Journal of
Integrative Neuroscience

J. Integr. Neurosci. **2024**; 23(7): 127
https://doi.org/10.31083/j.jin2307127

Systematic Review

Changes in Gut Microbiota in Patients with Multiple Sclerosis Based on 16s rRNA Gene Sequencing Technology: A Review and Meta-Analysis

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Academic Editor: Gernot Riedel
Submitted: 3 January 2024 Revised: 27 March 2024 Accepted: 10 April 2024 Published: 9 July 2024

Abstract

Background: This meta-analysis explores alterations in the gut microbiota of patients with Multiple Sclerosis (MS) using 16S ribosomal RNA (rRNA) gene sequencing. **Methods**: Adhering to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, our comprehensive review spanned major databases, including PubMed, Web of Science, Embase, Cochrane, and Ovid, targeting observational studies that implemented 16S rRNA gene sequencing on fecal specimens. The quality of these studies was meticulously evaluated using the Newcastle-Ottawa scale. **Results**: Our search yielded 26 relevant studies conducted between 2015-2022, encompassing 2885 participants. No significant differences were observed in alpha diversity indices (Shannon, Chao1, Operational Taxonomic Units (OTU), and Simpson) between MS patients and controls in general. Nonetheless, subgroup analyses according to disease activity using the Shannon index highlighted a significant decrease in microbial diversity during MS's active phase. Similarly, an evaluation focusing on MS phenotype revealed diminished diversity in individuals with relapsing-remitting MS (RRMS). Microbial composition analysis revealed no consistent increase in pro-inflammatory *Bacteroidetes* or decrease in anti-inflammatory *Firmicutes* within the MS cohort. **Conclusion**: The gut microbiome's role in MS presents a complex panorama, where alterations in microbial composition might hold greater significance to disease mechanisms than diversity changes. The impact of clinical factors such as disease activity and phenotype are moderately significant, underscoring the need for further research to elucidate these relationships. Prospective research should employ longitudinal methodologies to elucidate the chronological interplay among gut microbiota, disease evolution, and therapeutic strategies.

Keywords: multiple sclerosis; gut microbiota; 16S rRNA; meta-analysis

1. Background

Multiple sclerosis (MS), a chronic autoimmune and neurodegenerative disease, involves inflammatory demyelination and neuronal damage in the central nervous system [1]. Its complex etiology is thought to be influenced by genetics, environment, and lifestyle [2]. Recent studies have increasingly highlighted the significant role of gut microbiota in the pathogenesis of various neurological diseases [3]. Gut microbiota affects the immune system through metabolite production, mucosal barrier regulation, and modulation of immune cells and neurotransmitters [4,5]. Imbalances in gut microbiota may disrupt immune regulation, potentially exacerbating MS pathology.

Advancements in 16S ribosomal RNA (rRNA) gene sequencing technology have greatly enhanced our understanding of gut microbiota [6]. This technique, crucial for identifying and quantifying gut microbial communities, is applied in diverse fields, including microbiology and medicine, offering new perspectives on the microbiome's role in diseases like MS [7].

Research on MS has focused on analyzing the gut microbiota of patients and controls using this technology. However, inconsistencies in findings, influenced by factors such as sample size and geographical region, necessitate a systematic meta-analysis [8,9]. This study aims to conduct a meta-analysis using 16S rRNA gene sequencing to discern patterns in gut microbiota changes in MS patients, exploring their impact on disease progression.

2. Methods

The meta analysis was registered with PROSPERO No. CRD42023482457 and executed in compliance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (The PRISMA checklist can be found in **Supplementary Material-PRISMA_2020_checklist**) [10], as visually depicted in Fig. 1.

2.1 Literature Search

A comprehensive search was conducted in databases including PubMed, Web of Science, Embase, Cochrane, and Ovid. The search, extending up to July 3, 2023, focused on studies related to the gut microbiota in MS patients. Keywords utilized encompassed "multiple sclerosis", "gut mi-

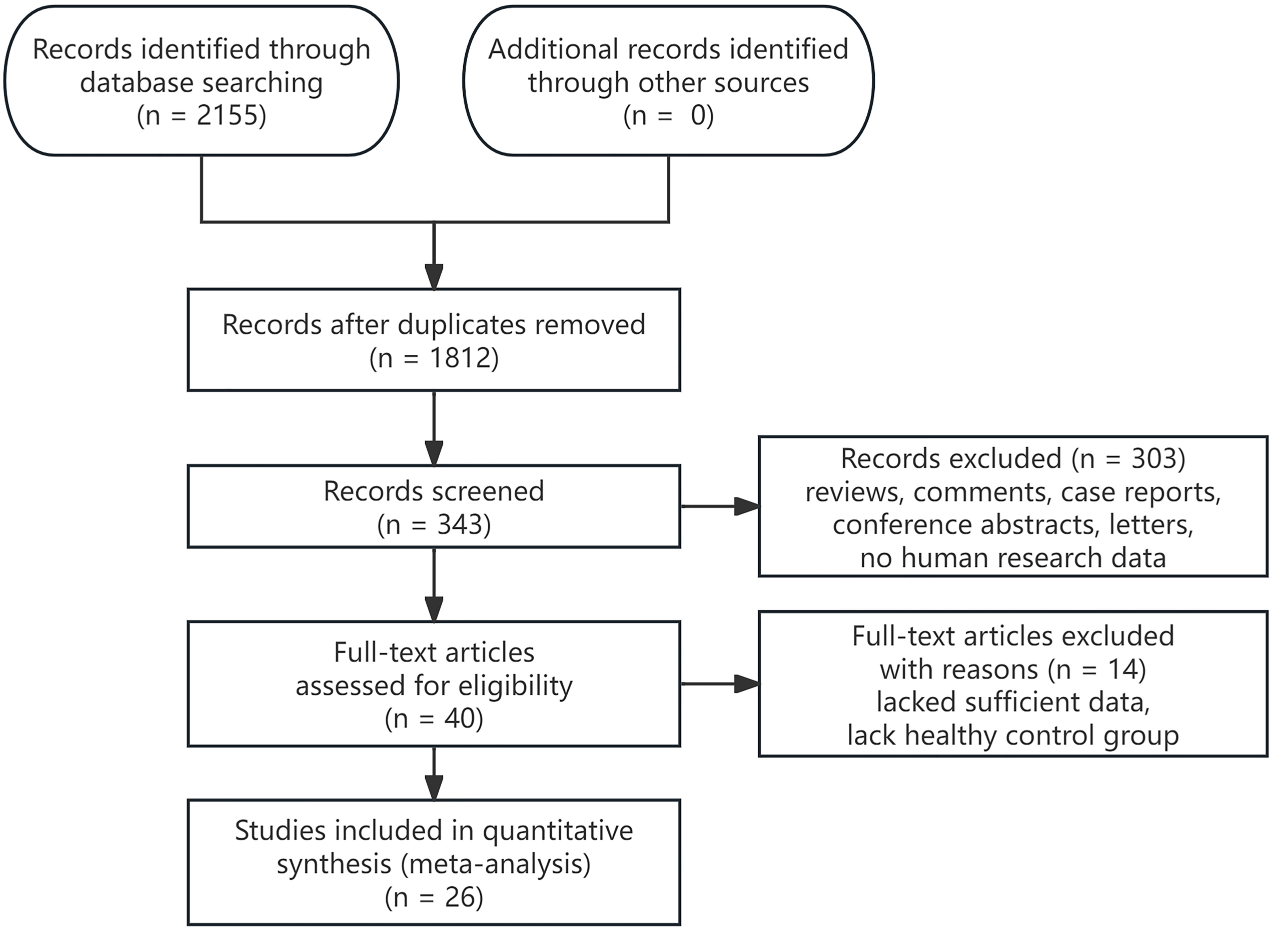

Fig. 1. Flowchart of the study selection process in accordance with PRISMA guidelines. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

crobiota", "gut microbiome", "16S rRNA", and related synonyms, without restrictions on language, region, or publication year. Additional literature was identified through manual reference checks to encompass studies that met our inclusion criteria.

2.2 Literature Screening

Inclusion criteria for literature selection were as follows:

- Observational studies (cross-sectional, cohort, or case-control).

- Studies employing 16S rRNA gene sequencing for fecal sample analysis of gut microbiota in patients.

- Provision of patients' demographic and clinical details (age, sex, disease type, duration, activity, medication, etc.).

- Data on gut microbiota composition and diversity (e.g., phyla, classes, orders, families, genera, species, and indices like Shannon, Simpson, Chao1).

- Presence of a healthy control group or comparative data with healthy subjects.

2.3 Exclusion Criteria Included

- Non-original works (reviews, comments, case reports, abstracts, letters).

- Studies on animals or *in vitro* experiments.

- Studies lacking sufficient data for meta-analysis or inaccessible supplementary data.

Two researchers (A and B) independently screened the literature, with a third researcher resolving disagreements.

2.4 Literature Quality Assessment

The Newcastle-Ottawa scale (NOS) was employed for quality assessment, scoring studies across selection, comparability, and outcome assessment. Scores ranged from 0 to 9, categorizing studies as high (7–9), medium (4–6), or low quality (0–3). Two researchers independently conducted this assessment, with a third researcher resolving disagreements through discussion or consensus.

2.5 Data Extraction

Data extraction involved:

- Study details (author, year, country, type).

- Subject demographics and clinical characteristics (sample size, age, sex, disease specifics, etc.).

Table 1. Basic characteristics of the included studies.

Study	Country	Study design	Total Sample size (%female)		Age at stool sample collection, years: mean (SD)		BMI mean (SD)		Mean EDSS (SD)	Disease duration at stool sampling, average months (SD)	Type of MS included into review	Steroids <1 month prior to stool sampling, n (%)	DMT use at stool sampling, n (%)	Disease activity	Outcomes	Sequencing platform	Extracted indicators
			Case group	Control group	Case group	Control group	Case group	Control group									
Bruijstens 2023 [11]	Netherlands	prospective cohort study	26 (65%)	24 (63%)	17.3 (2.3)	10.6 (5.6)	NA	NA	NA	30 (13.7)	NA	0 (0)	24 (92)	active (24/26; 92%)	Fecal microbiota	Illumina MiSeq platform	microbial composition
Chen 2016 [12]	USA	cross-sectional	12 (75%)	36 (61.1%)	39.3 (10.6)	40.3 (7.3)	32.7 (7.4)	27.8 (4.7)	NA	NA	RRMS	NA	12 (100%)	active (100%)	Fecal microbiota	Illumina paired-end sequencing	OTU, microbial composition
Choileáin 2020 [13]	USA	cross-sectional	26 (85%)	39 (69%)	42 (13)	45 (12)	29 (7)	27 (5)	NA	36 (87)	RRMS	NA	0 (0)	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, microbial composition
Cosorich 2017 [14]	Italy	cross-sectional	19 (57.9%)	17 (58.8%)	41 (2)	48 (3)	NA	NA	2.4 (1.4)	NA	RRMS	NA	19 (100%)	Active (10/19; 53%)	Fecal microbiota	Roche 454 platform	microbial composition
Cox 2021 [15]	USA	cross-sectional	199 (76.4%)	40 (70.0%)	49.3 (9.5)	45.4 (9.2)	27.1 (6.3)	28 (7.9)	1.8 (1.2)	195.6 (120)	RRMS	NA	155 (77.9%)	NA	Fecal microbiota	Illumina MiSeq platform	Shannon
Forbes 2018 [16]	Canada	cross-sectional	19 (73.7%)	23 (52.2%)	47.3 (NA)	32.4 (NA)	NA	NA	NA	NA	NA	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, Chao1, Simpson
Galluzzo 2021 [17]	Italy	case-control study	15 (46.7%)	15 (73.3%)	NA	NA	NA	NA	NA	NA	RRMS PPMS	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	OTU, microbial composition
Jangi 2016 [18]	USA	cross-sectional	60 (68%)	43 (86%)	49.7 (8.5)	42.2 (9.61)	NA	NA	1.2 (1.0)	12.8 (8.3)	RRMS	0 (0)	32 (53.3%)	Inactive (100%)	Fecal microbiota	Roche 454 platform	microbial composition
Kozhieva 2019 [19]	Russia	cross-sectional	15 (6)	15 (7)	45 (7.75)	23 (13.25)	22 (1.75)	24 (3.25)	NA	43.2 (9)	PPMS	0 (0)	0 (0)	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, Chao1, microbial composition
Miyake 2015 [7]	Japan	cross-sectional	20 (70%)	50 (54%)	36.0 (7.2)	27.2 (9.2)	NA	NA	NA	105 (73.4)	RRMS	0 (0)	13 (75%)	NA	Fecal microbiota	Roche 454 platform	Shannon, microbial composition
Moles 2022 [20]	Spain	cross-sectional	20 (80%)	20 (15%)	47.1 (0.15)	49.2 (0.15)	NA	NA	1.9 (4.81)	180 (1.78)	RRMS	0 (0)	16 (80%)	NA	Fecal microbiota	Ion Torrent	Shannon
Navarro-López 2022 [21]	Spain	cross-sectional	15 (86.7%)	15 (NA)	38.15 (8.08)	NA	27.01 (5.45)	NA	2.67 (1.5)	NA	RRMS	NA	NA	active (100%)	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, Chao1, Simpson, microbial composition
Oezguen 2019 [22]	Turkey	cross-sectional	13 (61.5%)	14 (28.5%)	39.1 (11.6)	37.8 (8.6)	NA	NA	3.5 (1.9)	120.1 (110.0)	RRMS	NA	NA	NA	Fecal microbiota	Roche 454 platform	OTU, Chao1, microbial composition
Pellizoni 2021 [23]	Brazil	cross-sectional	18 (88.9%)	18 (88.9%)	46.06 (11.83)	45.50 (11.03)	26.14 (4.01)	NA	3.61 (0.77)	125.88 (88.32)	RRMS	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	OTU, Simpson, microbial composition
Reynders 2020 [24]	Belgium	cross-sectional	24 (62.5)	120 (61.7)	44.5 (10.5)	49.0 (14.3)	23.6 (1.23)	23.7 (1)	2 (1.1)	6.0 (1.825)	RRMS	0 (0)	NA	active (100%)	Fecal microbiota	Illumina MiSeq platform	Shannon
Storm 2019 [25]	Norway	case-control study	34 (74%)	165 (63%)	46 (10.4)	47 (11.8)	24 (4.07)	25.8 (4.30)	1.56 (NA)	NA	RRMS	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, microbial composition
Tankou 2018 [26]	USA	case-control study	9 (55.6%)	13 (61.5%)	50 (10)	35 (14)	NA	NA	1.4 (0.9)	105.6 (78)	RRMS	0 (0)	7 (77.8%)	Inactive (100%)	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, microbial composition

Table 1. Continued.

Study	Country	Study design	Total Sample size (%female)		Age at stool sample collection, years: mean (SD)		BMI mean (SD)		Mean EDSS (SD)	Disease duration at stool sampling, average months (SD)	Type of MS included into review	Steroids $<$1 month prior to stool sampling, n (%)	DMT use at stool sampling, n (%)	Disease activity	Outcomes	Sequencing platform	Extracted indicators
			Case group	Control group	Case group	Control group	Case group	Control group									
Tremlett 2016 [27]	USA	case-control study	18 (55.6%)	17 (52.9%)	12.5 (4.4)	13.5 (3.08)	22.2 (5.66)	22.8 (7.10)	2 (1)	10.6 (6.34)	NA	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, Chao1
Tremlett 2016 [28]	USA	cross-sectional	15 (53 %)	9 (78 %)	11.9 (4.64)	13.8 (3.19)	NA	NA	NA	10 (5.25)	RRMS	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	OTU
Tremlett 2021 [29]	Canada-USA	case-control study	32 (75%)	36 (58%)	16.5 (3.7)	15.1 (3.44)	22.8 (5.63)	19.9 (4.18)	NA	18 (NA)	NA	23 (72%)	NA	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, Chao1, microbial composition
Troci 2022 [30]	Germany	longitudinal cohort study	14 (85.7%)	54 (59.3%)	46 (9.6)	47 (4.4)	NA	NA	NA	NA	NA	NA	NA	NA	Fecal microbiota	Illumina MiSeq platform	Chao1
Ventura 2019 [31]	USA	cross-sectional	45 (76%)	44 (64%)	31.8 (9)	37.1 (12.7)	NA	NA	NA	NA	NA	9 (20%)	NA	NA	Fecal microbiota	Illumina MiSeq platform	chao1, microbial composition
Yadav 2022 [32]	USA	cross-sectional	20 (75%)	33 (84%)	43 (7.7)	42 (14)	30 (7.9)	24 (3.7)	NA	NA	RRMS	0 (0)	16 (80%)	NA	Fecal microbiota	Illumina MiSeq platform	microbial composition
Zeng 2019 [33]	China	cross-sectional	34 (62%)	34 (62%)	29 (10.70)	35.18 (9.03)	21.39 (3.28)	21.72 (2.77)	NA	NA	RRMS	NA	NA	Active (26/34; 76%)	Fecal microbiota	Illumina MiSeq platform	Shannon, OTU, Chao1, microbial composition
Zhou 2020 [34]	USA	case-control study	437 (71.4%)	576 (34.9%)	45.8 (13.3)	50.6 (14.96)	NA	NA	1.77 (1.85)	114 (113.28)	RRMS	0 (0)	32.5 (74.4)	NA	Fecal microbiota	Illumina MiSeq platform	Shannon, Chao1
Zhou 2022 [35]	USA	case-control study	128 (71.1%)	128 (37.5%)	45 (13.33)	47.5 (14.63)	26.9 (4.07)	25.4 (4.30)	2 (2.4)	114 (113.28)	RRMS SPMS PPMS	0 (0)	0 (0)	NA	Fecal microbiota	Illumina MiSeq platform	microbial composition

BMI, Body Mass Index; EDSS, Expanded Disability Status Scale; MS, Multiple Sclerosis; RRMS, Relapsing-Remitting Multiple Sclerosis; DMT, Disease-Modifying Therapy; NA, Not Available; PPMS, Primary Progressive Multiple Sclerosis; SPMS, Secondary Progressive Multiple Sclerosis; OTU, Operational Taxonomic Unit; SD, standard deviation.

Table 2. Results of meta-analyses of gut microbiota diversity.

Outcome indicators	Effect size	95% confidence interval	Heterogeneity p value	I^2
Shannon index	0.06	–0.02, 0.14	$<$0.001	80.50%
Chao1 index	–3.28	–9.99, 3.44	0.017	58.80%
OTU index	–0.61	–11.18, 9.95	$<$0.001	87.20%
Simpson index	0.06	–0.13, 0.25	0.024	80.30%

- Gut microbiota composition and diversity data.
- Statistical analysis methods and results (means, standard deviations, etc.).

Two researchers independently undertook data extraction, resolving discrepancies through a third researcher.

2.6 Data Analysis

Data analysis was performed using Stata 15.0 (StataCorp LLC, College Station, TX, USA). The meta-analysis employed a random-effects model to account for study heterogeneity, using weighted mean difference (WMD) for diversity indices, and effect size for relative abundance to specifically address both the presence/absence and abundance of microbial taxa. This strategy, leveraging effect size analysis, integrates the natural logarithm of Odds Ratios (ln(OR)) for evaluating the probability and impact of specific bacterial presence in MS versus controls, and mean-based metrics for quantifying their average abundance. Heterogeneity was assessed using I^2 statistics and Q tests. Sensitivity and subgroup analyses explored heterogeneity sources. Publication bias was examined through funnel plots, Begg's, and Egger's tests. Crafted using Python's matplotlib library, the bar charts and forest plots serve distinct yet complementary roles in our analysis. The bar charts are dedicated to illustrating the relative effect sizes obtained from a literature review, effectively summarizing the range of findings across different studies. On the other hand, the forest plots focus on comparing the bacterial compositions within the gut microbiota and evaluating the likelihood and associated risks of specific microbial taxa presence in MS patients versus controls, as precisely determined by the meta-analysis results.

3. Results

3.1 Literature Search and Screening

As depicted in Fig. 1, our comprehensive literature search and meticulous screening process yielded 2155 pertinent articles. Following the exclusion of duplicates, a rigorous preliminary screening, and detailed full-text evaluation, a total of 26 articles were ultimately selected for inclusion in our meta-analysis [7,11–35].

3.2 Basic Characteristics and Quality Assessment of the Literature

Table 1 (Ref. [7,11–35]) delineates the fundamental characteristics and quality assessment of the incorporated studies. Predominantly cross-sectional in design, these studies spanned from 2015 to 2022, encompassing 13 countries with sample sizes totaling 2885 (1287 in the MS cohort and 1598 in the control group), varying from 22 to 1013 subjects. The quality assessment (**Supplementary Material-Table S1**) revealed that 20 studies were classified as high-quality, 6 as medium-quality, and none as low-quality.

3.3 Diversity of Gut Microbiota

The meta-analysis employed four distinct alpha diversity indices, namely the Shannon index, Simpson index, Chao1 index, and Operational Taxonomic Unit (OTU) index. The Shannon and Simpson indices integrate both species richness and evenness, whereas the Chao1 and OTU indices solely reflect species richness. Each index captures a unique facet of diversity. The Shannon index, an information-theoretic measure, encapsulates the aggregate of richness and evenness in a sample, with higher values indicating greater diversity. The Simpson index, rooted in probability, gauges the likelihood of randomly selected individuals belonging to the same species; lower values suggest increased diversity. The Chao1 index, a non-parametric estimator, considers the count of undetected species, with higher values denoting greater richness. The OTU index, based on sequence similarity, reflects the number of operational taxonomic units, with higher values signifying more diversity.

The studies reviewed did not find significant differences in gut microbiota diversity among MS patients when examining various diversity indices (Shannon, Chao1, OTU, and Simpson), as shown in Table 2 and Fig. 2 However, a more detailed analysis focusing on disease activity revealed a decrease in bacterial diversity during the active phase of MS, with a Weighted Mean Difference (WMD) of 0.49 and a 95% Confidence Interval (CI) of [0.31, 0.67] according to the Shannon index (**Supplementary Material-Fig. S6**). Similarly, an analysis based on disease phenotype, specifically within the relapsing-remitting MS (RRMS) group, showed a significant reduction in microbiota diversity (WMD 95% CI = 0.10 [0.01, 0.18]), as per the Shannon index (**Supplementary Material-Fig. S3**). However, subgroup analyses considering disease activity and phenotype through other indices (Chao1, OTU, Simpson) did not show any significant differences (**Supplementary Material-Figs. S11, S14, S19, S22, S27, S30**). Further subgroup analyses considering factors such as country, duration, platform, the Newcastle-Ottawa Scale (NOS), disease activity, and Body Mass Index (BMI) across the Shannon, Simpson, Chao1, and OTU indices also did not yield significant findings.

3.4 Composition of Bacterial Phyla, Genera, and Family

Our analysis based on data from at least two studies per bacterial composition, identified differences in gut microbiota between MS patients and healthy controls at various taxonomic levels (**Supplementary Material-Figs.**

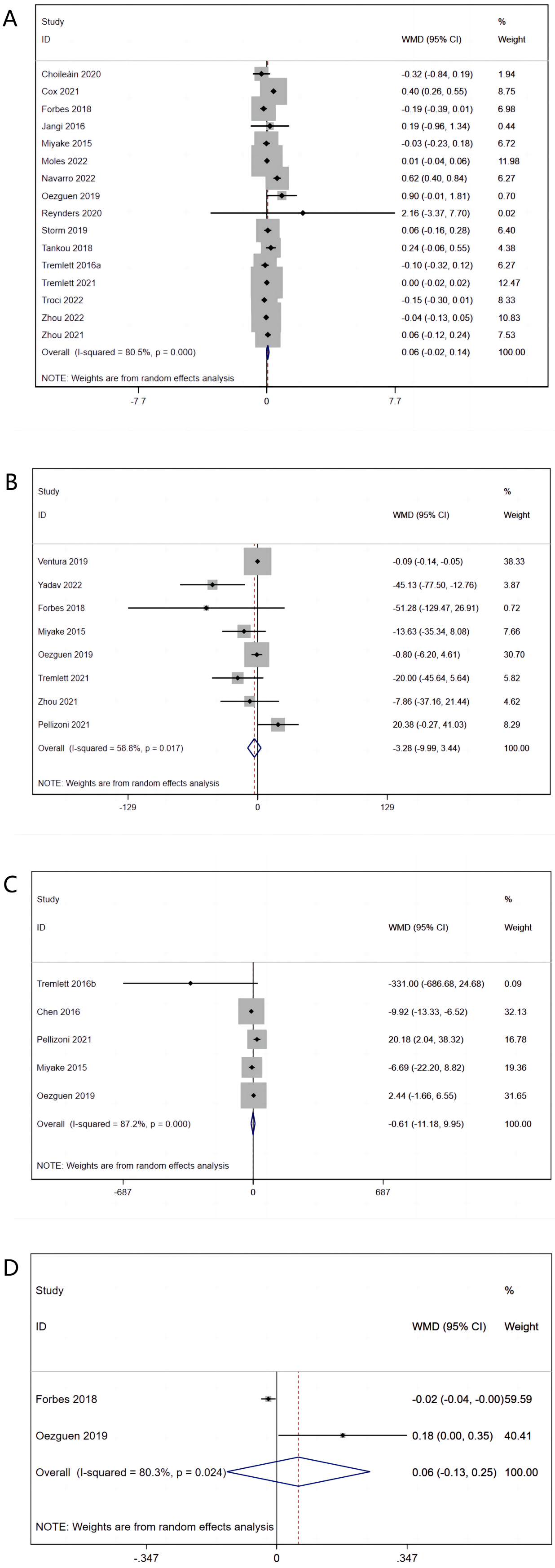

Fig. 2. Meta-analysis forest plot of gut microbiota alpha diversity. (A) Shannon. (B) Chao1. (C) OUT. (D) Simpson Indices. WMD, weighted mean difference; CI, Confidence Interval.

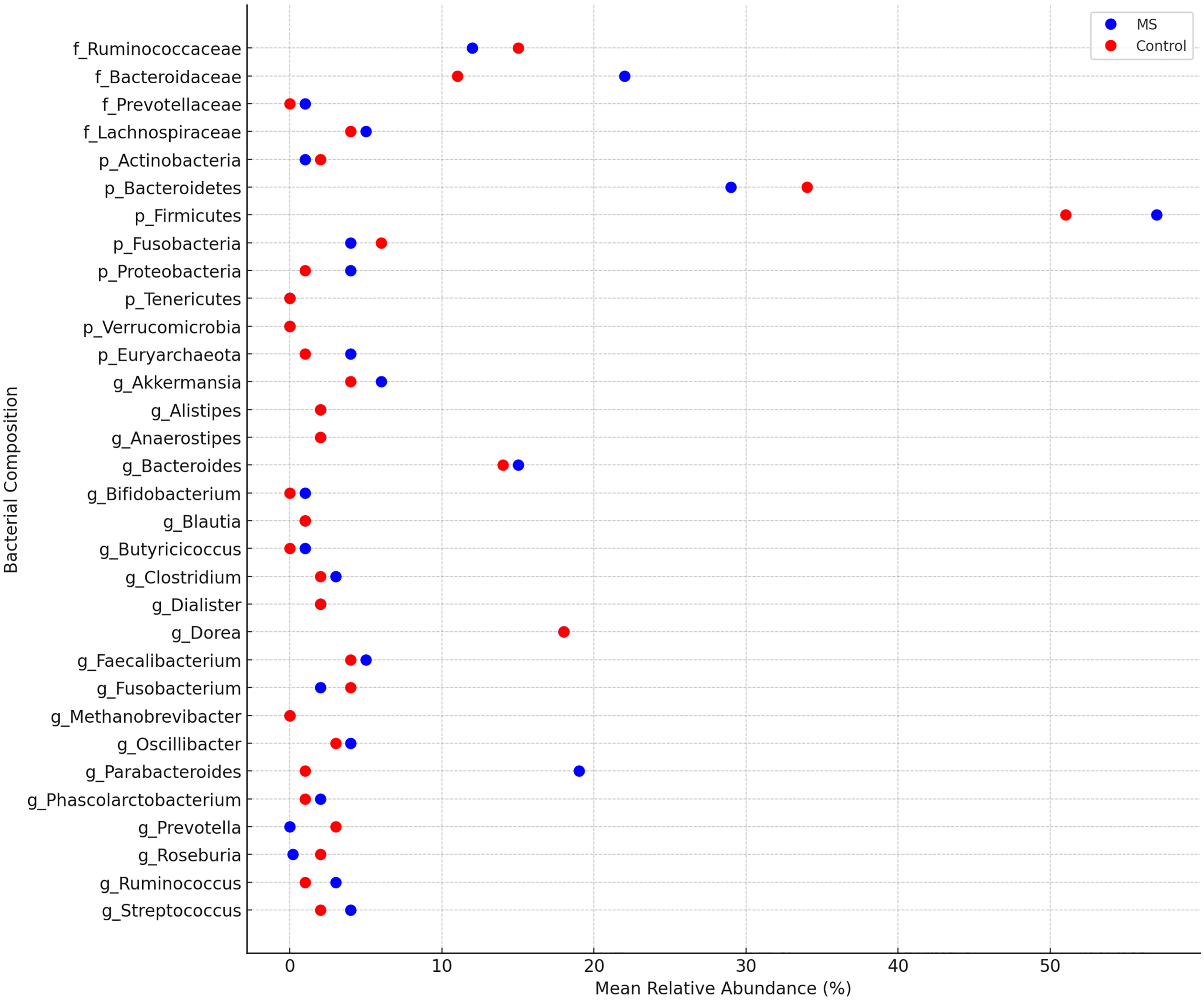

Fig. 3. Mean relative abundance of various gut bacterial taxa within MS patients and healthy controls. MS, Multiple Sclerosis; f, family; g, genus; p, phylum.

S33–S64). The family *Ruminococcaceae* was less abundant in the MS group, while families such as *Bacteroidaceae, Prevotellaceae, and Lachnospiraceae* were more prevalent. Increased abundance of phyla *Firmicutes, Proteobacteria, and Euryarchaeota* was noted in the MS cohort, whereas *Actinobacteria, Bacteroidetes, and Fusobacteria* were less prevalent. At the genus level, we observed an increased relative abundance of *Fusobacterium, Oscillibacter, Prevotella, and Roseburia* in MS patients, while genera including *Akkermansia, Bacteroides, Bifidobacterium, Faecalibacterium, Parabacteroides, Ruminococcus, Streptococcus, Clostridium, Phascolarctobacterium, and Butyricicoccus* were decreased. These findings are concisely summarized in Fig. 3.

3.5 Statistical Significance Analysis

As illustrated in Fig. 4 and **Supplementary Material-Figs. S65–S95**, numerous taxa display CIs that encompass the value of 0, indicating that the associations between these microbes and MS are not statistically significant based on the ln(OR)-based analysis alone. This suggests that definitive associations between these specific microbes and MS cannot be established from this analysis. However, certain microbial taxa demonstrate a positive association with health outcomes, such as *f_Prevotellaceae* and *p_Euryarchaeota*, characterized by ln(OR) values greater than 0 and CIs that do not include 0. This indicates a higher likelihood of these microbes being present in case groups. Conversely, other taxa, including *g_Methanobrevibacter* and *g_Dorea*, show ln(OR) values less than 0 with 95% CIs that also do not include 0, pointing towards a lower probability of their presence in case groups. This pattern may suggest a potential protective role against MS.

3.6 Comprehensive Review of Significant Findings

A review of studies revealing statistically significant differences in key microflora between the MS and healthy populations is illus-

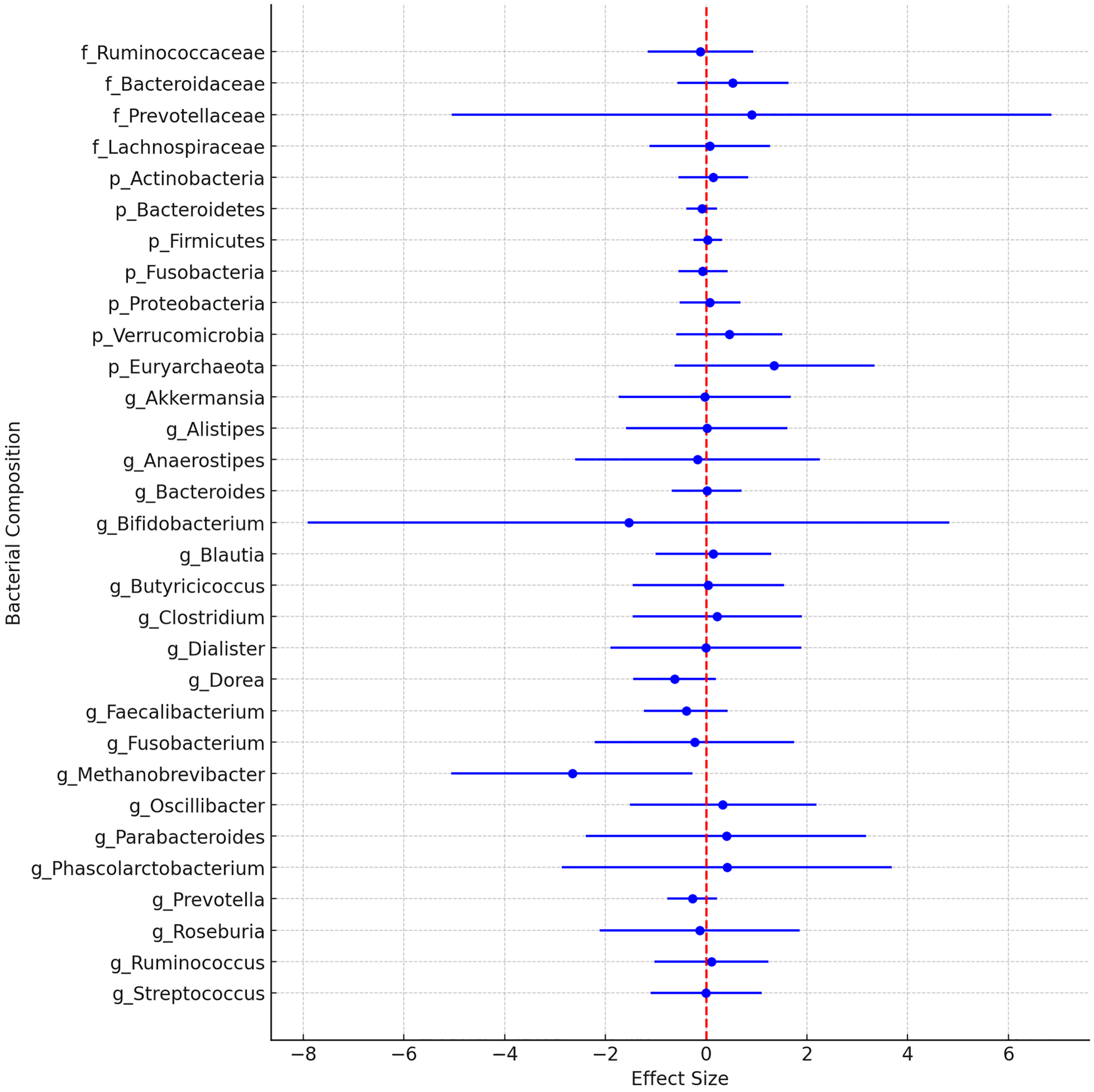

Fig. 4. Forest plot summarizing the pooled estimates of ln(OR) and 95% confidence intervals (CIs) for gut microbial taxa associations with MS. f, family; g, genus; p, phylum; ln(OR), logarithm of Odds Ratios.

trated in Fig. 5 The findings for *p_Actinobacteria*, *p_Firmicutes*, *g_Clostridium cluster IV*, *g_Butyricicoccus*, *g_Faecalicoccus*, *g_Lactobacillus*, *g_Methanobrevibacter*, *g_Parabacteroides*, *g_Sporobacter*, *g_Coprococcus*, and *g_Streptococcus* were inconsistent. Conversely, the results of *p_Bacteroidetes*, *g_Dorea*, *g_Flavobacterium*, *s_Akkermansia muciniphila* were all significantly more prevalent in the MS group compared to the healthy control group, whereas *f_Ruminococcaceae* and *g_Gemmiger* were more abundant in the healthy controls.

These results suggest a certain degree of dysbiosis or functional disturbance in the gut microbiota of MS patients. Notably, the findings regarding *p_Bacteroidetes* and *f_Ruminococcaceae* display a degree of consistency both in the meta-analysis and in the review of significant results of the included studies.

3.7 Heterogeneity and Sensitivity Analysis

Significant heterogeneity was detected among studies ($I^2 > 50\%$, Q-test $p < 0.1$). Sensitivity analysis, however, confirmed the stability of the meta-analysis outcomes for the Shannon, Chao1, and OTU indices, as excluding any single study did not significantly alter the results (**Supplementary Material-Figs. S29–S32**), except for the

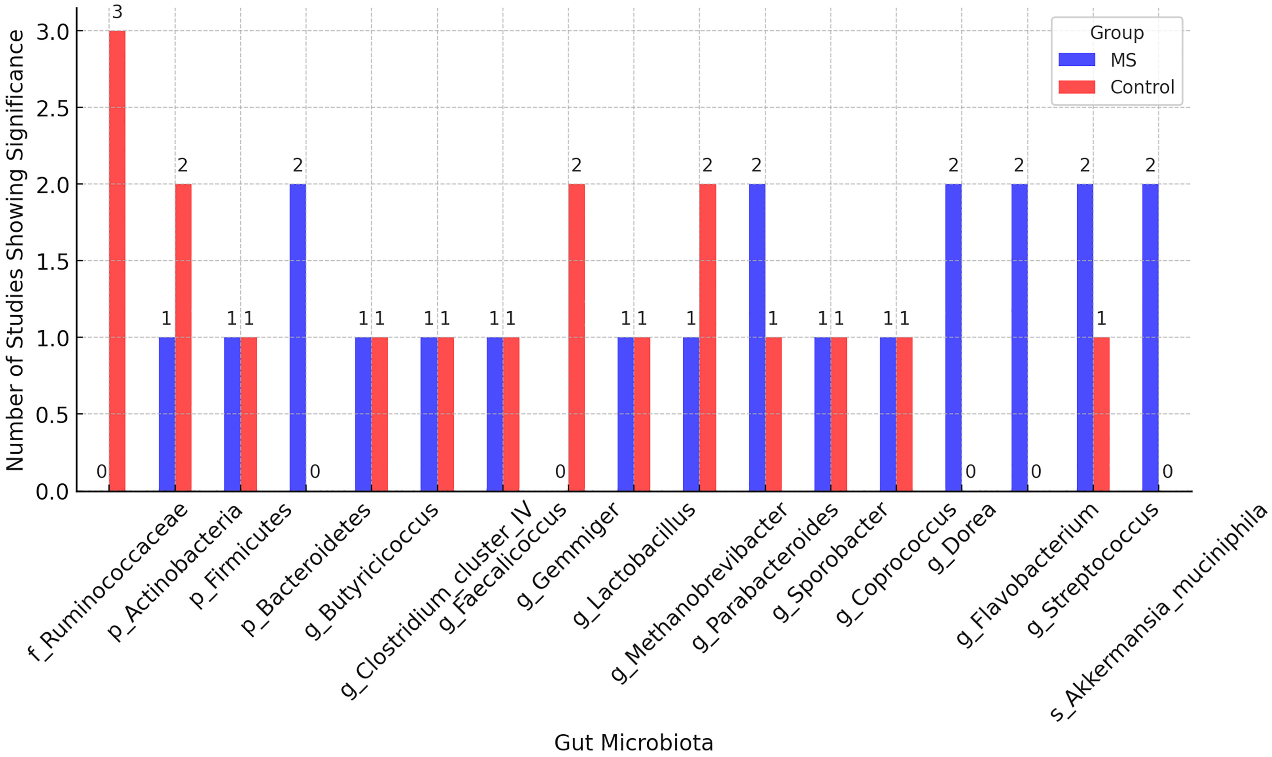

Fig. 5. Number of studies showing significant differences in key gut microbiota between the MS and Healthy Control Groups. s, specie.

Simpson index. Disease activity and phenotype were identified as primary sources of heterogeneity, as illustrated in **Supplementary Material-Figs. S1–S28**.

3.8 Publication Bias Analysis

Funnel plots and statistical tests, including Begg's and Egger's tests (Fig. 6 and Table 3, **Supplementary Material-Figs. S96–S126**), assessed publication bias. No significant bias was detected for most of the indices, suggesting that the studies included in the analysis were fairly representative and that publication bias was not a major concern. This enhances the confidence in the validity of the findings and strengthens the reliability of the meta-analysis results.

4. Discussion

Our meta-analysis, employing 16S rRNA gene sequencing, elucidates that the complexity of the gut microbiome in MS may not be captured by diversity indices alone. Although our findings align with the previous review [9], showing no significant differences in the Shannon, Chao1, OTU, and Simpson indices, our in-depth analyses based on disease activity and phenotype reveal pivotal narrative shifts.

We observed a notable decrease in bacterial diversity during the active phases of MS, as indicated by the Shannon index. This decrease suggests a link between disease exacerbation and microbial diversity reduction, highlighting the potential of microbial diversity as a biomarker for disease activity. Specifically, the reduction in diversity within the RRMS cohort underscores the dynamic microbial shifts accompanying distinct disease trajectories. Yet, the absence of significant differences in other diversity indices (Chao1, OTU, Simpson) across varied demographics and disease characteristics underscores the complex interplay of gut microbiota within MS pathology.

Analyzing the composition at the phylum, genus, and family levels revealed significant microbial alterations in MS, highlighting a disrupted microbial balance potentially contributing to disease mechanisms or reflecting underlying inflammatory processes. Notable changes include the differential abundance of certain families and phyla in MS patients compared to healthy controls. However, contrary to expectations, alterations in microbial composition did not consistently align with an increase in pro-inflammatory Bacteroidetes or a decrease in anti-inflammatory Firmicutes. This inconsistency suggests a complex relationship between microbial composition and MS pathophysiology.

At the genus level, the anticipated enrichment of Parabacteroides and reduction of Bifidobacterium did not consistently manifest in MS patients. The varied abundance of Methanobrevibacter and Akkermansia and the inconsistent decrease in Butyricimonas suggest that the role of specific bacteria in MS may be more intricate than previously thought [19,25,36].

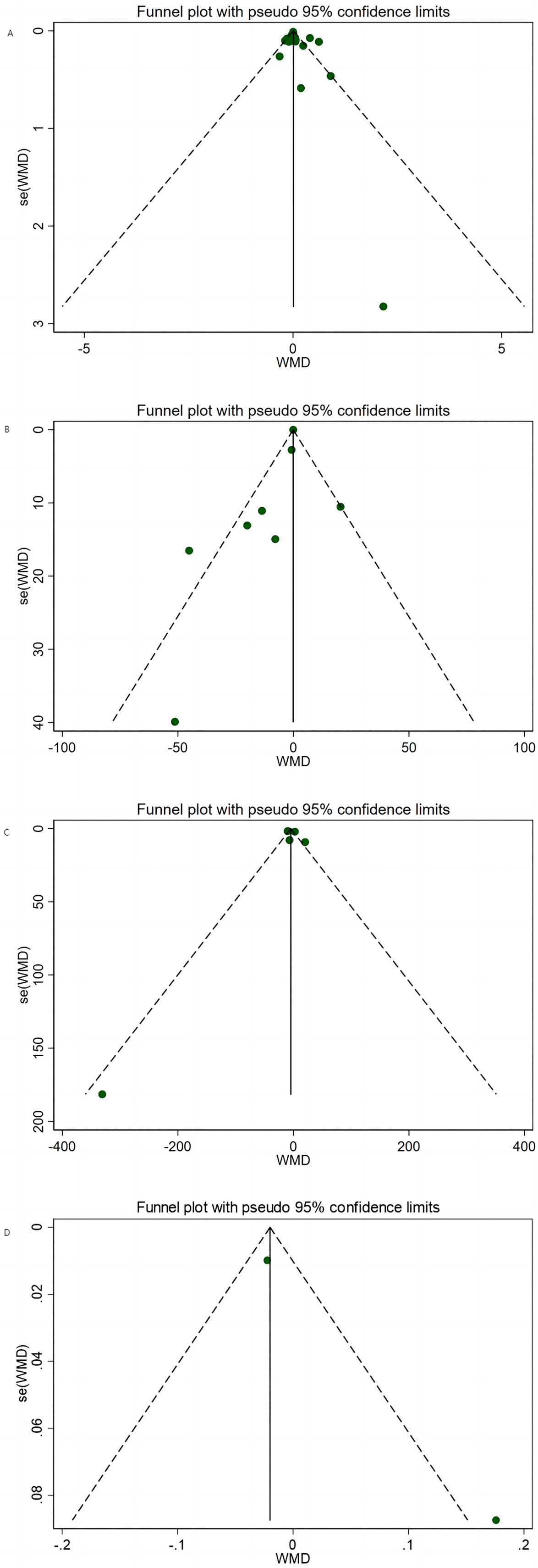

Fig. 6. Funnel plot of publication bias analysis result of gut microbiota alpha diversity. (A) Shannon. (B) Chao1. (C) OUT. (D) Simpson Indices.

Table 3. *p* value of Begg's test and Egger's test for meta analysis of gut microbiota and alpha diversities.

Outcome indicators	Begg's test	Egger's test
f_Ruminococcaceae	$p = 0.497$	$p = 0.315$
f_Bacteroidaceae	$p = 0.602$	$p = 0.560$
f_Prevotellaceae	$p = 0.317$	/
f_Lachnospiraceae	$p = 1.000$	$p = 0.445$
p_Actinobacteria	$p = 0.484$	$p = 0.357$
p_Bacteroidetes	$p = 1.000$	$p = 0.905$
p_Firmicutes	$p = 0.625$	$p = 0.904$
p_Fusobacteria	$p = 0.602$	$p = 0.200$
p_Proteobacteria	$p = 0.583$	$p = 0.438$
p_Euryarchaeota	$p = 0.297$	/
p_Verrucomicrobia	$p = 0.851$	$p = 0.600$
g_Akkermansia	$p = 0.602$	$p = 0.658$
g_Alistipes	$p = 0.117$	$p = 0.492$
g_Anaerostipes	$p = 0.317$	/
g_Bacteroides	$p = 0.624$	$p = 0.322$
g_Bifidobacterium	$p = 0.317$	/
g_Blautia	$p = 0.624$	$p = 0.312$
g_Butyricicoccus	$p = 0.317$	/
g_Clostridium	$p = 0.117$	$p = 0.612$
g_Dialister	$p = 0.117$	$p = 0.104$
g_Dorea	$p = 0.317$	/
g_Faecalibacterium	$p = 0.452$	$p = 0.151$
g_Fusobacterium	$p = 0.317$	/
g_Methanobrevibacter	$p = 0.497$	$p = 0.434$
g_Oscillibacter	$p = 0.602$	$p = 0.672$
g_Parabacteroides	$p = 0.602$	$p = 0.392$
g_Phascolarctobacterium	$p = 1.00$	/
g_Prevotella	$p = 0.621$	$p = 0.331$
g_Roseburia	$p = 0.317$	/
g_Ruminococcus *	$p = 0.050$	$p = 0.064$
g_Streptococcus	$p = 0.624$	$p = 0.466$
Shannon index	$p = 0.177$	$p = 0.286$
Simpson index	$p = 1.00$	/
Chao1 index *	$p = 0.048$	$p = 0.194$
OTU index	$p = 1.000$	$p = 0.889$

*Denotes that the *p*-value for either Begg's test or Egger's test was equal to or less than 0.05.

Incorporating clinical factors into our analysis revealed significant impacts of disease activity on the gut microbiome. Variations in bacterial abundance were evident between active RRMS patients and those with inactive disease. The study by Jangi suggest that immunomodulatory therapies might normalize some microbiome changes associated with MS, potentially contributing to therapeutic benefits [18]. However, the response to treatments like dimethyl fumarate appears varied, as Shah reported non-uniform changes in the gut mycobiome across MS patients [37]. Another study further stress the importance of microbiome studies in understanding the broad effects of immunomodulatory drugs [38]. These insights underline the need for further research to decipher the intricate mechanisms and full extent of these influences.

Recent research underscores the intricate connection between diet, the gut microbiome, and MS management. Dietary interventions, by modulating gut microbiota, play a crucial role in influencing inflammation and neuroinflammation [39,40], with studies pointing to the detrimental effects of Western diets [41] and the potential benefits of Mediterranean, low-carbohydrate, and fasting-mimicking diets. Additionally, the synergy of physical activity and dietary interventions opens new avenues for MS management [41].

Overall, our analysis of publication bias, indicating minimal bias, reinforces the credibility of our findings and their relevance in the broader MS research context. Despite facing limitations such as the scope and number of studies included, the diversity of study designs, and the observational nature of the data, our study highlights the need for more comprehensive, controlled longitudinal research. The absence of metagenomic and metatranscriptomic analyses to explore the microbiome's functional capacities points to an essential area for future investigation, crucial for understanding the metabolic pathways affected in MS and for developing microbiome-targeted therapeutic strategies.

5. Conclusion

In conclusion, while our analysis suggest that gut microbiota diversity may not significantly differ between MS patients and controls and do not support a simplified view of the gut microbiome in MS, they do emphasize the role of disease-related factors such as disease activity in shaping microbial communities. The potential for microbiome-targeted therapies in MS is promising, yet such approaches must be underpinned by a deeper understanding of the microbiome's interactions with host immunity and disease-modifying treatments. Future research should aim to delineate the temporal sequence of microbiome alterations, their impact on disease activity, and the influence of therapeutic interventions, guiding the development of tailored microbiome-focused treatments for MS.

Abbreviations

16S rRNA, 16S ribosomal RNA; BMI, Body Mass Index; CI, Confidence Interval; DMT, Disease-Modifying Therapy; EDSS, Expanded Disability Status Scale; f, family; g, genus; MS, Multiple Sclerosis; NA, Not Available; NOS, Newcastle-Ottawa Scale; OR, Odds Ratio; OTU, Operational Taxonomic Unit; p, phylum; PPMS, Primary Progressive Multiple Sclerosis; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO, International Prospective Register of Systematic Reviews; RRMS, Relapsing-Remitting Multiple Sclerosis; s, specie; SPMS, Secondary Progressive Multiple Sclerosis; SD, standard deviation; WMD, Weighted Mean Difference.

Availability of Data and Materials

Supplementary files supporting this study are included in the Supplementary Materials and are accessible through the online version of this article. For additional materials, please contact XYZ at zxycn86@outlook.com.

Author Contributions

Conceptualization, XYZ and YPH; Formal analysis, XYZ and ZL; Funding acquisition, YPH; Investigation, XYZ; Methodology, XYZ, ZQW and WWY; Writing—original draft, XYZ and ZL; Writing—review & editing, XYZ, ZQW and WWY. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors thank Home for Researchers (https://www.home-for-researchers.com/) for their writing assistance. We also used ChatGPT-3.5 for spelling and grammar checks. After using these tools, we thoroughly reviewed and edited the content, taking full responsibility for its accuracy and completeness.

Funding

This work was partially supported by the 2021 Basic Research Project of the Shenzhen Science, Technology, and Innovation Commission (ID: JCYJ20210324123414039, PI: YPH); the Scientific Research Projects of Medical and Health Institutions in Longhua District, Shenzhen (ID: 2022031, PI: YPH); and the Scientific Research Projects of Medical and Health Institutions in Longhua District, Shenzhen (ID: 2022027, PI: WWY).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2307127.

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