index | Chao1 is a nonparametric method for estimating the number of species in a community. The Chao richness estimator is based on the concept that rare species infer the most information about the number of missing species. | [4, 6] |
| PD whole tree / Faith Phylogenetic diversity | A quantitative measure of phylogenetic diversity, "PD", has been defined as the minimum total length of all the phylogenetic branches required to span a given set of taxa on the phylogenetic tree. | [4, 7] |
| Strong's dominance index | Strong's dominance index measures the maximum departure between the observed proportions and a perfectly even community. | [4, 8] |
| Pielou's evenness | Pielou's evenness is an index that measures diversity along with species richness. While species richness is the number of different species in a given area, evenness is the count of individuals of each species in an area. A calculated value of Pielou's evenness ranges from 0 (no evenness) to 1 (complete evenness). | [4, 9] |
| Sobs index | Sobs is the total number of species observed in a sample, or in a set of samples. | [4, 10] |
| ACE (Abundance-based coverage estimator) of species richness index | The ACE is a nonparametric method for estimating the number of species using sample coverage, which is defined as the sum of the probabilities of the observed species. By the ACE method the groups can be categorized as abundant and rare groups according to the observed frequencies. | [4, 11, 12] |
| Simpson index | Simpson's index is a weighted arithmetic mean of proportional abundance, used to measure the probability that two individuals randomly chosen from a sample belong to the same species. The index reflects both the richness (number of species) and evenness (distribution of individuals among species) within a sample. The value of the index (D) ranges between 0 (infinite diversity) and 1 (no diversity). To | [13–15] |

| | | |
|---|---|---|
| | emphasize diversity, the index is often transformed into Gini-Simpson index, the Simpson's index of Diversity (1-D) which ranges from 0 (maximum homogeneity) to 1 (maximum diversity). | |
| Inverse Simpson index | This is the inverse of Simpson dominance and is often used to measure species diversity. It gives an estimate of the effective number of equally abundant species that would result in the same level of dominance. A higher Simpson reciprocal dominance value signifies higher species diversity or richness, with a more even distribution of individuals among species. | [15, 16] |
| Shannon effective count | The number of equally-common species required to give a particular value of an index is called the "effective number of species". It provides an intuitive interpretation of diversity as the equivalent number of equally abundant species in a community. | [14, 16, 17] |
| **β-diversity indices** | **Definition** | **Reference** |
| Bray-Curtis (dis)similarity index | The Bray–Curtis dissimilarity is bounded between 0 and 1, where 0 means that the two sites have the same composition (i.e., they share all the species), and 1 means that the two sites do not share any species. At sites where BC is intermediate (e.g., BC = 0.5), this index differs from other commonly used indices. | [4, 18] |
| Euclidean distance | When two samples are compared, Euclidean distance puts more weight on differences in species abundances than on difference in species presences. As a result, two samples not sharing any species could appear more similar (with lower Euclidean distance) than two samples which share species, but the species largely differ in their abundances | [4, 19] |
| (un)weighted UniFrac distance | Both weighted (quantitative) and unweighted (qualitative) variants of UniFrac are widely used in microbial ecology, where the former accounts for the abundance of observed organisms, while the latter only considers their presence or absence. The distance is calculated between pairs of samples (each sample represents an organismal community). All taxa found in one or both samples are placed on a phylogenetic tree. A branch leading to taxa from both samples is marked as "shared" and branches leading to taxa which appear only in one sample are marked as "unshared". There is a weighted version of the UniFrac metric, which accounts for the relative abundance of each of the taxa within the communities. This is commonly used in metagenomic studies, where the number of metagenomic reads can be in the tens of thousands, and it is appropriate to 'bin' these reads into operational taxonomic units, or OTUs, which can then be dealt with as taxa within the UniFrac framework. | [4, 20] |
| Generalized UniFrac distance | The generalized UniFrac distances unifies weighted UniFrac and unweighted UniFrac distances. It covers a series of distances ranging from weighted to unweighted UniFrac by adjusting the weight on the branches. This approach is designed to offer a robust and versatile tool for detecting a broader spectrum of biologically significant changes in microbiome composition. | [21] |

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|---|---|---|
| Jensen-Shannon divergence | The Jensen-Shannon divergence is an asymmetric measure that quantifies the relative entropy or informational difference between two distributions. It provides a way to evaluate the distance between two data distributions, highlighting how distinct they are from one another. | [22] |
| Horn-Morisita distance metrics | The Horn-Morisita index evaluates the probability that individuals drawn from two separate vectors belong to different species, relative to drawing from each vector independently. It is applicable to both transformed counts and proportions. | [23] |
| Spearman correlation distance | The Spearman distance is based on the Spearman rank correlation coefficient, which evaluates the monotonic relationship between two variables. The Spearman distance is calculated as one minus the absolute value of the Spearman correlation coefficient, offering a measure of dissimilarity between ranked data. It is particularly useful when the data exhibit a monotonic association rather than a linear one. | |

Abbreviations: *OTU:* operational taxonomic unit; *PD*: phylogenetic diversity; *ACE:* abundance-based coverage estimator; *UniFrac*: unique fraction metric

α-diversity indices reflect the diversity of a single sample, measuring species richness (number of species) and/or distribution (evenness of species). Each alpha diversity index is calculated differently, depending on factors like how the presence or absence of certain rare species is assessed and interpreted. In contrast, β-diversity indices can be used to compare different samples and communities. It can consider both the overall abundance per sample and the abundance of each taxon [24]. In simple terms, α-diversity represents a within-sample diversity, while β-diversity describes similarity or dissimilarity between samples [25–27].

**Supplementary Methods S1** Detailed statistical description of the meta-analysis

As we assumed considerable between-study heterogeneity in all cases, a random-effects model was used to pool effect sizes in a frequentist framework.

In most studies, the quartiles of diversity index values could be extracted from box plots. Therefore, the effect size was expressed as the difference in medians (MedD) between groups (probiotic-treated minus control) instead of the usual mean difference. Based on the available data and our experience, we assume that the two are not significantly different. From the study MedD, we estimated the mean of median differences. Since only RCT studies were included, we used post-treatment data to estimate the difference between the treated and control groups.

Based on the available data (the symmetry of the lower and upper quartiles around the median, the similarity between the reported mean and median, and one study providing individual-level data), as well as our experience, it is a reasonable assumption that the distribution of diversity indices follows a normal distribution. Under this assumption, we estimated the differences in medians and their standard errors for each study using the quantile estimation method of the metamedian package [28], based on the available data (quartiles, as well as mean and standard deviation values). For the Lopez-Garcia study [29], individual-level data could be extracted from the plot, and the standard error was estimated using a bootstrap method.

We used the inverse variance weighting method for pooling MedDs. To estimate the heterogeneity variance measure $\tau 2$, the restricted maximum-likelihood estimator with the Q profile method for confidence interval, Veroniki et al. [30] was applied.

We used a Hartung-Knapp adjustment [31, 32] for CIs (if it is more conservative than the classical one, as recommended by Jackson et al.[33] as a hybrid method) and for prediction intervals. In case of subgroup (e.g., categorical moderator, as intervention type and categorized intervention time) analysis, we used a fixed-effects "plural" model (aka. mixed-effects model). We assumed that all subgroups share a different $\tau$. Although for practical reasons, if at least one

of the subgroups contains fewer than 6 studies, we use an assumption of the same τ across subgroups (recommended in Harrer et al. [34]).

In the case of meta-regression (e.g., continuous moderator, as time of intervention) analysis, a linear relation was assumed. A weighted least square method was used. The confidence interval and prediction interval estimate for the slope was based on the t-distribution. A Wald-type p-value for the slope and the meta-regression coefficient of determination ($R^2$*) correlation coefficient was also given.

Forest plots were used to graphically summarize the results. For meta-regression, bubble plots were used. The square or dot sizes on the plots refer to the weight in the random effects meta-analysis. The t-distribution-based method is used for the CI of MedD calculation in individual studies.

## Supplementary Results

### Figs. S1-3 Analyses of the Shannon diversity index – Sensitivity analysis, subgroups by risk of bias and meta-regression

As a sensitivity analysis, we performed a separate calculation with more restricted inclusion criteria without studies performed with cross-over design [35, 36] providing change data only [37] or with not no clear number of participants [38] (Fig. S1) (MedD = –0.08 [–0.20 to 0.04]).

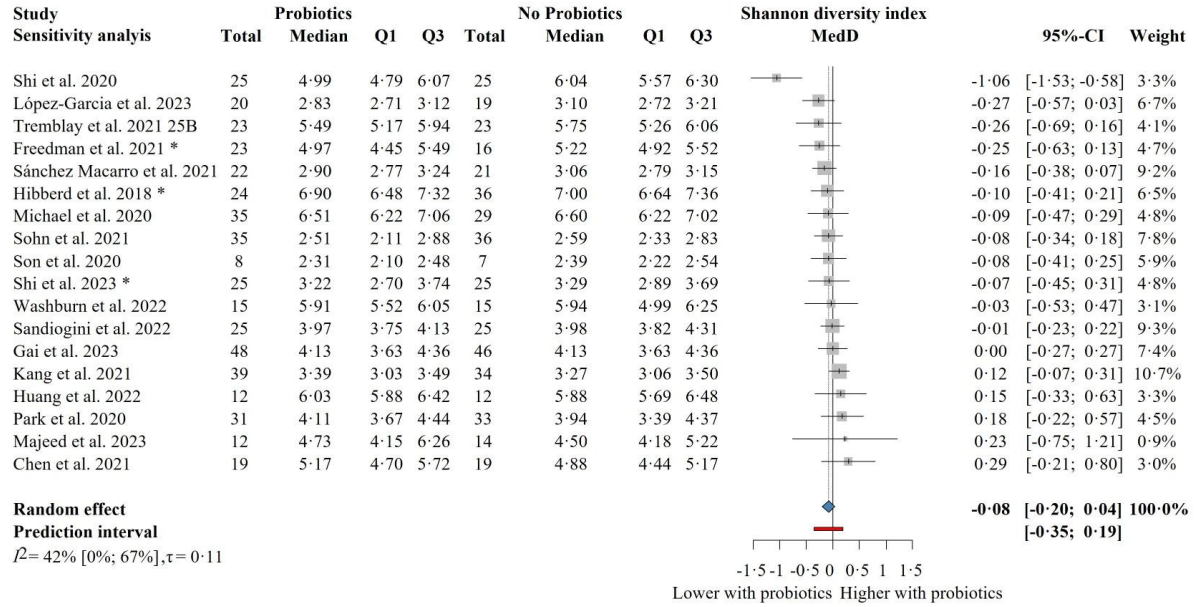



**Fig. S1** Additional sensitivity analysis for the more restricted analysis of Shannon diversity index.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

Furthermore, we analysed our data as subgroups based on the results of risk of bias assessment (high, some concerns, or low risk of bias). These results did not reveal any significant or clinically relevant difference between the intervention and control groups either. High risk of bias: MedD = –0.18 [–0.60 to 0.24]; Some concerns: MedD = –0.09 [–0.20 to 0.02]; Low risk of bias: MedD = –0.01 [–0.14 to 0.11] (Fig. S2).

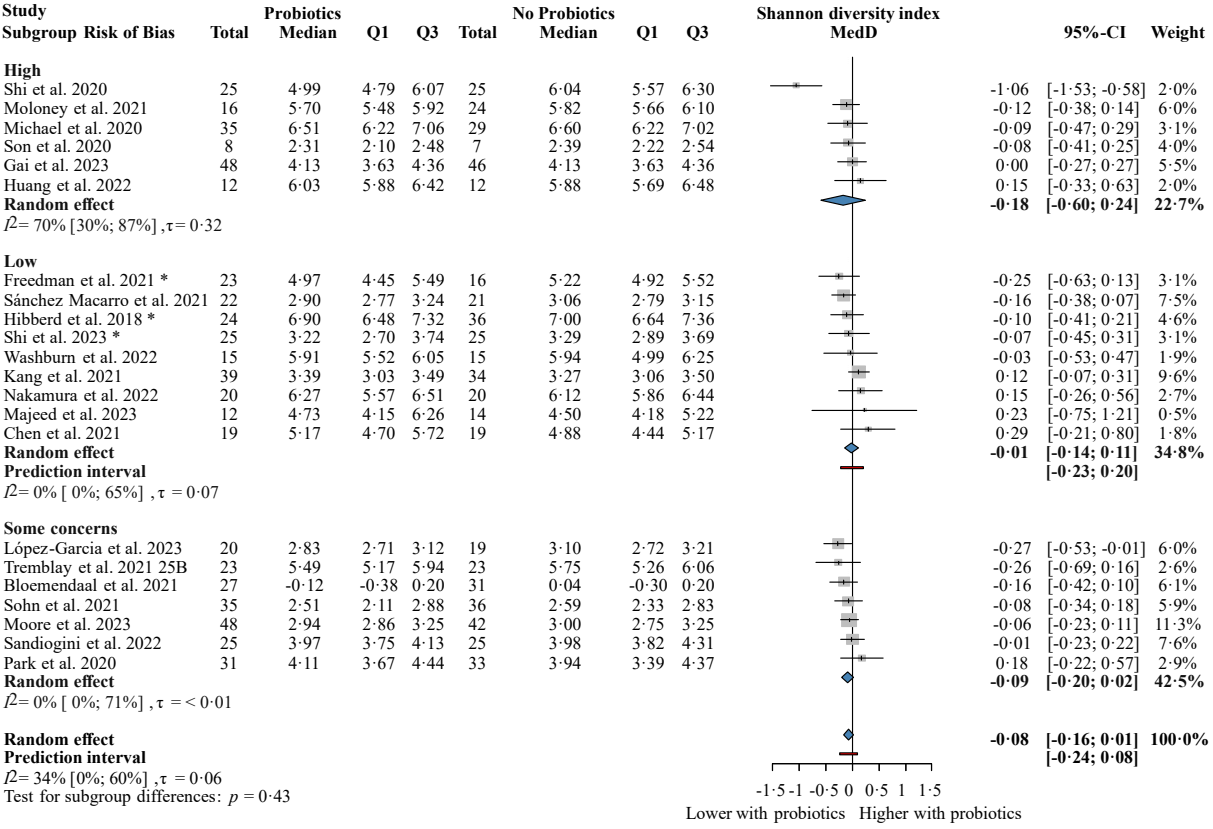



**Fig. S2** Additional sensitivity analysis for the subgroups based on risk of bias assessment for Shannon diversity index.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

Using the mixed-effects meta-regression model, we tested whether the duration of probiotic intervention significantly affected gut microbiota diversity compared to the control group. Assuming a linear relation, the estimated slope is 0, 95% CI [–0.01 to 0.02] [MedD value / weeks] (p-value: 0.5961). The $R^{2}$* value is 0%. The results indicated no significant association between intervention time and diversity outcomes, suggesting that the length of probiotic use did not meaningfully influence the observed effects (Fig. S3).

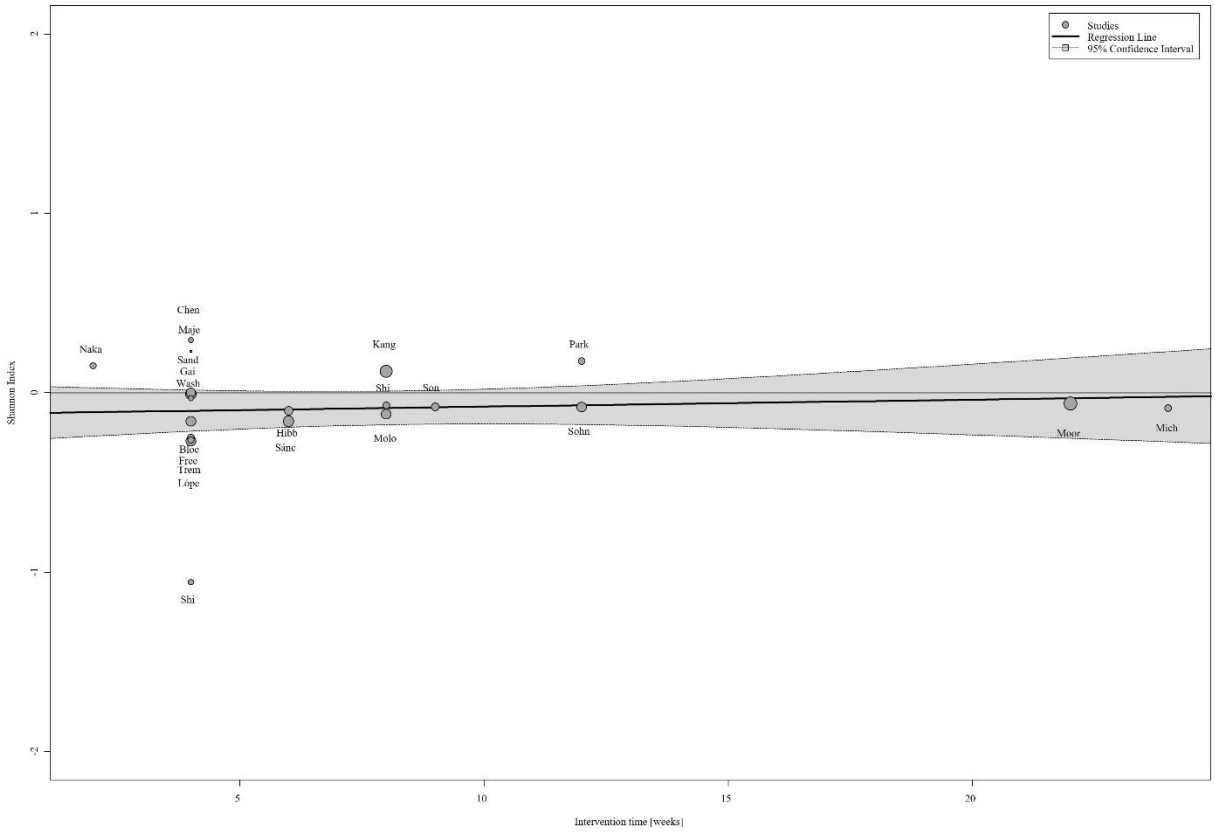



**Fig. S3** Meta-regression analysis investigating the relationship between intervention duration and the Shannon diversity index.

**Figs. S4-6 Analyses of the Observed OTUs diversity index – Sensitivity analysis, subgroups by risk of bias and meta-regression**

We did not identify significant difference when removing studies with not clear data on the number of participants [38], cross-over design [35] and change results [37] (Fig. S4).

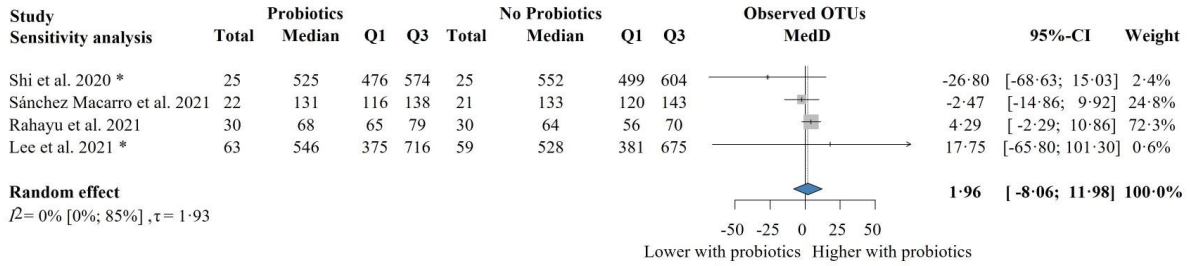



**Fig. S4** Additional sensitivity analysis for the more restricted analysis of Observed OTUs.

Abbreviations: OTU: Operational Taxonomic Unit; CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

Subgroup analysis based on the risk of bias assessment led to similar insignificant findings (Fig. S5).

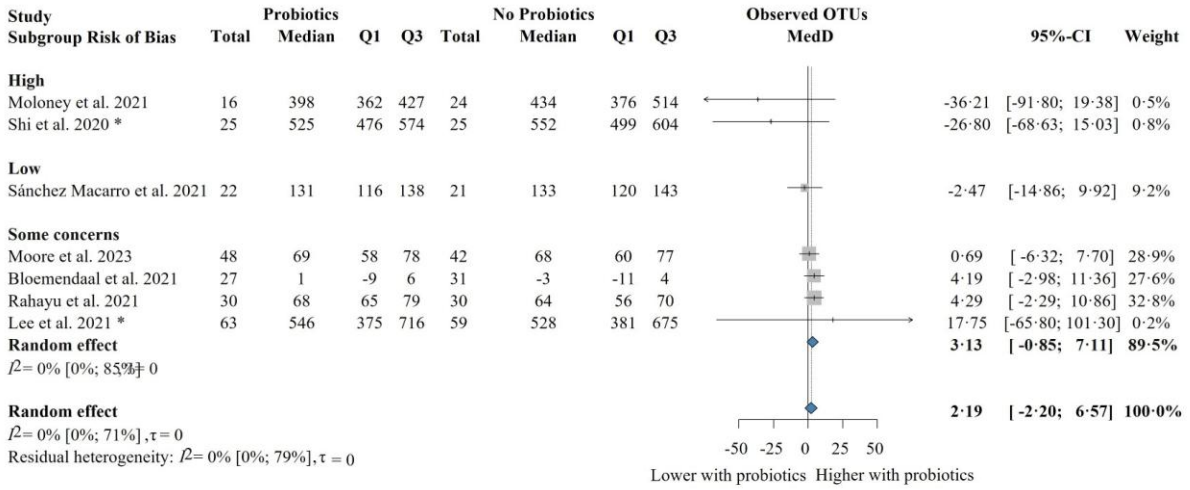



**Fig. S5** Additional sensitivity analysis for the subgroups based on risk of bias assessment for Observed OTUs.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

Using the mixed-effects meta-regression model, we tested whether the duration of probiotic intervention significantly affected gut microbiota diversity compared to the control group. Assuming a linear relation, the estimated slope is –0.06, 95% CI [–0.77 to 0.65] [MedD value / weeks] (p-value: 0.8367). The $R^{2*}$ value is 0%. The results indicated no significant association between intervention time and diversity outcomes, suggesting that the length of probiotic use did not meaningfully influence the observed effects (Fig. S6).

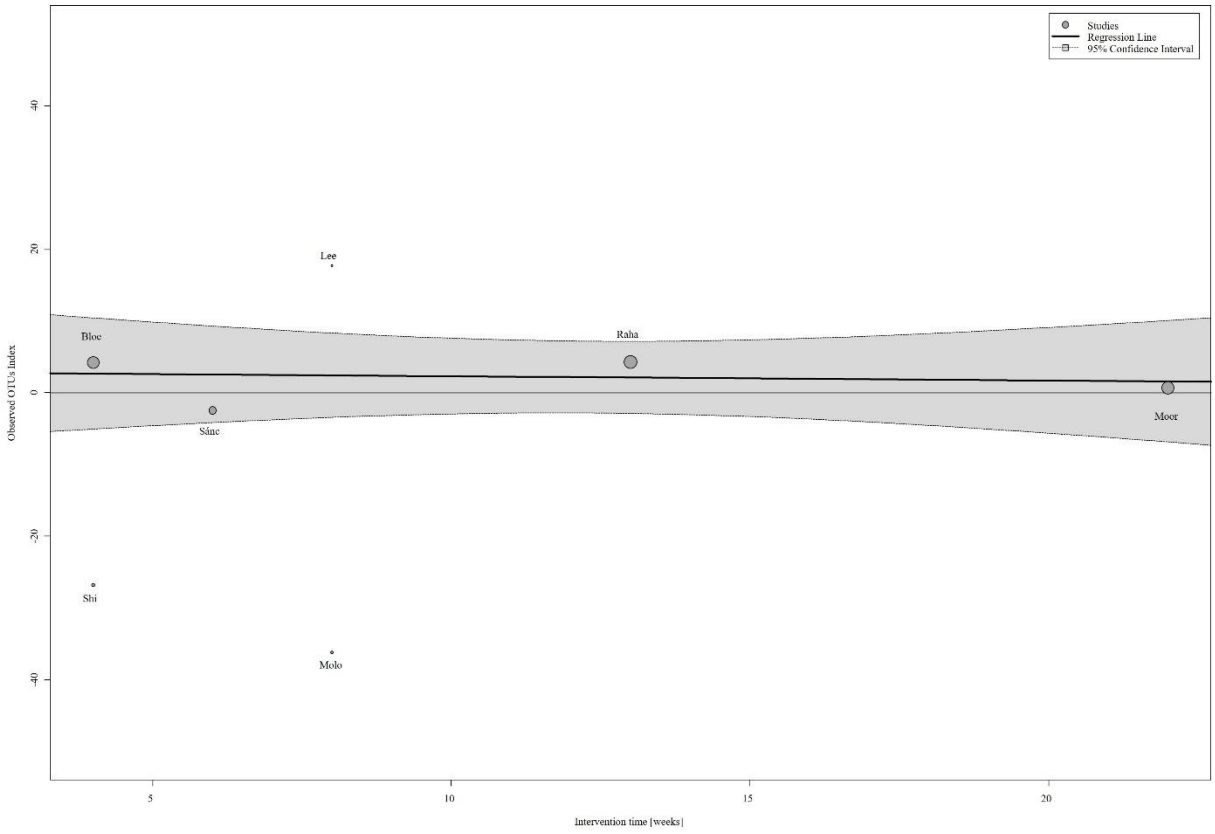



**Fig. S6** Meta-regression analysis investigating the relationship between intervention duration and the number of Observed OTUs.

**Figs. S7-9 Analyses of the Chao1 index – Sensitivity analysis, subgroups by risk of bias and meta-regression**

The more restricted sensitivity analysis removing studies with cross-over design [35, 39] revealed no significant or relevant difference between groups either (Fig. S7) (MedD = 1.02 [–25.00 to 27.03]).

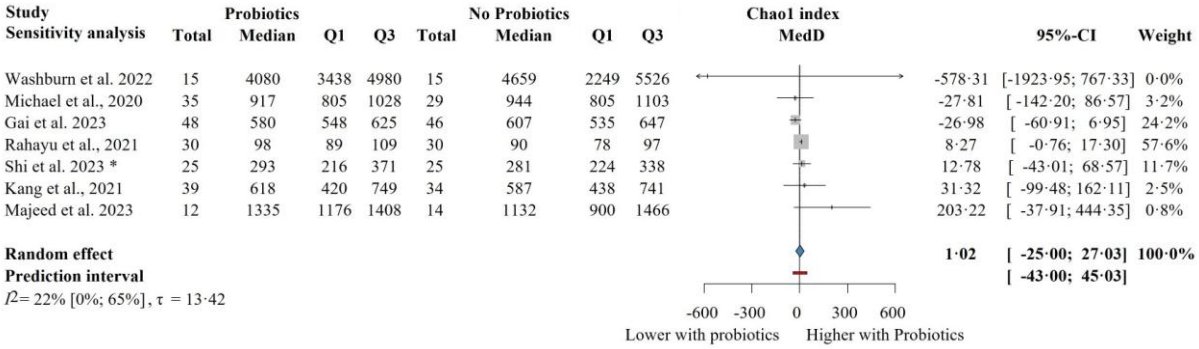



**Fig. S7** Additional sensitivity analysis for the more restricted analysis Chao1 index.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

The results of the additional subgroup analyses based on the risk of bias assessment are shown in Fig. S8, with no significant differences between the groups.

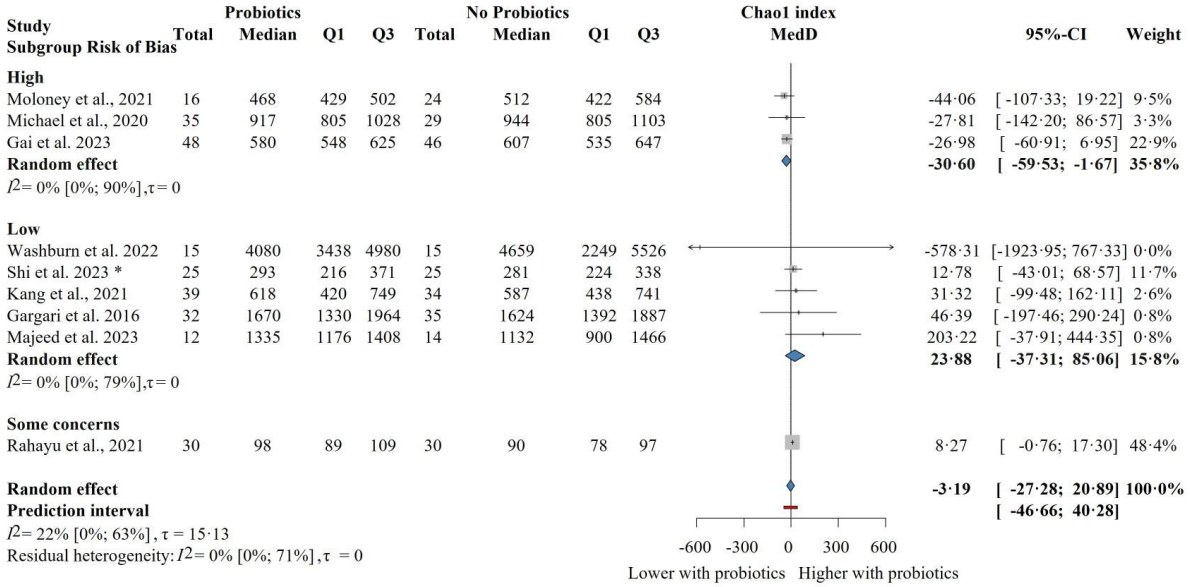



**Fig. S8** Additional sensitivity analysis for the subgroups based on the risk of bias assessment Chao1 index.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

Using the mixed-effects meta-regression model, we tested whether the duration of probiotic intervention significantly affected gut microbiota diversity compared to the control group. Assuming a linear relation, the estimated slope 1.75, 95% CI [–4.01 to 7.51] [MD value / weeks] (p-value: 0.4957). The $R^{2*}$ value is 41.3%. The results indicated no significant association between intervention time and diversity outcomes, suggesting that the length of probiotic use did not meaningfully influence the observed effects (Fig. S9).

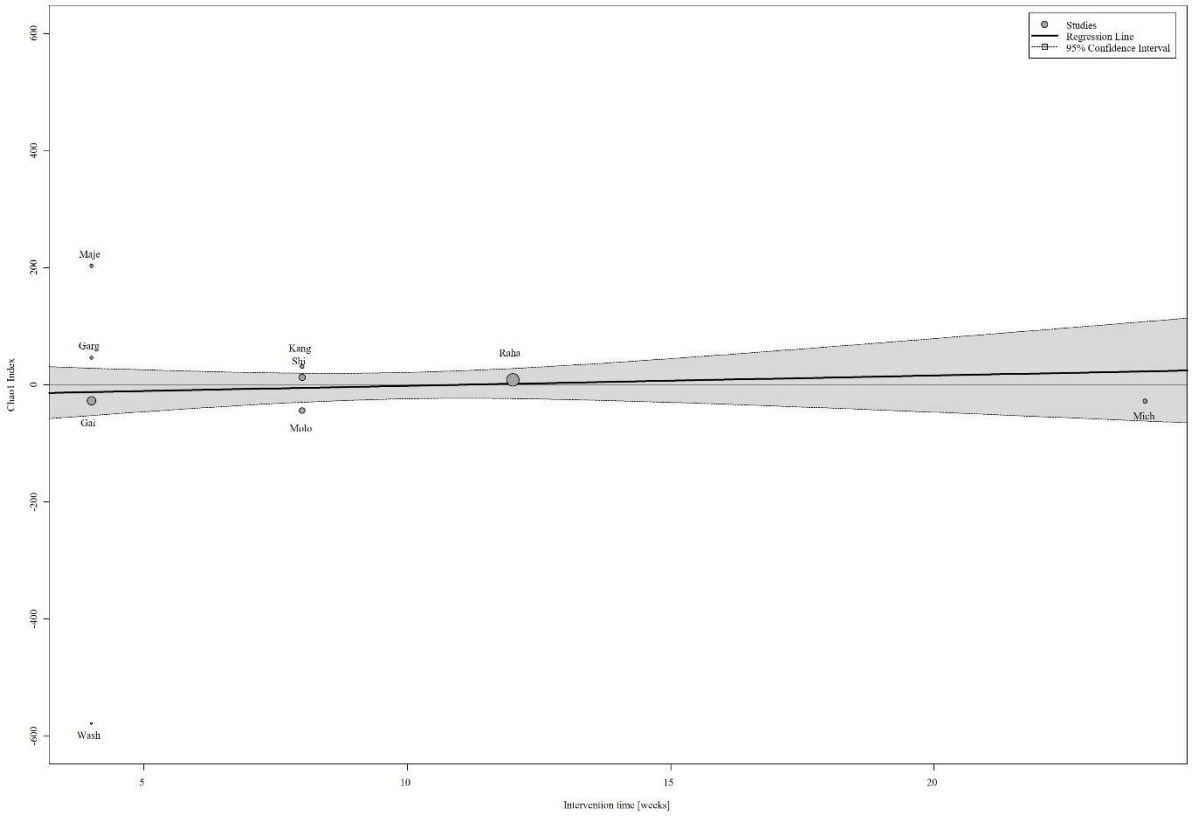



**Fig. S9** Meta-regression analysis investigating the relationship between intervention duration and the Chao1 index.

**Figs. S10-12 Analyses of the Simpson's Index of Diversity - Sensitivity analysis, subgroups by risk of bias and meta-regression**

In the sensitivity analysis we removed the study with cross-over design [35] and the one with no clear number of participants [38], but we did not reveal any effect of probiotics (Fig. S10).

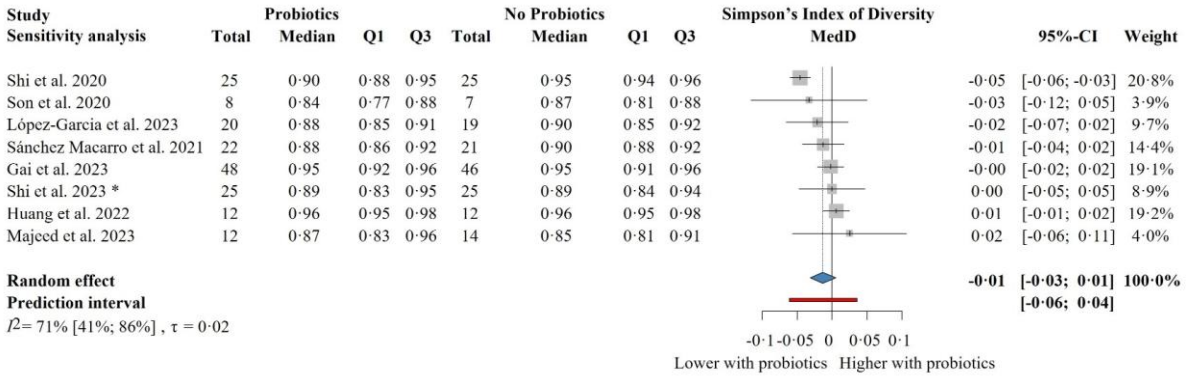



**Fig. S10** Additional sensitivity analysis for the more restricted analysis of Simpson's Index of Diversity.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

The subgroup analyses based on the risk of bias assessment did not reveal significant differences between probiotic and control groups (Fig. S11).

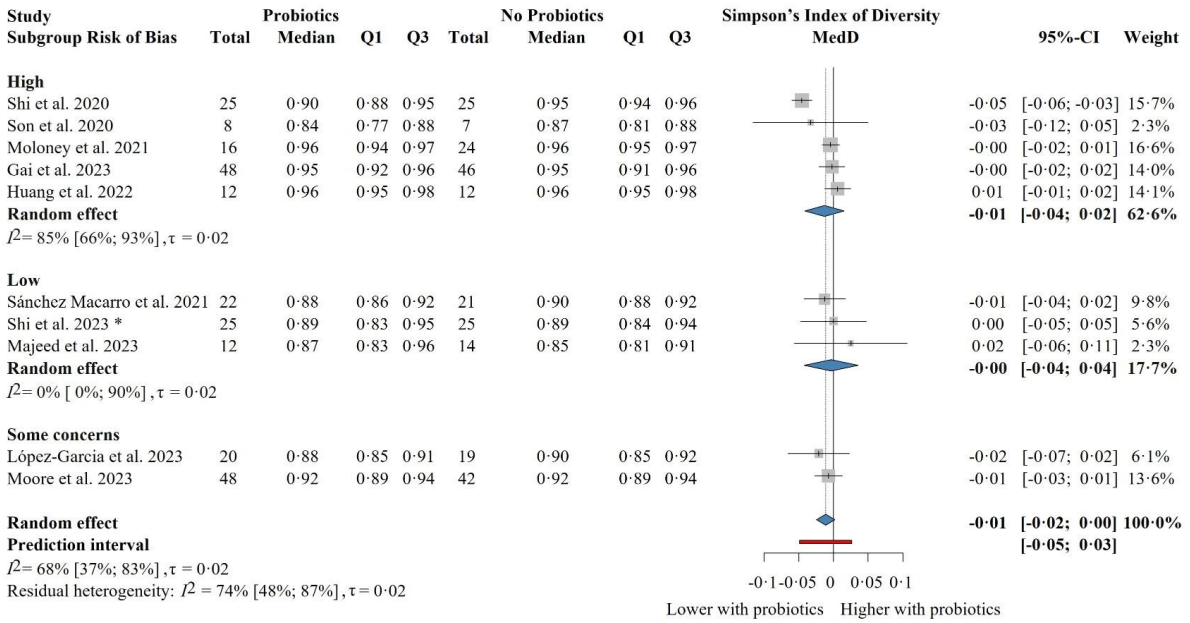



**Fig. S11** Additional sensitivity analysis for the subgroups based on risk of bias assessment of Simpson's Index of Diversity.

Abbreviations: CI: confidence interval; MedD: mean of median differences. Q1: first quartile; Q3: third quartile.

The "*" indicates that the median and q1, q3 are estimated from mean and standard deviation in that study.

Using the mixed-effects meta-regression model, we tested whether the duration of probiotic intervention significantly affected gut microbiota diversity compared to the control group. Assuming a linear relation, the estimated slope is 0, 95% CI [0 to 0] [MedD value / weeks] (p-value: 0.513). The $R^{2*}$ value is 0%. The results indicated no significant association between intervention time and diversity outcomes, suggesting that the length of probiotic use did not meaningfully influence the observed effects (Fig. S12).

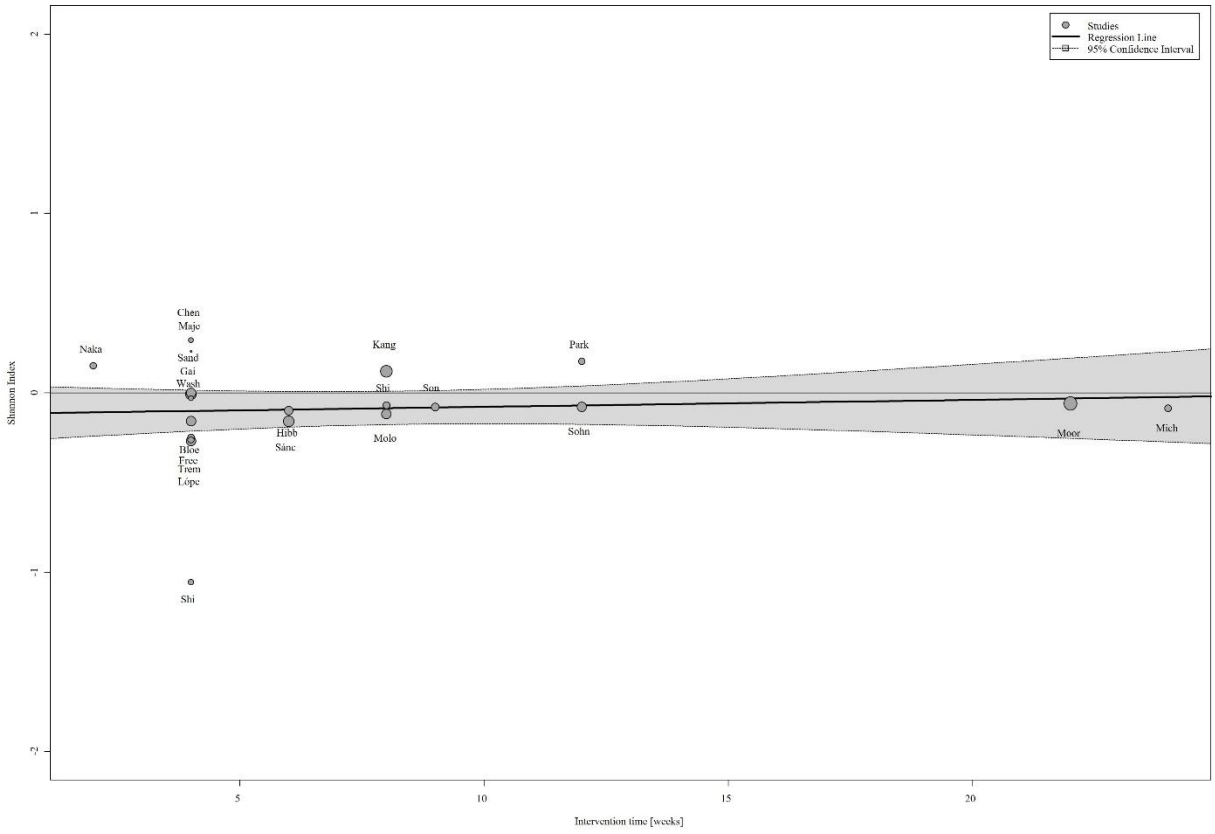



**Fig. S12** Meta-regression analysis investigating the relationship between intervention duration and the Simpson's Index of Diversity

**Figs. S13-20 Publication bias and leave-out analyses**

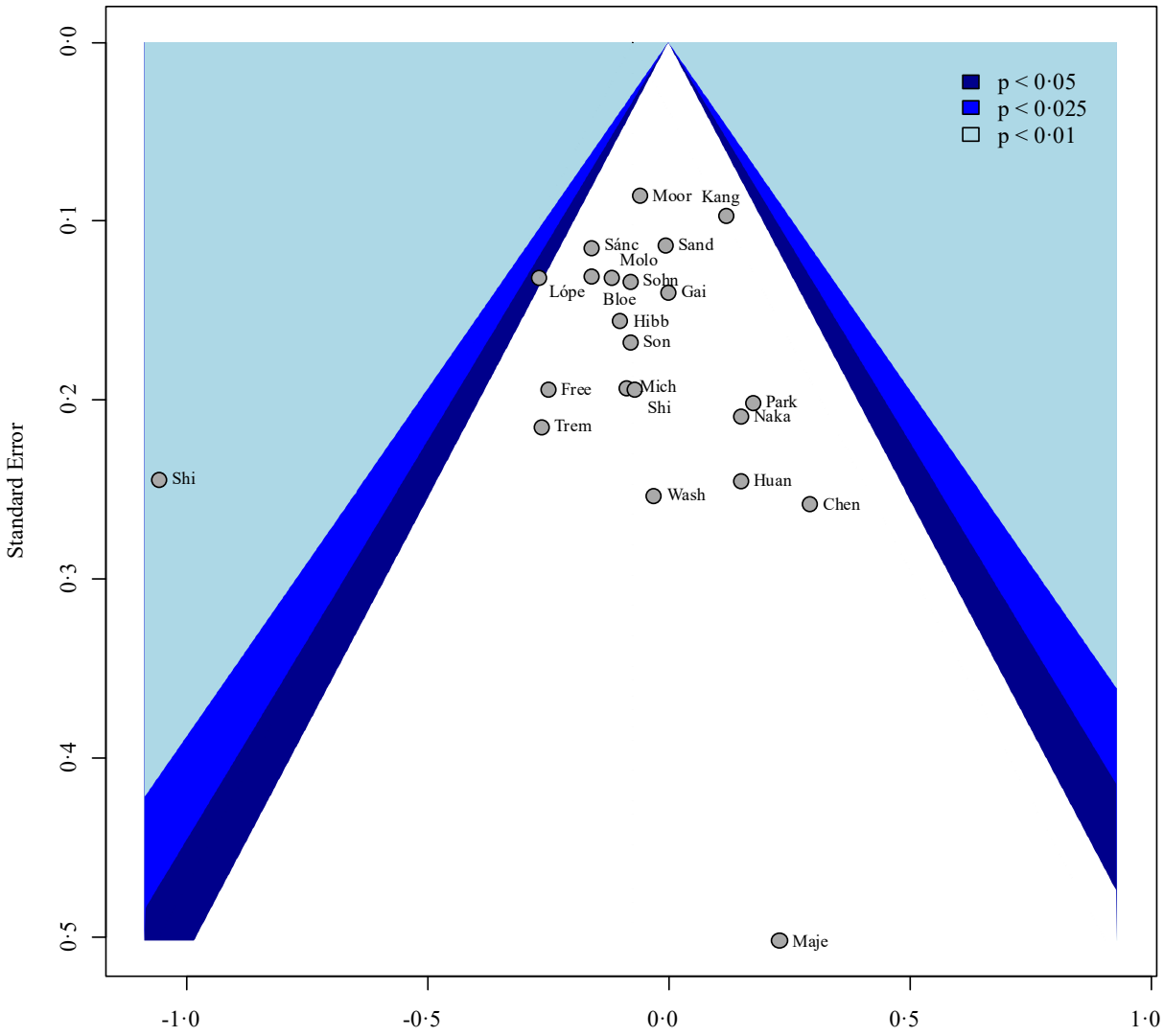



**Fig. S13** Funnel plot to assess publication bias for Shannon diversity index.

The points on the funnel plots for assessing publication bias represent the different studies. It shows the residuals on the x-axis against their corresponding standard errors. Here we could assess the small study bias: at the bottom of the funnel if the studies are distributed not symmetrically and out of the funnel, it indicates potential publication bias. Egger's test p-value is 0.6887.

In the leave-one-out analysis, the Kang et al. 2021 [40] study can be considered a statistically influential study. However, the analysis indicates that it does not clinically relevantly affect the point estimate of the effect size or its confidence interval (Fig. S14).

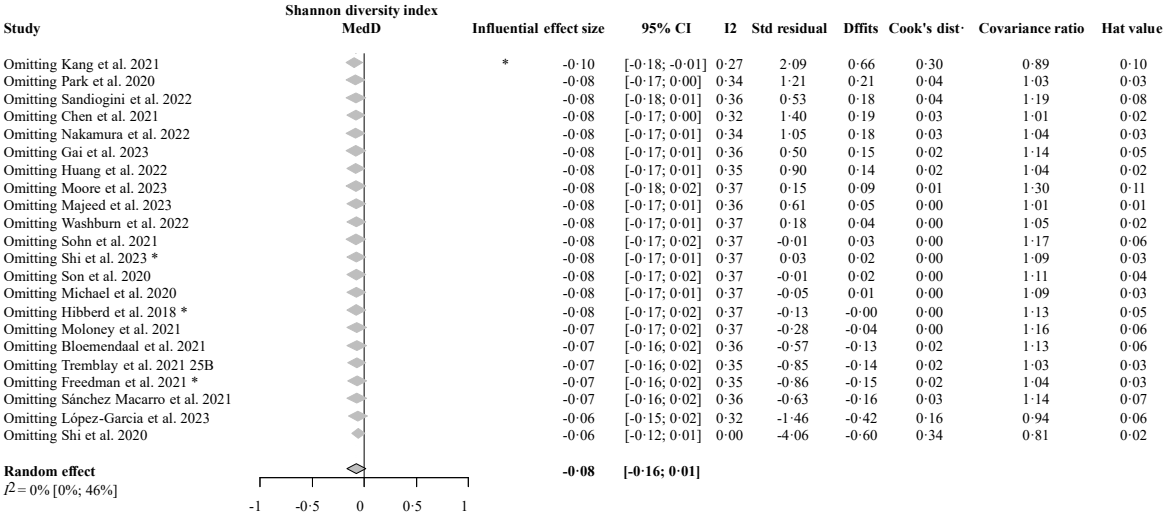



**Fig. S14** Additional leave-one-out analysis for Shannon diversity index.

Cases which are considered as possible influential with respect to any of the shown measures are marked with "*" at column "*Influential*". Note that the chosen cut-offs are (somewhat) arbitrary based on *dmetar* package. "*Effect size*": the pooled effect size without the given study. "*95% CI*": the 95% confidence interval of the pooled effect size without the given study. "$I^2$": the Higgins&Thomson $I^2$ heterogeneity value without the given study. "*Std residual*": the studentized residuals. It shows the deleted residual divided by its estimated standard deviation. "*Dffits*": the difference in fits. It quantifies the number of standard deviations that the fitted value changes without the given study. (Typical threshold is $3* \sqrt{(p/(k-p))}$, where *p* is the number of model coefficients and *k* is the number of cases). "*Cook's dist.*": Cook's distance. It depends on both the residual and leverage of the omitted study. (Typical threshold value is 2). "*Covariance ratio*": the covariance ratio. It shows the change in the determinant of the covariance matrix of the effect size. (Typical threshold value is 1). "*Hat value*": the value of the hat matrix without the given study. (Typical threshold is $3*p/k$)

Abbreviations: MedD: mean of median differences; CI: confidence interval.

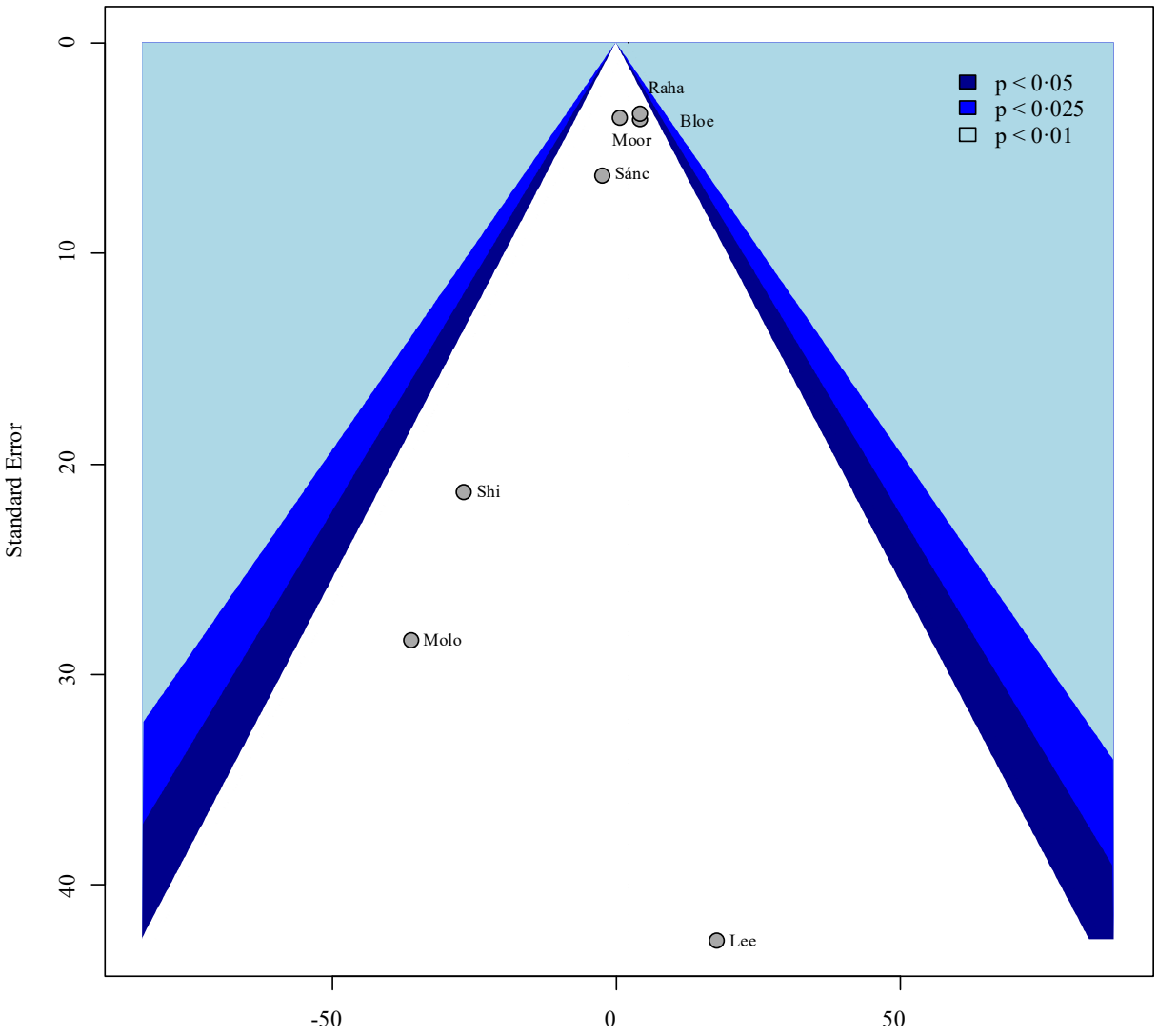



**Fig. S15** Funnel plot to assess publication bias for the Observed Operational Taxonomic Units (OTUs).

The points on the funnel plots for assessing publication bias represent the different studies. It shows the residuals on the x-axis against their corresponding standard errors. Here we could assess the small study bias: at the bottom of the funnel if the studies are distributed not symmetrically and out of the funnel, it indicates potential publication bias. Egger's test p-value is 0.1093.

In the leave-one-out analysis, the Shi et al. 2023 [41], Moloney et al. 2021 [35], and Gai et al. 2023 [42] studies can be considered a statistically influential study. However, the analysis indicates that they do not clinically relevantly affect the point estimate of the effect size or its confidence interval (Fig. S16).

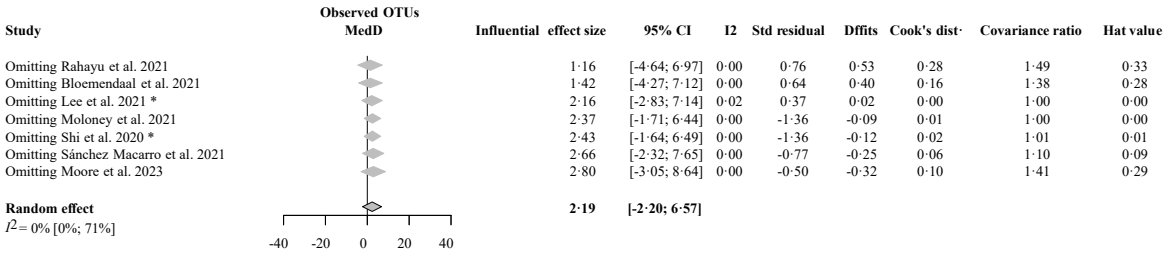



**Fig. S16** Additional leave-one-out analysis for the Observed Operational Taxonomic Units (OTUs).

Cases which are considered as possible influential with respect to any of the shown measures are marked with "*" at column "*Influential*". Note that the chosen cut-offs are (somewhat) arbitrary based on *dmetar* package. "*Effect size*": the pooled effect size without the given study. "*95% CI*": the 95% confidence interval of the pooled effect size without the given study. "$I^2$": the Higgins&Thomson $I^2$ heterogeneity value without the given study. "*Std residual*": the studentized residuals. It shows the deleted residual divided by its estimated standard deviation. "*Dffits*": the difference in fits. It quantifies the number of standard deviations that the fitted value changes without the given study. (Typical threshold is $3* \sqrt{(p/(k-p))}$, where $p$ is the number of model coefficients and $k$ is the number of cases). "*Cook's dist.*": Cook's distance. It depends on both the residual and leverage of the omitted study. (Typical threshold value is 2). "*Covariance ratio*": the covariance ratio. It shows the change in the determinant of the covariance matrix of the effect size. (Typical threshold value is 1). "*Hat value*": the value of the hat matrix without the given study. (Typical threshold is $3*p/k$)

Abbreviations: MedD: mean of median differences; CI: confidence interval.

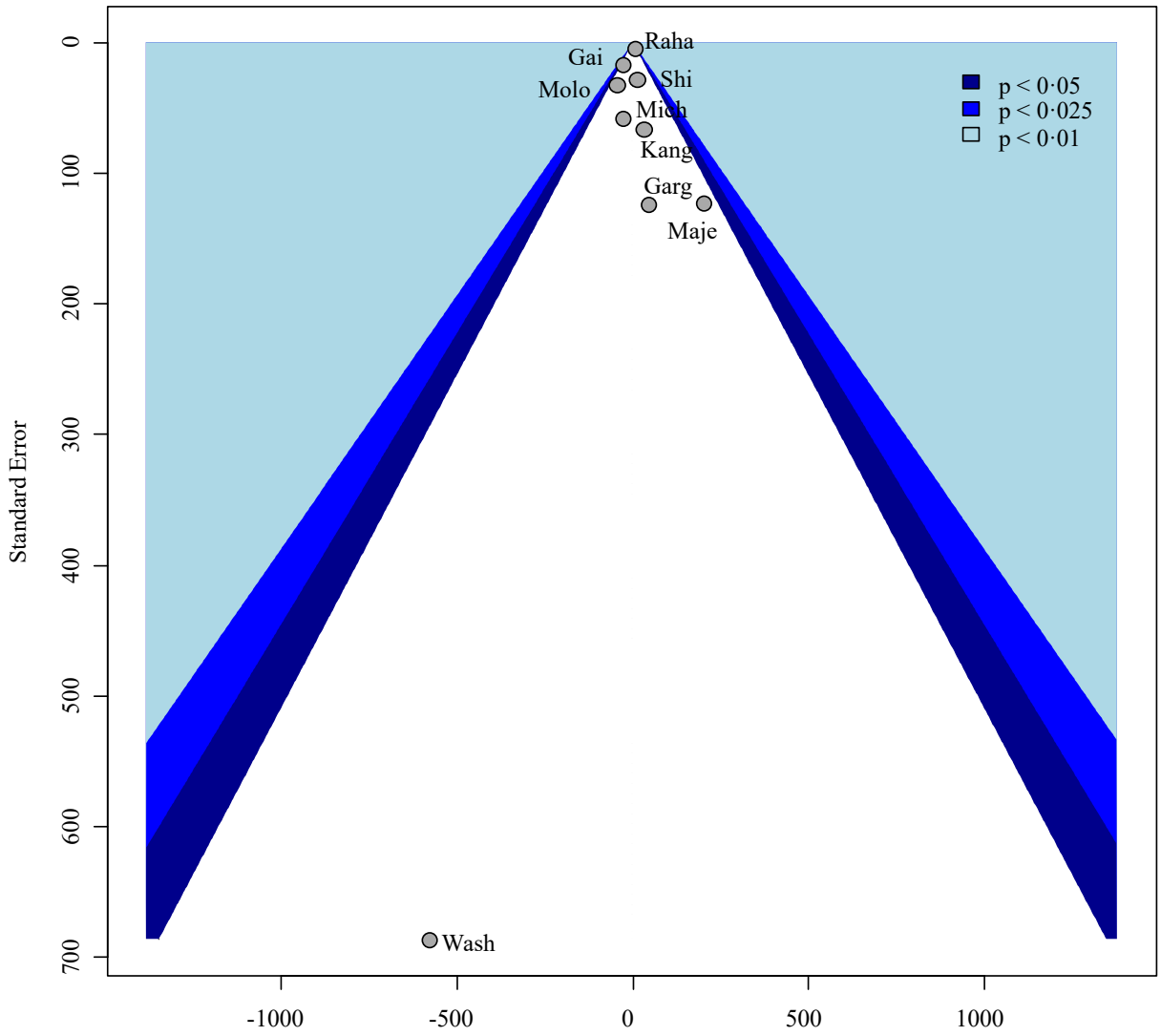



**Fig. S17** Funnel plot to assess publication bias for Chao1 index.

The points on the funnel plots for assessing publication bias represent the different studies. It shows the residuals on the x-axis against their corresponding standard errors. Here we could assess the small study bias: at the bottom of the funnel if the studies are distributed not symmetrically and out of the funnel, it indicates potential publication bias. Egger's test p-value is 0.5996.

In the leave-one-out analysis, the Shi et al. 2023 [41], Moloney et al. 2021 [35], and Gai et al. 2023 [42] studies can be considered a statistically influential study. However, the analysis indicates that they do not clinically relevantly affect the point estimate of the effect size or its confidence interval (Fig. S18).

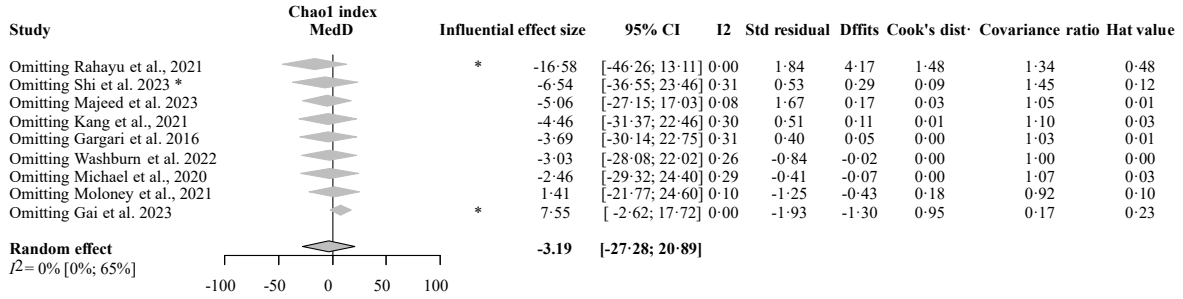



**Fig. S18** Additional leave-one-out analysis for Chao1 index.

Cases which are considered as possible influential with respect to any of the shown measures are marked with "*" at column "*Influential*". Note that the chosen cut-offs are (somewhat) arbitrary based on *dmetar* package. "*Effect size*": the pooled effect size without the given study. "*95% CI*": the 95% confidence interval of the pooled effect size without the given study. "$I^2$": the Higgins&Thomson $I^2$ heterogeneity value without the given study. "*Std residual*": the studentized residuals. It shows the deleted residual divided by its estimated standard deviation. "*Dffits*": the difference in fits. It quantifies the number of standard deviations that the fitted value changes without the given study. (Typical threshold is $3 * \sqrt{(p/(k-p))}$, where $p$ is the number of model coefficients and $k$ is the number of cases). "*Cook's dist.*": Cook's distance. It depends on both the residual and leverage of the omitted study. (Typical threshold value is 2). "*Covariance ratio*": the covariance ratio. It shows the change in the determinant of the covariance matrix of the effect size. (Typical threshold value is 1). "*Hat value*": the value of the hat matrix without the given study. (Typical threshold is $3*p/k$)

Abbreviations: MedD: mean of median differences; CI: confidence interval.

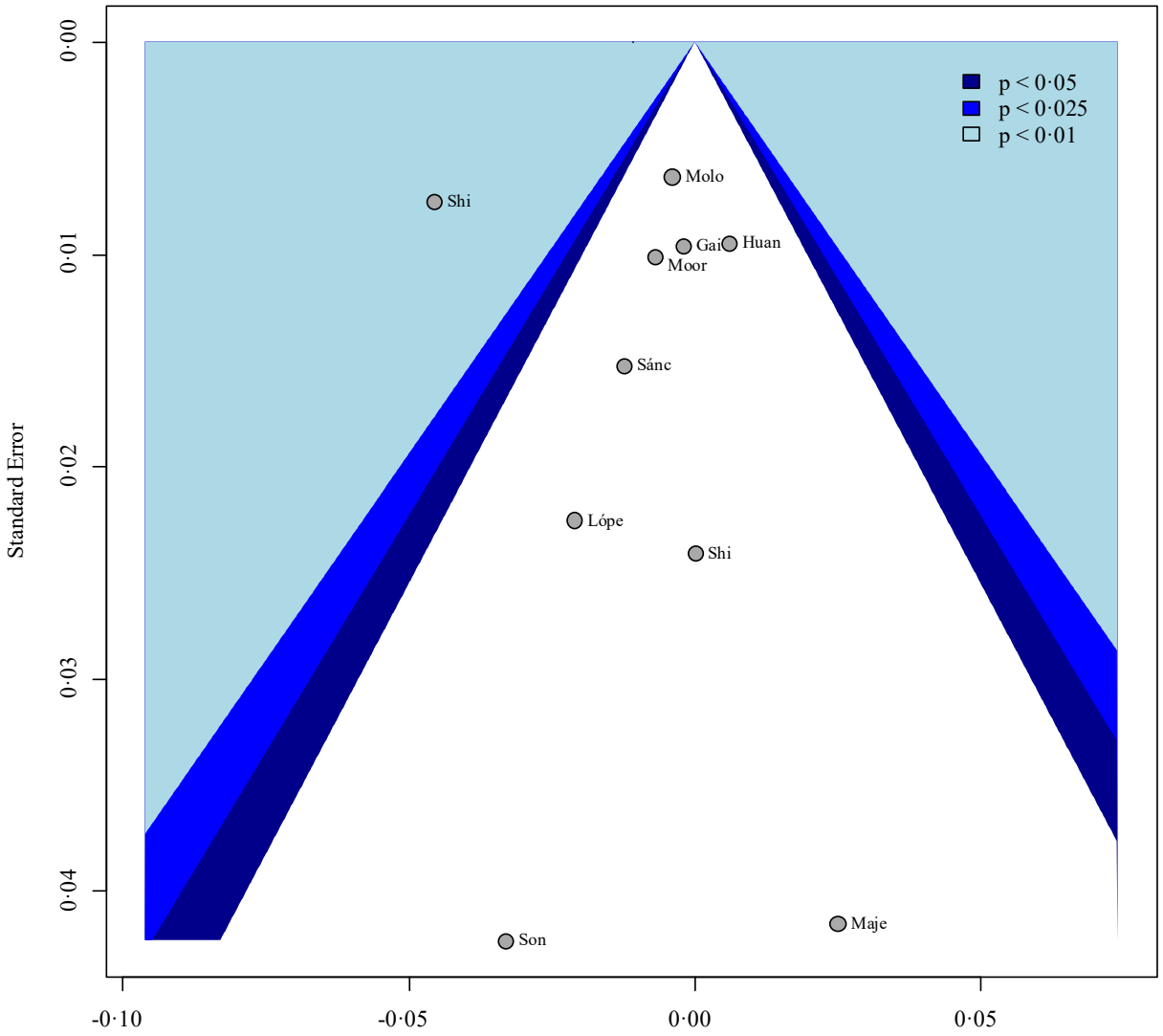



**Fig. S19** Funnel plot to assess publication bias for Simpson's Index of Diversity.

The points on the funnel plots for assessing publication bias represent the different studies. It shows the residuals on the x-axis against their corresponding standard errors. Here we could assess the small study bias: at the bottom of the funnel if the studies are distributed not symmetrically and out of the funnel, it indicates potential publication bias. Egger's test p-value is 0.7448.

In the leave-one-out analysis the Shi et al. 2023 [43] study can be considered a statistically influential study based on the $I^2$ value and the covariance ratio. However, the analysis indicates that it does not clinically relevantly affect the point estimate of the effect size or its confidence interval (Fig. S20).

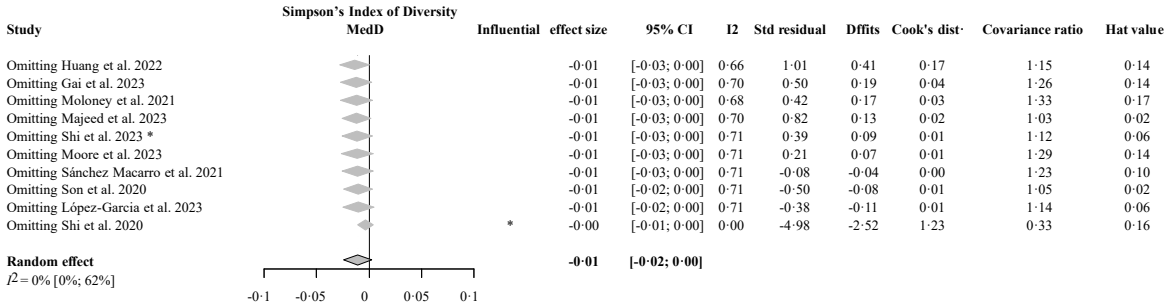


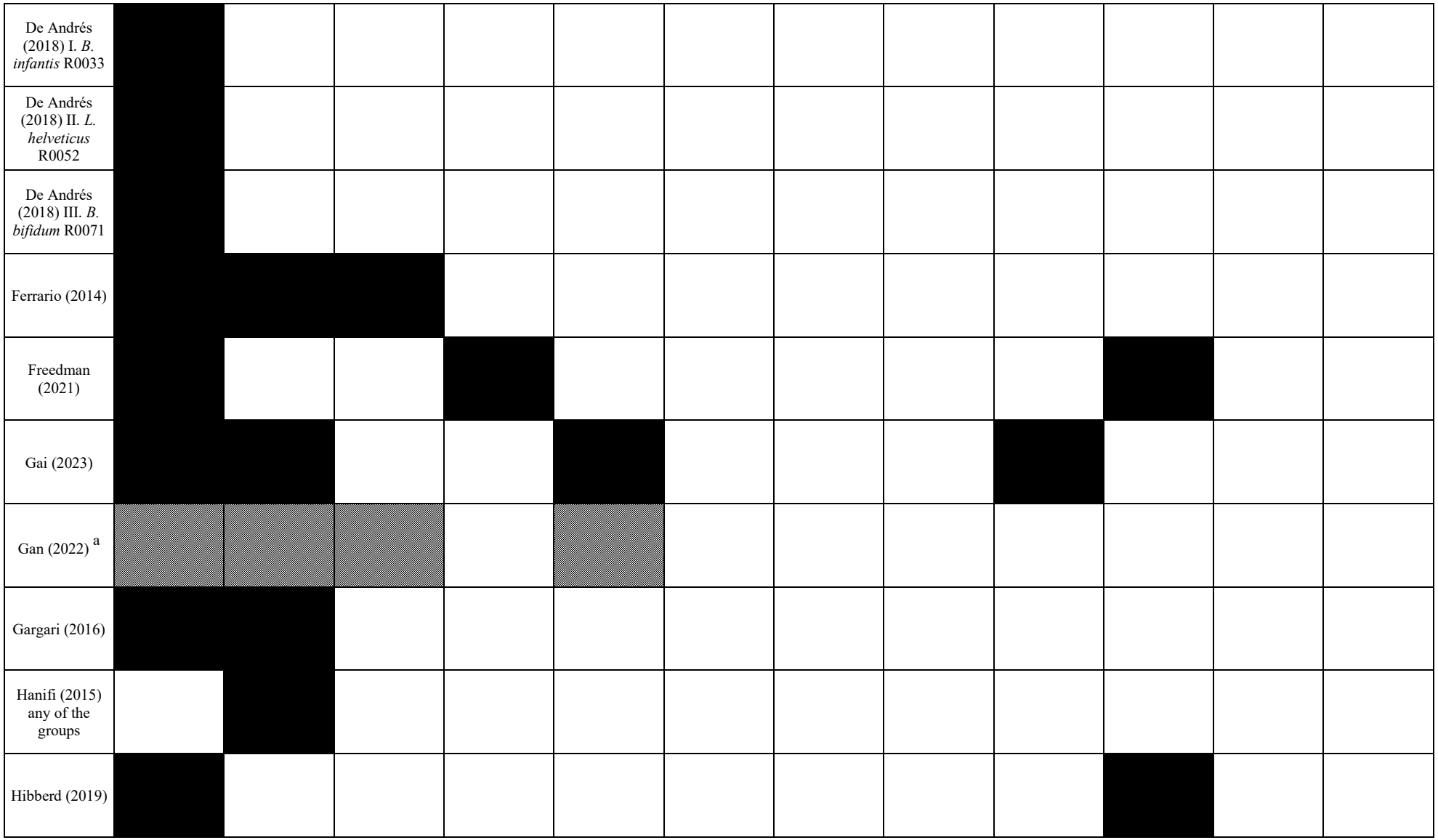

**Fig. S20** Additional leave-one-out analysis Simpson's Index of Diversity.

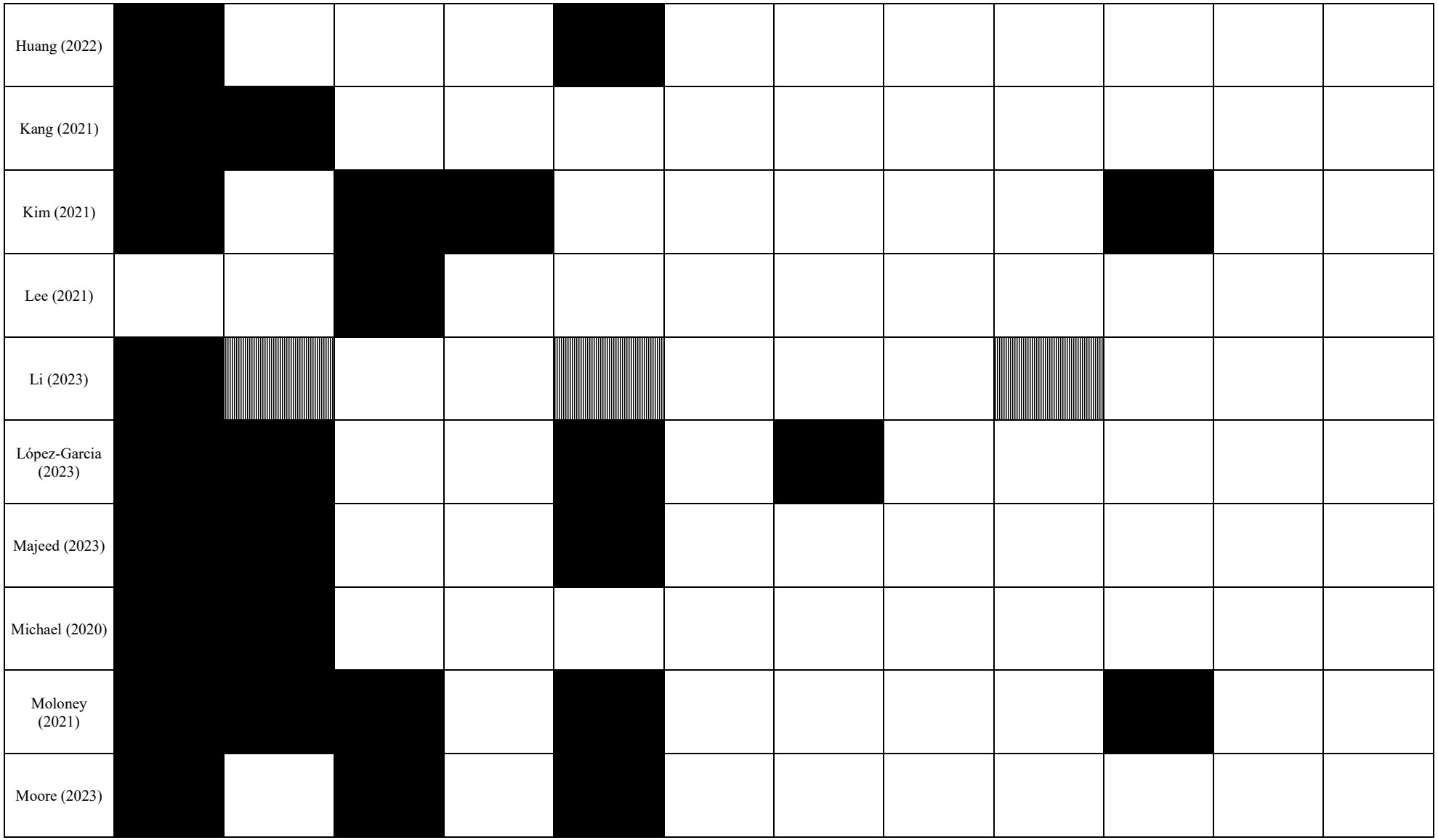
Cases which are considered as possible influential with respect to any of the shown measures are marked with "*" at column "*Influential*". Note that the chosen cut-offs are (somewhat) arbitrary based on *dmetar* package. "*Effect size*": the pooled effect size without the given study. "*95% CI*": the 95% confidence interval of the pooled effect size without the given study. "$I^2$": the Higgins&Thomson $I^2$ heterogeneity value without the given study. "*Std residual*": the studentized residuals. It shows the deleted residual divided by its estimated standard deviation. "*Dffits*": the difference in fits. It quantifies the number of standard deviations that the fitted value changes without the given study. (Typical threshold is $3* \sqrt{(p/(k-p))}$, where $p$ is the number of model coefficients and $k$ is the number of cases). "*Cook's dist.*": Cook's distance. It depends on both the residual and leverage of the omitted study. (Typical threshold value is 2). "*Covariance ratio*": the covariance ratio. It shows the change in the determinant of the covariance matrix of the effect size. (Typical threshold value is 1). "*Hat value*": the value of the hat matrix without the given study. (Typical threshold is $3*p/k$)

Abbreviations: MedD: mean of median differences; CI: confidence interval.

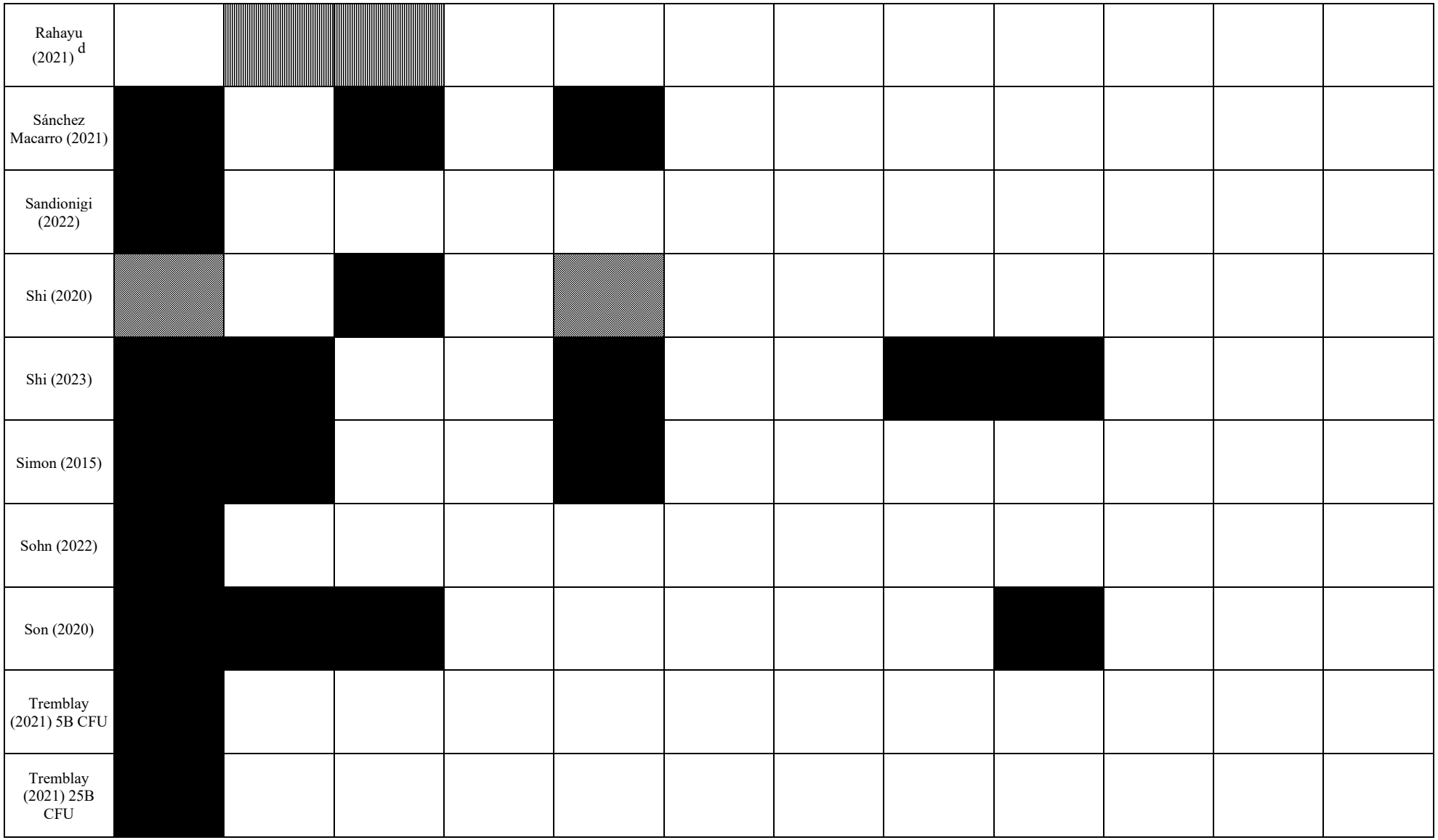
**Table S4** Changes in the microbiome α-diversity indices as measured after the intervention period.

| Study | Shannon diversity index | Chao1 index | Observed OTUs / Richness | Pielou's evenness | Simpson's Index of Diversity | Inverse Simpson index | Strong's dominance index | Sobs index | ACE index | Faiths Phylogenetic Diversity | Shannon effective count | Not specified |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Axelrod (2019) | ■ | ■ | | | ■ | | | | ■ | | | |
| Bagga (2018) | | | | | | ■ | | | | | | |
| Bazanella (2017) | | | ■ | | | | | | | | ■ | |
| Bloemendaal (2021) | ■ | | ■ | | | | | | | ■ | | |
| Boesmans (2018) | | ■ | | ■ | | | | | | | | |
| Castanet (2020) | | | | | | | | | | | | ■ |
| Chen (2021) | ■ | | | | | | | | | | | |
| Chen (2023) | ■ | | | | | | | | | | | |


De Andrés (2018) I. *B. infantis* R0033
De Andrés (2018) II. *L. helveticus* R0052
De Andrés (2018) III. *B. bifidum* R0071
Ferrario (2014)
Freedman (2021)
Gai (2023)
Gan (2022) [a]
Gargari (2016)
Hanifi (2015) any of the groups
Hibberd (2019)


Huang (2022)
Kang (2021)
Kim (2021)
Lee (2021)
Li (2023)
López-Garcia (2023)
Majeed (2023)
Michael (2020)
Moloney (2021)
Moore (2023)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nakamura (2022) | | | | | | | | | | | | |
| Pagliai (2023) [b] | | | | | | | | | | | | |
| Park (2020) | | | | | | | | | | | | |
| Paytuvi-Gallart (2020) | | | | | | | | | | | | |
| Plaza-Diaz (2015) *L. rhamnosus* CNCM I-4036 [c] | | | | | | | | | | | | |
| Plaza-Diaz (2015) *L. paracasei* CNCM I-4034 [c] | | | | | | | | | | | | |
| Plaza-Diaz (2015) *B. breve* CNCM I-4035 [c] | | | | | | | | | | | | |
| Plaza-Diaz (2015) *B. breve* CNCM I-4035 and *L. rhamnosus* CNCM I-4036 [c] | | | | | | | | | | | | |
| Qian (2020) | | | | | | | | | | | | |


Rahayu (2021) d
Sánchez Macarro (2021)
Sandionigi (2022)
Shi (2020)
Shi (2023)
Simon (2015)
Sohn (2022)
Son (2020)
Tremblay (2021) 5B CFU
Tremblay (2021) 25B CFU

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wahburn (2022) | | | | | | | | | | | | |
| Wischmeyer (2024) | | | | | | | | | | | | |

| | |
|---|---|
| | no significant difference in α-diversity between groups |
| | favors probiotics (higher α-diversity in the intervention group compared to baseline and/or to placebo) |
| | favors placebo (higher α-diversity in the control group compared to baseline and/or to probiotics) |
| | not applicable* |

Definitions for each outcome are detailed in Table S3.

[a] Gan et al. reported an increase in alpha diversity in the stools of the placebo group; however, did not specify which index was exactly affected.

[b] Instead of Observed OTUs, Observed Amplicon Sequence Variants were reported.

[c] No information about the placebo group.

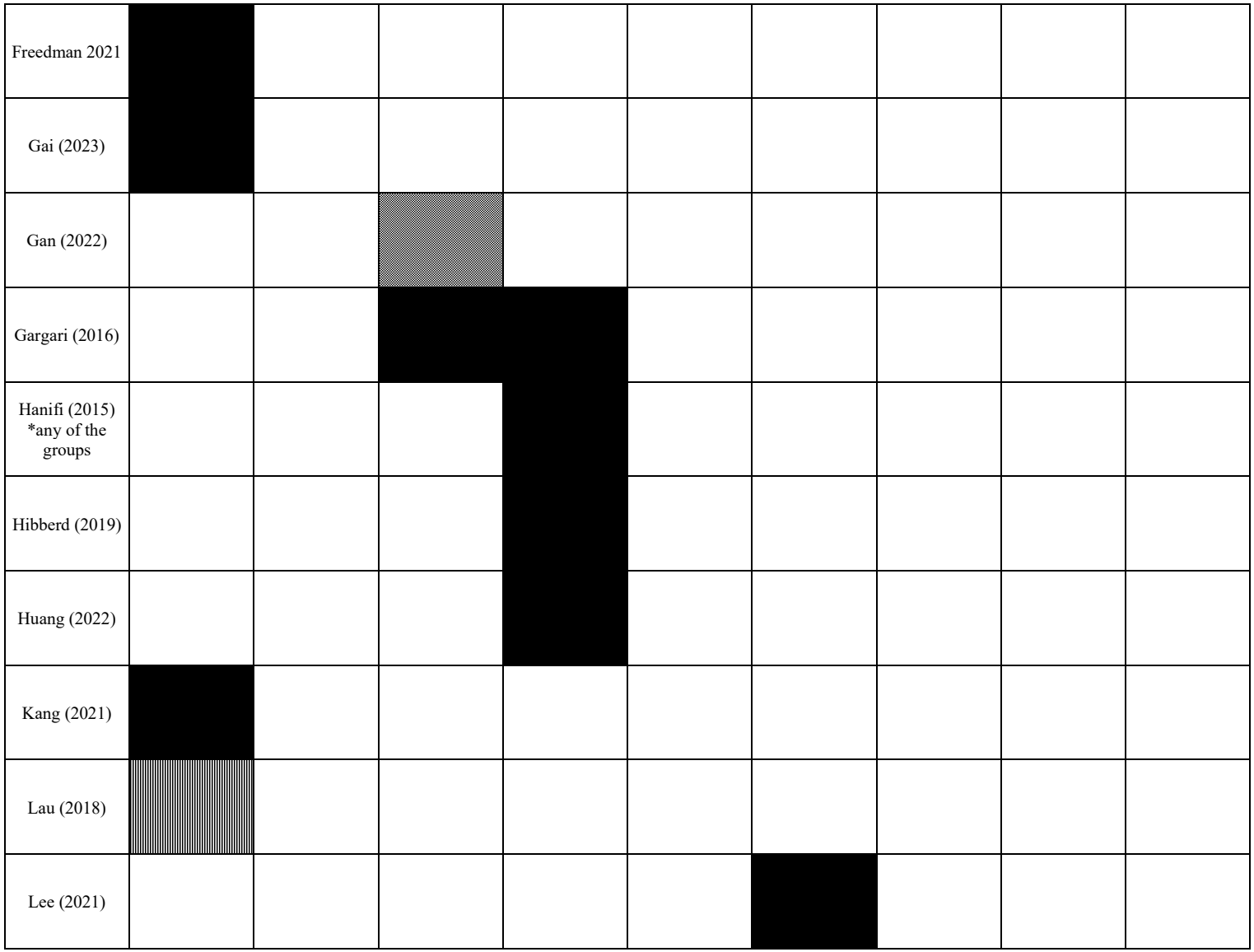
[d] Rahayu et al. reported significant increases in the probiotic group; however, a comparison with the placebo group was not performed. According to our comparative meta-analysis the mean of median differences were not significantly different in the two groups after the intervention period.

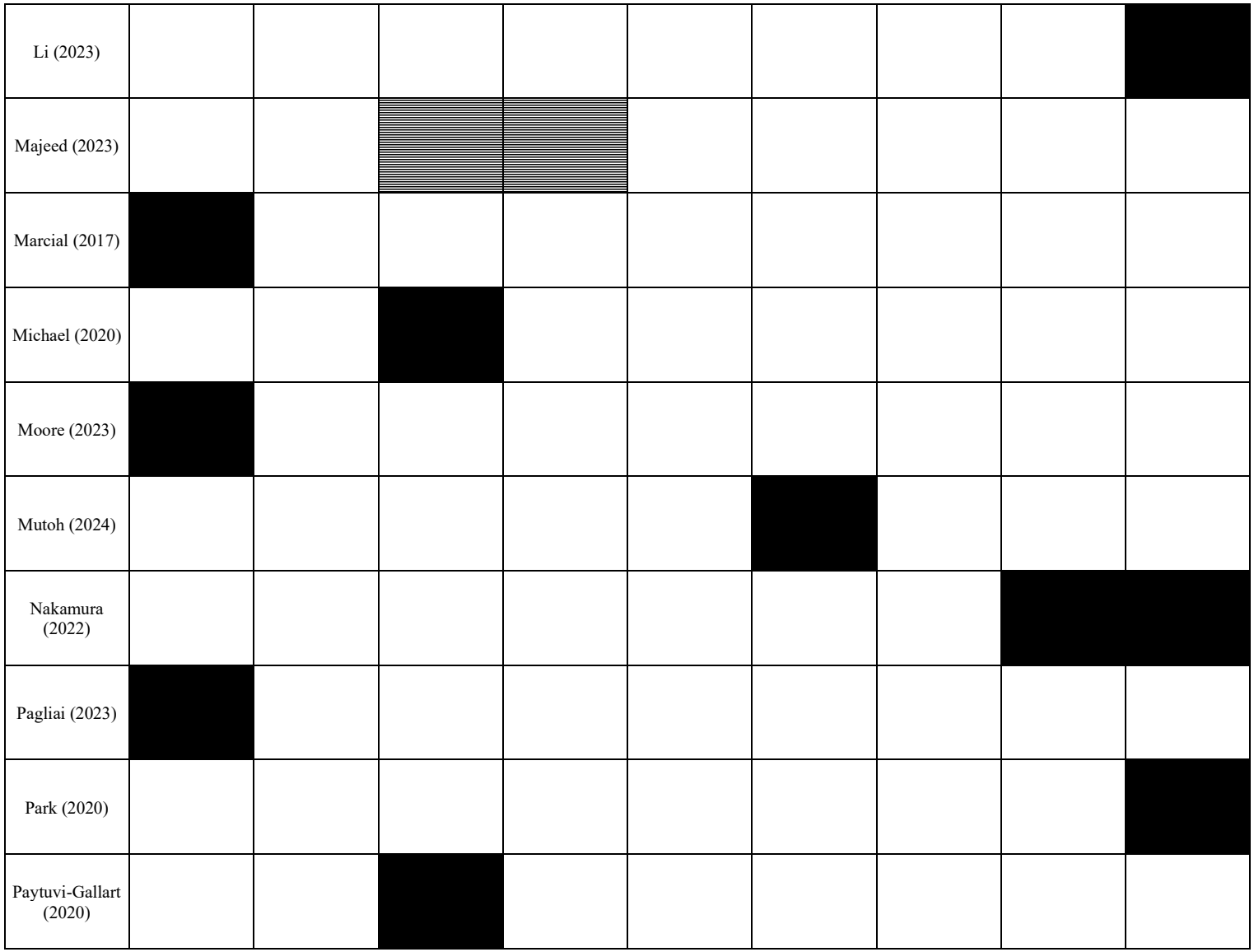
*If a study did not investigate a specific outcome, “not applicable” is indicated.

Abbreviations: OTU: operational taxonomic unit; ACE: Abundance-based coverage estimator

**Table S5** Changes in the microbiome β-diversity indices as measured after the intervention period.

| Study | Bray-Curtis (dis)similarity index | Euclidean distance | Weighted UniFrac distance | Unweighted UniFrac distance | Generalized UniFrac distance | Jensen-Shannon divergence | Morisita-Horn distance metrics. | Spearman correlation distance | Not clearly specified |
|---|---|---|---|---|---|---|---|---|---|
| Bagga (2018) | ■ | | | | | | | | |
| Bazanella (2017) | | | | | ■ | | | | |
| Bloemendaal (2021) | | | ■ | | | | | | |
| Boesmans (2018) | ■ | | | | | | | | |
| Castanet (2020) | | | | | | | | | ■ |
| Chen (2021) | ■ | | | | | | | | |
| Chen (2023) | ■ | | | | | | | | |
| Ferrario (2014) | | | ▒ | | | | | | |


Freedman 2021
Gai (2023)
Gan (2022)
Gargari (2016)
Hanifi (2015) *any of the groups
Hibberd (2019)
Huang (2022)
Kang (2021)
Lau (2018)
Lee (2021)


Li (2023)
Majeed (2023)
Marcial (2017)
Michael (2020)
Moore (2023)
Mutoh (2024)
Nakamura (2022)
Pagliai (2023)
Park (2020)
Paytuvi-Gallart (2020)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Plaza Diaz (2015) L.rhamnosus group [a] | | | | | | | | | |
| Plaza-Diaz (2015) L. paracasei CNCM I-4034 [a] | | | | | | | | | |
| Plaza-Diaz (2015) B. breve CNCM I-4035 [a] | | | | | | | | | |
| Plaza-Diaz (2015) B. breve CNCM I-4035 and L. rhamnosus CNCM I-4036 [a] | | | | | | | | | |
| Qian (2020) | | | | | | | | | |
| Sandionigi(2022) | | | | | | | | | |
| Shi (2020) | | | | | | | | | |
| Shi (2023) | | | | | | | | | |
| Simon (2015) | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sohn (2022) | | | | | | | | | |
| Tremblay (2021) 5B CFU | | | | | | | | | |
| Tremblay (2021) 25B CFU | | | | | | | | | |
| Washburn (2022) | | | | | | | | | |
| Wischmeyer (2024) | | | | | | | | | |

| | |
|---|---|
| | no significant difference in β-diversity between groups |
| | there is a significant change in the probiotic group compared to baseline |
| | there is a significant change in the placebo group compared to baseline |
| | there is a significant difference between the groups |
| | not applicable* |

Definitions for each outcome are detailed in Table S3.

[a] No information about placebo.

*If a study did not investigate a specific outcome, “not applicable” is indicated.

Abbreviations: UniFrac: unique fraction metric

**Tables S6-7 Risk of bias assessments**

**Table S6** Risk of bias assessment for parallel design studies.

| Risk of Bias assessment_Parallel | | D1 | D2 | D3 | D4 | D5 | Overall |
|---|---|---|---|---|---|---|---|
| Study | Aim | Randomization process | Deviations from the intended interventions | Missing outcome data | Measurement of the outcome | Selection of the reported result | |
| Bagga (2018) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | High | Low | Low | High |
| Bazanella (2017) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Bloemendaal (2021) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Some concerns | Some concerns |
| Castanet (2020) | adhering to intervention (the 'per-protocol' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| Chen (2021) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Chen (2023) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| De Andrés (2018) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Freedman (2021) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Gai (2023) | adhering to intervention (the 'per-protocol' effect) | Low | High | Low | Low | Some concerns | High |
| Hibberd (2019) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Huang (2022) | adhering to intervention (the 'per-protocol' effect) | Some concerns | High | Low | Low | Some concerns | High |
| Kang (2021) | assignment to intervention (the 'intention-to-treat' effect) | Low | Low | Low | Low | Low | Low |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lee (2021) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Some concerns |
| Li (2023) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| López-Garcia (2023) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Some concerns | Some concerns |
| Majeed (2023) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Michael (2020) | adhering to intervention (the 'per-protocol' effect) | Low | High | Low | Low | Low | High |
| Moore (2023) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| Park (2020) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| Paytuví-Gallart (2020) | adhering to intervention (the 'per-protocol' effect) | Low | High | Low | Low | Low | High |
| Rahayu (2021) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| Sánchez Macarro (2021) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |
| Sandionigi (2022) | assignment to intervention (the 'intention-to-treat' effect) | Low | Low | Low | Low | Some concerns | Some concerns |
| Shi (2023) | assignment to intervention (the 'intention-to-treat' effect) | Low | Low | Low | Low | Low | Low |
| Shi (2020) | assignment to intervention (the 'intention-to-treat' effect) | High | Some concerns | Low | Low | Some concerns | High |
| Sohn (2022) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| Son (2020) | adhering to intervention (the 'per-protocol' effect) | Low | High | Low | Low | Some concerns | High |
| Tremblay (2021) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Low | Low | Low | Some concerns |
| Washburn (2022) | adhering to intervention (the 'per-protocol' effect) | Low | Low | Low | Low | Low | Low |

**Table S7** Risk of bias assessment for cross-over design studies

| **Risk of Bias assessment_Cross-over** | | **D1** | **DS** | **D2** | **D3** | **D4** | **D5** | **Overall** |
|---|---|---|---|---|---|---|---|---|
| **Study** | **Aim** | Randomization process | Bias arising from period and carryover effects | Deviations from the intended interventions | Missing outcome data | Measurement of the outcome | Selection of the reported result | |
| Axelrod (2019) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | Some concerns | Low | Low | Some concerns | High |
| Moloney (2021) | assignment to intervention (the 'intention-to-treat' effect) | Low | Some concerns | High | Low | Low | Some concerns | High |

**Figs. S21-24** Risk of bias assessments

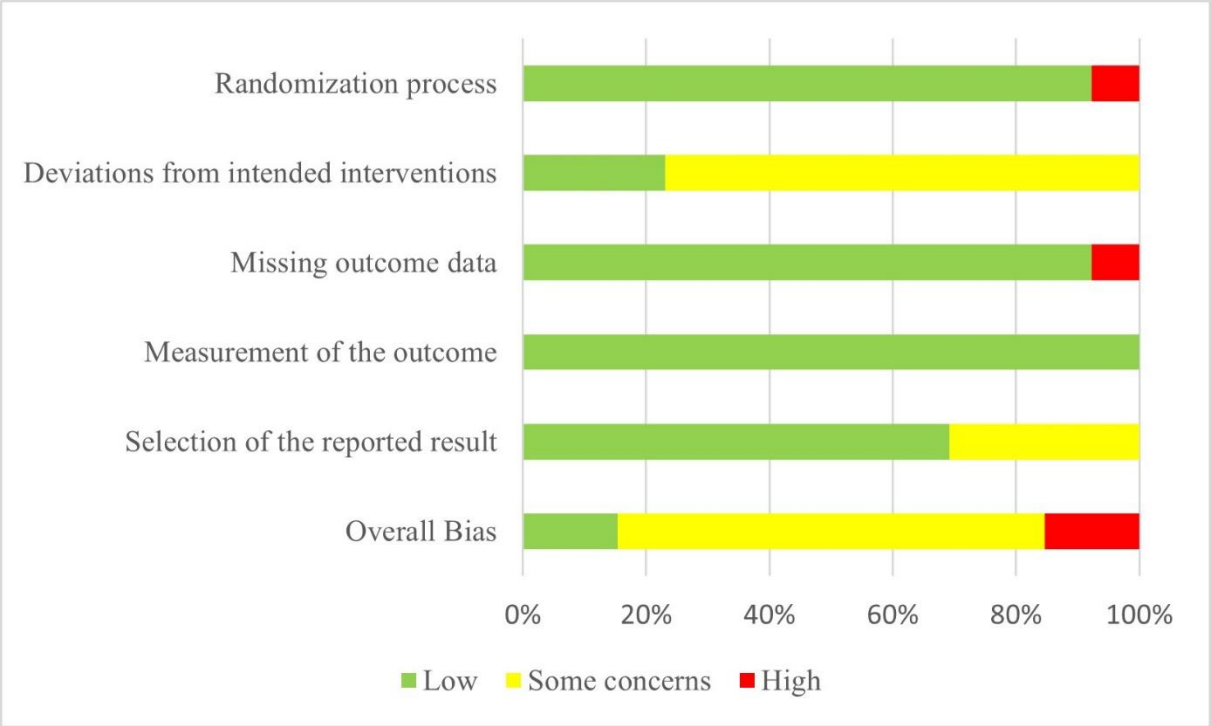



**Fig. S21** Risk of bias assessment for parallel design studies - Assignment to intervention (the 'intention-to-treat' effect).

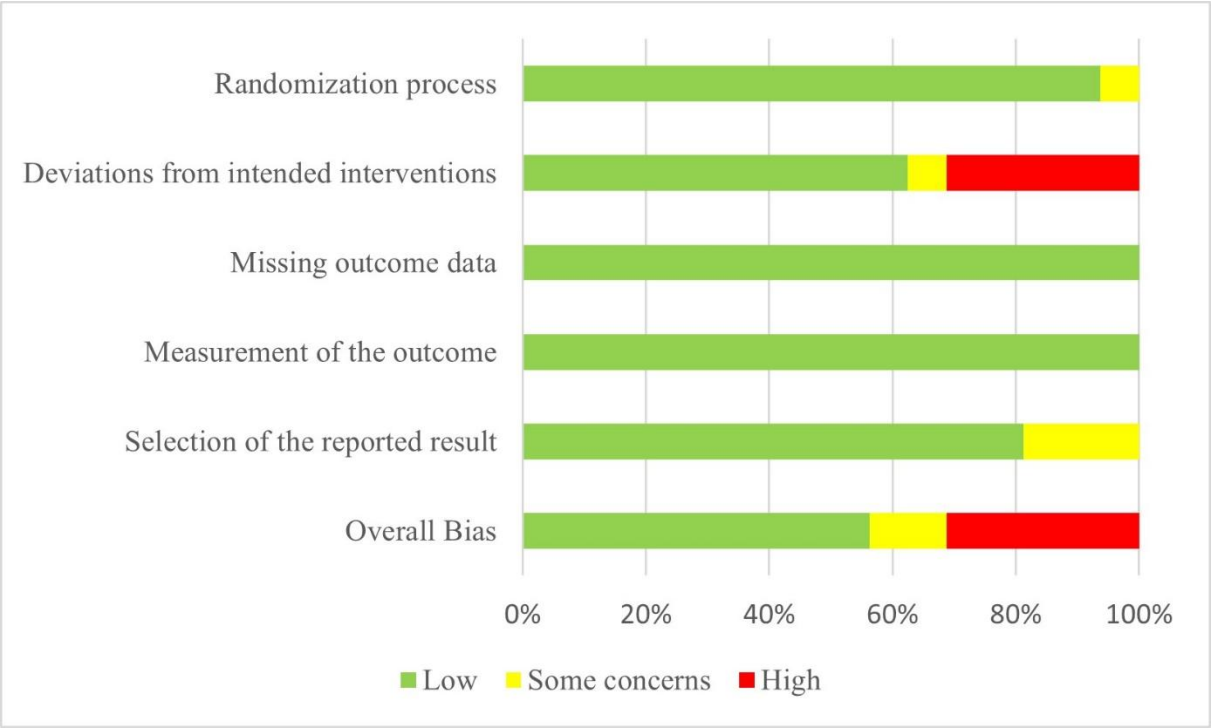



**Fig. S22** Risk of bias assessment for parallel design studies - Adhering to intervention (the 'per-protocol' effect).

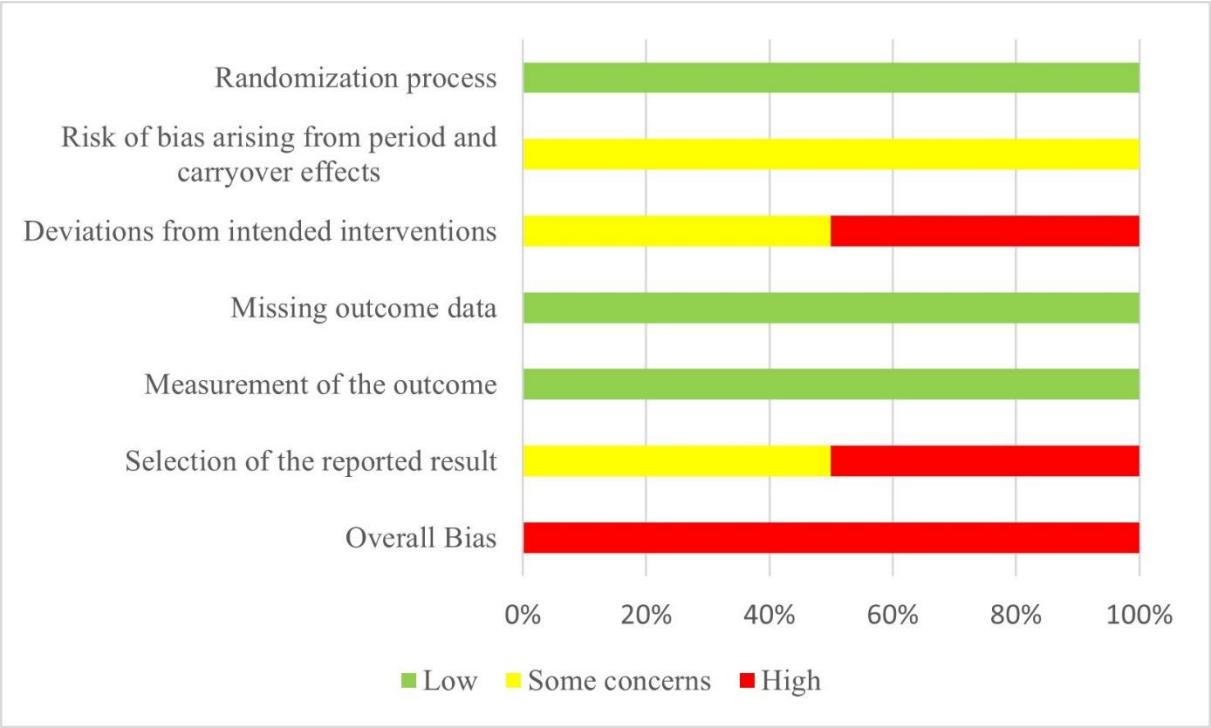



**Fig. S23** Risk of bias assessment for cross-over design studies - Assignment to intervention (the 'intention-to-treat' effect).

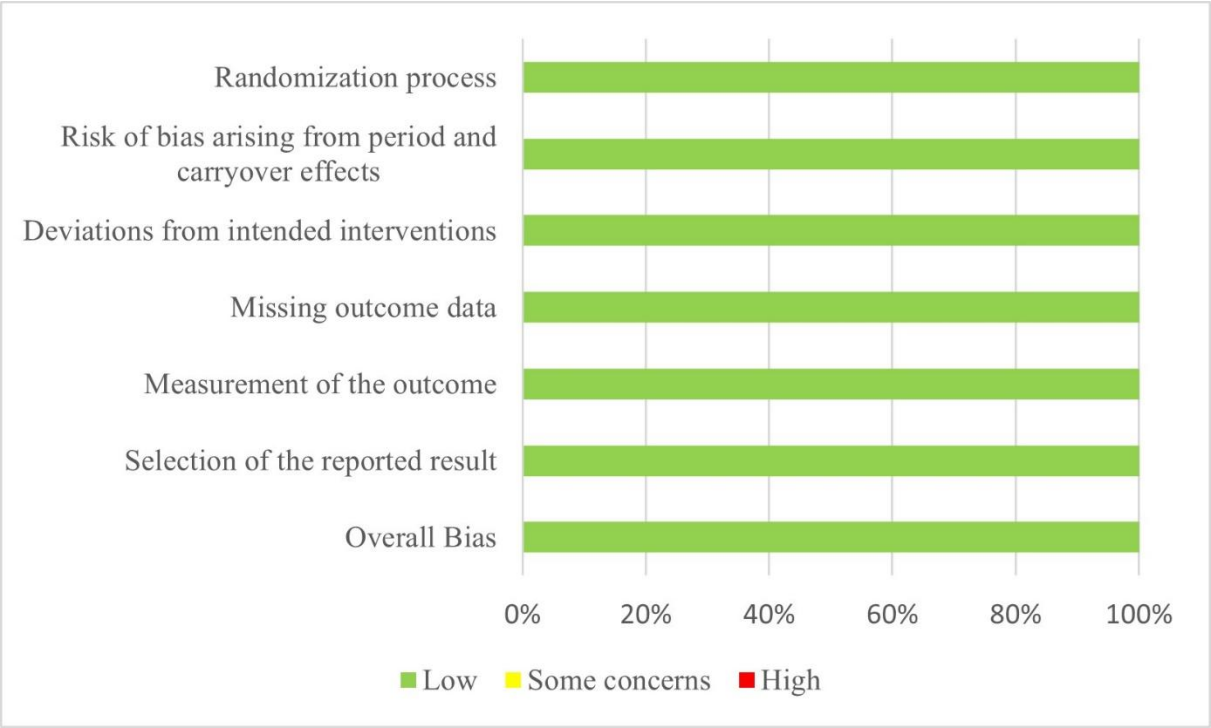



**Fig. S24** Risk of bias assessment for crossover design studies - Adhering to intervention (the 'per-protocol' effect).

**Table S8** GRADE assessment for the meta-analyses of Shannon, Observed OTUs, Chao1 and Simpson's Index of Diversity indices.

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | probiotics | control | Relative (95% CI) | Absolute (95% CI) | | |
| **Shannon diversity index (follow-up: range 2 weeks to 6 months; assessed with: 16S rRNA sequencing)** | | | | | | | | | | | | |
| 22 | randomised trials | serious[a] | not serious | not serious | serious[b] | none | 552 | 552 | - | MedD **0.08 lower** (0.16 lower to 0.01 higher) | ⊕⊕◯◯ Low[a,b] | IMPORTANT |
| **Observed OTUs (follow-up: range 4 weeks to 22 weeks; assessed with: 16S rRNA sequencing)** | | | | | | | | | | | | |
| 7 | randomised trials | serious[a] | not serious | not serious | serious[c] | none | 231 | 232 | - | MedD **2.19 higher** (2.2 lower to 6.57 higher) | ⊕⊕◯◯ Low[a,c] | IMPORTANT |
| **Chao1 index (follow-up: range 4 weeks to 24 weeks; assessed with: 16S rRNA sequencing)** | | | | | | | | | | | | |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | probiotics | control | Relative (95% CI) | Absolute (95% CI) | | |
| 9 | randomised trials | serious[a] | not serious | not serious | serious[d] | none | 252 | 252 | - | MedD **3.19 lower** (27.28 lower to 20.89 higher) | ⊕⊕◯◯ Low[a,d] | IMPORTANT |
| **Simpson's Index of Diversity (follow-up: range 4 weeks to 22 weeks; assessed with: 16S rRNA sequencing)** | | | | | | | | | | | | |
| 10 | randomised trials | serious[a] | not serious | not serious | not serious | none | 236 | 235 | - | MedD **0.01 lower** (0.02 lower to 0 ) | ⊕⊕⊕◯ Moderate[a] | IMPORTANT |

**CI:** confidence interval **MedD**: mean of median differences

**Explanations**

a. Although subgroup analysis did not demonstrate a difference in effect by risk of bias level, a substantial proportion of studies contributing to the evidence were classified as high or some concerns risk of bias, which may still downgrade the confidence in the estimate due to potential unmeasured sources of bias.

b. The total sample size exceeds standard optimal information size benchmarks for continuous outcomes, so sample size alone is sufficient. However, the pooled 95% confidence interval for the effect estimate is [-0.16; 0.01], which crosses the null value and includes both possible benefit and no effect. The prediction interval further demonstrates uncertainty, as it encompasses clinically relevant directions. As a result, the certainty of evidence is downgraded by one level for imprecision.

c. The total sample size for the outcome is limited, and the 95% confidence interval for the pooled effect estimate (-2.20 to 6.57) is wide, including both the possibility of a clinically important increase or decrease as well as no effect. Despite consistent effects across studies ($I^2 = 0\%$), the insufficient information size and broad confidence interval warrant downgrading the certainty of evidence by one level for imprecision.

d. The total sample size for the Chao1 index meta-analysis is limited, and the pooled confidence interval (-27.28 to 20.89) is wide, encompassing both possible harm, benefit, and no effect. The prediction interval is similarly broad, suggesting substantial uncertainty about the true effect. As a result, the certainty of evidence is downgraded for imprecision.

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