Gut Microbiome and Mental Health: Causation or Correlation?

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Abstract

The gut microbiome, a dynamic orchestrator of physiological and neuroimmune processes, influences mental health via the bidirectional microbiome-gut-brain axis. Although distinct microbial signatures are linked with psychiatric disorders like depression, anxiety, posttraumatic stress disorder, and schizophrenia, whether this relationship is causative, correlative, or a complex interplay remains unresolved. This review, therefore, examines this trichotomy, highlighting key mechanistic pathways - including microbial metabolites, immune modulation, and neural signalling - alongside challenges in disentangling causation from correlation. Clarifying this distinction elevates the gut microbiome from a curiosity to a cornerstone of personalised medicine. Furthermore, particular emphasis is placed on advancing methodological frameworks, fostering interdisciplinary collaboration, and addressing disparities in research that bias insights toward specific populations. Importantly, a clearer understanding of the microbiome's role in mental health could lead to novel therapies and its use as a predictive biomarker, ultimately charting a path toward more equitable and evidence-based approaches. This work underscores the transformative potential of clarifying the microbiome-gut-brain axis in addressing the global mental health burden.

Keywords

Gut Microbiome, Mental Health, Psychotropics, Depression, Microbiome-Gut-Brain Axis, Psychobiotics, Precision Psychiatry

Introduction

Once overlooked, the gut microbiome, a dynamic ecosystem of microorganisms in the gastrointestinal tract, has emerged as a critical player in human health. However, its role in psychiatric disorders remains unclear: *Does it drive mental health illness, merely mirror systemic changes, or is it a complex interplay of both?* Addressing this question is essential for guiding therapeutic strategies and clarifying our understanding of the mechanisms that underpin the intricate aetiology and pathophysiology of psychiatric disorders.

Central to this discussion is the microbiome-gut-brain (MGB) axis, a complex bidirectional network implicated in conditions including major depression, anxiety, posttraumatic stress disorder (PTSD), and schizophrenia[1-5]. Microbial metabolites (*e.g.*, short-chain fatty acids [SCFAs]), gut-derived immune mediators, and locally synthesised glucocorticoids shape neuroinflammatory and neuroendocrine pathways, while circulating stress hormones additionally modulate gut-brain signalling[1,2,4-6]. Beyond microbial metabolites and immune modulation, enteroendocrine cells (EECs) also critically contribute to gut-derived neuroendocrine signalling by influencing neural afferents and systemic hormone balance. Disruptions in these pathways have been linked to altered stress reactivity, mood dysregulation, and appetite disturbances, yet their precise role in psychiatric pathophysiology remains uncertain[7].

Despite such compelling mechanistic insights, human studies struggle to establish causality. Most evidence remains cross-sectional, limiting the ability to discern whether microbial shifts precede or follow psychiatric symptoms. Moreover, psychotropic medications significantly alter gut microbiota composition, further confounding interpretations[8,9]. Additionally, germ-free (GF) mouse models, commonly used to explore microbiome-brain interactions, exhibit

neurodevelopmental and behavioural endophenotypes relevant to mental health conditions even without microbiota, yet the relevance of these extreme phenotypes to psychiatric disorders remains uncertain[10,11]. Cross-sectional designs also obscure temporal dynamics while introducing potential confounding variables - genetic, environmental, and medication-related factors. Thus, addressing this causation-correlation dilemma is critical for both mechanistic understanding and therapeutic development, underscoring the need for rigorous, longitudinal, and mechanistically driven research approaches.

To move forward. a clearer conceptual framework is needed. At its core lies the fundamental trichotomy (illustrated in **Figure 1**):

- *Causal driver:* Microbiome shifts actively modulate neurobiological circuits governing mood, cognition, and behaviour.
- *Pathophysiological consequence:* Microbiome alterations arise as a result of psychiatric symptoms, diet, stress, or medication, reflecting but not initiating disease pathology.
- *Bidirectional interplay:* Gut microbial changes and psychiatric pathology influence one another in a feedback loop.

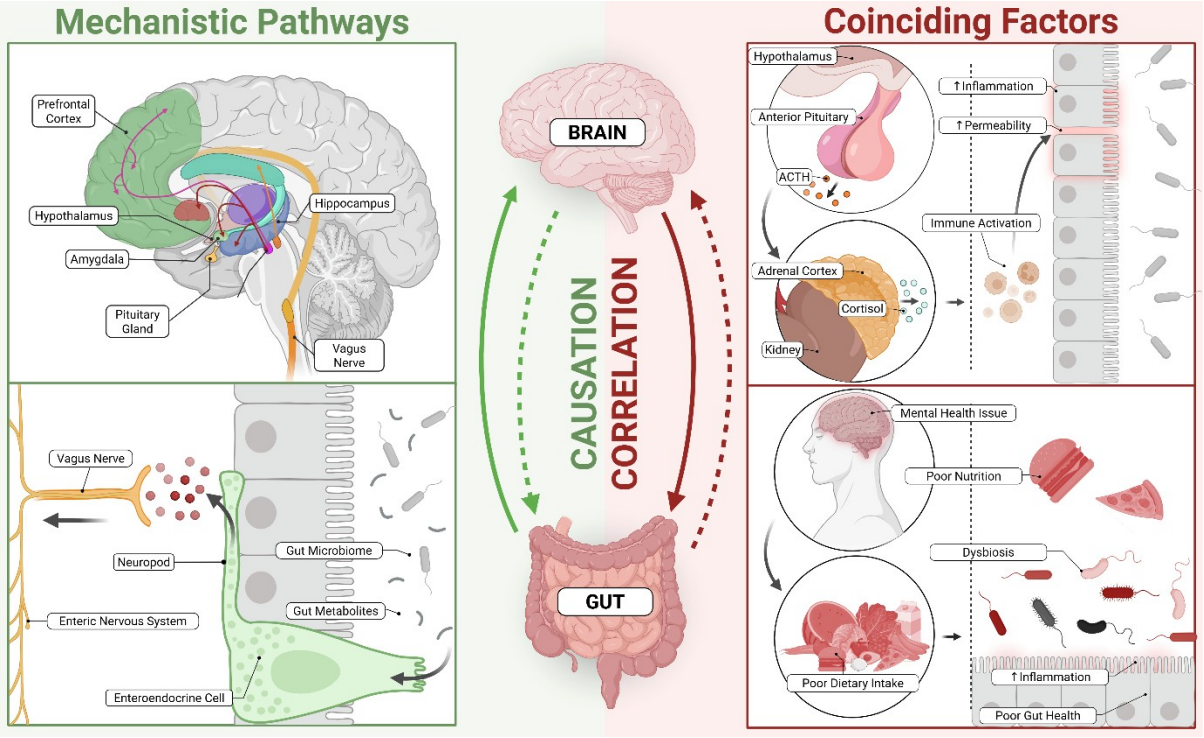


Figure 1. Schematic illustration of the two competing perspectives on the role of the gut microbiome in mental health. *The left panel (green) highlights mechanistic pathways that support a causative role, including microbial metabolite interactions with vagal and spinal afferents, neurotransmitter modulation, and direct effects on neural circuits implicated in psychiatric conditions. The right panel (red) presents coinciding factors that complicate causality and suggest a correlative role between the gut microbiome and mental health, such as systemic inflammation, stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation, and the influence of diet and lifestyle on microbial composition. The central diagram illustrates the bidirectional nature of microbiome-gut-brain axis communication, with dashed and solid arrows differentiating correlative versus causative influences, respectively.*

Clarifying this relationship has tangible implications for healthcare, particularly in reducing health disparities and advancing personalised medicine. Socioeconomically disadvantaged populations, disproportionately affected by psychiatric disorders, also exhibit microbiome

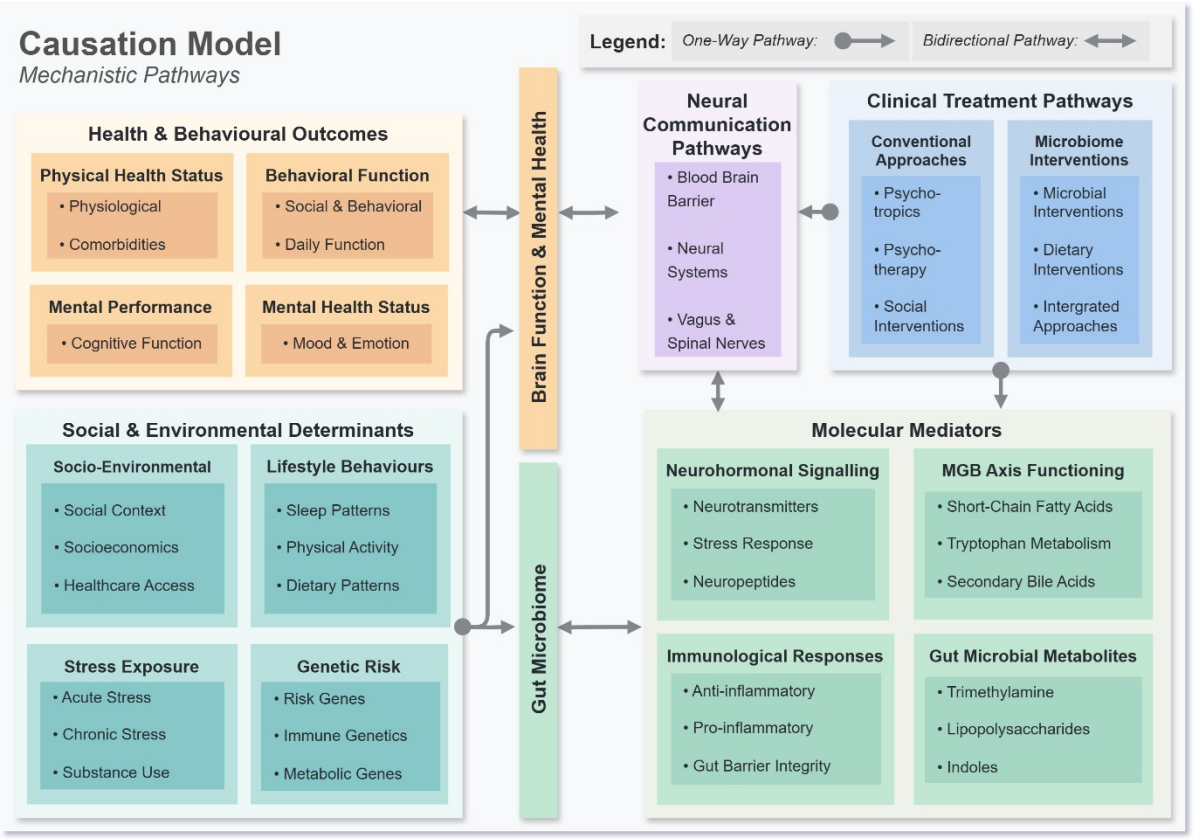
disruptions stemming from chronic stress, food insecurity, and environmental deprivation[12]. These factors, compounded by green space and health care inaccessibility, may further exacerbate neuroinflammatory and metabolic dysfunctions linked to mental illness[13]. If microbial causation is established, interventions like prebiotics, probiotics, and faecal microbiota transplantation (FMT) could serve as primary and/or adjunctive therapies[5,14-18]. Even if only correlative, microbiota shifts may serve as early risk biomarkers, aiding in precision psychiatry approaches. Nevertheless, a precise understanding would enable cost-effective, non-invasive, and clinically scalable interventions - an essential consideration given that current microbiome analyses remain prohibitively expensive and technically complex.

The aim of this review is to disentangle the current evidence underpinning gut microbiome–mental health interactions. By critically evaluating mechanistic studies, human clinical data, and methodological limitations, we seek to clarify whether, and under what circumstances, the gut microbiome serves as a causative agent, a consequence, or a bidirectional partner in psychiatric illness.

Causation: The Gut Microbiome as a Driver?

Accumulating evidence suggests the gut microbiome functions not merely as a passive biomarker but as an active driver of psychiatric pathophysiology, capable of initiating and modulating key neurobiological pathways. Establishing causation, however, remains challenging, particularly within complex systems like the MGB. A structured mechanistic framework isolating direct microbial influences on brain function is therefore essential**.** **Figure 2** presents a systems-level mechanistic framework of major pathways through which gut microbial communities directly influence brain function and psychiatric states. This model integrates key molecular mediators (SCFAs, bile acids, and tryptophan metabolites) which act as biochemical intermediaries in MGB signalling. These signals propagate through

neural (vagal and spinal afferents), immune (cytokine modulation), and metabolic (hormonal regulation) pathways, converging on neurobiological substrates implicated in psychiatric disorders. The framework accounts for environmental and genetic modulators shaping gut microbiota composition, illustrating potential intervention points where microbiome-targeted



therapies could disrupt pathological cascades.

Figure 2. Causation model - Mechanistic Pathways of Gut Microbiome Influence on Mental Health. *This systems-level framework illustrates the complex interactions through which the gut microbiome influences brain function and mental health outcomes. The diagram depicts a central bidirectional relationship between the gut microbiome and brain function and mental health. Upstream, social and environmental determinants, including socio-environmental factors, lifestyle behaviours, stress exposure, and genetic risk, are shown to modulate the gut microbiome. The gut microbiome is further depicted as influencing brain function and mental health through molecular mediators (neurohormonal signalling,*

Microbiota-Gut-Brain (MGB) axis functioning, immunological responses, and gut microbial metabolites) and neural communication pathways (blood-brain barrier, neural systems, and vagus and spinal nerves). These interconnected pathways contribute to various health and behavioural outcomes, encompassing physical health status, mental performance, behavioural function, and mental health status. Additionally, clinical treatment pathways, comprising conventional approaches (psychotropics, psychotherapy, and social interventions) and microbiome interventions (microbial therapies, dietary interventions, and integrated approaches), are presented as targeting specific nodes within this causal network, impacting both molecular mediators and neural communication pathways.

Mounting preclinical evidence suggests the gut microbiome can causally modulate brain function and behaviour. However, human evidence remains inconsistent. Below, we contrast preclinical findings with clinical data to clarify which pathways show translational promise and which remain speculative.

Box 1. Causation: Translating Microbial Mechanisms into Clinical Impact

Preclinical Evidence:

- *SCFAs:* Acetate, butyrate and propionate modulate neuroinflammation, blood-brain barrier integrity, and synaptic plasticity. Behavioural rescue is observed following supplementation[6,19-22].
- *Tryptophan metabolism:* Gut microbes regulate the kynurenine pathway vs serotonin synthesis; *Roseburia* colonisation reverses depressive phenotypes and restores 5-HT[21,23-26].
- *Neural circuits:* Vagal/spinal pathways mediate behavioural effects of FMT and probiotics; vagotomy abolishes these effects in mice[27-30].
- *FMT:* Depression-associated microbiota induce anxiety and anhedonia in germ-free rodents, replicating core psychiatric features[31-33].
- *Probiotics:* Specific strains modulate GABA receptors, HPA axis, and synaptic plasticity in vagus-dependent manner[5,15,34-36].

Clinical Evidence:

- *SCFAs:* Fibre-rich or psychobiotic diets enhance SCFA-producing taxa and associate with improved mood; direct SCFA-symptom links remain

unproven[4,22,37].

- *Tryptophan metabolism:* Depressed patients show altered 5-HT/kynurenine profiles; microbiota-tryptophan interactions inferred but not yet manipulated causally[24,26].
- *Neural circuits:* Vagal tone correlates with symptom severity, but no direct human demonstration of microbial modulation via these pathways[28,29].
- *FMT:* Some RCTs show transient mood improvements post-FMT; benefits often dissipate within months[38].
- *Probiotics:* Some trials show modest symptom relief or altered brain activation, but effects are often strain-specific, short-lived, or inconsistent across endpoints[5,39].

Translational Gaps:

- Human trials often underpowered, short-term, or rely on proxy outcomes[1,40].
- Few studies mechanistically validate microbial impact on brain circuits or metabolites *in vivo*[41].
- Causality remains best supported where microbial manipulation reverses symptoms, but replication in large, well-controlled trials is needed[41].

Mechanistic Pathways: Microbial Influences on the Brain

Short-Chain Fatty Acids

The causation hypothesis centres on the biochemical dialogue between the gut microbiome and the brain. Microbially derived SCFAs (*e.g.,* acetate, butyrate, and propionate) modulate neuroinflammation (via NLRP3 inflammasome inhibition), fortify the gut and blood-brain barriers (*e.g.*, through JAM-A/ZO-1 complex stabilisation), and shape synaptic plasticity (by enhancing LTP via cAMP/PKA-dependent CREB phosphorylation[19,37,42]. These properties position SCFAs as plausible mediators of microbiota–brain signalling in psychiatric disorders. SCFAs also modulate peripheral immune function and metabolic pathways, indirectly shaping MGB signalling[19,25].

Chronic low-grade inflammation is a recognised feature across psychiatric disorders, and SCFAs have been shown to modulate this axis by inducing IL-10–producing regulatory T cells and suppressing neuroinflammatory cytokines. In addition, butyrate selectively upregulates BDNF and Pgc1-α, while other SCFAs alter astrocyte gene expression in a sex-

dependent manner, highlighting neuroimmune and glial pathways through which SCFAs may influence mood and cognition[19,43-45]. These effects on inflammatory and barrier-related mechanisms are further illustrated in vascular depression models, where cerebral injury disrupts SCFA production and gut-brain signalling, as explored by Xiao et al. highlighting how chronic cerebral hypoperfusion impacts tryptophan metabolism[25]. In a bilateral common carotid artery occlusion (BCCAO) rat model, depressive-like behaviours emerged, including reduced sucrose preference (analogous to anhedonia, $p < 0.05$) and increased immobility in the forced swim and tail suspension tests (analogous to behavioural despair, $p < 0.01$). BCCAO induced gut dysbiosis, impaired motility, and weakened barrier integrity via reduced occludin, claudin-5, and ZO-1 expression ($p < 0.05$)[25]. Crucially, their experimental design and timeline indicate that vascular injury precedes the onset of gut perturbations, suggesting that microbiome alterations and mood phenotypes likely arise sequentially from the initial cerebral insult, rather than independently. Metabolomic and gene expression analyses revealed a shift toward kynurenine metabolism at the expense of 5-HT synthesis in both the gut and hippocampus. BCCAO rats had lower hippocampal and intestinal 5-HT levels ($p < 0.01$) alongside elevated kynurenine and quinolinic acid concentrations ($p < 0.01$), consistent with neuroinflammation and excitotoxicity and coincident with depletion of SCFA-producing bacteria (Prevotellaceae, Lachnospiraceae, *Roseburia*, *Blautia*) and significantly reduced serum SCFA levels ($p < 0.01$)[25]. Subsequent 21-day SCFA supplementation (acetate, propionate, butyrate) alleviated depressive-like behaviours, restored microbiota composition, and reinforced gut barrier integrity[25]. Treated BCCAO rats exhibited higher hippocampal 5-HT levels ($p < 0.05$) and reduced kynurenine pathway activation, marked by lower indoleamine 2,3-dioxygenase-1 (IDO1) and 3-hydroxyanthranilic acid 3,4-dioxygenase (3-HAO) expression ($p < 0.05$). SCFAs also lowered hippocampal oxidative stress (malondialdehyde, 8-oxo-deoxyguanosine; $p < 0.05$), suppressed NF-κB-mediated

196 neuroinflammation, and decreased microglial activation (Iba1$^+$, CD16$^+$, iNOS$^+$; $p < 0.01$)[25].
197 Such findings support a bidirectional model, wherein brain pathology drives gut dysfunction,
198 which in turn exacerbates neuroinflammation and depressive-like behaviours, highlighting a
199 MGB axis feedback loop that is therapeutically modifiable despite its top-down origin.

200 Butyrate also influences psychiatric-relevant pathways through epigenetic and receptor-
201 mediated mechanisms[19]. As a histone deacetylase (HDAC) inhibitor, it upregulates *Tet1*
202 expression in the prefrontal cortex, increasing DNA hydroxymethylation and boosting BDNF
203 levels, molecular changes closely associated with antidepressant effects and cognitive
204 resilience[46]. Butyrate also signals through GPCRs such as FFAR2 and FFAR3, modulating
205 neuroplasticity and immune tone in ways that may shape emotional regulation[47]. Notably,
206 while these effects are generally neuroprotective, SCFAs can also activate CD4$^+$ effector T
207 cells under specific conditions, suggesting a context-dependent role in neuroinflammation[48].

208 *Tryptophan Metabolism*

209 Beyond SCFAs, tryptophan also influences MGB functioning. While tryptophan is a 5-
210 hydroxytryptamine (5-HT), or serotonin precursor, altered microbial states can lead to its
211 breakdown via the kynurenine pathway, producing neuroactive metabolites linked to
212 neuroinflammation and neurotoxicity[26]. The gut microbiome modulates this pathway through
213 immune system-associated IDO1 activity or by altering tryptophan availability[49]. In the brain,
214 this pathway bifurcates in a cell-specific manner, astrocytes produce neuroprotective
215 kynurenic acid while microglia generate neurotoxic quinolinic acid, creating a
216 neuroinflammatory balance crucial for mental health[23,50]. Preclinical models demonstrate
217 causal links between gut microbiota and depressive pathophysiology[23,50]. Notably, while
218 serotonin orchestrates mood and cognition, over 90% originates from intestinal

enterochromaffin cells. GF mice exhibit markedly reduced 5-HT levels and concomitant depressive phenotypes, deficits reversed by microbial colonisation[51]. SCFAs further modulate colonic 5-HT production, establishing multiple mechanistic links between microbial metabolism and MGB signalling essential for mood regulation[51].

Clinically, individuals with depression exhibit reduced relative abundance of *Faecalibacterium, Roseburia, Collinsella, Blautia,* and *Phascolarctobacterium*. *Roseburia*, in particular, strongly predicted depression severity, with sertraline, a commonly prescribed selective serotonin reuptake inhibitor (SSRI), partially restoring microbial composition[52]. Zhou et al. extend these observations pre-clinically, showcasing that colonisation with *Roseburia intestinalis* directly increased 5-HT levels in both the brain and colon ($p < 0.001$) by upregulating tryptophan hydroxylase-1 and -2 (TPH1/2) expression[24]. Simultaneously, it suppressed the kynurenine pathway by downregulating IDO1 and 3HAO, reducing levels of neurotoxic quinolinic acid ($p < 0.01$) and 3-hydroxykynurenine ($p < 0.05$). *Roseburia* transplantation also preserved synaptic plasticity, increasing Drebrin, Synapsin-1, and PSD-95 expression ($p < 0.01$), and promoted astrocyte function whilst dampening CRS-induced microglial activation[24]. Furthermore, intestinal barrier integrity was protected, with increased occludin expression and reduced histological damage ($p < 0.001$)[24]. These investigations into SCFAs and tryptophan metabolism underscore the diverse and potent mechanisms by which the gut microbiome can exert a demonstrable causal influence on neural function.

Microbiome Interventions: Testing Causality in Mental Health

Faecal Microbiota Transplantation: Translational Insights

The most robust test of causality is through direct microbiome manipulation, such as FMT, the transfer of a whole microbiome sample to a recipient. FMT from individuals with

depression induces depressive-like behaviours in GF mice, while transplantation from healthy donors restores function[53,54]. Specifically, depressed donor FMT increases immobility (forced swim test: $p < 0.05$; tail suspension test: $p < 0.05$), reduces sucrose preference ($p < 0.05$), and alters hippocampal neurogenesis — changes that are commonly interpreted as modelling core behavioural and neurobiological features of depression in preclinical studies[55]. In tandem, transplantation of microbiota from depressed individuals was sufficient to induce depression-like behavioural responses, anxiety and increased inflammation in rats of normal health that had their microbiota depleted with antibiotics[54].

In contrast, clinical studies on FMT remain limited but show promising short-term effects. A systematic review of 28 studies examining the impacts of FMT on psychiatric symptoms, including preclinical ($n = 11$), preclinical with human donors ($n = 9$), and clinical trials ($n = 8$), highlighted a consistent trend of short-term behavioural and neurobiological changes across all study types[55]. Clinical trials, though limited by small sample sizes, showed transient improvements in depression (Hamilton Depression Rating Scale [HAM-D], $p < 0.05$) and anxiety (Hamilton Anxiety Rating Scale [HAM-A], $p < 0.05$) following FMT, with effects lasting 3–6 months before returning to baseline[55]. However, the magnitude and consistency of these effects varied considerably, with some studies reporting only marginal or non-significant changes. Mechanistically, FMT is hypothesised to directly influence psychiatric symptoms via modulation of neurotransmitter synthesis, immune signalling, and SCFA production[56]. Notably, long-term effects remain inconsistent or unclear, with symptoms often returning to baseline within 3–6 months[57]. This raises the question of whether a single FMT is sufficient for sustained benefits, particularly given the malleability of the gut microbiome. Unlike conventional treatments that require continuous administration, most clinical trials have relied on a single or short-course FMT protocol, which may not be adequate for long-

term microbiome restructuring[58]. Potential strategies to address this limitation include serial or maintenance FMT dosing, adjunctive prebiotics to promote donor strain engraftment, and dietary synchronisation protocols to stabilise microbial composition over time[18].

The variability in psychiatric outcomes suggests that factors such as donor microbiota composition, recipient baseline microbiome, host immune responses, and dietary influences may determine FMT efficacy[59,60]. Emerging evidence indicates that low engraftment efficiency, donor-recipient microbial incompatibility, or environmental factors like diet may contribute to symptom relapse, highlighting the need for personalised approaches to optimise FMT outcomes[32,61]. Thus, future trials may benefit from incorporating microbial compatibility scoring and functional profiling in donor-recipient matching, coupled with longitudinal multi-omics to monitor engraftment and mechanistic response. Despite promising findings, results are hampered by small sample sizes, heterogeneous methodologies, and insufficient long-term data. Larger, well-controlled trials are thus needed to establish FMT as a viable adjunctive treatment for psychiatric disorders and to determine whether ongoing or periodic administration might achieve lasting therapeutic effects. Additionally, environmental factors, diet, lifestyle, and external exposures, may influence the onset, persistence, and recurrence of pathological microbiome species, potentially confounding microbiome-behaviour interactions. Integrating these variables into future studies will be critical for distinguishing true causal relationships from external mediators of psychiatric outcomes. Carefully embedding these contextual variables into study design, via standardised dietary protocols, immune phenotyping, and baseline characterisation, will be critical for isolating true causal effects.

Dietary Intervention

Dietary interventions provide another compelling lens through which to examine causation. Increased dietary fibre intake or adherence to Mediterranean diets, rich in polyphenols and fermentable substrates, have been linked to improved mental health outcomes, likely mediated by enhanced SCFA production[62]. The 12-week randomised controlled trial, SMILES, tested a modified Mediterranean diet in patients with major depressive disorder (MDD), demonstrating significant reduction in Montgomery–Åsberg Depression Rating Scale (MADRS) scores compared to controls (mean change: −7.1 points, $p < 0.001$; Cohen's $d = -1.16$)[63]. Remission (MADRS < 10) was achieved in 32.3% of the dietary group versus 8.0% in controls ($p = 0.028$). Improvements were independent of body weight changes and correlated with dietary adherence ($\beta = -0.22$ per 10% improvement in diet score, $p < 0.001$). Anxiety (HAM-A, $p = 0.033$) and depression (HAM-D, $p = 0.032$) scores also improved significantly in the dietary group[63]. Thus, increasing evidence is now suggesting that the MGB axis may be extended to include diet[64].

Probiotic Supplementation

Probiotic evidence strengthens the causation argument, with specific *Lactobacillus* and *Bifidobacterium* strains exhibiting anxiolytic and antidepressant effects[5,36]. Preclinical studies demonstrate that supplementation with these strains attenuates stress responses and anxiety-like behaviour, concurrently modifying hypothalamic genes involved in synaptic plasticity via vagus nerve modulation[35,65]. Human trials, while less consistent, have nonetheless replicated select preclinical findings in clinical contexts, particularly among individuals with mild to moderate anxiety and depression[5,14,36]. However, strain-specific efficacy remains an

311 open question, as different probiotics exert distinct effects depending on host factors, baseline
312 microbiota composition, and dosing regimens. For example, in a recent RCT of probiotic
313 add-on therapy in patients with MDD, Schaub et al. administered a high-dose, multi-strain
314 probiotic (900 billion CFU/day) for four weeks alongside treatment-as-usual[34]. In the
315 modified intention-to-treat (mITT) sample (N=43), probiotic-treated participants showed a
316 significantly greater reduction in clinician-rated depressive symptoms (HAM-D) compared to
317 placebo, with a medium to large effect size from baseline to follow-up (d = 0.95). However,
318 no group*time interaction was observed in self-reported measures of depression (BDI),
319 anxiety (STAI), or gastrointestinal symptoms (GSRS), suggesting that symptom improvement
320 was not consistently detected across outcome types. Microbiome analysis revealed increased
321 relative abundance of *Lactobacillus* in the probiotic group (group*time interaction: $p < 0.05$),
322 with this change correlating negatively with HAM-D and BDI scores. Yet, overall microbiota
323 diversity remained stable in both groups, and no significant shifts in enterotype distribution
324 were detected[34]. On the neural level, the probiotic group exhibited decreased putamen
325 activation during neutral face processing (x = ±20, y = 16/6, z = 10/12, p<.001), a region
326 hyperactivated in MDD at baseline relative to healthy controls. Notably, this finding was
327 limited to within-group changes, was not observed for the pre-specified fearful face
328 condition, and did not survive between-group comparison[34]. Moreover, structural MRI
329 showed no robust group*time effects. Collectively, while this study represents a rigorous and
330 multimodal investigation of the MGB axis, its positive findings, particularly in imaging, must
331 be interpreted cautiously in light of several null results and modest sample size, outlining the
332 need for replication in larger, confirmatory trials[34].

333 Likewise, Allen et al. investigated the effects of *Bifidobacterium longum* 1714, a candidate
334 psychobiotic derived from preclinical screening, on stress, neurocognitive function, and brain

electrophysiology in a within-subjects crossover trial involving 22 healthy male volunteers[36]. Following four weeks of supplementation (1×10^9 CFU/day), cortisol output in response to a socially evaluated cold pressor test (SECPT) was significantly reduced compared to placebo, as indexed by lower area under the curve with respect to ground (AUCg; $p = 0.05$), although no time*condition interaction was detected[36]. Subjective anxiety increases post-stressor were significant in both baseline and placebo conditions, but not after probiotic treatment. Daily perceived stress was also lower in the probiotic condition (AUCg $p = 0.03$), yet these effects were transient, returning to baseline in the 2-week follow-up[36]. Cognitive testing showed a subtle improvement in visuospatial memory, with fewer errors on the Paired Associate Learning task post-*B. longum* 1714 ($p < 0.01$), though no enhancements were observed in sustained attention, social cognition, or emotional processing tasks[36]. EEG analysis indicated increased frontal (Fz) mobility and reduced θ power at Cz post-probiotic, suggesting altered prefrontal and midline cortical activation[36]. However, P300 event-related potential measures remained unchanged. Overall, while the study provides preliminary support for *B. longum* 1714's stress-attenuating and pro-cognitive effects, the results were modest, limited to specific outcomes, and based on a small, homogeneous sample. These findings further reinforce the need for replication in larger and more diverse populations, alongside comparative trials of strain-specific efficacy[36].

Microbiome-Gut-Brain Signalling: The Role of Vagal and Spinal Neural Pathways

The vagal and spinal nerves serve as another conduit through which microbiota-derived signals influence central nervous system neurotransmission[29]. Spinal afferents synapse in lamina I of the dorsal apex in the spinal cord, from where nociceptive and visceral sensory inputs are relayed to the mediodorsal thalamus, which integrates and transmits these signals to the medial orbitofrontal cortex (mOFC)[66-68]. The mOFC, a key region involved in reward

valuation and affective processing, projects to the nucleus accumbens, thereby modulating hedonic and motivational circuits[68]. This neuroanatomical pathway suggests gut-derived microbial signals influence not only stress-related behaviours but also exert broader effects on anhedonia, reward-seeking, and emotional regulation via the spinal-brain axis[66-68]. Vagotomy studies validate the functional importance of afferent vagal signalling, demonstrating that microbiota-driven behavioural changes require intact MGB communication[69]. Pre-clinical FMT studies from unpredictable chronic mild stress-derived mice to healthy recipients demonstrate that FMT can activate vagus and spinal afferent nerves, triggering deficits in hippocampal neurogenesis, neuroinflammation, and depressive-like behaviours[27]. Recipient mice showed decreased sucrose preference ($p = 0.01$), increased immobility in the forced swim test ($p = 0.0001$), and reduced hippocampal neurogenesis marked by fewer doublecortin-positive neurons ($p = 0.004$)[27]. Mechanistically, the gut microbiome changes induced 5-HT and dopamine deficits in the brainstem and hippocampus, with reduced expression of key neurogenic factors like BDNF and cyclic-AMP response element binding protein ([CREB] [$p < 0.05$]).

Importantly, subdiaphragmatic vagotomy abolished these effects, preventing behavioural deficits, hippocampal neurogenesis impairments, and neuroinflammatory responses[27]. Similarly, Bravo et al. investigated the effects of *Lactobacillus rhamnosus* JB-1 on emotional behaviour, stress responses, and GABA receptor expression in mice, highlighting the vagus nerve as a critical mediator of microbiota-brain communication[65]. Mice receiving *L. rhamnosus* JB-1 for 28 days exhibited significant reductions in stress-induced corticosterone levels ($p < 0.001$) and anxiety- and depression-related behaviours[65]. In the elevated plus-maze, probiotic-treated mice entered open arms significantly more frequently than controls ($p < 0.001$), indicating anxiolytic effects. Similarly, in the forced swim test, treated mice

displayed reduced immobility ($p < 0.01$), suggesting antidepressant-like properties[65]. Neurochemically, *L. rhamnosus* JB-1 modulated GABA receptor expression in a region-specific manner. Probiotic administration increased *GABAB1b* mRNA expression in the cingulate and prelimbic cortices ($p < 0.01$) but decreased expression in the amygdala, hippocampus, and locus coeruleus ($p < 0.01$)[65]. Additionally, *GABAA2* receptor expression was downregulated in the prefrontal cortex and amygdala ($p < 0.05$) but upregulated in the hippocampus ($p < 0.001$), aligning with improved stress resilience and mood regulation[65]. Crucially, these neurochemical and behavioural effects were absent in vagotomised mice, demonstrating that the vagus nerve is a necessary pathway for *L. rhamnosus* JB-1-mediated MGB modulation of mood[65]. Nevertheless, it is worth noting that vagotomy, either total or partial, was historically used as a treatment for peptic ulcer disease until the late 1970s yet reports of associated mood changes in these patients are scarce[28]. While this does not preclude a role for the vagus nerve in psychiatric modulation, it underscores the complexity of translating preclinical findings to human pathology.

Correlation: The Gut Microbiome as a Reflection?

Despite plausible mechanisms, current evidence often positions the microbiome as a correlative marker reflecting systemic and environmental factors rather than directly driving psychiatric pathology. **Figure 3** contextualises microbiome alterations within broader biopsychosocial determinants, presenting a comprehensive correlation model wherein the gut microbiome functions not as a primary pathological driver but as a biological barometer mirroring socioeconomic status, chronic stress, diet, and genetic predisposition. These shared influences create parallel trajectories between microbial composition and psychiatric outcomes. This framework offers a critical counterpoint to causal models, cautioning against simplistic interpretations of MGB associations while highlighting the potential utility of

microbiome signatures for diagnostic and prognostic applications - suggesting therapeutic strategies should be developed with clear understanding of whether microbial modifications can genuinely alter disease trajectories. In this context, certain boxes are shaded in grey to visually de-emphasise their role as direct causal agents within the correlational framing of the model, outlining their current status as associative rather than mechanistically proven pathways.

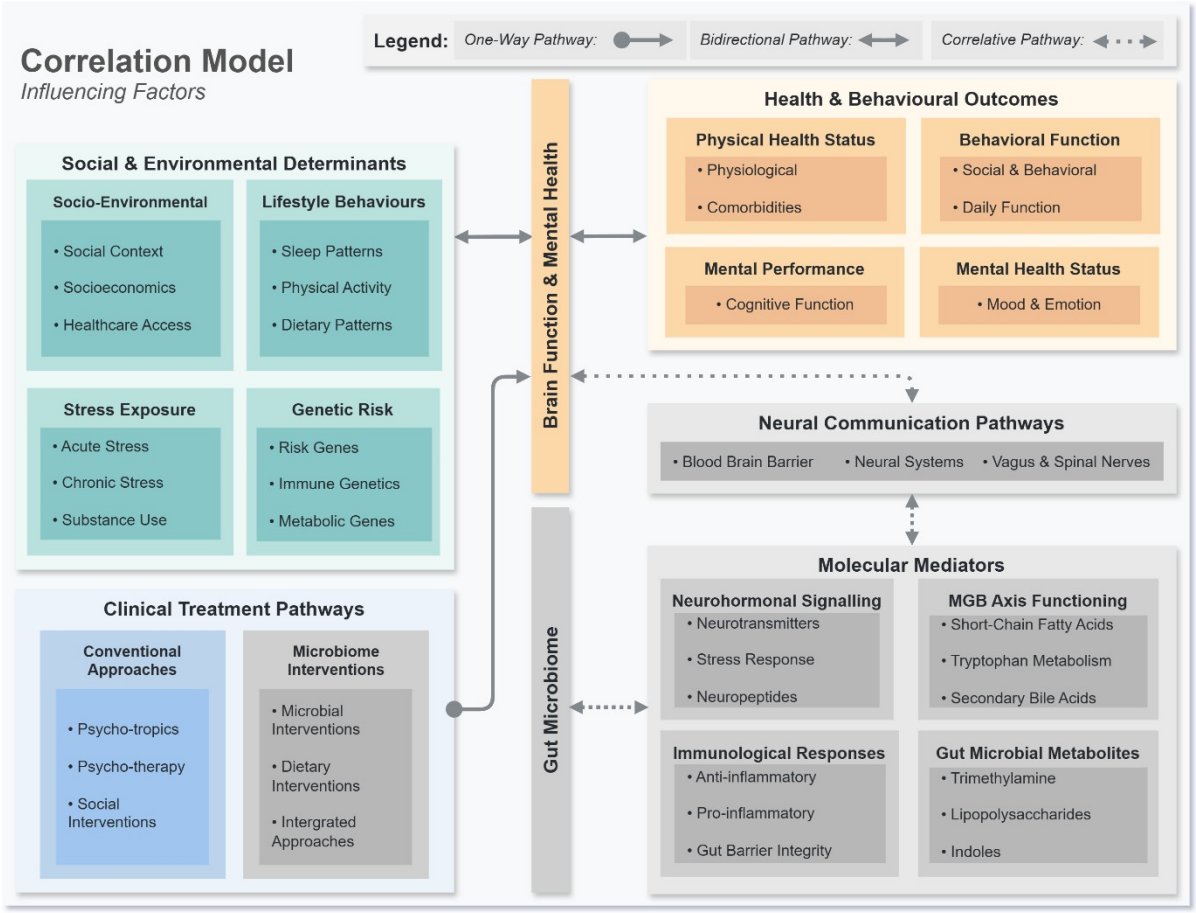


Figure 3. Correlation Model - Influencing Factors in Gut Microbiome-Mental Health Associations *This systems-level framework presents a correlation model illustrating associations between the gut microbiome and mental health outcomes, emphasising factors that may generate observed correlations without necessarily implying direct causality. The diagram highlights a bidirectional correlative pathway (indicated by a dotted line) between*

the gut microbiome and brain function and mental health. Social and environmental determinants, including socio-environmental factors, lifestyle behaviours, stress exposure, and genetic risk, are shown to independently influence both the gut microbiome and brain function and mental health. In this model, clinical treatment pathways comprising conventional approaches (psychotropics, psychotherapy, and social interventions) are considered the only suitable treatment approach for modulating brain function to treat mental health, with the grey shading for microbiome interventions (microbial therapies, dietary interventions, and integrated approaches) de-emphasising their role as primary treatment approach within this specific correlational framework. Neural communication pathways and molecular mediators are depicted as associated factors, acknowledging their biological relevance in the context of brain function and mental health, but shaded grey to de-emphasise their direct role in causally influencing mental health outcomes. These pathways contribute to various health and behavioural outcomes, encompassing physical health status, mental performance, behavioural function, and mental health status. This model suggests that shared upstream influences and independent effects of interventions may lead to the correlations observed between gut microbial profiles and neuropsychiatric manifestations.

In many cases, the gut microbiome reflects, rather than drives, psychiatric pathology. Box 2 highlights domains where associations are robust, yet causality remains uncertain or unsupported.

Box 2. Correlation: Microbial Shifts as a Mirror, Not Driver?

Preclinical Evidence:

- *Stress and inflammation:* Chronic stress disrupts microbial composition and increases gut permeability; endotoxin translocation triggers neuroinflammation[4,24,27,47,70].

- *Psychotropics:* SSRIs and antipsychotics reshape microbial profiles in rodents, often mirroring side effects such as weight gain or metabolic disturbance[71,72]. - *Environmental exposures:* Diet, housing, and isolation influence microbiota and behaviour in animal models, confounding inferences of causality[73].
Clinical Evidence: - *Microbial diversity:* Reduced α-diversity seen in depression and anxiety, but findings vary by study and population[74]. - *Systemic inflammation:* Depression is associated with elevated LPS antibodies and pro-inflammatory cytokines, consistent with gut barrier dysfunction[75]. - *Psychotropic use:* Medications significantly alter microbiota; some taxa associate with treatment response but may reflect secondary systemic effects[72]. - *Sociodemographic factors:* Low socioeconomic status, poor diet, and reduced green space access correlate with both psychiatric risk and microbial dysbiosis[76].
Interpretive Considerations: - Correlations may reflect shared upstream factors (*e.g.* diet, stress, SES) that simultaneously affect microbiota and mental health[73,77]. - Confounders, diet, stress, medication, often affect both microbiota and mood[73]. - Microbiome profiles may hold diagnostic value, but inferring causality from correlation remains premature without mechanistic or temporal validation.

Correlative Evidence: Signatures of Psychiatric Conditions

Reductions in gut microbiome α-diversity (microbial richness and evenness) are frequently observed in psychiatric conditions such as depression, anxiety, and schizophrenia[78,79]. However, precise and reproducible microbial signatures are lacking, precluding their use as reliable biomarkers, and the question of causality remains unresolved.

Several mechanisms may underlie these specific microbiome differences[78,80]. For instance, stress-induced HPA axis hyperactivation can impair gut barrier integrity and alter microbial composition, typically decreasing *Lactobacillus* and increasing *Clostridium*, as seen in animal and human studies[81]. However, a systematic review of 13 human studies (median *n*: 70, median age: 28) examining stress and the gut microbiome found mixed results[39]. While most studies showed no significant associations with overall α or β diversity, some reported negative correlations between psychological stress and specific α-diversity metrics (Shannon,

Chao 1, Simpson) and the β-diversity metric weighted UniFrac ($p < 0.05$)[39]. It is important to acknowledge that a reduction in diversity may not always signify a negative shift in the microbiome, particularly if accompanied by an increase in specific beneficial bacteria[82]. Nevertheless, at the taxonomic level, stress was consistently associated with decreased abundance of *Lachnospiraceae, Lachnospira, Phascolarctobacterium, Sutterella*, and *Veillonella*, and increased abundance of *Methanobrevibacter, Rhodococcus*, and *Roseburia* ($p < 0.05$)[39]. However, study variability and limited sample sizes preclude definitive conclusions regarding the influence of age, sex, and ethnicity[39,83].

Similarly, systemic inflammation, which has been associated with depression, may shape the microbiome, mirroring disease progression without directly driving it[43]. Multiple reviews, including Peirce and Alviña, have synthesised evidence demonstrating that psychological stress increases intestinal permeability, leading to bacterial endotoxin translocation into circulation and subsequent endotoxemia[84]. Psychological stress increased intestinal permeability, leading to bacterial endotoxin translocation into circulation and subsequent endotoxemia[85]. This peripheral immune activation triggered central neuroinflammation, correlating with increased plasma pro-inflammatory cytokine levels (*e.g.,* IL-6, TNF-α) in individuals with mood disorders, possibly via vagus nerve modulation[84]. Notably, chronic stress-induced hypercortisolaemia compromises intestinal tight junction integrity via activation of myosin light chain kinase (MLCK), resulting in further gut barrier dysfunction and exacerbating bacterial translocation[85]. Elevated circulating endotoxin levels have been directly correlated with depressive symptoms, as evidenced by increased IgA and IgM immune responses against lipopolysaccharides (LPS) of commensal bacteria in depressed individuals[85]. Additionally, endotoxemia-induced neuroinflammation has been mechanistically linked to persistent activation of the HPA axis, leading to glucocorticoid

resistance in immune cells and an aberrant cytokine milieu, further perpetuating the inflammatory state associated with depression[85]. Longitudinal studies confirmed that elevated inflammatory markers predicted future depression risk, while anti-inflammatory interventions, for example, non-steroidal anti-inflammatory drugs (NSAIDs), improved depressive symptoms ($p < 0.05$)[84]. These observations suggest that the microbiome may act as a biological barometer, reflecting physiological changes underlying mental health conditions rather than initiating them.

Crucially, broader biopsychosocial factors, socioeconomic status, diet quality, and chronic stress, function as established determinants of both mental health and microbiota composition[77]. Dietary patterns hold particular relevance, shaping microbial diversity while influencing inflammation, metabolic health, and neuroimmune signalling[86]. Individuals with depression frequently consume diets deficient in fibre and abundant in ultra-processed foods, both independently linked to microbiome alterations and deteriorated mental health outcomes[38]. Within dietary patterns, carbohydrate consumption demonstrates a nuanced relationship with depression risk. Mendelian randomization analysis suggests a causal association between higher relative carbohydrate intake and reduced depression risk, with a one-standard-deviation increase in carbohydrate-derived calories linked to a 58% reduction in depression risk (OR = 0.42, 95% CI: 0.28–0.62; $p = 1.49 \times 10^{-5}$)[87]. This effect persists after adjusting for other dietary components and appears partially mediated by body mass index, suggesting metabolic pathway involvement. Given these intricate bidirectional influences between diet, microbiota composition, inflammation, and mental health, future research must prioritise disentangling these relationships with mechanistic precision.

Psychotropics and the Microbiome: A Bidirectional Influence

Psychotropic treatments reshape the microbiome, introducing a major confounding factor that complicates efforts to establish causality between microbiome alterations and psychiatric outcomes[71,79,88,89]. In particular, atypical antipsychotics (*e.g.*, olanzapine) and SSRIs induce significant microbial shifts[71]. Olanzapine specifically has been associated with increased levels of Firmicutes and reduced Bacteroidetes, changes that parallel the metabolic side effects commonly experienced by patients[90]. Similarly, SSRIs have been reported to modulate gut microbiota in ways that align with symptom improvement[91]. For example, escitalopram (mean dose: 16.33 ± 3.46 mg/day for 4-6 weeks) has shown the capacity to significantly increase α-diversity (Shannon index: 3.57 ± 0.07 vs. 2.99 ± 0.09 in controls, $p < 0.05$). Post 6 weeks of treatment, α-diversity decreased (3.17 ± 0.11), approaching levels observed in healthy controls, suggesting partial microbiota normalisation[91]. Beta-diversity analysis revealed distinct gut microbiota compositions between participants with MDD and non-MDD controls ($p = 0.001$), with some post-treatment patients' microbiota shifting closer to controls, though others retained depression-associated profiles[91].

Likewise, Gao et al. examined the relationship between gut microbial composition and the efficacy of SSRIs in 62 first-episode, drug-naïve persons with MDD. Participants were treated with SSRIs for eight weeks and classified as responders (≥50% reduction in HAM-D scores, $n = 37$) or treatment-resistant ($n = 25$)[92]. Gut microbiota diversity and community composition were analysed using 16S rRNA gene amplicon sequencing, and correlation analysis assessed microbial associations with antidepressant response. A- and β- diversity analyses revealed significant differences in gut microbial community composition between responders, non-responders, and healthy controls ($p < 0.05$)[92]. Responders exhibited increased relative abundance of *Blautia*, *Bifidobacterium*, and *Coprococcus*, which correlated

positively with treatment efficacy (*r*: 0.361, *p*: 0.004 for *Blautia*; *r*: 0.332, *p*: 0.008 for *Bifidobacterium*; *r*: 0.361, *p*: 0.004 for *Coprococcus*)[92]. Logistic regression and receiver operating characteristic analysis demonstrated that these bacterial genera predicted treatment response with high accuracy (AUC: 0.931, sensitivity: 87.9%, specificity: 92.0%).

These observations suggest microbial shifts may track treatment responses rather than mediate them. Psychotropic-induced microbiome changes likely reflect broader systemic effects— altered metabolism or immune modulation, but may also result from direct interactions, as SSRIs undergo first-pass metabolism in the gastrointestinal tract[92]. While this responsiveness highlights the microbiome's potential as a treatment monitoring tool, it demands caution when interpreting such changes as causal evidence. Different SSRIs exert distinct effects on gut microbial composition — fluoxetine and escitalopram for instance have divergent impacts, suggesting antidepressant efficacy unlikely operates primarily through gut microbiota without considering microbiome-mediated influences on drug metabolism or absorption[93]. Similarly, broad-spectrum antibiotics induce profound microbial diversity shifts without typically precipitating significant mood disturbances, despite weeks-long recovery periods[94]. These findings raise a fundamental question: does decreased microbial diversity merely confer susceptibility, or does it, like other risk factors, shape vulnerability through its interplay with broader biological and environmental forces, modulating psychiatric disorder risk and resilience in predisposed individuals?

Future Research Directions: Key Questions and Gaps

To advance understanding of the gut microbiome's role in mental health, research must critically examine overarching assumptions and refine specific hypotheses underpinning causative and correlative models. In **Table 1**, we outline priority questions that will address key knowledge gaps and offer actionable directions for the field.

Table 1: Key Knowledge Gaps in the Microbiome-Gut-Brain Axis Field.

Domain	Key Research Themes and Representative Questions
Causality vs Correlation	– Can targeted manipulation of specific microbes or metabolites reliably induce or alleviate psychiatric symptoms? – Do microbiome alterations precede, follow, or co-occur with symptom onset across illness stages? – Are microbiome signatures more strongly linked to dimensional symptoms (*e.g.*, anxiety, anhedonia) than to categorical diagnoses? – Can microbiome features predict or enhance psychotherapy outcomes?
Mechanistic Understanding	– How do microbial metabolites (*e.g.*, SCFAs, tryptophan derivatives), immune mediators, and neural signals shape psychiatric symptoms? – Can microbiome or metabolite profiles differentiate between psychiatric subtypes (*e.g.*, melancholic vs atypical depression)? – Do non-bacterial taxa (*e.g.*, fungi, viruses, archaea) contribute to pathogenesis independently of bacterial communities? – How does sex influence microbiota-related mechanisms across mental health disorders?
Developmental Influences	– How do microbiome disruptions during prenatal, early postnatal, early childhood, or adolescent periods affect long-term mental health outcomes? – What is the role of maternal microbiota, mental health, and psychotropic use in shaping offspring neurodevelopment? – Do paternal microbiomes and environmental exposures impact early microbial colonisation and later psychiatric risk? – Can early-life interventions (*e.g.*, prebiotics, probiotics) alter developmental trajectories or foster resilience?

Environmental Modulation	– How do stress, diet, physical activity, and medications interact to influence microbiome stability in psychiatric conditions? – What is the cumulative impact of chronic exposures (*e.g.*, social isolation, air pollution, polypharmacy)? – How do shared living environments (*e.g.*, families, households) affect microbiome-related psychological resilience or risk? – Can environmental microbiota or access to green space influence collective mental health?
Treatment Implications	– Can baseline microbiome profiles guide treatment selection or predict response to psychotropics? – Are microbiome changes required for treatment efficacy, or are they secondary effects? – Can microbiome-targeted interventions (*e.g.*, FMT, prebiotics, probiotics, synbiotics) improve treatment outcomes or reduce side effects?
Neuroimmune Interactions	– How do gut microbiota influence neuroinflammatory signalling pathways in psychiatric disorders? – Do early microbial exposures programme long-term immune responses that affect mental health risk? – Can targeting microbiota–immune interactions yield new therapeutic strategies for inflammation-linked psychiatric conditions?

The Translational Gap: Addressing the Lack of Clinical Evidence

While promising interventions such as FMT and probiotics suggest a role for the gut microbiome in psychiatric disorders, defining causality remains a salient challenge in the field. Crucially, much of the current mechanistic insight stems from preclinical models, which, while invaluable, do not always translate reliably to clinical settings[40]. Human trials have often failed to replicate the magnitude or consistency of preclinical effects, highlighting a need for rigorous frameworks that explicitly account for translational divergence. Traditional frameworks, including Hill's Criteria for Causality and Koch's Postulates, provide guiding principles but necessitate adaptation to the unique complexities of the MGB axis[95-99]. Here, in **Table 2**, we propose a structured framework that integrates epidemiological, mechanistic, and interventional evidence to assess whether microbiome alterations causally contribute to psychiatric pathophysiology. This table harnesses core elements of classical causality frameworks with contemporary microbiome research approaches, providing a roadmap for distinguishing correlation from true causal influence[95,97].

Table 2: Establishing Causality in the Microbiome-Gut-Brain Axis: A Psychiatry-Specific Framework

Causality Criterion	Application to MGB Axis & Psychiatry	Example of Strong Evidence Required
Temporal Relationship	Microbiome alterations must precede psychiatric symptom onset.	Longitudinal human studies showing microbial shifts in at-risk individuals before disease onset.
Consistency	Findings must be replicated across diverse populations, studies, and models.	Meta-analyses of microbiome studies across depression, schizophrenia, PTSD and anxiety cohorts.
Specificity	Psychiatric disorders should have unique microbiome	Case-control studies showing disorder-specific microbial

	signatures distinct from general dysbiosis.	taxa independent of lifestyle factors.
Biological Gradient	A proportional relationship should exist between microbiome changes and psychiatric severity.	Microbiota transplants from mild vs. severe depression patients show graded symptom transfer in GF mice.
Experimental Evidence	Direct microbiome manipulation should influence psychiatric outcomes.	Randomised controlled trials showing microbiome-targeted interventions (FMT, probiotics, prebiotics and synbiotics) alleviate symptoms with mechanistic validation.
Plausibility	Established mechanistic pathways link microbiota to neural function.	SCFAs, tryptophan metabolites, spinal and vagal modulation influencing stress resilience in animal/human models.
Coherence	Evidence should align with known biological mechanisms.	Microbiome influences on neuroinflammation match established psychiatric pathophysiology.

Notably, the strength of evidence across these criteria remains uneven between preclinical and clinical models. While experimental and mechanistic data in animal models fulfil several causality benchmarks, clinical studies often yield inconsistent or short-lived effects, and null findings are underreported[40]. For instance, probiotics demonstrating vagus-dependent behavioural effects in rodents frequently show modest or non-significant changes in human trials across self-report, clinical, and neuroimaging endpoints. For instance, the aforementioned randomised controlled trial by Schaub et al., only noted one imaging outcome (putamen activation during neutral face processing) reached within-group significance, while other primary symptom measures, microbiota shifts, and mechanistic targets remained unchanged[34]. This asymmetry in evidence strength underscores the importance of contextualising causality claims according to the model used and avoiding overinterpretation of preliminary clinical signals.

While the proposed framework provides a structured approach to assessing causality, its effectiveness ultimately depends on rigorous study design and methodological consistency. Clarifying the role of the gut microbiome in mental health requires research that is equitable, accessible, and reproducible, directly addressing the challenge of disentangling causation from correlation.

Standardising Study Designs

The over-representation of cross-sectional designs limits our ability to discern whether microbiome changes drive mental health outcomes or merely reflect them[100]. To differentiate between causative mechanisms and correlational patterns, prospective longitudinal cohort studies are essential[101]. Establishing causality, in particular, necessitates several methodological improvements, including:

- *Adequately powered studies:* Small sample sizes constrain statistical power and reproducibility in many microbiome-mental health studies. Larger, well-powered investigations are essential to distinguish true associations from random variability and build a robust evidence base. To assist investigators in planning adequately powered microbiome-mental health studies, we provide a practical guide in Box 3.

Box 3. Recommendations for Conducting Power Calculations in Microbiome-Mental Health Studies

1. **Clarify Causal Assumptions Early**
   - Clearly define whether microbial features are expected to influence mental health *bottom-up*, *top-down*, or both[102].
   - Directionality determines the statistical role of microbiome data (*e.g.*, predictor, mediator, outcome) and affects model structure and required sample size[103].
2. **Engage with Bioinformaticians and Biostatisticians Early**
   - Power calculations in this space often require tailored, simulation-based approaches that fall outside typical statistical workflows.
   - Engaging with biostatisticians or bioinformaticians during the design phase can prevent overpromising, underpowering, or misinterpreting association as causation. This is particularly important in small cohorts or mechanistic studies where design decisions cannot be fixed *post hoc*.
3. **Select and Prioritise Endpoints in Each Domain**
   - **Microbiome Domain:** Endpoints may include ecosystem-level features, such as alpha diversity, taxonomic features, pathway-level inferences, or metabolomic readouts (*e.g.*, short-chain fatty acids, tryptophan derivatives). Each has distinct variability and detection thresholds[102].
   - **Mental Health Domain:** Outcomes may involve clinical scales (*e.g.*, PANSS, HAM-D), imaging readouts, or physiological biomarkers[104].
4. **Account for Microbiome-Specific Statistical Properties**
   - Compositionality, sparsity, and high inter-individual variability mean that effect sizes are not always stable or comparable across studies[40,73].
   - Power calculations should account for covariates like diet, medication use, and socioeconomic status, which often account for large portions of explained variance[73].
5. **Leverage Simulation-Based Powering for Causal Designs**
   - Standard formula-based power tools often fail under complex, high-dimensional, or causal models[104].
   - Simulation-based approaches allow researchers to explore the sample size needed under realistic assumptions about confounding, measurement error, and temporal structure[104].

- *Sequential resolution for causality:* While large-scale longitudinal studies present challenges, pre- and post-intervention designs, tracking microbial shifts in response to targeted treatments or environmental changes, offer a feasible approach. This helps determine whether microbiome alterations precede, follow, or coincide with psychiatric symptom changes, providing a more practical framework for assessing causality.

- *Refining preclinical models:* Unlike metabolic conditions with clear biomarkers, psychiatric disorders develop gradually and unpredictably, complicating prospective sampling[105]. High interindividual variability limits rodent-to-human translation. Moreover, while mechanisms such as SCFA signalling or vagal modulation are reproducible in animal models, their relevance in humans remains less certain: vagal tone correlates with psychiatric symptoms, but direct microbial modulation of vagal pathways has not been demonstrated in humans[40]. These limitations necessitate psychiatric-specific frameworks incorporating translational benchmarks such as imaging endpoints, behavioural harmonisation, and parallel mechanistic readouts across species.
- *Uniform sampling, analysis, and reporting protocols:* Variability in stool collection, preservation, and analysis methodology introduces confounders that obscure causal interpretations[106]. Standardised protocols, accounting for variables such as diet, psychotropic drug usage, drug administration timing, and symptom fluctuations, and reporting (*e.g.*, STORMS guidelines) are critical to ensure comparability across studies[107].

To further fortify causal inference, comprehensive metadata on stress exposure, trauma history, psychotropic medication usage, and neurodevelopmental trajectories is essential[108]. However, the logistical and financial burden of collecting such extensive data in large-scale studies must be acknowledged. Expansive initiatives such as the ABCD study exemplify the feasibility of integrating biospecimen collection with detailed neurodevelopmental assessments, yet the complexity, cost, and data management challenges are substantial[109]. A pragmatic approach may involve identifying a minimum viable dataset - capturing the most critical variables with the lowest complexity, to balance feasibility with scientific rigour. In

parallel, systematic inclusion of null and mixed findings in evidence syntheses is critical to avoid inflated estimates of microbiome impact and provide a more balanced view of translational viability. Future research should aim to streamline metadata collection while maintaining sufficient depth to contextualise microbiome-mental health interactions, ensuring methodological advancements remain both impactful and scalable.

In large observational studies, capturing extensive metadata may prove impractical due to logistical constraints. Nevertheless, controlled clinical trials or targeted cohort studies can integrate detailed data on stress exposure, trauma history, medication usage, and neurodevelopmental trajectories to disentangle confounding factors. Ecological Momentary Assessment (EMA) presents a feasible alternative for capturing real-time data in naturalistic settings, mitigating recall bias while maintaining scalability[108]. Combining standardised psychiatric assessments with microbiome profiling in clinical trials could enhance data quality, providing nuanced, causally informative interpretations of microbiome-mental health relationships within a biopsychosocial framework.

Incorporating Advanced Statistical Approaches

In light of the logistical and temporal constraints of large-scale longitudinal studies, advanced statistical frameworks offer a critical adjunct for strengthening causal inference in microbiome–mental health research[110]. These methods enable researchers to dissect causal pathways using observational data, provided assumptions are explicitly modelled and appropriately interrogated. Key methodological innovations include:

- *Causal Discovery and Structural Causal Models:* Frameworks such as structural causal models (SCMs) and directed acyclic graphs (DAGs) allow researchers to explicitly model assumptions and clarify hypothesised causal pathways[41]. Algorithms

like Fast Causal Inference (FCI) can estimate directed edges from observational data while accounting for latent confounding, feedback loops, and sampling bias[41]. Recent applications to large microbiome datasets have identified microbial taxa such as *Eggerthella* and *Hungatella* as potential causal contributors to depression, illustrating the translational value of such tools[41].

- *Probabilistic causal effect estimation:* Beyond identifying causal links, techniques such as the partial ancestral graphs (PAGs) algorithms quantify the magnitude of causal effects using observational data alone[41]. These methods allow estimation of interventional probabilities without requiring randomised trials, provided model assumptions are rigorously tested. This opens avenues for hypothesis generation, microbial target prioritisation, and simulation of intervention effects.
- *Limitations of traditional instrumental variable approaches:* Methods such as Mendelian randomisation (MR) can be valuable but are often constrained by weak instruments, horizontal pleiotropy, and insufficient resolution of microbiome genome-wide association study data[111]. Structural approaches offer a more flexible alternative, particularly in psychiatric populations where genetic-microbiome-behaviour interactions are complex and frequently non-linear.
- *Powering causal inference studies:* Traditional sample size calculations often do not apply to high-dimensional, graph-based methods[112]. Simulation-based power analysis, pilot-informed effect size calibration, and sparsity-aware techniques (*e.g.*, permutation tests, bootstrapping, or synthetic DAGs) are increasingly necessary to ensure appropriate model fit and reproducibility[41]. Special consideration must be given to compositional data structure, zero inflation, and the risk of overfitting in small or noisy datasets[113].

Powering causal inference studies remains an underdeveloped but critical frontier[114]. Unlike traditional power calculations based on *t*-tests or regression, causal inference methods such as FCI or PAGs require tailored strategies that account for high dimensionality, latent confounding, and graph complexity[41]. Simulation-based approaches using synthetic DAGs, where known causal structures are constructed and used to generate synthetic data, allow researchers to empirically test how sample size, noise, sparsity, or missing data affect causal identifiability and effect estimation[41]. These simulations provide a practical means of calibrating study design, informing the minimum viable sample size and metadata completeness needed to reliably recover causal effects in realistic microbiome-psychiatry settings.

Transparency and Open Science Practices

Reproducibility is essential, yet inconsistent analyses and limited data sharing obstruct validation. Three key infrastructure improvements are required to address these challenges:

- *Comprehensive patient phenotyping:* Rigorous characterisation of psychiatric diagnoses is essential for drawing meaningful conclusions, as relying solely on self-reported symptom measures risks misclassification due to the dynamic nature of mood and cognition. Standardised clinician-administered scales (*e.g.,* PANSS for schizophrenia, HAM-D for depression) remain the diagnostic gold standard[115,116]. Nevertheless, advances in neuroimaging and multi-omics profiling can provide deeper insights into underlying biological mechanisms, refining our understanding of disease pathophysiology and its links to the microbiome.
- *Unified data analysis pipelines:* Transparent, standardised bioinformatics workflows that consistently handle confounders and batch effects prove essential. These pipelines

should prioritise identifying reproducible microbial features across datasets, strengthening the evidence base for causative associations.

- *Centralised open-access repositories:* A global, standardised database integrating microbiome profiles with psychiatric phenotypes, neuroimaging, and polygenic risk scores would enable meta-analyses that strengthen causality claims[117]. However, ethical considerations, including data privacy and consent, must underpin such initiatives. Additionally, privacy-preserving frameworks, like federated learning, could enable broad collaboration while safeguarding participant confidentiality.
- *Ethically responsible AI-assisted analysis*: Machine learning models are increasingly used to identify microbial biomarkers, yet biased training data and black-box methodologies can obscure interpretations. Transparent, explainable AI frameworks that prioritise interpretability and reproducibility should be standardised to preclude misleading associations.

Inclusive and Representative Research

Microbiome research remains skewed toward high-income populations, thereby limiting global generalisability. Consequently, the following are imperative:

- *Recruiting a diverse cohort:* Studies on the role of the gut microbiome in psychiatric disorders disproportionately focus on certain populations, with a meta-analysis indicating that 71% of human microbiome samples originate from Europe, the United States, and Canada[118]. Expanding participant diversity is indispensable for capturing the full spectrum of microbiome-mental health interactions[77]. While socioeconomic background represents one consideration, broader factors, including diet, antibiotic exposure, and lifestyle, may play more direct roles in shaping microbiome-associated psychiatric risk[73]. Variations in diet, physical activity, and environmental exposures

across populations provide critical insights into causative mechanisms. Notably, exercise has emerged as a key modulator of gut microbiota composition, potentially through myokine signalling[119]. Future studies should account for individual differences in participant lifestyles when analysing microbiome-mental health interactions.

- *Tailored interventions for equity:* Causal mechanisms may not operate uniformly across populations. Thus, therapies such as probiotics or dietary changes must be evaluated within the context of population-specific microbiome characteristics to ensure equitable efficacy.
- *Capturing early-life microbiome signatures*: The gut microbiome undergoes critical developmental windows during infancy and childhood, shaping neurodevelopmental trajectories[120]. Factors such as C-section delivery, breastfeeding, antibiotic exposure, and maternal stress may program lifelong vulnerability to psychiatric disorders via MGB axis alterations. Future studies should prioritise longitudinal birth cohorts to determine whether early microbiome disruptions predispose individuals to later psychiatric outcomes.

Developing Mental Health-Specific Reporting Standards

The complexity of microbiome research necessitates clear and consistent reporting practices. Existing frameworks, such as the STORMS checklist for microbiome studies, provide a foundation, but specific adaptations are needed for mental health research[107].

- *Integration of clinical metrics:* Future reporting guidelines should mandate rigorous documentation of psychiatric diagnoses (*e.g.*, DSM-5, ICD-11) alongside clinician-administered symptom scales. Additionally, capturing key environmental factors, such

as socioeconomic status, medication adherence, and diet, will be essential for contextualising microbiome associations without redundancy.

- *Causation-oriented data sharing:* Making supplementary materials, including raw data, sequencing workflows, and analysis scripts, publicly available would foster hypothesis testing that prioritises causal interpretations.

Conclusion and Prospects

Causality between the gut microbiome and mental health is increasingly supported, particularly for bottom-up mechanisms (*i.e.*, gut to brain), and should no longer be treated as speculative. While much of the human evidence remains unresolved, reflecting the microbiome's sensitivity to systemic influences, preclinical studies consistently demonstrate that microbial metabolites and MGB signalling can drive endophenotypes relevant to neuropsychiatric changes. SCFA signalling, tryptophan metabolism, and behavioural transfer via FMT have shown reproducible, reversible effects on behaviour and brain function. Top-down influences (*i.e.*, brain to gut), including stress, inflammation, and psychotropic exposure, are also well-established across models, underscoring a bidirectional framework where microbiome and host influence each other in dynamic feedback loops.

Causality is most compelling where microbial disruption induces neuroimmune or behavioural change that is reversible through targeted interventions. Early trials of diet, probiotics, and FMT offer promising, if preliminary, translational signals. However, large-scale, longitudinal studies with mechanistic endpoints are essential to determine whether microbial changes precede, follow, or co-evolve with psychiatric symptoms.

Crucially, exploring the bidirectional relationship between psychotropic treatments and microbiome alterations is vital: *Do psychotropics reshape the microbiome, or do microbial*

changes mediate therapeutic effects? Or is it a complex interplay of both? Robust *in vivo* and *in vitro* models are needed to address these interactions and determine if microbiome-targeted interventions, like probiotics, independently enhance mental health outcomes. Current evidence suggests these dynamics vary by drug class, host profile, and disorder subtype, highlighting the need for stratified research frameworks.

However, a multidisciplinary approach is essential. Integrating psychiatry's clinical insights, microbiology's functional analyses, bioinformatics' dataset processing, and pharmacology's drug-microbiome interaction studies will distinguish causative mechanisms from systemic influences. Immunology, endocrinology, and health economics must also be integrated for a holistic understanding of the microbiome's impact on mental health.

Equitable clinical translation must remain a guiding principle. Scalable, non-invasive therapies like diet-based interventions or prebiotic formulations should account for socioeconomic and cultural contexts. Thus, implementing culturally sensitive, multi-modal strategies will be essential to ensuring microbiome-based advances benefit global populations rather than exacerbate disparities in mental health care.

As the field moves forward, the focus must shift from debating causality to rigorously testing it, clarifying when, where, and for whom microbiome-targeted interventions are effective. Progress will not be defined by resolving the debate alone, but by translating mechanistic insight into timely, accessible interventions that address the biological and psychosocial dimensions of mental health.

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