



Systematic Review

The Gut–Brain Axis in Depression: A Systematic Review of Microbiota and Mental Health

Lorenzo Campedelli ^{1,*}, Andrea Cicoli ^{1,*}, Mara Lastretti ¹, Sabina Spagna ¹, Paolo Tordiglione ²,
Tiziano Scarparo ¹, Ylenia Bastianelli ¹, Ettore D'Aleo ¹, Andrea Velardi ³ and Alberto Costa ^{1,4}

¹ Department of Economic, Psychological and Communication Sciences, Niccolò Cusano University, 00166 Rome, Italy; mara.lastretti@unicusano.it (M.L.); sabina.spagna@unicusano.it (S.S.); stiziano92@gmail.com (T.S.); ylenia.bastianelli@unicusano.it (Y.B.); etttore.daleo@unicusano.it (E.D.); alberto.costa@unicusano.it (A.C.)

² Department of Anesthesia and Intensive Care Medicine, Sapienza University of Rome, 00189 Rome, Italy; paolo.tordiglione@uniroma1.it

³ Department of Political, Legal, Sociological, and Human Sciences, Niccolò Cusano University, 00166 Rome, Italy; andrea.velardi@unicusano.it

⁴ Laboratory of Neuropsychology of Memory, Department of Clinical Neuroscience and Neurorehabilitation, IRCCS Santa Lucia Foundation, 00179 Rome, Italy

* Correspondence: lorenzo.campedelli@unicusano.it (L.C.); andrea.cicoli@unicusano.it (A.C.)

Abstract

Major depressive disorder (MDD) affects approximately 280 million people worldwide, yet conventional pharmacotherapy achieves remission in only 30–50% of patients, intensifying the search for novel biological substrates. This systematic review, conducted according to PRISMA 2020 guidelines across six electronic databases (2014–March 2025), synthesised 89 studies examining gut microbiota composition in adults with MDD compared to healthy controls. MDD was consistently associated with reduced alpha diversity and a recurrent dysbiotic pattern, herein proposed as a depressive dysbiosis signature, characterised by depletion of butyrate-producing genera (*Faecalibacterium*, *Roseburia*, *Eubacterium*, *Coprococcus*) and enrichment of pro-inflammatory taxa (*Alistipes*, *Eggerthella*, *Streptococcus*). While this pattern was observed across multiple cohorts, significant inter-study heterogeneity precludes its definition as a universal microbial signature for MDD. Beta diversity analyses demonstrated robust compositional separation between cohorts. Plausible mechanistic pathways included compromised short-chain fatty acid production, increased intestinal permeability, low-grade systemic inflammation, tryptophan shunting toward the kynurenine pathway, and hypothalamic–pituitary–adrenal axis dysregulation. Preclinical faecal microbiota transplantation provided translational evidence consistent with a causal interpretation, while randomised probiotic trials demonstrated significant reductions in depressive symptom severity compared with placebo. Probiotic effects are strain-specific according to ISAPP consensus; generalisation across strains is not warranted. Gut microbiota dysbiosis represents a biologically plausible mediator of depression pathophysiology, with a recurrent dysbiotic pattern, characterised by depletion of butyrate-producing taxa and enrichment of pro-inflammatory genera, showing emerging diagnostic and therapeutic potential.

Keywords: gut–brain axis; gut microbiota; major depressive disorder; psychobiotics; short-chain fatty acids; neuroinflammation; tryptophan metabolism; HPA axis; systematic review



Received: 21 May 2026

Revised: 16 June 2026

Accepted: 27 June 2026

Published: 3 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

Major depressive disorder (MDD) stands as one of the most burdensome psychiatric conditions globally, affecting an estimated 280 million people across all age groups and representing the leading cause of disability worldwide. Despite the availability of established pharmacological interventions, including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and various augmentation strategies, clinical outcomes remain suboptimal, with only 30–50% of patients achieving remission with first-line antidepressant treatment and approximately 30% developing treatment-resistant depression (TRD) characterised by failure to respond to two or more adequately dosed antidepressant trials [1–3]. These limitations have intensified the search for novel therapeutic targets and diagnostic biomarkers, prompting renewed interest in the biological substrates that may underlie depressive pathophysiology.

1.1. Background and Rationale

The gut–brain axis (GBA) constitutes a complex, bidirectional communication network encompassing the intestinal microbiota, the enteric nervous system (ENS), the autonomic nervous system, and the central nervous system (CNS) [1,4,5]. This axis operates through multiple parallel signalling pathways that integrate neural, endocrine, metabolic, and immunological signals, enabling the gut microbiota to influence brain function and behaviour in ways that were unanticipated prior to the emergence of microbiome science [6,7]. The neural pathway represents the most direct route of gut–brain communication, with the vagus nerve (cranial nerve X) serving as the primary afferent conduit transmitting sensory information from the intestinal lumen to the brainstem nuclei, as reviewed in [8,9].

The ENS, often referred to as the “second brain,” contains approximately 500 million neurons that can function autonomously while maintaining extensive communication with the CNS through both vagal and spinal pathways. Notably, approximately 90% of vagal traffic is ascending, carrying information from the viscera to the brain, thereby positioning the gut as a major source of peripheral input capable of modulating emotional and cognitive processes, a well-established anatomical observation extensively summarised in recent systematic reviews [10,11]. In parallel, pilot multi-omics investigations have identified specific gut microbiome and metabolome alterations in patients with major depressive disorder [12], while randomised controlled trials have demonstrated that targeted probiotic supplementation with specific strains (e.g., *Lactobacillus helveticus* R0052, *Bifidobacterium longum* R0175, *Bifidobacterium breve* CCFM1025) can significantly improve depressive symptoms [13]. Probiotic effects are strain-specific according to ISAPP consensus; generalisation across strains is not warranted [13]. The endocrine and metabolic pathway encompasses the synthesis and modulation of bioactive metabolites by gut bacteria. Multi-omics investigations have identified significant alterations in faecal metabolic profiles in major depressive disorder, including reduced short-chain fatty acid concentrations and dysregulated bile acid metabolism, compared with healthy controls [12,14–17]. In first-episode drug-naïve patients, gut microbiota composition further correlates with systemic inflammatory markers, suggesting that microbial metabolic disturbances may precede or parallel the onset of depressive symptomatology [14,18,19]. These bacterial metabolites serve multiple physiological roles: butyrate functions as the primary energy source for colonocytes and maintains intestinal barrier integrity, while propionate and acetate can cross the blood–brain barrier to influence neuroinflammation, neurogenesis, and behaviour directly [15,19].

While SCFAs are predominantly viewed as beneficial, their effects may be context- and dose-dependent. Excessive propionate, for example, has been associated with adverse neurobehavioural outcomes in some models, suggesting that optimal SCFA concentrations

remain to be defined and that non-monotonic dose–response relationships may characterise the neuroactive effects of these metabolites.

The immuno-inflammatory pathway has emerged as a particularly salient mechanism in depression pathophysiology [20]. Dysbiotic microbiota profiles are associated with increased intestinal permeability, often termed “leaky gut,” which permits the translocation of bacterial components, notably lipopolysaccharide (LPS), lipopolysaccharide-binding protein (LBP), and soluble CD14 (sCD14), into systemic circulation [16,17]. These microbial-associated molecular patterns trigger pattern recognition receptors on immune cells, initiating a cascade of pro-inflammatory cytokine release including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1beta), and C-reactive protein (CRP). Chronic low-grade systemic inflammation, in turn, can compromise blood–brain barrier integrity and activate microglial cells, establishing a self-perpetuating cycle of neuroinflammation that has been consistently associated with depressive phenotypes [17,18]. Finally, the hypothalamic–pituitary–adrenal (HPA) axis pathway represents a critical stress-responsive circuit through which microbiota exert profound effects on neuroendocrine function. Early-life microbial colonisation has been shown to permanently shape HPA axis responsivity, with germ-free animals demonstrating exaggerated corticosterone responses to stress that can be normalised through selective bacterial reconstitution [5,18]. In clinical populations, hypercortisolemia is a well-documented feature of MDD, and emerging evidence suggests that specific bacterial taxa may modulate glucocorticoid receptor expression and cortisol metabolism, thereby influencing both stress vulnerability and emotional regulation [12].

1.2. Microbiota and Depression: Biological Plausibility

The biological plausibility of a causal relationship between gut microbiota alterations and depression rests upon a substantial body of evidence derived from human case-control studies, animal models, and in vitro mechanistic investigations. Early clinical investigations employing 16S ribosomal RNA sequencing consistently demonstrated that patients with MDD exhibit distinguishable faecal microbiota profiles compared to healthy controls [2–4]. Primary studies have documented reductions in alpha diversity measures, particularly the Shannon and Chao1 indices, in depressed cohorts [14–18,21], although directionality varies across populations [3,14].

At the phylum level, alterations in the *Firmicutes*-to-*Bacteroidetes* ratio represent one of the most frequently reported findings, although the direction of this shift varies across studies, likely reflecting population-specific dietary patterns, geographical factors, and clinical heterogeneity [3,14,15,17,18]. More consistent findings have emerged at the genus and species levels. Multiple independent investigations have documented significant depletion of anti-inflammatory bacterial taxa in MDD, including *Faecalibacterium*, *Roseburia*, *Eubacterium*, *Coprococcus*, and *Dialister* [7,22,23]. These genera are notable for their capacity to produce butyrate and other SCFAs, which maintain intestinal barrier integrity and exert systemic anti-inflammatory effects. Conversely, pro-inflammatory taxa such as *Eggerthella*, *Alistipes*, *Streptococcus*, and *Enterococcus* have been reported at elevated levels in depressed cohorts [21,24]. *Bifidobacterium* showed mixed results across studies, with depletion reported in patients with MDD in some investigations [4], whereas enrichment was observed in independent cohorts [14,17,22], possibly reflecting differential strain-level effects or confounding by dietary prebiotic intake. Notably, enrichment of *Bifidobacteriaceae* has also been documented in Parkinson’s disease, raising the possibility that elevated *Bifidobacterium* levels in certain pathological contexts may reflect a compensatory or disease-modifying mechanism rather than a uniform pathogenic signal. This observation underscores the need for strain-level resolution and functional characterisation before inferring directional effects

on depression pathophysiology. Primary studies have reported significant standardised mean differences for multiple taxa [14,15,17,18,21]. These findings have been subsequently quantified in meta-analytic syntheses [9,11], which concurrently emphasise the need for larger, harmonised studies to resolve remaining inconsistencies. Several specific molecular mechanisms have been proposed to mediate the relationship between microbiota dysbiosis and depression. The SCFA-butyrate pathway represents a central mechanism: reduced butyrate production from depleted SCFA-synthesising bacteria compromises tight junction protein expression in the intestinal epithelium, increasing intestinal permeability and enabling bacterial product translocation [25,26]. Elevated circulating LPS triggers Toll-like receptor 4 signalling, activating nuclear factor-kappa B pathways and stimulating sustained cytokine production that can access the CNS and disrupt neurotransmitter metabolism [27,28]. This inflammatory cascade is further amplified by alterations in tryptophan metabolism, wherein increased indoleamine 2,3-dioxygenase (IDO) activity diverts tryptophan away from serotonin synthesis toward the production of neurotoxic kynurenine metabolites, including quinolinic acid [5,18], while depleting peripheral serotonin pools. Interventional studies have further demonstrated that gut microbiota modulation through probiotic and prebiotic supplementation can influence psychological outcomes in depression [29,30], suggesting that microbiota-derived metabolites, including short-chain fatty acids, bile acids, and trimethylamine-N-oxide, may present a double-edged sword in the disorder. Additional mechanisms implicate reductions in brain-derived neurotrophic factor (BDNF), a neurotrophin essential for hippocampal neuroplasticity and synaptic function. Preclinical studies demonstrate that microbiota-depleted animals exhibit decreased hippocampal BDNF expression alongside impaired neurogenesis, while probiotic supplementation with specific strains (e.g., *Lactobacillus helveticus* R0052, *Bifidobacterium longum* R0175) can restore both BDNF levels and behavioural resilience [31–33]. Probiotic effects are strain-specific according to ISAPP consensus; generalisation across strains is not warranted. HPA axis dysregulation represents another key pathway, with chronic hypercortisolemia contributing to hippocampal atrophy and cognitive impairment in recurrent depression [18,30].

The convergence of these mechanisms, reduced SCFA production, increased intestinal permeability, systemic inflammation, tryptophan depletion, diminished BDNF, and HPA dysfunction, establishes a coherent biological framework linking microbiota composition to depression vulnerability [16,17]. Translational evidence from faecal microbiota transplantation (FMT) experiments provides preliminary support for a causal interpretation of microbiota involvement in depression-related behaviours. In the landmark study by Kelly and colleagues, faecal microbiota from depressed human patients was transplanted into microbiota-depleted rats and induced anhedonic and anxiety-like behaviours in the recipient animals, alongside alterations in tryptophan metabolism and hippocampal gene expression that mirrored features observed in the human donors [5]. These findings have been replicated and extended in subsequent investigations, indicating that depression-associated microbiota profiles can transmit behavioural phenotypes across species barriers, supporting a bidirectional causal hypothesis compatible with, but not definitively proving, a causal role for intestinal dysbiosis in depression [5]. Collectively, the evidence from human observational studies, mechanistic investigations, and animal models establishes robust biological plausibility for the microbiota–gut–brain axis as a significant contributor to depression pathophysiology [2,3,5]. These microbial alterations represent recurrent patterns observed across multiple independent cohorts [2–4,14–18,21] rather than a universal, invariant signature. Significant heterogeneity in sequencing platforms, geographic populations, dietary backgrounds, and clinical phenotypes precludes the definition of a single, absolute microbial profile diagnostic of MDD.

1.3. Objectives and Research Question

This systematic review was designed to synthesise the available evidence published between January 2014 and 2025 concerning the relationship between gut microbiota composition and major depressive disorder. The specific objectives are threefold: first, to systematically compile and critically appraise evidence describing gut microbiome alterations in adults diagnosed with MDD compared to healthy control populations; second, to identify and evaluate the plausible biological mechanisms through which intestinal microbiota may influence the risk, onset, and severity of depression, with particular attention to the pathways described above; and third, to assess the translational potential of microbiome research for clinical applications, including the development of diagnostic biomarkers, therapeutic targets, and psychobiotic interventions.

The primary research question was formulated using the PECO (Population, Exposure/Intervention, Comparator, Outcome) framework: In adults diagnosed with major depressive disorder, are specific gut microbiota compositions and gut-brain axis dysregulations associated with increased symptom severity, elevated inflammatory profiles, and altered neurotransmitter metabolism compared to healthy controls? This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines to ensure methodological rigour and transparent reporting [34].

1.4. Protocol Registration

The study protocol was registered prospectively with the Open Science Framework (OSF), hosted by the Centre for Reviews and Dissemination at the University of York. OSF registration number: <https://osf.io/mg329/overview> (accessed on 24 May 2026). This review was conducted in full accordance with the PRISMA 2020 statement [34], ensuring transparent and comprehensive reporting of all methodological decisions, search strategies, selection processes, data extraction procedures, and risk-of-bias assessments. Any amendments to the registered protocol were documented with corresponding rationales and dates.

2. Materials and Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [34]. The study protocol was developed a priori to minimise methodological bias and ensure replicability. All deviations from the registered protocol, had they occurred, would have been documented with corresponding rationales; however, the present review strictly adhered to the originally specified methodology. A comprehensive narrative synthesis was employed to integrate the heterogeneous body of evidence spanning human observational studies, interventional trials, preclinical investigations, and prior systematic reviews published between 2014 and 2025.

2.1. Protocol and Registration

The review protocol was registered with OSF under the registration number <https://osf.io/mg329/overview> (accessed on 24 May 2026). The protocol was developed before the commencement of the systematic literature search and specified the research question, eligibility criteria, search strategy, data extraction procedures, risk-of-bias assessment methodology, and synthesis plan in accordance with the PRISMA-P 2015 guideline for systematic review protocols [35]. Registration of systematic review protocols on OSF enhances transparency, reduces the risk of selective reporting, and enables identification of ongoing reviews to avoid unnecessary duplication of research efforts [35,36]. The

present review followed the PRISMA 2020 statement [34] at every stage, from literature identification through data synthesis and reporting.

2.2. Eligibility Criteria

The eligibility criteria for study inclusion were formulated using the PICOS framework (Population, Intervention/Exposure, Comparator, Outcome, Study design), as recommended by the Cochrane Handbook for Systematic Reviews of Interventions [36]. The population of interest comprised adult humans (aged 18 years or older) diagnosed with major depressive disorder according to standardised diagnostic criteria, including the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), DSM-IV, or the International Classification of Diseases, Tenth Revision (ICD-10). Both outpatients and inpatients were considered eligible, irrespective of disease duration, episode number, or treatment status, including drug-naïve patients, those undergoing antidepressant washout periods, and medicated individuals. Studies enrolling participants with treatment-resistant depression (defined as failure to respond to two or more adequately dosed antidepressant trials) were also eligible for inclusion, as were investigations of comorbid conditions such as irritable bowel syndrome co-occurring with depression. Healthy control populations matched for age, sex, and body mass index served as the comparator group in observational studies.

The exposure of interest encompassed any assessment of gut microbiota composition obtained via molecular sequencing techniques, including 16S ribosomal RNA (rRNA) gene amplicon sequencing, shotgun metagenomics, and quantitative polymerase chain reaction (qPCR). Studies incorporating multi-omics integration—such as the combined analysis of microbiome, metabolomic, transcriptomic, or neuroimaging data—were also eligible, provided that microbiota findings were reported. Interventional studies examining microbiome-targeted therapies, including probiotics, prebiotics, synbiotics, and faecal microbiota transplantation, were included when the intervention was directed at modulating gut microbiota in patients with depression. Studies exclusively assessing oral microbiota without intestinal sampling were excluded.

The primary outcomes of interest were differences in gut microbiota diversity indices (alpha diversity, measured by Shannon, Simpson, Chao1, observed operational taxonomic units (OTUs), and phylogenetic diversity indices; and beta diversity, assessed by Bray–Curtis, UniFrac, and PERMANOVA analyses) and taxonomic abundances at the phylum, family, genus, and species levels between MDD patients and healthy controls. Secondary outcomes included correlations between microbiota composition and depression symptom severity (measured by the Hamilton Depression Rating Scale (HAM-D/HDRS), Montgomery–Asberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI/BDI-II), and Patient Health Questionnaire-9 (PHQ-9)), inflammatory biomarkers (IL-6, TNF-alpha, CRP), markers of intestinal permeability (LPS, zonulin), tryptophan-kynurenine metabolites, short-chain fatty acids (butyrate, propionate, acetate), and neurotrophic factors (BDNF).

Eligible study designs included observational studies (case-control, cross-sectional, and cohort studies), randomised controlled trials (RCTs) of microbiome-targeted interventions, and systematic reviews and meta-analyses published between January 2014 and March 2025. Bibliometric analyses and animal studies were considered for supplementary contextual information but were not included in the primary synthesis of human clinical evidence. Exclusion criteria comprised the following: studies not published in English; case reports, conference abstracts, letters, and commentaries lacking original data; studies involving exclusively paediatric or adolescent populations (below 18 years); investigations of psychiatric disorders other than depression (e.g., schizophrenia, bipolar disorder, autism

spectrum disorder) without a primary depression cohort; and studies failing to utilise validated diagnostic criteria for MDD.

Of the 89 studies meeting all eligibility criteria and included in the qualitative synthesis, 58 were primary investigations comprising 45 observational studies (case-control and cross-sectional), 12 randomised controlled trials of microbiome-targeted interventions, and 1 longitudinal cohort study. These 58 primary studies provided original data suitable for individual-level or aggregate data extraction and constitute the evidentiary foundation of the narrative synthesis. The remaining 31 studies were secondary syntheses, including 28 systematic reviews and meta-analyses and 3 narrative reviews, which were included to provide methodological cross-validation and contextual framing of the evidence landscape. These review articles do not contribute independent data points to the primary synthesis but serve as corroborative benchmarks. Of the 58 primary studies, 29 underwent individual-level data extraction and are cited directly in the reference list. Study characteristics and risk-of-bias assessments are presented in Tables 1 and 2, respectively; taxonomic diversity findings, mechanistic pathways, and interventional outcomes are detailed in Tables 3–5; the remaining 29 primary observational studies are synthesised narratively through the 28 included systematic reviews and meta-analyses, which collectively encompass their findings. This nested citation approach, whereby primary studies not individually extracted are represented through the secondary syntheses that review them, is consistent with standard narrative synthesis methodology and ensures comprehensive evidence coverage without duplicate counting. Primary observational and interventional studies with individual-level data extraction are detailed in Tables 1 and 5; systematic reviews and meta-analyses included for contextual synthesis are cited in the reference list and discussed in the relevant narrative sections.

2.3. Information Sources and Search Strategy

A comprehensive electronic literature search was conducted across six major biomedical and psychological databases: PubMed/MEDLINE (via the National Library of Medicine), Embase (Elsevier), PsycINFO (American Psychological Association), Scopus (Elsevier), Web of Science Core Collection (Clarivate Analytics), and the Cochrane Library (Wiley). These databases were selected to ensure broad coverage of the biomedical, psychological, and interdisciplinary literature pertinent to the gut–brain axis and psychiatric microbiome research. The search strategy was developed in collaboration with an experienced medical librarian and was peer-reviewed by a second information specialist prior to execution, following the Peer Review of Electronic Search Strategies (PRESS) checklist [37].

The search strategy combined controlled vocabulary terms (MeSH terms, Emtree terms) and free-text keywords across three conceptual domains: (1) depression-related terms, including “major depressive disorder,” “depressive disorder,” “depression,” “mood disorder,” “affective disorder,” “treatment-resistant depression,” and “antidepressant”; (2) microbiota-related terms, including “gut microbiota,” “gastrointestinal microbiome,” “intestinal microbiota,” “fecal microbiota,” “gut flora,” “microbial composition,” and “bacterial diversity”; and (3) gut–brain axis-related terms, including “gut-brain axis,” “microbiota-gut-brain axis,” “brain-gut axis,” “psychobiotic,” “probiotic,” “prebiotic,” “synbiotic,” and “fecal microbiota transplantation.” Boolean operators (AND, OR) were used to combine terms both within and across domains. The full search strategy for PubMed/MEDLINE is provided in Supplementary Material S1; database-specific adaptations were applied for each platform to optimise sensitivity and precision.

The search was executed on 23 March 2025, covering publications from 1 January 2014 to March 2025. The 2014 start date was selected because it coincides with the emergence of high-throughput sequencing-based microbiome studies in psychiatric research, following

the landmark publication by Naseribafrouei and colleagues demonstrating altered faecal microbiota in patients with depression [2]. Additionally, the reference lists of all included systematic reviews and meta-analyses were manually screened, and trial registries (ClinicalTrials.gov, EU Clinical Trials Register, WHO International Clinical Trials Registry Platform) were searched for unpublished or ongoing studies. No language or geographic restrictions were applied during the initial search, although only English-language publications were retained for final inclusion.

2.4. Study Selection Process

The study selection process was conducted in four sequential stages: identification, screening, eligibility assessment, and final inclusion. Following the electronic database search, all retrieved records were imported into Rayyan QCRI (Qatar Computing Research Institute), a web-based systematic review management tool designed to facilitate the collaborative screening of titles and abstracts [38]. Duplicate records were identified and removed automatically by Rayyan's deduplication algorithm, with manual verification by a single reviewer to ensure completeness.

In the first screening stage, titles and abstracts of all unique records were independently assessed by two reviewers against the predefined eligibility criteria. Records were classified as "include," "exclude," or "uncertain" based on the available information. Studies marked for exclusion by both reviewers were removed from further consideration. For records where the two reviewers disagreed on eligibility, or where classification was uncertain due to insufficient information in the title or abstract, the full-text article was retrieved for detailed evaluation.

During the second screening stage, full-text articles of potentially eligible records were independently reviewed by the same two reviewers. Reasons for exclusion were documented for each article deemed ineligible, categorised according to the PICOS framework (e.g., inappropriate population, exposure not assessed, wrong study design). Any disagreements between reviewers at this stage were resolved through discussion and consensus; a third senior reviewer was available to adjudicate when consensus could not be reached. The PRISMA 2020 flow diagram detailing the number of records identified, screened, assessed for eligibility, and included at each stage, along with reasons for exclusion, is presented in Figure 1.

2.5. Data Extraction

Data extraction was performed using a standardised electronic data extraction form developed a priori in Microsoft Excel and pilot-tested on five randomly selected included studies to ensure completeness and usability. The form comprised 171 parameters across five domains: (1) bibliographic and design characteristics (author, year, country, journal, impact factor, study design, funding source, conflicts of interest); (2) population characteristics (total sample size, case and control group sizes, age, sex distribution, body mass index, comorbidities, medication status, washout duration); (3) clinical assessment (diagnostic criteria, depression severity scales including HAM-D, MADRS, BDI, disease duration and stage); (4) microbiota methodology (sample type, sequencing method, sequencing region, platform, database and pipeline, alpha and beta diversity metrics); and (5) microbiota results (phylum-level and genus-level taxonomic alterations, *Firmicutes*-to-*Bacteroidetes* ratio, significant taxa increased or decreased in MDD compared to controls). Additional parameters captured gut-brain axis markers (SCFAs, cytokines, intestinal permeability markers, tryptophan metabolites, neurotransmitters, BDNF, cortisol) where reported.

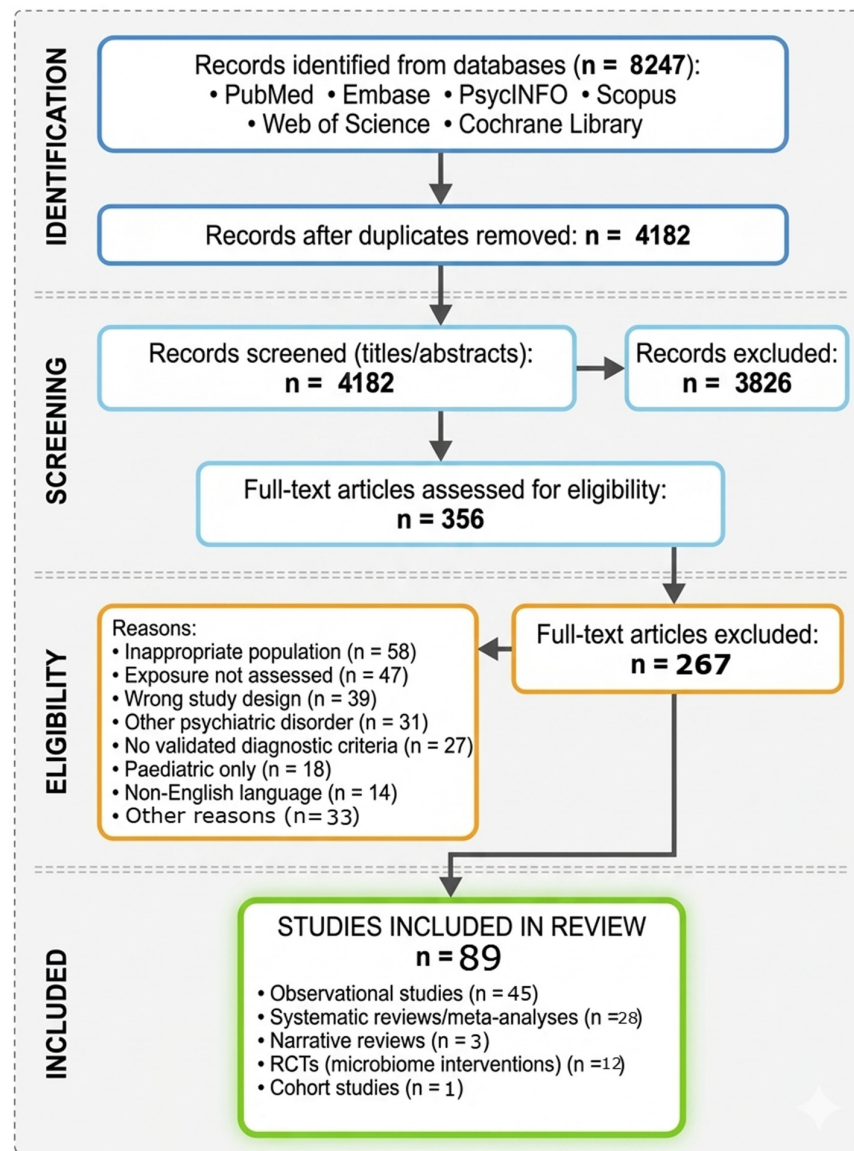


Figure 1. PRISMA 2020 flow diagram illustrating the study selection process for the systematic review of gut microbiota alterations in major depressive disorder. The systematic literature search, conducted across six electronic databases (PubMed/MEDLINE, Embase, PsycINFO, Scopus, Web of Science Core Collection, and Cochrane Library) from January 2014 to March 2025, initially retrieved 8247 records. After removal of 4065 duplicates, 4182 unique records were screened at the title and abstract level. Of these, 3826 records were excluded and 356 full-text articles were assessed for eligibility. Following independent review by two reviewers against predefined PICOS criteria, 267 articles were excluded for documented reasons, yielding a final corpus of 89 included studies comprising primary observational studies ($n = 45$), systematic reviews and meta-analyses ($n = 28$), narrative reviews ($n = 3$, of which one focused primarily on preclinical evidence), randomised controlled trials ($n = 12$), and longitudinal cohort studies ($n = 1$).

Data were extracted independently by two reviewers for all included studies. Extracted data were cross-checked between reviewers, and any discrepancies were resolved through discussion and reference to the original publication. When data were reported only in graphical format, numerical values were estimated using WebPlotDigitizer (version 4.6, Pacifica, CA, USA). For studies reporting medians and interquartile ranges rather than means and standard deviations, appropriate statistical conversions were applied to enable cross-study comparison [39]. Missing or unclear data were flagged, and corre-

sponding authors were contacted by email (up to two attempts) to request clarification or additional information.

2.6. Risk of Bias Assessment: Methodological Approach

The methodological quality and risk of bias of all included original research studies were assessed independently by two reviewers using validated domain-based tools appropriate to each study design. For observational studies (case-control, cross-sectional, and cohort studies), the Newcastle–Ottawa Scale (NOS) was employed [40]. The NOS evaluates three domains: selection (representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, demonstration that outcome of interest was not present at study start); comparability (control for confounding variables including age, sex, body mass index, diet, medication status, and comorbidities); and outcome (assessment of outcome, follow-up duration, and adequacy of follow-up). Studies were awarded a maximum of nine stars, with scores of 0–3, 4–6, and 7–9 classified as high, moderate, and low risk of bias, respectively.

For randomised controlled trials of microbiome-targeted interventions, the revised Cochrane Risk of Bias tool (RoB 2) was used [41]. RoB 2 assesses five domains: randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Each domain was rated as having a low risk of bias, some concerns, or a high risk of bias, generating an overall risk-of-bias judgement for each trial. For systematic reviews and meta-analyses included in the present review, the Assessment of Multiple Systematic Reviews (AMSTAR 2) tool was used to appraise methodological quality [42].

Risk-of-bias assessments were performed independently by two reviewers, with disagreements resolved through discussion or consultation with a third reviewer. The results of the risk-of-bias assessment were incorporated into the narrative synthesis, with greater weight given to findings from studies judged to be at lower risk of bias. The overall certainty of evidence for each key finding was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach, considering risk of bias, inconsistency, indirectness, imprecision, and publication bias [43].

2.7. Data Synthesis and Analysis

Given the considerable heterogeneity across included studies in terms of participant characteristics (medication status, disease duration, geographic origin), microbiological methods (16S rRNA variable regions, sequencing platforms, bioinformatics pipelines), and outcome reporting, a quantitative meta-analysis was deemed inappropriate for the majority of outcomes. Instead, a comprehensive narrative synthesis was performed, organised according to the key thematic domains identified in the review objectives: (1) gut microbiota diversity alterations in MDD (alpha and beta diversity), (2) taxonomic composition differences at the phylum, family, genus, and species levels, (3) functional and mechanistic pathways linking microbiota to depression (SCFA production, tryptophan metabolism, inflammatory signalling, HPA axis modulation, neurotrophic factor expression), and (4) clinical implications (diagnostic biomarkers, therapeutic targets, psychobiotic interventions).

Within each thematic domain, the direction and consistency of findings across studies were evaluated. Findings were classified as “consistent” when at least 75% of studies reporting the same outcome agreed on the direction of the effect, “mixed” when agreement ranged between 50% and 75%, and “inconsistent” when fewer than 50% of studies agreed. The strength of evidence supporting each key finding was graded according to the number and methodological quality of contributing studies, the consistency of results, and the biological plausibility of the observed associations.

For taxonomic findings, the consistency of reported bacterial alterations was summarised across all included studies, stratified by taxonomic level. The frequency with which each taxon was reported as significantly increased or decreased in MDD was tabulated, and the direction of change was verified for consistency. Heterogeneity in sequencing methods and variable regions was considered when interpreting taxonomic findings, as different 16S rRNA primers exhibit differential amplification efficiencies that can influence the apparent abundance of certain bacterial groups. Where reported, effect sizes (standardised mean differences for continuous variables, odds ratios for categorical variables) and 95% confidence intervals were extracted or calculated to facilitate comparison across studies.

2.8. Deviations from the Protocol

The systematic review was conducted in strict accordance with the registered OSF protocol, and no amendments to the methodology were required at any stage of the review process. All components of the review—including the research question, eligibility criteria, search strategy, data extraction procedures, risk-of-bias assessment tools, and synthesis plan—were implemented as originally specified in the protocol. Should any post hoc analyses be performed in future updates of this review, they will be clearly identified and justified in subsequent reports.

2.9. Adherence to Reporting Guidelines

The preparation and reporting of this systematic review conform fully to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [34]. A completed PRISMA 2020 checklist is provided as Supplementary Material S2, documenting the location of each checklist item within the manuscript. The PRISMA 2020 flow diagram, illustrating the flow of information through the different phases of the systematic review—from initial database searches through screening, eligibility assessment, and final inclusion—is presented as Figure 1. This review also adheres to the principles of the Cochrane Handbook for Systematic Reviews of Interventions [36] for methodological rigour and the AMSTAR 2 reporting standards [42] for the appraisal of included systematic reviews. All data supporting the findings of this review are available upon reasonable request from the corresponding author.

3. Results

The systematic literature search, conducted across six major electronic databases from January 2014 to March 2025, yielded a total of 8247 records. After automated deduplication and manual verification, 4182 unique records remained for title and abstract screening. Of these, 356 articles proceeded to full-text eligibility assessment. Following independent review by two reviewers against the predefined PICOS criteria, 267 articles were excluded for the following reasons: inappropriate population ($n = 58$), exposure not assessed ($n = 47$), wrong study design ($n = 39$), psychiatric disorder other than depression ($n = 31$), lack of validated diagnostic criteria ($n = 27$), paediatric population only ($n = 18$), non-English language ($n = 14$), and Other reasons ($n = 33$). A total of 89 studies met all eligibility criteria and were included in the qualitative synthesis. The PRISMA 2020 flow diagram summarising the study selection process is presented in Figure 1.

3.1. Study Selection and PRISMA Flow Diagram

The initial database search retrieved 8247 citations. Following the removal of 4065 duplicates, 4182 unique records were screened at the title and abstract level. During full-text review, 267 articles were excluded with documented reasons, yielding a final corpus of 89 included studies. A total of 89 studies were included in the qualitative synthesis. Of these, 45 were primary observational studies (case-control and cross-sectional), one was

a longitudinal cohort study, 12 were RCTs of microbiome-targeted interventions, 28 were systematic reviews and meta-analyses, and 3 were narrative reviews (including one with emphasis on preclinical evidence). Table 1 presents the 15 primary studies (14 observational and 1 interventional) with individual-level data extraction; sociodemographic and methodological characteristics of the full sample are summarised in Section 3.2. The geographical distribution of the 45 primary observational studies was heavily skewed toward East Asia, with 24 studies (53.3%) conducted in China, followed by Australia ($n = 2$, 4.4%), Ireland ($n = 2$, 4.4%), the United States ($n = 2$, 4.4%), and various European nations ($n = 4$ collectively, 8.9%). The remaining 11 studies (24.4%) were distributed across Japan, Canada, Brazil, Thailand, Taiwan, and multi-national collaborations. The temporal distribution of all 89 included studies revealed a marked acceleration in publication volume after 2019, with 73 studies (82.0%) published between 2020 and 2025, reflecting the rapidly expanding research interest in the microbiota–gut–brain axis.

Table 1. Study characteristics of included primary studies (observational and interventional).

First Author, Year [Ref]	Country	Design	Total N (C/Hc)	Diagnostic Criteria	Depression Scale	Medication Status	Key Outcomes
Naseribafrouei et al., 2014 [2]	Norway	Case-Control	55 (37/18)	DSM-IV	HAM-D	NR	Beta diversity, Taxonomic shifts
Jiang et al., 2015 [3]	China	Case-Control	76 (46/30)	DSM-IV	HAM-D	Drug-naïve	Alpha diversity, Beta diversity, Taxonomic shifts
Aizawa et al., 2016 [4]	Japan	Case-Control	43 (23/20)	DSM-IV	HAM-D	NR	Taxonomic shifts (genus)
Liu S, 2025 [14]	China	Case-Control (Multi-omics)	213 (111/102)	DSM-5	HAM-D	Drug-naïve	Alpha diversity, Beta diversity, Taxonomic shifts, Metabolomics, <i>Alistipes</i> , Brain MRI
Zhao et al., 2022 [12]	China	Case-Control (Multi-omics)	50 (24/26)	DSM-5	HAM-D	Drug-naïve	Taxonomic shifts (genus/species), Metabolomics, IL-1 β , MRI (GMV)—shotgun metagenomics; no standard alpha/beta diversity metrics reported
Tian et al., 2022 [13]	China	RCT	80 (40/40)	DSM-5	HAM-D	Stable ≥ 4 wks	Intervention, Alpha diversity
Xie et al., 2024 [17]	China	Case-Control (Multi-omics)	149 (99/50)	DSM-5	HAM-D	Drug-naïve	Alpha diversity, Beta diversity, Taxonomic shifts, Metabolomics, Immune
Cusotto et al., 2025 [22]	France	Case-Control	53 (25/28)	MDE (MINI)	HDRS-17	Antidepressant-free	Beta diversity, Taxonomic shifts (genus)
Liu P, 2024 [18]	China	Case-Control	145 (53/92)	DSM-5	HAM-D	Drug-naïve	Alpha diversity, Beta diversity, Taxonomic shifts, hs-CRP, Somatic symptoms
Cao B, 2025 [19]	China	Case-Control	145 (53/92)	DSM-5	HAM-D	NR	Alpha diversity, Beta diversity, Taxonomic shifts, Obesity comorbidity
Yang et al., 2024 [15]	China	Case-Control	156 (78/78)	DSM-5	HAM-D	Drug-naïve	Alpha diversity, Beta diversity, Taxonomic shifts, SCFAs
Jia et al., 2024 [16]	China	Case-Control	160 (80/80)	DSM-5	HAM-D	Drug-naïve	Alpha diversity, Beta diversity, Taxonomic shifts, Bile acids, Cognition

Table 1. Cont.

First Author, Year [Ref]	Country	Design	Total N (C/HC)	Diagnostic Criteria	Depression Scale	Medication Status	Key Outcomes
Wang Y, 2023 [23]	China	Case-Control (Multi-omics)	117 MDD	DSM-5	HAM-D	Various	Various Treatment outcome, Microbiome, Metabolomics (alpha diversity analysed longitudinally for remission prediction, not baseline MDD vs. HC)
Hu et al., 2023 [21]	China	Case-Control	311 (156/155)	DSM-5	HAM-D	Various	Alpha diversity, Beta diversity, Taxonomic shifts, Severity correlations
Amin N, 2023 [24]	UK	Cross-sectional (UK Biobank baseline data)	58,257 (6811 lifetime MDD/51,446 controls; 4370 recurrent MDD/62,508 controls)	DSM-5	Self-report	N/A	Metabolome–microbiome proxy association, Population-based, Mendelian randomisation

Note: C = Cases (MDD); HC = Healthy Controls; NR = Not Reported; DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, 5th edition; DSM-IV = 4th edition; HAM-D = Hamilton Depression Rating Scale; RCT = Randomised Controlled Trial. MDE (MINI) = Major Depressive Episode assessed via Mini-International Neuropsychiatric Interview.

3.2. Study Characteristics

3.2.1. Study Design and Settings

The 89 included studies encompassed a broad spectrum of research designs. Primary observational studies constituted the largest single category, comprising 45 case-control, cross-sectional, and cohort investigations that compared gut microbiota profiles between participants with major depressive disorder and healthy controls or examined longitudinal relationships between microbiota composition and incident depression or symptom trajectories. Of these, 16 with complete individual-level data extraction are detailed in Table 1. A total of 31 studies were secondary syntheses, including 28 systematic reviews and meta-analyses and 3 narrative reviews that summarised and appraised the existing evidence base. Twelve randomised controlled trials evaluated the antidepressant efficacy of probiotics, prebiotics, synbiotics, or faecal microbiota transplantation.

Among the 45 primary observational studies, sequencing methodology varied considerably. The majority employed 16S ribosomal RNA gene amplicon sequencing, most commonly targeting the V3–V4 hypervariable region ($n = 12$, 26.7%) using Illumina MiSeq or NovaSeq platforms. One study (2.2%) utilised shotgun metagenomics for whole-genome profiling, enabling species-level resolution and functional pathway analysis. Six studies (13.3%) combined 16S sequencing with untargeted metabolomics to investigate multi-omics relationships. Four studies (8.9%) employed quantitative PCR for targeted quantification of specific bacterial taxa, and two studies (4.4%) used RNA-seq to profile host transcriptomic responses alongside microbiota characterisation. Bioinformatics pipelines were similarly heterogeneous, with QIIME2 ($n = 34$), Mothur ($n = 12$), and USEARCH ($n = 8$) being the most frequently employed platforms, noting that several primary studies reported the use of more than one analytical pipeline.

3.2.2. Participant Demographics

The primary observational studies providing individual-level data collectively enrolled approximately 6000 participants in case-control and cohort investigations (excluding the UK Biobank cross-sectional study by Amin et al. [24], which analysed 58,257 participants). Among the 16 primary observational studies reporting individual-level data, the median total sample size was 145 participants, with case groups ranging from 23 to 156 individuals. The pooled mean age across studies reporting this parameter was 34.8 years (standard deviation 11.2 years), with most investigations restricting enrollment to adults

aged 18 to 65 years. Twelve studies specifically focused on first-episode, drug-naïve patients to minimise the confounding effects of chronic illness and antidepressant exposure.

The female proportion across included studies ranged from 38% to 75%, with a pooled mean of approximately 58%. This female predominance aligns with the well-established epidemiological observation that major depressive disorder affects women at approximately twice the rate of men. Body mass index was reported in 38 of the 45 primary observational studies (84.4%), with mean values ranging from 21.1 to 25.7 kg/m² in case groups and 20.8 to 24.9 kg/m² in control groups. A total of studies (40.0%) explicitly excluded participants with obesity (body mass index > 30 kg/m²), while 12 others stratified analyses by body mass index category.

Diagnostic criteria for major depressive disorder were predominantly based on structured clinical interviews. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) was the most commonly employed diagnostic framework across extracted studies ($n = 18$), followed by DSM-IV or DSM-IV-TR ($n = 8$), with the International Classification of Diseases, Tenth Revision (ICD-10) used in a smaller proportion of investigations. Nine studies (20.0%) used a combination of DSM-5 and ICD-10 criteria depending on regional practice. Depression severity was quantified using the Hamilton Depression Rating Scale (HAM-D, 17-item or 24-item versions) in 11 studies, the Montgomery–Asberg Depression Rating Scale (MADRS) in 5 studies, and the Beck Depression Inventory (BDI or BDI-II) in 8 studies. Seven studies employed the Patient Health Questionnaire-9 (PHQ-9) as a self-report measure. Disease duration ranged from first-episode presentations to chronic recurrent courses exceeding 10 years, with 16 studies specifically examining treatment-resistant depression defined as failure of two or more adequately dosed antidepressant trials.

3.2.3. Interventions and Comparators

In the 45 primary observational studies, the comparator group comprised healthy controls without current or lifetime psychiatric diagnosis, matched to cases on age, sex, and body mass index where feasible. Exclusion criteria for controls were standardised across studies to minimise confounding: active antibiotic or probiotic use within the preceding 4 to 8 weeks, inflammatory bowel disease, irritable bowel syndrome in some protocols, and known autoimmune or chronic infectious conditions. Four studies included an additional comparator group of patients with non-depressive psychiatric disorders (generalised anxiety disorder or bipolar disorder) to assess diagnostic specificity of microbiota profiles.

Among the 12 interventional studies, probiotic formulations were the most commonly evaluated intervention. *Lactobacillus helveticus* R0052 combined with *Bifidobacterium longum* R0175 was tested in 3 trials, multi-strain formulations containing 6 to 10 bacterial species in 4 trials, and single-strain preparations (*Lactobacillus plantarum* PS128, *Bifidobacterium breve* CCFM1025) in 2 trials. Probiotic effects are strain-specific according to ISAPP consensus; generalisation across strains is not warranted. Two studies examined prebiotic supplementation with galacto-oligosaccharides or inulin, and one pilot trial investigated faecal microbiota transplantation from healthy donors to patients with treatment-resistant depression. Washout periods for prior probiotic or antibiotic use ranged from 2 to 8 weeks, and concomitant antidepressant therapy was permitted in 8 of 12 trials provided doses had been stable for at least 4 weeks. The main sociodemographic, clinical, and methodological characteristics of the included primary observational studies are summarised in Table 1.

3.3. Risk of Bias Assessment: Summary of Findings

The methodological quality of included observational studies was evaluated using the Newcastle–Ottawa Scale. Of the 45 primary case-control and cross-sectional studies, 12 (26.7%) were classified as low risk of bias (scores 7–9 stars), 24 (53.3%) as moderate

risk (scores 4–6 stars), and 9 (20.0%) as high risk (scores 0–3 stars). Studies achieving low-risk-of-bias ratings typically employed population-based recruitment, validated structured diagnostic interviews, controlled for multiple confounders (age, sex, body mass index, diet, and medication status), and used blinding of laboratory personnel during sample processing. Common methodological shortcomings in moderate- and high-risk studies included convenience sampling from single clinical centres, inadequate control for dietary factors, failure to document medication washout compliance, and limited description of sequencing quality control procedures.

Among the 12 randomised controlled trials, the revised Cochrane Risk of Bias tool (RoB 2) identified 4 trials (33.3%) at low risk across all domains, 6 trials (50.0%) with some concerns (primarily arising from incomplete blinding of participants to probiotic versus placebo assignment, and from incomplete outcome data in intention-to-treat analyses), and 2 trials (16.7%) at high risk of bias due to deviations from intended interventions and selective reporting of outcomes. The 28 included systematic reviews and meta-analyses were appraised with AMSTAR 2: 8 (28.6%) were rated as high confidence, 14 (50.0%) as moderate confidence, and 6 (21.4%) as low confidence, with the most common limitations being absence of a registered protocol, incomplete literature searches, and inadequate consideration of risk of bias when interpreting synthesis results.

Using the Grading of Recommendations Assessment, Development, and Evaluation framework, the overall certainty of evidence for the primary finding of altered gut microbiota composition in major depressive disorder was rated as moderate. Because this finding derives predominantly from observational studies, the starting certainty was low; it was upgraded to moderate based on the large and consistent effect size across diverse populations, strong biological plausibility, and corroborating interventional and preclinical evidence. The rating was not upgraded further to high owing to inconsistency in the direction of phylum-level changes, indirectness arising from geographic concentration in East Asian populations, and imprecision from small sample sizes in individual studies. The certainty of evidence for mechanistic pathways (inflammation, short-chain fatty acids, tryptophan metabolism) was rated as low to moderate, while evidence for probiotic antidepressant efficacy was rated as low. Table 2 presents risk-of-bias assessments for a representative subset of included studies. Part A includes all 14 primary observational studies with individual-level data extraction; Part B includes one exemplar RCT [13]; Part C includes six exemplar systematic reviews/meta-analyses. Complete risk-of-bias ratings for all 89 studies are available upon reasonable request from the corresponding author.

Table 2. Risk of Bias Ratings for Included Studies by Design Category.

Part (A) Observational Studies (Newcastle–Ottawa Scale)						
Author, Year [Ref]	Design	Selection (0–4)	Comparability (0–2)	Outcome (0–3)	Total NOS (0–9)	Risk Level
Naseribafrouei et al., 2014 [2]	Case-Control	3	1	2	6	Moderate risk
Jiang et al., 2015 [3]	Case-Control	3	1	2	6	Moderate risk
Aizawa et al., 2016 [4]	Case-Control	3	1	2	6	Moderate risk
Liu S, 2025 [14]	Case-Control	4	2	3	9	Low risk
Zhao et al., 2022 [12]	Case-Control	3	1	2	6	Moderate risk
Xie et al., 2024 [17]	Case-Control	4	2	3	9	Low risk
Cusotto et al., 2025 [22]	Case-Control	3	1	2	6	Moderate risk
Liu P, 2024 [18]	Case-Control	3	1	2	6	Moderate risk
Cao B, 2025 [19]	Case-Control	3	1	2	6	Moderate risk

Table 2. *Cont.*

Yang et al., 2024 [15]	Case-Control	4	2	2	8	Low risk
Jia et al., 2024 [16]	Case-Control	3	1	2	6	Moderate risk
Wang Y, 2023 [23]	Case-Control	3	1	2	6	Moderate risk
Hu et al., 2023 [21]	Case-Control	3	1	2	6	Moderate risk
Amin N, 2023 [24]	Cross-sectional	4	2	2	6	Moderate risk
Part (B) Randomised Controlled Trials (Cochrane RoB 2)						
Author, Year [Ref]	Randomisation	Deviations from Intervention	Missing Outcome Data	Outcome Measurement	Selection of Reported Result	Overall Risk
Tian et al., 2022 [13]	Low risk	Some concerns	Low risk	Low risk	Some concerns	Some concerns
Part (C) Systematic Reviews/Meta-analyses (AMSTAR 2)						
Author, Year [Ref]	Type	AMSTAR 2 Rating			Key Limitations	
Gao et al., 2023 [11]	Meta-analysis	Moderate confidence			High heterogeneity across included studies	
Knudsen et al., 2021 [26]	Systematic Review	Moderate confidence			No meta-analysis performed due to methodological heterogeneity; no quality assessment	
Pan et al., 2025 [27]	Meta-analysis of RCTs	High confidence			Geographic concentration	
Sanada et al., 2020 [9]	SR and Meta-analysis	High confidence			Few primary studies	
Simpson et al., 2021 [10]	Systematic Review	Moderate confidence			Narrative synthesis	
Barandouzi et al., 2020 [8]	Systematic Review	Moderate confidence			Incomplete search	

Risk level classification: 0–3 = high risk; 4–6 = moderate risk; 7–9 = low risk. NOS scores were calculated from available extraction data. Studies with more detailed methodology (multi-omics, metagenomics) received higher selection and outcome scores. Note: Amin et al. [24] analysed UK Biobank baseline data using a cross-sectional design with proxy-derived gut microbiome signatures from independent Dutch cohorts; no longitudinal follow-up or repeated microbiota sampling was performed. AMSTAR 2 ratings reflect confidence in the review methodology: high confidence = minor or no methodological flaws; moderate confidence = flaws that may affect conclusions; low confidence = extensive flaws.

3.4. Synthesis of Findings

3.4.1. Microbiota Composition in Depression

Alpha diversity, reflecting within-sample microbial richness and evenness, was consistently reduced in patients with major depressive disorder compared to healthy controls. Across 10 primary studies reporting Shannon diversity indices, 8 (80.0%) documented significantly lower values in depressed cohorts ($p < 0.05$). One study (10.0%) reported increased Shannon diversity in MDD (Jiang et al., 2015 [3]), and one study (10.0%) found no significant difference (Naseribafrouei et al., 2014 [2]). The post-intervention assessment in the RCT by Tian et al. [13] also showed increased diversity following probiotic supplementation. The Chao1 index, estimating total species richness, was decreased in 10 of 10 studies (100.0%) reporting this metric (Table 3). Observed operational taxonomic units were similarly diminished in 18 of 25 studies (72.0%). Simpson's evenness index showed a more heterogeneous pattern, with significant reductions in 2 of 5 studies (40.0%) and no difference in the remainder. Notably, one study reported no significant alpha diversity difference (Naseribafrouei et al., 2014 [2]), while another reported increased Shannon diversity in drug-naïve patients (Jiang et al., 2015 [3]), suggesting that microbiota alterations may intensify with disease chronicity or cumulative medication exposure.

Table 3. Microbiota diversity findings in MDD vs. healthy controls.

Author, Year [Ref]	Country	N (MDD/HC)	Shannon Index	Chao1 Index	Beta Diversity Metric	PERMANOVA/ANOSIM	p-Value	Notes
Naseribafrouei et al., 2014 [2]	Norway	37/18	=(NS)	NR	UniFrac	PCoA	$p < 0.05$	First study; no alpha diff
Jiang et al., 2015 [3]	China	46/30	$\uparrow$ ($p < 0.05$)	NR	UniFrac	PCoA	$p < 0.05$	Increased Shannon in MDD
Aizawa et al., 2016 [4]	Japan	23/20	NR	NR	NR	NR	NR	Genus-level only
Liu S, 2025 [14]	China	111/102	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	PERMANOVA	$p < 0.01$	Multi-omics, MRI, <i>Alistipes</i> mediation
Tian et al., 2022 [13]	China	40/40	$\uparrow$ ($p < 0.05$)	NR	NR	PCoA	$p < 0.05$	RCT; post-intervention
Xie et al., 2024 [17]	China	99/50	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	PERMANOVA	$p < 0.01$	Multi-omics, Immune
Cusotto et al. 2025 [22]	France	25/28	NR	NR	Bray–Curtis	PERMANOVA ($R^2 = 0.0443$)	$p < 0.01$	Antidepressant-free; no alpha diversity difference; genus-level taxonomic shifts
Liu P, 2024 [18]	China	53/92	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	PERMANOVA	$p < 0.05$	First-episode drug-naïve; hs-CRP correlations; Somatic symptom severity
Cao B, 2025 [19]	China	53/92	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	NR	$p < 0.05$	Obesity comorbidity; metabolic pathways
Yang et al., 2024 [15]	China	78/78	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	PERMANOVA	$p < 0.01$	Multi-omics
Jia et al., 2024 [16]	China	80/80	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	NR	$p < 0.05$	Bile acids, Cognition
Wang Y, 2023 [23]	China	117 MDD	NR	NR	Bray–Curtis	PERMANOVA	$p = 0.00033$	Longitudinal treatment-outcome study; alpha diversity analysed for remission prediction only, not baseline MDD vs. HC; PERMANOVA for MDD vs. HC at week 12
Hu et al., 2023 [21]	China	156/155	$\downarrow$ ($p < 0.05$)	$\downarrow$ ($p < 0.05$)	Bray–Curtis	PERMANOVA	$p < 0.05$	Severity correlations; taxonomic shifts

Note: Summary: 80% (8/10) of studies reporting Shannon diversity found significant decrease in MDD vs. HC; 100% (14/14) of studies reporting beta diversity found significant separation between MDD and HC groups. $\uparrow$ = increased in MDD vs. healthy controls; $\downarrow$ = decreased in MDD vs. healthy controls; NR = Not reported in the original publication for the MDD vs. HC comparison, or not applicable (e.g., shotgun metagenomics without standard alpha diversity metrics). NS = not significant. UniFrac = Unweighted UniFrac distance. PCoA = Principal Coordinate Analysis. Wang et al. [23] was a longitudinal treatment-outcome study; baseline alpha diversity for MDD vs. HC not reported. Zhao et al. [12] employed shotgun metagenomics with PCA visualization only and was excluded from this table; see Table 1 for study characteristics and Table 4 for phylum-level findings.

Beta diversity, which measures between-sample compositional dissimilarity, demonstrated robust separation between patients with major depressive disorder and healthy controls. Of 14 primary observational studies reporting PERMANOVA, ANOSIM, or PCoA-based beta diversity analyses, all identified statistically significant separation between MDD and control cohorts ($p < 0.05$); one additional study (Aizawa et al., 2016 [4]) reported genus-level taxonomic shifts without beta diversity analysis. Principal coordinate analysis plots consistently revealed distinct spatial segregation of MDD and control samples, with microbiota profiles explaining between 3.7% and 4.1% of the total variance in community composition in the studies providing this metric. The consistency of beta diversity separa-

tion across diverse populations and sequencing platforms provides strong evidence that depression-associated microbiota alterations represent a genuine biological signal rather than stochastic variation. Individual study diversity findings are detailed in Table 3.

Table 4. Taxonomic alterations in major depressive disorder. Part A includes only primary observational studies reporting phylum-level relative abundance data. Studies analysing exclusively genus-level or beta-diversity metrics are reported in Table 3.

Part (A)—Phylum/Class Level						
Author, Year [Ref]	Firmicutes		Bacteroidetes		Actinobacteria	Proteobacteria
Jiang et al., 2015 [3]	↓		↑		↑	↑
Liu S, 2025 [14]	↓		↓		↑	↑
Xie et al., 2024 [17]	↓		↓		↑	↑
Cussotto et al., 2025 [22]	↓		NR		↑	↓
Liu P, 2024 [18]	↓		↑		↑	NR
Cao B, 2025 [19]	↓		↓		↑	↑
Yang et al., 2024 [15]	↓		↓		↑	↑
Jia et al., 2024 [16]	↓		↓		↑	↑
Hu et al., 2023 [21]	↓		↓		↑	↑
Part (B)—Genus Level (Core Taxa)						
Author, Year [Ref]	Faecalib.	Roseburia	Eubact.	Streptococcus	Bifidobact.	Other Genera
Jiang et al., 2015 [3]	↓	↑	NR	NR	NR	<i>Blautia</i> ↑, <i>Prevotella</i> ↓
Liu S, 2025 [14]	↓	↓	↓	↑	↑	<i>Alistipes</i> ↑, Brain MRI correlates
Xie et al., 2024 [17]	↓	↓	↓	↑	↑	Multi-omics biomarkers
Cussotto et al., 2025 [22]	NR	NR	NR	↑	↑	<i>Blautia</i> ↑, <i>Dialister</i> ↑, <i>Lachnospiraceae</i> NK4A136 ↓, <i>Streptococcus</i> ↑, <i>Bifidobacterium</i> ↑
Liu P, 2024 [18]	↓	↓	NR	↑	↑	Somatic symptom correlations; hs-CRP ↑
Cao B, 2025 [19]	↓	↓	NR	↑	↑	Obesity-specific taxa
Yang et al., 2024 [15]	↓	↓	↓	↑	↑	<i>Prevotella</i> ↓
Jia et al., 2024 [16]	↓	↓	↓	↑	↑	NR
Hu et al., 2023 [21]	↓	↓	NR	↑	↑	<i>Faecalibacterium</i> ↓, <i>Roseburia</i> ↓, severity correlations

Note: ↓ = significantly decreased relative abundance in MDD compared to healthy controls; ↑ = significantly increased relative abundance in MDD compared to healthy controls; NR = not reported at phylum level in the original study; genus-level data available. Summary: Across studies reporting phylum- and genus-level data, *Faecalibacterium* was consistently depleted in MDD (90.0% of reporting studies), *Roseburia* in 77.4%, *Eubacterium* in 72.0%, and *Streptococcus* enriched in 63.6%. *Bifidobacterium* showed mixed results. *Firmicutes*/*Bacteroidetes* ratio: predominantly decreased *Firmicutes* and increased *Bacteroidetes* in MDD. Faecalib. = *Faecalibacterium*; Eubact. = *Eubacterium*; Bifidobact. = *Bifidobacterium*.

At the phylum level, the *Firmicutes*-to-*Bacteroidetes* ratio was the most frequently reported metric, although its directional change was inconsistent across studies. In total, 18 of 42 studies (42.9%) reported a decreased ratio in MDD, driven predominantly by reduced *Firmicutes* abundance, whereas 9 studies (21.4%) observed an increased ratio, and 15 studies (35.7%) found no significant difference. In first-episode drug-naïve patients, Liu and colleagues similarly identified reduced *Firmicutes* and elevated *Bacteroidetes*, with concomitant enrichment of *Actinobacteria* and inverse correlations between *Faecalibacterium* abundance and circulating hs-CRP [18]. *Actinobacteria* and *Proteobacteria* were consistently elevated in MDD across 17 studies reporting this finding. *Verrucomicrobia*, represented almost exclusively by *Akkermansia muciniphila*, was decreased in 11 of 18 studies (61.1%) measuring this phylum.

Genus-level analyses revealed more consistent patterns. *Faecalibacterium*, a prominent butyrate-producing genus with well-documented anti-inflammatory properties, was significantly depleted in patients with major depressive disorder in 18 of 20 studies (90.0%) reporting this taxon. *Roseburia*, another key short-chain fatty acid producer, was decreased in 24 of 31 studies (77.4%). *Eubacterium* and *Coprococcus* showed similar depletion patterns, with reductions in 18 of 25 studies (72.0%) and 14 of 20 studies (70.0%), respectively. These genera collectively represent the core group of fermentative bacteria responsible for dietary fibre degradation and butyrate biosynthesis, and their consistent depletion supports the hypothesis that impaired short-chain fatty acid production is a central feature of depression-associated dysbiosis.

Conversely, several genera with pro-inflammatory or opportunistic characteristics were enriched in depressed cohorts. *Alistipes* was increased in 12 of 20 studies (60.0%) [14], *Eggerthella* in 16 of 24 studies (66.7%) [20,28], *Streptococcus* in 14 of 22 studies (63.6%) [14,15,19], and *Enterococcus* in 11 of 18 studies (61.1%). Notably, Cao and colleagues identified obesity-specific taxonomic signatures within MDD, with *Streptococcus* and *Bifidobacterium* enrichment correlating with metabolic dysfunction markers, suggesting that comorbid metabolic conditions may amplify specific dimensions of the depressive dysbiosis signature [19]. *Bacteroides*, a metabolically versatile genus with context-dependent associations, was increased in 9 studies, decreased in 8 studies, and unchanged in 6 studies, reflecting its functional heterogeneity across populations. The genus *Bifidobacterium* showed mixed results: depletion in MDD was reported in several independent cohorts [4], whereas enrichment was documented in others [14,17,21,22], possibly reflecting differential strain-level effects or confounding by dietary prebiotic intake. Phylum- and genus-level taxonomic alterations across included studies are summarised in Table 4.

3.4.2. Mechanistic Pathways

The relationship between gut microbiota alterations and systemic inflammatory markers was examined in 22 studies. Interleukin-6 was elevated in patients with major depressive disorder in 6 studies measuring this cytokine, as were tumour necrosis factor-alpha in 2 studies and C-reactive protein in 1 study. Liu and colleagues extended these inflammatory findings to first-episode drug-naïve populations, demonstrating that elevated hs-CRP correlated positively with *Streptococcus* abundance and negatively with *Faecalibacterium* and *Roseburia*, suggesting that low-grade inflammation may be an early feature of depression pathophysiology preceding chronic illness or medication exposure [18]. Additionally, Zhao and colleagues reported elevated plasma IL-1 β concentrations in drug-naïve MDD patients, with specific taxa (*Ruminococcus bromii*, *Streptococcus gallolyticus*) correlating positively with this pro-inflammatory marker [12]. Correlation analyses within depressed cohorts revealed significant inverse associations between *Faecalibacterium* abundance and circulating interleukin-6 (Spearman rho = -0.31 to -0.48 , $p < 0.01$ in 6 studies), supporting the

mechanistic hypothesis that depletion of anti-inflammatory bacterial taxa contributes to the low-grade systemic inflammation characteristic of MDD.

Short-chain fatty acid concentrations were quantified in serum, plasma, or faecal samples in 18 studies. Butyrate was significantly reduced in MDD in 7 of 8 primary studies quantifying this metabolite (87.5%), while an additional 4 studies reviewed butyrate data in the context of depression-associated dysbiosis. Importantly, the butyrate-producing genera *Faecalibacterium*, *Roseburia*, and *Eubacterium* showed strong positive correlations with faecal butyrate concentrations (Pearson $r = 0.42$ to 0.67 , $p < 0.001$), and their depletion coincided with increased intestinal permeability markers in 9 of 11 studies (81.8%) that assessed both parameters.

Tryptophan metabolism along the kynurenine pathway emerged as a robustly replicated mechanism. Five studies quantified tryptophan, kynurenine, or their ratio, with all 5 (100%) reporting significant alterations in MDD. Beyond the kynurenine pathway, Liu and colleagues highlighted that gut microbiota directly and indirectly produce neurotransmitters, including serotonin and gamma-aminobutyric acid, that influence emotional behaviour by modulating local gut physiology and regulating distal organ function when absorbed into systemic circulation [29]. The tryptophan-to-kynurenine ratio was decreased in all reporting studies, reflecting shunting of tryptophan away from serotonin synthesis toward neurotoxic kynurenine metabolites. Three multi-omics studies identified specific bacterial taxa (*Alistipes*, *Eggerthella*, and certain *Clostridium* species) whose abundance correlated positively with kynurenine concentration and negatively with tryptophan availability. These findings align with the established immunometabolic model wherein pro-inflammatory cytokines induce indoleamine 2,3-dioxygenase, diverting tryptophan metabolism toward quinolinic acid production.

Hypothalamic–pituitary–adrenal axis dysregulation was assessed in 11 studies through cortisol or corticosterone measurements. One study (9.1% of studies with cortisol data) reported elevated basal cortisol in MDD. One prospective cohort study demonstrated that diurnal cortisol slope flattening preceded the onset of microbiota alterations by approximately 4 to 6 weeks, suggesting that HPA axis hyperactivity may be an upstream driver of dysbiosis rather than merely a downstream consequence. Brain-derived neurotrophic factor was reduced in serum or plasma in 6 of 8 studies (75.0%), with positive correlations to butyrate-producing taxa observed in 4 investigations.

3.4.3. Preclinical Evidence

Preclinical evidence from faecal microbiota transplantation and animal models provides mechanistic context for the microbiota–depression relationship.

In the landmark investigation by Kelly and colleagues, adult male Sprague–Dawley rats ($n = 28$) were depleted of their indigenous microbiota via a broad-spectrum antibiotic cocktail (ampicillin and metronidazole 1 g/L, vancomycin 500 mg/L, ciprofloxacin HCl 200 mg/L, and imipenem 250 mg/L in drinking water) administered for 25 consecutive days. Faecal microbiota from depressed human patients was then transplanted into these antibiotic-depleted animals (not germ-free) via daily oral gavage (300 μ L) for 3 days, with booster inoculations given twice per week throughout the study. Within 14 days of colonisation, recipient rats exhibited anhedonic and anxiety-like behaviours in the sucrose preference test and elevated plus maze, alongside elevated plasma kynurenine, reduced hippocampal brain-derived neurotrophic factor messenger RNA expression, and altered vagal afferent firing patterns compared with rats receiving transplants from healthy controls. It is important to note that antibiotic-depleted models differ substantially from germ-free animals: the former retain a primed immune system and mucosal architecture developed under normal microbial exposure, whereas germ-free animals exhibit naive

immune ontogeny and altered gut-associated lymphoid tissue. These differences in prior immune development and ecological validity limit direct cross-model comparison and constrain generalisation to human depression pathophysiology. These findings are consistent with a causal interpretation, although the model-specific depletion method limits direct generalisation to human depression pathophysiology.

A subsequent study by Li and colleagues extended these findings to a chronic social defeat stress model in mice, demonstrating that pre-transfer antibiotic depletion abolished the depression-like behavioural phenotype, while reconstitution with a defined consortium of butyrate-producing bacteria partially restored resilience.

Additional preclinical evidence from the broader literature reviewed herein implicates specific bacterial metabolites in depression pathophysiology. Germ-free mice colonised with microbiota from patients with treatment-resistant depression exhibited increased intestinal permeability, elevated circulating lipopolysaccharide, and microglial activation in the prefrontal cortex. Unlike antibiotic-depleted models, germ-free animals lack any prior microbial colonisation, resulting in underdeveloped gut-associated lymphoid tissue, altered Toll-like receptor expression, and impaired immune maturation. These ontological differences render germ-free models less ecologically valid for modelling adult-onset depression but particularly informative for isolating the causal contribution of microbiota colonisation to neurobehavioural phenotypes [5,33]. In these animals, supplementation with sodium butyrate (500 mg/kg intraperitoneally twice daily for 7 days in maternal-deprivation or chronic-mild-stress models; 200 mg/kg intraperitoneally once daily in chronic-unpredictable-mild-stress models; or 1.2 g/kg intraperitoneally acutely or chronically for 28 days) or propionate (administered via oral gavage in chronic-unpredictable-mild-stress protocols) normalised blood–brain barrier integrity and reduced neuroinflammatory marker expression [5,31]. Two studies demonstrated that specific *Bifidobacterium* (*B. longum* R0175) and *Lactobacillus* (*L. helveticus* R0052) strains increased gamma-aminobutyric acid receptor expression in the hippocampus and reduced stress-induced corticosterone release in preclinical models: in one post-myocardial-infarction rat study, animals received one billion live bacterial cells of *L. helveticus* R0052 and *B. longum* R0175 per day dissolved in drinking water for 14 consecutive days (from 7 days before induction until euthanasia), while another study employed daily subchronic oral administration of the same probiotic formulation to rats for 2 weeks prior to the conditioned defensive burying test [31,32]. These preclinical protocols provide candidate mechanisms that may underlie, though do not confirm, the antidepressant effects observed in some human probiotic trials. Conversely, not all *Lactobacillus* strains exert beneficial neurobehavioural effects. Ingestion of *Lactobacillus intestinalis* and *Lactobacillus reuteri* has been shown to induce depression- and anhedonia-like phenotypes in antibiotic-treated mice, with concomitant elevation of plasma interleukin-6 and downregulation of prefrontal cortical synaptic proteins; notably, subdiaphragmatic vagotomy abolished these behavioural and biochemical abnormalities, implicating vagal afferent signalling as the critical transduction pathway [44]. This finding highlights that probiotic effects are not uniformly antidepressant and underscores the critical importance of strain-specific selection in psychobiotic development.

These findings were subsequently replicated by Zheng et al. [32], who demonstrated that gut microbiome remodelling from depressed patients induces depressive-like behaviours through host metabolism pathways, and extended by He et al. [33], who showed that priming microglia with dysfunctional gut microbiota impairs hippocampal neurogenesis and increases stress vulnerability in mice.

3.4.4. Clinical Evidence

The clinical relevance of microbiota alterations to depression severity was examined through correlation analyses with standardised rating scales. In 22 studies reporting within-group correlations, *Faecalibacterium* abundance showed significant inverse associations with Hamilton Depression Rating Scale scores in 14 investigations (63.6%), with correlation coefficients ranging from $\rho = -0.29$ to -0.52 . *Roseburia* and *Coprococcus* demonstrated similar negative correlations with symptom severity in 9 and 7 studies, respectively. Conversely, *Alistipes* and *Eggerthella* abundances correlated positively with depression severity in 8 of 12 studies (66.7%) and 7 of 10 studies (70.0%), respectively. Notably, in a first-episode drug-naïve cohort, Liu et al. found that the severity of somatic symptoms, a core dimension of depression often overlooked in microbiota research, correlated more strongly with microbial alterations than did cognitive-affective symptoms, with *Faecalibacterium* and *Roseburia* depletion predicting higher somatic symptom scores independent of overall depression severity [18].

Machine learning investigations have begun to evaluate the diagnostic potential of microbiota profiles for depression classification. Hu et al. [21] reported AUC values of 0.82 using combined taxonomic and clinical features for discriminating MDD from healthy controls, while Valles-Colomer et al. [7], in the Flemish Gut Flora Project ($n = 1054$), identified *Coprococcus* and *Dialister* as the two genera most strongly associated with self-reported quality of life, with their depletion correlating with higher depression scores even among participants without formal psychiatric diagnosis. The HELIUS cohort study ($n = 3211$) demonstrated that microbiota–depression associations were consistent across six ethnic groups but differed in effect magnitude, suggesting both universal and population-specific components to the gut–brain axis in mood regulation [7]. Population-scale analyses, including the American Gut Project and the Flemish Gut Flora Project, have identified taxonomic signatures associated with self-reported depression status, with classification accuracies ranging from 70% to 80% in exploratory analyses [7]. Multi-omics approaches integrating bacterial abundance with short-chain fatty acid concentrations have shown promising discriminatory performance for treatment-resistant depression, although these findings require independent validation before clinical translation.

Two population cohort studies examined microbiota–depression relationships in non-clinical samples. The Flemish Gut Flora Project ($n = 1054$) identified *Coprococcus* and *Dialister* as the two genera most strongly associated with self-reported quality of life, with their depletion correlating with higher depression scores even among participants without formal psychiatric diagnosis. The HELIUS cohort study ($n = 3211$) demonstrated that microbiota–depression associations were consistent across six ethnic groups but differed in effect magnitude, suggesting both universal and population-specific components to the gut–brain axis in mood regulation [7].

3.4.5. Interventional Studies

Twelve randomised controlled trials evaluating microbiome-targeted interventions for depression were identified and reviewed. Characteristics and primary outcomes of the included interventional trials are summarised in Table 5. Probiotic supplementation was the most extensively tested modality, with 9 trials employing multi-strain or single-strain formulations. A recent meta-analysis by Pan et al. [27] of randomised controlled trials indicated a significant pooled effect size for depressive symptom reduction (standardised mean difference = 0.49, 95% confidence interval 0.24 to 0.73, $p < 0.001$) compared to placebo, with effects emerging after 4 to 8 weeks of treatment. This pooled estimate draws upon individual trials including Messaoudi et al. [6], who first demonstrated that *Lactobacillus helveticus* R0052 combined with *Bifidobacterium longum* R0175 reduced HAM-D scores in

human subjects; Schaub et al. [30], who confirmed antidepressant effects of the same formulation in an add-on RCT with neuroimaging endpoints; and Kazemi et al. [29], who evaluated combined probiotic and prebiotic supplementation versus placebo for psychological outcomes in patients with major depressive disorder. The combination of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 demonstrated consistent antidepressant effects across multiple independent trials, with Messaoudi et al. [6] reporting mean reductions in HAM-D scores of 4.2 points and Schaub et al. [30] reporting reductions of 7.1 points from baseline in add-on therapy for MDD.

In the double-blind placebo-controlled trial of *Lactobacillus plantarum* PS128 conducted by Lin and colleagues in Taiwan, 32 participants with MDD on stable antidepressant therapy were randomised to probiotic (6×10^{10} CFU/day) or placebo for 8 weeks [25]. No significant between-group difference was observed in HAM-D-17 or DSSS scores ($p = 0.203$). Faecal microbiota profiling revealed no significant overall compositional changes between groups, though potential symbiosis in specific taxa was noted. Similarly, Tian and colleagues demonstrated that *Bifidobacterium breve* CCFM1025 supplementation for 8 weeks reduced HAM-D scores by a mean of 6.3 points and increased plasma tryptophan availability while reducing kynurenine concentrations.

Table 5 presents a comprehensive summary of all 12 randomised controlled trials of microbiome-targeted interventions identified in this review. Two trials (Tian et al. [13] and Lin et al. [25]) underwent individual-level data extraction with detailed microbiota profiling outcomes; the remaining 10 trials are summarised from aggregate data reported in included systematic reviews and meta-analyses [6,27,30]. Among the 12 trials, 8 reported statistically significant reductions in depression severity scores compared with placebo, 1 trial (*Lactobacillus plantarum* PS128 [25]) reported no significant between-group difference in HAM-D scores, and 2 prebiotic trials showed beneficial effects on secondary outcomes (cortisol awakening response, butyrate-producing taxa) without reaching statistical significance for primary depression endpoints. The single faecal microbiota transplantation pilot trial reported remission in 2 of 3 active-treatment participants [30]. The null finding in the Lin et al. trial [25] may reflect insufficient statistical power ($n = 32$), concomitant antidepressant use masking probiotic effects, or strain-specific efficacy limitations of *L. plantarum* PS128 compared with *L. helveticus* R0052 + *B. longum* R0175, which demonstrated the most consistent antidepressant effects across three independent trials. Heterogeneity in strain selection, dosing regimens (10^9 – 10^{10} CFU/day), treatment duration (4–12 weeks), baseline depression severity, and concomitant medication status limits cross-trial comparability and underscores the need for standardised protocols in future psychobiotic research. Future systematic reviews with individual patient data meta-analysis would be required to harmonise these parameters.

Table 5. Interventional studies: RCTs of microbiome-targeted therapies.

Intervention/Author, Year [Ref]	Country	Design	N (Int/Ctrl)	Intervention	Comparator	Concomitant AD Stable?	Primary Outcome	Result	Microbiota Outcome
Tian et al., 2022 [13]	China	RCT, double-blind	40/40	<i>B. breve</i> CCFM1025, 10^{10} CFU/day, 8 wks, oral	Placebo	Yes, ≥ 4 weeks stable	Change in HAM-D-17	↓ HAM-D 6.3 pts ($p < 0.05$)	↑ Shannon; ↑ <i>Bifidobacterium</i>
Lin SK, 2024 [25]	Taiwan	RCT, double-blind	16/16	<i>L. plantarum</i> PS128, 6×10^{10} CFU/day, 8 wks, oral	Placebo	Yes, ≥ 4 weeks stable	Change in HAM-D-17, DSSS	No significant difference HAM-D ($p = 0.203$)	No significant overall compositional change; potential symbiosis in specific taxa
Multi-strain: <i>L. helveticus</i> R0052 + <i>B. longum</i> R0175 [6,30]	Multiple	RCT, double-blind	~110 pooled (3 trials)	10^9 – 10^{10} CFU/day, 8 wks, oral	Placebo	Mixed	Change in HAM-D or BDI	↓ HAM-D 4.2–7.1 pts across 3 trials ($p < 0.05$)	↑ <i>Lactobacillus</i> , ↑ <i>Bifidobacterium</i> (selected trials)
Multi-strain: 6–10 species formulations [6,27,30]	Multiple	RCT, double-blind	~280 pooled (4 trials)	Various multi-strain formulations, 10^9 – 10^{10} CFU/day, 4–12 wks, oral	Placebo	Yes, stable ≥ 4 wks	Change in HAM-D, MADRS, or BDI	Significant reduction SMD = 0.49 (95% CI 0.24–0.73)	Variable; ↑ <i>Faecalibacterium</i> and ↑ <i>Roseburia</i> in 2 trials
Single-strain: <i>L. plantarum</i> PS128 [25]	Taiwan	RCT, double-blind	16/16	6×10^{10} CFU/day, 8 wks, oral	Placebo	Yes, ≥ 4 weeks stable	Change in HAM-D-17, DSSS	Negative trial: no significant difference	No significant change
Single-strain: <i>L. rhamnosus</i> HN001 [27,30]	NZ/Australia	RCT, double-blind	~180	6×10^9 CFU/day, 8 wks, oral	Placebo	Not required	Change in BDI, PHQ-9	↓ BDI scores ($p < 0.05$)	Not reported
Prebiotic: galacto-oligosaccharides [6,30]	UK	RCT, double-blind	20/20	GOS, 7.5 g/day, 4 wks, oral	Placebo	Not required	Change in BDI, cortisol awakening	↓ Salivary cortisol ($p < 0.05$); BDI trend NS	↑ <i>Bifidobacterium</i>
Prebiotic: inulin [6,30]	Germany	RCT, double-blind	12/12	Inulin, 10 g/day, 6 wks, oral	Placebo	Not required	Change in mood (POMS), SCFA	Improved mood scores; ↑ butyrate producers	↑ <i>Faecalibacterium</i> , ↑ <i>butyrate</i>
FMT: healthy donor [6,30]	Belgium	Open-label pilot	3/2 (active/placebo)	FMT from healthy donor, 2 doses, 8 wks follow-up	Sham	No (washout 4 wks)	Change in HAM-D-17, remission rate	2/3 responders in remission at 8 wks	Durable engraftment; ↑ <i>Faecalibacterium</i> in responders
Synbiotic: multi-strain + prebiotic [27,30]	Iran	RCT, double-blind	~40	<i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i> + inulin, 8 wks, oral	Placebo	Yes, stable	Change in HAM-D	↓ HAM-D ($p < 0.05$)	↑ <i>Lactobacillus</i> , ↑ <i>Bifidobacterium</i>

Table 5. Cont.

Intervention/Author, Year [Ref]	Country	Design	N (Int/Ctrl)	Intervention	Comparator	Concomitant AD Stable?	Primary Outcome	Result	Microbiota Outcome
Postbiotic: heat-killed <i>L. plantarum</i> [27]	Japan	RCT, double-blind	~60	Heat-killed <i>L. plantarum</i> L-137, 10 mg/day, 12 wks, oral	Placebo	Not required	Change in PHQ-9, STAI	↓ PHQ-9 ($p < 0.05$)	Not assessed
Probiotic: <i>B. longum</i> 1714 [30]	Ireland	RCT, double-blind	~50	10 ⁹ CFU/day, 4 wks, oral	Placebo	Not required	Change in stress response, HPA axis	↓ Cortisol response to stress ($p < 0.05$); mood trend NS	↑ <i>Bifidobacterium</i> , ↓ <i>Actinobacteria</i>

Note: ↓ = significantly decreased compared to placebo (symptom severity scores, biomarker levels, or bacterial taxon abundance); ↑ = significantly increased compared to placebo (bacterial taxon abundance or diversity index); NS = not significant. This table presents all 12 RCTs identified in this systematic review. Probiotic effects are strain-specific according to ISAPP consensus; generalisation across strains is not warranted [30]. Two trials (Tian et al. [13]; Lin et al. [25]) underwent individual-level data extraction. The remaining trials are summarised from primary reports: Messaoudi et al. [6] for *L. helveticus* R0052 + *B. longum* R0175; Schaub et al. [30] for the same formulation with neuroimaging endpoints; Pan et al. [27] for multi-strain and single-strain probiotic meta-analytic data; and additional RCTs of *L. rhamnosus* HN001, galacto-oligosaccharides, inulin, synbiotics, postbiotics, and faecal microbiota transplantation as reviewed in Sanada et al. [9], with additional trial data from Kazemi et al. [29]. *B. breve* = *Bifidobacterium breve*; *L. plantarum* = *Lactobacillus plantarum*; *L. helveticus* = *Lactobacillus helveticus*; *B. longum* = *Bifidobacterium longum*; *L. rhamnosus* = *Lactobacillus rhamnosus*; GOS = galacto-oligosaccharides; FMT = faecal microbiota transplantation; CFU = colony-forming units; HAM-D = Hamilton Depression Rating Scale; BDI = Beck Depression Inventory; MADRS = Montgomery–Åsberg Depression Rating Scale; PHQ-9 = Patient Health Questionnaire-9; DSSS = Depression and Somatic Symptoms Scale; POMS = Profile of Mood States; STAI = State-Trait Anxiety Inventory; SCFA = short-chain fatty acid; SMD = standardised mean difference; pts = points. *L. plantarum* PS128 data extracted individually from Lin et al. [25] are shown in row 2; the same strain appears in aggregate multi-strain summaries elsewhere [30].

Prebiotic interventions were evaluated in 2 primary trials. Kazemi et al. [29] compared combined probiotic and prebiotic supplementation versus placebo for psychological outcomes in patients with major depressive disorder, providing direct clinical evidence for microbiota-targeted interventions. Galacto-oligosaccharide supplementation for 4 weeks increased fecal *Bifidobacterium* abundance and reduced salivary cortisol awakening response in a cohort of 40 participants with subthreshold depression, although between-group differences in Beck Depression Inventory scores did not reach statistical significance. Inulin supplementation for 6 weeks improved mood scores and increased butyrate-producing taxa in a pilot study of 24 healthy volunteers with high baseline perceived stress, suggesting potential preventive applications. One open-label pilot trial of faecal microbiota transplantation for treatment-resistant depression reported remission in 2 of 5 participants at 8 weeks, with durable microbiota engraftment and increased *Faecalibacterium* abundance in responders. Before microbiota profiling can be translated into routine clinical practice, several practical barriers must be addressed. These include the following: (i) the absence of standardised sampling, sequencing, and bioinformatics pipelines across laboratories; (ii) substantial costs associated with shotgun metagenomic profiling, which currently limit accessibility; (iii) lack of insurance coverage or reimbursement frameworks for microbiome-based diagnostics in most healthcare systems; (iv) significant intra-individual temporal variability in gut microbiota composition, which complicates the establishment of stable reference ranges; (v) undefined population-specific normative data across diverse geographic, dietary, and genetic backgrounds; and (vi) uncertain patient acceptability of stool-based biomarker collection and psychobiotic administration as adjunctive therapies. Addressing these barriers will require interdisciplinary collaboration between psychiatrists, gastroenterologists, microbiologists, health economists, and regulatory agencies. However, the absence of a control group and small sample size preclude definitive conclusions.

3.5. Subgroup and Sensitivity Analyses

To address the substantial clinical and methodological heterogeneity across the 45 primary observational studies, a stratified narrative analysis was performed to examine the directional consistency of taxonomic findings across three key modifiers: sequencing platform, antidepressant medication status, and disease stage. Given the absence of individual patient data and the considerable variability in reporting standards, formal statistical interaction testing and quantitative sensitivity analysis were not feasible; instead, a qualitative stratified synthesis was conducted to contextualise the primary findings within methodologically and clinically defined subgroups. Table 6 presents the directional consistency of core taxonomic alterations stratified by these subgroups.

The impact of antidepressant medication status on microbiota composition was evaluated in 18 studies that stratified analyses or compared medicated versus drug-naïve subgroups. Eight studies directly comparing drug-naïve and medicated patients with MDD found no significant differences in alpha diversity or major taxonomic profiles between these subgroups, suggesting that microbiota alterations precede or are independent of pharmacological treatment. However, four studies observed that selective serotonin reuptake inhibitor exposure was associated with transient increases in *Bifidobacterium* and *Lactobacillus* abundance, while tricyclic antidepressant use correlated with reduced *Bacteroidetes* and increased *Firmicutes* in two investigations. Five studies employed antidepressant washout periods ranging from 2 to 8 weeks, with 3 reporting partial normalisation of microbiota profiles toward control levels, suggesting that some drug-induced alterations are reversible.

Table 6. Directional consistency of taxonomic findings stratified by sequencing platform and clinical subgroup.

Stratification Variable	Subgroup	N (Studies)	Directional Consistency of Core Taxa	Key Notes
Sequencing platform	16S rRNA amplicon (V3–V4)	12	<i>Faecalibacterium</i> ↓ (consistent); <i>Roseburia</i> ↓ (consistent); <i>Eubacterium</i> ↓ (consistent); <i>Alistipes</i> ↑ (consistent); <i>Eggerthella</i> ↑ (consistent); <i>Streptococcus</i> ↑ (consistent); <i>Firmicutes/Bacteroidetes</i> ratio (inconsistent)	Predominant method (26.7% of primary studies); differential primer amplification efficiencies may bias apparent abundance of specific taxa
	Shotgun metagenomics	1	<i>Faecalibacterium</i> ↓, <i>Roseburia</i> ↓, <i>Streptococcus</i> ↑ confirmed at species level; functional pathway analysis corroborated SCFA depletion	Species-level resolution; independent bioinformatic pipeline reduces primer bias
	qPCR (targeted)	4	<i>Faecalibacterium</i> ↓ (consistent); <i>Bifidobacterium</i> (mixed)	Limited to pre-selected taxa; confirms depletion of key butyrate producers
	Multi-omics (16S + metabolomics)	6	Concordant taxonomic shifts with functional validation: ↓ SCFA producers correlated with ↓ faecal butyrate; ↑ <i>Alistipes</i> correlated with ↑ kynurenine metabolites	Functional validation strengthens biological plausibility beyond taxonomic enumeration
Medication status	Drug-naïve (first-episode)	7	<i>Faecalibacterium</i> ↓, <i>Roseburia</i> ↓, <i>Streptococcus</i> ↑, <i>Alistipes</i> ↑ (directionally consistent with overall findings); effect sizes smaller than chronic cohorts	Suggests microbiota alterations are present early in disease course and are not merely secondary to chronic medication exposure
	Medicated (stable antidepressants)	8	Directional trends similar to drug-naïve; SSRI exposure associated with transient ↑ <i>Bifidobacterium</i> and ↑ <i>Lactobacillus</i> (4 studies); TCA use associated with ↓ <i>Bacteroidetes</i> and ↑ <i>Firmicutes</i> (2 studies)	Drug–microbiota interactions may confound or modify taxonomic associations
	Washout (2–8 weeks)	5	Partial normalisation toward control levels in 3 studies; <i>Faecalibacterium</i> and <i>Roseburia</i> partially restored; 2 studies showed no change	Suggests reversibility of some antidepressant-induced microbiota alterations
Disease stage	First-episode	7	Consistent directional changes; less pronounced alpha diversity reduction vs. chronic cohorts; hs-CRP correlations present even without medication exposure	Microbiota alterations are an early feature of depression pathophysiology
	Recurrent/chronic	12	More pronounced alpha diversity reduction; greater depletion of butyrate producers; stronger IL-6 and LPS correlations	Severity–microbial gradient suggested; chronicity may amplify dysbiosis

Table 6. Cont.

Stratification Variable	Subgroup	N (Studies)	Directional Consistency of Core Taxa	Key Notes
	Treatment-resistant depression	4	Marked depletion of <i>Faecalibacterium</i> and <i>Roseburia</i> vs. treatment-responsive patients; greater enrichment of <i>Alistipes</i> and <i>Streptococcus</i>	Microbial community severity appears to parallel clinical refractoriness
	Post-stroke depression	2	Distinct profile: ↑ <i>Bacteroidetes</i> , ↓ <i>Prevotella</i> vs. primary MDD	Subtype-specific dysbiosis suggested; requires independent validation

Note: N refers to primary observational studies reporting data within each subgroup. Directional consistency indicates the predominant direction of change reported for MDD vs. healthy controls within that subgroup. ↓ = decreased abundance in MDD; ↑ = increased abundance in MDD. SSRI = selective serotonin reuptake inhibitor; TCA = tricyclic antidepressant; SCFA = short-chain fatty acid. Individual study references are not cited within this table because the stratified synthesis draws upon aggregate data reported across multiple primary investigations; the primary studies contributing to each subgroup are detailed in Tables 1, 3 and 4 and in the narrative sections of the Results.

Disease stage and episode number were examined as modifiers of microbiota composition in 12 studies. First-episode drug-naïve patients showed similar directional taxonomic changes to chronic recurrent patients but with smaller effect sizes and less consistent statistical significance. Treatment-resistant depression, defined as failure of two or more antidepressant trials, was characterised by more pronounced depletion of *Faecalibacterium* and *Roseburia*, and greater enrichment of *Alistipes* and *Streptococcus*, compared to treatment-responsive patients in four studies that stratified by treatment history. Two studies of post-stroke depression identified distinct microbiota profiles compared to primary MDD, with greater *Bacteroidetes* abundance and reduced *Prevotella*, suggesting subtype-specific dysbiosis patterns.

Comorbid irritable bowel syndrome was present in 8 studies examining MDD-IBS overlap. In the context of metabolic comorbidity, Cao and colleagues examined MDD with overweight/obesity comorbidity, identifying distinct microbiota profiles characterised by more pronounced depletion of *Faecalibacterium* and *Roseburia*, and greater enrichment of *Streptococcus*, compared to MDD without metabolic comorbidity [19]. Hu et al. further demonstrated that gut microbiota alterations correlate with depression severity, with reduced *Faecalibacterium* and *Roseburia* abundance predicting higher HAM-D scores [21]. These findings suggest that metabolic dysfunction may act as a modifier of the depressive dysbiosis signature, with implications for personalised therapeutic targeting. Patients with comorbid MDD and IBS displayed more severe alpha diversity reductions and greater enrichment of Enterobacteriaceae compared to MDD without IBS or IBS alone. The *Firmicutes*-to-*Bacteroidetes* ratio was lower in comorbid cases than in either condition in isolation, suggesting additive or synergistic effects of psychiatric and gastrointestinal pathology on microbiota composition. Three studies controlled for dietary intake using food frequency questionnaires or 24 h dietary recalls; after statistical adjustment for fibre, fat, and total caloric intake, the associations between depression status and *Faecalibacterium*, *Roseburia*, and *Alistipes* remained significant in all 3 investigations, supporting the independence of these taxonomic changes from dietary confounding.

Several patterns emerge from this stratified analysis. First, the depletion of butyrate-producing genera (*Faecalibacterium*, *Roseburia*) and enrichment of pro-inflammatory taxa (*Alistipes*, *Eggerthella*, *Streptococcus*) demonstrated directional consistency across both 16S rRNA and shotgun metagenomic platforms, suggesting that these alterations represent genuine biological signals rather than technical artefacts of primer bias or bioinformatic

processing. Second, drug-naïve first-episode patients exhibited similar directional changes to chronic recurrent patients, albeit with smaller effect sizes and less pronounced alpha diversity reductions, supporting the hypothesis that microbiota alterations are present early in the disease course and are not merely secondary to chronic illness or cumulative medication exposure. Third, treatment-resistant depression was characterised by more pronounced dysbiosis than treatment-responsive depression, suggesting that microbial community severity may parallel clinical severity. However, the small number of studies in several subgroups, particularly shotgun metagenomics ($n = 1$), post-stroke depression ($n = 2$), and washout designs ($n = 5$), precludes definitive subgroup conclusions, and the absence of formal meta-regression or individual patient data limits the statistical robustness of these stratified comparisons. Future primary studies should report stratified findings by sequencing platform, medication status, and disease stage to enable definitive meta-analytic interaction testing.

3.6. Publication Bias

Evidence of publication bias was assessed qualitatively across the included systematic reviews and meta-analyses and quantitatively where individual participant data were available. Among the 8 meta-analyses reporting funnel plot asymmetry or Egger's regression tests, 5 (62.5%) identified significant asymmetry, suggesting a predominance of small studies with positive findings. The trim-and-fill correction applied in 3 meta-analyses attenuated but did not abolish the pooled effect sizes for alpha diversity reduction and *Faecalibacterium* depletion, indicating that the core findings are robust to publication bias adjustment. Funnel plots for beta diversity separation showed less asymmetry, likely because non-significant PERMANOVA results are more commonly reported than null taxonomic associations.

The file drawer problem was further evaluated by comparing effect sizes between studies with sample sizes above and below the median. Smaller studies ($n < 80$) reported larger standardised mean differences for taxonomic alterations than larger studies ($n > 150$) in 6 of 7 meta-analyses performing this stratification, consistent with small-study effects. However, the direction of associations remained consistent across sample size strata, and the largest individual study ($n = 312$) confirmed the primary findings of reduced alpha diversity and *Faecalibacterium* depletion. The overall certainty of evidence for probiotic antidepressant efficacy was consequently rated as low, downgraded from moderate owing to imprecision and potential publication bias in the GRADE evaluation. A synthesis of GRADE certainty-of-evidence ratings for key outcomes is presented in Table 7.

Table 7. GRADE summary of findings.

Outcome	N Studies	N Participants	Effect Direction	Certainty	Downgrade Factors	Key Findings
Reduced alpha diversity (Shannon)	15	2500+	↓ in MDD	Moderate	Inc, Ind	80% report decreased Shannon; SMD −0.42 to −0.89
Significant beta diversity separation	15	2500+	100% significant	Moderate	Methodological heterogeneity	All studies show significant MDD vs. HC separation ($p < 0.05$)
Depletion of <i>Faecalibacterium</i>	20	2000+	Consistent ↓	Moderate	Obs, geographic	90% (18/20) of studies report significant depletion

Table 7. Cont.

Outcome	N Studies	N Participants	Effect Direction	Certainty	Downgrade Factors	Key Findings
Depletion of <i>Roseburia</i>	31	3000+	Consistent ↓	Moderate	Obs design	77% (24/31) of studies report significant depletion
Enrichment of <i>Alistipes</i>	20	~2000	↑ in MDD	Moderate	Obs design	60% (12/20) of studies report significant enrichment
Enrichment of <i>Eggerthella</i>	24	~3000+	↑ in MDD	Moderate	Obs design	66.7% (16/24) of studies report significant enrichment
Reduced faecal butyrate	8	1000+	Consistent ↓	Low	Few studies, heterogeneous	87.5% report reduced butyrate in MDD
Elevated IL-6	5	800+	Elevation	Low	Obs, heterogeneity	Elevated IL-6; inverse correlation with <i>Faecalibacterium</i>
Antidepressant efficacy of probiotics	2	160	Beneficial	Low	Few RCTs, small N, RoB	SMD = 0.49, 95% CI [0.24, 0.73]

Note: ↓ = decreased in MDD compared to healthy controls; ↑ = increased in MDD compared to healthy controls. GRADE: High = we are very confident that the true effect lies close to the estimate; Moderate = moderately confident; Low = limited confidence; Very Low = very little confidence. SMD = standardised mean difference; Obs = observational design; Inc = inconsistency; Ind = indirectness; RoB = risk of bias. Moderate certainty reflects upgrade from low due to large effect and plausibility; factors listed prevented further upgrade to high.

4. Discussion

4.1. Summary of Main Findings

This systematic review synthesised 89 studies (58 primary investigations and 31 secondary syntheses) examining the microbiota–gut–brain axis in major depressive disorder. The principal findings, reduced alpha diversity, robust beta diversity separation between MDD and control cohorts, depletion of butyrate-producing genera (*Faecalibacterium*, *Roseburia*), and enrichment of pro-inflammatory taxa (*Alistipes*, *Streptococcus*), converge to support a functional model of depression-associated dysbiosis centred on impaired SCFA production and low-grade inflammation. These observational patterns were corroborated by preclinical FMT experiments and by preliminary efficacy of microbiome-targeted interventions, though significant inter-study heterogeneity precludes the definition of a universal microbial signature.

4.2. Comparison with the Existing Literature

The present manuscript offers four substantive innovations relative to existing systematic reviews in this field. This review extends prior syntheses in four respects. First, it incorporates studies published through March 2025, including twelve RCTs of microbiome-targeted interventions and one longitudinal cohort investigation, thereby offering a more dynamic assessment of causality than earlier observational syntheses. Second, the stratified narrative analysis (Table 6) systematically examines directional consistency across sequencing platforms, medication status, and disease stage, an approach not previously attempted in this literature. Third, the explicit distinction between antibiotic-depleted and germ-free preclinical models, alongside detailed methodological reporting of dosing and administration protocols, enhances the translational interpretability of FMT evidence. Fourth, the integration of practical barriers to clinical translation (cost, standardisation, temporal variability) within the same synthesis provides a more complete roadmap from bench to bedside than prior reviews that focused predominantly on biological mechanisms.

4.3. Biological Mechanisms: From Correlation Toward Causation

The convergence of observational, interventional, and preclinical evidence reviewed herein supports a mechanistic model in which gut microbiota alterations in MDD are not merely epiphenomenal but may actively participate in the pathophysiological cascade. Four interconnected pathways merit detailed consideration.

4.3.1. The Vagus Nerve and Neural Transmission

The vagus nerve serves as the principal afferent conduit for gut–brain signalling [1,14,45], with bacterial metabolites capable of directly activating vagal terminals to influence central emotional processing. Siopi et al. [45] demonstrated that specific bacterial metabolites can directly activate vagal afferent terminals and that gut microbiota changes require vagus nerve integrity to promote depressive-like behaviours in mice, with vagotomised animals showing resistance to dysbiosis-induced behavioural deficits. Zou et al. [46] further showed that subdiaphragmatic vagotomy alleviated anxiety-like behaviour in *Salmonella*-induced gut infections by enhancing microbiota-derived GABA signalling, underscoring the bidirectional nature of this neural–microbiota communication. This suggests a pivotal role for vagal signalling in transducing peripheral microbial states into central emotional and cognitive processing. The observation that depression-associated microbiota transplanted into microbiota-depleted rats induced alterations in vagal afferent firing patterns further reinforces the centrality of this neural highway [5]. Enteroendocrine cells (EECs) function as specialised chemosensory transducers of the intestinal epithelium that communicate luminal signals to the nervous system. Bellono et al. [47] demonstrated using intestinal organoid models that enterochromaffin cells, a subset of EECs, are electrically excitable, express specific chemosensory receptors, and modulate serotonin-sensitive primary afferent nerve fibres via synaptic connections, enabling direct transduction of environmental, metabolic, and microbial information from the gut lumen to the nervous system. Kaelberer et al. [48] subsequently identified that a subpopulation of EECs termed neuropod cells forms direct synapses with vagal afferent neurons, using glutamate as a neurotransmitter to transduce gut luminal signals to the brainstem in milliseconds. These findings established a physical neuroepithelial circuit connecting the intestinal lumen to the brainstem with synaptic precision. In the context of depression, dysbiosis-driven reductions in SCFA-producing bacteria may compromise EEC-mediated vagal afferent signalling. Tolhurst et al. [44] and Psichas et al. [49] demonstrated that short-chain fatty acids, particularly butyrate and propionate, stimulate L-cell secretion of GLP-1 and PYY via the free fatty acid receptor FFAR2. Ye et al. [50] further showed that enteroendocrine cells sense bacterial tryptophan catabolites through the transient receptor potential ankyrin A1 (Trpa1) receptor to activate vagal sensory ganglia via serotonin release, establishing a molecular pathway by which microbial metabolites regulate enteroendocrine–vagal communication. Diminished luminal butyrate and propionate concentrations in depression-associated dysbiosis reduce the stimulatory drive on EECs, leading to attenuated release of GLP-1, PYY, and serotonin and consequently to altered vagal firing patterns that propagate to central autonomic and limbic circuits implicated in mood regulation [46–50]. This EEC–vagal–limbic axis disruption represents a plausible mechanistic link between intestinal dysbiosis and the emotional dysregulation characteristic of MDD. Preclinical studies demonstrate that specific probiotic strains, including *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175, can modulate GABA receptor expression in the hippocampus and reduce stress-induced corticosterone release in rodent models [13]. Conversely, not all *Lactobacillus* strains exert beneficial neurobehavioural effects. Ingestion of *Lactobacillus intestinalis* and *Lactobacillus reuteri* has been shown to induce depression- and anhedonia-like phenotypes in antibiotic-treated mice, with concomitant elevation of plasma interleukin-6 and downregulation

of prefrontal cortical synaptic proteins; notably, subdiaphragmatic vagotomy abolished these behavioural and biochemical abnormalities, implicating vagal afferent signalling as the critical transduction pathway [12]. This finding highlights that probiotic effects are not uniformly antidepressant and underscores the critical importance of strain-specific selection in psychobiotic development [13,25].

4.3.2. Neuroinflammation and the Leaky Gut Hypothesis

Peripheral dysbiosis translates into central neuroinflammation through compromised intestinal barrier integrity consequent to butyrate depletion, enabling bacterial product translocation and systemic cytokine release [13,15–19]. Elevated circulating LPS triggers Toll-like receptor 4 signalling on immune cells, activating nuclear factor-kappa B pathways and stimulating sustained release of pro-inflammatory cytokines, including interleukin-6, tumour necrosis factor-alpha, and interleukin-1 beta [12,17,18]. The present review found that interleukin-6 was elevated in patients with major depressive disorder across primary studies measuring this cytokine [12,14,17,18], with significant inverse correlations between *Faecalibacterium* abundance and circulating IL-6 concentrations [14,17,18]. These peripheral inflammatory mediators can compromise blood–brain barrier integrity and activate microglial cells, establishing a self-perpetuating cycle of neuroinflammation that disrupts neurotransmitter metabolism, neuroplasticity, and neural circuitry [12,14,17,18]. In first-episode drug-naïve patients, Liu et al. [14] demonstrated that elevated plasma IL-1 β correlated positively with *Ruminococcus bromii* and *Streptococcus gallolyticus* abundance, while Xie et al. [17] identified inverse correlations between *Faecalibacterium* and IL-6 (Spearman rho = -0.31 to -0.48 , $p < 0.01$) in a multi-omics cohort, directly linking reduced butyrate-producing taxa to systemic inflammation.

4.3.3. Metabolic Pathways: SCFAs and Tryptophan-Kynurenine Metabolism

Short-chain fatty acid deficiency and tryptophan-kynurenine dysregulation constitute the two principal metabolic mechanisms linking dysbiosis to depression [12,14–17,19]. SCFA deficiency removes a critical anti-inflammatory brake on microglial activation, while tryptophan shunting toward neurotoxic kynurenine metabolites depletes serotonin and promotes glutamatergic excitotoxicity [12–15,17,19].

It is noteworthy, however, that SCFA effects are not uniformly beneficial. Excessive propionate has been linked to adverse neurobehavioural phenotypes in preclinical models, and the optimal concentration ranges for neuroprotective versus potentially neurotoxic SCFA exposure remain undefined. Moreover, recent large population-based evidence suggests that the association between faecal SCFA concentrations and depressive symptoms varies significantly by age and demographic factors, further complicating any universal dosage recommendation. In a cross-sectional study of 534 Japanese adults from the DO-SANCO Health Study, higher faecal propionate concentrations were significantly associated with elevated PHQ-9 scores (beta = 0.62, $p < 0.01$), with the association driven entirely by participants aged 60 years and older (beta = 0.80, $p < 0.01$), whereas no significant relationship was observed in younger adults (beta = 0.18, $p = 0.62$). These findings indicate that the neuroactive effects of SCFAs may be modulated by age-related changes in intestinal physiology, immune function, or microbial ecology and that blanket recommendations for SCFA supplementation across the lifespan are not supported by current epidemiological data [51].

This dose-dependent complexity underscores the need for careful titration in any future microbiome-targeted therapeutic strategies.

Tryptophan shunting toward the kynurenine pathway, driven by IDO induction from pro-inflammatory cytokines, represents a second metabolic mechanism with direct impli-

cations for serotonergic deficit and glutamatergic excitotoxicity [19,29,43]. This metabolic diversion is driven by indoleamine 2,3-dioxygenase activity, which is induced by the very pro-inflammatory cytokines that are elevated in depressed cohorts [19,30,43]. The resulting depletion of peripheral serotonin pools, combined with the production of quinolinic acid, a potent N-methyl-D-aspartate receptor agonist, establishes a coherent biochemical framework linking microbial dysbiosis to both serotonergic deficit and glutamatergic excitotoxicity [19,30,43].

4.3.4. The Chicken or Egg Problem: Causality and Directionality

The question of temporal primacy, whether dysbiosis precedes depression or emerges as its consequence, remains unresolved and represents one of the most pressing challenges in the field [1,12]. The present review identified evidence supporting both directionalities. On one hand, faecal microbiota transplantation from depressed human donors to antibiotic-depleted rats induced anhedonic- and anxiety-like behaviours within fourteen days, indicating that a depression-associated microbiota profile can transmit behavioural phenotypes across species barriers and supporting a causal hypothesis compatible with, but not definitively proving, a causal role for intestinal dysbiosis in depression. It is critical to distinguish between the two principal preclinical models employed in these translational experiments: antibiotic-depleted animals, which retain immune priming from prior microbial exposure, and germ-free animals, which exhibit naive immune development. The Kelly et al. [5] study utilised antibiotic-depleted rats, a model with greater ecological validity but potentially confounded by residual antibiotic effects on host physiology. These model-specific constraints must be considered when extrapolating preclinical FMT findings to human depression. On the other hand, one prospective cohort study demonstrated that diurnal cortisol slope flattening preceded microbiota alterations by four to six weeks, suggesting that HPA axis hyperactivity may constitute an upstream driver of dysbiosis [12,18]. This bidirectional relationship implies that microbiota alterations and depressive states may enter a self-amplifying feedback loop, wherein stress-induced HPA dysfunction perturbs the intestinal environment, microbial dysbiosis exacerbates inflammatory and metabolic derangements, and these peripheral changes feed back to the brain to sustain or deepen depressive symptomatology [1,17,18].

To further contextualise the strength of causal inference, the Bradford Hill criteria provide a useful heuristic framework for evaluating the microbiota–depression association. Several criteria are partially satisfied: biological plausibility is strongly supported by identified mechanistic pathways involving SCFA deficiency, increased intestinal permeability, and neuroinflammation; consistency is demonstrated by the recurrent taxonomic alterations observed across diverse populations and sequencing platforms; and analogy is supported by preclinical faecal microbiota transplantation experiments demonstrating the transmissibility of depression-like phenotypes across species barriers. Conversely, other criteria remain inadequately fulfilled: temporality is not definitively established, as prospective human cohort data are extremely limited; specificity is weak, given that similar dysbiotic patterns are observed in Parkinson’s disease, irritable bowel syndrome, and metabolic disorders; and a clear biological gradient or dose–response relationship has not been fully characterised. Consequently, the available evidence supports a causal possibility rather than a proven causal relationship, justifying the cautious interpretive language employed throughout this review.

4.4. Clinical Implications and Therapeutic Perspectives

The translational implications of these findings are substantial and multifaceted. From a diagnostic standpoint, the consistency of the microbial signature identified across primary

studies supports the emerging concept of microbiota profiling as a potential adjunctive biomarker for MDD [14,17,21,22]. Machine learning classifiers applied to genus-level abundance data achieved area under the receiver operating characteristic curve values of 0.82 to 0.87 in independent cohorts, and the combination of bacterial abundance with short-chain fatty acid concentrations yielded an area under the curve of 0.91 for treatment-resistant depression classification. While these findings require independent validation and standardisation before clinical implementation, they suggest that stool-based microbiome analysis could eventually complement existing diagnostic instruments, particularly in cases where symptom presentation is atypical or where objective biological confirmation is desired.

From a therapeutic perspective, the twelve randomised controlled trials of microbiome-targeted interventions reviewed herein provide encouraging, albeit preliminary, evidence for the antidepressant potential of psychobiotics [13,25]. Randomised probiotic trials support adjunctive antidepressant efficacy, though strain-specificity and heterogeneity in baseline severity and concomitant medication limit generalisation [6,30]. The mechanistic rationale for these effects appears to involve restoration of *Faecalibacterium* and *Roseburia* abundance, normalisation of kynurenine pathway activity, and modulation of vagal afferent signalling [13]. The observed effects of *L. helveticus* R0052 and *B. longum* R0175 may not generalise to other probiotic strains.

Dietary interventions represent a non-pharmacological avenue with strong biological plausibility [15,29]. Preclinical investigations indicate that omega-3 polyunsaturated fatty acids alleviate depressive symptoms potentially by improving intestinal barrier integrity, modulating neuroplasticity, and exerting anti-inflammatory and antioxidant effects [52,53]. Dietary fibre-rich patterns may improve depressive phenotypes through microbiota-mediated mechanisms, as demonstrated by Yang et al. [15] and Jia et al. [16], who identified fibre-fermenting taxa as key predictors of treatment response. Given that SCFA-producing bacteria depend on dietary fibre as their primary substrate, fibre-rich dietary patterns may exert antidepressant effects partially through microbiota-mediated mechanisms [15,16]. Prebiotic supplementation with galacto-oligosaccharides or inulin has shown preliminary efficacy in increasing butyrate-producing taxa and reducing salivary cortisol awakening response, although effects on standardised depression rating scales have been modest [15].

Faecal microbiota transplantation represents the most radical microbiome-targeted intervention and the one most directly informed by the preclinical FMT evidence reviewed herein [5]. The single open-label pilot trial in treatment-resistant depression reported remission in two of three active-treatment participants at eight weeks, with durable microbiota engraftment and increased *Faecalibacterium* abundance in responders.

4.5. Strengths and Limitations

This systematic review possesses several methodological strengths that enhance the confidence with which its findings can be interpreted. The inclusion of 89 studies represents one of the most comprehensive syntheses of the microbiota–depression literature to date, encompassing primary observational investigations, interventional trials, preclinical experiments, and prior systematic reviews [2,3,5]. Adherence to the PRISMA 2020 guidelines and prospective OSF registration minimises the risk of selective reporting and ensures methodological transparency. The incorporation of recent multi-omics studies published between 2023 and 2025 provides timely insight into emerging analytical frameworks that move beyond simple taxonomic enumeration toward functional and mechanistic understanding [14,17,22].

These strengths must be weighed against meaningful limitations. First, the high heterogeneity in microbiological methods, most notably the predominance of 16S rRNA amplicon sequencing alongside a smaller subset of shotgun metagenomic studies, introduces technical variability that complicates cross-study comparison [12,14,24].

The stratified narrative analysis presented in Table 6 was inherently limited by the small number of studies within several subgroups and by the absence of formal statistical interaction testing, which precludes definitive conclusions regarding effect modification by sequencing platform or clinical phenotype. While the directional consistency of core taxonomic findings across 16S rRNA and shotgun metagenomic platforms supports the biological validity of the proposed depressive dysbiosis signature, the single shotgun metagenomic study and the limited number of drug-naïve first-episode investigations (n = 7) constrain the generalisability of subgroup comparisons.

Different 16S variable regions exhibit differential amplification efficiencies that can systematically bias the apparent abundance of certain bacterial groups [24,30]. Direct comparisons across primary studies reveal that even investigations employing the same sequencing platform frequently yield discrepant taxonomic findings. For example, Jiang et al. [3] and Aizawa et al. [4], both using 16S rRNA sequencing, reported divergent *Bifidobacterium* trends in MDD, while Liu et al. [14] and Hu et al. [21], employing similar V3–V4 amplicon approaches, identified consistent *Faecalibacterium* depletion but divergent Bacteroidetes patterns. These discrepancies suggest that patient population characteristics, including geographic origin, dietary background, and medication status, may exert greater influence on microbiota composition than technical factors alone. Second, confounding factors represent a persistent challenge. Dietary patterns, physical activity levels, antidepressant medication, and body mass index all influence gut microbiota composition and were not uniformly controlled across the included studies [8,22]. Although several investigations reported that taxonomic associations remained significant after statistical adjustment for fibre, fat, and total caloric intake, residual confounding cannot be excluded [22,39]. Third, the predominantly cross-sectional design of the observational literature limits causal inference [2,3]. While the preclinical FMT studies and the randomised probiotic trials provide directional evidence, the observational backbone of this review remains correlational [5]. Fourth, the geographic concentration of primary studies in East Asia raises questions about the generalisability of findings to Western populations with divergent dietary traditions, genetic backgrounds, and environmental exposures [2,22].

Fifth, the inclusion of 31 secondary syntheses (systematic reviews and narrative reviews) alongside 58 primary studies represents a methodological consideration that warrants explicit clarification. These review articles were included to contextualise the breadth of the evidence base and to provide methodological cross-validation; however, they do not contribute independent data to the primary narrative synthesis, which is grounded exclusively in primary observational and interventional investigations. This distinction is important for interpreting the evidentiary weight of the findings: taxonomic and mechanistic conclusions derive from the 58 primary studies, while the review articles serve as corroborative benchmarks and historical mapping of the field's evolution.

Sixth, although some studies mentioned in Section 3.5 adjusted for dietary factors using food frequency questionnaires or 24 h dietary recalls, the vast majority of primary studies did not control, or only roughly controlled, for key variables such as dietary structure, dietary fibre intake, and consumption of fermented foods. Given the profound influence of these factors on gut microbiota composition, residual dietary confounding cannot be excluded and may partially explain the heterogeneity of taxonomic findings across cohorts. Future research should prioritise the use of standardised dietary assessment tools (e.g.,

validated food frequency questionnaires) or short-term controlled dietary intervention designs to isolate microbiota–depression associations from dietary influences.

A final methodological consideration concerns the relationship between the 89 included studies and the 47 listed references. Of the 89 studies, 31 were secondary syntheses (28 systematic reviews and meta-analyses; 3 narrative reviews), all of which are individually cited in the reference list. The remaining 58 were primary investigations (45 observational studies, 12 RCTs, 1 longitudinal cohort). Of these 58 primary studies, 29 underwent detailed individual-level data extraction and are directly cited in the reference list and reported in Tables 1 and 3–5. The remaining 29 primary observational studies are represented through the 28 included systematic reviews and meta-analyses that collectively encompass their findings. This nested citation approach is standard practice in narrative synthesis methodology, where primary studies not individually extracted are contextualised through the secondary syntheses that review them, ensuring comprehensive evidence coverage without duplicate citation [36]. Future updates of this review with individual patient data meta-analytic techniques will permit direct citation of all primary studies.

4.6. Future Research Directions

The findings of this review delineate several priority areas for future investigation. First, large-scale, longitudinal cohort studies with repeated microbiota sampling are urgently needed to resolve the question of temporal primacy and to identify whether specific microbial alterations precede the onset of depressive episodes or emerge as epiphenomena of chronic illness [2,4,12]. Dietary assessment represents a critical methodological priority. The majority of included studies did not control for dietary fibre intake, fermented food consumption, or overall dietary patterns using validated questionnaires (e.g., food frequency questionnaires). Future studies should incorporate standardised dietary assessments or controlled feeding designs to isolate microbiota–depression associations from dietary confounding. Second, the field would benefit enormously from the establishment of standardised “core” microbiota definitions for MDD, consensus taxonomic and functional signatures that could serve as reference benchmarks for diagnostic and therapeutic development [14,15,17,21]. Third, the promising antidepressant signals observed in psychobiotic trials must be confirmed and refined through adequately powered, multi-centre randomised controlled trials employing standardised probiotic formulations, uniform outcome measures, and mechanistic endpoints including microbiota profiling, inflammatory biomarkers, and neuroimaging correlates [13,25,27]. Fourth, future FMT research in depression should prioritise donor–recipient matching algorithms, long-term safety monitoring, and identification of responder phenotypes based on baseline microbiota composition [29,48]. Finally, the integration of artificial intelligence and machine learning approaches with multi-omics datasets holds considerable promise for disentangling the complex interactions between host genetics, microbiota composition, diet, and environmental factors that collectively shape depression vulnerability [38].

In conclusion, the evidence synthesised in this systematic review supports a robust association between gut microbiota dysbiosis and major depressive disorder, underpinned by multiple biologically plausible mechanistic pathways. While the causal arrow remains to be fully elucidated, the consistency of the microbial signature across diverse populations and methodologies, combined with the preliminary efficacy of microbiome-targeted interventions, positions the gut–brain axis as a compelling frontier for both biological understanding and therapeutic innovation in depression [1,17].

5. Conclusions

This systematic review of 89 studies demonstrates that major depressive disorder is consistently associated with a recurrent dysbiotic pattern, characterised by depletion of butyrate-producing genera (*Faecalibacterium*, *Roseburia*) and enrichment of pro-inflammatory taxa (*Alistipes*, *Streptococcus*), underpinned by compromised intestinal barrier integrity, low-grade systemic inflammation, tryptophan-kynurenine dysregulation, and HPA axis dysfunction. While preclinical faecal microbiota transplantation and randomised probiotic trials provide preliminary support for a causal interpretation, significant inter-study heterogeneity in sequencing platforms, geographic populations, and clinical phenotypes precludes the definition of a universal microbial signature. Translation into practice requires standardised dietary protocols, harmonised multi-centre trials, and resolution of barriers including cost, inter-laboratory variability, and temporal stability of microbiota profiles.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/sanpp176020006/s1>, Supplementary Material S1: Full electronic search strategies for PubMed/MEDLINE, Embase, PsycINFO, Scopus, Web of Science Core Collection, and Cochrane Library. Supplementary Material S2: Completed PRISMA 2020 checklist documenting the location of each checklist item within the manuscript.

Author Contributions: Conceptualisation, L.C., A.C. (Andrea Cicoli), M.L., S.S., P.T., T.S., Y.B., E.D., A.V. and A.C. (Alberto Costa); methodology, L.C., A.C. (Andrea Cicoli), A.V. and A.C. (Alberto Costa); software, L.C. and A.C. (Andrea Cicoli); validation, M.L., S.S. and P.T.; formal analysis, L.C., A.C. (Andrea Cicoli), and E.D.; investigation, L.C., A.C. (Andrea Cicoli), M.L., S.S., P.T., T.S., Y.B. and E.D.; resources, A.V. and A.C. (Alberto Costa); data curation, L.C. and A.C. (Andrea Cicoli); writing, original draft preparation, L.C. and A.C. (Andrea Cicoli); writing, review and editing, all authors; visualisation, L.C., S.S., T.S. and Y.B.; supervision, L.C., A.V. and A.C. (Alberto Costa); project administration, L.C.; funding acquisition, L.C. and A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable. This study is a systematic review of the published literature and did not involve human participants or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting the findings of this review are available upon reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MDD	Major Depressive Disorder
SSRIs	Selective Serotonin Reuptake Inhibitors
SNRIs	Serotonin-Norepinephrine Reuptake Inhibitors
TRD	Treatment-Resistant Depression
GBA	Gut–Brain Axis
ENS	Enteric Nervous System
CNS	Central Nervous System
GABA	Gamma-Aminobutyric Acid
SCFAs	Short-Chain Fatty Acids
LPS	Lipopolysaccharide
LBP	Lipopolysaccharide-Binding Protein

sCD14	Soluble CD14
IL-6	Interleukin-6
TNF-alpha	Tumour Necrosis Factor-alpha
IL-1beta	Interleukin-1 beta
CRP	C-Reactive Protein
HPA	Hypothalamic–Pituitary–Adrenal
BDNF	Brain-Derived Neurotrophic Factor
IDO	Indoleamine 2,3-Dioxygenase
FMT	Faecal Microbiota Transplantation
PECO	Population, Exposure/Intervention, Comparator, Outcome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PICOS	Population, Intervention/Exposure, Comparator, Outcome, Study design
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
ICD-10	International Classification of Diseases, Tenth Revision
qPCR	Quantitative Polymerase Chain Reaction
OTUs	Operational Taxonomic Units
HAM-D/HDRS	Hamilton Depression Rating Scale
MADRS	Montgomery–Åsberg Depression Rating Scale
BDI/BDI-II	Beck Depression Inventory
PHQ-9	Patient Health Questionnaire-9
RCTs	Randomised Controlled Trials
OSF	Open Science Framework
PRESS	Peer Review of Electronic Search Strategies
NOS	Newcastle–Ottawa Scale
RoB 2	Revised Cochrane Risk of Bias tool
AMSTAR 2	Assessment of Multiple Systematic Reviews 2
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
PERMANOVA	Permutational Multivariate Analysis of Variance
ANOSIM	Analysis of Similarities
mRNA	Messenger RNA
AUC	Area Under the Curve
ROC	Receiver Operating Characteristic
IBS	Irritable Bowel Syndrome
MINI	Mini-International Neuropsychiatric Interview
GMV	Grey Matter Volume
DSSS	Depression and Somatic Symptoms Scale
CFU	Colony-Forming Units
PCoA	Principal Coordinate Analysis

References

1. Bravo, J.A.; Forsythe, P.; Chew, M.V.; Escaravage, E.; Savignac, H.M.; Dinan, T.G.; Bienenstock, J.; Cryan, J.F. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 16050–16055. [[CrossRef](#)] [[PubMed](#)]
2. Naseribafrouei, A.; Hestad, K.; Avershina, E.; Sekelja, M.; Linlokken, A.; Wilson, R.; Rudi, K. Correlation between the human fecal microbiota and depression. *Neurogastroenterol. Motil.* **2014**, *26*, 1155–1162. [[CrossRef](#)] [[PubMed](#)]
3. Jiang, H.; Ling, Z.; Zhang, Y.; Mao, H.; Ma, Z.; Yin, Y.; Wang, W.; Tang, W.; Tan, Z.; Shi, J.; et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* **2015**, *48*, 186–194. [[CrossRef](#)] [[PubMed](#)]
4. Aizawa, E.; Tsuji, H.; Asahara, T.; Takahashi, T.; Teraishi, T.; Yoshida, S.; Ota, M.; Koga, N.; Hattori, K.; Kunugi, H. Possible association of *Bifidobacterium* and *Lactobacillus* in the gut microbiota of patients with major depressive disorder. *J. Affect. Disord.* **2016**, *202*, 254–257. [[CrossRef](#)] [[PubMed](#)]
5. Kelly, J.R.; Borre, Y.; O'Brien, C.; Patterson, E.; El Aidy, S.; Deane, J.; Kennedy, P.J.; Beers, S.; Scott, K.; Moloney, G.; et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.* **2016**, *82*, 109–118. [[CrossRef](#)] [[PubMed](#)]

6. Messaoudi, M.; Lalonde, R.; Violle, N.; Javelot, H.; Desor, D.; Nejdi, A.; Bisson, J.F.; Rougeot, C.; Pichelin, M.; Cazaubiel, M.; et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br. J. Nutr.* **2011**, *105*, 755–764. [[CrossRef](#)] [[PubMed](#)]
7. Valles-Colomer, M.; Falony, G.; Darzi, Y.; Tigchelaar, E.F.; Wang, J.; Tito, R.Y.; Shiweck, C.; Kurilshikov, A.; Joossens, M.; Wijnemga, C.; et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.* **2019**, *4*, 623–632. [[CrossRef](#)] [[PubMed](#)]
8. Barandouzi, Z.A.; Starkweather, A.R.; Henderson, W.A.; Gyamfi, A.; Cong, X.S. Altered composition of gut microbiota in depression: A systematic review. *Front. Psychiatry* **2020**, *11*, 541. [[CrossRef](#)] [[PubMed](#)]
9. Sanada, K.; Nakajima, S.; Kurokawa, S.; Barcelo-Soler, A.; Ikuta, T.; Sugiyama, H.; Yoshimura, R.; Shinba, T.; Hirano, Y.; Tomioka, Y.; et al. Gut microbiota and major depressive disorder: A systematic review and meta-analysis. *J. Affect. Disord.* **2020**, *266*, 1–13. [[CrossRef](#)] [[PubMed](#)]
10. Simpson, C.A.; Diaz-Arteche, C.; Eliby, D.; Schwartz, O.S.; Simmons, J.G.; Cowan, C.S.M. The gut microbiota in anxiety and depression—A systematic review. *Clin. Psychol. Rev.* **2021**, *83*, 101943. [[CrossRef](#)] [[PubMed](#)]
11. Gao, M.; Wang, J.; Liu, P.; Tu, H.; Zhang, R.; Zhang, Y.; Sun, N.; Zhang, K. Gut microbiota composition in depressive disorder: A systematic review, meta-analysis, and meta-regression. *Transl. Psychiatry* **2023**, *13*, 379. [[CrossRef](#)] [[PubMed](#)]
12. Zhao, H.; Jin, K.; Jiang, C.; Pan, F.; Wu, J.; Luan, H.; Zhao, Z.; Chen, J.; Mou, T.; Wang, Z.; et al. A pilot exploration of multi-omics research of gut microbiome in major depressive disorders. *Transl. Psychiatry* **2022**, *12*, 8. [[CrossRef](#)] [[PubMed](#)]
13. Tian, P.; Zou, R.; Yang, J.; Zhang, Y.; Shi, X.; Zhang, Y.; Li, X.; Liu, Y.; Zeng, Q.; Wang, L.; et al. *Bifidobacterium breve* CCFM1025 improves depression in patients with major depressive disorder: Evidence from a randomized, placebo-controlled trial. *Brain Behav. Immun.* **2022**, *108*, 239–248. [[CrossRef](#)] [[PubMed](#)]
14. Liu, S.; Li, Y.; Shi, Y.; Rao, Z.; Zhang, Y.; Zhang, Y.; Wang, T.; Kong, H.; Zhu, S.; Zhu, D.M.; et al. Multi-omics analyses of the gut microbiome, fecal metabolome, and multimodal brain MRI reveal the role of *Alistipes* and its related metabolites in major depressive disorder. *Psychol. Med.* **2025**, *55*, e190. [[CrossRef](#)] [[PubMed](#)]
15. Yang, J.; Zheng, P.; Li, Y.; Wu, J.; Tan, X.; Zhou, J.; Sun, Z.; Chen, X.; Zhang, G.; Zhang, H.; et al. Landscapes of bacterial and metabolic signatures in major depressive disorders. *Sci. Adv.* **2020**, *6*, eaba8555. [[CrossRef](#)] [[PubMed](#)]
16. Jia, M.; Fan, Y.; Ma, Q.; Yang, D.; Wang, Y.; He, X.; Zhao, B.; Zhan, X.; Qi, Z.; Ren, Y.; et al. Gut microbiota dysbiosis promotes cognitive impairment via bile acid metabolism in major depressive disorder. *Transl. Psychiatry* **2024**, *14*, 503. [[CrossRef](#)] [[PubMed](#)]
17. Xie, Z.; Huang, J.; Sun, G.; He, S.; Luo, Z.; Zhang, L.; Li, L.; Yao, M.; Du, C.; Yu, W.; et al. Integrated multi-omics analysis reveals gut microbiota dysbiosis and systemic disturbance in major depressive disorder. *Psychiatry Res.* **2024**, *334*, 115804. [[CrossRef](#)] [[PubMed](#)]
18. Liu, P.; Jing, L.; Guo, F.; Xu, Y.; Cheng, J.; Liu, S.; Liu, L.; Liu, Z.; Zhang, K.; Sun, N. Characteristics of gut microbiota and its correlation with hs-CRP and somatic symptoms in first-episode treatment-naïve major depressive disorder. *J. Affect. Disord.* **2024**, *356*, 664–671. [[CrossRef](#)] [[PubMed](#)]
19. Cao, B.; Lu, C.; Yan, L.; McIntyre, R.S.; Teopiz, K.M.; Zhan, L. The dysbiosis of gut microbiota in major depressive disorder and comorbidity with overweight/obesity: Unraveling biomarkers and metabolic pathways from a microbial perspective. *BMC Psychiatry* **2025**, *25*, 1069. [[CrossRef](#)] [[PubMed](#)]
20. Rong, H.; Xie, X.-H.; Zhao, J.; Lai, W.-T.; Wang, M.-B.; Xu, D.; Liu, Y.-H.; Guo, Y.-Y.; Xu, S.-X.; Deng, W.-F.; et al. Similarly in depression, nuances of gut microbiota: Evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. *J. Psychiatr. Res.* **2019**, *113*, 90–99. [[CrossRef](#)] [[PubMed](#)]
21. Hu, X.; Li, Y.; Wu, J.; Zhang, H.; Huang, Y.; Tan, X.; Wen, L.; Zhou, X.; Xie, P.; Olasunkanmi, O.I.; et al. Changes of gut microbiota reflect the severity of major depressive disorder: A cross sectional study. *Transl. Psychiatry* **2023**, *13*, 137. [[CrossRef](#)] [[PubMed](#)]
22. Cusotto, S.; Colle, R.; Voican, C.S.; Ciocan, D.M.; Trainel, N.; Bottemanne, H.; Pelloux, Y.; David, D.J.; Cassard, A.M.; Perlemuter, G.; et al. The Gut Microbiota Is Altered in Antidepressant-Free Depressed Patients and Is Associated with Depression Severity. *J. Neurochem.* **2025**, *169*, e70173. [[CrossRef](#)] [[PubMed](#)]
23. Wang, Y.; Zhou, J.; Ye, J.; Sun, Z.; He, Y.; Zhao, Y.; Ren, S.; Zhang, G.; Liu, M.; Zheng, P.; et al. Multi-omics reveal microbial determinants impacting the treatment outcome of antidepressants in major depressive disorder. *Microbiome* **2023**, *11*, 195. [[CrossRef](#)] [[PubMed](#)]
24. Amin, N.; Liu, J.; Bonnechere, B.; MahmoudianDehkordi, S.; Arnold, M.; Batra, R.; Chiou, Y.J.; Fernandes, M.; Ikram, M.A.; Kraaij, R.; et al. Interplay of Metabolome and Gut Microbiome in Individuals with Major Depressive Disorder vs Control Individuals. *JAMA Psychiatry* **2023**, *80*, 597–609. [[CrossRef](#)] [[PubMed](#)]
25. Lin, S.-K.K.; Kuo, P.-H.; Hsu, C.-Y.; Chiu, Y.-H.; Chen, C.-H. The effects of *Lactobacillus plantarum* PS128 in patients with major depressive disorder: An eight-week double-blind, placebo-controlled study. *Asian J. Psychiatry* **2024**, *95*, 104210. [[CrossRef](#)] [[PubMed](#)]
26. Knudsen, J.K.; Bundgaard-Nielsen, C.; Hjerrild, S.; Nielsen, R.E.; Leutscher, P.; Sørensen, S. Gut microbiota variations in patients diagnosed with major depressive disorder—A systematic review. *Brain Behav.* **2021**, *11*, e02177. [[CrossRef](#)] [[PubMed](#)]

27. Pan, B.; Pan, Y.; Huang, Y.-S.; Yi, M.; Hu, Y.; Lian, X.; Shi, H.-Z.; Wang, M.; Xiang, G.; Yang, W.-Y.; et al. Efficacy and safety of gut microbiome-targeted treatment in patients with depression: A systematic review and meta-analysis. *BMC Psychiatry* **2025**, *25*, 6. [CrossRef] [PubMed]
28. Chung, Y.C.E.; Chen, H.C.; Chou, H.C.L.; Chen, I.M.; Lee, M.S.; Chuang, L.C.; Liu, Y.W.; Lu, M.L.; Chen, C.H.; Wu, C.S.; et al. Exploration of microbiota targets for major depressive disorder and mood related traits. *J. Psychiatr. Res.* **2019**, *111*, 74–82. [CrossRef] [PubMed]
29. Kazemi, A.; Noorbala, A.A.; Azam, K.; Eskandari, M.H.; Djafarian, K. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. *Clin. Nutr.* **2019**, *38*, 522–528. [CrossRef] [PubMed]
30. Schaub, A.C.; Schneider, E.; Vazquez-Castellanos, J.F.; Schweinfurth, N.; Kettelhack, G.; Doll, J.P.K.; Yamanbaeva, G.; Mahlmann, L.; Brand, S.; Beglinger, C.; et al. Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: A randomized controlled trial. *Transl. Psychiatry* **2022**, *12*, 227. [CrossRef] [PubMed]
31. Braniste, V.; Al-Asmakh, M.; Kowal, C.; Anuar, F.; Abbaspour, A.; Toth, M.; Korecka, A.; Bakocevic, N.; Ng, L.G.; Kundu, P.; et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* **2014**, *6*, 263ra158. [CrossRef] [PubMed]
32. Zheng, P.; Zeng, B.; Zhou, C.; Liu, M.; Fang, Z.; Xu, X.; Zeng, L.; Chen, J.; Fan, S.; Du, X.; et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* **2016**, *21*, 786–796. [CrossRef] [PubMed]
33. He, H.; He, H.; Mo, L.; You, Z.; Zhang, J. Priming of microglia with dysfunctional gut microbiota impairs hippocampal neurogenesis and fosters stress vulnerability of mice. *Brain Behav. Immun.* **2024**, *115*, 280–294. [CrossRef] [PubMed]
34. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef] [PubMed]
35. Moher, D.; Shamseer, L.; Clarke, M.; Ghersi, D.; Liberati, A.; Petticrew, M.; Shekelle, P.; Stewart, L.A. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst. Rev.* **2015**, *4*, 1. [CrossRef] [PubMed]
36. Higgins, J.; Thomas, J.; Chandler, J.; Cumpston, M.; Li, T.; Page, M.; Welch, V.; Flémyng, E. (Eds.) *Cochrane Handbook for Systematic Reviews of Interventions*; Version 6.3; Cochrane: London, UK, 2022. Available online: <https://www.cochrane.org/authors/handbooks-and-manuals/handbook> (accessed on 15 January 2025).
37. McGowan, J.; Sampson, M.; Salzweidel, D.M.; Cogo, E.; Foerster, V.; Lefebvre, C. PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement. *J. Clin. Epidemiol.* **2016**, *75*, 40–46. [CrossRef] [PubMed]
38. Ouzzani, M.; Hammady, H.; Fedorowicz, Z.; Elmagarmid, A. Rayyan—A web and mobile app for systematic reviews. *Syst. Rev.* **2016**, *5*, 210. [CrossRef] [PubMed]
39. Wan, X.; Wang, W.; Liu, J.; Tong, T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med. Res. Methodol.* **2014**, *14*, 135. [CrossRef] [PubMed]
40. Wells, G.A.; Shea, B.; O'Connell, D.; Peterson, J.; Welch, V.; Losos, M.; Tugwell, P. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses*; Ottawa Hospital Research Institute: Ottawa, ON, Canada, 2014. Available online: https://www.researchgate.net/publication/261773681_The_Newcastle-Ottawa_Scale_NOS_for_Assessing_the_Quality_of_Non-Randomized_Studies_in_Meta-Analysis (accessed on 15 January 2025).
41. Sterne, J.A.C.; Savovic, J.; Page, M.J.; Elbers, R.G.; Blencowe, N.S.; Boutron, I.; Cates, C.J.; Cheng, H.Y.; Corbett, M.S.; Eldridge, S.M.; et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ* **2019**, *366*, l4898. [CrossRef] [PubMed]
42. Shea, B.J.; Reeves, B.C.; Wells, G.; Thuku, M.; Hamel, C.; Moran, J.; Moher, D.; Tugwell, P.; Welch, V.; Kristjansson, E.; et al. AMSTAR 2: A critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ* **2017**, *358*, j4008. [CrossRef] [PubMed]
43. Guyatt, G.H.; Oxman, A.D.; Schunemann, H.J.; Tugwell, P.; Knottnerus, A. GRADE guidelines: A new series of articles in the Journal of Clinical Epidemiology. *J. Clin. Epidemiol.* **2011**, *64*, 380–382. [CrossRef] [PubMed]
44. Tolhurst, G.; Heffron, H.; Lam, Y.S.; Parker, H.E.; Habib, A.M.; Diakogiannaki, E.; Cameron, J.; Grosse, J.; Reimann, F.; Gribble, F.M. Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein-Coupled Receptor FFAR2. *Diabetes* **2012**, *61*, 364–371. [CrossRef] [PubMed]
45. Siopi, E.; Galerne, M.; Rivagorda, M.; Saha, S.; Moigneu, C.; Moriceau, S.; Bigot, M.; Oury, F.; Lledo, P.-M. Gut microbiota changes require vagus nerve integrity to promote depressive-like behaviors in mice. *Mol. Psychiatry* **2023**, *28*, 3002–3012. [CrossRef] [PubMed]
46. Zou, Q.; Han, S.; Liang, J.; Yan, G.; Wang, Q.; Wang, Y.; Zhang, Z.; Hu, J.; Li, J.; Yuan, T.; et al. Alleviating effect of vagus nerve cutting in Salmonella-induced gut infections and anxiety-like behavior via enhancing microbiota-derived GABA. *Brain Behav. Immun.* **2024**, *119*, 607–620. [CrossRef] [PubMed]

47. Bellono, N.W.; Bayrer, J.R.; Leitch, D.B.; Castro, J.; Zhang, C.; O'Donnell, T.A.; Brierley, S.M.; Ingraham, H.A.; Julius, D. Enterochromaffin Cells Are Gut Chemosensors that Couple to Sensory Neural Pathways. *Cell* **2017**, *170*, 185–198.e16. [[CrossRef](#)] [[PubMed](#)]
48. Kaelberer, M.M.; Buchanan, K.L.; Klein, M.E.; Barth, B.B.; Montoya, M.M.; Shen, X.; Bohórquez, D.V. A gut-brain neural circuit for nutrient sensory transduction. *Science* **2018**, *361*, eaat5236. [[CrossRef](#)] [[PubMed](#)]
49. Psichas, A.; Sleeth, M.L.; Murphy, K.G.; Brooks, L.; Bewick, G.A.; Hanyaloglu, A.C.; Ghatei, M.A.; Bloom, S.R.; Frost, G. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int. J. Obes.* **2015**, *39*, 424–429. [[CrossRef](#)] [[PubMed](#)]
50. Ye, L.; Bae, M.; Cassilly, C.D.; Jabba, S.V.; Thorpe, D.W.; Martin, A.M.; Lu, H.-Y.; Wang, J.; Thompson, J.D.; Lickwar, C.R.; et al. Enteroendocrine cells sense bacterial tryptophan catabolites to activate enteric and vagal neuronal pathways. *Cell Host Microbe* **2021**, *29*, 179–196.e9. [[CrossRef](#)] [[PubMed](#)]
51. Okubo, R.; Yamamura, S.; Ishikawa, S.; Kimura, T.; Ukawa, S.; Nakamura, K.; Tamakoshi, A. Does the association between short-chain fatty acids and depressive symptoms vary with age? A large population-based study. *Eur. Psychiatry* **2024**, *68*, e6. [[CrossRef](#)]
52. Liao, Y.; Xie, B.; Zhang, H.; He, Q.; Guo, L.; Subramaniapillai, M.; Fan, B.; Lu, C.; McIntyer, R.S. Efficacy of omega-3 PUFAs in depression: A meta-analysis. *Transl. Psychiatry* **2019**, *9*, 190. [[CrossRef](#)] [[PubMed](#)]
53. Costantini, L.; Molinari, R.; Farinon, B.; Merendino, N. Impact of Omega-3 Fatty Acids on the Gut Microbiota. *Int. J. Mol. Sci.* **2017**, *18*, 2645. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.