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Altered oral health and microbiota in drug-free patients with schizophrenia

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Abstract

Background The oral microbiota is associated with neuro-psychiatric disorders. However, there is presently inadequate comprehension regarding the correlation between schizophrenia and the oral microbiota. Moreover, patients with schizophrenia frequently exhibit poor oral health, potentially influencing research outcomes. Therefore, this study aims to investigate changes in the oral microbiota and oral health status in drug-free schizophrenia patients.

Methods Oral microbiota samples were collected from 50 drug-free patients with schizophrenia and 50 healthy controls (HCs). The downstream microbiota analysis was based on Illumina sequencing of the V3-V4 hypervariable region of the 16 S rRNA gene.

Results The alpha diversity of SCZ group is increased, such as the Shannon index ($p < 0.001$) and Simpson index ($p = 0.004$), while the community structure also displays variance compared to the HC group ($p < 0.001$). Key discriminative taxa were found in LEfSe analysis, including the phyla *Fusobacteriota*, *Firmicutes*, and *Actinobacteriota*. The differential taxa and microbial functions showed a strong correlation with clinical oral conditions. Further analysis demonstrated that models based on the entire oral microbiota effectively distinguished SCZ patients from HC (AUC = 0.97).

Conclusions The significant changes in the microbiota of Drug-free SCZ patients appear to be closely associated with the poor oral environment.

Highlights

- Sample: 50 HCs & 50 drug-free SCZ patients recruited to reduce medication impact.
- Employed comprehensive oral assessments and microbiota sequencing.
- SCZ patients showed poorer oral health and altered oral microbiota.
- Oral microbiota changes correlated with oral health in SCZ patients.

Keywords Schizophrenia, Oral microbiota, Drug-free, Oral health, 16S rRNA sequencing

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Background

The microbiota plays a pivotal role in human health. The oral cavity, serving as a natural microbial habitat, hosts over 700 microbial species, and the periodontal tissues exhibit intricate anatomical and tissue structures that create favorable conditions for microbial growth due to their physicochemical characteristics [1]. The oral microbiota not only affects oral health but is also closely related to overall health [2, 3].

There exists a significant relationship between oral microbiota and neuropsychiatric disorders. Recent research suggests a potential causal connection between chronic periodontitis and the development of Alzheimer's disease (AD) [4]. Notably, *Porphyromonas gingivalis*, a bacterium associated with gum disease, coexists with species that can disrupt the host's immune system, leading to systemic inflammatory reactions and causing various metabolic and inflammatory complications. Studies have revealed that *Porphyromonas gingivalis* is present not only in the oral cavity of AD patients but also in their brains, subsequent in vivo and in vitro studies have demonstrated its neurotoxicity, inducing typical brain changes in AD mouse models [5]. Longitudinal studies also indicate that elevated levels of oral anaerobic bacteria and middle intestinal anaerobic rod antibody levels at baseline are associated with cognitive impairment ten years later [6]. Beyond AD, there exists a correlation between oral microbiota and various other neurological and psychiatric conditions [7–10]. Several studies have identified a distinct oral microbiome composition in patients with Parkinson's disease (PD), with microbiome diversity metrics correlating with the severity of non-motor autonomic symptoms [11–13]. Patients with bulbar-onset amyotrophic lateral sclerosis also exhibit severe oral microbiota dysbiosis, such as a decreased oral Firmicutes/Bacteroidetes ratio. Additionally, increased microbial translocation to blood is associated with more severe disease symptoms [14].

Schizophrenia (SCZ) is a complex disorder characterized by cognitive, emotional, and behavioral symptoms. Its pathophysiological mechanisms involve neurotransmitter imbalances, immune system dysregulation, genetic factors, and neurodevelopmental abnormalities [15, 16]. In microbiological research, studies have highlighted dysbiosis and bacterial translocation in individuals with SCZ, which are associated with neurological damage and autoimmune responses [17, 18]. Evidence suggested that individuals with SCZ harbor a distinct gut microbiota, some of which may be linked to psychotic symptoms and inflammation [19, 20]. Furthermore, transplantation of gut microbiota from untreated individuals with SCZ into germ-free mice has been shown to induce behavioral abnormalities [21, 22].

However, the exploration of the oral microbiota in individuals with SCZ is currently limited. A preliminary study conducted on a small sample suggests that the oral microbiota of individuals with SCZ tends to be more fragile and exhibits lower diversity [23]. Another study found strong correlations of oral bacterial taxa with cytokines and hippocampal gliosis, dysmyelination, and excitatory neurotransmission, highlighted the relative importance of the oral microbiota in peripheral and hippocampal inflammatory pathways [24]. Moreover, SCZ patients exhibited elevated levels of peripheral pro-inflammatory cytokines and chemokines, which were associated with changes in the abundance of the oral fungi *Candida* and *Malassezia* [25], highlighted the relative importance of the oral microbiota in peripheral and hippocampal inflammatory pathways. Nonetheless, the specific mechanisms remain poorly characterized. Moreover, research indicates that individuals with SCZ often experience compromised oral health compared to the general population, manifested through severe dental caries, tooth loss, decay, and poor periodontal conditions [26, 27]. Poor oral environments and inadequate oral hygiene habits can significantly influence the oral microbiota, leading to a notable increase in the relative abundance of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Filifactor alocis*, and potential cariogenic *Leptotrichia* species, among others [28–31]. Although inconsistent oral environments may potentially influence the consistency of results in oral microbiota studies, there is still a lack of rigorously designed studies specifically addressing the oral microbiota of individuals with SCZ and its related influencing factors.

Hence, the primary objectives of this study are to: (1) Explore the disparities in oral microbiota and oral hygiene conditions between drug-free schizophrenia patients and healthy controls; (2) Investigate potential correlations between oral microbiota and oral hygiene status.

Methods

Participant selection

All participants were recruited between February 2022 and April 2023 (Clinical trial number - not applicable). Inclusion criteria for participants with SCZ were as follows: (1) Individuals diagnosed with SCZ (age between 18 and 60); (2) Meeting the ICD-10 F20 diagnostic criteria for SCZ; (3) No use of antipsychotic medications in the last 3 months; (4) Demonstrating a full understanding of the therapy, agreeing to participate voluntarily, and providing informed consent either personally or through a legal guardian. The exclusion criteria were: (1) Undergoing dental cleaning, oral surgery, orthodontic procedures, or similar operations in the last 3 months; (2) Receiving oral or systemic antibiotics in the last month;

(3) Presenting severe oral diseases such as significant congenital oral deformities or oral cancer; (4) Suffering from severe or unstable immune or inflammatory disorders; (5) Unable to cooperate with oral microbiota sampling; (6) Unable to adhere to the study protocol.

Inclusion criteria for healthy controls (HC) were as follows: (1) No prior diagnosis of psychiatric or psychological disorders; (2) Absence of severe or unstable major illnesses (e.g., Parkinson's disease, multiple sclerosis, stroke, substance dependence). Exclusion criteria for HC were same as the SCZ group.

Clinical measures

General information was collected at baseline, and symptomatic severity was assessed using the Positive and Negative Syndrome Scale (PANSS) [32], and oral hygiene status was evaluated using the oral assessment scale, oral check questionnaire and other index as follows.

Dental caries index: The Decayed, Missing, and Filled Teeth (DMFT) index represents the subject's historical and current encounters with dental caries [33]. The DMFT index is categorized based on the obtained values into three groups: low (0–12), moderate (13–19), and high (20–28).

O'Leary Index: This index indicates the presence or absence of plaque on tooth surfaces (buccal/cheek, lingual/palatal, proximal/distal surfaces) from the gingival margin to the crown using a plaque-disclosing solution [34]. The index is calculated by dividing the sum of tooth surfaces with plaque by the total number of tooth surfaces, then multiplying by 100%. Results are classified as good (20%), fair (20–40%), and poor (40%).

Health Motivation Index: This index evaluates patients' attitudes toward oral hygiene using the tooth brushing frequency indicator. It defines brushing habits as adequate (brushing at least once a day), inadequate (only rinsing after meals), or none (never cleaning teeth).

Oral Mucosal Lesion Index: This index provides examination findings regarding the soft tissues of the oral mucosa, recorded as either present or absent.

The detailed oral assessment scale and oral check questionnaire are provided in the supplementary materials.

Sample collection

Oral Swab Sample Collection Method: Rinse the mouth thoroughly with approximately 50 ml of water for about 10 s, then spit it out. Insert the swab into the mouth, ensuring the swab head makes full contact with the inner cheek and mucosa of the upper and lower dental arches. Apply brushing pressure while moving the swab up and down and simultaneously rotating it, ensuring full contact with the oral mucosa. Repeat this action for 1 min. Three oral swabs were collected for each participant. After sampling, the samples were immediately store samples at -80 °C.

Sample size calculation

Considering that previous studies have consistently reported significant differences in oral microbiota between patients with schizophrenia and healthy controls, we selected effect sizes of Cohen's $d = 0.5$, 0.6, 0.7, and 0.8 with a power of 0.8. This resulted in sample sizes of 128, 90, 68, and 52, respectively. Taking into account potential dropouts, we ultimately decided on a sample size of 100 individuals (50 each for healthy controls and individuals with schizophrenia). The calculation was using G*Power software (ver. 3.1.9.7) [35].

DNA extraction and 16 S sequencing

Total DNA was extracted using the DNA extraction kit (Model: DZ314, FINDROP Corporation, Guangdong, China) according to the manufacturer's instructions. Subsequently, the V3-V4 regions of the 16 S rRNA gene were selected for polymerase chain reaction amplification using 338 F/806R. The NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) was used for library construction. The MiSeq2500 PE300 system (Illumina) was used for sequencing.

Data analysis

Microbial sequences were processed using QIIME2 (version 2023.9) [36]. Amplicon sequence variant (ASV) representative sequences and abundance information was obtained using the DADA2 [37] with the following parameters: forward and reverse reads were trimmed by removing the first 20 bases to eliminate potential primer sequences and low-quality bases. Reads were truncated at 250 bp for both forward and reverse directions based on quality score profiles to remove low-quality bases at the ends before denoising. The taxonomic classification of ASVs was done by using pre-trained classifier (a scikit-learn naive Bayes machine-learning classifier) against Greengenes database [38] as provided by Qiime2 (https://docs.qiime2.org/2023.9/data-resources/). A phylogenetic tree was generated using MAFFT and FastTree method [39, 40]. Downstream analyses were performed and visualized through microeco packages (version 1.4.0) [41] and ggplot2 package [42] in R (version 4.2.0, R Project for Statistical Computing). The rarefaction curve (S.Figure 1) was employed to demonstrate sequencing depth. With increasing sequencing, the curve tends to plateau, indicating that sufficient sequencing depth has been achieved. Samples were rarefied to 12,852 sequences per sample. Alpha-diversity, including observed species, ACE, Chao 1, Shannon, and Simpson index were calculated to measure dissimilarities in richness and evenness of microbial community. Comparisons

of alpha diversity between groups were performed using the Wilcoxon rank-sum test. Principal coordinates analysis (PCoA) based on the Bray-Curtis index was used for beta-diversity analysis, with statistical significance tested using the analysis of similarities (ANOSIM) analysis (10000 permutations). Linear discriminant analysis (LDA) effect size (LEfSe) test [43] was performed with an LDA threshold of 3.0 and significance level of $p < 0.01$ to identify the different taxa in the two groups. Use redundancy analysis (RDA) to demonstrate the impact of environmental variables on microbial communities. To gain deeper insights into the influence of environmental factors on microbiota, we utilized the functional annotation of prokaryotic taxa (FAPROTAX) algorithm [44] to predict the functional contributions of microbiota to their host environments. Subsequently, spearman correlation analysis was conducted between environmental factors and microbial function. Then, a random forest model was constructed to investigate whether the oral microbiota could serve as potential biomarkers relevant to SCZ patients. Feature selection was performed using the Boruta algorithm with a maximum of 300 runs and a *p*-value threshold of 0.01, combined with a random forest model that utilized a number of 500 trees. Stratified sampling was applied with 75% of the dataset al.located to the training set. The false discovery rate (FDR) correction (Benjamini and Hochberg method) [45] was conducted to accommodate false positive results.

Table 1 Subjects' demographics data

Characteristic	HC, $N=50$[1]	SCZ, $N=50$[1]	*p*-value[2]
Sex			0.280
Female	18 / 50 (36%)	13 / 50 (26%)	
Male	32 / 50 (64%)	37 / 50 (74%)	
Age	33.2 (11.6)	37.0 (8.2)	0.061
Education level			< 0.001
Primary Or Middle	6 / 50 (12%)	38 / 50 (76%)	
High School	2 / 50 (4.0%)	9 / 50 (18%)	
Undergraduate	41 / 50 (82%)	3 / 50 (6.0%)	
Postgraduate	1 / 50 (2.0%)	0 / 50 (0%)	
Smoking (Yes/No)	12 / 50 (24%)	12 / 50 (24%)	> 0.999
Smoking years	5.0 (10.1)	2.5 (4.7)	0.110
Cigarettes number / day	2.9 (6.6)	5.8 (11.8)	0.136
Alcohol abuse (Yes/No)	1 / 50 (2.0%)	2 / 50 (4.0%)	> 0.999
Alcohol abuse years	0.6 (4.2)	0.6 (3.0)	0.978
Age of onset		25.1 (7.9)	
PANSS-positive		15.0 (8.3)	
PANSS-negative		16.5 (6.9)	
PANSS-general psychopathology		29.8 (9.4)	
PANSS-total score		61.3 (21.1)	
Oral assessment scale	5.1 (0.4)	6.8 (1.8)	< 0.001
Oral check questionnaire	8.1 (0.4)	10.4 (2.5)	< 0.001
DMFT			0.002
High	0 / 50 (0%)	1 / 50 (2.0%)	
Low	49 / 50 (98%)	38 / 50 (76%)	
Moderate	1 / 50 (2.0%)	11 / 50 (22%)	
O'Leary Index			< 0.001
Excellent	50 / 50 (100%)	15 / 50 (30%)	
Good	0 / 50 (0%)	35 / 50 (70%)	
Health Motivation Index			< 0.001
Brush teeth at least once a day	50 / 50 (100%)	35 / 50 (70%)	
Never clean teeth	0 / 50 (0%)	4 / 50 (8.0%)	
Only rinsing after meals	0 / 50 (0%)	11 / 50 (22%)	
Oral Mucosal Lesion	3 / 50 (6.0%)	9 / 50 (18%)	0.065

[1]n / N (%); Mean (SD); [2]Pearson's Chi-squared test, Welch Two Sample t-test, Fisher's exact test

Clinical statistical analysis

R was used for statistical analysis of the demographic and clinical characteristics. Categorical variables were assessed with the chi-squared test or Fisher's exact test. For continuous data, Welch's two-sample *t*-tests was employed. The statistical significance level was set at a two-tailed $p < 0.05$.

Results

Demographic and clinical characteristics

Considering the interaction between antipsychotic drugs and the microbiota [46–48], drug-free patients were recruited. Ultimately, 50 patients and 50 healthy controls were enrolled (Table 1). There were no significant differences in age, sex ratio, smoking or alcohol abuse between patients and HCs (all $p > 0.05$; Table 1). Patients with SCZ were less educated ($p < 0.001$). To comprehensively characterize the oral environment of the subjects, a series of assessments were conducted. Results from the oral assessment scale ($p < 0.001$) and Oral check questionnaire ($p < 0.001$) both indicated poorer oral environmental health among patients. Furthermore, additional assessments revealed a low frequency of tooth brushing ($p < 0.001$), along with a higher incidence of cavities ($p = 0.002$) and dental plaque ($p < 0.001$) in the patient group.

Diversity

Alpha-diversity was compared using the Wilcoxon rank-sum test, revealing significant differences in observed species (Fig. 1a, $p < 0.001$), ACE index (Fig. 1b, $p < 0.001$), Chao1 index (Fig. 1c, $p < 0.001$), Shannon index (Fig. 1d, $p < 0.001$) and Simpson index (Fig. 1e, $p = 0.004$), between groups (S.Table 1). Overall differences in community structure between groups were measured using Bray-Curtis analysis (Fig. 1f-g). The PCoA plot demonstrated a significant dissimilarity in microbial community between the two groups (ANOSIM statistic $R = 0.137$, $p < 0.001$), the difference of PCo1 is not significant between the two groups, but the difference of PCo2 is significant between

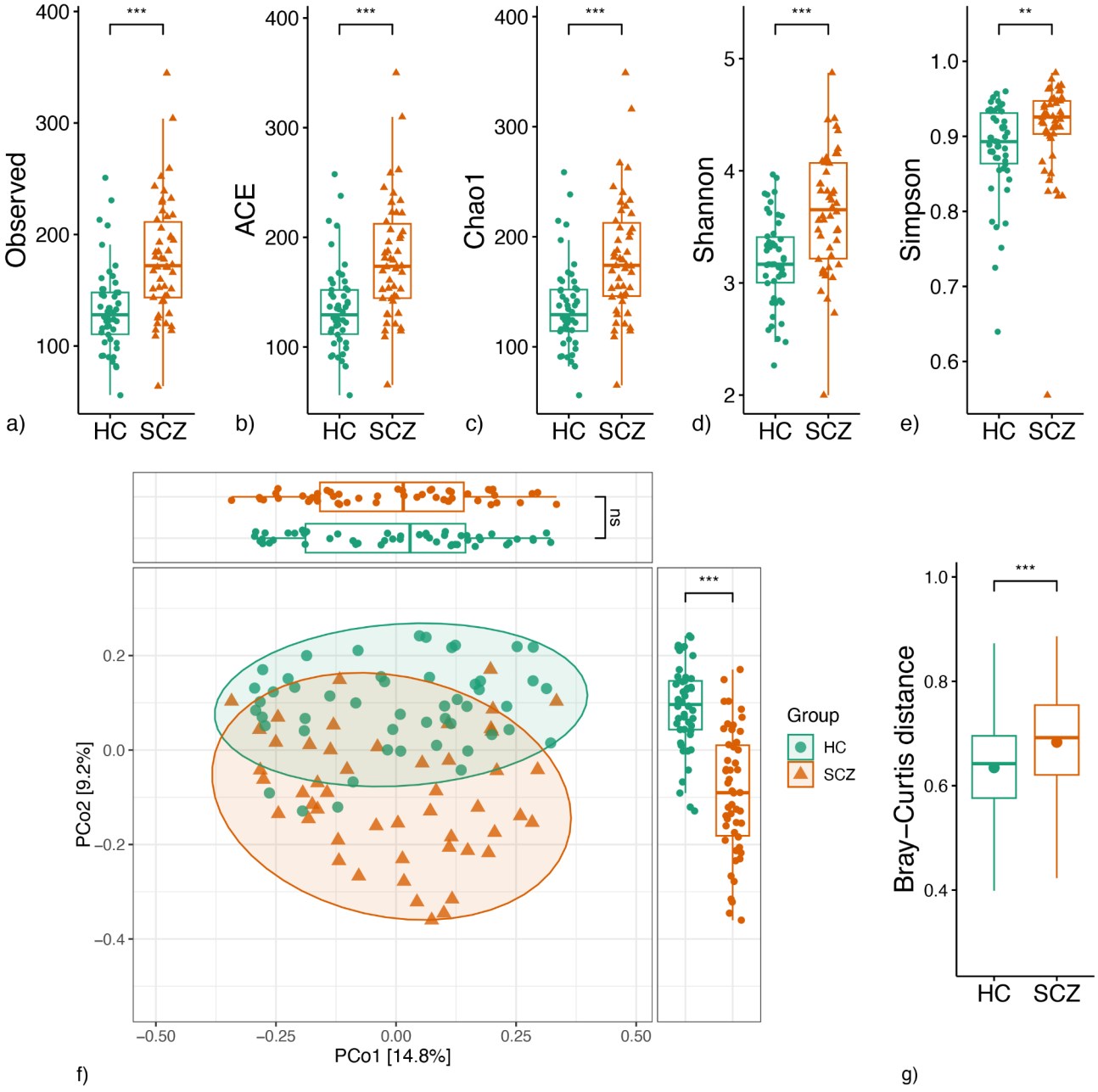

Fig. 1 Alterations of oral microbiota diversity in SCZ patients. (**a-e**) Comparison of alpha-diversity between SCZ patients and HCs. The x-axis represents the HCs (green) and SCZ (yellow) groups, the y-axis represents the value of observed species (**a**), ACE index (x), Chao1 index (**c**), Shannon index (**d**) and Simpson index (**e**). (**f**) Beta-diversity as a PCoA plot based on Bray-Curtis dissimilarity. (**g**) Comparison of Bray-Curtis distance between SCZs and HCs

the two groups. The difference of Bray-Curtis distance is also significant between the two groups.

Alterations of oral microbiota

The most prevalent phylum observed between the two groups were *Firmicutes*, *Proteobacteria* and *Bacteroidota*, and different relative abundances could be observed (Fig. 2a). A Venn diagram was made to define the unique and overlapping distribution of ASVs in two groups. A total of 2555 ASVs were identified, of which 821 ASVs were common, representing a relative abundance of 93.3%, indicating that dominant taxa were shared between groups (Fig. 2b). Among others, 1175 ASVs could be detected only in SCZ, while 559 ASVs were unique to the HC group. Differential taxa between SCZ and HC were identified by the LEfSe approach (LDA score > 3.0 and *FDR. p*-value < 0.05), and key discriminative taxa were shown (Fig. 2c). At the phylum level, the abundance of *Fusobacteriota*, a phylogenetically distinct clade within the phylum *Firmicutes*, *spirochaetota*,

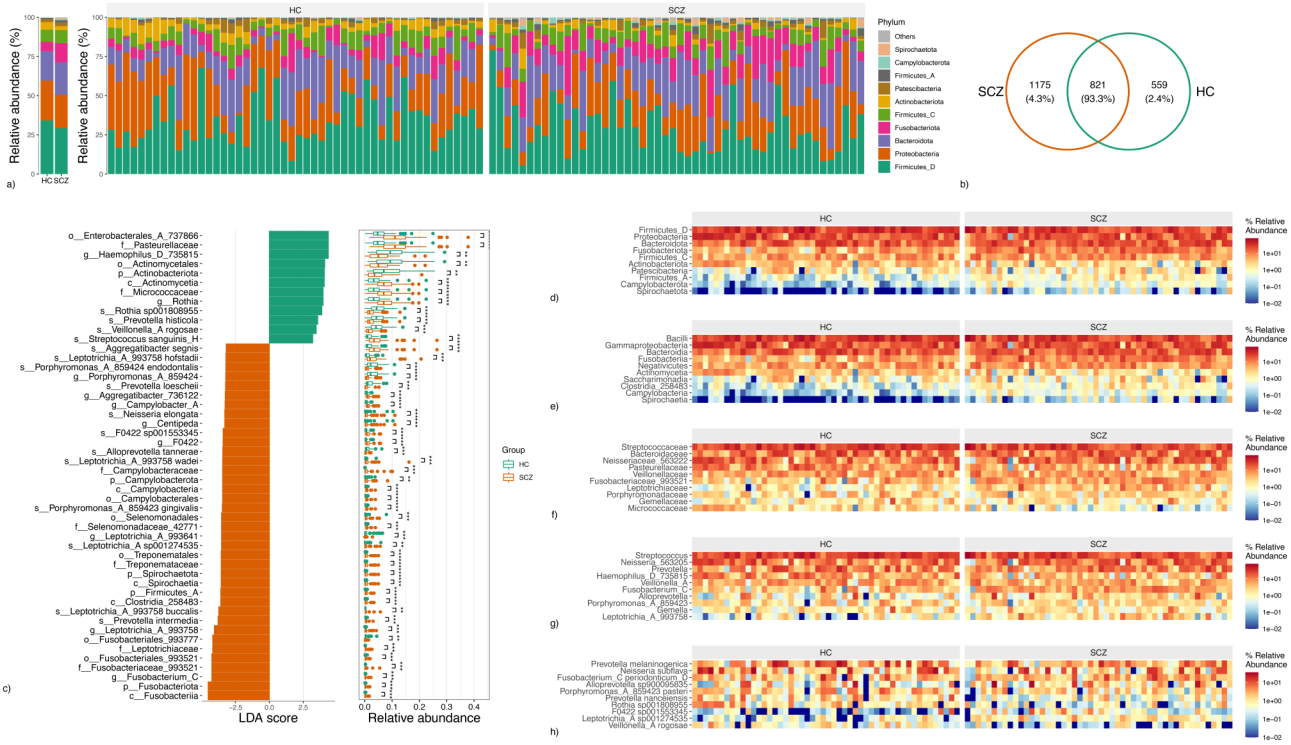

Fig. 2 Alterations of oral microbiota in SCZ patients. (**a**) Community barplot analysis. Relative abundance of the dominant taxa in the oral microbiota of SCZ patients and the HCs group (at phylum level). (**b**) Venn diagram of shared and unique ASVs in SCZ and HC groups, illustrating both the quantity and relative abundance. (**c**) Taxonomic biomarkers found by LEfSe in HC (green) and SCZ (yellow). Only taxa with *FDR*. $p < 0.05$ and LDA score (log10) ≥ 3.0 are shown. The right histogram displayed the relative abundance of taxonomic biomarkers in the two groups. (**d-h**) Heatmap of the relative abundance of the most differentially abundant taxa ranking in the top ten at the phylum level (**d**), class level (**e**), family level (**f**), genus level (**g**) and ASV level (**h**)

campylobacterota increased in SCZ patients vs. HCs, whereas *Actinobacteriota* decreased. Further visualization using a heatmap depicted the relative abundance of the top 10 taxa with the most significant differences across different taxonomic levels (Fig. 2d-h).

Relationship between environmental factors and microbiota

Redundancy analysis (RDA) was performed to elucidate the factors driving changes in microbial community structures (Fig. 3). The results highlighted the significant impact of clinical oral conditions on bacterial composition. With arrows of environmental factors are at acute angles to each other, there were high correlation between these clinical oral conditions. Besides, taxa exhibiting the most noteworthy differences across various taxonomic levels demonstrated a robust correlation with clinical oral conditions. In detail, *streptococcaceae*, *proteobacteria*, *streptococcus*, *gammaproteobacteria* displayed a pronounced negative correlation with clinical oral conditions; whereas *bacteroidota*, *bacteroidia*, *leptotrichiaceae*, fusobacterium exhibited a positive association. Further analysis indicates that the microbial function is notably influenced by pertinent environmental factors. Specifically, the environmental scores of the oral gums and palate show positive correlations with microbial functions such as sulfite respiration, sulfur respiration, nitrite respiration, nitrite ammonification, nitrate ammonification, and sulfate respiration. The environmental scores of gums, tooth, and tongue are positively correlated with microbial functions including fumarate respiration, nitrate respiration, and nitrogen respiration, and are negatively correlated with functions such as aerobic chemoheterotrophy, human pathogens – meningitis, human pathogens – septicemia, human-associated pathogens, animal parasites or symbionts, and nitrate reduction (Fig. 3g).

Predictor performance of microbiota

We further investigated whether the oral microbiota could serve as potential biomarkers relevant to SCZ patients using random forest classifier. The model demonstrated a sensitivity of 92% and a specificity of 86%, with the accuracy of 88%. As showed in Fig. 4a and b, in receiver operating characteristics (ROC) analysis, the discriminating models based on the entire oral microbiota effectively distinguished SCZ from HC (AUC = 0.97); and the HC from SCZ (AUC = 0.97). The three microbial features that contributed most to this classifier were *Alloprevotella tannerae*, a phylogenetically distinct clade within

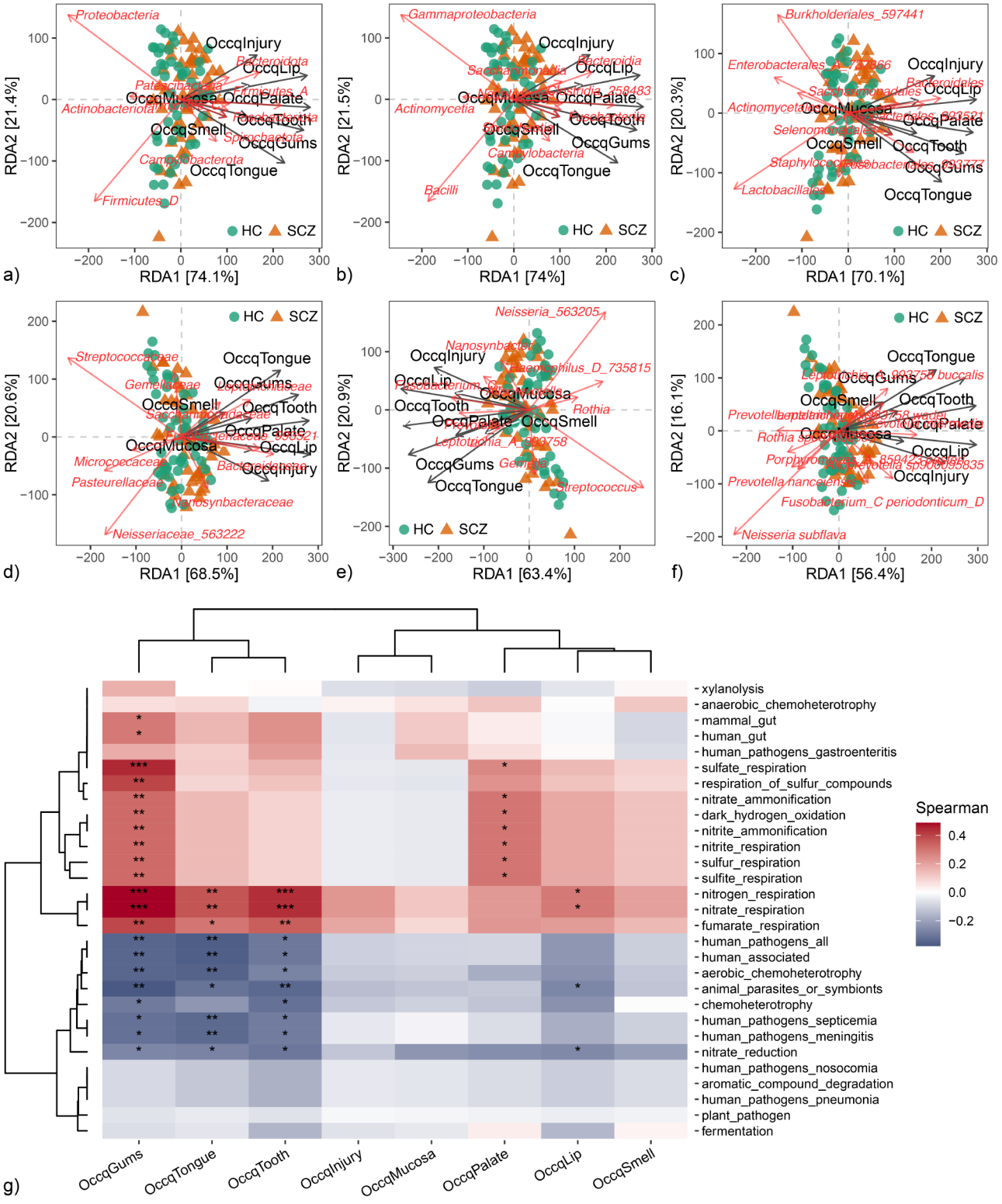

Fig. 3 (See legend on next page.)

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Fig. 3 The impact of environmental factors on microbiota. (**a-f**) Redundancy analysis (RDA) of the relationship between clinical scales' scores and microbial community structure at phylum level (**a**), class level (**b**), order (**c**), family level (**d**), genus (**e**), and species level (**f**). In RDA analysis, the yellow arrows represent key taxa, whereas clinical scales' scores are shown with dark arrows. The scores of the clinical scales correspond to the different subcategories of the Oral Check Questionnaire, which assess the overall oral environment, including aspects such as smell and injury, as well as specific areas of the mouth, such as the mucosa, gums, tongue, teeth, lips, and palate. Correlation between clinical scales' scores and RDA axes are shown by both length and angle of arrows. (**g**) Heat map of correlation coefficients of clinical scales' scores and microbial function based on predictions from the 16 S rRNA data. The x-axis shows the clinical scales' scores. The y-axis shows microbial function predicted by using the Functional Annotation of Procaryotic Taxa (FAPROTAX) annotation database [44]. Red squares indicate positive correlations, while blue squares indicate negative correlations. The statistical significance is denoted on the squares (*$p < 0.05$)

the genus *Campylobacter*, closely related to the species *C. showae*, and *Prevotella intermedia* (S.Figure 2, S.Table 2). The model utilizing microbiota data could accurately distinguish SCZ patients from HCs, suggesting the potential application of microbiota information in patient identification. We also presented the boxplots of relative abundances for the top 10 significant taxa in Fig. 4c–l, with all Wilcoxon rank-sum test p-values < 0.001.

Discussion

This study enrolled 50 drug-free individuals with SCZ and 50 HCs, revealing not only an adverse oral health status in individuals with SCZ but also notable alterations in microbial diversity and community structure. Differences in abundance were noted for specific taxa, including the phyla *Fusobacteriota*, a phylogenetically distinct clade within the phylum *Firmicutes*, and *Actinobacteriota*. Moreover, the identified differential taxa and microbial functions exhibited a strong correlation with clinical oral conditions. Further ROC analysis highlighted the potential of oral microbiota information in identifying individuals with SCZ.

Significant lower oral health conditions in SCZ group

Individuals with SCZ often experience poorer oral health compared to the general population [49]. One strength of this study lies in the comprehensive investigation of both the oral hygiene status and microbiota of individuals. As anticipated, our research observed that patients had worse oral environmental health, with a higher prevalence of cavities and dental plaque. The poorer oral health of patients with schizophrenia may be attributed not only to the side effects of medications but also to lifestyle factors, including inadequate dental hygiene and smoking [50]. Moreover, another potential correlation lies in schizophrenia's connection to immune system dysregulation. Patients with SCZ consistently exhibit elevated concentrations of interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor α, and C-reactive protein compared to healthy controls [51]. This chronic low-grade inflammatory state associated with SCZ may contribute to immune system abnormalities, potentially heightening susceptibility to systemic diseases and local inflammation [52, 53], which could also impact oral health. In conclusion, individuals diagnosed with SCZ typically display poorer oral hygiene, which may influence the oral microbiota [54].

The different oral microbiota in SCZ group

SCZ is a multisystemic disorder affecting the entire body, and abundant evidence has demonstrated various manifestations of the microbiota's impact on the brain [22, 55, 56]. The human microbiota may serve as the foundation for the etiology and pathophysiology of diseases. For complex systemic disease such as SCZ, gaining an understanding of pathways intersecting with the bidirectional microbiota-brain axis is expected to yield novel therapeutic approaches [57]. Significant alterations were observed in microbial diversity and community structure among individuals with SCZ, consistent with some previous studies [58–61]. A shotgun metagenomic study with a small sample size reported contrasting results, finding that the controls exhibited a higher diversity of oropharyngeal microbiota compared to individuals with SCZ [23]. Nevertheless, our results, in line with prior research focusing on oral conditions, indicate that individuals with SCZ exhibit inferior oral hygiene and a heightened incidence of periodontal diseases [62, 63]. Indeed, periodontal diseases are associated with increases in diversity of the microbiota, thought to be the consequence of additional nutrients derived from host tissue damage and increasing physical space as the gingival crevice deepens [64, 65]. In conclusion, the undocumented oral conditions, as well as variations in sequencing methods, sample sizes, and sampling locations, may collectively contribute to the disparities in research outcomes.

Additionally, the oral cavity and the intestine are the parts of the physiological structure with continuous relationship. Other studies focusing on gut microbiota have also identified substantial differences in the gut microbiota of individuals with SCZ compared to HC [19, 60, 66, 67]. The causal relationship between this significant symbiotic microbiota imbalance in SCZ and the disease itself remains unclear. On the one hand, the microbiota is influenced by the patient's lifestyle, and the products of the disturbed microbiota may also directly contribute to the specific signs, symptoms, and etiology of disease [57]. However, it is intriguing that the gut microbiota transplantation can transfer phenotypes. Germ-free mice receiving SCZ microbiota fecal transplants displayed

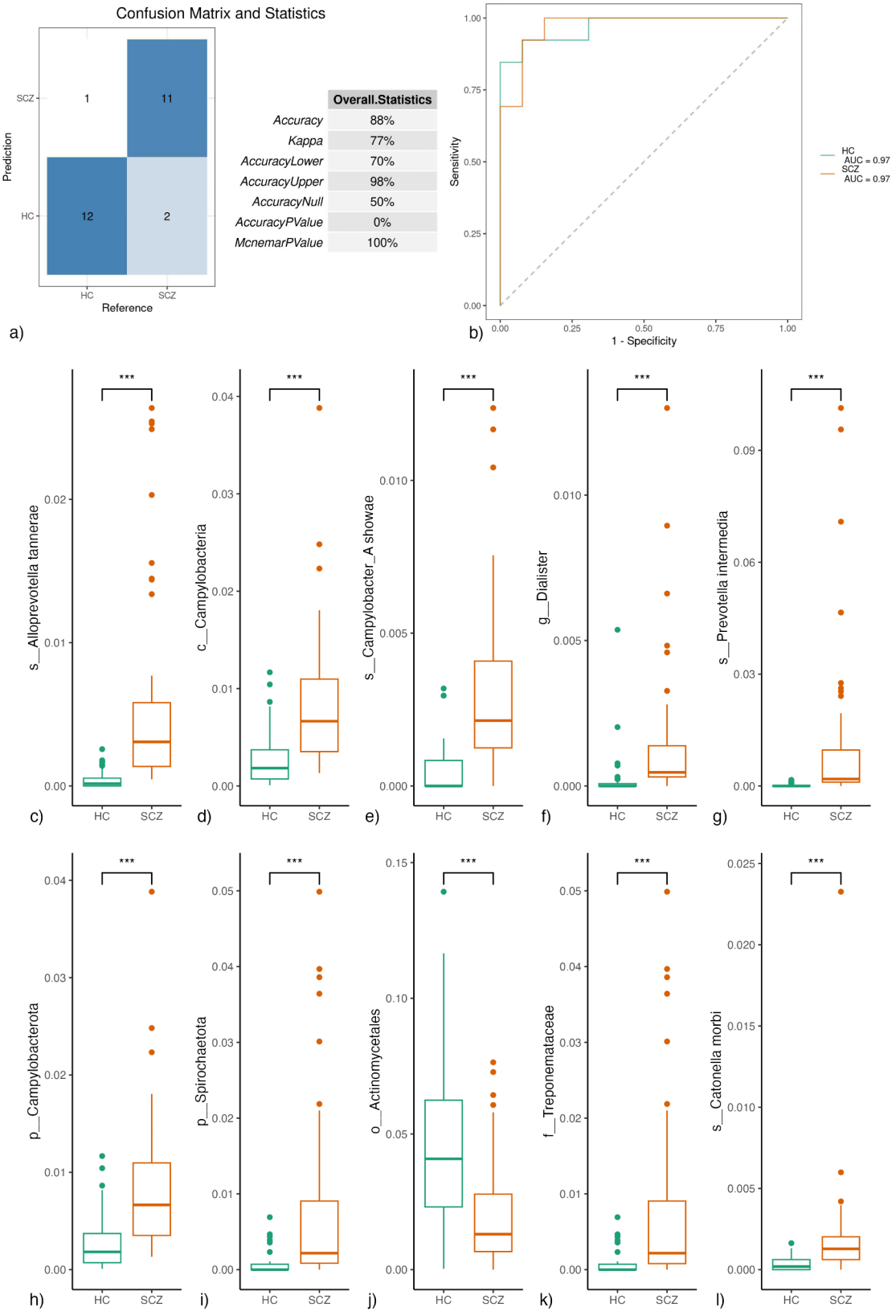

Fig. 4 Predictor performance of microbiota in SCZ patients and HCs. (**a**) Confusion Matrix and Statistics. (**b**) Receiver operating characteristics (ROC) characteristics for the random forest model. (c–l) The boxplots of the relative abundances of the top 10 taxa based on importance rankings in the random forest model

SCZ-relevant behaviors and had lower glutamate and higher glutamine and GABA in the hippocampus [22]. These findings imply a potential causal link between microbiota and certain disease symptoms. Since the pathogenicity of gut microbiota in SCZ patients has been demonstrated to a certain extent, whether oral microbiota have similar properties remains to be explored.

Noticed ASVs in SCZ

Notably, distinct differences in taxonomic abundance, particularly within the phyla *Fusobacteriota*, *Firmicutes*, *Actinobacteriota*, were observed between the two groups. The phylum Firmicutes, further categorized into three phylogenetically distinct clades within the phylum *Firmicutes*, were predominant in the general population and were found to be enriched in the salivary and intestinal microbiota of individuals with SCZ [23, 61, 68, 69]. Similarly, *Fusobacteria*, recognized as common opportunistic oral bacteria associated with various infections [70, 71], displayed a higher relative abundance in SCZ patients, and showed a positive correlation with cognitive function, as evidenced by a previous study focusing on the gut microbiota [72]. In the case of *Actinobacteriota*, another prevalent taxon in the general population, alterations in its abundance were noted in the gut microbiota of individuals with SCZ [73]. Further analysis employing Mendelian randomization revealed causal associations between the class *Actinobacteria* and SCZ [74], providing an intriguing insight into the microbiota's role in SCZ.

The random forest classifier identified several significant microbial features in the SCZ group. *Prevotella intermedia*, a well-known oral pathogen, is not only a major cause of oral diseases such as periodontitis and oral cysts [75–77], but is also linked to systemic conditions like rheumatoid arthritis, adverse pregnancy outcomes, and neurological disorders [78, 79]. For example, in middle-aged and older adults, *Prevotella intermedia* has been negatively associated with cognitive function [80]. The bacterial load of *Prevotella intermedia* is also significantly higher in AD patients [79]. In fact, antibody levels against *Prevotella intermedia* were elevated in AD patients years before cognitive impairment manifested [6]. The inflammatory response triggered by oral pathogens may lead to chronic systemic inflammation, which, in turn, exacerbates central nervous system inflammation and promotes neuropsychiatric disorder progression through complex pathways [81, 82]. *Alloprevotella tannerae* and a phylogenetically distinct clade within the genus *Campylobacter*, closely related to the species *C. showae*, are both Gram-negative bacteria with limited research to date [83]. *Alloprevotella tannerae* has been associated with dentine caries [84, 85] and endodontic infections [86]. The genus *Campylobacter* is currently considered opportunistic oral commensals associated with poor oral health [87] and has been observed to show a significant increase in the oral microbiota of children with autism [88]. Furthermore, other species within this genus have been shown to mediate high immunostimulatory activity, exhibiting robust toll-like receptor (TLR4) stimulatory activity [89], which may lead to excessive inflammatory cytokine secretion and host immune system dysregulation [90, 91].

In random forest analysis, features derived from the microbial community exhibited exceptionally high diagnostic accuracy, with AUC values of 0.97. Given the robust performance of random forest models and the portable, non-invasive nature of oral sampling, the oral microbial community presents itself as a promising tool for clinical diagnosis. Such a high AUC raised concerns about potential overfitting. Therefore, we re-examined the top 10 important taxa and found that these taxa exhibited significant differences between the groups, effectively distinguishing between patients with SCZ and healthy controls. Furthermore, the differential microbes with higher LDA values in LEfSe analysis were mostly identified in the ROC model as taxa with high importance, such as *Alloprevotella tannerae*, a phylogenetically distinct clade within the genus *Campylobacter*, *Campylobacteria*, *Fusobacteriia*, *Rothia*, thereby reflecting the reliability of the results to a considerable extent. However, it is important to note that although both random forest analysis and LEfSe identified taxa with significant differences between the groups, the cross-sectional nature of this study cannot provide definitive evidence to establish these taxa as diagnostic markers for distinguishing patients with SCZ from HCs. A more appropriate approach would be to treat these significant taxa as valuable prior knowledge to guide future research and inform the design of more targeted studies.

Noteworthy, subjects in this study were mainly young and middle-aged drug-free individuals. A recent study investigated oral dysbiosis in Chinese elderly patients with schizophrenia, and identified schizophrenia-associated oral dysbiosis characterized by increased *Streptococcus* and *Fusobacterium*, as well as decreased *Prevotella* and *Veillonella* [59]. Some differential taxa found in these previous studies [23, 59] were not identified in our results, which may be attributed to differences in the study population. The age and oral medication of the subjects may affect the composition of their microbiota [92–95]. Differences in specific oral microbiota taxa vary with age [93]. Similarly, there are sex- and age-related trajectories in the human gut microbiota that are shared between populations of different ethnicities [96]. Although related research is lacking in schizophrenia, studies have demonstrated that there are age-specific differential changes in gut microbiota composition in patients with depression, differential taxa including *Clostridium*, *Streptococcus* and

others [97, 98]. On the other hand, antipsychotic treatment could lead to decreased alpha-diversity as well as increased abundance of the *Actinobacteria* phylum, and decreased abundance in *Clostridium* and *Lactobacillus* in gut microbiota [48, 99, 100]. Thus, our research on a drug-free population could reflect the situation without the disturbance of antipsychotic treatment and offer an accurate view of the oral microbiota in the pathophysiology of schizophrenia.

Poorer oral health in SCZ: possible associations with microbiota?

Additionally, clinical oral conditions displayed a strong correlation with the taxa showcasing the most noteworthy differences and the associated microbial functions. The microbiota community proved highly susceptible to environmental influences. Individuals with SCZ exhibit distinct lifestyles compared to HCs, potentially resulting in changes in the microbiota. Studies on the gut microbiota have recognized the importance of potential confounding factor [101]. Nevertheless, researches concentrated on the oral microbiota often overlook relevant oral conditions, potentially leading to exaggerated or heterogeneous conclusions. Hence, our study meticulously documented relevant scales and conducted analyses to evaluate the impact of the oral environment on the microbiota. Subsequent analysis showed that environmental factors may account for the majority of observed differences between groups. Given that altered lifestyle is a symptom of SCZ, it is crucial to acknowledge that the most taxonomic differences between groups may result from environmental factors. Therefore, a challenge is on mitigating the influence of environmental factors and identifying microbial taxa independently associated with SCZ. To address this issue, integrating new analytical methods or utilizing longitudinal cohorts becomes imperative.

Limitations

Limitations of this research are as follows. Firstly, this study did not employ metagenomic sequencing, which offers higher resolution compared to 16 S rRNA sequencing. However, given the concern of host contamination, we ultimately opted for 16 S sequencing [102]. Deep sequencing should be contemplated once the pertinent issues are adequately addressed. Secondly, as a cross-sectional study, it cannot establish causal relationships between diseases, lifestyles, and microbiota. Changes in the lifestyle of individuals with SCZ may be closely related to the microbiota. Additionally, due to limitations in sample size, subgroup analysis of oral microbial communities in SCZ patients with varying oral environments was not conducted. Therefore, an updated experimental design is warranted, which includes specific analytical methods, expands sample sizes, and incorporates longitudinal studies to pinpoint specific taxonomic groups independently associated with the disease. Additionally, participants willing to undergo the trial represent only a subset of SCZ individuals, and due to the characteristics of SCZ, outcomes for patients with poorer social functioning may be even severe, necessitating further targeted experimental design. Furthermore, whole oral swabs represent a mixed state within the oral cavity. Considering challenges in obtaining specific samples like dental plaque, especially from subjects such as those with SCZ, we initially used oral swabs to obtain a more general result. The next step would involve more detailed research, such as further distinguishing saliva, mucosa, dental plaque, hard palate, etc. Regarding the strains of bacteria that did not yield positive results in this study, it's essential to understand that their absence doesn't necessarily imply no differences between groups. It's plausible that the current sample size is insufficient to detect potentially subtle yet meaningful differences. Additionally, limitations in conditions prevented us from conducting more in-depth research using cultivation methods for the differential bacteria found in this study. In conclusion, we aim for this study to serve as a foundational reference for future research in this area, encouraging more thorough exploration and understanding of the microbiota's role in SCZ.

Conclusions

Drug-free SCZ patients exhibit a less favorable oral environment, along with significant changes in microbial diversity, community structure, and microbial abundance. The abundance of significantly altered microbiota appears to be closely intertwined with the oral environment, warranting further exploration.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12888-025-06633-6.

Supplementary Material 1

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Author contributions

Huawei Huang: Investigation, Methodology, Data Curation, Funding acquisition, Writing - Review & Editing; Naiyan Yang: Investigation, Methodology, Data Curation, Funding acquisition, Writing - Review & Editing; Mian-mian Chen: Writing - Original Draft, Writing - Review & Editing; Xiaoting Chen, Wei Chen, Xiaoping Li, Yuchun Chen, Zhengang Deng, and Wenbing Zhou: Investigation, Data Curation; Shu-xian Xu: Writing - Original Draft, Writing - Review & Editing; Xin-hui Xie: Conceptualization, Methodology, Formal analysis, Visualization, Project administration, Supervision, Writing - Review & Editing.

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Data availability
The raw sequence data have been deposited in the Genome Sequence in National Genomics Data Center (GSA: CRA015747, https://ngdc.cncb.ac.cn/gsa).

Declarations

Ethical approval and consent to participate
This study was conducted at Huizhou Mental Health Center (the Second People's Hospital of Huizhou) in accordance with the Declaration of Helsinki (revised edition, 2013) [103]. The Human Ethics Committee of Huizhou Mental Health Center approved the study protocol. Patients or their legal guardians provided informed consents and all participants could withdraw from the trial at any time for any reason. This report follows the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [104].

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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