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Probing the oral-brain connection: oral microbiome patterns in a large community cohort with anxiety, depression, and trauma symptoms, and periodontal outcomes

Stefanie Malan-Müller[1,2,3,4]✉, Rebeca Vidal[1,3,4,5], Esther O'Shea[1,3,4,5], Eduardo Montero[6,7], Elena Figuero[6,7], Iñaki Zorrilla[2,8], Javier de Diego-Adeliño[2,9,10,11], Marta Cano[2,9], Maria Paz García-Portilla[2,12], Ana González-Pinto[2,8] and Juan C. Leza[1,2,3,4]

The role of the oral microbiome in mental health has recently been appreciated within the proposed oral-brain axis. This study examined the structure and composition of the salivary microbiome in a large-scale population-based cohort of individuals reporting mental health symptoms ($n = 306$) compared to mentally healthy controls ($n = 164$) using 16S rRNA sequencing. Mental health symptoms were evaluated using validated questionnaires and included depression, anxiety, and posttraumatic stress disorder (PTSD), with accompanying periodontal outcomes. Participants also indicated current or previous diagnoses of anxiety, depression, periodontitis, and gingivitis. Mental and periodontal health variables influenced the overall composition of the oral microbiome. PTSD symptoms correlated with a lower clr-transformed relative abundance of *Haemophilus sputorum* and a higher clr-transformed relative abundance of *Prevotella histicola*. The clr-transformed relative abundance of *P. histicola* was also positively associated with depressive scores and negatively associated with psychological quality of life. Anxiety disorder diagnosis was associated with a lower clr-transformed relative abundance of *Neisseria elongate* and a higher clr-transformed relative abundance of *Oribacterium asaccharolyticum*. A higher clr-transformed relative abundance of *Shuttleworthia* and lower clr-transformed relative abundance of *Capnocytophaga* were evident in those who reported a clinical periodontitis diagnosis. Higher *Eggerthia* and lower *Haemophilus parainfluenzae* clr-transformed relative abundances were associated with reported clinical periodontitis diagnoses and psychotherapeutic efficacy. Functional prediction analysis revealed a potential role for tryptophan metabolism/degradation in the oral-brain axis, which was confirmed by lower plasma serotonin levels across symptomatic groups. This study sheds light on the intricate interplay between oral microbiota, periodontal and mental health outcomes, and a potential role for tryptophan metabolism in the proposed oral-brain axis, emphasizing the need for further exploration to pave the way for novel therapeutic interventions and predicting therapeutic response.

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INTRODUCTION

Mental health disorders place a heavy burden on patients, families, societies, and global economies. In 2019, an estimated 418 million disability-adjusted life years (DALYs) could be attributable to mental disorders [1]. In 2017, depression was the leading cause of disability globally, with an estimated 322 million people living with depression [2], whilst about 260 million people suffered from anxiety disorders, and many suffered with additional comorbidities [3]. Mental disorders were the leading cause of the health-related burden of disease, and to worsen the situation, the COVID-19 pandemic has left in its wake a steep rise in the global prevalence of anxiety and depressive disorders [4].

Several factors, including economic insecurity, work-related stress, collective trauma, inequality, modern lifestyles, global events, and environmental factors, have likely contributed to the increased prevalence of mental health disorders [5]. Modern lifestyles, characterized by high stress levels, processed diets, excessive sanitation practices, and antibiotic use, alongside environmental changes like increased pollutants, climate change, and urbanization, have shifted human microbiota towards an industrialized state [6]. These microbiota alterations, coupled with the loss of specific functional attributes, may lead to suboptimal disease-promoting microbial communities, worsening compromised mental health [6].

The burden of mental health disorders is compounded by treatment limitations such as non-adherence, treatment

[1]Department of Pharmacology and Toxicology, Faculty of Medicine, University Complutense Madrid (UCM), Madrid, Spain. [2]Biomedical Research Network Centre in Mental Health (CIBERSAM), Institute of Health Carlos III (ISCIII), Madrid, Spain. [3]Hospital 12 de Octubre Research Institute (Imas12), Madrid, Spain. [4]Instituto Universitario de Investigación Neuroquímica (IUIN-UCM), Madrid, Spain. [5]Red de Investigación en Atención Primaria de Adicciones (RIAPAd-ISCIII), Madrid, Spain. [6]ETEP (Etiology and Therapy of Periodontal and Peri-implant Diseases) Research Group, UCM, Madrid, Spain. [7]Department of Dental Clinical Specialties, Faculty of Dentistry, UCM, Madrid, Spain. [8]BIOARABA, Department of Psychiatry, Hospital Universitario de Alava, UPV/EHU, Vitoria, Spain. [9]Sant Pau Mental Health Research Group, Institut de Recerca Sant Pau (IR Sant Pau), Barcelona, Spain. [10]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. [11]Autonomous University of Barcelona (UAB), Barcelona, Spain. [12]Department of Psychiatry, Universidad de Oviedo, Servicio de Psiquiatría, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain. ✉email: smalanmu@ucm.es

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resistance, and relapse [7–9], highlighting the necessity for innovative treatment modalities. The human holobiont, comprising the human host and its symbiotic microorganisms, plays a crucial role in health and disease [10–12]. While much attention has been given to the gut-brain axis, emerging evidence suggests that the oral microbiota, a less explored niche, may also influence the central nervous system (CNS) and behavior [13, 14].

The oral cavity hosts a diverse array of bacteria, and dysregulation can lead to disease. Periodontitis, a chronic bacterial infection affecting nearly half the global population [15], triggers systemic inflammation through pro-inflammatory cytokine release and invasion by periodontal keystone pathogens like *Porphyromonas gingivalis* (*P. gingivalis*) [16]. Periodontitis not only contributes to chronic inflammatory conditions like atherosclerosis, diabetes, and cardiovascular diseases [17, 18] but also shows associations with psychiatric disorders [19], suggesting involvement in the oral-brain axis. A longitudinal study spanning 10 years found a higher incidence of subsequent depression in individuals with periodontitis compared to those without [20], indicating a potential causal relationship between periodontitis and major depression. Additionally, recent research has identified specific bacterial taxa implicated in periodontal disease as well as anxiety, depressive disorders, and trauma-related disorders [21].

Pathogenic periodontal bacteria can impact the CNS through various pathways, both directly and indirectly [21]. Direct routes include bloodstream transmission or areas with compromised blood-brain barrier (BBB) integrity [22]. Indirectly, they induce pro-inflammatory cytokine production, activating endothelial cells expressing tumor necrosis factor (TNF)-α and interleukin-1 (IL-1) β receptors, which signal perivascular macrophages, leading to neuroinflammation [23]. Keystone pathogens widen intercellular spaces in periodontal pockets, causing epithelial rupture and a "leaky mouth" [24], facilitating lipopolysaccharide (LPS) access to circulation, activating the immune system and the hypothalamic pituitary adrenal (HPA) axis, influencing CNS function [23, 25]. Other entry points include circumventricular organs, the choroid plexus [22, 26], and olfactory/trigeminal nerves [27]. Brain-resident microglia can be influenced by periodontal bacteria via leptomeninges [25]. Periodontal pathogens also affect gut microbial composition/function directly via enteral or indirectly via hematogenous transmission [28].

Clinical data linking the oral microbiome to mental health disorders are limited. A study employing genetic association analysis and Mendelian randomization found significant associations and causal effects between salivary-tongue dorsum microbiome interactions and anxiety/depression, with *Eggerthia* notably linked to both conditions across multiple databases [29]. A clinical case study reported the pathogenic potential of *Eggerthia catenaformis*, which started as a submandibular abscess that spread hematogenously, subsequently causing a perihepatic abscess and severe clinical sepsis [30]. Another study involving adolescents ($n = 66$) observed differing abundances of *Actinomyces*, *Spirochaetaceae*, *Fusobacterium*, and *Treponema* in individuals with symptoms of anxiety and depression [31]. Wingfield et al. compared the oral microbial composition in depressed young adults ($n = 40$), identifying 21 bacterial taxa with varying levels compared to controls, including higher abundances of *Neisseria spp.* and *Prevotella nigrescens* [32]. *P. nigrescens* show moderate pathogenicity [33] and can produce mannose polysaccharides, which promote chronic inflammatory processes, including altered leukocyte phagocytosis and invasion of host barriers [34, 35].

Although limited mechanistic data is currently available to explain the links between these oral bacteria and mental health outcomes, an oral-brain axis has been proposed, where periodontal bacteria can directly reach and influence the brain via several pathways. Periodontitis can indirectly impact the CNS through pro-inflammatory cytokines [36], which activate endothelial cells expressing TNF-a and IL-1 receptors. This activation signals perivascular macrophages, which in turn activate microglia, leading to neuroinflammation [37, 38]. Additionally, periodontitis can cause a leaky periodontium, allowing LPS to enter the systemic circulation. This can activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in elevated stress hormones or neurotransmitters [39], thereby influencing mental health outcomes. Larger studies encompassing diverse age groups and gathering microbiome and mechanistic data longitudinally are needed to elucidate the role of the oral-brain axis in anxiety and depression.

This study aimed to enhance the limited understanding of how periodontal health can impact mental health through the oral microbiome. We characterized the salivary microbiome, predicted the functional potential of salivary taxa, and measured plasma levels of tryptophan metabolites to confirm these predictions in individuals exhibiting symptoms of anxiety, depression, and PTSD along with periodontal outcomes. These findings could lay the foundation for targeting the oral microbiome and improving periodontal health as a novel approach to enhancing mental health outcomes.

METHODS

Study participant evaluation and enrollment

This cohort comprised two Spanish study populations from PsicoBioma ($n = 186$, March 2021 - Jan 2022) and TRIAD ($n = 284$, Nov 2021 - Dec 2022), both population-based microbiome projects. PsicoBioma recruited participants from Spain, while TRIAD recruited from Madrid, Barcelona, Vitoria, and Oviedo municipalities (allowing blood collection). Both cohorts provided saliva samples and completed similar online questionnaires. TRIAD also provided blood samples for plasma analysis and completed a more comprehensive periodontal health questionnaire. The research adhered to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for human experiments, and data processing followed Spanish Organic Law 3/2018 on Personal Data Protection and Digital Rights Guarantee (BOE 16673 of 6 Dec 2018) and its 17th Additional Provision. Approval was obtained from the Ethics Committees of Hospital Clínico San Carlos (Madrid), Medical Research Ethics Committee of Asturias, Basque Medicine Research Ethics Committee, and Drug Research Ethics Committee of Hospital de la Santa Creu i Sant Pau (PSQ-19-2 C.I. 196/474-E). All research participants provided online, written informed consent.

The study recruited healthy controls, participants with a current/previous diagnosis of anxiety, depressive, or trauma-related disorders, or individuals who were experiencing these symptoms. Spanish residents, 18 years or older, who were proficient in reading and understanding Spanish were included. Individuals who used antibiotics within the previous six months, and those diagnosed with any *other* major psychiatric disorders including psychotic disorders, personality disorders, or neurodegenerative disorders, were excluded.

Demographic and clinical data

Demographic, health, and clinical data were collected using a secure online questionnaire. Psychological evaluations relied on standardized self-report questionnaires validated for the Spanish population; this study focused on symptoms rather than formal diagnoses. However, participants also indicated on the questionnaire whether they had a previous/current clinical diagnosis of anxiety or depression ("diagnosis" henceforth refers to clinical diagnoses, and "symptoms" refers to self-report questionnaire data). Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression (CESD) scale; state and trait anxiety symptoms were evaluated using the state-trait anxiety inventory (STAI). The posttraumatic stress disorder (PTSD) Checklist for the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5, PCL-5), and the Childhood Trauma Questionnaire-Short Form (CTQ-SF) evaluated trauma exposure. Additionally, quality of life was measured using the World Health Organization Quality Of Life Questionnaire (WHOQOL).

Psychiatric symptoms were determined based on the following criteria. Depressive symptoms: CESD scores of 16 – 24 indicated mild and 25 – 55 severe depressive symptoms [40]. PTSD symptoms: PCL-5 score > 33 + more than 3 symptom clusters [41]. State anxiety symptoms: STAI-S scores > 41; trait anxiety symptoms: STAI-T scores > 45; CTQ-SF [42] total score

was used to evaluate the severity of childhood maltreatment.

The TRIAD cohort completed a periodontal health questionnaire [43] (validated for the Spanish population [44]) to predict severe periodontitis, according to Montero et al. [44] Specifically, severe periodontitis was defined according to three criteria: (i) the Centers for Disease Control/American Academy of Periodontology (CDC/AAP) case definition [45], henceforth referred to as *SeverePerioCDCAAP*; (ii) the presence of ≥ 50% of teeth with clinical attachment level (CAL) ≥ 5 mm, henceforth referred to as *TeethCAL5*; (iii) the presence of ≥ 25% of teeth with probing pocket depth (PPD) ≥ 6 mm, henceforth referred to as *TeethPPD6*. In addition, participants also indicated whether they had a previous/current clinical diagnosis of periodontitis and/or gingivitis ("diagnosis" refers to a self-reported clinical diagnosis, and "predicted severe periodontitis" refers to self-reported questionnaire data).

Blood collection and processing

Whole blood (10 ml) was collected from 282 of the TRIAD participants, using BD Vacutainer® EDTA tubes. Blood was centrifuged at 1800 rpm for 10 minutes at room temperature, and the resulting supernatant (plasma) was transferred into clean 1.5 ml Eppendorf tubes for storage at −80 °C for later use.

Kynurenine, tryptophan, and serotonin quantification in plasma

Plasma levels of kynurenine (KYN), tryptophan (TRP), and serotonin (5-HT) were measured using high-performance liquid chromatography (HPLC). TRP and 5-HT were detected fluorometrically at excitation/emission wavelengths of 270/360 nm and 290/398 nm, respectively (Waters 2475, Multi fluorescence Detector; Waters, Milford, MA, USA). The ratios of KYN or 5-HT to TRP concentrations were calculated and used as a measure of TRP degradation (see Supplementary Materials for details).

Bacterial DNA extraction, 16S rRNA gene sequencing and analysis

Participants self-collected saliva samples in DNA/RNA Shield Safe Collect Saliva Collection tubes (Zymo Research, Irvine, California, USA), from which microbial DNA was extracted (ZymoBIOMICS DNA Miniprep Kit, Zymo Research). Bacterial 16S rRNA gene V3-4 amplicons were generated, using previously described primers [46] and sequenced (2 × 300 bp paired-end) (Center de Regulació Genòmica, Barcelona, Spain), on the Illumina NextSeq2000 platform (see Supplementary Material for details).

Quality control of FASTQ sequencing files was performed using fastqc and multiqc. Raw sequence reads were de-replicated and de-noised to combine identical reads into amplicon sequence variants (ASVs) [47], and construct consensus quality profiles for each combined set of sequences (*dada2* version 3.11 [48]). Following chimera removal, a consensus paired-end reads file was generated for feature construction and downstream analysis. Taxonomic binning of classified sequences was built using a local copy of the Ribosomal Database Project (RDP) Classifier (Train Set 19 [49]), and normalized data were produced from the relative abundance of taxa present in each sample. A feature table of 54,817 unique ASVs with an average read length of 391 nucleotides in 470 samples was consequently constructed (following pre-processing, the minimum number of reads per sample was 18,868, and the average number of reads per sample was 95,763).

Statistical analyses

Data was analyzed using bioinformatics and statistical analysis packages in *R* [47], including the packages *dada2* (version 3.18 [48]), *vegan* (version 2.6.4 [50]), *phyloseq* (version 1.46.0 [51]), *ggplot2* (version 3.4.4 [52]), and *CoDaSeq* (version 0.99.7 [53, 54]. For clinical and demographic data, continuous variables were summarized as means (M) and standard deviations (SD) if normally distributed or as medians and interquartile ranges (IQRs) if non-normally distributed. To assess differences in the metadata variables between symptomatic and control groups, Student's *t*-tests and Wilcoxon rank sum tests were used to assess differences between normally and non-normally distributed data (normality tested using Shapiro-Wilk Normality Test), respectively. Categorical data were summarized as counts (*n*) and percentages, and χ^2 or Fisher exact tests were used to assess differences between groups, where appropriate. Significance was defined as $p \leq 0.05$.

The Simpson index (which takes both richness and evenness into account), Chao1 (estimator of species richness), and Pielou's evenness index were used to evaluate α-diversity [55]. Taxa were agglomerated to genus level, assigning species-level where possible. Data was transformed to relative abundance out of 100 to account for differences in total depth per sample. Variance filtering was performed (*genefilter* function, version 1.84.0), which removed taxa with the lowest 40% variance. Abundance matrices were centered log-ratio (clr)-transformed, using the minimum proportional abundance detected for each taxon for the imputation of zeros. The ordination of community variation was visualized using multidimensional scaling (MDS) of genus-level Aitchison distances. The *capscale* function (*vegan* package) [50] was used to determine the contribution of metadata variables to microbiome community variation.

The ASV table was filtered to retain taxa observed in at least 15% of participants. Associations between taxonomic abundance and metadata variables were analyzed using a linear modeling approach (*fw_glm* function, *Tjazi* package) [56]) on the clr-transformed and filtered relative taxonomic abundances, whilst adjusting for covariates that were associated with taxonomic abundance in our dataset, including age, body mass index (BMI), smoking status, and cholesterol medication use. Other potential covariates were tested using linear modeling, but did not have an association with taxonomic abundance, these included sex, alcohol use, psychotropic medication use, weekly consumption of processed food, green vegetables, and fruits, and whether filtered or unfiltered water was consumed. We performed false discovery rate (FDR) correction using the Benjamini-Hochberg procedure and significance was defined as $q \leq 0.1$. Throughout the manuscript, we refer to *relative abundance*, which denotes filtered, clr-transformed relative taxonomic abundances for brevity. Medians (*mdn*) and interquartile ranges (IQRs) are also reported.

We utilized PICRUSt2 [57] to predict the CNS-related functional potential of oral taxa, by focusing on gut-brain modules (GBMs) [58]. Associations between GBMs and mental health outcomes as well as periodontal outcomes were tested using the same linear modeling approach as previously described, with significance set at $q \leq 0.1$.

Post hoc power analysis

Following the completion of the study, a post hoc power analysis was conducted to evaluate the statistical power achieved with the final sample size. The analysis was performed using the Power & Sample Sizes Tool for Case-Control Microbiome Studies [59], which incorporates Monte Carlo simulations with 100 replications and an alpha level of 0.1. Firstly, we computed the power across a range of sample sizes to understand how varying the sample size influences the power of the study. Secondly, we specified the number of controls as $n = 164$ (mentally healthy controls) and the number of cases as $n = 306$ (the symptomatic cohort). The outcomes of the simulation are reported in terms of the Wald statistic, the Wilcoxon–Mann–Whitney (WMW) test, and the average Wilcoxon–Mann–Whitney (WMW avg) test.

RESULTS

Clinical and demographic characteristics

Clinical and demographic characteristics of the depressive ($n = 148$), state ($n = 256$) and trait anxiety ($n = 281$), and PTSD ($n = 73$) symptomatic cohorts (according to criteria described in the Methods and Materials), and healthy controls (no significant mental health symptoms) ($n = 164$) are described in Tables 1–4. In our cohort of 470 individuals, 306 presented with at least one or a combination of the aforementioned symptoms. Females constituted 72% of our cohort, and the median age was 40 years. Comorbidity of these psychiatric symptoms was common; of the 232 individuals who had both state and trait anxiety symptoms, 133 of them also had depressive symptoms (57.3%), 67 had comorbid PTSD symptoms (28.9%), and 52 (22.4%) had PTSD and depressive symptoms. Of the 148 individuals with depressive symptoms, 144 (97.3%) also had trait anxiety symptoms, and 54 (36.5%) had symptoms of PTSD.

A total of 34 (7%) individuals had a current clinical diagnosis of periodontitis, and 30 (11%) had a gingivitis diagnosis. The self-reported periodontal health questionnaire administered to most of the TRIAD cohort ($n = 196$) (unavailable for the PsicoBioma cohort) showed 81 individuals (41%) had probable severe periodontitis using TeethPPD6 criteria, 93 (47%) had it based on the TeethCAL5 criteria, and 106 (54%) had it based on the Centers for Disease Control (CDC)/American Academy of Periodontology (AAP) criteria (SeverePerioCDCAAP).

Table 1. Comparative statistics of continuous and categorical variables for the depressive symptom vs. healthy control cohorts.

	Total cohort ($n = 470$)		Healthy controls ($n = 164$)		Depressive symptom cohort ($n = 148$)		*p*-value
	median	IQR	median	IQR	median	IQR	
	n	%	*n*	%	*n*	%	
Age	40.0	31.0–50.0	38.5	30.0–47.5	40.0	33.0–51.0	NS
BMI	22.8	20.7–25.4	22.8	20.9–25.4	22.9	20.4–26.0	NS
CTQ total score	34.0	29.0–44.0	29.0	27.0–34.0	43.5	34.0–55.3	**<0.001**
WHOQOL Physical health	14.3	12.0–16.6	16.6	14.9–17.9	12.0	9.7–13.1	**<0.001**
WHOQOL Psychological health	13.0	10.0–15.3	16.0	14.0–17.3	9.3	8.0–11.3	**<0.001**
WHOQOL Social relationships	12.7	9.3–14.7	14.7	13.3–17.3	9.3	6.7–12.0	**<0.001**
WHOQOL Environment	15.0	12.5–16.5	16.5	15.0–18.0	13.0	11.0–14.6	**<0.001**
WHOQOL Total score	7.0	5.0–8.0	8.0	7.0–9.0	5.0	4.0–6.0	**<0.001**
PCL Total score	17.5	5.0–35.0	5.0	1.0–11.5	35.0	21.0–47.0	**<0.001**
CESD total score	16.0	8.0–30.0	6.0	2.0–10.0	35.0	31.0–41.0	**<0.001**
STAIS total score	44.0	35.0–54.0	32.0	27.8–36.0	57.0	50.0–62.0	**<0.001**
STAIT total score	49.0	40.0–58.0	37.0	33.0–41.0	59.0	55.0–64.0	**<0.001**
LPS concentration	0.2	0.1–0.2	0.2	0.1–0.2	0.2	0.1–0.3	NS
KYN (log)	0.3	0.2–0.5	0.4	0.2–0.5	0.3	0.1–0.5	NS
5HT (log)	−0.7	−2.2–0.2	−0.5	−1.1–0.0	−1.3	−2.8–0.4	**<0.001**
TRP (log)	3.8	3.6–3.9	3.8	3.6–3.9	3.8	3.6–3.9	NS
KYN/TRP (log)	3.5	3.3–3.7	3.5	3.3–3.8	3.4	3.3–3.6	NS
5HT/TRP (log)	2.5	1.0–3.0	2.6	2.1–3.2	1.9	0.3–2.7	**<0.001**
Female	341	73%	110	67%	113	76%	NS
Gingivitis diagnosis[a]	30	11%	6	9%	12	10%	NS
Periodontitis diagnosis	34	7%	11	7%	10	7%	NS
IBS/Coeliac/Crohn's diagnosis	78	17%	18	11%	29	20%	**<0.05**
Psychotherapeutic Response							
Unresponsive	29	9%	3	4%	19	15%	**<0.001**
Responsive	195	61%	65	77%	65	51%	
Somewhat responsive	98	30%	16	19%	44	34%	
Psychoactive medication[a]	117	44%	12	21%	68	61%	**<0.001**
Smoking status (yes)	120	26%	32	20%	45	30%	**<0.05**
PTSD symptoms	73	16%	0	0%	54	36%	**<0.001**
State anxiety symptoms	256	54%	0	0%	136	92%	**<0.001**
Trait anxiety symptoms	281	60%	0	0%	144	97%	**<0.001**
Predicted periodontitis							
TeethPPD6[a]	81	41%	21	49%	29	36%	NS
SeverePerioCDCAAP[a]	106	54%	27	63%	37	46%	NS
TeethCAL5[a]	93	47%	24	56%	35	43%	NS

TeethPPD6, SeverePerioCDCAAP, TeethCAL5 data available for $n = 196$ of the total cohort, $n = 43$ of HC, and $n = 81$ of the depressive symptom cohort; Gingivitis diagnosis data available for $n = 284$ of the total cohort, $n = 66$ of HC, and $n = 117$ of the depressive symptom cohort.

5HT serotonin, *BMI* body mass index, *CESD* Center for Epidemiologic Studies Depression scale, *CTQ* childhood trauma questionnaire, *HC* healthy controls, *IBS* Irritable bowel syndrome, *IQR* interquartile range, *KYN* kynurenine, *LPS* lipopolysaccharide, *PCL-5* Posttraumatic Stress Disorder Checklist for DSM-5, *PTSD* posttraumatic stress disorder, *SeverePerioCDCAAP* Centers for Disease Control/American Academy of Periodontology (CDC/AAP) case definition, *sd* standard deviation, *STAI* state-trait anxiety inventory, *TeetchCAL5* probable severe periodontitis based on the "≥50% of teeth with clinical attachment level (CAL) ≥5 mm" criteria, *TeethPPD6* probable severe periodontitis based on the "≥25% of teeth with probing pocket depth (PPD) ≥6 mm" criteria, *TRP* tryptophan, *WHOQOL* World Health Organization Quality of Life assessment.

[a]Psychoactive medication data available for $n = 256$ of the total cohort, $n = 58$ of the HC, and $n = 111$ of the depressive symptom cohort. Bold values indicate statistical significance.

Post hoc power analysis

The power analysis conducted over a range of sample sizes (30 to 200 participants) indicated that a sample size of 200 participants would achieve 92% power to detect differences. Secondly, using the specified sample sizes of 164 controls and 306 cases, the simulation yielded the following outcomes: Wald Statistic = 0.98, Wilcoxon–Mann–Whitney (WMW) Test = 1, Average Wilcoxon-Mann-Whitney (WMW avg) Test = 0.798. Indicating that that the power to detect differences in microbiome composition between the case and control groups is 98%. The Wilcoxon-Mann-Whitney test value of 1 also indicates a high level of power, confirming the robustness of the test in detecting significant differences. The average WMW test value of 0.798 reflects the average performance of the WMW test across the simulations.

Table 2. Comparative statistics of continuous and categorical variables for the trait anxiety symptom vs. healthy control cohorts.

	Total cohort ($n = 470$)		Healthy controls ($n = 164$)		Trait anxiety symptoms cohort ($n = 281$)		*p*-value
	median	IQR	median	IQR	median	IQR	
	n	%	*n*	%	*n*	%	
Age	40.0	31.0–50.0	38.5	30.0–47.5	40.0	31.0–49.0	NS
BMI	22.8	20.7–25.4	22.8	20.9–25.4	22.8	20.4–25.4	NS
CTQ total score	34.0	29.0–44.0	29.0	27.0–34.0	39.0	31.0–49.0	**<0.001**
WHOQOL Physical health	14.3	12.0–16.6	16.6	14.9–17.9	13.1	10.9–14.9	**<0.001**
WHOQOL Psychological health	13.0	10.0–15.3	16.0	14.0–17.3	11.3	9.3–13.3	**<0.001**
WHOQOL Social relationships	12.7	9.3–14.7	14.7	13.3–17.3	10.7	8.0–13.3	**<0.001**
WHOQOL Environment	15.0	12.5–16.5	16.5	15.0–18.0	13.5	11.5–15.5	**<0.001**
WHOQOL Total score	7.0	5.0–8.0	8.0	7.0–9.0	6.0	4.0–7.0	**<0.001**
PCL Total score	17.5	5.0–35.0	5.0	1.0–11.5	26.0	13.0–41.8	**<0.001**
CESD total score	16.0	8.0–30.0	6.0	2.0–10.0	25.0	17.0–35.0	**<0.001**
STAIS total score	44.0	35.0–54.0	32.0	27.8–36.0	52.0	44.0–58.0	**<0.001**
STAIT total score	49.0	40.0–58.0	37.0	33.0–41.0	56.0	51.0–61.0	**<0.001**
LPS concentration	0.2	0.1–0.2	0.2	0.1–0.2	0.2	0.1–0.2	NS
KYN (log)	0.3	0.2–0.5	0.4	0.2–0.5	0.3	0.1–0.5	NS
5HT (log)	−0.7	−2.2–0.2	−0.5	−1.1–0.0	0.4	0.1–0.8	**<0.005**
TRP (log)	3.8	3.6–3.9	3.8	3.6–3.9	3.8	3.6–3.9	NS
KYN/TRP (log)	3.5	3.3–3.7	3.5	3.3–3.8	3.4	3.3–3.7	NS
5HT/TRP (log)	2.5	1.0–3.0	2.6	2.1–3.2	2.4	0.7–2.9	**<0.05**
Female	341	73%	110	67%	213	76%	**<0.05**
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Psychotherapeutic Response							
Unresponsive	29	9%	3	4%	26	12%	**<0.001**
Responsive	195	61%	65	77%	119	53%	
Somewhat responsive	98	30%	16	19%	78	35%	
Psychoactive medication[a]	117	44%	12	21%	100	52%	**<0.001**
Smoking status (yes)	120	26%	32	20%	79	28%	**0.050**
PTSD symptoms	73	16%	0	0%	72	26%	**<0.001**
State anxiety symptoms	256	54%	0	0%	232	83%	**<0.001**
Trait anxiety symptoms	281	60%	0	0%	281	100%	**<0.001**
Predicted periodontitis							
TeethPPD6[a]	81	41%	21	49%	56	39%	NS
SeverePerioCDCAAP[a]	106	54%	27	63%	75	52%	NS
TeethCAL5[a]	93	47%	24	56%	65	45%	NS

TeethPPD6, SeverePerioCDCAAP, TeethCAL5 data available for $n = 196$ of the total cohort, $n = 43$ of HC, and $n = 143$ of the trait anxiety symptoms cohort. Gingivitis diagnosis data available for $n = 284$ of the total cohort, $n = 66$ of HC, and $n = 204$ of the trait anxiety symptoms cohort.

5HT serotonin, *BMI* body mass index, *CESD* Center for Epidemiologic Studies Depression scale, *CTQ* childhood trauma questionnaire, *HC* healthy controls, *IBS* Irritable bowel syndrome, *IQR* interquartile range, *KYN* kynurenine, *LPS* lipopolysaccharide, *PCL-5* Posttraumatic Stress Disorder Checklist for DSM-5, *PTSD* posttraumatic stress disorder, *SeverePerioCDCAAP* Centers for Disease Control/American Academy of Periodontology (CDC/AAP) case definition, *sd* standard deviation, *STAI* state-trait anxiety inventory, *TeetchCAL5* probable severe periodontitis based on the "≥50% of teeth with clinical attachment level (CAL) ≥5 mm" criteria, *TeethPPD6* probable severe periodontitis based on the "≥25% of teeth with probing pocket depth (PPD) ≥6 mm" criteria, *TRP* tryptophan, *WHOQOL* World Health Organization Quality of Life assessment.

[a]Psychoactive medication data available for $n = 256$ of the total cohort, $n = 58$ of HC, and $n = 194$ of the trait anxiety symptoms cohort. Bold values indicate statistical significance.

Periodontal and mental health variables influence oral microbiome community composition

The Simpson alpha diversity index showed no significant differences between the mental health symptomatic groups and controls. Individuals who experienced higher levels of childhood physical neglect had lower Pielou's evenness scores ($mdn = 0.713$, IQR = 0.05 versus $mdn = 0.72$, IQR = 0.05, highest neglect quartile versus lowest) (Spearman's rank correlation test, $p = 0.03$, $rho = -0.10$, $n = 470$). Individuals with receding gums had a lower Simpson alpha diversity index ($mdn = 0.94$, IQR = 0.03 versus $mdn = 0.95$, IQR = 0.02) (Wilcoxon rank-sum tests, $p = 0.005$, $r = 0.17$, $n = 284$), and Pielou's evenness scores ($mdn = 0.70$, IQR = 0.06 versus $mdn = 0.72$, IQR = 0.05) (Wilcoxon rank-sum tests, $p = 0.02$, $r = 0.13$, $n = 284$) compared to those without.

Table 3. Comparative statistics of continuous and categorical variables for the state anxiety symptom vs. healthy control cohorts.

	Total cohort ($n = 470$)		Healthy controls ($n = 164$)		State anxiety symptoms cohort ($n = 256$)		*p*-value
	median	IQR	median	IQR	median	IQR	
	n	%	*n*	%	*n*	%	
Age	40.0	31.0–50.0	38.5	30.0–47.5	41.0	32.0–50.0	NS
BMI	22.8	20.7–25.4	22.8	20.9–25.4	22.9	20.7–25.2	NS
CTQ total score	34.0	29.0–44.0	29.0	27.0–34.0	39.0	31.0–51.0	**<0.001**
WHOQOL Physical health	14.3	12.0–16.6	16.6	14.9–17.9	12.6	10.9–14.3	**<0.001**
WHOQOL Psychological health	13.0	10.0–15.3	16.0	14.0–17.3	10.7	8.7–12.7	**<0.001**
WHOQOL Social relationships	12.7	9.3–14.7	14.7	13.3–17.3	10.7	8.0–13.3	**<0.001**
WHOQOL Environment	15.0	12.5–16.5	16.5	15.0–18.0	13.5	11.5–15.5	**<0.001**
WHOQOL Total score	7.0	5.0–8.0	8.0	7.0–9.0	6.0	4.0–7.0	**<0.001**
PCL Total score	17.5	5.0–35.0	5.0	1.0–11.5	28.0	14.0–42.0	**<0.001**
CESD total score	16.0	8.0–30.0	6.0	2.0–10.0	26.0	17.0–35.0	**<0.001**
STAIS total score	44.0	35.0–54.0	32.0	27.8–36.0	53.0	47.0–59.0	**<0.001**
STAIT total score	49.0	40.0–58.0	37.0	33.0–41.0	56.0	51.0–61.0	**<0.001**
LPS concentration	0.2	0.1–0.2	0.2	0.1–0.2	0.2	0.1–0.2	NS
KYN (log)	0.3	0.2–0.5	0.4	0.2–0.5	0.4	0.2–0.5	NS
5HT (log)	−0.7	−2.2–0.2	−0.5	−1.1–0.0	-0.5	−1.1–0.0	**<0.005**
TRP (log)	3.8	3.6–3.9	3.8	3.6–3.9	3.8	3.6–3.9	NS
KYN/TRP (log)	3.5	3.3–3.7	3.5	3.3–3.8	3.5	3.3–3.8	NS
5HT/TRP (log)	2.5	1.0–3.0	2.6	2.1–3.2	2.6	2.1–3.2	**<0.05**
Female	341	73%	110	67%	192	75%	NS
Gingivitis diagnosis[a]	30	11%	6	9%	21	11%	NS
Periodontitis diagnosis	34	7%	11	7%	18	7%	NS
IBS/Coeliac/Crohn's diagnosis	78	17%	18	11%	55	21%	**<0.05**
Psychotherapeutic Response							
Unresponsive	29	9%	3	4%	25	12%	**<0.001**
Responsive	195	61%	65	77%	105	52%	
Somewhat responsive	98	30%	16	19%	72	36%	
Psychoactive medication[a]	117	44%	12	21%	87	50%	**<0.001**
Smoking status (yes)	120	26%	32	20%	76	30%	**<0.05**
PTSD symptoms	73	16%	0	0%	68	27%	**<0.001**
State anxiety symptoms	256	54%	0	0%	256	100%	**<0.001**
Trait anxiety symptoms	281	60%	0	0%	232	91%	**<0.001**
Predicted periodontitis							
TeethPPD6[a]	81	41%	21	49%	55	41%	NS
SeverePerioCDCAAP[a]	106	54%	27	63%	70	53%	NS
TeethCAL5[a]	93	47%	24	56%	63	47%	NS

TeethPPD6, SeverePerioCDCAAP, TeethCAL5 data available for $n = 196$ of the total cohort, $n = 43$ of HC, and $n = 133$ for state anxiety symptoms cohort; Gingivitis diagnosis data available for $n = 284$ of the total cohort, $n = 66$ of HC, and n = 186 of the state anxiety symptoms cohort.
5HT serotonin, *BMI* body mass index, *CESD* Center for Epidemiologic Studies Depression scale, *CTQ* childhood trauma questionnaire, *HC* healthy controls, *IBS* Irritable bowel syndrome, *IQR* interquartile range, *KYN* kynurenine, *LPS* lipopolysaccharide, *PCL-5* Posttraumatic Stress Disorder Checklist for DSM-5, *PTSD* posttraumatic stress disorder, *SeverePerioCDCAAP* Centers for Disease Control/American Academy of Periodontology (CDC/AAP) case definition, *sd* standard deviation, *STAI* state-trait anxiety inventory, *TeetchCAL5* probable severe periodontitis based on the "≥50% of teeth with clinical attachment level (CAL) ≥5 mm" criteria, *TeethPPD6* probable severe periodontitis based on the "≥25% of teeth with probing pocket depth (PPD) ≥6 mm" criteria, *TRP* tryptophan, *WHOQOL* World Health Organization Quality of Life assessment.
[a]Psychoactive medicationdata available for $n = 256$ of the total cohort, $n = 58$ of HC, and $n = 175$ of state anxiety symptoms cohort. Bold values indicate statistical significance.

Individuals with self-reported malocclusion had lower Simpson's diversity ($mdn = 0.93$, IQR = 0.02 versus $mdn = 0.95$, IQR = 0.02) (Wilcoxon rank-sum tests, $p = 0.014$, $r = 0.18$, $n = 183$), Chao1 richness ($mdn = 122$, IQR = 19.5 versus $mdn = 129.5$, IQR = 22.25) (Wilcoxon rank-sum tests, $p = 0.005$, $r = 0.2$, $n = 183$), and Pielou's evenness scores ($mdn = 0.70$, IQR = 0.04 versus $mdn = 0.72$, IQR = 0.06) (Wilcoxon rank-sum tests, $p = 0.05$, $r = 0.15$, $n = 183$) compared to those without. Higher Chao1 richness scores were evident in those reporting bleeding and inflamed gums ($mdn =$ 132, IQR = 27 versus $mdn = 125$, IQR = 19.5) (Wilcoxon rank-sum tests, $p = 0.003$, $r = 0.17$, $n = 284$), and also for those with a clinical diagnosis of gingivitis ($mdn = 135.5$, IQR = 26.75 versus $mdn =$

Table 4. Comparative statistics of continuous and categorical variables for the PTSD symptom vs. healthy control cohorts.

	Total cohort ($n = 470$)		Healthy controls ($n = 164$)		PTSD symptoms cohort ($n = 73$)		*p*-value
	median	IQR	median	IQR	median	IQR	
	n	%	*n*	%	mean *n*	± sd %	
Age	40.0	31.0–50.0	38.5	30.0–47.5	40.6	± 12.5	NS
BMI	22.8	20.7–25.4	22.8	20.9–25.4	21.7	19.7–24.7	**<0.05**
CTQ total score	34.0	29.0–44.0	29.0	27.0–34.0	45.0	34.0–59.0	**<0.001**
WHOQOL Physical health	14.3	12.0–16.6	16.6	14.9–17.9	11.9	± 2.9	**<0.001**
WHOQOL Psychological health	13.0	10.0–15.3	16.0	14.0–17.3	10.2	± 2.9	**<0.001**
WHOQOL Social relationships	12.7	9.3–14.7	14.7	13.3–17.3	10.7	8.0–13.3	**<0.001**
WHOQOL Environment	15.0	12.5–16.5	16.5	15.0–18.0	13.0	11.0–15.5	**<0.001**
WHOQOL Total score	7.0	5.0–8.0	8.0	7.0–9.0	6.0	4.0–6.0	**<0.001**
PCL Total score	17.5	5.0–35.0	5.0	1.0–11.5	46.0	38.0–54.0	**<0.001**
CESD total score	16.0	8.0–30.0	6.0	2.0–10.0	32.0	± 2.9	**<0.001**
STAIS total score	44.0	35.0–54.0	32.0	27.8–36.0	56.0	± 2.9	**<0.001**
STAIT total score	49.0	40.0–58.0	37.0	33.0–41.0	59.0	± 2.9	**<0.001**
LPS concentration	0.2	0.1–0.2	0.2	0.1–0.2	0.2	0.1–0.2	NS
KYN (log)	0.3	0.2–0.5	0.4	0.2–0.5	0.3	0.1–0.4	NS
5HT (log)	−0.7	−2.2–0.2	−0.5	−1.1–0.0	−2.0	−2.9–0.5	**<0.001**
TRP (log)	3.8	3.6–3.9	3.8	3.6–3.9	3.7	3.6–3.8	NS
KYN/TRP (log)	3.5	3.3–3.7	3.5	3.3–3.8	3.5	3.3–3.6	NS
5HT/TRP (log)	2.5	1.0–3.0	2.6	2.1–3.2	1.3	0.2–2.8	**<0.01**
Female	341	73%	110	67%	63	86%	**<0.01**
Gingivitis diagnosis[a]	30	11%	6	9%	9	16%	NS
Periodontitis diagnosis	34	7%	11	7%	6	8%	NS
IBS/Coeliac/Crohn's diagnosis	78	17%	18	11%	15	21%	NS
Psychotherapeutic Response							
Unresponsive	29	9%	3	4%	5	8%	**<0.05**
Responsive	195	61%	65	77%	35	54%	
Somewhat responsive	98	30%	16	19%	25	38%	
Psychoactive medication[a]	117	44%	12	21%	34	62%	**<0.001**
Smoking status (yes)	120	26%	32	20%	22	30%	NS
PTSD symptoms	73	16%	0	0%	73	100%	**<0.001**
State anxiety symptoms	256	54%	0	0%	68	93%	**<0.001**
Trait anxiety symptoms	281	60%	0	0%	72	99%	**<0.001**
Predicted periodontitis							
TeethPPD6[a]	81	41%	21	49%	14	36%	NS
SeverePerioCDCAAP[a]	106	54%	27	63%	21	54%	NS
TeethCAL5[a]	93	47%	24	56%	19	49%	NS

TeethPPD6 SeverePerioCDCAAP, TeethCAL5 data available for $n = 196$ of the total cohort, $n = 43$ of HC, and, n = 39 of the PTSD symptoms cohort; Gingivitis diagnosis data available for $n = 284$ of the total cohort, $n = 66$ of HC, and $n = 57$ of the PTSD cohort

5HT serotonin, *BMI* body mass index, *CESD* Center for Epidemiologic Studies Depression scale, *CTQ* childhood trauma questionnaire, *HC* healthy controls, *IBS* Irritable bowel syndrome, *IQR* interquartile range, *KYN* kynurenine, *LPS* lipopolysaccharide, *PCL-5* Posttraumatic Stress Disorder Checklist for DSM-5, *PTSD* posttraumatic stress disorder, *SeverePerioCDCAAP* Centers for Disease Control/American Academy of Periodontology (CDC/AAP) case definition, *sd* standard deviation, *STAI* state-trait anxiety inventory, *TeetchCAL5* probable severe periodontitis based on the "≥50% of teeth with clinical attachment level (CAL) ≥5 mm" criteria, *TeethPPD6* probable severe periodontitis based on the "≥25% of teeth with probing pocket depth (PPD) ≥6 mm" criteria, *TRP* tryptophan, *WHOQOL* World Health Organization Quality of Life assessment.

[a]Psychoactive medication data available for $n = 256$ of the total cohort, $n = 58$ of the HC, and $n = 55$ of the PTSD symptoms cohort. Bold values indicate statistical significance.

128, IQR = 21.75) (Wilcoxon rank-sum tests, $p = 0.013$, $r = 0.15$, $n = 284$).

Several variables influenced the overall oral microbial composition (β-diversity), with smoking status eliciting the largest effect, followed by age, living environment (city, town, or rural setting), and alveolar bone loss. The most significant subset ($R^2 \geq 0.002$ and $q \leq 0.1$) of variables is illustrated in Fig. 1 (Supplementary Table 1 contains the full set of significant variables).

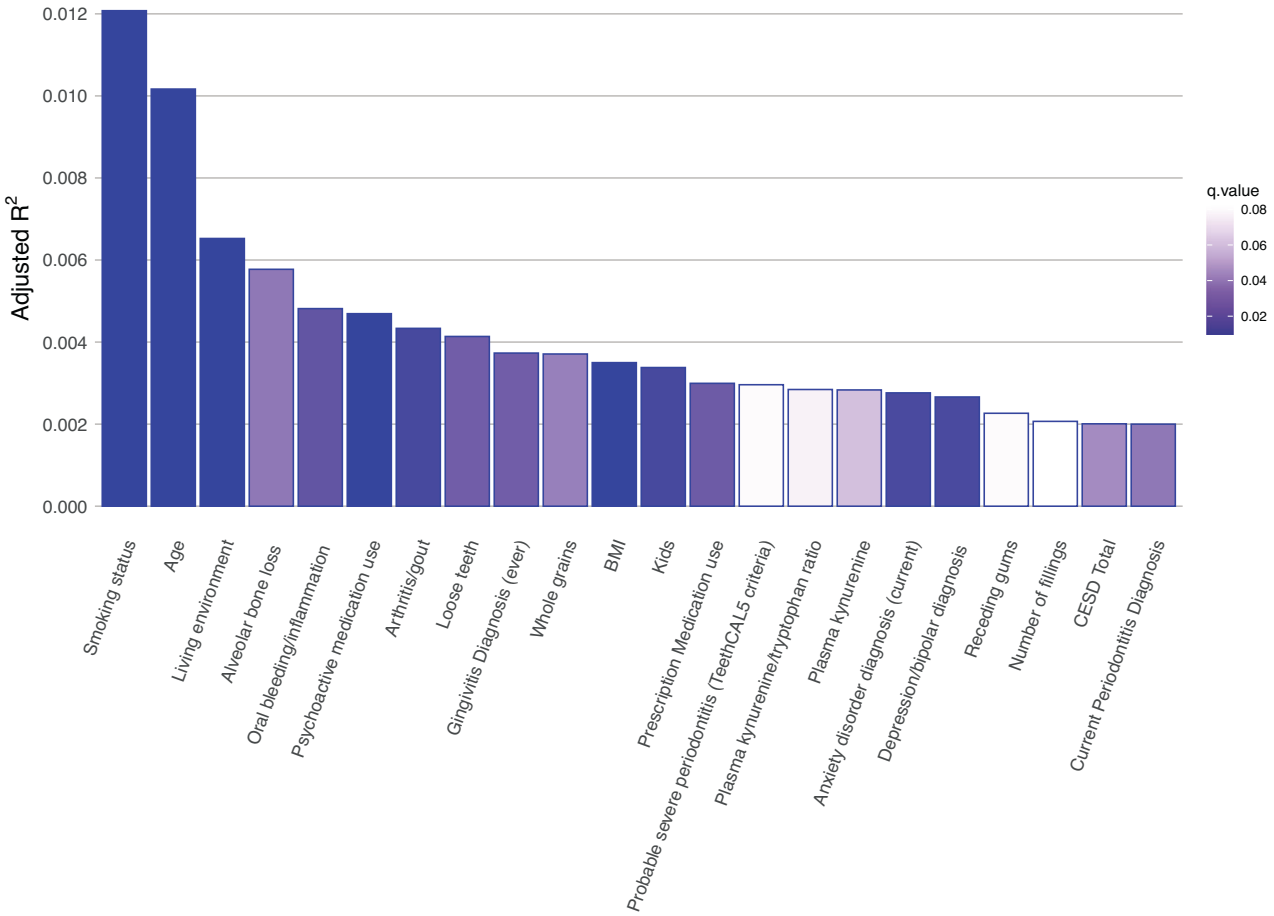

Fig. 1 Effect sizes of variables that had a statistically significant effect on the oral microbiome community variation (distance-based redundancy analysis (dbRDA) on genus-level Aitchison distance) in our cohort ($n = 470$). Color intensity is proportional to the *q*-values (False Discovery Rate (FDR) corrected *p*-values); adjusted R^2 effect sizes are indicated on the y-axis. Certain variables were not available for the entire cohort: gingivitis diagnosis (ever), oral bleeding or inflammation, loose teeth, consumption of dietary whole grains ($n = 284$); alveolar bone loss ($n = 184$), psychoactive medication use ($n = 265$), and having arthritis or gout ($n = 282$). BMI - body mass index, CESD - Center for Epidemiologic Studies Depression, Whole grains – weekly whole grain consumption, TeetchCAL5 criteria - probable severe periodontitis based on the "≥50% of teeth with clinical attachment level (CAL) ≥ 5 mm" criteria.

Oral taxa associated with trauma, mental health outcomes, and psychological quality of life

Our symptomatic cohort reported significantly higher levels of childhood trauma compared to controls. Individuals who reported high levels of emotional neglect had significantly higher relative abundance (clr-transformed) of *Streptococcus mutans* ($mdn = -2.52$, IQR = 2.12) compared to those with low levels or no emotional neglect ($mdn = -2.68$, IQR = 0.8) (GLM $q = 0.07$, $\beta = -0.6$, $n = 470$) (Fig. 2a). Individuals with PTSD symptoms ($n = 73$) had significantly lower clr-transformed relative abundance of *Haemophilus sputorum* (*H. sputorum*) ($mdn = -1.92$, IQR = 3.07 versus $mdn = 0.62$, IQR = 3.94) (GLM, $q = 0.09$, $\beta = -1.2$, $n = 237$) and higher clr-transformed relative abundance of *Prevotella histicola* (*P. histicola*) (mdn = −2.92, SD = 3.07, IQR = 5.80 versus mdn = −3.25, SD = 2.38, IQR = 3.46) (GLM, $q = 0.09$, $\beta = 1.6$, $n = 237$), compared to mentally healthy controls ($n = 164$) (Fig. 2b).

Those with a current anxiety disorder diagnosis ($n = 134$) harbored significantly lower clr-transformed relative abundance of *Neisseria* ($mdn = 3.71$, IQR = 2.15 versus $mdn = 4.28$, IQR = 1.74) (GLM, $q = 0.001$, $\beta = -0.96$, $n = 470$), specifically *Neisseria elongate* (*N. elongate*) ($mdn = -0.52$, IQR = 4.74 versus $mdn = 0.39$, IQR = 2.94) (GLM, $q = 0.09$, $\beta = -0.94$, $n = 470$) and significantly higher clr-transformed relative abundance of *Oribacterium asaccharolyticum* (*O. asaccharolyticum*) ($mdn = 0.73$, IQR = 1.52 versus $mdn = 0.25$, IQR = 2.19) (GLM $q = 0.03$, $\beta = 0.53$, $n = 470$) compared to those without a current diagnosis ($n = 336$) (Fig. 2c). Interestingly, higher relative abundance (clr-transformed) of *P. histicola* was also evident in individuals with higher CESD depressive scores ($mdn = -3.17$, IQR = 4.68) (GLM $q = 0.05$, $\beta = 0.04$, $n = 470$) (Fig. 2d) and those with poor psychological quality of life scores ($mdn = -3.17$, IQR = 4.68) (GLM $q = 0.08$, $\beta = -0.2$, $n = 470$) (Fig. 2e). Individuals with higher CESD scores also had a higher clr-transformed relative abundance of *Lancefieldella* ($mdn = 1.98$, IQR = 1.34) (GLM $q = 0.05$, $\beta = 0.01$, $n = 470$) and *O. asaccharolyticum* ($mdn = 0.37$, IQR = 2.01) (GLM $q = 0.05$, $\beta = 0.02$, $n = 470$), however, the effect sizes were relatively small. Supplementary Table 2 contains all statistical results and Fig. 2f provides a graphical summary.

Oral microbiome signatures related to periodontal health

Several oral taxa were associated with periodontal health variables (Fig. 3, Supplementary Table 3). The clr-transformed relative abundance of *Shuttleworthia* was higher in participants with a self-reported periodontitis diagnosis and those with predicted severe periodontitis based on the TeethCAL5 criteria. The clr-transformed relative abundance of *Capnocytophaga* was lower in participants with a self-reported clinical periodontitis diagnosis and those with predicted severe periodontitis based on the TeethPPD6 criteria. Several common taxa were differentially

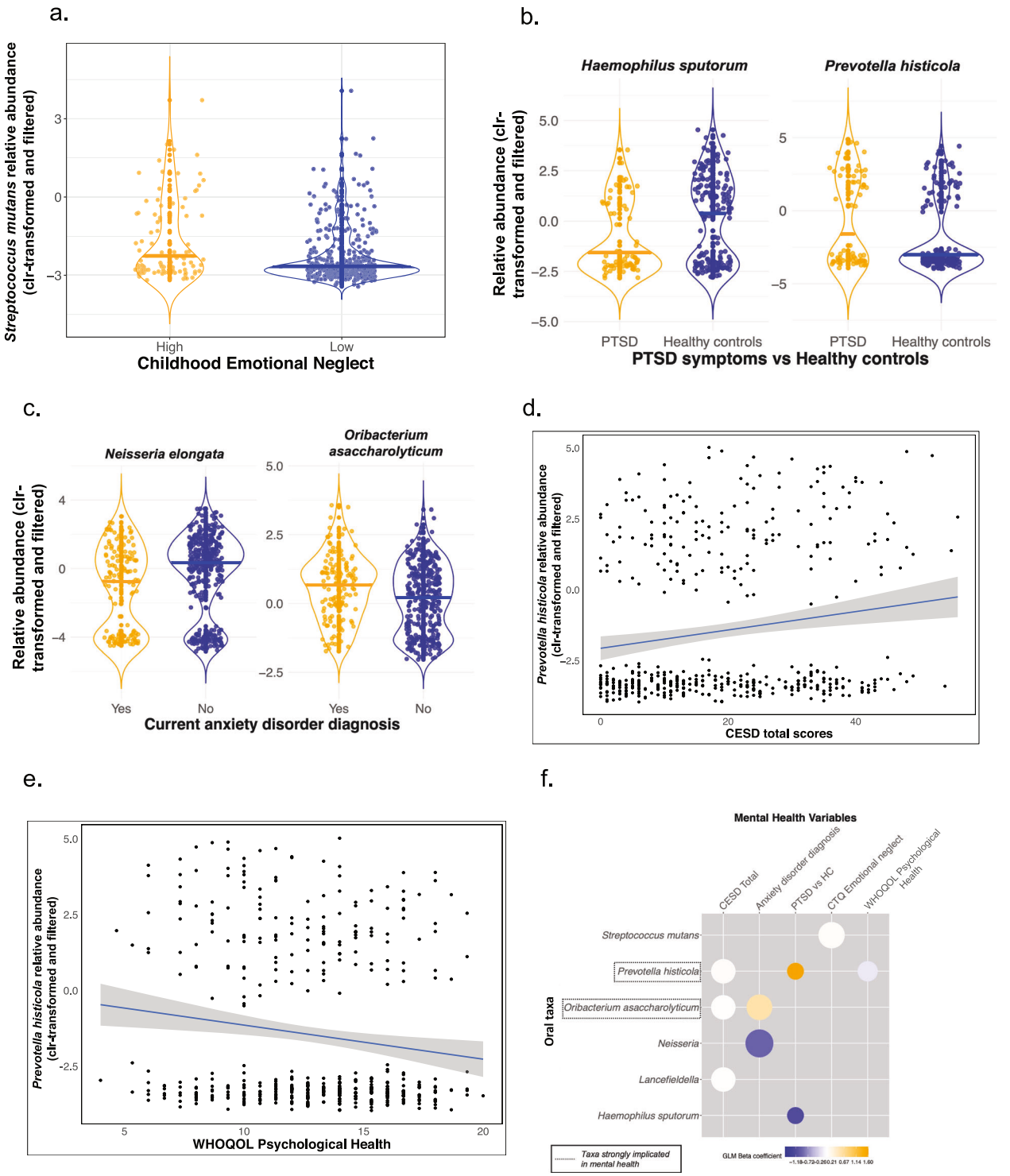

Fig. 2 The relative abundance of oral taxa associated with mental health outcomes, trauma, and well-being. a Higher relative abundance of *S. mutans* in individuals who experienced childhood emotional neglect. **b** Lower relative abundance of *H. sputorum* and higher relative abundance of *P. histicola* in individuals with symptoms of PTSD compared to controls. **c** Individuals with a current anxiety disorder diagnosis had lower levels of *N. elongata* and higher levels of *O. asaccharolyticum*. **d** The relative abundance of *P. histicola* was positively associated with CESD depressive scores and (**e**) negatively associated with World Health Organization Quality Of Life (WHOQOL) scores for domain 2. **f** Summary graphic highlighting common taxa associated with mental health outcomes, trauma, and well-being (positive associations are indicated in yellow tones, negative associations in blue tones, color intensity is proportional to standardized GLM β coefficients, and point size is proportional to the *q*-values (FDR corrected *p*-values)). Horizontal lines on the violin plots indicate the median and the thicker part of the violin around the median represents the interquartile range (IQR). Significance $q \leq 0.1$ (only statistically significant taxa are illustrated). Relative abundance is the clr-transformed and filtered relative abundance values. *H. sputorum* - *Haemophilus sputorum*, *P. histicola* - *Prevotella histicola*, *N. elongata* - *Neisseria elongate*, *O. asaccharolyticum* - *Oribacterium asaccharolyticum*, CESD - Center for Epidemiologic Studies Depression, PTSD - posttraumatic stress disorder, GLM - generalized linear model.

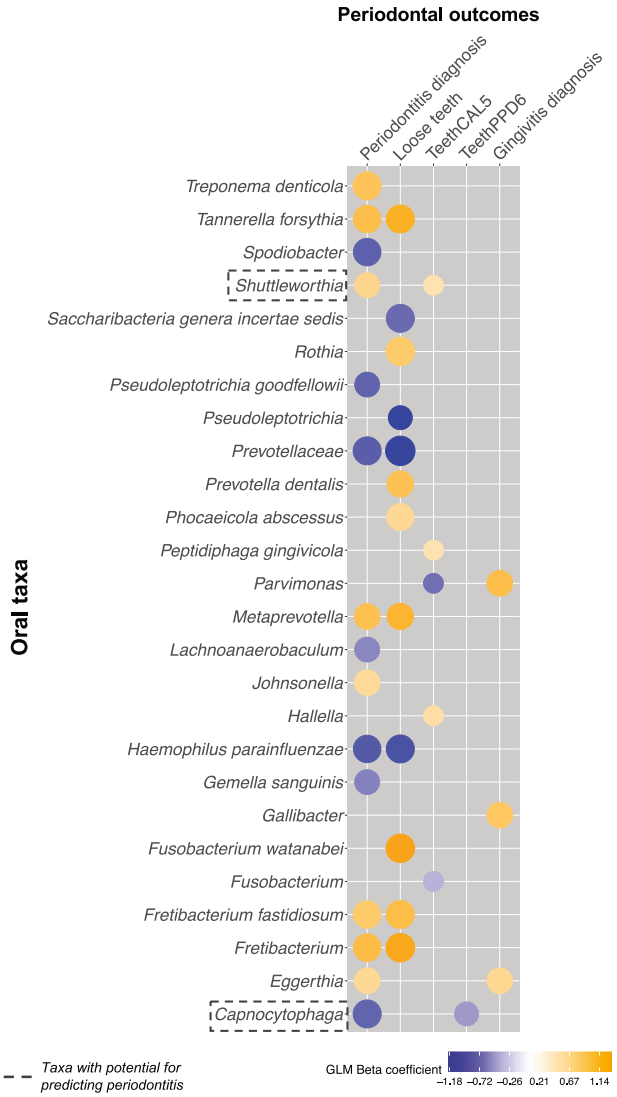

Fig. 3 Summary graphic highlighting common taxa associated with periodontal outcomes (self-reported clinical diagnosis of current periodontitis/gingivitis available for all participants, $n = 470$; questionnaire data to predict severe periodontitis available for $n = 196$ [48%] of the participants). Positive associations are in shades of yellow, negative associations in shades of blue, and color intensity is proportional to standardized GLM β coefficients, and point size is proportional to the *q*-values (FDR corrected *p*-values). Periodontitis diagnosis ($n = 34$, 7%) – participants reported having a current clinical diagnosis of periodontitis; Gingivitis diagnosis ($n = 30$, 11%) – participants reported having a current clinical diagnosis of gingivitis; TeetchCAL5 - probable severe periodontitis based on the "≥50% of teeth with clinical attachment level (CAL) ≥ 5 mm" criteria ($n = 93$, 47%); TeethPPD6 - probable severe periodontitis based on the "≥25% of teeth with probing pocket depth (PPD) ≥ 6 mm" criteria ($n = 81$, 41.3%).

abundant in those with a self-reported clinical periodontitis diagnosis and those who reported loose teeth (a symptom of periodontitis), including a higher clr-transformed relative abundance of *Tannerella forsythia*, *Metaprevotella*, *Fretibacterium fastidiosum*, and lower clr-transformed relative abundance of *Prevotellaceae* and *Haemophilus parainfluenzae*. Three taxa had a higher relative abundance (clr-transformed) in participants with a self-reported clinical gingivitis diagnosis, *Parvimonas*, *Gallibacter*, and *Eggerthia*; *Eggerthia* was the only common taxon between periodontitis and gingivitis diagnosis, whose clr-transformed relative abundance was higher in both diagnoses.

Functional potential of the oral microbiome: possible implications for mental health outcomes

Functional prediction revealed lower tryptophan metabolism/degradation in individuals with PTSD symptoms ($mdn = -0.68$, IQR = 1.98 versus $mdn = -0.42$, IQR = 2.17) (GLM, $q = 0.02$, $\beta = -0.8$, $n = 470$), those who experienced higher levels of childhood trauma ($mdn = -0.35$, IQR = 2.22) (GLM, $q = 0.09$, $\beta = -0.01$, $n = 470$), and those with lower quality of life relating to personal relationships) ($mdn = -0.35$, IQR = 2.22) (GLM, $q = 0.06$, $\beta = 0.06$, $n = 470$). Supplementary Table 4 contains all statistical results for predicted GBMs associated with periodontal variables. Interestingly, lower metabolism/degradation of tryptophan was also predicted in individuals with predicted severe periodontitis based on the TeethCAL5 criteria ($n = 93$) ($mdn = -0.40$, IQR = 1.70 versus $mdn = -0.01$, IQR = 1.29) (GLM, $q = 0.07$, $\beta = -0.52$, $n = 196$) and TeethPPD6 criteria ($n = 81$) ($mdn = -0.40$, IQR = 1.67 versus $mdn = -0.02$, IQR = 1.37) (GLM, $q = 0.02$, $\beta = -0.6$, $n = 196$,). Supplementary Table 5 contains all statistical results for GBMs associated with periodontal variables and Fig. 4 illustrates the full set of predicted GBMs linked to severe periodontitis, mental health, childhood trauma, and quality of life.

Plasma measures

Analyses to confirm lower TRP metabolism/degradation revealed lower plasma levels of 5-HT and the ratio of 5-HT/TRP in individuals with depressive symptoms (Wilcoxon rank-sum test, $p < 0.01$, mean difference (MD) = 0.8, and $p < 0.01$, MD = 0.9, $n = 282$ respectively), state anxiety symptoms ($p < 0.01$, MD = 0.6, and $p < 0.01$, MD = 0.5, $n = 282$, respectively), trait anxiety symptoms ($p < 0.01$, MD = 0.6, and $p < 0.01$, MD = 0.6, $n = 282$ respectively), and PTSD symptoms ($p < 0.01$, MD = 1.0, and $p < 0.01$, MD = 0.9, $n = 282$ respectively) compared to healthy controls (Fig. 5).

Oral microbes and therapeutic response

Interestingly, two taxa associated with a current self-reported clinical diagnosis of periodontitis and/or gingivitis were also associated with self-reported efficacy of psychotherapy, namely *Eggerthia* and *Haemophilus parainfluenza*. *Eggerthia* was present at a higher relative abundance (clr-transformed) in those with a current self-reported clinical diagnosis of periodontitis and/or gingivitis and in individuals with poor self-reported psychotherapeutic efficacy ($n = 29$) (GLM $q = 0.12$, $r = -0.62$, $n = 322$), whereas the clr-transformed relative abundance of *H. parainfluenza* was lower in these individuals (GLM $q = 0.12$, $r = 0.60$, $n = 322$), although the association did not reach the threshold for statistical significance of $q \leq 0.1$.

DISCUSSION

This study represents one of the largest oral microbiome investigations in mental health, to date. The composition of the overall oral microbiome was significantly impacted by several mental health variables (including clinical diagnoses of anxiety disorders or depression), as well as periodontal symptoms, predicted severe periodontitis, and self-reported clinical diagnoses of gingivitis and/or periodontitis. These findings correlate with previous research highlighting the significant effects of mental [32] and periodontal [60, 61] health on the oral microbiome beta diversity. Various additional factors shaped the oral microbiome composition, aligning with earlier research emphasizing the impact of factors such as smoking [62], BMI [63], age [60], arthritis [64], gout [65], and geographic location [66] on the oral microbiome.

None of the mental health variables or self-reported periodontal outcomes influenced Simpson's diversity. Earlier studies also failed to detect differences in alpha diversity between individuals with depression and anxiety compared to controls [31]. We detected

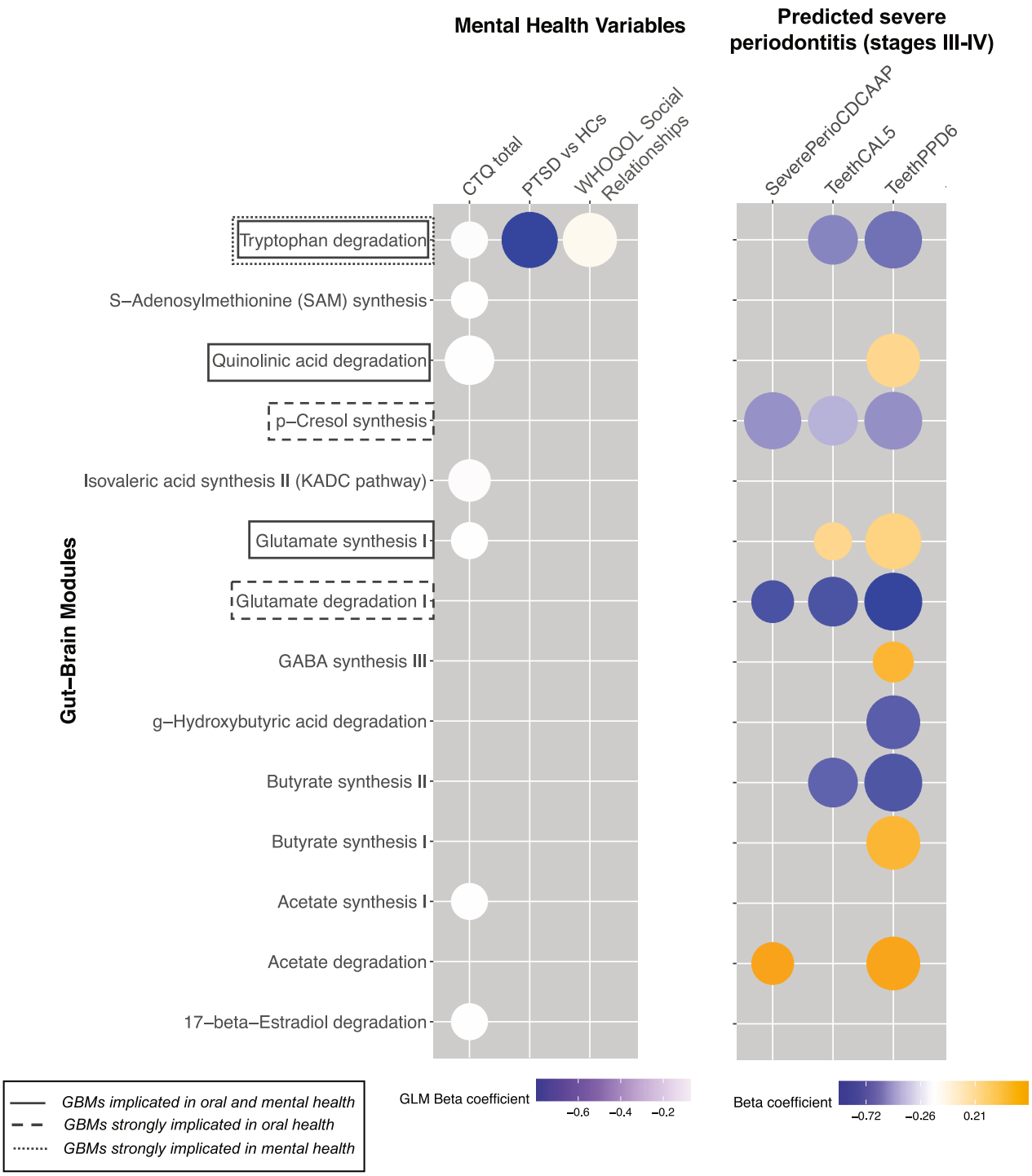

Fig. 4 Shared (solid line boxes) and unique (dotted line boxes) gut-brain modules (GBMs) associated with predicted severe periodontitis (stages III-IV) as well as mental health, childhood trauma, and quality of life variables. Positive correlations are displayed in yellow tones and negative correlations in blue tones (white tones indicate values close to zero). Color intensity is proportional to standardized GLM β coefficients, and point size is proportional to the *q*-values (FDR corrected *p*-values).

lower Pielou's evenness scores in individuals who experienced higher levels of childhood physical neglect. One study reported no significant difference between Simpson and Shannon diversity indices of the oral microbiome among those who experienced early life trauma [67], but these diversity metrics evaluate richness and evenness, which could explain the discrepant results.

Lower diversity was evident among individuals with self-reported receding gums (Simpson's diversity and Pielou's evenness), and malocclusion (Simpson's diversity, Chao1 richness, and Pielou's evenness), whilst higher Chao1 richness scores were noted in those reporting bleeding and inflamed gums and a reported clinical diagnosis of gingivitis. Receding gums could be a symptom of periodontitis, and bleeding and inflamed gums are the main symptoms of gingivitis. Alpha diversity findings in periodontitis patients have shown contradictory results, with some studies reporting no differences between periodontitis patients

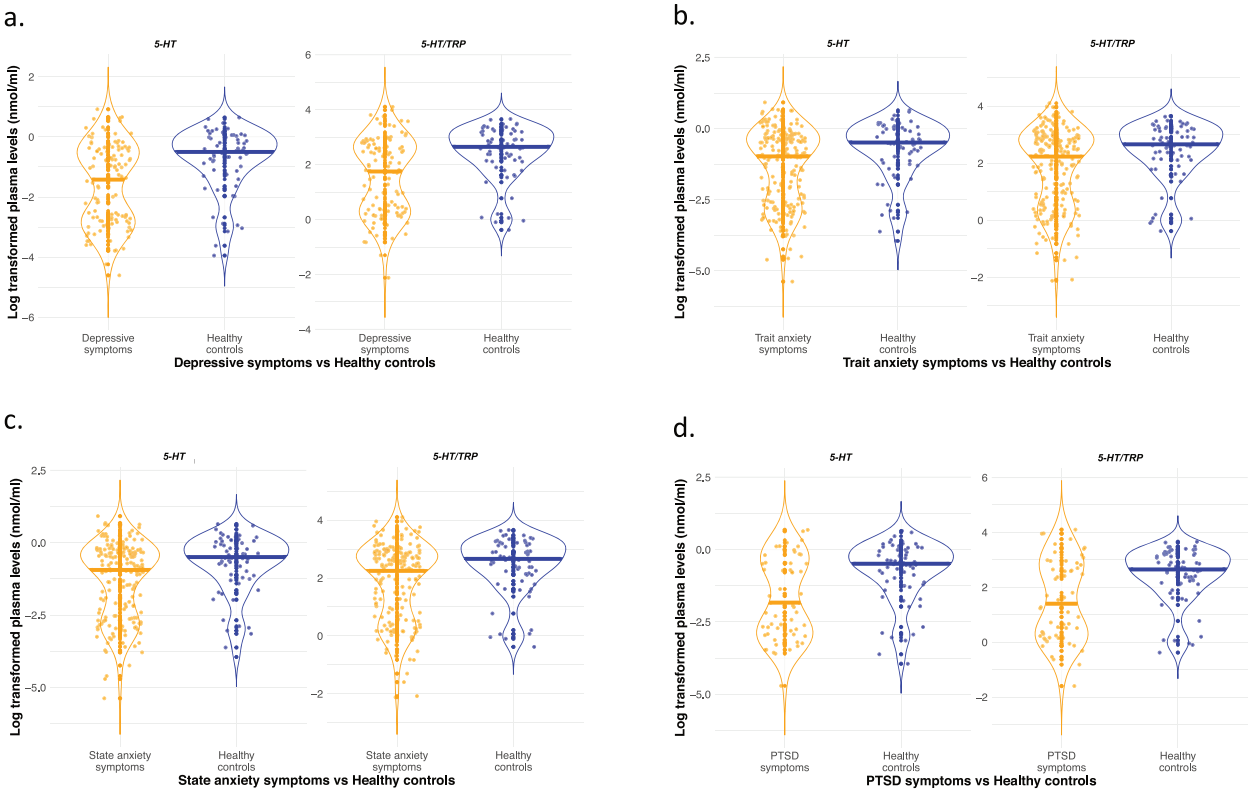

Fig. 5 Log transformed plasma levels (nmol/ml) of serotonin (5-HT) and the ratio of serotonin/tryptophan (5-HT/TRP) in participants with self-reported mental health symptoms. Lower plasma levels of 5-HT and 5-HT/TRP in participants with (**a**) depressive, (**b**) trait anxiety, (**c**) state anxiety, and (**d**) PTSD symptoms had lower plasma levels of 5-HT and 5-HT/TRP compared to healthy controls. Horizontal lines on the violin plots indicate the median, and the thicker part of the violin around the median represents the interquartile range (IQR). Significance $p < 0.01$. PTSD posttraumatic stress disorder.

and controls [61], and others noting higher [68] or lower diversity [69] in periodontitis patients. The latter study reported the loss of diversity in oral microbiota between healthy individuals, patients with stable periodontitis, or patients with progressing periodontitis, reporting a strong association between lower alpha diversity in the oral microbiota and the progression of periodontitis, illustrating the potential of using oral microbial alpha diversity as a predictor of periodontitis [69]. These conflicting results require further investigation, particularly through studies involving large cohorts or meta-analyses of combined cohorts. Other studies have also reported higher alpha diversity (Chao1 and Shannon indices) in gingivitis patients compared to controls [70]. Concerning malocclusion, one recent study also reported lower Simpson's diversity and Chao1 richness in individuals with malocclusion undergoing orthodontic treatment [71].

Several taxa were associated with mental health, trauma, and well-being. Due to the limited data currently available for the oral microbiome and mental health outcomes, we will discuss our findings in the context of the current literature available on the oral microbiome in mental health disorders. We recognize that mental health disorders are distinct from one another, although some share common symptoms. Precisely because of this, identifying commonalities in the abundance of oral taxa across different disorders could provide further insights into the transdiagnostic role of these taxa in mental health symptoms.

P. histicola is of particular interest, with a higher clr-transformed relative abundance in individuals with PTSD symptoms, those with higher CESD scores, and those with poorer interpersonal quality of life. *Prevotella* is the second most common bacteria dominating the oral cavity and this diverse genus includes several species. *P. histicola* is a facultative oral pathogen, which can cause pathologies such as caries and periodontitis [72, 73]. Although no data is currently available on the relationship between *Prevotella* species and PTSD or depression, a negative association was previously reported between the relative abundance of *Prevotella* and distress [74]. Importantly, *Prevotella* is a genus strongly associated with waking samples, and the majority of our samples were collected close to waking, which could explain the discrepancies between the findings. Furthermore, the majority of studies report on genus level, whereas our finding is for the species *P. histicola*, and research has shown that species from the same genus could fulfill vastly different roles [75, 76], therefore future studies should aim for higher resolution of taxonomic classification to disentangle taxonomic functions and disease associations.

We noted a lower clr-transformed relative abundance of *N. elongata* in individuals with a clinical diagnosis of anxiety disorders. Although no literature is available regarding the association of this taxon and anxiety disorders, similar observations were made in patients with schizophrenia and mania [77], whilst research in young adults linked depression with a higher relative abundance of *Neisseria spp* [32]. *Neisseria* species, integral members of the oropharyngeal flora [78], play crucial roles in maintaining oral [79] and cardiovascular health [79, 80]. Their presence correlates with good oral health, attributed to their aerobic, nitrite-reducing capabilities essential for gum health [79–81]. Moreover, *Neisseria*-dominated oral microbiomes exhibit a reduced likelihood of hosting the cariogenic pathogen *S. mutans* [82]. Notably, we detected a higher clr-transformed relative abundance of *S. mutans* in individuals reporting childhood emotional neglect, a known risk factor for mental health disorders. These findings underscore the intricate interplay between oral microbial composition, mental health outcomes, and early life

adversity. The involvement of cross-feeding and interactions among microbial taxa adds complexity to understanding comorbidity and risk factors in mental health conditions.

Individuals with an anxiety disorder diagnosis and higher CESD scores harbored a higher clr-transformed relative abundance of *O. asaccharolyticum*. This correlates with the higher relative abundance reported in elderly people receiving treatment for anxiety, depression, and insomnia [83]. Furthermore, a higher relative abundance of the *Odoribacter* genus was also noted in the gut microbiomes of patients with depression [84], a preclinical model of depression [85], and individuals with periodontal disease [86]. A higher relative abundance of the *Odoribacter* genus was also detected in the oral microbiome of periodontitis patients [87]. These findings underscore the potential interconnectivity between oral and gut microbiomes and taxa implicated in both mental and periodontal health, with implications for the oral-gut-brain axis. Furthermore, in a randomized, double-blind placebo-controlled trial, synbiotics reduced both systemic inflammation and systemic lupus erythematosus disease activity, whilst simultaneously also depleting *O. asaccharolyticum* from the microbiome [88], suggesting pathogenicity and a potential therapeutic target to facilitate anti-inflammatory effects, which warrant further investigation.

Participants with PTSD symptoms had a lower clr-transformed relative abundance of *H. sputorum*, correlating with findings in young adults with depression [32]. *Haemophilus* is a nitrate-reducing genus, and therefore, higher abundances are associated with good oral health. This taxon is also depleted in the oral and gut microbiomes of individuals with rheumatoid arthritis (RA), which also correlated with higher levels of serum autoantibodies [89], suggesting a potential involvement in autoimmunity and inflammation. Interestingly, PTSD is associated with RA, with female PTSD patients having a 76% higher risk of developing RA [90]. Levels of *Haemophilus* in the oral and gut microbiomes could therefore be involved in this comorbidity, possibly via its immunomodulatory effects.

The clr-transformed relative abundance of several taxa was associated with periodontal outcomes; *Shuttleworthia* and *Capnocytophaga* were associated with a self-reported clinical diagnosis of periodontitis and predicted severe periodontitis. Previous studies reported similar findings in periodontitis [87, 91] and gingivitis patients [92]. *Shuttleworthia* and *Capnocytophaga* should therefore be investigated as potential non-invasive, salivary microbiome markers of periodontitis.

Eggerthia was more prevalent in those with a periodontitis/gingivitis diagnosis, correlating with previous reports in periodontitis [93], and gingivitis patients [70]. These findings suggest that salivary levels of *Eggerthia* should be investigated as an early, non-invasive indicator of periodontal health problems. We however did not detect differences in the clr-transformed relative abundance of certain keystone periodontitis-associated taxa, including *Porphyromonas gingivalis*, which could be attributed to analyzing saliva samples and not periodontal pocket samples [94].

While no shared oral taxa were associated with both mental and periodontal health, we found a common functional pathway: metabolism/degradation of TRP. This pathway was diminished in individuals with PTSD symptoms, those who experienced childhood trauma, those with poor interpersonal quality of life, and those with predicted periodontitis. Decreased degradation of TRP through the 5-HT pathway could result in lower 5-HT levels and higher TRP levels. Our data revealed reduced plasma levels of 5-HT and 5-HT/TRP ratios in all symptomatic groups compared to healthy controls. Decreased 5-HT/TRP ratios may result from lower 5-HT levels and increased TRP levels. Directly measuring 5-HT levels in plasma can be challenging due to factors like its short half-life and difficulties in accurately measuring relatively low 5-HT plasma levels [95]. Therefore, the 5-HT/TRP ratio allows for an indirect assessment of serotonin synthesis capacity, which may indicate alterations in serotonin function in the CNS [96, 97].

Lower levels of 5-HT align with previous research on depression [98] and PTSD [99]. Serotonin-mediated neurotransmission is implicated in anxiety disorders, although its relationship is complex due to the diversity of anxiety disorders [100]. Serotonin is crucial for CNS development and function [101, 102], yet it also affects oral health. Psychotropic drugs like selective serotonin reuptake inhibitors (SSRIs) can reduce the salivary flow rate and cause xerostomia (dry mouth) [103], affecting oral cleansing and tooth decay prevention [104]. While our study didn't find statistically significant differences in 5-HT levels in those with clinical diagnosis or predicted severe periodontitis, altered serotonin levels could influence oral health.

Although serotonin is vital for mental health, most of its production (~90%) occurs in the gastrointestinal tract [105], influencing various physiological processes beyond the CNS, including colonic motility [106]. While our findings suggest potential oral microbiota involvement in TRP metabolism and systemic serotonin levels, systemic levels don't directly reflect CNS levels due to the BBB. Psychotropic medications, like SSRIs, may have affected CNS serotonin levels in this cohort. Nevertheless, our study underscores the importance of the serotonergic system in mental and oral health, suggesting avenues for further research into oral microbiota, TRP metabolism, and serotonin production interplay. Understanding these relationships could lead to novel therapeutic approaches for mental health disorders associated with serotonin dysregulation.

Identifying treatment response markers is crucial to lighten the burden of disease and enhance treatment efficacy. Although oral taxa were not associated with psychoactive medication use, a higher clr-transformed relative abundance of *Eggerthia* and a lower clr-transformed relative abundance of *H. parainfluenza* was evident in individuals reporting poor psychotherapeutic efficacy and those with a self-reported periodontitis diagnosis, hinting at a potential effect of oral health on treatment efficacy. These associations narrowly missed statistical significance, and warrants further investigation. Data on oral microbiome and treatment response associations are limited, however, research suggests a causal effect of elevated levels of *Eggerthia* on anxiety and depression [29]; links between lower levels of *H. parainfluenza* and generalized anxiety disorder [107], and higher levels of this taxon in individuals with periodontitis + IBS [108]. These taxa are good candidates to explore in future longitudinal treatment outcome studies, especially in patients with periodontal health problems.

Limitations of this study include the absence of clinical assessments of anxiety, depression, PTSD, periodontitis, and gingivitis. Instead, validated questionnaires were used to assess symptoms, in addition, participants reported current diagnoses of any of these disorders/conditions. Mental health disorders are complex, with varying symptom presentations even among individuals diagnosed with the same disorder. Understanding these disorders in this context is crucial. Additionally, diagnoses and treatment strategies are informed by symptoms rather than rigid diagnostic criteria, with associations with biological markers often correlating more strongly with symptoms [109]. Further oral microbiome studies in well-defined clinical samples are warranted to compare findings to self-reported symptom cohorts.

Our analyses accounted for several covariates that showed associations with clr-transformed relative taxonomic abundance in our cohort. Although we tested an extensive list of potential confounders, there may be additional confounders that we were unable to test and incorporate into our models. Furthermore, it is important to be cautious of overfitting the models by including too many covariates, as this can reduce the ability to detect significant associations [110].

Although species-level assignment was possible for several taxa, it should be noted that species-level identification is less reliable for targeted 16S rRNA sequencing compared to full-length 16S rRNA sequencing or shotgun metagenomic sequencing.

Different oral niches harbor distinct microbiomes. This study investigated self-collected saliva samples as a proxy for the oral microbiome. Samples from the periodontal pocket would be ideal for studying the microbiome related to periodontitis. However, the aim of this study was mental health outcomes whilst considering self-reported periodontal health outcomes. Furthermore, studies have shown that saliva samples were the most stable within-subjects (temporal) as well as between-subjects [111]. Lastly, our cross-sectional study can only report on microbial associations with symptomology; future longitudinal studies are needed to infer causality between the oral microbiome and mental health symptoms.

This study reveals a compelling connection between the composition of oral microbiota, mental health conditions, early life experiences, as well as periodontal outcomes. By identifying taxa and functional pathways potentially involved in both mental and oral health, our findings contribute to the growing body of evidence linking oral microbiota to mental and oral health outcomes. These results suggest a complex interplay between microbial composition, systemic neuromodulators, and health outcomes, and highlight potential avenues for further research. Understanding these relationships offers promising avenues for integrated approaches to promote oral and psychological resilience, emphasizing the importance of considering both oral and mental health within a holistic framework of care.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, [S Malan-Müller], upon reasonable request. The sequencing data have been deposited with links to BioProject accession number PRJNA1162741 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

REFERENCES

1. Arias D, Saxena S, Verguet S. Quantifying the global burden of mental disorders and their economic value. eClinicalMedicine. 2022. https://doi.org/10.1016/j.eclinm.2022.101675.
2. Friedrich MJ. Depression is the leading cause of disability around the world. JAMA. 2017;317:1517.
3. Depression and Other Common Mental Disorders. https://www.who.int/publications-detail-redirect/depression-global-health-estimates. Accessed 23 Feb 2024.
4. Santomauro DF, Herrera AMM, Shadid J, Zheng P, Ashbaugh C, Pigott DM, et al. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. Lancet. 2021;398:1700–12.
5. Hidaka BH. Depression as a disease of modernity: explanations for increasing prevalence. J Affect Disord. 2012;140:205–14.
6. Sonnenburg JL, Sonnenburg ED. Vulnerability of the industrialized microbiota. Science. 2019;366:eaaw9255.
7. Johnston KM, Powell LC, Anderson IM, Szabo S, Cline S. The burden of treatment-resistant depression: a systematic review of the economic and quality of life literature. J Affect Disord. 2019;242:195–210.
8. Taylor S, Abramowitz JS, McKay D. Non-adherence and non-response in the treatment of anxiety disorders. J Anxiety Disord. 2012;26:583–9.
9. Cooper C, Bebbington P, King M, Brugha T, Meltzer H, Bhugra D, et al. Why people do not take their psychotropic drugs as prescribed: results of the 2000 National Psychiatric Morbidity Survey. Acta Psychiatr Scand. 2007;116:47–53.
10. Salvucci E. Microbiome, holobiont and the net of life. Crit Rev Microbiol. 2016;42:485–94.
11. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Curr Opin Gastroenterol. 2015;31:69–75.
12. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. Neurogastroenterol Motil. 2012;24:405–13.
13. Martín-Hernández D, Caso JR, Bris ÁG, Maus SR, Madrigal JLM, García-Bueno B, et al. Bacterial translocation affects intracellular neuroinflammatory pathways in a depression-like model in rats. Neuropharmacology. 2016;103:122–33.
14. Leira Y, Domínguez C, Seoane J, Seoane-Romero J, Pías-Peleteiro JM, Takkouche B, et al. Is periodontal disease associated with Alzheimer's disease? A systematic review with meta-analysis. Neuroepidemiology. 2017;48:21–31.
15. GBD 2017 Oral Disorders Collaborators, Bernabe E, Marcenes W, Hernandez CR, Bailey J, Abreu LG, Alipour V, et al. Global, regional, and national levels and trends in burden of oral conditions from 1990 to 2017: a systematic analysis for the global burden of disease 2017 study. J Dent Res. 2020;99:362–73.
16. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15:30–44.
17. Velsko IM, Chukkapalli SS, Rivera MF, Lee J-Y, Chen H, Zheng D, et al. Active invasion of oral and aortic tissues by porphyromonas gingivalis in mice causally links periodontitis and atherosclerosis. PLoS One. 2014. https://doi.org/10.1371/journal.pone.0097811.
18. Sanz M, Marco Del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and cardiovascular diseases: consensus report. J Clin Periodontol. 2020;47:268–88.
19. Hashioka S, Inoue K, Miyaoka T, Hayashida M, Wake R, Oh-Nishi A, et al. The possible causal link of periodontitis to neuropsychiatric disorders: more than psychosocial mechanisms. Int J Mol Sci. 2019. https://doi.org/10.3390/ijms20153723.
20. Hsu C-C, Hsu Y-C, Chen H-J, Lin C-C, Chang K-H, Lee C-Y, et al. Association of periodontitis and subsequent depression: a nationwide population-based study. Medicine (Baltimore). 2015;94:e2347.
21. Martínez M, Postolache TT, García-Bueno B, Leza JC, Figuero E, Lowry CA, et al. The role of the oral microbiota related to periodontal diseases in anxiety, mood and trauma- and stress-related disorders. Front Psychiatry. 2022;12:814177.
22. Solár P, Zamani A, Kubíčková L, Dubový P, Joukal M. Choroid plexus and the blood–cerebrospinal fluid barrier in disease. Fluids Barriers CNS. 2020;17:35.
23. Martínez M, Martín-Hernández D, Virto L, MacDowell KS, Montero E, González-Bris Á, et al. Periodontal diseases and depression: a pre-clinical in vivo study. J Clin Periodontol. https://doi.org/10.1111/jcpe.13420.
24. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89:S74–84.
25. Liu Y, Wu Z, Zhang X, Ni J, Yu W, Zhou Y, et al. Leptomeningeal cells transduce peripheral macrophages inflammatory signal to microglia in reponse to Porphyromonas gingivalis LPS. Mediators Inflamm. 2013;2013:407562.
26. Kamer AR, Dasanayake AP, Craig RG, Glodzik-Sobanska L, Bry M, de Leon MJ. Alzheimer's disease and peripheral infections: the possible contribution from periodontal infections, model and hypothesis. J Alzheimers Dis. 2008;13:437–49.
27. Yu X-C, Yang J-J, Jin B-H, Xu H-L, Zhang H-Y, Xiao J, et al. A strategy for bypassing the blood-brain barrier: facial intradermal brain-targeted delivery via the trigeminal nerve. J Control Release. 2017;258:22–33.
28. Kitamoto S, Nagao-Kitamoto H, Hein R, Schmidt TM, Kamada N. The bacterial connection between the oral cavity and the gut diseases. J Dent Res. 2020;99:1021–9.
29. Li C, Wen Y, Cheng S, Zhang H, Meng P, Zhang F. A genetic association study reveals the relationship between the oral microbiome and anxiety and depression symptoms. Front Psychiatry. 2022. https://doi.org/10.3389/fpsyt.2022.960756.
30. Sakkas A, Nolte I, Heil S, Mayer B, Kargus S, Mischkowski RA, et al. Eggerthia catenaformis infection originating from a dental abscess causes severe intestinal complications and osteomyelitis of the jaw. GMS Interdiscip Plast Reconstr Surg DGPW. 2021;10:Doc02.
31. Simpson CA, Adler C, du Plessis MR, Landau ER, Dashper SG, Reynolds EC, et al. Oral microbiome composition, but not diversity, is associated with adolescent anxiety and depression symptoms. Physiol Behav. 2020;226:113126.
32. Wingfield B, Lapsley C, McDowell A, Miliotis G, McLafferty M, O'Neill SM, et al. Variations in the oral microbiome are associated with depression in young adults. Sci Rep. 2021;11:15009.
33. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25:134–44.
34. Yamane K, Yamanaka T, Yamamoto N, Furukawa T, Fukushima H, Walker CB, et al. A novel exopolysaccharide from a clinical isolate of Prevotella nigrescens: purification, chemical characterization and possible role in modifying human leukocyte phagocytosis. Oral Microbiol Immunol. 2005;20:1–9.
35. Yamanaka T, Yamane K, Furukawa T, Matsumoto-Mashimo C, Sugimori C, Nambu T, et al. Comparison of the virulence of exopolysaccharide-producing Prevotella intermedia to exopolysaccharide non-producing periodontopathic organisms. BMC Infect Dis. 2011;11:228.
36. D'Aiuto F, Nibali L, Parkar M, Patel K, Suvan J, Donos N. Oxidative stress, systemic inflammation, and severe periodontitis. J Dent Res. 2010;89:1241–6.
37. Perry VH. The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease. Brain Behav Immun. 2004;18:407–13.
38. D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factorα signaling during peripheral organ inflammation. J Neurosci. 2009;29:2089–102.
39. Karrow NA. Activation of the hypothalamic–pituitary–adrenal axis and autonomic nervous system during inflammation and altered programming of the

neuroendocrine–immune axis during fetal and neonatal development: Lessons learned from the model inflammagen, lipopolysaccharide. Brain Behav Immun. 2006;20:144–58.
40. Vilagut G, Forero CG, Barbaglia G, Alonso J. Screening for depression in the general population with the center for epidemiologic studies depression (CES-D): a systematic review with meta-analysis. PLoS One. 2016;11:e0155431.
41. Blevins CA, Weathers FW, Davis MT, Witte TK, Domino JL. The posttraumatic stress disorder checklist for DSM-5 (PCL-5): development and initial psychometric evaluation. J Trauma Stress. 2015;28:489–98.
42. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, et al. Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry. 1994;151:1132–6.
43. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Beck JD, et al. Self-reported measures for surveillance of periodontitis. J Dent Res. 2013;92:1041–7.
44. Montero E, La Rosa M, Montanya E, Calle-Pascual AL, Genco RJ, Sanz M, et al. Validation of self-reported measures of periodontitis in a Spanish Population. J Periodontal Res. 2020;55:400–9.
45. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. J Periodontol. 2012;83:1449–54.
46. Willis JR, González-Torres P, Pittis AA, Bejarano LA, Cozzuto L, Andreu-Somavilla N, et al. Citizen science charts two major "stomatotypes" in the oral microbiome of adolescents and reveals links with habits and drinking water composition. Microbiome. 2018;6:218.
47. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria: 2020. https://www.R-project.org/.
48. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
49. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014;42:D633–42.
50. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara B, et al. Vegan: community ecology package. R Package Version 22-1. 2015;2:1–2.
51. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8:e61217.
52. Wickham, H. ggplot2: Elegant graphics for data analysis. ISBN 978-3-319-24277-4. Springer-Verlag New York: 2016. https://ggplot2.tidyverse.org.
53. Gloor GB, Reid G. Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. Can J Microbiol. 2016;62:692–703.
54. Gloor GB, Wu JR, Pawlowsky-Glahn V, Egozcue JJ. It's all relative: analyzing microbiome data as compositions. Ann Epidemiol. 2016;26:322–9.
55. Haegeman B, Hamelin J, Moriarty J, Neal P, Dushoff J, Weitz JS. Robust estimation of microbial diversity in theory and in practice. ISME J. 2013;7:1092–101.
56. Bastiaanssen TFS, Quinn TP, Loughman A. Bugs as features (part 1): concepts and foundations for the compositional data analysis of the microbiome–gut–brain axis. Nat Ment Health. 2023;1:930–8.
57. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38:685–8.
58. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 2019;4:623–32.
59. Mattiello F, Verbist B, Faust K, Raes J, Shannon WD, Bijnens L, et al. A web application for sample size and power calculation in case-control microbiome studies. Bioinformatics. 2016;32:2038–40.
60. Schwartz JL, Peña N, Kawar N, Zhang A, Callahan N, Robles SJ, et al. Old age and other factors associated with salivary microbiome variation. BMC Oral Health. 2021;21:490.
61. Kim Y-T, Jeong J, Mun S, Yun K, Han K, Jeong S-N. Comparison of the oral microbial composition between healthy individuals and periodontitis patients in different oral sampling sites using 16S metagenome profiling. J Periodontal Implant Sci. 2022;52:394–410.
62. Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, et al. Cigarette smoking and the oral microbiome in a large study of American adults. ISME J. 2016;10:2435–46.
63. Wu Y, Chi X, Zhang Q, Chen F, Deng X. Characterization of the salivary microbiome in people with obesity. PeerJ. 2018;6:e4458.
64. Chen B, Zhao Y, Li S, Yang L, Wang H, Wang T, et al. Variations in oral microbiome profiles in rheumatoid arthritis and osteoarthritis with potential biomarkers for arthritis screening. Sci Rep. 2018;8:17126.
65. Chu Y, Sun S, Huang Y, Gao Q, Xie X, Wang P, et al. Metagenomic analysis revealed the potential role of gut microbiome in gout. npj Biofilms Microbiomes. 2021;7:1–13.
66. Ma G, Qiao Y, Shi H, Zhou J, Li Y. Comparison of the oral microbiota structure among people from the same ethnic group living in different environments. Biomed Res Int. 2022;2022:6544497.
67. Charalambous EG, Mériaux SB, Guebels P, Muller CP, Leenen FAD, Elwenspoek MMC, et al. Early-life adversity leaves its imprint on the oral microbiome for more than 20 years and is associated with long-term immune changes. Int J Mol Sci. 2021;22:12682.
68. Ortiz AP, Acosta-Pagán KT, Oramas-Sepúlveda C, Castañeda-Avila MA, Vilanova-Cuevas B, Ramos-Cartagena JM, et al. Oral microbiota and periodontitis severity among Hispanic adults. Front Cell Infect Microbiol. 2022. https://doi.org/10.3389/fcimb.2022.965159.
69. Ai D, Huang R, Wen J, Li C, Zhu J, Xia LC. Integrated metagenomic data analysis demonstrates that a loss of diversity in oral microbiota is associated with periodontitis. BMC Genomics. 2017;18:1041.
70. Deng K, Ouyang XY, Chu Y, Zhang Q. Subgingival microbiome of gingivitis in Chinese undergraduates. Chin J Dent Res. 2017;20:145–52.
71. Zheng J, Wang X, Zhang T, Jiang J, Wu J. Comparative characterization of supragingival plaque microbiomes in malocclusion adult female patients undergoing orthodontic treatment with removable aligners or fixed appliances: a descriptive cross-sectional study. Front Cell Infect Microbiol. 2024. https://doi.org/10.3389/fcimb.2024.1350181.
72. Alauzet C, Marchandin H, Lozniewski A. New insights into Prevotella diversity and medical microbiology. Future Microbiol. 2010;5:1695–718.
73. Takeshita T, Kageyama S, Furuta M, Tsuboi H, Takeuchi K, Shibata Y, et al. Bacterial diversity in saliva and oral health-related conditions: the Hisayama Study. Sci Rep. 2016;6:22164.
74. Kohn JN, Kosciolek T, Marotz C, Aleti G, Guay-Ross RN, Hong S-H, et al. Differing salivary microbiome diversity, community and diurnal rhythmicity in association with affective state and peripheral inflammation in adults. Brain Behav Immun. 2020;87:591–602.
75. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, et al. Genomic variation landscape of the human gut microbiome. Nature. 2013;493:45–50.
76. Liao H, Ji Y, Sun Y. High-resolution strain-level microbiome composition analysis from short reads. Microbiome. 2023;11:183.
77. Yolken R, Prandovszky E, Severance EG, Hatfield G, Dickerson F. The oropharyngeal microbiome is altered in individuals with schizophrenia and mania. Schizophr Res. 2021;234:51–7.
78. Liu G, Tang CM, Exley RM. Non-pathogenic Neisseria: members of an abundant, multi-habitat, diverse genus. Microbiology. 2015;161:1297–312.
79. Rosier BT, Takahashi N, Zaura E, Krom BP, Martínez-Espinosa RM, van Breda SGJ, et al. The importance of nitrate reduction for oral health. J Dent Res. 2022;101:887–97.
80. Rosier BT, Buetas E, Moya-Gonzalvez EM, Artacho A, Mira A. Nitrate as a potential prebiotic for the oral microbiome. Sci Rep. 2020;10:12895.
81. Burne RA, Marquis RE. Alkali production by oral bacteria and protection against dental caries. FEMS Microbiol Lett. 2000;193:1–6.
82. Maurer B, The 5 most abundant and common oral bacteria and what they mean for your health. In: Bristle. https://www.bristlehealth.com/blogs/oral-microbiome/the-5-most-abundant-and-common-oral-bacteria-and-what-they-mean-for-your-health. 2022. (Accessed 22 Mar 2024).
83. Pesantes N, Barberá A, Pérez-Rocher B, Artacho A, Vargas SL, Moya A, et al. Influence of mental health medication on microbiota in the elderly population in the Valencian region. Front Microbiol. 2023;14:1094071.
84. Liu P, Gao M, Liu Z, Zhang Y, Tu H, Lei L, et al. Gut microbiome composition linked to inflammatory factors and cognitive functions in first-episode, drug-naive major depressive disorder patients. Front Neurosci. 2021;15:800764.
85. Zhang M, Li A, Yang Q, Li J, Wang L, Liu X, et al. Beneficial effect of alkaloids from Sophora alopecuroides L. on CUMS-Induced depression model mice via modulating gut microbiota. Front Cell Infect Microbiol. 2021. https://doi.org/10.3389/fcimb.2021.665159.
86. Lourenço TGB, Spencer SJ, Alm EJ, Colombo APV. Defining the gut microbiota in individuals with periodontal diseases: an exploratory study. J Oral Microbiol. 2018;10:1487741.
87. Liu S, Xie G, Chen M, He Y, Yu W, Chen X, et al. Oral microbial dysbiosis in patients with periodontitis and chronic obstructive pulmonary disease. Front Cell Infect Microbiol. 2023;13:1121399.
88. Widhani A, Djauzi S, Suyatna FD, Dewi BE. Changes in gut microbiota and systemic inflammation after synbiotic supplementation in patients with systemic lupus erythematosus: a randomized, double-blind, placebo-controlled trial. Cells. 2022;11:3419.
89. Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med. 2015;21:895–905.
90. Lee YC, Agnew-Blais J, Malspeis S, Keyes K, Costenbader K, Kubzansky LD, et al. Posttraumatic stress disorder and risk for incident rheumatoid arthritis. Arthritis Care Res (Hoboken). 2016;68:292–8.

91. Holdeman LV, Moore WEC, Cato EP, Burmeister JA, Palcanis KG, Ranney RR. Distribution of Capnocytophaga in periodontal microfloras. J Periodontal Res. 1985;20:475–83.
92. Idate U, Bhat K, Kulkarni R, Kumbar V, Pattar G. Identification of Capnocytophaga species from oral cavity of healthy individuals and patients with chronic periodontitis using phenotypic tests. JCRI. 2018;5:173–7.
93. Yu X-L, Chan Y, Zhuang L, Lai H-C, Lang NP, Keung Leung W, et al. Intra-oral single-site comparisons of periodontal and peri-implant microbiota in health and disease. Clin Oral Implants Res. 2019;30:760–76.
94. Haffajee AD, Socransky SS. Effect of sampling strategy on the false-negative rate for detection of selected subgingival species. Oral Microbiol Immunol. 1992;7:57–59.
95. Boadle-Biber MC. Regulation of serotonin synthesis. Prog Biophys Mol Biol. 1993;60:1–15.
96. Russo S, Kema IP, Bosker F, Haavik J, Korf J. Tryptophan as an evolutionarily conserved signal to brain serotonin: molecular evidence and psychiatric implications. World J Biol Psychiatry. 2009;10:258–68.
97. Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological dependence on plasma tryptophan levels. Science. 1971;173:149–52.
98. Trujillo-Hernández PE, Sáenz-Galindo A, Saucedo-Cárdenas O, Villarreal-Reyna MLÁ, Salinas-Santander MA, Carrillo-Cervantes AL, et al. Depressive symptoms are associated with low serotonin levels in plasma but are not 5-HTTLPR genotype dependent in older adults. Span J Psychol. 2021;24:e28.
99. Ogłodek EA. Changes in the serum concentration levels of serotonin, tryptophan and cortisol among stress-resilient and stress-susceptible individuals after experiencing traumatic stress. Int J Environ Res Public Health. 2022;19:16517.
100. Stein DJ, Stahl S. Serotonin and anxiety: current models. Int Clin Psychopharmacol. 2000;15:S1–6.
101. Lin S-H, Lee L-T, Yang YK. Serotonin and mental disorders: a concise review on molecular neuroimaging evidence. Clin Psychopharmacol Neurosci. 2014;12:196–202.
102. Stanley M, Mann JJ. Increased serotonin-2 binding sites in frontal cortex of suicide victims. Lancet. 1983;1:214–6.
103. Daly C. Oral and dental effects of antidepressants. Aust Prescr. 2016;39:84.
104. Hopcraft MS, Tan C. Xerostomia: an update for clinicians. Aust Dent J. 2010;55:238–44.
105. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Curr Opin Endocrinol Diab Obes. 2013;20:14–21.
106. Kendig DM, Grider JR. Serotonin and colonic motility. Neurogastroenterol Motil. 2015;27:899–905.
107. Guo X, Lin F, Yang F, Chen J, Cai W, Zou T. Gut microbiome characteristics of comorbid generalized anxiety disorder and functional gastrointestinal disease: correlation with alexithymia and personality traits. Front Psychiatry. 2022. https://doi.org/10.3389/fpsyt.2022.946808.
108. Sohn J, Li L, Zhang L, Genco RJ, Falkner KL, Tettelin H, et al. Periodontal disease is associated with increased gut colonization of pathogenic Haemophilus parainfluenzae in patients with Crohn's disease. Cell Rep. 2023;42:112120.
109. Marshall M. The hidden links between mental disorders. Nature. 2020;581:19–21.
110. Babyak MA. What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. Psychosom Med. 2004;66:411–21.
111. Pandey D, Szczesniak M, Maclean J, Yim HCH, Zhang F, Graham P, et al. Dysbiosis in head and neck cancer: determining optimal sampling site for oral microbiome collection. Pathogens. 2022;11:1550.

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AUTHOR CONTRIBUTIONS

SMM conceptualized the study, and performed microbiome and plasma-related experimentation, data curation, formal analysis, and writing of the manuscript. RV and EO performed HPLC analyses and manuscript editing. EM and EF performed periodontal health questionnaire scoring and assisted with manuscript writing and editing. IZ, JDDA, MC, MPGP, and AGP, assisted with participant recruitment, blood collection, sample preparation, and manuscript editing. JCL provided expert supervision and guidance, including project administration, conceptual direction, and manuscript writing and editing.

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COMPETING INTERESTS

The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Stefanie Malan-Müller.