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To cite this article: Siobhan P. Moran, Bob T. Rosier, Fiona L. Henriquez & Mia C. Burleigh (2024) The effects of nitrate on the oral microbiome: a systematic review investigating prebiotic potential, Journal of Oral Microbiology, 16:1, 2322228, DOI: [10.1080/20002297.2024.2322228](https://doi.org/10.1080/20002297.2024.2322228)

To link to this article: <https://doi.org/10.1080/20002297.2024.2322228>



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Published online: 27 Feb 2024.



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## The effects of nitrate on the oral microbiome: a systematic review investigating prebiotic potential

Siobhan P. Moran<sup>a</sup>, Bob T. Rosier<sup>b</sup>, Fiona L. Henriquez<sup>a</sup> and Mia C. Burleigh<sup>a</sup>

<sup>a</sup>School of Health and Life Sciences, University of the West of Scotland, Blantyre, UK; <sup>b</sup>Department of Health and Genomics, Center for Advanced Research in Public Health, FISABIO Foundation, Valencia, Spain

### ABSTRACT

**Background:** Nitrate ( $\text{NO}_3^-$ ) has been suggested as a prebiotic for oral health. Evidence indicates dietary nitrate and nitrate supplements can increase the proportion of bacterial genera associated with positive oral health whilst reducing bacteria implicated in oral disease(s). In contrast, chlorhexidine-containing mouthwashes, which are commonly used to treat oral infections, promote dysbiosis of the natural microflora and may induce antimicrobial resistance.

**Methods:** A systematic review of the literature was undertaken, surrounding the effects of nitrate on the oral microbiota.

**Results:** Overall,  $n = 12$  *in vivo* and *in vitro* studies found acute and chronic nitrate exposure increased (representatives of) health-associated *Neisseria* and *Rothia* (67% and 58% of studies, respectively) whilst reducing periodontal disease-associated *Prevotella* (33%). Additionally, caries-associated *Veillonella* and *Streptococcus* decreased (25% for both genera). Nitrate also altered oral microbiome metabolism, causing an increase in pH levels ( $n = 5$ ), which is beneficial to limit caries development. Secondary findings highlighted the benefits of nitrate for systemic health ( $n = 5$ ).

**Conclusions:** More clinical trials are required to confirm the impact of nitrate on oral communities. However, these findings support the hypothesis that nitrate could be used as an oral health prebiotic. Future studies should investigate whether chlorhexidine-containing mouthwashes could be replaced or complemented by a nitrate-rich diet or nitrate supplementation.

### ARTICLE HISTORY

Received 20 September 2023  
Revised 12 February 2024  
Accepted 15 February 2024

### KEYWORDS



Nitrate; nitrite; nitric oxide; periodontal disease; chlorhexidine; antimicrobial resistance

## Introduction


Dietary  $\text{NO}_3^-$  is a polyatomic ion that naturally occurs in vegetables, particularly leafy greens (lettuce, kale and spinach) and beetroots [1–6]. Additionally,  $\text{NO}_3^-$  is commonly added to processed meats for flavouring, preservation and antimicrobial purposes [2]. Evidence suggests  $\text{NO}_3^-$  added to processed meats can lead to the formation of carcinogenic nitrosamines [4]. However,  $\approx 80\%$  of dietary nitrate is obtained from vegetables which are considered anticarcinogenic [4,7]. Moreover, plant-based  $\text{NO}_3^-$  exerts positive effects on oral and systemic health [1,2,4].  $\text{NO}_3^-$  consumption leads to the production of nitric oxide (NO), a bioactive molecule associated with host defence, neuronal communication, improved vascular and metabolic health, and improved exercise performance [1,4–7]. Importantly, NO is a potent vasodilator that regulates blood pressure (BP) and blood flow within tissues and organs. High  $\text{NO}_3^-$  diets are associated with a reduced risk of cardiovascular disease (CVD), diabetes and cognitive impairment, as increased NO bioavailability enhances the delivery of oxygen and nutrients to body systems [4,6]. Interestingly, NO can also be produced endogenously from L-arginine and NADPH in

an oxygen-dependent reaction involving NO synthase enzymes (NOS), of which there are three main types (endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS)) [8]. Unfortunately, endogenous NO production becomes less efficient with age [3–5]. However, dietary  $\text{NO}_3^-$  is reduced to NO via the nitrate-nitrite-nitric oxide pathway ( $\text{NO}_3^-$ - $\text{NO}_2^-$ -NO) (Figure 1) [1,3,4,6]. This process is facilitated by nitrate-reducing bacteria (NRB) inside the oral cavity, including *Rothia* and *Neisseria*, which are associated with positive oral health [7,8]. High-nitrate diets have been shown to increase health-associated genera and decrease the number of disease-associated bacteria [7,8].

In contrast, some bacteria are linked to the development of dental caries (e.g. *Lactobacillus* and cariogenic representatives of *Streptococcus*, *Veillonella* and *Actinomyces*), whilst others are linked to the development of halitosis and periodontitis (e.g. *Porphyromonas*, *Fusobacterium* and *Prevotella*) [7,8]. Cariogenic bacteria ferment carbohydrates (e.g. glucose and sucrose) into organic acids (e.g. lactic acid) which can decrease oral pH and cause demineralisation of tooth enamel [6,7]. Studies have shown  $\text{NO}_3^-$  limits

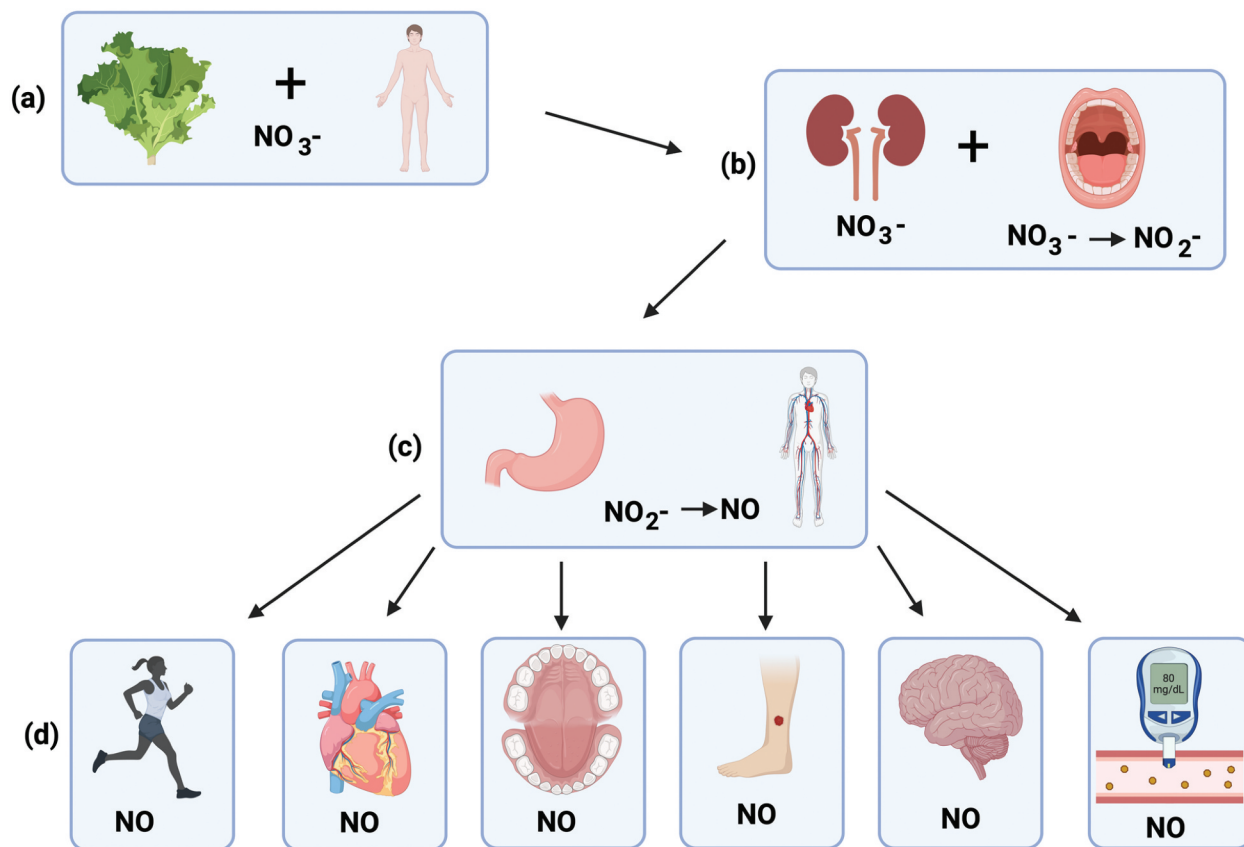
**CONTACT** Mia C. Burleigh  [mia.burleigh@uws.ac.uk](mailto:mia.burleigh@uws.ac.uk)  School of Health and Life Sciences, University of the West of Scotland, Blantyre, Scotland G72 0LH, UK

This article has been republished with minor changes. These changes do not impact the academic content of the article.

 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/20002297.2024.2322228>

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**Figure 1.** Schematic representation of the  $\text{NO}_3^-$ - $\text{NO}_2^-$ - $\text{NO}$  pathway (a) Nitrate-rich vegetables are consumed,  $\text{NO}_3^-$  enters the GI tract and is absorbed into the bloodstream (b) about 75% of  $\text{NO}_3^-$  is excreted in urine, whilst approximately 25% is concentrated by the salivary glands (facilitated by the transporter protein sialin) and is reduced to  $\text{NO}_2^-$  by bacteria inside the oral cavity (c)  $\text{NO}_2^-$  is then swallowed and reduced to  $\text{NO}$  in the stomach and/or other body tissues after entering the systemic circulation (d)  $\text{NO}$  induces health benefits, including improvements to exercise performance, cardiovascular health, oral health, wound healing, cognitive function and blood glucose levels (created with BioRender.com).

oral acidification via lactic acid/proton consumption during denitrification and nitrite reduction to ammonium by oral bacteria, which can prevent caries development [4,6–8]. Additionally, periodontal disease (PD) is a chronic oral infection that affects 20–50% of the global population [9–11]. PD is characterised by chronic inflammation, bleeding gums and destruction of the periodontium; the supporting tissue of the teeth [11]. Although the condition is multifactorial, complex biofilms (dental plaque) containing a large diversity of bacterial species contribute to PD manifestation and progression [7,9,10]. Interestingly, some studies have found PD patients have high concentrations of  $\text{NO}$  in their saliva and gingival crevicular fluid, potentially due to excessive iNOS activation driven by proinflammatory cytokines which can contribute to tissue damage [8,11]. In contrast, dietary  $\text{NO}_3^-$  appears to induce a controlled, low-level production of  $\text{NO}$  that is beneficial for oral health [7,8].

Treatment for oral disease(s) typically involves mechanical plaque removal and the use of chlorhexidine-containing mouthwashes (CHX) to reduce gingival inflammation [9,10]. Unfortunately, prolonged

use of CHX mouthwash can disrupts the oral microbiota, displacing  $\text{NO}_3^-$  reducing species and lowering oral pH, in addition to negatively affecting the expected BP reduction following an  $\text{NO}_3^-$  dose [4,8]. Moreover, evidence suggests that antimicrobial resistance (AMR) is developing against antibacterial mouthwash, thus promoting cross-resistance to antibiotics [4,10,12]. Worryingly, annual deaths related to AMR are expected to reach 10 million yearly by 2050 [13]. Thus, the emergence of resistant strains can induce a life-threatening infection inside a compromised host and/or spread environmentally [10,13,14]. In terms of periodontitis, CHX resistance would enable disease-causing bacteria to increase and exacerbate oral disease progression [7,10].

Several studies have suggested that dietary  $\text{NO}_3^-$  should be investigated as a prebiotic for oral health, as a mechanism to prevent or treat oral disease(s) [4,7,15–18]. Prebiotics are food components that promote the growth of beneficial microorganisms [7]. Using  $\text{NO}_3^-$  as a prebiotic could reduce the over-reliance on CHX mouthwashes by giving health-associated species a growth advantage and potentially

limiting the growth of disease-associated species [4,7]. To address this hypothesis, a systematic review of the literature was performed with the scope to answer the following research question: *What are the effects of nitrate on the oral microbiota?*

## Methodology

### Overview

Evidence presented in this review to satisfy the research aim was derived from clinical and *in vitro* studies. A narrative synthesis will highlight the effects of  $\text{NO}_3^-$  on the oral microbiome.

### Search strategy

This review was conducted following PRISMA (2020) guidelines [19]. Four databases were accessed (Table 1). All relevant papers came from peer-reviewed journal articles. The search criteria narrowly focused on the effects of  $\text{NO}_3^-$  on oral microorganisms. A date restriction was applied (2012–2022) to find the most up to date information relevant to the topic. Key words combined with Boolean operators and search strings were used to confine search results to the topic of interest (Table 1). Mendeley and Microsoft Excel were used for record keeping. A PRISMA diagram is shown in the Results section.

### Inclusion and exclusion criteria

Material to be included in the review: 1) Peer-reviewed *in vitro* and *in vivo* studies analysing the effect of  $\text{NO}_3^-$  on oral community composition and activity 2) Studies published between 2012–2022 3) no restrictions for country, participant gender, age and race.

Material to be excluded from the review: 1) Studies that did not analyse complex oral microbiota samples (saliva, tongue samples or dental plaque) 2) duplicate research papers 3) non-relevant articles and/or articles containing insufficient information to answer the research question 4) non-English language articles 5) book chapters, conference notes, reviews and theses 6) articles with no full-text access.

### Quality assessment checks

Relevant studies were analysed against a Scottish Intercollegiate Guidelines Network flowchart (2022) to determine study type [20,21] (Appendix A). Studies were established to be randomised control trials (RCTs), Quasi-experiments (non-randomised control studies (non-RCS) and pre-post interventions), cross-sectional studies, and *in vitro* analyses. All studies were quality assessed against critical appraisal tools to determine validity and suitability.

Each RCT was quality assessed against two critical appraisal tools provided by the Center for Evidence-Based Management and Critical Appraisal Skills Programme [22,23]. Quasi-experimental studies were critically appraised against tools provided by the British Medical Journal and The Joanna Briggs Institute [24,25]. Similarly, cross sectional studies were quality assessed against two appraisal tools provided by the Center for Evidence-Based Management and STROBE [26,27]. Lastly, *in vitro* studies were critically appraised using a checklist provided by the University of Exeter and The QUIN tool [28,29] (See appendix B-I). All tools had a checklist format and contained key questions highlighted in Table 2.

Most tools consisted of optional answers, including 'yes', 'no', 'unclear' or 'can't tell'. Good quality studies should return a majority of 'yes' answers. If

**Table 1.** Search strategy used during systematic review.

Main Concept	Keywords/key terms	Boolean Operators	Databases
Search Strategy			
The effects of nitrate on oral microbiome composition and activity	nitrate effects impact "oral microbiome" "oral microbiota"	AND OR	Scopus NCBI Science Direct Google Scholar
Search string 1: (effects OR impact) AND (nitrate) AND ("oral microbiome" OR "oral microbiota")			

**Table 2.** Common quality assessment questions for RCTs, quasi-experiments, cross-sectional studies and *in vitro* analyses.

Typical critical appraisal questions
Did the study have a focused research question?
Was the study design valid?
Was the sample size adequate and target population representative?
Was there a clear method of selection/randomisation and/or a control group?
Was the intervention/exposure clearly reported?
Were outcomes measured reliably and are results clear and unbiased?
Have confounders been identified?
Will the results help locally/target populations?

appraisal results caused uncertainty regarding inclusion or exclusion, the project team were consulted for a second opinion. Overall, selected papers had a good critical appraisal result.

### Data collection and narrative synthesis

A summary table was created in Microsoft Word to store key information from each study (Table 3). Key trends, similarities, and differences between studies were explored and an overall conclusion relating to the research question was reached.

## Results

### Prisma diagram and summary table

Applying the search strategy outlined in Section 2.3 returned 3133 potential articles (Figure 2). This total was reduced to 1943 after removing 1190 duplicates. Thereafter, title and abstract screening removed a further 1745 non-related articles, leaving 198 articles to be fully screened. Subsequently, a further 188 papers met the exclusion criteria (section 2.4). Thus, 10 articles satisfied the inclusion guidelines and featured in the review. Additionally, 2 further papers were found through screening the reference lists of eligible articles. Overall, 12 studies satisfied the inclusion criteria (Table 3).

### Study characteristics

Several study types were identified from this systematic approach: 7 were randomised control trials (RCTs), 3 were *in vitro* analyses and 2 demonstrated a Quasi experimental design (Table 4). Moreover, studies were highly variable by location. Additionally, 11 of the studies reported that  $\text{NO}_3^-$  induced oral microbiome changes, with 8 emphasising  $\text{NO}_3^-$  had a positive impact on oral health. Lastly, several studies ( $n=6$ ) reported  $\text{NO}_3^-$ -associated systemic benefits, predominantly improvements to cardiovascular health.

### Laboratory investigations

The most common laboratory procedures for  $\text{NO}_3^-/\text{NO}_2^-$  quantification included chemiluminescence, chromatography, the Griess colorimetric method and the RQflex® reflectometer. Additionally, 16s rRNA gene Illumina Sequencing was predominantly used for bacteria identification/quantification. Typically, the V3-V4 region of the 16s rRNA gene was amplified, using, the 341F and 806R or 805R primer set (Table 5).

### Bacteria constituents

Several bacterial genera were identified across all studies, including gram-positive and gram-negative genera, with and without nitrate producing capacity (NPC). Moreover, some genera contained spp. that are associated with oral disease and dental caries (Table 6).

### Nitrate supplementation induced modifications in oral communities

Post  $\text{NO}_3^-$  supplementation, there is an increase in bacterial spp. associated with good oral health, whilst a decrease is observed in genera linked to oral disease (Table 7). For instance, *Neisseria* and *Rothia* increased the most, at 67% and 58%, respectively (Table 6). In terms of percentage decreases, *Prevotella* and *Veillonella* decreased the most across all studies, at 33% and 25%, respectively.

Additionally, eleven studies reported an increase in NRB associated with eubiosis, whilst five studies reported a decrease in bacterial species associated dysbiosis (Table 8). Interestingly, five studies reported both an increase in NRB associated with eubiosis and a decrease in bacterial genera associated with dysbiosis. Lastly, five studies reported oral pH changes post  $\text{NO}_3^-$  treatment.

### Additional findings

Five studies reported other *in vivo* health benefits, including improvements to cardiovascular and cognitive health (Table 9). Moreover, one study emphasised that  $\text{NO}_3^-$  supplementation could be used for plaque control or to treat periodontitis. Lastly, a study discussed the detrimental impact of antiseptic mouthwash on oral communities (Table 9).

## Discussion

### $\text{NO}_3^-$ promotes eubiosis inside the oral cavity

$\text{NO}_3^-$  supplementation increased oral health-associated NRB, particularly *Rothia* and *Neisseria*, whilst reducing generally disease-associated *Prevotella*, *Veillonella* and *Streptococcus* (Table 7). Collectively, most studies ( $n=11$ ) reported an increase in NRB associated with eubiosis and/or a decrease in bacterial spp. associated with dysbiosis, post  $\text{NO}_3^-$  supplementation (Table 8). Eubiosis refers to a balanced symbiotic relationship between oral microbes [40–42]. In contrast, dysbiosis occurs when insults to oral ecology, such as sugar consumption, smoking, poor dental hygiene or antimicrobial mouthwash, disrupt this commensal relationship, which causes disease-associated bacteria to increase [41–46]. In PD, dysbiosis activates an excessive



**Table 3.** Studies assessing NO<sub>3</sub><sup>-</sup> supplementation and oral ecology (2012–2022).

First author, Year and journal	Study Type and Location	Aim/participant information	Laboratory investigation(s)	Results/Findings	Author's Conclusions
(1) Velmurugan <i>et al.</i> , 2016 The American Journal of Clinical Nutrition [30]	<i>In vivo</i> : randomised, double-blind, placebo-controlled parallel study (UK)	The effects of dietary NO <sub>3</sub> <sup>-</sup> on vascular and platelet function, and the oral microbiome <i>n</i> = 67 hypercholesterolemic M and F participants Age: 18–80 yrs old BMI: 18.5–40 kg/m <sup>2</sup>	BR juice vs PL (6-wk, once-daily) <b>Analyses:</b> Vascular and platelet function, BP, saliva, urine and plasma NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> levels and oral microbiome (saliva) <b>Methods:</b> FMD, aPWV, portable BP device, flow cytometry, chemiluminescence and 16s rRNA gene Illumina Sequencing	In the treatment group, FMD and aPWV improved ( <i>p</i> < 0.001 and <i>p</i> = 0.06, respectively); platelet monocyte aggregates significantly reduced ( <i>p</i> < 0.004); <i>Rothia mucilaginosa</i> increased and <i>Neisseria flavescens</i> significantly increased ( <i>p</i> < 0.01)	Chronic dietary NO <sub>3</sub> <sup>-</sup> consumption improves vascular function in hypercholesterolemic individuals; changes are related to alterations in the oral microbiome/increased levels of NRB
(2) Koopman <i>et al.</i> , 2016 Microbial Ecology [31]	<i>In vitro</i> study (Netherlands)	Assessing the effects of dietary NO <sub>3</sub> <sup>-</sup> on oral ecology and biochemistry MAM biofilm model inoculated with stimulated saliva from a healthy M and F donor Age: 20–30 yrs old	<i>n</i> = 4 microcosms received NO <sub>3</sub> <sup>-</sup> , all received weekly NO <sub>3</sub> <sup>-</sup> dose and sucrose pulse (4-wk exp) <b>Analyses:</b> NO <sub>3</sub> <sup>-</sup> and NO <sub>2</sub> <sup>-</sup> concentrations, oral pH, short chain fatty acid production and bacterial composition <b>Methods:</b> Capillary electrophoresis, pH meter and 16s rRNA gene Illumina Sequencing	In one donor, NO <sub>3</sub> <sup>-</sup> supplementation significantly increased <i>Neisseria</i> on a genus-level. In both donors, <i>Neisseria</i> OTUs were associated with NO <sub>3</sub> <sup>-</sup> supplementation and in one donor, <i>Veillonella</i> OTUs. Butyrate levels were lower after nitrate addition in NO <sub>3</sub> <sup>-</sup> receiving microcosms ( <i>p</i> < 0.05)	NO <sub>3</sub> <sup>-</sup> affects the composition/biochemistry of oral microbiomes, but changes vary depending on the inoculum (donor)
(3) Vanhatalo <i>et al.</i> , 2018 Free Radical Biology and Medicine [32]	<i>In vivo</i> : randomised, double-blind, cross-over trial (UK)	Examining relationships between oral ecology and physiological indices of NO bioavailability, and assessing the impact of NO <sub>3</sub> <sup>-</sup> supplementation on these variables <i>n</i> = 18 normotensive M and F Age: 19–22 yrs old; 70–79 yrs old	BR juice vs PL (10 days, twice-daily) <b>Analyses:</b> oral ecology (tongue and saliva), plasma, NO <sub>3</sub> <sup>-</sup> and NO <sub>2</sub> <sup>-</sup> concentrations, BP and arterial stiffness <b>Methods:</b> 16s rRNA gene Illumina Sequencing, chemiluminescence, automated sphigmomanometer MAP measurements and PWV	NO <sub>3</sub> <sup>-</sup> supplementation increased NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> plasma concentrations ( <i>p</i> < 0.05); <i>Rothia/Neisseria</i> genera increased and <i>Prevotella/Veillonella</i> decreased vs PL ( <i>p</i> < 0.05). SBP and MAP were significantly lower in the older age group vs PL ( <i>p</i> < 0.05)	Dietary NO <sub>3</sub> <sup>-</sup> altered the oral microbiota and changes in oral ecology positively impacted NO homeostasis and vascular health
(4) Ashworth <i>et al.</i> , 2019 Free Radical Biology and Medicine [33]	<i>In vivo</i> : non-randomised, single blinded, cross-over trial (UK)	Evaluating dietary NO <sub>3</sub> <sup>-</sup> intake in vegetarians vs omnivores and the impact of AM on NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> levels, RMR, BP and the oral microbiome. Healthy M and F participants <i>n</i> = 22 vegetarians <i>n</i> = 19 omnivores Age: 18–45 yrs old	PL vs Corsodyl (2-wk trial) <b>Analyses:</b> RMR, BP, plasma and salivary NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> concentrations, saliva pH, blood glucose and lactate levels, and oral ecology (saliva) <b>Methods:</b> RMR calculations, oscillometric device, chemiluminescence, biochemistry analyser, digital pH meter and 16s rRNA gene Illumina Sequencing	No significant differences in NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> concentrations, NRC, and oral ecology between groups following PL ( <i>p</i> > 0.05). AM decreased NO <sub>2</sub> <sup>-</sup> levels ( <i>p</i> < 0.05), NRC and oral pH, and increased salivary lactate and glucose levels in both groups (all <i>p</i> < 0.001) ( <i>Prevotella</i> , <i>Actinomyces</i> , <i>Rothia</i> and <i>Fusobacterium</i> genera decreased ( <i>p</i> < 0.05))	Dietary NO <sub>3</sub> <sup>-</sup> consumption in vegetarians vs omnivores was not statistically different.

(Continued)

Table 3. (Continued).

First author, Year and journal	Study Type and Location	Aim/participant information	Laboratory investigation(s)	Results/Findings	Author's Conclusions
(5) Burleigh <i>et al.</i> , 2019 Nitric Oxide [15]	<i>In vivo</i> placebo-controlled, single-blind randomised cross over trial (UK)	Analysing the effects of dietary NO <sub>3</sub> <sup>-</sup> on oral microbiome composition and NRC, and assessing vascular responses to acute NO <sub>3</sub> <sup>-</sup> ingestion. n = 11 healthy M Age: 30 ± 7 yrs old BM: 86.9 ± 14.1kg	BR juice vs PL (7 days, twice daily) <b>Analyses:</b> plasma and salivary NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> concentrations, microbiome (tongue), oral pH, HR, BP and endothelial functioning <b>Methods:</b> chemiluminescence, 16s rRNA gene Illumina Sequencing, pH-meter, FMD, automated BP monitor, telemetry and MAP measurements	Plasma/saliva NO <sub>3</sub> <sup>-</sup> and NO <sub>2</sub> <sup>-</sup> levels and salivary pH increased post NO <sub>3</sub> <sup>-</sup> supplementation (p<0.05). <i>Prevotella</i> , <i>Actinomyces</i> and <i>Streptococcus</i> genera decreased in the BR group; <i>Neisseria subflava</i> increased in both groups compared to baseline, but this increase was most significant for the BR group (p = 0.001) Profound CV <sub>6</sub> variations in saliva and plasma NO markers, and NR bacteria at baseline vs post BR ingestion. <i>Neisseria subflava</i> was negatively associated with peak saliva/plasma NO <sub>2</sub> <sup>-</sup> levels; <i>Rothia mucilaginosa</i> and <i>Haemophilus parainfluenzae</i> were positively and negatively associated with between-trial peak saliva/plasma NO <sub>2</sub> <sup>-</sup> levels (all p < 0.05). BR ingestion reduced BP	Dietary NO <sub>3</sub> <sup>-</sup> altered the oral microbiome in favour of oral health, but changes did not enhance NRC or vascular responses to acute NO <sub>3</sub> <sup>-</sup> ingestion  There is profound CV <sub>6</sub> in oral NR bacterial abundance on the tongue and NO metabolites in plasma/saliva pre and post-ingestion of BR
(6) Liddle <i>et al.</i> , 2019 Nitric Oxide [34]	<i>In vivo</i> : before-and-after study (UK)	Analysing CV <sub>6</sub> , CV <sub>A</sub> and CD for plasma and saliva NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> concentrations pre and post-ingestion of BR juice, and assessing the CV <sub>6</sub> of NR bacteria on the tongue n = 10 Recreationally active M Age: 25 ± 5 yrs old BM: 81 ± 11 kg	<b>Analyses:</b> salivary, plasma and urine NO <sub>2</sub> <sup>-</sup> and NO <sub>3</sub> <sup>-</sup> concentrations, BP, MAP, microbiome (tongue) <b>Methods:</b> chemiluminescence, oscillometric device, MAP measurements and 16s rRNA gene Illumina Sequencing	NO <sub>3</sub> <sup>-</sup> increased ammonium and pH levels, and decreased lactate production (all p<0.01) NO <sub>3</sub> <sup>-</sup> increased abundance of <i>Neisseria</i> and <i>Rothia</i> genera after 5h (p<0.01) and reduced <i>Streptococcus</i> , <i>Oribacterium</i> , <i>Veillonella</i> , <i>Fusobacterium</i> , <i>Leptotrichia</i> , <i>Prevotella</i> , <i>Alloprevotella</i> and <i>Porphyromonas</i> after 5 and/or 9h (p<0.05)	NO <sub>3</sub> <sup>-</sup> significantly altered the oral environment to benefit the host and could serve as a prebiotic for oral health
(7) Rosier <i>et al.</i> , 2020 Scientific Reports [7]	<i>In vitro</i> study (Spain)	Establishing the short-term effect of a growth medium containing NO <sub>3</sub> <sup>-</sup> dose on oral biofilm growth, composition and biochemistry n = 12 saliva samples from healthy donors	Samples were incubated in nutrient-rich media with or without NO <sub>3</sub> <sup>-</sup> and examined at 5h and 9h <b>Analyses:</b> NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> levels, ammonium, lactate and pH measurements; protein and DNA quantification <b>Methods:</b> Reflectoquant reflectometer, Bradford protein assay and 16s rRNA gene Illumina Sequencing	Biofilm NRC increased when adding isolates compared to controls (p<0.05). Adding NO <sub>3</sub> <sup>-</sup> or NO <sub>3</sub> <sup>-</sup> + isolates caused a smaller decrease in pH (p<0.05) and some isolates in combination with NO <sub>3</sub> <sup>-</sup> increased lactate consumption. A positive correlation between NRC and ammonium production was observed, even in samples without NO <sub>3</sub> <sup>-</sup> (p<0.01)	Oral communities with reduced NRC could benefit from a NR probiotic or symbiotic combination (NO <sub>3</sub> <sup>-</sup> and NR probiotic)
(8) Rosier <i>et al.</i> , 2020 Frontiers in Microbiology [35]	<i>In vitro</i> study (Spain)	Isolating oral NR bacteria and assessing their <i>in vitro</i> probiotic capabilities <b>Probiotic isolation:</b> n = 5 healthy M and F donors Age: 23–32 yrs old <b>Saliva donors:</b> n = 9 healthy M and F donors Age: 23–45 yrs old	6 <i>Rothia</i> sp. underwent <i>in vitro</i> biofilm tests (saliva biofilms). <b>Analyses:</b> NRC, bacterial identification; NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> levels, ammonium, lactate and pH measurements <b>Methods:</b> cell culture, Griess reaction, 16s rRNA gene Illumina Sequencing, Reflectoquant reflectometer and the Nessler method		

(Continued)

Table 3. (Continued).

First author, Year and journal	Study Type and Location	Aim/participant information	Laboratory investigation(s)	Results/Findings	Author's Conclusions
(9) Vanhatalo <i>et al.</i> , 2021 Redox Biology [36]	<i>In vivo</i> : randomised, placebo-controlled cross-over trial (UK)	Assessing relationships between cognitive and physiological traits and clusters of co-occurring oral bacteria n = 30 elderly M and F Age: 70–80 yrs old	BR juice vs PL (10 day trial) <b>Analyses:</b> plasma NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> levels, microbiome (saliva), cognitive function, BP, arterial stiffness, exercise assessments, muscle function, brain matter <b>Methods:</b> chemiluminescence, 16s rRNA gene Illumina Sequencing, WGCNA, cognitive function tests, BP measurement, PWV, MAP measurements, VO <sub>2</sub> max, p-MRS of quadriceps and H-MRS of brain	<i>Rothia-Streptococcus</i> microbiome module increased after NO <sub>3</sub> <sup>-</sup> supplementation and showed stable associations with cardiovascular indices of health; <i>Neisseria-Haemophilus</i> module increased and showed stable associations with cognitive indices of health. Co-occurring <i>Prevotella-Veillonella</i> diminished after NO <sub>3</sub> <sup>-</sup> ingestion	NO <sub>3</sub> <sup>-</sup> sensitive oral microbiome modules could be potential pre- and probiotic targets to modify age-associated cardiovascular and cognitive impairments
(10) Sinha <i>et al.</i> , 2021 Environmental Research [37]	<i>In vivo</i> : randomised control trial (USA)	Analysing the effects of NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> processed meat and nitrate-enriched drinking water on salivary microbial populations n = 63 healthy M and F volunteers Age: 18–70 yrs old BMI: 18–25 kg/m <sup>2</sup>	2 groups: 3 meat intervention phases and 1 meat intervention phase + nitrate-enriched water (7-week trial) <b>Analyses:</b> urinary NO <sub>3</sub> <sup>-</sup> /creatinine levels, microbiome (Saliva) <b>Methods:</b> ion chromatography and mass spectrometry, Beckman clinical analyser (colorimetric method) and 16s rRNA gene Illumina Sequencing	Urinary NO <sub>3</sub> <sup>-</sup> levels were reduced during the phytochemical-enriched-low- NO <sub>2</sub> <sup>-</sup> meat diet and poultry diet compared to baseline (p < 0.009). NO <sub>3</sub> <sup>-</sup> rich water induced changes to salivary microbiome (p < 0.001). <i>Neisseria</i> , <i>Kingella</i> , <i>Lautropia</i> , <i>Alloprevotella</i> and <i>Haemophilus</i> genera increased; <i>Streptococcus</i> decreased (FDR < 0.05)	Drinking NO <sub>3</sub> <sup>-</sup> enriched water increases oral NR bacteria abundance, which can subsequently increase NOC production
(11) Rosier <i>et al.</i> , 2021 Frontiers in Cellular and Infection Microbiology [38]	<i>In vivo</i> : placebo-controlled, blinded, cross-over trial (study 1) and before-and-after study (study 2 & 3) (Spain)	Evaluating the ability of NO <sub>3</sub> <sup>-</sup> to reduce oral acidification caused by sugar fermentation <i>in vivo</i> <b>Study 1:</b> n = 12 healthy M and F participants, age: 25–60 yrs old <b>Study 2:</b> n = 6 healthy M and F participants, age: 24–46 yrs old <b>Study 3:</b> n = 6 healthy M and F participants, age: 25–33 yrs old	Sucrose rinses 1, 2 and 4h after NO <sub>3</sub> <sup>-</sup> ingestion (BR vs placebo) <b>Analyses:</b> salivary NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> concentrations, microbiome (saliva), pH levels, lactate and ammonium measurements <b>Methods:</b> Reflectoquant reflectometer, 16s rRNA gene Illumina Sequencing, the Nessler method and lactate colorimetric assay	<b>Sucrose rinse 4hrs:</b> NO <sub>3</sub> <sup>-</sup> supplementation reduced lactate production (p < 0.05), increased pH and <i>Rothia</i> genera abundance (p < 0.05). Relative abundances of <i>Neisseria</i> and <i>Rothia</i> negatively correlated with lactate production, and <i>Neisseria</i> abundance positively correlated with pH post sucrose intake (p < 0.05)	NO <sub>3</sub> <sup>-</sup> can limit oral acidification induced by sugar fermentation, predominantly due to lactate usage by NR bacteria
(12) Jockel-Schneider <i>et al.</i> , 2021 Journal of Periodontology [17] (continuation Jockel-Schneider <i>et al.</i> , 2016 [16])	<i>In vivo</i> : randomised control trial (Germany)	Evaluating the impact of a NO <sub>3</sub> <sup>-</sup> -rich diet on NO <sub>3</sub> <sup>-</sup> fermenting bacterial abundance within the pocket microbiota n = 37 periodontal recall patients with gingival inflammation and reduced periodontium	lettuce juice vs PL (14 days, three times daily) <b>Analyses:</b> subgingival microbial samples (pre and post plaque removal) and salivary NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> levels <b>Methods:</b> anion exchange chromatography and 16s rRNA gene Illumina Sequencing	No baseline differences in bacterial diversity between the BR and PL group (p > 0.05) NO <sub>3</sub> <sup>-</sup> supplementation induced significant increases in <i>Rothia</i> and <i>Neisseria</i> genera (p < 0.05) and significantly altered alpha and beta-diversity (p < 0.05) compared to PL group	NO <sub>3</sub> <sup>-</sup> supplementation in periodontal recall patients reduced gingival inflammation and induced significant changes to subgingival microbial populations; NO <sub>3</sub> <sup>-</sup> could be used as an adjunct therapy to plaque removal to control gingival inflammation

M = male, F = female, BMI = body mass index, BR = beetroot, PL = placebo, wk = week, FMD = flow-mediated dilation, aPWV = aortic pulse wave velocity, BP=blood pressure, rRNA = ribosomal ribonucleic acid, NRB = nitrate reducing bacteria, MAM = multiplex artificial mouth, exp = experiment, MAP = mean arterial pressure, PWV = pulse wave velocity, SBP = systolic blood pressure, AM = antibacterial mouthwash, RMR = resting metabolic rate, NRC = nitrate reducing capacity, HR = heart rate, CVB= within-person biological variation, CVA = analytical imprecision, CD = critical difference, NR = nitrate reducing, WGCNA = weighted gene co-expression network analysis, VO2 max = maximal oxygen consumption, p-MRS = phosphorus magnetic resonance spectroscopy, H-MRS = proton magnetic resonance spectroscopy, NOC = N-nitroso compounds, OTU = operational taxonomic unit.



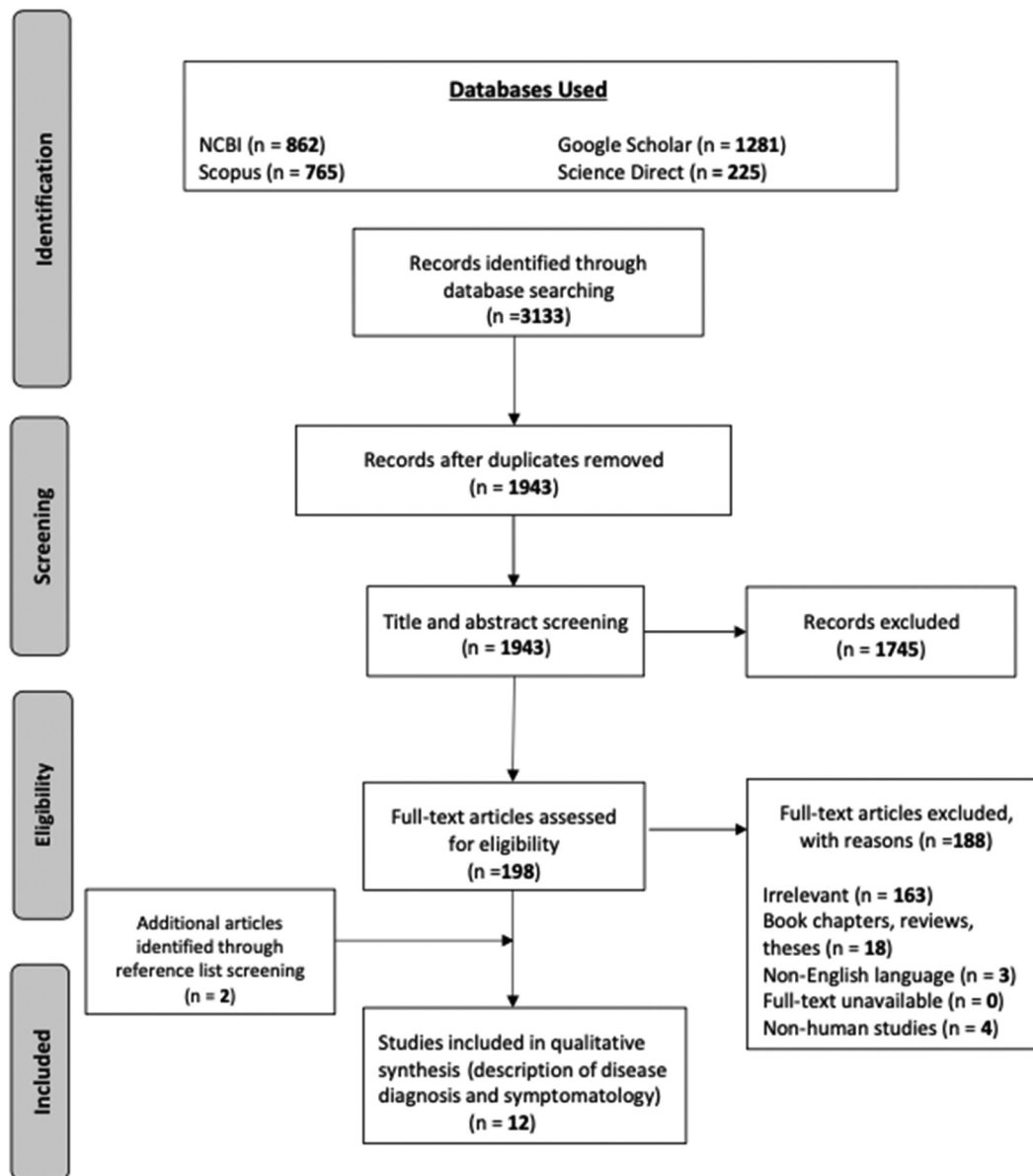


Figure 2. PRISMA flow diagram.

Table 4. Summary of study characteristic (n = 12).

Study type	
RCTs	7
<i>in vitro</i>	3
Quasi exp (non-RCTs and pre-post interventions)	2
Location	
UK	6
Spain	3
USA	1
Netherlands	1
Germany	1
NO <sub>3</sub> <sup>-</sup> altered the oral microbiome	
Yes	11
impact on oral health	
Positive	8
*Unspecified	4
NO <sub>3</sub> <sup>-</sup> and other health benefits	
CV health	5
Cognitive health	1

\*studies were investigating the impact of NO<sub>3</sub><sup>-</sup> induced oral microbiome changes on other parameters (e.g. cardiovascular health), and did not directly analyse the positive or negative impact of NO<sub>3</sub><sup>-</sup> on oral health.

inflammatory response that destroys host tissues, while in dental caries, dysbiosis can contribute to acidification and enamel demineralisation [11,41]. Overall, NO<sub>2</sub><sup>-</sup> and NO production via the NO<sub>3</sub><sup>-</sup>-NO<sub>2</sub><sup>-</sup>-NO pathway promotes microbial homeostasis by exerting antibacterial effects against certain disease-associated bacteria, potentially maintaining a balanced microenvironment that lowers oral disease risk [8]. Thus, findings from this review indicate NO<sub>3</sub><sup>-</sup> can alter the oral microbiota in favour of oral health, highlighting that it may have a protective role against the development of oral disease, or the potential to be used as a therapeutic intervention.

Additionally, studies that measured oral pH (n=5) found acute and chronic NO<sub>3</sub><sup>-</sup> consumption beneficially altered oral biochemistry, by increasing salivary

**Table 5.** Most common laboratory procedures across all studies.

NO <sub>3</sub> <sup>-</sup> and NO <sub>2</sub> <sup>-</sup> Quantification	Bacterial ID/quantification (primers)
Chemiluminescence, The Griess colorimetric method, chromatography and Reflectoquant RQflex®	16s rRNA gene Illumnia Sequencing (V3-V4 region)
	341F (forward primer): 5'-CCTACGGGNGGCWGCAG-3'
	805R (reverse primer): 5'-GACTACHVGGGTATCTAATCC-3'
	806R (reverse primer): 5'-GGACTACHVGGGTWTCTAAT-3'

**Table 6.** Bacterial taxa identified across all studies.

Genus	Species	NPC	Disease-associated*
<b>Gram positive</b>			
<i>Lactobacillus</i>			Dental caries
<i>Streptococcus</i>		Yes	Dental caries
<i>Rothia</i>	<i>mucilaginoso</i>	Yes	
<i>Actinomyces</i>		Yes	Dental caries
<i>Oribacterium</i>			
<b>Gram negative</b>			
<i>Neisseria</i>	<i>flavescens/subflava</i>	Yes	
<i>Veillonella</i>		Yes	Dental caries
<i>Prevotella</i>		Yes	Periodontitis
<i>Fusobacterium</i>			Periodontitis
<i>Haemophilus</i>	<i>parainfluenzae</i>	Yes	
<i>Leptotrichia</i>			Periodontitis
<i>Alloprevotella</i>			Periodontitis
<i>Porphyromonas</i>			Periodontitis
<i>Kingella</i>		Yes	
<i>Lautropia</i>			

A search was performed on Bacterial Feature Finder (<http://csbg.cnb.csic.es/BaFF>) to determine microbiological characteristics [39]. NPC, and disease status were determined based on recent findings in the literature [4,6–8,17,38]. NPC = nitrite-production capacity, meaning bacteria have been found to produce nitrite in the presence of nitrate [6]. \*Disease-associated on a genus level. Note that some genera are clearly disease-associated (i.e. they generally increase in caries or periodontitis) but can contain health-associated species and strains, thus, future studies should test oral microbiome changes to at least species-level.

**Table 7.** Bacterial population increase versus decrease post NO<sub>3</sub><sup>-</sup> supplementation.

Bacterial genera that increase	Percent of studies (n = 12)
<i>Neisseria</i> (incl. <i>flavescens</i> and <i>subflava</i> spp.)	67%
<i>Rothia</i> (incl. <i>mucilaginoso</i> sp.)	58%
<i>Haemophilus</i>	17%
<i>Alloprevotella/Prevotella</i>	8%
<i>Veillonella</i>	8%
<i>Kingella</i>	8%
<i>Lautropia</i>	8%
<i>Streptococcus</i>	8%
<b>Bacterial genera that decrease</b>	
<i>Alloprevotella/Prevotella</i>	33%
<i>Veillonella</i>	25%
<i>Streptococcus</i>	25%
<i>Fusobacterium</i>	8%
<i>Oribacterium</i>	8%
<i>Porphyromonas</i>	8%
<i>Leptotrichia</i>	8%
<i>Actinomyces</i>	8%
<i>Haemophilus parainfluenza</i>	8%
<i>Neisseria subflava</i>	8%

**Table 8.** NO<sub>3</sub><sup>-</sup> supplementation and oral community composition/activity.

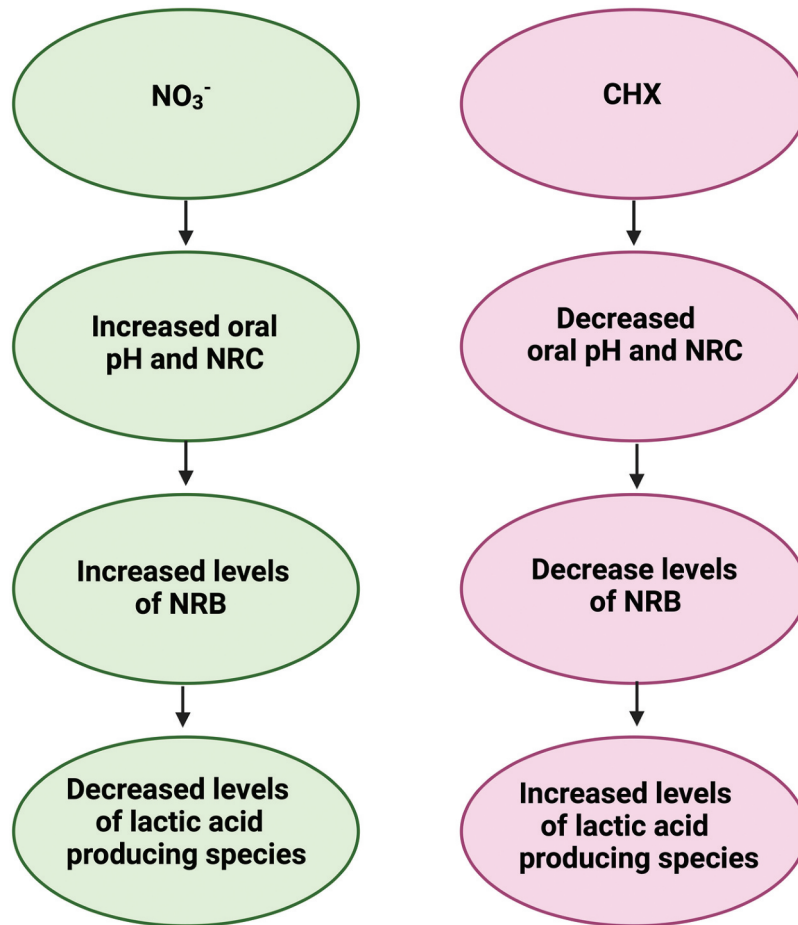
Impact of NO <sub>3</sub> <sup>-</sup> supplementation on oral health	Studies (n = 12)	Ref.
Studies reporting an increase in NRB associated with eubiosis	11	[7,15,17,30–32,34–38]
Studies reporting a decrease in bacterial species associated with dysbiosis	5	[7,15,32,36,37]
Studies reporting an increase in NRB associated with eubiosis and decrease in bacterial species associated with dysbiosis	5	[7,15,32,36,37]
Studies reporting an increase in salivary pH levels and/or lactate consumption	5	[7,15,31,35,38]

pH levels and/or enhancing lactic acid/lactate consumption [7,15,31,35,38]. These findings coincide with other studies which have shown acute increases in oral pH post NO<sub>3</sub><sup>-</sup> consumption [18,47]. An increase in pH combined with a decrease in *Veillonella* and *Streptococcus* is a positive change from a caries perspective; *Streptococcus* is a carbohydrate fermenting genus whilst *Veillonella* consumes the lactic acid produced by *Streptococci*, thus causing a consistent increase in both genera in the supragingival plaque of individuals with caries [48]. Nevertheless, these genera also contain health-associated species (e.g. *S. dentisani*) that can decrease during caries development [49]. Future studies should explore the effects of NO<sub>3</sub><sup>-</sup> on the oral microbiota at species level. Overall, oral bacteria consume protons and lactic acid during denitrification and nitrite reduction to ammonium which increases pH levels, limits oral acidification and inhibits the growth of cariogenic representatives [7,35,38].

Importantly, one study in this review emphasised that NO<sub>3</sub><sup>-</sup> could be used as a therapeutic intervention for plaque control or periodontal treatment [17]. Jockel-Schneider *et al.*, [16,17] found lettuce juice consumption, coupled with gingival debridement, in PD patients reduced gingival inflammation and significantly altered the subgingival microbiome to benefit oral health; NR *Rothia* and *Neisseria* genera significantly increased. Changes were associated with a significant increase in mean salivary NO<sub>3</sub><sup>-</sup> levels. Thus, NO<sub>3</sub><sup>-</sup> supplementation should be explored as an adjunct therapy to treat periodontitis [17]. This idea is supported by a recent study in which subgingival plaque of PD patients was grown *in vitro* and NO<sub>3</sub><sup>-</sup> decreased biofilm formation, the levels of periodontitis-associated species and the dysbiosis index [50]. Overall, NO<sub>3</sub><sup>-</sup> alters the oral environment to benefit the host, which makes it a strong candidate as a prebiotic for oral health ([Figure 3, left side] [7,8,16,17].

**Table 9.** NO<sub>3</sub><sup>-</sup> supplementation and other health outcomes.

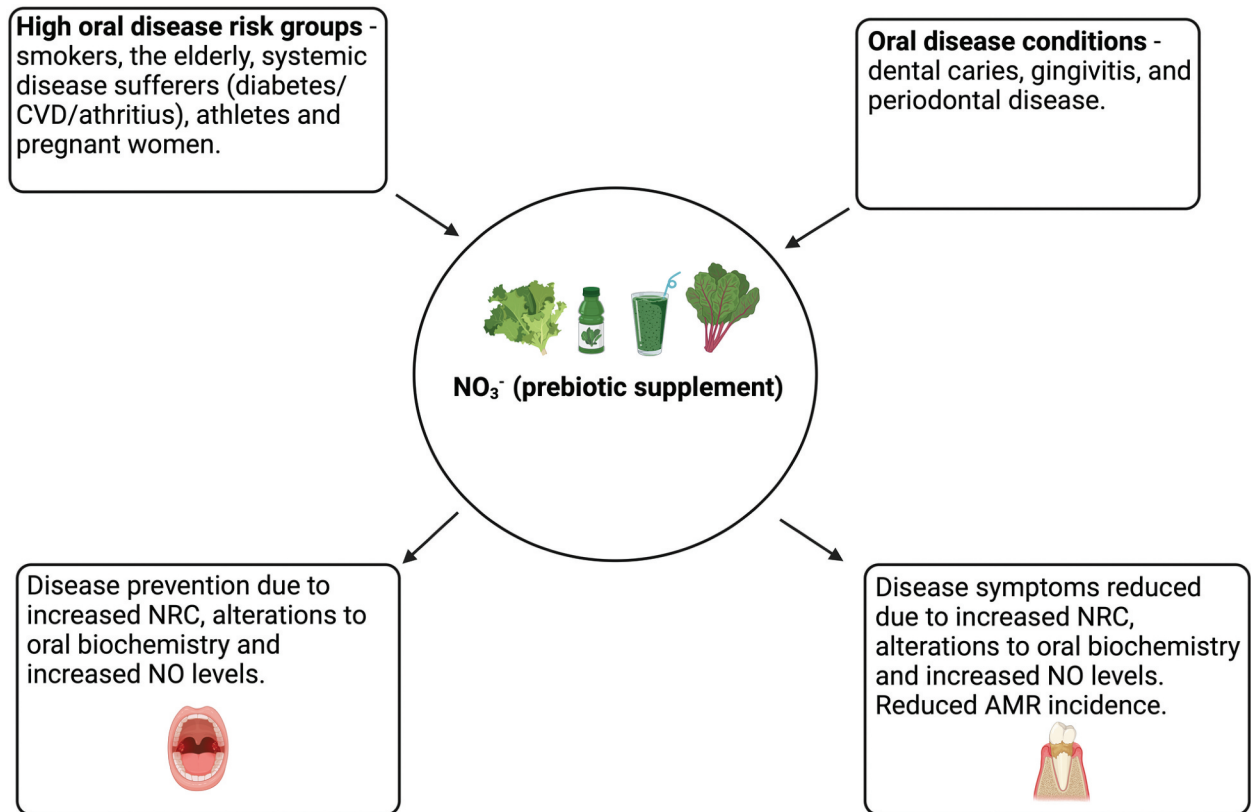
NO <sub>3</sub> <sup>-</sup> and <i>in vivo</i> health effects	Studies ( <i>n</i> = 12)	Ref.
NO <sub>3</sub> <sup>-</sup> and systemic health benefits (CV and cognitive)	5	[15,30,32,34,36]
NO <sub>3</sub> <sup>-</sup> as a treatment for oral disease	1	[17]
Antiseptic mouthwash exacerbates dysbiosis	1	[33]

**Figure 3.** The effects of NO<sub>3</sub><sup>-</sup> vs CHX on the oral environment: (left) summary based on findings in this review (right) summary based on (limited) current literature examining the effects of CHX on the oral environment (created with BioRender.com).

### Advantages of NO<sub>3</sub><sup>-</sup> supplementation over the use of chlorhexidine mouthwash

CHX inhibits plaque formation and exerts antibacterial action against various gram-positive and gram-negative bacteria [45,51,52]. In this review, Ashworth *et al.*, [33] showed CHX mouthwash decreased NRC and oral pH, whilst significantly increasing salivary lactate and glucose levels. Other studies have reported similar findings (Figure 3, right side). Chatzigiannidou *et al.*, [53] found that prolonged use of CHX-containing mouthwash altered salivary pH and increased lactic-acid producing species. Moreover, Bescos *et al.*, [45] showed 7-day use of a CHX mouth rinse increased *Streptococcus* spp. abundance and reduced levels of *Actinomyces* spp., whilst also decreasing salivary pH and increasing lactate/glucose levels. Additionally, studies have shown CHX-containing mouth rinses

are inducing resistance. Kulik *et al.*, [54] detected two- to fourfold increases in minimum inhibitory concentrations (MIC's) of *P. gingivalis* against sub-inhibitory concentrations of CHX after  $\approx$  30 passages. Wang *et al.*, [55] found CHX induced one to two-fold MIC increases in *F. nucleatum* and *P. gingivalis*. Thus, CHX-containing mouthwash can induce adaptation in oral bacteria and can also facilitate the development of cross-resistance to other antiseptics, including antibiotics, thus exacerbating AMR occurrence [10,12,52]. In contrast, NO<sub>3</sub><sup>-</sup> exerts antibacterial action through NO<sub>2</sub><sup>-</sup> and NO production, and resistance has not yet been observed [56]. Moreover, NO is significantly less toxic to human gingival fibroblasts than clinical concentrations of CHX [56,57]. Figure 3 compares and contrasts the effects of NO<sub>3</sub><sup>-</sup> and CHX on the oral environment.



**Figure 4.** NO<sub>3</sub><sup>-</sup> as a prebiotic for oral health. NO<sub>3</sub><sup>-</sup> supplementation could be used in high risk groups to prevent oral disease (left side) or be used as a treatment method for individuals with oral disease(s) (right side) (created with BioRender.com).

### NO<sub>3</sub><sup>-</sup> and secondary findings

As discussed, NO<sub>3</sub><sup>-</sup> reduction improves both oral and systemic health [1,4,6]. NO is a well-known vasodilator that decreases arterial stiffness and blood pressure [5,58–61]. The positive effects of NO on blood pressure have been well documented [1,3,4,40,62–64]. Webb *et al.*, [62] showed a dose of beetroot juice (500ml) could significantly reduce systolic blood pressure (SBP) and diastolic blood pressure (DBP) by  $\approx 10$  mm Hg and  $\approx 8$  mm Hg, respectively. Moreover, Kenjale *et al.*, [63] showed beetroot juice could significantly reduce DBP in subjects with peripheral arterial disease vs placebo. Similarly, studies have shown NO has positive effects on vascular function. Endothelial dysfunction is associated with arterial stiffness and impaired blood flow [61,64]. Acute (2–6 hours; 68–583 mg) and chronic (7–42 days; 300–650 mg/d) NO<sub>3</sub><sup>-</sup> consumption can significantly reduce arterial stiffness [59]. Additionally, another study showed consuming spinach soup for 7 days significantly reduced arterial stiffness and BP [65]. In contrast, CHX mouthwash has been shown to negate the BP lowering effects of NO<sub>3</sub><sup>-</sup> [4].

In this review, five studies emphasised that oral microbiome changes were associated with cardiovascular health improvements, namely improvements to BP, mean arterial pressure (MAP), flow-mediated dilation (FMD), pulse wave velocity (PWV) and platelet

aggregation [15,30,32,34,36]. Vanhatalo *et al.*, [32] showed that dietary NO<sub>3</sub><sup>-</sup> supplementation reduced BP and improved MAP in older participants, whilst another clinical study suggested that NO<sub>3</sub><sup>-</sup> sensitive microbial modules could serve as pre and probiotic targets to alter age-associated cardiovascular impairments [32,36]. A lowering of 5 mmHg in SBP can reduce the risk of CVD death caused by stroke and heart disease by 14% and 9%, respectively [5]. Furthermore, two studies reported improvements to FMD and PWV following NO<sub>3</sub><sup>-</sup> consumption, whilst another trial recorded BP lowering effects [15,30,34]. Additionally, one study reported NO<sub>3</sub><sup>-</sup> supplementation significantly lowered platelet aggregation [30]. Thus, evidence suggests dietary NO<sub>3</sub><sup>-</sup> has cardioprotective benefits which are associated with oral microbiome-related changes post NO<sub>3</sub><sup>-</sup> ingestion.

As previously highlighted, non-plant-based sources of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> (processed meats and drinking water) can induce carcinogenesis and, in infants, increase the risk of methemoglobinemia [2,66]. A study in this review emphasised that organic sources of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> can cause the formation of carcinogenic *N*-nitroso compounds (NOCs) linked to colorectal cancer [37]. However, high levels of antioxidants in vegetables counteract nitrosation [4]. Furthermore, an individual's daily NO<sub>3</sub><sup>-</sup> intake is mostly derived from fruits and vegetables and generally exceeds acceptable daily intake levels (ADI's) (3.7mg NO<sub>3</sub><sup>-</sup>/kg/day),



as established by The World Health Organisation [2,66]. Given the positive systemic and oral health benefits of inorganic  $\text{NO}_3^-$ , perhaps ADI's should be reevaluated to reflect  $\text{NO}_3^-/\text{NO}_2^-$  source.

### ***NO<sub>3</sub><sup>-</sup> as a prebiotic for oral health***

Throughout, connections between dietary  $\text{NO}_3^-$  consumption and positive oral health have been highlighted. In total,  $n=11$  studies showed  $\text{NO}_3^-$  supplementation increased NR health-associated bacteria and/or reduced levels of common oral pathogens, thus establishing eubiosis and reducing oral disease risk [7,15,17,30–32,34–38]. Additionally,  $n=5$  studies showed  $\text{NO}_3^-$  consumption modified oral biochemistry by increasing pH levels and reducing lactate production, thus also minimising dysbiosis [7,15,31,35,38]. Lastly, one study showed  $\text{NO}_3^-$  decreased gingival inflammation in patients with chronic gingivitis [16,17].

In contrast, evidence suggests CHX-containing mouthwashes used for periodontal treatment could stimulate dysbiosis and caries development, and could be exacerbating AMR [10,12,51,54,55]. Given the positive effects of vegetable-based sources of  $\text{NO}_3^-$  and the low risk of emerging NO resistance in oral pathogens [56], evidence in this review indicates  $\text{NO}_3^-$  could be used as an effective prebiotic to promote oral health. Overall,  $\text{NO}_3^-$  could serve as a preventative strategy against PD in high-risk groups or as a therapeutic intervention in individuals with PD (Figure 4). Although more studies are required to confirm the effects of  $\text{NO}_3^-$  and secondary metabolites on oral commensals, evidence provided in this review strongly indicates that  $\text{NO}_3^-$  could be used to simulate eubiosis and oral health.

### **Conclusion**

This review has evaluated the impact of  $\text{NO}_3^-$  on the oral microbiome. The findings indicate that dietary  $\text{NO}_3^-$  increases oral health-associated bacterial genera, including *Rothia* and *Neisseria*, whilst reducing bacteria implicated in oral disease, including *Prevotella* and *Veillonella* spp.  $\text{NO}_3^-$  also beneficially alters the biochemical environment, by increasing oral pH and reducing dental caries risk. Additionally, the link between oral and systemic benefits was highlighted, particularly in relation to  $\text{NO}_3^-$  associated cardioprotective benefits. Further *in vivo* studies are required to uncover the mechanisms underlying the beneficial effects of  $\text{NO}_3^-$  on the oral microbiome; however, the evidence presented in this review indicates dietary  $\text{NO}_3^-$  could be used as a prebiotic for oral health.

### **Highlights**

- This systematic review evaluated the effects of nitrate on the oral microbiome.
- Dietary nitrate and nitrate supplements increased oral health-associated genera, particularly *Rothia* and *Neisseria*.
- The genus *Prevotella*, which is normally associated with periodontal disease and halitosis, decreased post-nitrate consumption.
- Nitrate also increased oral pH, while decreasing the levels of *Streptococcus* and *Veillonella*, which is a positive change from a caries perspective.
- Oral health benefits were linked to systemic benefits, particularly improvements to markers of cardiovascular disease risk.
- Overall, nitrate could be considered as a prebiotic for oral health.

### **Disclosure statement**

No potential conflict of interest was reported by the author(s).

### **Funding**

This research received no external funding.

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