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Differences in the subgingival microbial composition associated with alcohol intake: A systematic review

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ABSTRACT

Objectives: This study aimed to conduct a systematic evaluation of the literature on whether individuals exposed to alcohol intake present differences in the subgingival microbial composition compared to those unexposed.
Methods: Five databases (MEDLINE, EMBASE, LILACS, SCOPUS and Web of Science) and one source of grey literature (Google Scholar) were searched by two independent reviewers up to December 2022 according to pre-specified eligibility criteria. No restrictions were imposed regarding the date and language of publication and the periodontal status of the participants. The Newcastle-Ottawa Scale was used for methodological quality appraisal and a narrative synthesis was performed.
Results: Eight cross-sectional studies and one cross-sectional analysis nested in a cohort were considered for qualitative analysis, including data of 4636 individuals. Overall, the studies exhibited considerable heterogeneity in terms of characteristics of the participants and microbiological methods. Four studies have high methodological quality. Exposed individuals have higher overall quantity of periodontal pathogens in shallow and moderate to deep pockets. Findings on richness, relative abundance, alpha- and beta-diversity were limited and inconclusive.
Conclusion: The subgingival microbiota of individuals exposed to alcohol intake has higher overall quantity of red (i.e., *P. gingivalis*) and orange-complex (i.e., *F. nucleatum*) bacteria when compared to those unexposed.

1. Introduction

Periodontitis, in its current definition, is a chronic inflammatory disease associated with dysbiotic dental biofilms and characterized by progressive destruction of the tooth-supporting apparatus.[1] Recently, the theory of multi-causation acting in its aetiology has gained support – that is, several causal components may cooperatively drive the periodontal destruction, and the disease development and progression are influenced by the interplay between genetic, lifestyle, socioeconomic, contextual and even unknown factors,[2,3] which ultimately affect the inflammatory profile and drives a shift in microbial composition, specially at (but not restricted to) the subgingival environment.[4,5]

Among lifestyle factors related to periodontitis, alcohol intake has shown to be associated with its occurrence in cross-sectional studies,[6–8] and some pathways were suggested to explain this association: besides the fact that ethanol is a substrate for bacterial metabolism,[9,10] alcohol use overtime may create a favourable environment with sufficient energy for the growth of anaerobic periodontal pathogens due to disturbed host-bacterium interactions[11] and depletion of microbiota associated with periodontal health.[12] Although five systematic reviews had explored the impact of alcohol consumption on periodontal status,[13–17] it is not clear whether the described exposure impacts the presence or levels/proportions/overall quantity of specific pathogens, as well as the homeostatic integrity of the polymicrobial biofilm, which are likely to be altered prior to the development of clinically detectable disease. Currently, little is known regarding exogenous exposures that cause dysbiosis of the subgingival microbiota.

Moreover, given the recent findings from Finland suggesting no

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association between type of alcoholic beverage, frequency, and volume of ingestion with incidence of periodontal pockets,[18,19] contrasting with previous cohorts,[20,21] it is relevant to step back and examine a potential path linking exposure to disease occurrence. Therefore, we aimed to systematically evaluate the literature on whether individuals exposed to alcohol intake have differences in the subgingival microbial composition compared to their unexposed counterparts.

2. Materials and methods

This systematic review was reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[22] and conducted based on the Cochrane Handbook for Systematic Reviews of Interventions with modifications for a review of exposures.[23] The protocol is available at the following link: https://osf.io/h3ua6/.

2.1. Eligibility criteria

We aimed to answer the following focused question: Do individuals aged 15 years or more, with or without periodontitis (Population), exposed to high levels of alcohol consumption or alcohol use disorders (AUD) (Exposure), show differences in the composition of the subgingival microbiota (Outcome), when compared with those lifetime abstainers (those having not drunk in the entire lifetime), current non-drinkers (those having not drunk in the previous 12 months) or exposed to lower levels of alcohol (Comparator)?

We imposed no restriction regarding language, publication date, microbial diagnostic and sample collection methods used. We excluded case reports, reviews, letters to the editor, conference abstracts, preprints, reports that did not perform any statistical analysis for microbiological data between exposed and unexposed groups and which investigated samples from any other origin. In this respect, we must acknowledge that although saliva has been proposed as an alternative to subgingival biofilm for studying the oral microbiota associated with periodontitis, recent evidence suggested that one-time saliva sampling cannot replace subgingival plaque for microbial analysis of red-complex bacteria in patients with periodontitis.[24] Moreover, salivary microbiota has been also strongly linked to oral cancer[25] and, as our main interest was to investigate the mechanism through which alcohol intake impact the periodontitis occurrence via microbiota composition, we included only studies that assessed the microbiome at subgingival environment.

Importantly, the primary outcome was the count (frequency/proportions/overall quantity) of specific pathogens or relative abundance and, as secondary outcomes, we planned to include findings on pathogens presence (qualitative measure), richness (number of species), alpha- [a measure to evaluate the richness and evenness (how well each specie is represented)] and beta-diversity (a measure of interindividual diversity that assesses the similarity of communities).

2.2. Information sources and search strategy

Data search was performed in The US National Library of Medicine (MEDLINE-PubMed), EMBASE, LILACS, SCOPUS, and Web of Science databases up to 26 December 2022. The reference lists of the included studies were hand-searched to identify additional relevant papers. An additional search was performed in Google Scholar (first 300 most relevant hits) to address grey literature.

We designed a structured search strategy (Online Resource 1) combining both controlled and free text terms. We consulted some systematic reviews addressing similar research questions[26–29] to retrieve important search terms and validate our strategy.

2.3. Selection process

The titles and abstracts of the reports retrieved from the searches were screened by two independent reviewers (LMO and FBZ) for the preselection of those that potentially met the eligibility criteria. Retrieved records were classified as “include”, “exclude” or “uncertain” and the Mendeley Desktop 1.19.8 (England) was used to group and manage the references.

Thereafter, full-text versions of potentially relevant papers were obtained. Studies fulfilling all eligibility criteria were screened again by two independent reviewers (LMO and FBZ) and processed for data extraction. Divergences of opinion among the reviewers were resolved by discussion. The judgement of an additional reviewer (RPA) was considered decisive if a disagreement persisted.

2.4. Data collection and items

One reviewer (LMO) extracted data on study’s identification (first author, design, year and location of publication), sample characteristics (sex and age), exposure definitions (including reported cut offs and information on recall and lifetime use), microbial sampling (index sites and teeth) and diagnostic [targeted or next-generation techniques (NGS)] methods, microbiological outcomes and study’s main findings. A second reviewer (FBZ) supported this assessment checking the process.

2.5. Methodological quality assessment

As only cross-sectional analyses were retrieved and included, we used an adapted version of the Newcastle-Ottawa Scale to rate the methodological quality.[30] Each item on the scale is scored with one star, except for exposure and outcome assessments and comparability, which can be given up to two stars. Therefore, the maximum score for each study was 10.

Although measurements of volume and frequency of alcohol consumption are recurrent in the literature, the instability of this approach may impair its validity.[31] Thus, we considered only AUD questionnaires [i.e., Alcohol Use Disorders Identification Test (AUDIT) or Cut Down, Annoyed, Guilty, Eye-opener (CAGE)] as validated measurement tools. Smoking and periodontal status were set as the most important confounding factors.

The described assessment was performed independently by two reviewers (LMO and FBZ), and disagreements were resolved through discussion. If consensus was not reached, a third judgement (RPA) was considered.

2.6. Data synthesis

The assessed microbiological outcomes were heterogeneous and meta-analysis was not feasible. In this perspective, we perform a narrative synthesis considering the following structure: (a) study selection and (b) descriptive qualitative analysis on characteristics of the included studies. Data were pooled into an evidence table according to the year of publication to determine the quantity of data.

3. Results

The electronic search strategy provided 640 unique records. After initial screening (titles and abstracts), 14 full-text publications were comprehensively evaluated and five were excluded (Online Resource 2). Thus, nine studies[11,32–39] were included in this systematic review. Fig. 1 displays the review workflow.

3.1. Study characteristics

Table 1 presents the main characteristics of the included studies. A total of 4636 individuals were evaluated, with age range varying from 16 to 83 years, and all studies presented cross-sectional analyses. Five papers stated the ethnicity of the participants.[32,35,36,38,39] Three studies assessed the volume and frequency of alcohol consumption,[32,37,38] two only the frequency[35,36] and three measured AUD.[11,33,34] In one,

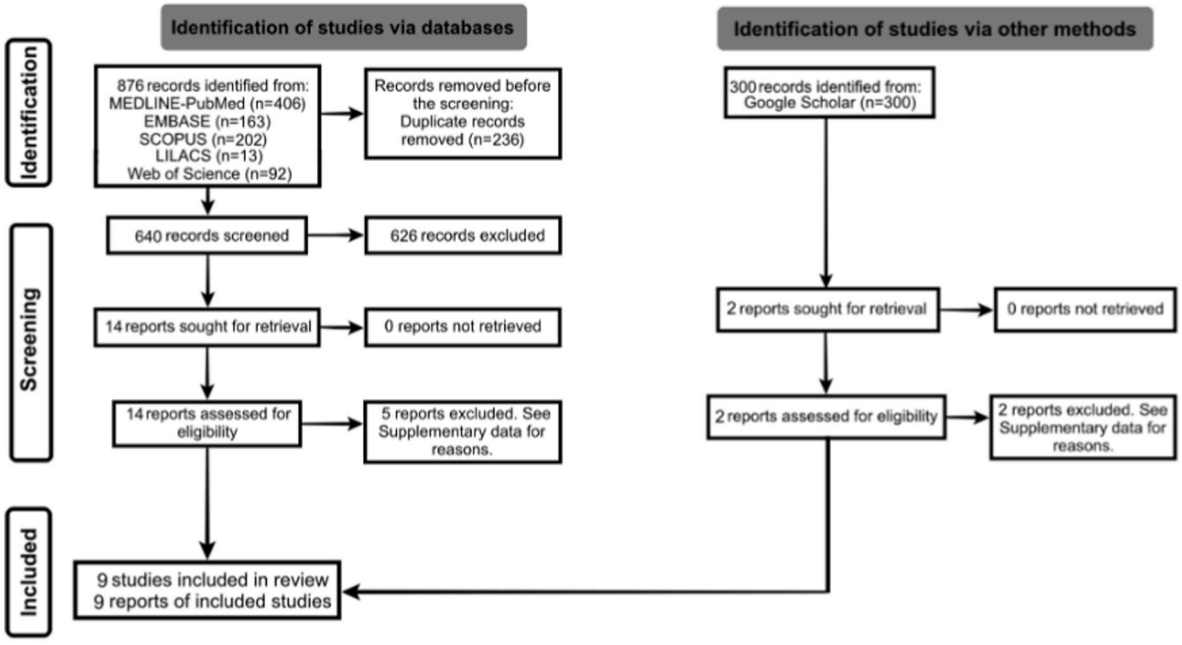

Fig. 1. PRISMA flow diagram that was followed in this review.

however, there was no clear report on alcohol exposure definition.[39] Subgingival plaque was collected with sterile paper points[11,34,37–39] or curettes.[33,35,36] Only one study used an open-ended approach as microbial diagnostic test,[36] all other papers used targeted techniques.

Eligibility criteria differed between studies. Four studies set the number of present teeth as an inclusion criterion: ≥ 27,[36] ≥ 15,[33] ≥ 14[11] and ≥ 6[38]; and most excluded patients that required antibiotic treatment or had received it in the past months.[11,32,33,36,37,39] Exclusions of systemically impaired and periodontitis patients were reported in two[33,37] and one study,[36] respectively, and other included only individuals diagnosed with generalized chronic periodontitis.[34] Three studies restricted the inclusion to patients that had not been submitted to periodontal therapy in the preceding 3[34,39] and 6 months[33] to the commencement of data collection.

3.2. Methodological quality assessment

Summarized results of the methodological quality appraisal are displayed in Table 2. Four studies present high methodological quality (≥7 stars).[11,32,33,35] Only two studies provided data on non-response rate[33,35] and five justified the sample size.[11,32,33,35,38] Five studies controlled the analyses for both smoking and periodontal status[11,32,33,35,39] and three did not describe measurements of association in details.[32,36,39]

3.3. Syntheses

Table 1 provides summarized information on the main findings of each study. We reported the syntheses according to the different microbiological outcomes assessed.

3.3.1. Overall quantity of target bacteria

Three studies evaluated the impact of AUD on proportions/frequency/overall quantity of subgingival bacteria and increased taxa of *Fusobacterium nucleatum*,[11,33] *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*[33,34] were shown in exposed individuals. A significant difference in the subgingival microbial composition was consistently detected when comparing exposed and unexposed patients with periodontitis.[11,33,34] Nevertheless, such difference was not consistent when comparing exposed and unexposed patients without periodontitis – while Amaral et al. (2011)[14] found significant higher counts of *Capnocytophaga sputigena, F. nucleatum nucleatum, F. nucleatum vincentii, Gemella morbilorum, Neisseria mucosa, P. gingivalis, Streptococcus gordonii* and *Tannerella forsythia* and lower counts of *Streptococcus anginosus* and *Pseudomonas aeruginosa* in sites with probing pocket depth (PPD) < 4 mm, Lages et al. (2015)[11] detected no significant difference for counts of *A. actinomycetemcomitans, Eikenella corrodens, F. nucleatum, P. gingivalis* and *Prevotella intermedia* (in this study, periodontitis was defined as ≥ 4 teeth with ≥1 site with PPD ≥4 mm and clinical attachment loss ≥3 mm). Besides that, when comparing sites with probing pocket depth (PPD) < 4 mm from the exposed group and sites with PPD ≥4 mm from the unexposed group, statistically significant higher counts of *C. sputigena, F. nucleatum nucleatum, F. nucleatum vincentii, G. morbilorum, N. mucosa, P. gingivalis, S. anginosus, S. gordonii* and *T. forsythia* were detected in the AUD group.[33]

Analyses controlling for the periodontal and smoking status were performed in three[11,33,34] and two studies,[11,33] respectively. Importantly, the definition of the unexposed group was poorly described in one[34] and the risk of contamination is uncertain.

When comparing the proportion of subgingival bacteria according to the frequency of alcohol consumption, those exposed at least twice a month have significantly higher frequency of *P. gingivalis* than their counterparts.[35] Since the unexposed group was composed by both current abstainers and those who consumed alcohol less than twice a month, contamination is likely.

3.3.2. Presence of target bacteria

Five[32–34,38,39] out of seven studies detected no statistically significant difference between groups for the presence of different bacteria. The remaining two studies present contradictory results – although in one the consumption of alcohol at least twice a month was significantly associated with the presence of *P. gingivalis*,[35] in the other the ingestion of a couple of glasses of red wine every day for at least two years was associated with lower prevalence of *Actinomyces naeslundii, Dialister pneumosites, F. nucleatum, Magasphera micromuciformis, Peptostreptococcus micros, Peptostreptococcus anaerobius, P. gingivalis, P. intermedia, Rothia dentocariosa* and *Treponema denticola*.[37]

3.3.3. Richness, relative abundance, alpha- and beta-diversity

In one study, the exposed group showed fewer denaturing gradient

Table 1
Characteristics of the included studies.

Identification	Sample characteristics	Comparison groups	Sampling and microbial diagnostic methods	Microbiological outcome	Main findings and commentaries
Umeda et al., 1998; United States; Cross-sectional;	199; ♂: 82 ♀: 117 Age range: 16–83 years; Mean age (stratified by ethnicity): 46.4 [16–73 (Caucasians)] 53.0 [21–79 (African-Americans)] 36.0 [17–83 (Asian-Americans)] 42.3 [16–72 (Hispanics)];	Reference group (N = 104): None (unclear criteria); Exposed groups: Slight [unclear criteria (N = 82)] Medium [unclear criteria (N = 10)] Heavy [unclear criteria (N = 3)];	The four deepest periodontal pockets, preferably each from a different quadrant; PCR[a];	Presence: *A. actinomycetemcomitans, B. forsythus, P. gingivalis, P. intermedia, P. nigrescens, T. denticola;*	*There was no statistically significant difference between groups regarding the occurrence of any microorganism; *Analysis adjusted for age, sex, ethnicity, income, smoking status, PPD[b] and CAL[c];
Tezal et al., 2001; United States; Cross-sectional;	1371; ♂: 661 ♀: 710 Age range: 25–74 years; Mean age: NR;	Reference group: <5 drinks/week (N = 1136) or < 10 drinks/week (N = 1215); Exposed group: ≥5 drinks/week (N = 235) or ≥ 10 drinks/week (N = 156);	Mesiobuccal sites of Ramfjord teeth and right first premolar, right lateral incisor, left first molar at maxilla, and left first premolar, left lateral incisor, right first molar at mandibula; Immunofluorescence assay;	Presence: *A. actinomycetemcomitans, B. forsythus, C. rectus, Capnocytophaga* species, *E. saburreum, F. nucleatum, P. gingivalis, P. intermedia;*	*There was no statistically significant difference between groups regarding the occurrence of any microorganism; *Analysis adjusted for age, sex, smoking status, diabetes and CAL;
Signoretto et al., 2010; Italy; Cross-sectional;	75; ♂: 31 ♀: 44 Age range: 22–65 years;	Reference group (N = 27): Current abstainers or occasionally wine drinkers; Exposed group (N = 17): Couple of glasses (200 ml each) of red wine every day for at least two years;	Lingual or buccal site of mandibular molar (either left or right); PCR-DGGE[d];	Richness: number of DGGE bands; Presence: *A. naeslundii, D. pneumosites, F. nucleatum, M. micromuciformis, P. micros, P. anaerobius, P. gingivalis, P. intermedia, R. dentocariosa, T. denticola;*	*The samples from the exposed group had fewer bands; *The exposed group showed statistically significant lower prevalence of individuals presenting any of the target microorganisms; *Analyses adjusted for age and sex;
Amaral et al., 2011; Brazil; Cross-sectional;	98; ♂: 98 ♀: 0 Age range: 30–60 years; Median age (stratified by AUD[e] occurrence): 42 (unexposed) 46 (exposed);	Reference group (N = 49): CAGE[f] score <2; Exposed group (N = 49): ICD-10 diagnostic criteria;	Sites with PPD <4 mm and ≥4 mm; Checkerboard DNA-DNA hybridization;	Presence and proportions: *A. actinomycetemcomitans, A. baumannii, A. gerencseriae, A. israelii, A. odontolyticus, A. naeslundii I, A. oris, C. gingivalis, C. ochraceae, C. rectus, C. sputigena, C. showae, E. nodatum, E. corrodens, E. saburreum, E. faecalis, E. coli, F. nucleatum nucleatum, F. periodonticum, F. nucleatum polymorphum, F. nucleatum vincentii, G. morbilorum, H. pylor, L. buccalis, N. mucosa, P. micra, P. gingivalis, P. aeruginosa, P. intermedia, P. melaninogenica, P. nigrescens, P. acnes, S. noxia, S. aureus, S. anginosus, S. constellatus, S. gordonii, S. intermedius, S. oralis, S. mitis, S. sanguinis, T. forsythia, T. denticola, V. parvula;*	*There was no statistically significant difference in the presence of any of the target bacteria by AUD; *Overall, the exposed group showed statistically significant higher counts of *A. actinomycetemcomitans, F. nucleatum nucleatum* and *P. gingivalis*; *In sites with PPD <4 mm, the exposed group showed statistically significant higher counts of *C. sputigena, F. nucleatum nucleatum, F. nucleatum vincentii, G. morbilorum, N. mucosa, P. gingivalis, S. gordonii* and *T. forsythia* and lower counts of *S. anginosus* and *P. aeruginosa*; *In sites with PPD ≥4 mm, the exposed group showed statistically significant higher counts of *C. sputigena, F. nucleatum nucleatum, F. nucleatum vincentii, G. morbilorum, N. mucosa, P. gingivalis, S. anginosus, S. gordonii* and *T. forsythia*; *When comparing sites with PPD <4 mm from the exposed group and sites with PPD ≥4 mm from the reference group, the exposed group showed statistically significant higher counts of *C. sputigena, F. nucleatum nucleatum, F. nucleatum vincentii, G. morbilorum, N. mucosa, P. gingivalis, S. anginosus, S. gordonii* and *T. forsythia*; *Groups were balanced for smoking and those presenting diabetes were not included. The exposed group

(*continued on next page*)

Table 1 (*continued*)

Identification	Sample characteristics	Comparison groups	Sampling and microbial diagnostic methods	Microbiological outcome	Main findings and commentaries
					presented statistically significant higher means of PPD and CAL;
Lages et al., 2015; Brazil; Cross-sectional;	88; ♂: 47 ♀: 41 Age range: 35–55 years; Mean age: 47.3 ± 5.7;	Reference group (N = 44): AUDIT[g] score = 0 CAGE score = 0 Frequency of consumption: never or less than once a month; Exposed group (N = 44): AUDIT score >8 CAGE score ≥2 Frequency of consumption: ≥4 times a week;	Deepest pocket per tooth associated with BoP[h] (non-contiguous dental surfaces) in six teeth; Real-time PCR;	Proportions: *A. actinomycetemcomitans, E. corrodens, F. nucleatum, P. gingivalis, P. intermedia;*	*In patients with periodontitis (N = 44), those exposed to AUD showed higher proportions of *E. corrodens, F. nucleatum* and *P. intermedia* than those unexposed; *In patients without periodontitis (N = 44), there was no statistically significant difference in the proportions of any of the target bacteria by AUD; *Those exposed to AUD presented statistically significant worse periodontal status; *Groups were balanced for smoking, diabetes, body mass index, income and education;
Sender-Janeczek & Zietek, 2016; Poland; Cross-sectional;	50; ♂: 35 ♀: 15 Age range: 21–73 years; Mean age: NR;	Reference group (N = 25): Unclear criteria (patients that did not have any alcohol-related problems in their medical history); Exposed group (N = 25): Unclear criteria (hospitalized patients with alcohol dependence);	Four deepest sites (PPD ≥4 mm and CAL ≥3 mm); Real-time PCR;	Presence and proportions: *A. actinomycetemcomitans, P. gingivalis, T. denticola, T. forsythia;*	*There was no statistically significant difference in the presence of any of the target bacteria by AUD; *There was no statistically significant correlation between the amount of alcohol consumption and presence of any of the target bacteria; *Patients with alcohol dependence showed higher proportions of *A. actinomycetemcomitans, P. gingivalis* and *T. denticola* than their counterparts; *All patients were diagnosed with generalized periodontitis (>30% of sites with CAL ≥3 mm and PPD ≥4 mm); *Duration of alcohol dependence: 12.8 ± 11.8;
Mai et al., 2016; United States; Cross-sectional analysis nested in a cohort study;	1252; ♂: 0 ♀: 1252 Age range: 53–83 years; Mean age: 66.6 ± 7.0;	Mean ounces/day over the last year;	Mesiobuccal sites of Ramfjord teeth and right first premolar, right lateral incisor, left first molar at maxilla, and left first premolar, left lateral incisor, right first molar at mandibula; Immunofluorescence assay;	Presence: *C. rectus, F. nucleatum, P. gingivalis, P. intermedia* and *T. forsythia;*	*There was no statistically significant difference in the presence of any of the target bacteria by volume of alcohol consumption;
Yu et al., 2017; United States; Cross-sectional;	43; ♂: 35 ♀: 15 Age range: 21–73 years; Median age (stratified by smoking status): 34 [28–50 (non-smokers)] 45 [33–49 (smokers)];	Reference group (N = 37): Rarely consumers; Exposed group (N = 6): Weekly consumers;	Mesiobuccal sites of Ramfjord teeth; 16S rRNA sequencing;	Alpha diversity: Shannon index; Beta-diversity: PCoA[i] based on unweighted and weighted UniFrac distance; Relative abundance;	*There was no statistically significant difference in any outcome by frequency of alcohol consumption; *Patients diagnosed with periodontitis were excluded;
Torrungruang et al., 2020; Thailand; Cross-sectional;	1460; ♂: 1044 ♀: 416 Age range: 39–66 years; Mean age: 47.3 ± 4.4;	Reference group (N = 1003): Frequency of consumption of less than twice a month or quitters; Exposed group (N = 457): Frequency of consumption of at least twice a month;	Mesiobuccal surfaces in the right quadrants and the mesiolingual surfaces in the left quadrants; 16S rDNA-based real-time PCR;	Presence and proportions: *P. gingivalis*;	*Drinkers had 1.4-fold increased odds (95% CI: 1.0–2.0) of detecting *P. gingivalis* compared to their counterparts; *Drinkers present higher proportions of *P. gingivalis* than their counterparts; *Analyses adjusted for age, sex, smoking status, diabetes, education and genetic polymorphism (*FokI*);

[a] PCR: Polymerase Chain Reaction.
[b] PPD: Probing pocket depth.
[c] CAL: Clinical attachment loss.
[d] DGGE: Denaturing gradient gel electrophoresis.
[e] AUD: Alcohol use disorders.
[f] CAGE: Cut Down, Annoyed, Guilty, Eye-opener.
[g] AUDIT: Alcohol Use Disorders Identification Test.
[h] BoP: Bleeding on probing.

[i] PCoA: Principal Coordinate Analysis.

Table 2
Quality assessment of the included studies using the Newcastle-Ottawa Scale.

Study	NOS domains			Scores
	Selection	Comparability	Outcome	
Umeda et al., 1998	–	**	**	4
Tezal et al., 2001	***	**	**	7
Signoretto et al., 2010	*	*	***	5
Amaral et al., 2011	****	**	***	9
Lages et al., 2015	***	**	***	8
Sender-Janeczek & Zietek, 2016	**	*	***	6
Mai et al., 2016	**	–	***	5
Yu et al., 2017	–	*	**	3
Torrungruang et al., 2020	****	**	***	9

gel electrophoresis bands than those unexposed, indicating reduced species richness.[37] On the other hand, no significant difference was found for relative abundance, alpha and beta-diversity when comparing weekly and rarely alcohol consumers.[36]

4. Discussion

Recent systematic reviews have shown that alcohol intake, in its distinct definitions, is associated with higher prevalence[13–16] and, in some scenarios, incidence of periodontitis,[17] although the mechanistic link behind this association needs to be further explored. To the best of our knowledge, this is the first systematic review conducted to evaluate the impact of alcohol consumption and AUD on the composition of subgingival microbiota among individuals with and without periodontitis. In this respect, we comprehensively searched records indexed in six biomedical databases and the entire review process was performed according to a pre-established protocol to minimize biases. There is considerable variance between the selected studies in terms of characteristics of the participants - that may also act as confounders in the association studied (i.e., age, periodontal and smoking status) - sampling and diagnostic methods, all of which collectively affect the applicability of evidence. Given the described high heterogeneity across the selected studies, the findings were presented under the form of a narrative synthesis. In addition, since all included studies present cross-sectional analyses, we cannot infer causality in the results. The fact that more than half of the papers included in this review have low methodological quality reveals the challenge of obtaining representative samples and controlling confounding factors in studies investigating the subgingival microbiome.

Overall, the results suggest that those exposed to higher frequency[35] of alcohol consumption, and mainly AUD,[11,33,34] have higher levels of some periodontal pathogens (i.e., *F. nucleatum, P. gingivalis* and *A. actinomycetemcomitans*) and such difference seems to be more pronounced when comparing patients with periodontitis,[11,34] although clear data on gingivitis and periodontal health are absent. It is also expected that shallow pockets of exposed individuals harbor higher proportions of red and orange microbial complexes [i.e., *F. nucleatum*, *P. gingivalis* and *T. forsythia*[40]] when compared not only with shallow but with moderate to deep pockets from those unexposed.[33] The absence of statistically significant difference between groups regarding the presence of such bacteria was not surprised,[32–34,38,39] since these microorganisms were found to be present in ecological sites, even in states of periodontal health, at very low abundance.[41] Lastly, further studies using NGS are required to draw conclusions on richness, alpha and beta-diversity of the subgingival microbiota associated with alcohol abuse.

The exact molecular mechanisms by which alcohol intake act in the subgingival microbial ecotype are not yet clear. Recently, studies investigating mouthwash[12,42] and salivary[43] samples identified features of a dysbiotic community such as decreased abundance of commensals, overall loss of diversity and enriched pathogenic taxa in heavy drinkers. The increased proportions of specific periodontal pathogens caused by ethanol are possibly a result of the decreased commensals (i.e, *Lactobacillales*, *Streptococcus* and *Rothia*) levels[12,44] and/or inflammation-related disturbances (which are expected to act on periodontal pocketing) associated with the immune subversion on the gingival crevicular fluid, ultimately impairing the host-microbial balance.[45] Previous studies also reported impaired neutrophil function[46] and reduced monocyte production of cytokines[47] following alcohol abuse, factors that contribute to increased bacterial proliferation and penetration. This complex interplay sheds light that the association of alcohol intake and periodontitis is driven by a shift in the subgingival microbial composition of those exposed.

Curiously, despite the finding of higher proportions of red and orange complex bacteria at the subgingival environment of AUD patients, differences on specific taxa were found between studies. For instance, increased proportions of *P. gingivalis* were consistent in the included literature,[33–35] except in one study that found no significant difference between groups regarding this pathogen but detected increased levels of *P. intermedia* and *F. nucleatum*.[11] In this respect, *P. intermedia* was also investigated in the study conducted by Amaral et al. (2011)[14]; however, there was no significant difference between groups regarding this target bacteria. Such divergences may be explained due to high inter-individual variance when dealing with physiologic or pathologic microbiome, possibly because differences in consumed food, age, and ethnic background.[48] Furthermore, even though both studies[11,33] controlled the analyses for the periodontal condition – either by comparing exposed and unexposed groups stratified by the presence of periodontitis or evaluating the composition of subgingival microbiota at shallow and moderate to deep pockets – in Lages et al. (2015),[11] the sample was collected from the deepest pockets associated with bleeding on probing and, therefore, one may hypothesize that even in individuals without the described case definition of periodontitis, the sample collection could have been done in moderate to deep pockets, mitigating the difference between groups.

Considering the complexity and variability of the microbiota within and amongst subjects, particularly in the levels of periodontal pathogens, the use of methods that are limited to a small range of microorganisms and samples may lead to incomplete or conflicting results.[49] Moreover, the actual knowledge on the role of microbiota on periodontitis pathogenesis supports the hypothesis that the entire community acts as a collective pathogenic unit.[41] Therefore, we consider that next step on this field should be to make more studies using NGS techniques. In these approaches, diversity indexes (i.e., Chao 1, Shannon and Simpson index) consider not only the number of species found (richness), but also the evenness of species distribution.[27] Only one included study used 16S rRNA sequencing for microbial analysis, and no difference was detected between rarely and weekly alcohol drinkers regarding alpha and beta-diversity and relative abundance.[36] This finding may be attributable to contamination of both risk and reference categories, since those rarely consumers might drink higher quantities in rare episodes and weekly consumers might drunk very low doses in a unique week episode. Such measurement bias is an important concern in alcohol research because makes the effect of heavy drinking less obvious.[50]

Lastly, the role of specific beverages should be further investigated. The consumption of couple of glasses of red wine every day was associated with lower prevalence of periodontal pathogens.[37] In this context, a similar protective effect of wine consumption on periodontitis occurrence was also detected.[51] Thus, the amount of alcohol consumed by type of beverage seems to provide more relevant information on the

predictive role of alcohol intake on subgingival microbiota composition. In addition, improvements on reporting quality are needed since the report of all included studies was suboptimal considering a recent guidance on sample metadata for recording in oral microbiome studies.[52] Altogether, the described aspects may clarify the role of alcohol intake on the dysbiosis of the subgingival microbiota.

In conclusion, individuals exposed to alcohol intake present differences on subgingival microbial composition compared with their counterparts. Alcohol drinkers, especially those with periodontitis, have higher proportions of red (i.e., *P. gingivalis*) and orange-complex (i.e., *F. nucleatum*) bacteria in shallow and moderate to deep pockets.

CRediT authorship contribution statement

Leandro Machado Oliveira: Conceptualization, Data curation, Formal analysis, Writing – Original draft. Raquel Pippi Antoniazzi: Writing – Review & editing. Flávio Fernando Demarco: Writing – Review & editing. Fabrício Batistin Zanatta: Data curation, Writing – Review & editing.

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Availability of data and materials

Authors are available to share any data upon request.

Code availability

Not applicable.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Declaration of competing interest

The authors declare no conflict of interest.

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