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Authors	Simpson, Carra A.;Diaz-Arteche, Carmela;Eliby, Djamila;Schwartz, Orli S.;Simmons, Julian G.;Cowan, Caitlin S. M.
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The gut microbiota in anxiety and depression – a systematic review

Carra A. Simpson^{a,b*}, Carmela Diaz-Arteche^b, Djamila Eliby^{a,b}, Orli S. Schwartz^c, Julian G. Simmons^{a,b†}, Caitlin S. M. Cowan^{d†}

† joint senior authorship

^a Melbourne School of Psychological Sciences, The University of Melbourne, VIC, Australia.

^b Melbourne Neuropsychiatry Centre, The University of Melbourne and Melbourne Health, VIC, Australia.

^c Orygen; Centre for Youth Mental Health, The University of Melbourne, VIC, Australia.

^d APC Microbiome Ireland, University College Cork, Ireland.

Correspondence

*Carra A. Simpson, <https://orcid.org/0000-0002-8281-5881>

Melbourne School of Psychological Sciences, 12th floor Redmond Barry Building, The University of Melbourne, VIC 3010, Australia. carra.simpson@unimelb.edu.au

Phone: +61 8344 1845

Abstract

Growing evidence indicates the community of microorganisms throughout the gastrointestinal tract, (i.e., gut microbiota), is associated with anxiety and depressive disorders. We present the first systematic review of the gut microbiota in anxiety disorders, along with an update in depression. Consideration of shared underlying features is essential due to the high rates of comorbidity. Systematic searches, following PRISMA guidelines, identified 26 studies (two case-control comparisons of the gut microbiota in generalized anxiety disorder, 17 in depression, one incorporating both anxiety/depression, and five including symptom-only measures). Alpha and beta diversity findings were inconsistent; however, differences in bacterial taxa indicated disorders may be characterized by higher abundance of proinflammatory species (e.g., *Enterobacteriaceae* and *Desulfovibrio*), and lower short-chain fatty acid producing-bacteria (e.g., *Faecalibacterium*). Several taxa, and their mechanisms of action, may relate to anxiety and depression pathophysiology via communication of peripheral inflammation to the brain. Although the gut microbiota remains a promising target for prevention and therapy, future research should assess confounders, particularly diet and psychotropics, and should examine microorganism function.

Keywords: Anxiety disorders, depression, microbiome, gut-brain axis, microbiota

The gut microbiota in anxiety and depression – a systematic review

Anxiety and depressive disorders are ubiquitous and debilitating psychiatric conditions that collectively affect close to 10% of the global population every year (World Health Organization, 2017). The World Health Organization (2019) estimates the global loss in productivity due to anxiety and depressive disorders amounts to \$1 trillion USD per year – a trajectory expected to rise (Doran & Kinchin, 2019). Although engagement with psychotherapeutic and psychotropic treatments has increased over the past several decades (Olfson, Druss, & Marcus, 2015; Stephenson, Karanges, & McGregor, 2012), the prevalence and burden of anxiety and depressive disorders remains unchanged (Jorm, Patten, Brugha, & Mojtabai, 2017). Furthermore, there is substantial variation in response to existing treatments, which are overall efficacious in less than half of diagnosed patients (Casacalenda, Perry, & Looper, 2002; Cipriani et al., 2018). Accordingly, in order to develop more effective treatment targets, there is an urgent need to gain new insight into the underlying pathophysiology of anxiety and depressive disorders. The high comorbidity between internalizing disorders has been cited as evidence for possible shared physiological processes, risk factors, and illness trajectories (Kotov et al., 2017). One such promising area of research is the microbiota-gut-brain axis, which may elucidate shared pathophysiology.

A growing body of research describes the bidirectional communication between the gut microbiota – the ecosystem of trillions of bacteria, viruses, archaea and fungi, along with their collective gene pool – with the host's central nervous system (CNS; Dinan & Cryan, 2015, 2017; Rieder, Wisniewski, Alderman, & Campbell, 2017). This biochemical signaling pathway, also known as the gut-brain-axis, is thought to influence cognitive functioning and mood via neural, metabolic, hormonal, and immune-mediated mechanisms (Foster & McVey Neufeld, 2013). The gut microbiota is a key regulator within the gut-brain-axis: bacterial species regulate the production of neurotransmitters and their precursors (e.g., serotonin, GABA, tryptophan), and can secrete and upregulate essential proteins and metabolites involved in neuropeptide and gut hormone release, such as short-chain fatty acids (SCFAs; e.g., *Faecalibacterium prausnitzii* and *Clostridium leptum*) and brain-derived neurotrophic factor (BDNF; e.g., *Bifidobacterium*; Bercik et al., 2010; O'Sullivan et al., 2011; Parada Venegas et al., 2019). Furthermore, vagal and spinal afferent pathways mediate neural communication between gut microbes and the CNS, and the gut microbiota modulates immune signaling from gut to brain, via cytokine induction (Dinan & Cryan, 2017; Foster, Rinaman, & Cryan, 2017).

The extant literature indicates that gut microbes may also be involved in the development and function of the hypothalamic-pituitary-adrenal (HPA) axis, which coordinates the adaptive stress response in the body (Foster et al., 2017; Sudo et al., 2004). Dysregulated HPA axis signaling is implicated in anxiety and depressive disorders, typically associated with higher levels of cortisol and inflammatory mediators that lead to a sustained proinflammatory state (Keller et al., 2017; Winter, Hart, Charlesworth, & Sharpley, 2018). Not only can the gut microbiota contribute to increases in cortisol and inflammation (Kamada, Seo, Chen, & Núñez, 2013), proinflammatory states may compound microbiota alterations via deleterious effects on gastrointestinal health. Excessive levels of circulating cortisol and inflammatory mediators increase intestinal permeability, thus allowing Gram-negative bacteria to translocate into the bloodstream which may induce chronic CNS inflammation (i.e., bacteria which contain an additional lipopolysaccharide exterior membrane, associated with inflammation in high concentrations; Foster et al., 2017; T.-T. Huang et al., 2019). This suggests that microbiota-driven inflammatory responses may contribute to affective disorders, due in part to increased intestinal permeability. Similarly, gastrointestinal conditions suspected to involve alterations in the gut microbiota and intestinal permeability co-occur at remarkably high rates with psychiatric disorders (e.g., irritable bowel syndrome; Simpson, Mu, Haslam, Schwartz, & Simmons, 2020). Hence, the role of the gut microbiota in mood regulation and emotional processing, via the gut-brain-axis, may be of particular relevance to anxiety and depression etiology.

Given the role of gastrointestinal bacteria in the bidirectional communication between the gut and the brain, recent studies have focused on characterizing gut microbiota composition in anxiety and depression. Preclinical models highlight gut microbiota disturbances in rodents exhibiting anxiety- and depressive-like behaviors, and report normalization of both behavioral and microbial alterations after bacterial probiotic administration (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014; Mayer, Tillisch, & Gupta, 2015). Extension of this research into humans has been relatively slow prior to the last several years. Reviews have highlighted gut microbiota alterations in clinical depressive disorders relative to healthy control groups (Cheung et al., 2019; T.-T. Huang et al., 2019; Sanada et al., 2020); however, findings related to the diversity of microbial communities in depression are inconsistent, and it is unclear whether specific bacterial taxa drive group differences (Cheung et al., 2019; T.-T. Huang et al., 2019; Sanada et al., 2020). Existing reviews have also inadequately considered research quality and the effects of confounders, particularly diet and psychotropic medication (Simpson, Schwartz, & Simmons, 2020).

The present systematic review provides an essential update of the expanding literature characterizing the gut microbiota in depressive disorders, and provides the first systematic review in anxiety disorders. This paper also aims to integrate evidence to examine whether these highly comorbid conditions share underlying microbial features, and to critically appraise the effect of methodological inconsistencies and confounding factors. A more nuanced understanding of the pathophysiology of anxiety and depressive disorders may inform future diagnosis and treatment options in these common and debilitating psychiatric conditions.

Method

Search strategy

Systematic searches were conducted in March 2020 following PRISMA guidelines (Moher, Liberati, Tetzlaff, Altman, & Group, 2009). The MEDLINE (Ovid), Embase, PsycINFO, and PubMed databases were searched to capture human studies which i) assessed the gut microbiota composition in anxiety or depressive disorders, or ii) investigated associations between the gut microbiota and anxiety/depression symptom measures in healthy participants or relevant conditions (i.e., anxiety and depressive disorders). Comprehensive search terms are presented in Appendix A.

Study selection

Titles and abstracts were independently reviewed by two authors (CS and CDA) using Covidence technology (www.covidence.org). A third author (JS) was consulted to resolve inconsistencies in screening decisions. Studies were required to be published in a peer-reviewed journal and written in languages spoken by authors performing filtering and extraction (English, French, Spanish). Studies were excluded if they examined the gut microbiota and anxiety/depression symptoms solely in another psychiatric disorder or disease, or if they assessed the effect of an intervention without reporting relevant baseline measurements.

Data extraction

Data was extracted by two independent authors (CS and CDA) and confirmed by a third (DE). Information gathered for each study included demographics, sample characteristics, method of gut microbiota estimation, anxiety/depression measures, confounding variables, specimen processing (collection, storage, DNA extraction), methods of microbial data pre-processing, and relevant results (analyses which compared the gut microbiota between cases and controls, or assessed associations between anxiety/depression symptoms and the gut microbiota).

Quality assessment of studies

The internal validity of studies was examined against the National Institutes of Health Study Quality Assessment Tools (National Heart Blood and Lung Institute, 2019). Studies were rated as “Good”, “Fair”, or “Poor” quality by two authors (CS and DE). Differences in ratings were resolved by a third author (CDA).

Results

Characteristics of included studies

Comprehensive screening yielded 1216 studies after duplicate removal (Figure 1). A total of 26 studies met inclusion criteria, including 17 clinical case-control studies that compared the gut microbiota of controls to participants with a depressive disorder, two studies which compared individuals with generalized anxiety disorder (GAD) to controls, and one study which compared participants with depression, anxiety, or comorbid anxiety/depressive disorder and controls. The remaining six studies assessed associations between anxiety or depression symptoms and the gut microbiota in the general population ($n=1$), in major depressive disorder (MDD) with no control group ($n=1$), in mothers selected from two lower socioeconomic communities ($n=1$), and in healthy community samples ($n=3$). The filtering process is depicted in Figure 1 and Table 1 provides a summary of the included studies.

[INSERT FIGURE 1 HERE, COLOR ONLINE ONLY]

[INSERT TABLE 1 HERE]

Methodological summary

Various sequencing workflows were employed by the reviewed literature to estimate gut microbiota composition. Amplicon 16S rRNA gene sequencing was used by most studies ($n=20$), one of which also included shotgun metagenomics, and two which validated 16S rRNA gene sequencing findings using qPCR. Two other studies employed shotgun metagenomics to comprehensively sample all microbial genes (Lai et al., 2019; Rong et al., 2019). Of the remaining four techniques, RT-qPCR was used by one study to quantify *Bifidobacterium* and *Lactobacillus* counts (Aizawa et al., 2016), and oligonucleotide probes for *Bifidobacterium* spp. and *Lactobacillus* spp. were used by another (Heym et al., 2019). One study performed single nucleotide polymorphism genotyping and microbiota-related gene set enrichment analysis (Cheng et al., 2019), and one study performed comparative

metaproteomics analysis coupled with tandem mass spectrometry (Z. Chen et al., 2018). Additional methodological information, including sample collection and storage, DNA extraction methods, hypervariable regions sequenced, and microbial data pre-processing methods are presented in Appendix B.

Quality assessment

An assessment of study quality revealed that only three studies were rated as ‘Good’. These studies sufficiently described inclusion and exclusion criteria, applied consistent screening and diagnostic methods in their categorization of cases and controls, and considered a number of confounding variables. Most studies were rated as ‘Fair’ ($n=16$), indicating susceptibility to bias without sufficient evidence that these limitations invalidated results. Seven studies were rated as ‘Poor’, indicating a significant risk of bias. These studies had unclear methods for recruitment, varied screening/categorization methods for cases and controls, insufficient consideration of confounding variables, or included self-reported diagnosis as the primary method for determining anxiety or depression group status. Detailed assessment of the included studies’ internal validity is presented in Appendix C. A quantitative meta-analysis was not performed due to the disparate sequencing and bioinformatics techniques used (Appendix B).

Anxiety and depression measures and diagnostic tools

Methods for defining clinical groups

A total of 23 studies analyzed the gut microbiota of clinical groups with anxiety or depression (Table 1). The majority of studies utilized a gold standard clinical interview or diagnostic criteria to define groups ($n=20$; e.g., DSM-IV/5, ICD-10th revision, Mini Neuropsychiatric Interview, Structured Clinical Interview for the DSM). One study included two large cohorts who were defined as having a depressive disorder by their general practitioner or using a self-reported diagnosis (Valles-Colomer et al., 2019). The remaining two studies utilized self-reported measures to stratify cases and controls, including a clinical cut-off score on the Hamilton Depression Rating Scale (J. Chen et al., 2018) and self-report of an anxiety or depression diagnosis by a general medical practitioner (Jackson et al., 2018).

Symptom-level questionnaire measures

Most studies included self-report symptom questionnaires in addition to group comparisons ($n=17$), although only six included case-control studies explicitly assessed associations between microbial taxa abundance and internalizing symptoms at a continuous level. Five studies included self-report questionnaires as their only measure of anxiety or depression. A small number of case-control studies also focused at a diagnosis level only

($n=4$). Anxiety and depression symptoms were quantified using 11 different self-report questionnaires, of which the 17- and 21-item versions of the Hamilton Depression Rating Scale ($n=10$) and the Beck Depression Inventory ($n=6$) were the most common.

Definition of microbial indices utilized in the extant literature

Characterization of the gut microbiota involved a multi-faceted approach in most studies, usually including a measure of both alpha and beta diversity. Alpha diversity provides a summary statistic of the microbial community, whereby higher alpha diversity indicates a greater number of species (i.e., “richness”), with more even representation (i.e., “evenness”), and/or greater biodiversity according to the ancestral dissimilarity of species (i.e., “phylogenetic diversity”; Figure 2). The computation of alpha diversity indices (e.g., Shannon, Simpson’s, and Faith’s phylogenetic diversity) vary in their consideration and weighting of these factors, but overall, alpha diversity is often used as a proxy for community stability and function (Shade, 2017). The alpha diversity indices utilized in the reviewed literature are described in Appendix D.

[INSERT FIGURE 2 HERE, IN COLOR ONLINE ONLY]

While alpha diversity is estimated for each participant separately, beta diversity is an inter-individual measure that examines similarity of communities relative to the other samples analyzed (Figure 3). Dimension reduction techniques are employed to visualize data on a smaller number of axes (e.g., using principal coordinates analysis [PCoA]), whereby samples closer together are more similar in their microbial composition. This can be used to examine whether participants in the same group (e.g., with a depressive disorder) cluster together in multidimensional space by their microbiota, but separately from another group (e.g., controls). Just as for alpha diversity, different measures of beta diversity (e.g., utilizing weighted vs. unweighted UniFrac distances) emphasize different factors (e.g., evenness, phylogeny). Machine learning/clustering methods can also be utilized to analyze whether groups can be differentiated based on their microbial communities (e.g., hierarchical clustering, random forest models). The varying approaches used to calculate beta diversity in the reviewed literature are summarized in Appendix D.

[INSERT FIGURE 3 HERE, IN COLOR ONLINE ONLY]

Gut microbiota in anxiety and depression

The present review identified 13 studies which compared gut microbiota composition of participants with MDD relative to controls (Aizawa et al., 2016; J. Chen et al., 2018; Z. Chen et al., 2018; Chung et al., 2019; Y. Huang et al., 2018; Jiang et al., 2015; Kelly et al., 2016; Lai et al., 2019; Lin et al., 2017; Mason et al., 2020; Rong et al., 2019; P. Zheng et al., 2016), or analyzed MDD-associated microbial genes in a clinical group without controls (Cheng et al., 2019). Of these, the study by Mason et al. (2020) included comparisons of participants with MDD, comorbid MDD and GAD/Anxiety not otherwise specified (NOS), GAD/Anxiety NOS alone, and controls, and is therefore discussed in several sections. An additional six studies examined the microbiota of participants with a depressive disorder, without specifying the type of depression (Chahwan et al., 2019; Liu et al., 2016; Naseribafrouei et al., 2014; Szczesniak, Hestad, Hanssen, & Rudi, 2016; Valles-Colomer et al., 2019; Vinberg et al., 2019).

Three studies compared the gut microbiota of participants with GAD to controls (Y. Chen et al., 2019; Jiang et al., 2018; Mason et al., 2020). As well as performing whole group comparisons, Jiang et al. (2018) performed subgroup analyses by stratifying participants with GAD into those currently taking psychotropic medication, and those who had never been on medication (“treatment-naïve”; Jiang et al., 2018).

Four studies examined associations between microbiota composition and anxiety and depression using self-report symptom questionnaires as their only measure of psychopathology (Jackson et al., 2018; Kleiman et al., 2017; Naudé et al., 2020; Taylor et al., 2019), and one study assessed microbial associations with depression symptoms (Heym et al., 2019). The results of all studies are presented in Appendix D.

Alpha diversity

Case-control studies in depressive disorders. A total of 13 case-control studies in depressive disorders examined alpha diversity indices incorporating richness and evenness. The Shannon index was the most widely examined, reported in 11 studies. Seven studies found no difference between MDD and control groups across all examined indices (Chahwan et al., 2019; Chung et al., 2019; Kelly et al., 2016; Mason et al., 2020; Naseribafrouei et al., 2014; Valles-Colomer et al., 2019; Vinberg et al., 2019). Two studies found lower alpha diversity in depressive disorders using the Shannon index (Y. Huang et al., 2018; Liu et al., 2016). The remaining three studies reported inconsistent findings across indices (Jiang et al., 2015; Lai et al., 2019; Rong et al., 2019).

Total number of microbial species, as estimated by community richness, was examined by nine studies. Number of amplicon sequence variants/operational taxonomic

units (ASVs/OTUs) was the most widely examined community richness index ($n=7$). Four studies found lower richness in depressive disorders relative to controls across a number of indices (Y. Huang et al., 2018; Kelly et al., 2016; Rong et al., 2019; Vinberg et al., 2019), although five studies found no difference (Chahwan et al., 2019; Chung et al., 2019; Jiang et al., 2015; Naseribafrouei et al., 2014; P. Zheng et al., 2016).

Five studies investigated phylogenetic diversity. Two studies observed lower phylogenetic diversity in depression (Y. Huang et al., 2018; Kelly et al., 2016), although three studies found no difference (J. Chen et al., 2018; Chung et al., 2019; P. Zheng et al., 2016). Results for the most commonly reported indices are displayed visually in Figure 4.

Case control studies in anxiety disorders. Alpha diversity indices incorporating richness and evenness were examined in all three studies comparing the gut microbiota of participants with GAD to controls (Figure 4). The Shannon index was reported in all three studies (exclusively or in combination with other indices; Y. Chen et al., 2019; Jiang et al., 2018; Mason et al., 2020). Alpha diversity did not differ between participants with depressive disorders (GAD or Anxiety NOS) and controls in three studies (Y. Chen et al., 2019; Jiang et al., 2018; Mason et al., 2020). Jiang et al. (2018) observed higher alpha diversity in participants with GAD who were treatment naïve (Simpson's Index), a finding not observed in the medicated group.

Community richness was examined in two studies which compared participants with GAD to controls (Y. Chen et al., 2019; Jiang et al., 2018). Participants with GAD had lower richness compared to controls (ACE index; Y. Chen et al., 2019; Jiang et al., 2018). Phylogenetic diversity was not analyzed.

Anxiety/depression symptoms. Three studies which investigated cross-sectional associations between depression symptoms did not report alpha diversity indices considering richness and evenness. In the four studies that did, no significant associations were observed between richness/evenness indices with depression symptoms (Jackson et al., 2018; Kleiman et al., 2017; Mason et al., 2020; Naudé et al., 2020). No studies investigated community richness and depression symptoms. One study investigated phylogenetic diversity, but found no association with depression symptoms (Jackson et al., 2018).

Four of the five studies which incorporated anxiety symptom measures investigated associations with alpha diversity indices incorporating richness and evenness. All four studies reported no association between the Shannon index and anxiety symptoms (Jackson et al., 2018; Kleiman et al., 2017; Mason et al., 2020; Naudé et al., 2020). Only one study

investigated phylogenetic diversity, observing no association (Jackson et al., 2018). Richness was not examined.

[INSERT FIGURE 4 HERE, IN COLOR ONLINE ONLY]

Beta diversity

Case-control studies in depressive disorders. Of the 19 case-control studies which examined the gut microbiota of participants with a depressive disorder, 13 analyzed beta diversity. Eight studies found significant differences in beta diversity between participants with a depressive disorder and controls, as indicated by group clustering on PCoA (J. Chen et al., 2018; Chung et al., 2019; Y. Huang et al., 2018; Kelly et al., 2016; Lai et al., 2019; Lin et al., 2017; P. Zheng et al., 2016). Highlighting variation based on the distance measure employed, Huang et al. (2018) found significant group differences using weighted UniFrac, but not using unweighted UniFrac (Y. Huang et al., 2018). Five studies found no difference in beta diversity in participants with a depressive disorder relative to controls (Chahwan et al., 2019; Jiang et al., 2015; Mason et al., 2020; Rong et al., 2019; Vinberg et al., 2019). One of these studies found no difference between participants with depression, anxiety, comorbid anxiety and depression, and controls on PCoA when stratified by diagnosis (weighted UniFrac), but hierarchical clustering of beta diversity identified two participant groups associated with anhedonia scores derived from self-report questionnaires (weighted UniFrac; Mason et al., 2020). This finding indicates the choice of clinical stratification criteria is an important consideration (i.e., based on clinical diagnoses versus questionnaires).

Case-control studies in anxiety disorders. Beta diversity, analyzed using PCoA, indicated the overall microbial composition of GAD patients deviated from that of controls in two studies (Y. Chen et al., 2019; Jiang et al., 2018). One study found no difference in beta diversity between participants with an anxiety disorder, MDD, comorbid anxiety/MDD, or controls (Mason et al., 2020).

Anxiety/depression symptoms. Anxiety and depression symptoms were associated with beta diversity distances in one study (Jackson et al., 2018), and anhedonia scores were associated with beta diversity in another (Mason et al., 2020). The remaining three studies found no association between beta diversity and symptoms (Douglas et al., 2019; Kleiman et al., 2017; Naudé et al., 2020).

Taxonomic findings

Case-control studies in depressive disorders.

A large number of bacterial taxa were significantly different in their abundance between clinical and control groups (Figure 5), although a smaller number were more consistently implicated.

[INSERT FIGURE 5, IN COLOR ONLINE ONLY]

Of the 19 studies which analyzed the gut microbiota in depressive disorders, the phylum Actinobacteria was higher in MDD in six studies (J. Chen et al., 2018; Z. Chen et al., 2018; Chung et al., 2019; Lai et al., 2019; Rong et al., 2019; P. Zheng et al., 2016) and positively correlated with depression symptoms (J. Chen et al., 2018), although its abundance was lower in one study relative to controls (Jiang et al., 2015). A lower abundance of Bacteroidetes in MDD was observed in seven studies (J. Chen et al., 2018; Z. Chen et al., 2018; Chung et al., 2019; Lai et al., 2019; Lin et al., 2017; Rong et al., 2019; P. Zheng et al., 2016), although the opposite was observed in two studies (Jiang et al., 2015; Liu et al., 2016).

At the order level, Enterobacteriales was higher in three studies, one in MDD/depression (Jiang et al., 2015) and two in GAD (Y. Chen et al., 2019; Jiang et al., 2018). Within this order, the relative abundance of the family *Enterobacteriaceae* was also higher in four MDD/depression studies (Y. Chen et al., 2019; Jiang et al., 2015, 2018; Rong et al., 2019), and was positively correlated with depression symptoms in another (Taylor et al., 2019). A further study found a lower relative abundance of *Enterobacteriaceae* in MDD (Z. Chen et al., 2018); however, of the studies which found significant differences in *Enterobacteriaceae*, this was one of only two studies that did not exclude for antibiotics or probiotics prior to sample collection (at least one month). It also did not exclude for psychotropic medication, although this was inconsistently considered amongst all studies.

At the family level, the abundance of *Prevotellaceae* was lower in four studies of MDD relative to controls (Z. Chen et al., 2018; Chung et al., 2019; Jiang et al., 2015; Kelly et al., 2016). Consistently, depression symptoms were also negatively correlated with *Prevotellaceae* (Chung et al., 2019). *Bifidobacteriaceae* was also higher in MDD in five studies (J. Chen et al., 2018 [females only]; Z. Chen et al., 2018; Chung et al., 2019; Lai et al., 2019; Rong et al., 2019), although was lower in one study examining depression (Jackson et al., 2018), and negatively correlated with anxiety symptoms in another (Taylor et al., 2019, [females only]). Reported group differences in the relative abundance of *Lachnospiraceae*

were also inconsistent, with five studies finding higher abundance in MDD/depressive disorders (J. Chen et al., 2018 [females only]; Z. Chen et al., 2018; Cheng et al., 2019; Chung et al., 2019; Jackson et al., 2018), two finding lower (Y. Huang et al., 2018; Jiang et al., 2015), and one study reporting a negative correlation with depression symptoms (J. Chen et al., 2018 [males only]). Abundance of the family *Coriobacteriaceae* was higher in three studies in depression relative to controls (J. Chen et al., 2018 [females only]; Rong et al., 2019; P. Zheng et al., 2016), and positively correlated with depressive symptoms in two studies in females only (J. Chen et al., 2018; Taylor et al., 2019). Further indicating interactions between *Coriobacteriaceae* and sex, the opposite direction relationship was observed in males (i.e., lower abundance; J. Chen et al., 2018). Finally, the relative abundance of *Streptococcaceae* was higher in depressive disorders in three studies (Chung et al., 2019; Jackson et al., 2018; P. Zheng et al., 2016), although was negatively correlated with depression symptoms in another in females only (J. Chen et al., 2018)

At the genus level, four studies reported lower *Sutterella* in depressive disorders (J. Chen et al., 2018; Chung et al., 2019; Liu et al., 2016; P. Zheng et al., 2016), and one reported a negative correlation with depression symptoms (Chung et al., 2019). Similarly, *Faecalibacterium* was lower in MDD/depression in five studies (Y. Huang et al., 2018; Jiang et al., 2015; Liu et al., 2016; Valles-Colomer et al., 2019; P. Zheng et al., 2016), and was negatively correlated with depression symptoms (Jiang et al., 2015). In contrast, one study found a higher abundance in MDD, although only in females (J. Chen et al., 2018). Within the *Coriobacteriaceae* family, three genera were higher in MDD/depression, including *Eggerthella* in six studies (J. Chen et al., 2018 [females only]; Chung et al., 2019; Kelly et al., 2016; Lai et al., 2019; Rong et al., 2019; P. Zheng et al., 2016), *Olsenella* in three studies (J. Chen et al., 2018; Lai et al., 2019; P. Zheng et al., 2016), and *Collinsella* in one study (P. Zheng et al., 2016), which was also positively correlated in two studies (J. Chen et al., 2018 [females only]; Taylor et al., 2019 [males only]). *Lactobacillus* abundance was higher in MDD/depression relative to controls in four studies (Lai et al., 2019; Rong et al., 2019; Valles-Colomer et al., 2019; P. Zheng et al., 2016), although was negatively correlated with depression symptoms in one study in females only (Taylor et al., 2019). Moreover, lower *Clostridium cluster XIVa* was reported (P. Zheng et al., 2016), which was also negatively correlated with depression symptoms (J. Chen et al., 2018 [females only]; Mason et al., 2020). The genus *Oscillibacter* was higher in MDD in three studies (Jiang et al., 2015; Lai et al., 2019; Rong et al., 2019), although the opposite relationship was observed in one study (Liu et al., 2016). Several genera within the *Lachnospiraceae* family were also differentially

abundant, although varying in their direction: four studies reported higher *Blautia* in MDD (J. Chen et al., 2018 [females only]; Chung et al., 2019; Jiang et al., 2015; P. Zheng et al., 2016) and its abundance was positively correlated with depression symptoms (Chung et al., 2019); the opposite relationship was observed in two studies (Y. Huang et al., 2018; Liu et al., 2016). Four studies observed lower *Coprococcus* in depression (Y. Huang et al., 2018; Liu et al., 2016; Valles-Colomer et al., 2019 [medicated cohort only]; P. Zheng et al., 2016). A higher abundance of *Erysipelotrichaceae incertae sedis* was reported in depression in four studies (J. Chen et al., 2018 [males only]; Chung et al., 2019; Jiang et al., 2015; P. Zheng et al., 2016), as well as higher *Holdemania* in three studies of depression (Chung et al., 2019; Kelly et al., 2016; Valles-Colomer et al., 2019 [medicated cohort only]). The genus *Streptococcus* was also reported to be higher in MDD in four studies (Chung et al., 2019; Lin et al., 2017; Rong et al., 2019; P. Zheng et al., 2016), although a negative correlation between depression symptoms was also reported in females only (J. Chen et al., 2018). Three studies observed higher *Desulfovibrio* in MDD relative to controls (J. Chen et al., 2018 [females only]; Cheng et al., 2019; Szczesniak et al., 2016). Finally, higher levels of *Paraprevotella* were observed in two studies in depression (Kelly et al., 2016; Liu et al., 2016), and its abundance was positively correlated with depression symptoms (Taylor et al., 2019).

Fewer studies of depressive disorders examined the microbiota with sufficient taxonomic resolution to report differences at the species level. Nevertheless, two case-control studies independently reported a number of overlapping findings, including a higher relative abundance of *Acidaminococcus intestini*, *Bifidobacterium adolescentis*, *Bifidobacterium dentium*, *Bifidobacterium longum*, *Clostridium saccharolyticum*, *Megasphaera elsdenii*, and *Oscillibacter valericigenes* in MDD relative to controls (Lai et al., 2019; Rong et al., 2019).

Case-control studies in anxiety disorders. Two studies found several consistent taxonomic differences between participants with GAD relative to controls, including higher Enterobacterales, *Bacteroidaceae*, *Escherichia/Shigella*, *Bacteroides*, *Tyzerella*, and lower Firmicutes, Mollicutes, *Prevotellaceae*, *Ruminococcaceae*, *Subdoligranulum*, *Coprococcus* and *Dialister* in patients (Y. Chen et al., 2019; Jiang et al., 2018). A third study observed a lower abundance of *Bacteroides* in the anxiety/depression comorbidity relative to a depression only group (Mason et al., 2020).

Differential taxa observed in studies comparing the gut microbiota in case-control studies in anxiety were subsequently considered alongside findings reported in depression. A total of six studies observed lower *Prevotellaceae* relative to controls, including four studies in MDD (Z. Chen et al., 2018; Chung et al., 2019; Jiang et al., 2015; Kelly et al., 2016), as

was observed in the two case-control studies in GAD reported above (Y. Chen et al., 2019; Jiang et al., 2018). Two studies observed higher *Escherichia/Shigella* in GAD (Y. Chen et al., 2019; Jiang et al., 2018), and a positive correlation between anxiety symptoms and its abundance in one study (Y. Chen et al., 2019). In contrast, two studies observed a lower abundance of this genus in MDD/depression (Jiang et al., 2015; Liu et al., 2016), although one study did observe a positive correlation with depression symptoms (Y. Chen et al., 2019). The genera *Faecalibacterium* and *Sutterella* were also lower in GAD (Jiang et al., 2018), as was separately observed in five and four studies in depressive disorders, respectively. The genus *Eggerthella* was positively correlated with both anxiety and depression symptoms (Chung et al., 2019), in line with a higher abundance observed in six studies in MDD. Moreover, *Lactobacillus* was higher in GAD compared to controls (Jiang et al., 2018), and positively correlated with anxiety symptoms (Taylor et al., 2019). Finally, one study observed a negative correlation between *Clostridium XIVa* and anxiety symptoms (Mason et al., 2020), one study observed a positive correlation between anxiety symptoms and *Holdemania* (Chung et al., 2019), and one study observed a lower relative abundance of *Megamonas* in GAD (Y. Chen et al., 2019), findings also observed in depressive disorders.

Discussion

The present systematic review provides an essential update of studies characterizing the gut microbiota in anxiety and depressive disorders, and captures the large number of studies published in recent years. Of note, the body of literature has nearly doubled since the last systematic review of the gut microbiota in depressive disorders (Cheung et al., 2019). This review also extends a recent summary focused on the gut microbiota in MDD-only (Sanada et al., 2020), by considering depressive disorders more widely, appraising research quality, and examining associations with symptom measures (Simpson, Schwartz, et al., 2020). To the best of our knowledge, it is also the first systematic review of the gut microbiota in anxiety disorders, thus facilitating investigation of possible shared microbial features between these highly comorbid groups of internalizing disorders.

Qualitative synthesis of this literature revealed that alpha and beta diversity were widely investigated by the included studies; however, differences in these community-wide measures were inconsistent. For alpha diversity, the direction of any significant effect was, with one exception, in the direction of reduced alpha diversity in the clinical group, but such differences were only observed in less than half of all analyses. Similarly, less than half of beta diversity comparisons reported a significant difference between clinical and control groups. Instead, differences in the gut microbiota between cases and controls appeared to be

localized to specific microbial taxa. In the following sections, we will discuss each of these findings in more detail, then attempt to synthesize the functional relevance of the reported taxonomic differences. We will reflect on the limitations of the extant literature in order to subsequently describe important considerations for future research.

Inconsistent diversity findings

The use of alpha diversity indices in biomedical research stems from the assumption that “higher diversity is somehow more meritorious ecologically”, and that diversity of species provides a proxy for microbial function and stability that is assumed to be favorable for the host (Shade, 2017). Consistently, lower diversity in patients relative to controls has been reported in a number of diseases and mental health disorders (Ai et al., 2019; Gong, Gong, Wang, Yu, & Dong, 2016; B. Ma et al., 2019; Nguyen, Hathaway, Kosciolk, Knight, & Jeste, 2019; Prehn-Kristensen et al., 2018). Nevertheless, the present review reveals that this conclusion is, at present, unfounded in anxiety and depression: more case-control studies found no difference in alpha diversity between anxiety/depression groups and controls ($n=10$), compared to those who found lower alpha diversity in anxiety/depression across all measured indices ($n=2$). Our conclusions are supported by a recent meta-analysis of including a very small number of case-control studies in MDD, which also found no significant difference in alpha diversity (Sanada et al., 2020). Furthermore, the present review revealed that estimated shared phylogenetic structure of the gut microbiota between groups, as assessed by beta diversity, had little consensus; ten studies reported beta diversity differences in anxiety or depressive disorders relative to controls, although six studies found no such difference.

The usefulness of gastrointestinal microbial diversity as a proxy for host health has been widely critiqued, with research recognizing that host-microbe interactions are more complex than can be modelled by simply quantifying the number of bacterial species present in a community (Gerritsen, Smidt, Rijkers, & De Vos, 2011; Shade, 2017). Indeed, several recent systematic syntheses have reported no clear consensus with regards to alpha diversity in mental health and neurological/developmental conditions relative to controls, including in Parkinson’s disease (Nuzum et al., 2020), autism spectrum disorder (Ho et al., 2020), bipolar disorder, and schizophrenia (Nguyen et al., 2019). A number of intrinsic and extrinsic/environmental factors also contribute to variation in baseline microbial diversity, thus complexing its measurement, including medication, body mass index, age, sex, diet, and early-life exposures (e.g., delivery method, breastfeeding; De Filippo et al., 2010; Hollister et al., 2015; Leeming, Johnson, Spector, & Roy, 2019; Z. Ma & Li, 2019; Reese & Dunn, 2018;

Commented [1]: I assume bipolar disorder was discussed with schizophrenia in the same paper (which is why I changed the order so the and could be between them, but a very minor point so feel free to change it back) If not, then add ref for bipolar disorder?

Tanaka & Nakayama, 2017). Quality assessment revealed only two studies considered all essential confounders identified by authors (i.e., diet, medication use, and body mass index). Studies investigating the gut microbiota in anxiety and depression should take into consideration these factors to better disentangle disorder-specific effects from baseline individual differences in diversity. Moreover, existing multivariate analyses which compare beta diversity between groups often involve dichotomization based on absence or presence of a condition (PCoA/NMDS). Research in anxiety and depressive disorders thus encounters several methodological challenges not faced by conditions with defined biomarkers, including the disparate methods by which clinical groups are defined (e.g., self-reported symptom levels, psychiatrist diagnosis, clinical interviews). Accordingly, incorporation of bias due to nosology requires consideration in future research.

Differences in specific microbial members in case-control studies

Although diversity findings were inconsistent, specific bacterial taxa were implicated in studies which compared gut microbiota of clinical groups relative to controls. Among the most consistent findings was lower abundance of Bacteroidetes, *Prevotellaceae*, *Faecalibacterium*, *Coprococcus*, and *Sutterella*, as well as a higher abundance of Actinobacteria and *Eggerthella* in participants with MDD/depressive disorders relative to controls. Participants with GAD also had a lower abundance of Firmicutes, *Ruminococcaceae*, *Subdoligranulum*, and *Dialister*, and a higher abundance of Enterobacteriales, *Enterobacteriaceae*, and *Escherichia/Shigella*, albeit in a small number of studies. Several taxa were implicated across both anxiety and depression, including lower *Prevotellaceae*, *Faecalibacterium*, *Sutterella*, and *Dialister*, as well as higher *Lactobacillus*. It is worth noting that there was a large number of studies which found no significant difference in these taxa, although this may be more reflective of low power and methodological issues, rather than true inconsistency between groups (further discussed below). Several mechanisms by which these taxa may be associated with depression and anxiety will be discussed herein, focusing on increased proinflammatory communication via the gut-brain-axis, as well as differences in how studies considered methodological confounders which may modulate microbial composition.

The role of inflammation in the gut-brain-axis

A number of taxa reported to have a higher relative abundance in clinical anxiety and depression are associated with gastrointestinal inflammation (i.e., Enterobacteriales, *Enterobacteriaceae*, *Eggerthella*, *Desulfovibrio*; Belizário, Faintuch, & Garay-Malpartida, 2018; Devkota et al., 2012; Loubinoux, Bronowicki, Pereira, Mougénel, & Le Faou, 2002;

Pedersen et al., 2018). Our study also observed higher *Bifidobacteriaceae/Bifidobacterium* in depressive disorders, which is of particular interest given that specific strains are commonly considered *anti*-inflammatory and used as probiotics (Saez-Lara, Gomez-Llorente, Plaza-Diaz, & Gil, 2015). A recent study reported mono-colonization of mice with human *Bifidobacterium*-rich microbiota had higher proinflammatory Th17 intestinal cells compared to mice colonized with *Bifidobacterium*-depleted microbiota (Ang et al., 2020). Similarly, a higher abundance of *Bifidobacterium* abundance has been associated with inflammatory bowel disease, indicating specific strains may have inflammatory potential (Wang et al., 2014).

Inflammation has been widely suggested as a contributor to the pathogenesis of depression and anxiety disorders (Raison, Capuron, & Miller, 2006; Vogelzangs et al., 2012). These hypotheses stem from the observation of higher levels of acute phase proteins and proinflammatory cytokines in otherwise medically healthy individuals with an anxiety or depressive disorder (DellaGioia & Hannestad, 2010; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Pitsavos et al., 2006). Consistently, changes in the gut microbiota, and the resultant cascade of proinflammatory communication (Figure 6), is a possible contributor to the immune dysregulation observed in mental health disorders. Peripheral inflammation may be associated with brain function via signaling across the blood-brain-barrier (BBB) and permeation of immune cells into the brain (DellaGioia & Hannestad, 2010). Inflammatory signals also activate vagal afferents which relay information to the brain from the enteric nervous system, inducing a local CNS increase of noradrenaline and acetylcholine to keep inflammation at bay (Andersson, 2005). Moreover, infiltration of lipopolysaccharides and bacterial metabolites may activate innate resistance receptors, resulting in CNS inflammation (Sochocka et al., 2019). Longer-term induction of this system is hypothesized to impair downregulation of HPA axis hormones, contributing to sustained high levels of circulating cortisol in anxiety and depression (Madeeh Hashmi, Awais Aftab, Mazhar, Umair, & Butt, 2013).

The potential for microbiota-mediated inflammation in anxiety and depression is not only indicated by the increase in inflammation-associated microbial members, but may be further exacerbated by a loss of species that secrete anti-inflammatory metabolic products. The present review revealed a reduction of bacterial species which secrete the anti-inflammatory SCFAs in anxiety/depression groups relative to controls, including *Faecalibacterium*, *Coprococcus*, *Clostridium XIVa* species which can produce butyrate (Sokol et al., 2008; Vital, Karch, & Pieper, 2017), and *Megamonas* which can produce

acetate and propionate (Sakon, Nagai, Morotomi, & Tanaka, 2008). A reduction in SCFA-producing species, and their resultant products, may contribute to dysregulated immune function. Consistently, butyrate has been implicated in reinstating BBB integrity in germ-free mice by increasing expression of tight junction proteins, and also by attenuating neuronal deficits (Stilling et al., 2016). Lower levels of butyrate have also been associated with increased intestinal permeability (Plöger et al., 2012; Stilling et al., 2016), as well as higher levels of several taxa which were observed with greater abundance in participants with anxiety and depression (e.g., Enterobacterales, *Desulfovibrio*, *Eggerthella*; Maldonado-Contreras et al., 2017; Pedersen et al., 2018; Wexler, 2007). Accordingly, a lower concentration of butyrate, and the implications this has for mucosal barrier integrity, increases the likelihood of translocation of microorganisms and their products into the bloodstream. Containment of luminal contents is essential to maintain balance between the host immune system, as permeability facilitates increased translocation of bacteria, their endotoxins (lipopolysaccharides), and other metabolites into the bloodstream, which significantly impact organs throughout the body (Bischoff et al., 2014). Similar mechanisms may underlie the association between the gut microbiota with anxiety and depression, as microbial metabolites have been associated with BBB permeability and neuroinflammation (Pedersen et al., 2018), and the administration of lipopolysaccharides induces symptoms that resemble idiopathic depression (DellaGioia & Hannestad, 2010). Future research should investigate whether specific microbial groups, and their metabolic products, are associated with reported inflammation observed in anxiety and depressive disorders.

[INSERT FIGURE 6, IN COLOR ONLINE ONLY]

Translational studies have begun to target microbiota-immune-brain dysregulation in internalizing disorders, with results supporting the possibility of microbially-mediated inflammation. Inflammation and colonic barrier integrity have been demonstrated to improve following supplementation with probiotic bacterial strains, albeit in functional gastrointestinal disorder cohorts (Schmulson & Chang, 2011). Benefits of butyrate-producing species on anxiety and depression-like symptoms have also been observed in preclinical models (*Faecalibacterium prausnitzii*, *B. longrum* and *B. breve*; Hao, Wang, Guo, & Liu, 2019; Tian, Wang, Zhao, Zhang, & Chen, 2019). High soluble fiber diets promoting butyrate-producing genera have also been associated with decreased proinflammatory cytokines and anxiety symptoms (Bourassa, Alim, Bultman, & Ratan, 2016). An improved understanding of

gut microbiota function in anxiety and depressive disorders may inform the direction for targeted and personalized interventions.

Exogenous sources of inter-study heterogeneity

Antidepressants

A range of exogenous factors were not adequately considered by studies, including psychotropic medication. Previous reviews have highlighted higher *Lachnospiraceae* in depression (Cheung et al., 2019; T.-T. Huang et al., 2019), although the present review observed this finding primarily in studies which failed to exclude for psychiatric medication (J. Chen et al., 2018; Z. Chen et al., 2018; Cheng et al., 2019; Chung et al., 2019; Jackson et al., 2018). To our knowledge, no studies have examined the effects of anti-depressants on *Lachnospiraceae* in humans; however, a recent study reported *Lachnospiraceae* species were more abundant in SSRI-treated mice compared to controls (Lyte, Daniels, & Schmitz-Esser, 2019). One reviewed study also highlighted that higher *Lactobacillus*, a member of the *Lachnospiraceae* family, was not significant after controlling for medication (Valles-Colomer et al., 2019). Psychotropic medication remains a significant source of inter-study variation which requires consideration in future studies, particularly if the aim is to understand the gut microbiota with regards to anxiety and depression pathophysiology.

Diet

The present review revealed a higher abundance of Actinobacteria in MDD relative to controls (J. Chen et al., 2018; Z. Chen et al., 2018; Chung et al., 2019; Rong et al., 2019; P. Zheng et al., 2016), in line with a previous review (T.-T. Huang et al., 2019). Consumption of a high-fat and animal protein diet has also been associated with elevated Actinobacteria (Rinninella et al., 2019), and the majority of studies which reported differences in Actinobacteria did not control for diet (Y. Chen et al., 2019; Z. Chen et al., 2018; Rong et al., 2019; P. Zheng et al., 2016). Further investigations which adequately model dietary intake are required to disentangle whether associations are driven by dietary intake or independently associated with MDD. Similarly, a large number of studies in both depressive disorders and GAD reported lower abundance of the family *Prevotellaceae* compared to controls (Y. Chen et al., 2019; Z. Chen et al., 2018; Chung et al., 2019; Jiang et al., 2015, 2018; Kelly et al., 2016). This bacterial family consists of a variety of genera associated with plant polysaccharide and mucin glycoprotein degradation (Flint, Scott, Duncan, Louis, & Forano, 2012). Complex interactions between low carbohydrate intake and deficiencies in disaccharide metabolism have previously been hypothesized to explain reduced *Prevotellaceae* in participants with autism (Kang et al., 2013). Accordingly, further research

is required to understand how dietary intake may be associated with the lower abundance of *Prevotellaceae* observed in anxiety and depressive disorders. Understanding the complex relationships between diet, the microbiota and internalizing symptoms is essential, as dietary intervention may also provide promising non-invasive point of intervention (Jacka et al., 2017).

Sex

Finally, there are clear sex differences in the prevalence of anxiety and depressive disorders, both being more common in females than males (World Health Organization, 2017). Sex differences in gut microbiota composition have also been suggested to underlie susceptibility to gut-microbiota mediated conditions in females (Z. Ma & Li, 2019). As most reviewed studies failed to consider biological sex, we did not have the power to differentiate this in terms of microbiota-gut-brain axis contribution. In the few studies that examined males and females separately, significant differences were observed, sometimes with effects in opposite directions (e.g., *Lachnospiraceae*, *Coriobacteriaceae* and *Erysipelotrichaceae incertae sedis*; J. Chen et al., 2018). This is another likely factor contributing to heterogeneity across studies.

Heterogeneity and limitations in existing methodologies

The present review revealed seven of the 26 studies had a significant risk of bias. Poorer ratings were primarily due to unclear or inconsistent methods for recruitment, screening, and categorization of cases and controls, as well as insufficient consideration of confounding variables and the use of self-reported diagnosis as the primary method for determining anxiety/depression status. All case-control studies were also cross-sectional in nature (or assessed a longitudinal intervention not of interest), and only one study investigated relevant associations at multiple timepoints. Longitudinal studies are required to disentangle disorder-specific effects from baseline individual differences, and provide an indication of the direction of causality in the associations between anxiety, depression and the gut microbiota. The current literature is not able to disentangle whether differences in the gut microbiota are a cause or a consequence of disorder presence.

Despite technological advancements in recent years, microbiota research continues to face methodological challenges. The largest sources of inter-study variability remains heterogeneity in laboratory processing (e.g., DNA extraction kits, hypervariable region, sequencing platform), and in data pre-processing and analysis – the studies reviewed herein were no exception (Appendix B). Pre-processing techniques were particularly varied (e.g., taxonomic library for sequence comparison and quality control criteria for sequence reads).

Researchers are encouraged to report all decisions and follow standardized bioinformatics and analysis pipelines, where applicable (e.g., Earth Microbiome Project; QIIME2; Caporaso et al., 2010; Marotz et al., 2017).

There are several additional factors that complicate replication and comparison of microbiota studies. There is ongoing discovery of novel bacterial species and strains, and constant updates reflect said changes in nomenclature or phylogenetic reclassification. A recent example includes the reclassification of *Lactobacillus*, among which 23 novel genera have been described and over 250 species now belong to a different genus (J. Zheng et al., 2020). Researchers must remain privy to constant updates to correctly interpret results of different taxa, which may in reality reflect the same genus/species.

Finally, another notable methodological inconsistency included the method by which clinical groups were defined, including semi-structured interviews using varying diagnostic criteria (e.g., DSM-IV/5, ICD-10), symptom cut-off scores on questionnaires, or a self-reported history of anxiety or depression. This is problematic in downstream analyses, which often involve dichotomization into disorder present/absent groupings. Researchers should carefully consider how groups are defined, an additional consideration for mental health disorders not essential for medical conditions with clear biomarkers.

Limitations of the present review

The present review has some limitations which should be considered in future syntheses. Our searches aimed to capture studies examining gut microbiota composition, rather than species' function. Functional redundancy is often reported, whereby populations can cover identical functions and secrete the same metabolic products, despite varying in their microbial composition (Heintz-Buschart & Wilmes, 2018). Accordingly, methods which examine microbial function are needed to more accurately capture host-microbe interactions. Moreover, the most commonly utilized method for estimating the gut microbiota composition within the reviewed literature was 16S rRNA gene sequencing, which is unable to distinguish between species and some phylogenetically similar genera (Janda & Abbott, 2007). Future reviews should investigate altered microbial function (e.g., using metabolomics and metagenomics), particularly if lower SCFA production and elevated peripheral inflammation remain central hypotheses for understanding associations between the gut microbiota and mental health. Furthermore, a meta-analysis was not feasible due to disparate sequencing and bioinformatics techniques. As this body of research expands, meta-analysis will be essential to disentangle whether null findings reflect an absence of associations, or are due to insufficient statistical power in light of small sample sizes. Studies are encouraged to deposit

raw sequencing reads to enable application of standardized pre-processing pipelines, taxonomic re-assignment against uniform libraries, and re-analysis using standardized techniques.

Conclusions

A growing body of literature has investigated the gut microbiota in anxiety and depressive disorders, with an aim to elucidate underlying microbial associations and inform future diagnosis and treatment avenues in these prevalent and burdensome disorders. Although diversity findings are inconsistent, a number of taxa warrant further investigation. Differential abundance analyses indicated that anxiety and depressive disorders may be characterized by a higher relative abundance of proinflammatory species, and a lower abundance of SCFA-producing bacteria. Further research is required to disentangle the contribution of potential confounding factors towards these associations, particularly diet and psychotropic medication. A summary of methodologies employed in the extant literature reveals significant variation in pre-processing and the down-stream bioinformatics employed, which complicates generalization of findings. Future research should prioritize employing standardized pre-processing and analytic pipelines. Moreover, longitudinal research is required to disentangle cause-effect and elucidate microbial interventions in these ubiquitous and debilitating psychiatric disorders. Specific differences in the composition of the microbiota in anxiety and depressive disorders continues to provide promising direction for new disease prevention and therapy targets.

Declarations of interest: None

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Figure captions

Figure 1. PRISMA flow diagram depicting the screening process for inclusion of studies in the qualitative synthesis.

Figure 2. A. Conceptual representation of alpha diversity, which is used to measure within-sample complexity. Different metrics place different emphasis on the various components of alpha diversity: richness (i.e., number of different community members), evenness (i.e., equal representation of those members), and phylogenetic diversity (i.e., relatedness of community members). *B.* To evaluate the relationship between alpha diversity and clinical symptoms, bioinformatics analyses can be conducted to statistically test for group differences or correlations with symptom scores.

Figure 3. A. Conceptual representation of beta diversity, which is used to measure between-group differences in microbiota communities. *B.* Selected bioinformatics analyses: principal coordinates analysis [PCoA] and other multidimensional scaling techniques are used (together with complementary statistical analyses) to visualize group differences based on beta diversity measures, while machine learning approaches test whether it is possible to classify participants into groups (e.g., clinical vs. control) based on beta diversity (i.e., microbiota features).

Figure 4. Summary of results of alpha diversity analyses by type of metric (richness: observed OTUs/ASVs; richness & evenness: Shannon index; phylogenetic diversity: Faith's PD) in group comparisons of participants with *A.* depressive disorders or *B.* anxiety

disorders, relative to respective control groups. Inconsistent results were found, with a minority of studies reporting significantly (sig.) higher alpha diversity, more studies reporting significantly lower alpha diversity, but the majority of studies reporting no significant difference (n.s.) or not reporting an analysis/result for the specific metric.

Figure 5. Taxonomic differences/associations with microbial taxa observed in at least two reviewed studies (at the phylum, order, family and genus levels), whereby \uparrow = higher relative abundance in clinical groups (i.e., anxiety, depression or MDD), \downarrow = lower relative abundance in clinical groups, $\overline{\otimes}$ = positive association between symptoms and taxa (anxiety, depression or both combined [mixed]), \otimes = negative association between symptoms and taxa. Studies: 1. Aizawa et al. (2016); 2. Chahwan et al. (2019); 3. Z. Chen et al. (2018); 4. J. Chen et al. (2018); 5. Chen et al. (2019); 6. Cheng et al. (2019); 7. Chung et al. (2019); 8. Heym et al. (2019); 9. Huang et al. (2018); 10. Jackson et al. (2018); 11. Jiang et al. (2015); 12. Jiang et al. (2018); 13. Kelly et al. (2016); 14. Kleiman et al. (2017); 15. Lai et al. (2019); 16. Lin et al. (2017); 17. Liu et al. (2016); 18. Mason et al. (2020); 19. Naseribafrouei et al. (2014); 20. Naudé et al. (2019); 21. Rong et al. (2019); 22. Szczesniak et al. (2016); 23. Taylor et al. (2019); 24. Valles-Colomer et al. (2019); 25. Vinberg et al. (2019); 26. Zheng et al. (2016).

Figure 6. An inflammatory gastrointestinal state is associated with higher levels of proinflammatory bacterial species, as well as a lower relative abundance of short-chain fatty acid (SCFA) producing species which ordinarily assist to maintain integrity of the intestinal barrier. The resultant compromised intestinal barrier allows bacterial translocation and higher levels of immune mediators. Peripheral inflammation communicated to the brain is associated with a disruption in hypothalamic-pituitary-adrenal (HPA) axis regulation, resulting in higher circulating levels of stress hormones, including cortisol. CRH = corticotropin-releasing hormone; ACTH = adrenocorticotrophic hormone

Table captions

Table 1. Summary of Studies Included in the Systematic Review