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# Association Between Dietary Patterns and Subgingival Microbiota: Results From the Oral Infections, Glucose Intolerance, and Insulin Resistance Study (ORIGINS)

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**Keywords:** diet | microbial diversity | oral microbiome | subgingival plaque | α-diversity

## ABSTRACT

**Objective:** To study the association between dietary patterns and subgingival microbiota.

**Methods:** Participants ($n = 651$) who were enrolled in the Oral Infections, Glucose Intolerance, and Insulin Resistance Study (ORIGINS) with subgingival plaque sampling ($n = 890$ plaques) and a dietary assessment were included. 16S rRNA gene amplicon sequences of subgingival plaque from sites with either probing depth <4 or ≥4 mm were processed separately and used to obtain α-diversity metrics (Faith, Shannon, Simpson, Observed) and taxa ratios (*Red Complex* to *Corynebacterium* [RCLR], *Treponema to Corynebacterium* [TCLR], and *Treponema to Neisseria* [TNLR]). Food frequency questionnaires (FFQs) were processed to calculate Alternate Healthy Eating Index (AHEI) and A Priori Diet Quality Score (APDQS) scores. Mixed regression models examined the mean levels of microbial metrics across quartiles of diet quality. Means ± standard errors are reported along with *p*-values.

**Results:** In multivariable models assessing the association between diet scores and α-diversity metrics, higher AHEI values were significantly associated with lower Faith ($p$-value = 0.01) and Observed ($p$-value = 0.04) diversity values; similar findings were observed for APDQS ($p$-value = 0.01, $p$-value = 0.04). In multivariable models assessing the association between diet scores (AHEI and APDQS) and taxa ratios (RCLR, TCLR and TNLR), as the AHEI quartile increased, all taxa ratios decreased significantly as follows: −1.06 ± 0.093 in Q1 to −1.34 ± 0.099 in Q4 (RCLR), −0.43 ± 0.077 in Q1 to −0.64 ± 0.083 in Q4 (TCLR)

and −0.09 ± 0.083 in Q1 to −0.38 ± 0.089 in Q4 (TNLR), respectively. In contrast, as the APDQS quartiles increased, only TNLR decreased significantly from −0.08 ± 0.085 in Q1 to −0.34 ± 0.091 in Q4.
**Conclusion:** Diets rich in fruits, vegetables, whole grains and other nutritionally rich plant foods are associated with lower oral microbial diversity and favourable ratios of pathogenic to commensal microbiota.

## 1 | Introduction

The oral cavity harbours a wide range of diverse microorganisms, creating an ecological community known as the oral microbiome (Lamont, Koo, and Hajishengallis 2018). Prior research has extensively characterized how microbial dysbiosis (i.e., imbalance) contributes to both oral diseases (e.g., caries and periodontitis) and systemic ones (e.g., diabetes, cardiovascular disease and stroke) (X. Li et al. 2000; Kebschull, Demmer, and Papapanou 2010; Socransky and Haffajee 2005). In states of microbial homeostasis, oral microorganisms help protect against pathogens and harmful external stimuli, promote pH recovery and inhibit the proliferation of periodontal pathogens and cariogenic species, contributing to resilience against oral pathologic conditions and their sequalae (Radaic and Kapila 2021; Van Dyke, Bartold, and Reynolds 2020).

Diet plays a major role in health, and substantial evidence exists demonstrating that an unhealthy diet (often characterized by high consumption of red meat, saturated fat, refined grains and added sugars, and/or low consumption of fruits, vegetables and poly- and mono-unsaturated fats) is related to a variety of chronic diseases including cancers, cardiometabolic disease, renal disease and dementia (Mueller and Appel 2017). Evidence is also accumulating linking diet to periodontal disease (Khocht et al. 2021; Tennert et al. 2020; Vach et al. 2022; Martinon et al. 2021; Salazar et al. 2018; DeMayo et al. 2021; A. Li et al. 2023), although only limited research exists exploring the relationship between diet and the subgingival microbiome.

Dietary intake can influence endogenous environments through systemic circulation of nutrients, warranting further investigation of its effects on the oral microbiota (Kato et al. 2017). Several studies assessing short- (Johnson et al. 2019; David et al. 2014; De Filippis et al. 2016) and long-term (Bolte et al. 2021; Asnicar et al. 2021; Wu et al. 2011) influences of diet on the gut microbiome have found that dietary patterns correspond with microbial composition (Asnicar et al. 2021). Short-term dietary interventions have been shown to alter gut microbiota diversity quickly in humans, although these alterations are transient and do not persist for more than a few days after removal of the intervention (David et al. 2014), suggesting that there are other host factors controlling microbial ecological homeostasis that extend beyond diet (Roager et al. 2016). In contrast, long-term dietary patterns have been shown to be associated with distinct compositional differences (Wu et al. 2011; Choi et al. 2022). In the context of the oral microbiome, a nascent literature is emerging, suggesting that diet might influence oral microbial composition (Khocht et al. 2021; Tennert et al. 2020; Vach et al. 2022). However, prior studies had small sample sizes ($n = 39$ participants in the largest prior publication; Khocht et al. 2021) and only two prior studies, to our knowledge, evaluated the microbial composition of subgingival plaque (Khocht et al. 2021; Woelber et al. 2019; A. Li et al. 2023).

Diet quality scores, such as the Alternate Healthy Eating Index (AHEI) and A Priori Diet Quality Score (APDQS), make it possible to quantify diet quality. AHEI was constructed using evidence-based recommendations that incorporate foods and nutrients to predict the risk of chronic diseases (Al-Ibrahim and Jackson 2019). The index seeks to capture specific dietary patterns and eating behaviours that have been associated consistently with lower risk for chronic diseases in clinical and epidemiological investigations (McCullough et al. 2002). APDQS is a food-based scoring measure that has recently been developed to reflect overall diet quality, and APDQS rewards reductions in the consumption of processed foods, red meat, sweet/salty foods and whole fat dairy, and increased consumption of seeds, white meat, plants and low-fat dairy (Sijtsma et al. 2012). Both measures have been validated (Nettleton et al. 2008; Chiuve et al. 2012; McCullough and Willett 2006).

In the present study, we investigate the cross-sectional association between dietary patterns and the subgingival microbiota among participants enrolled in the Oral Infections Glucose Intolerance and Insulin Resistance Study (ORIGINS). We hypothesized that healthier diets would be associated with altered oral microbial diversity and specific taxa ratios.

## 2 | Methods

### 2.1 | Study Population

ORIGINS is a prospective cohort study at the Columbia University Medical Center in New York City. Participants enrolled between January 2016 and January 2020 ($n = 814$) were included in the current analysis. The inclusion criteria for ORIGINS participants were as follows: (i) aged 20–55 years; (ii) had no diabetes mellitus (T1 or T2) based on the participant's self-report of no previously diagnosed disease, HbA1c values <6.5% and fasting plasma glucose <126 mg/dL; and (iii) had no history of myocardial infarction, congestive heart failure, stroke or chronic inflammatory conditions based, again, on the participant's self-report. A total of $n = 782$ participants completed a dietary questionnaire via electronic survey at the time of their baseline enrollment. After removing individuals with missing periodontal measures, demographic information or diet information, 651 participants who contributed a total of 890 subgingival plaque samples remained in the analysis. Female participants with food energy values ≤600 or ≥6000 kcal and males with food energy values ≤800 or ≥8000 kcal were excluded (Choi et al. 2020; Banna et al. 2017; Lo Siou et al. 2021).

### 2.2 | Ethics Statement

All participants in ORIGINS provided written informed consent prior to study enrollment. The Institutional Review Boards

**Summary**

- Growing evidence suggests that dietary patterns are related to periodontal health outcomes. Diet has emerged as a potential influencer of oral microbial diversity, but this relationship is not well studied.
- We found that healthier diets (driven by higher nut/legume and whole grain intake) were associated with lower oral microbial diversity and favourable ratios of pathogenic to commensal microbiota.
- Diet interventions may serve as a viable approach in promoting oral eubiosis, thus promoting periodontal health.

at Columbia University (AAAD2521) and the University of Minnesota (STUDY00002673) approved the use of health data for the purposes of scientific research.

## 2.3 | Dietary Assessment

Dietary data were collected using the National Cancer Institute's Diet History Questionnaire 1 (DHQ-1), which queries the frequency of consumption and portion size for 124 food items over the past 12 months (Csizmadi et al. 2007). This food frequency questionnaire (FFQ) has previously been validated and found to provide valid nutrient intake estimates (Kipnis et al. 2003; Subar et al. 2001, 2003; Thompson et al. 2002). Of the participants, 96.1% completed the FFQ. DietCalc (2007) (National Cancer Institute 2005) was used to calculate nutrient level data from the FFQ. This dietary data was subsequently operationalized using SAS (version 9.4) to calculate the AHEI and APDQS. Briefly, the AHEI was scored on the basis of intake of 11 dietary components (fruits, vegetables, nuts, red meat, sugar sweetened beverages, omega-3 fatty acids, polyunsaturated fats, trans fats, alcohol, whole grains and sodium), which were summed for a total AHEI score (Al-Ibrahim and Jackson 2019; Kirkpatrick et al. 2018). APDQS was calculated on the basis of intake of 'positive foods' (i.e., those postulated to be associated with reduced cardiovascular disease risk) such as green vegetables, fruits, lean fish, low fat dairy products and whole grains; 'neutral foods' (i.e., those irrelevant to or with uncertain cardiovascular disease risk) such as eggs, lean meat, shellfish and potatoes; and 'negative foods' (i.e., those postulated to be associated with higher cardiovascular disease risk) such as fried potatoes, high-fat and processed meat, desserts, pastries, full-fat dairy products and soft drinks (Sijtsma et al. 2012). The total and subcomponent AHEI score and APDQS were modelled in quartiles. Quartile 1 consisted of participants with the lowest total and subcomponent diet variable scores for AHEI and APDQS, respectively (AHEI quartile 1: 16.36–37.11, AHEI quartile 2: 37.11–45.64, AHEI quartile 3: 45.64–54.39 and AHEI quartile 4: 54.39–82.49; APDQS quartile 1: 27–54, APDQS quartile 2: 54–60.57, APDQS quartile 3: 60.57–69, and APDQS quartile 4: 69–96). Higher values of either score represent a theoretically more favourable diet pattern, rich in fruits, vegetables, whole grains and nuts. Lower values of either score represent a theoretically less favourable diet patten, rich in processed foods, sugar-sweetened beverages and sodium.

## 2.4 | Periodontal Assessments and Plaque Collection

Dental examiners conducted full-mouth periodontal examinations as previously described (Demmer et al. 2015, 2019). Periodontal probing depth and attachment loss were measured at six sites per tooth with a UNC-15 manual probe. Periodontitis was defined according to the CDC/AAP classification (Eke et al. 2012). Subgingival plaque was collected from up to eight predetermined sites per participant. Plaque samples were collected from the mesiolingual site in the upper teeth and mesio-buccal site in the lower teeth, preserved in 750 μL of MoBio buffer and stored at −80°C following the Human Microbiome Project protocol (The Human Microbiome Project Consortium 2012). Sites of plaque collection were as follows and in accordance with the Human Microbiome Project protocol: six plaques were collected from the following six index teeth: two molar teeth (#3 and #19), two premolar teeth (#12 and #28) and two incisor teeth (#9 and #25). If index teeth were missing, the next most anterior tooth in the same quadrant was selected. Two additional biofilm samples were collected from the two deepest pockets identified in the full mouth in any interproximal site (if they were not one of the index sites). Plaque samples were preserved in MoBio tubes. To be considered as a moderate/deep pocket, the probing depth was required to be ≥4 mm, while probing depth <4 mm was considered within the range of the normal periodontal sulcus. After DNA extraction from individual plaques, DNA was pooled together based on clinical status (PD <4 vs. ≥4 mm). Thus, each participant contributed at most two pooled subgingival samples to the analysis. Periodontitis was treated as a continuous and categorical variable, classified as a binary variable, that is, non/mild periodontitis and moderate/severe periodontitis.

## 2.5 | Oral Microbiota Assessment

### 2.5.1 | DNA Extraction

Microbial DNA was extracted using the MasterPure Gram Positive DNA Positive Purification kit (Lucigen).

### 2.5.2 | 16S rRNA Sequencing and Taxonomic Classification

Sequencing of the 16S rRNA gene was conducted per the HOMINGS methodology (Human Oral Microbiome Identification Using Next Generation Sequencing), designed specifically for oral taxa to generate species-level information. A modified protocol was used as previously described (Gomes et al. 2015): 16S rDNA (50 ng) was amplified with 341F/806R universal primers (V3–V4 region), and polymerase chain reaction products were purified with AMPure beads; 100 ng of each library was pooled, gel-purified and quantified with a bioanalyzer; and 12pM of the library mixture was spiked with 20% PhiX and run on a MiSeq (Illumina) platform. The 16S data curation pipeline has been outlined previously (Marotz et al. 2022). Overall, 18,531,931 sequences were generated for the final analysis (median, 75,977 sequences per sample). Sequence reads were taxonomically classified with two approaches. First, a customized BLAST program (ProbeSeq for HOMINGS) blasted the

16S rRNA reads against species-specific 16S rRNA-based oligonucleotide 'probes' (Mougeot et al. 2017). Quality-filtered forward-read sequences were denoised using Deblur (Marotz et al. 2017) with default parameters. Samples with less than 1000 quality-filtered reads were removed from downstream analysis.

The multinomial regression tool Songbird (Morton et al. 2019) was used to identify differentially abundant microbes in periodontal pockets <4 versus ≥4 mm. Each amplicon sequence variant (ASV) was assigned a differential, where higher scores reflect relative enrichment in periodontal pockets <4 mm, and low scores reflect relative enrichment in periodontal pockets ≥4 mm. The phylogenetic relationship among these ASVs and their associated Songbird differentials was visualized with EMPress (Chen et al. 2018). ASVs from the genus *Treponema* tended to have low (disease-associated) differentials, while ASVs from the genus *Corynebacterium* tended to have high (health-associated) differentials. Second, a DADA2 workflow was applied to identify 'exact sequence variants', and taxonomy was assigned using the Silva Projects (version 128) reference database (Callahan, McMurdie, and Holmes 2017; Callahan et al. 2016).

#### 2.5.3 | α-Diversity

α-Diversity is the mean diversity of species within a sample (Whittaker 1972). Four different measures of α-diversity were calculated: Faith phylogenetic diversity (Faith), Shannon index (Shannon), Simpson's index (Simpson) and Observed species (Observed). Faith accounts for phylogenetic relatedness (Faith 2018); Shannon is an information statistic index, assuming all species are represented in a sample and that they are randomly sampled (Thukral et al. 2019); while Simpson is a dominance index, giving more weight to common or dominant species (Thukral et al. 2019), and Observed is the simplest measure of richness, that is, the number of species observed in the sample (Edgar and Flyvbjerg 2018).

### 2.6 | Statistical Analyses

Participant characteristics were described using means ± standard deviation (SD), frequencies and percentages. A Pearson correlation matrix containing α-diversity metrics and taxa ratios is presented to illustrate the relationships between the different measures. Mixed regression models were used to examine the associations between quartiles of diet quality and α-diversity metrics (using quartile 1 as the reference group). Given that each participant could contribute up to two pooled subgingival samples, mixed models were used to account for the within-person correlation present within samples contributed by the same participant. Means ± standard errors (SE) are reported along with *p*-values. Models were constructed for each diet quality score as follows: Model 1: total caloric intake, age, gender, race/ethnicity, education, smoking, body mass index, prediabetes, periodontitis status. Similar linear regressions were also performed separately among periodontal pockets <4 mm and periodontal pockets ≥4 mm.

α-Diversity metrics that were statistically significantly associated with the diet score (AHEI and/or APDQS) were then further evaluated to examine whether the metric was associated with the different components that make up each diet score. Mixed regression models were used to examine the associations of quartiles of each dietary component with α-diversity metrics (using quartile 1 as the reference group). Results were presented as means ± SE for two models: α model 1: crude, and α model 2: age, gender, race/ethnicity, education, smoking, total caloric intake, body mass index, prediabetes, periodontal status and total AHEI score or total APDQS score depending on which diet score was being assessed.

The multinomial regression tool Songbird (Morton et al. 2019) was used to identify differentially abundant microbes in high versus low diet scores. Each ASV was assigned a differential score, where higher scores reflect relative enrichment in higher (better/healthier) AHEI and APDQS, and low scores reflect relative enrichment in lower (worse) AHEI and APDQS scores. To further characterize ASVs associated with pockets <4 versus ≥4 mm, we plotted the Songbird differentials from each ASV using the interactive tool Qurro (X. Li et al. 2000) to visualize feature rankings and log-ratios. Prior research (Marotz et al. 2022; Kageyama et al. 2017; Socransky et al. 1998) and findings from Qurro have identified three different taxa ratios that are predictive of systemic disease: the Red Complex (consisting of known periodontopathic bacteria *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*) to *Corynebacterium* (RCLR); *Treponema* to *Corynebacterium* (TCLR) and *Treponema* to *Neisseria* (TNLR).

All log-ratios of taxa were calculated as pseudo-counts to deal with zero inflation. Mixed regression models were used to examine the associations of quartiles of diet quality with taxa ratios (using quartile 1 as the reference group). Means ± SEs were reported along with *p*-values. Five models were constructed for each diet quality score as follows: diet model 1: total caloric intake; diet model 2: model 1 + age, gender, race/ethnicity, education; diet model 3: model 2 + smoking; diet model 4: model 3 + body mass index, prediabetes; diet model 5: model 4 + periodontitis status. All analyses were performed with R (version 3.5.3).

## 3 | Results

### 3.1 | Study Participants

The average age of participants was 31.3 ± 9.2 (median age 27.7) with 72% being women, 31% White, 28% Hispanic, 14% Black and 27% other. Eighty-seven percent of participants never smoked, 51% had a 4-year college education and 59% were underweight/normal weight. The mean BMI was 25.5 ± 5.9 kg/m$^2$ and the total caloric intake was 1742 ± 942 kcal, with 9.9% having prediabetes, 9.6% having hypertension and 28% having moderate/severe periodontitis (Table 1). General characteristics of study participants according to quartiles of Faith are presented in Table 1. Age, BMI and periodontitis significantly increased with increasing quartiles of Faith.

### 3.2 | Microbial Diversity Metrics and Diet Score

The correlations between α-diversity metrics and taxa ratios are presented in Table 2. Faith and Observed are highly correlated ($r = 0.90$, $p < 0.0001$) as are Simpson and Shannon ($r = 0.85$, $p < 0.0001$). The three taxa ratios (RCLR, TCLR and TNLR) are

**TABLE 1** | General demographics for ORIGINS participants, mean ± SD or % and mean ± SE for Faith or %.

| | All ($n = 651$) | Q1 Faith ($N = 163$) | Q2 Faith ($N = 163$) | Q3 Faith ($N = 162$) | Q4 Faith ($N = 163$) |
|---|---|---|---|---|---|
| AHEI score | 46.4 ± 12.1 | 48.4 ± 0.94 | 46.3 ± 0.94 | 47.6 ± 0.95 | 43. ± 0.94*,** |
| APDQS score | 61.1 ± 11.6 | 63.5 ± 0.90 | 61.0 ± 0.90 | 61.5 ± 0.90 | 58.6 ± 0.90*,** |
| Age (years) | 31.3 ± 9.2 | 30.7 ± 0.72 | 30.8 ± 0.72 | 30.4 ± 0.72 | 33.2 ± 0.72*,** |
| Sex | | | | | |
| Male | 27.6 | 27.0 | 28.8 | 21.0 | 33.7 |
| Female | 72.4 | 73.0 | 71.2 | 79.0 | 66.3 |
| Race | | | | | |
| Hispanic | 27.6 | 21.5 | 27.6 | 24.7 | 36.8* |
| White | 30.6 | 42.9 | 26.4 | 33.3 | 19.6 |
| Black | 14.0 | 11.0 | 15.3 | 13.0 | 16.6 |
| Other | 27.8 | 24.5 | 30.7 | 29.0 | 27.0 |
| Education | | | | | |
| <College | 21.2 | 14.1 | 17.2 | 17.9 | 35.6* |
| 4-Year college | 51.3 | 50.9 | 60.7 | 54.3 | 39.3 |
| Graduate | 27.5 | 35.0 | 22.1 | 27.8 | 25.1 |
| Smoking | | | | | |
| Never | 86.9 | 85.9 | 92.0 | 87.7 | 82.2 |
| Former | 6.5 | 7.4 | 4.3 | 6.2 | 8.0 |
| Current | 6.6 | 6.7 | 3.7 | 6.2 | 9.8 |
| Weight status | | | | | |
| Underweight/ normal | 58.4 | 65.0 | 60.9 | 65.8 | 41.9* |
| Overweight | 24.3 | 18.8 | 24.2 | 18.6 | 35.6 |
| Obese | 17.3 | 16.3 | 14.9 | 15.5 | 22.5 |
| BMI | 25.5 ± 5.9 | 24.6 ± 0.46 | 25.1 ± 0.46 | 25.0 ± 0.46 | 27.2 ± 0.46*,** |
| Total caloric intake | 1742 ± 942.2 | 1686 ± 73.8 | 1747 ± 73.8 | 1708 ± 74.1 | 1828 ± 73.8 |
| Prediabetes | | | | | |
| Yes | 9.9 | 8.6 | 9.3 | 9.3 | 12.3 |
| Hypertension | | | | | |
| Yes | 9.6 | 8.0 | 8.1 | 8.0 | 14.4 |
| Periodontitis | 0.64 ± 0.93 | 1.53 ± 0.071 | 1.44 ± 0.071 | 1.52 ± 0.071 | 2.06 ± 0.071*,** |
| Periodontitis | | | | | |
| None/mild | 72.0 | 75.5 | 82.2 | 75.3 | 55.2* |
| Moderate/severe | 28.0 | 24.5 | 17.8 | 24.7 | 44.8 |

*Note:* $N = 651$; quartiles mean ± SE.
*$p < 0.05$ for any difference in category.
**$p < 0.05$ for linear trend.

most strongly correlated with Faith diversity and less strongly with Shannon, Observed or Simpson (Table 2).

In the fully adjusted analysis including all periodontal sites, which controlled for total caloric intake, age, gender, race/ethnicity, education, smoking status, BMI, prediabetes and periodontal status, Faith and Observed diversity were significantly associated with AHEI. As the AHEI quartiles increased, mean values of Faith were lower ([15.2 ± 0.31 in quartile 1 to 14.5 ± 0.33 in quartile 4,

**TABLE 2** | Correlation matrix of α-diversity metrics and three taxa ratios assessed in this study.

| | Shannon | Observed | Faith | Simpson | TCLR | RCLR | TNLR |
|---|---|---|---|---|---|---|---|
| Shannon | 1 | **0.785** | **0.647** | **0.851** | **0.194** | **0.189** | **0.253** |
| Observed | **0.785** | 1 | **0.899** | **0.473** | **0.365** | **0.385** | **0.408** |
| Faith | **0.647** | **0.899** | 1 | **0.359** | **0.581** | **0.621** | **0.604** |
| Simpson | **0.851** | **0.473** | **0.359** | 1 | 0.034 | 0.029 | **0.101** |
| TCLR_pseudo[a] | **0.194** | **0.365** | **0.581** | 0.034 | 1 | **0.948** | **0.751** |
| RCLR_pseudo[b] | **0.189** | **0.385** | **0.621** | 0.029 | **0.948** | 1 | **0.726** |
| TNLR_pseudo[c] | **0.253** | **0.408** | **0.604** | **0.101** | **0.751** | **0.726** | 1 |

*Note:* Bolded correlation coefficients *p*-values <0.05.
[a]TCLR: Log-ratio of taxa of *Treponema to Corynebacterium* calculated as pseudo-counts to deal with zero inflation.
[b]RCLR: Log-ratio of taxa of *Red Complex to Corynebacterium* calculated as pseudo-counts to deal with zero inflation.
[c]TNLR: Log-ratio of taxa of *Treponema to Neisseria* calculated as pseudo-counts to deal with zero-inflation.

$p=0.01$] Table 3). Similar patterns were observed for Observed ([198 ± 5.77 in quartile 1 to 187 ± 6.11 in quartile 4, $p=0.04$] Table 3). Other α-diversity metrics also decreased with increasing quartiles of AHEI, but none was statistically significant (Table 3). These trends were generally consistent when analysing plaques from periodontal sites <4 versus ≥4 mm separately. Among periodontal sites with <4 mm probing depth, as the AHEI quartile increased, mean values of Shannon ($p=0.02$), Observed ($p=0.01$) and Faith diversity ($p=0.01$) decreased significantly. The same trend persisted among ≥4 mm pockets (as the AHEI quartile increased, the mean values of α-diversity decreased), although the trend was only statistically significant for Faith ($p=0.02$).

In the fully adjusted model including all periodontal sites, higher APDQS was associated with lower Faith: scores decreased significantly from 15.5 ± 0.31 in quartile 1 to 14.6 ± 0.33 in quartile 4 ($p$ for trend = 0.01, Table 3). Findings were similar for Observed, where diversity scores decreased from 201 ± 5.82 in quartile 1 to 189 ± 6.21 in quartile 4 ($p$ for trend = 0.04, Table 3). Among the <4 mm probing depth sites, as APDQS quartile increased, the mean values of Observed ($p=0.03$) and Faith diversity ($p=0.01$) decreased significantly. The same trend persisted among ≥4 mm sites (as APDQS quartile increased, the mean values of α-diversity decreased), although the trend was not significant. Lastly, β-diversity was not associated with either diet score after full multivariable adjustment (data not shown).

### 3.3 | Microbial Diversity Metrics and Diet Score Components

The association between Faith and components of the AHEI diet score are presented in Table 4. Of the 11 components, only nuts/legumes consumption was statistically significantly associated with Faith diversity after adjustment. Components of the APDQS score were not significantly associated with Faith diversity in the fully adjusted model (data not shown).

### 3.4 | Taxa Ratios and Diet Score

In Songbird analyses of taxa-level associations which account for microbiota compositionality, higher diet quality was generally associated with lower relative abundance of ASVs from the genera *Treponema* and *Red Complex* taxa, and higher relative abundance of ASVs from the genera *Corynebacterium* and *Neisseria* (Figure 1). Among all ASV ratios, the inverse association between ASV log-ratios and AHEI score was strongest in <4 mm probing depth sites and not statistically significant in ≥4 mm sites for the *Treponema:Corynebacterium* and *Treponema:Neisseria* ratios (Figure 1). Similar patterns were observed for the APDQS score (Figure S1).

The relationship between the same taxa ratios TCLR, TNLR and RCLR were then assessed across quartiles of AHEI. As AHEI quartiles increased, TCLR, TNLR and RCLR decreased significantly (−0.43 ± 0.08 in quartile 1 to −0.64 ± 0.08 in quartile 4; 0.09 ± 0.08 in quartile 1 to −0.38 ± 0.089 in quartile 4; and −1.06 ± 0.09 in quartile 1 to −1.34 ± 0.1 in quartile 4, respectively) (Table 5). Similarly, as APDQS quartiles increased, TNLR decreased significantly (−0.08 ± 0.1 in quartile 1 to −0.34 ± 0.09 in quartile 4) (Table 5). However, as APDQS quartiles increased, TCLR and RCLR decreased though not significantly (−0.43 ± 0.078 in quartile 1 to −0.56 ± 0.084 in quartile 4, $p$-value = 0.33 and −1.05 ± 0.093 in quartile 1 to −1.23 ± 0.101 in quartile 4, $p$-value = 0.19), respectively (Table 5).

## 4 | Discussion

We observed that higher diet quality as assessed via AHEI or ADPQS was associated with reduced subgingival microbial α-diversity. Findings were strongest among Observed and Faith diversity indices. Among sites with <4 mm probing depth, higher AHEI diet quality was additionally related to reduced Shannon. Additionally, higher diet quality was associated with lower ratios of pathogenic-associated to health-associated microbiota. These findings remained after multivariable adjustment for potential confounders.

In a previous publication from ORIGINS, neither AHEI or APDQS was associated with mean probing depth, mean attachment loss or periodontitis, although increased AHEI was found to be modestly associated with reduced bleeding on probing (DeMayo et al. 2021). These findings were consistent at the level of food groups with only nuts, red meat and trans-fatty acid consumption being related to probing depth and bleeding on probing but not attachment

**TABLE 3** | Association between alternative healthy eating index score or a priori diet quality score and α-diversity metrics, mean ± SE.

| | First quartile AHEI ($n = 223$) | Second quartile AHEI ($n = 222$) | Third quartile AHEI ($n = 222$) | Fourth quartile AHEI ($n = 223$) | *p*-value* |
|---|---|---|---|---|---|
| Shannon | | | | | |
| All sites | 5.38 ± 0.08 | 5.42 ± 0.08 | 5.29 ± 0.08 | 5.29 ± 0.08 | 0.12 |
| Shallow | 5.41 ± 0.08 | 5.45 ± 0.09 | 5.25 ± 0.09 | 5.27 ± 0.09 | 0.02 |
| Moderate/deep | 5.41 ± 0.16 | 5.42 ± 0.17 | 5.43 ± 0.16 | 5.32 ± 0.19 | 0.67 |
| Observed | | | | | |
| All sites | 198 ± 5.77 | 197 ± 6.22 | 189 ± 6.12 | 187 ± 6.11 | 0.04 |
| Shallow | 198 ± 6.14 | 199 ± 6.68 | 185 ± 6.53 | 185 ± 6.46 | 0.01 |
| Moderate/deep | 214 ± 10.3 | 208 ± 10.9 | 206 ± 10.6 | 197 ± 12.0 | 0.15 |
| Faith | | | | | |
| All sites | 15.2 ± 0.31 | 15.3 ± 0.33 | 14.8 ± 0.33 | 14.5 ± 0.33 | 0.01 |
| Shallow | 15.0 ± 0.32 | 15.1 ± 0.35 | 14.4 ± 0.34 | 14.3 ± 0.34 | 0.01 |
| Moderate/deep | 17.2 ± 0.56 | 17.0 ± 0.59 | 16.4 ± 0.58 | 16.0 ± 0.65 | 0.02 |
| Simpson | | | | | |
| All sites | 0.940 ± 0.006 | 0.946 ± 0.006 | 0.938 ± 0.006 | 0.936 ± 0.006 | 0.33 |
| Shallow | 0.945 ± 0.006 | 0.948 ± 0.007 | 0.936 ± 0.006 | 0.937 ± 0.006 | 0.08 |
| Moderate/deep | 0.932 ± 0.013 | 0.941 ± 0.013 | 0.944 ± 0.013 | 0.927 ± 0.015 | 0.98 |
| | **First quartile APDQS ($n = 246$)** | **Second quartile APDQS ($n = 214$)** | **Third quartile APDQS ($n = 230$)** | **Fourth quartile APDQS ($n = 200$)** | ***p*-value*** |
| Shannon | | | | | |
| All sites | 5.42 ± 0.08 | 5.33 ± 0.08 | 5.30 ± 0.08 | 5.29 ± 0.08 | 0.09 |
| Shallow | 5.44 ± 0.08 | 5.31 ± 0.09 | 5.30 ± 0.09 | 5.28 ± 0.09 | 0.07 |
| Moderate/deep | 5.47 ± 0.16 | 5.47 ± 0.17 | 5.33 ± 0.17 | 5.34 ± 0.18 | 0.29 |
| Observed | | | | | |
| All sites | 201 ± 5.82 | 191 ± 5.97 | 189 ± 6.10 | 189 ± 6.21 | 0.04 |
| Shallow | 202 ± 6.20 | 188 ± 6.37 | 189 ± 6.52 | 188 ± 6.58 | 0.03 |
| Moderate/deep | 215 ± 10.3 | 212 ± 11.0 | 201 ± 10.7 | 202 ± 11.8 | 0.10 |
| Faith | | | | | |
| All sites | 15.5 ± 0.31 | 14.8 ± 0.32 | 14.8 ± 0.33 | 14.6 ± 0.33 | 0.01 |
| Shallow | 15.3 ± 0.32 | 14.4 ± 0.33 | 14.6 ± 0.34 | 14.4 ± 0.34 | 0.01 |
| Moderate/deep | 17.2 ± 0.56 | 16.9 ± 0.60 | 16.4 ± 0.58 | 16.4 ± 0.65 | 0.08 |
| Simpson | | | | | |
| All sites | 0.942 ± 0.006 | 0.941 ± 0.006 | 0.939 ± 0.006 | 0.935 ± 0.006 | 0.18 |
| Shallow | 0.945 ± 0.006 | 0.943 ± 0.006 | 0.941 ± 0.006 | 0.935 ± 0.006 | 0.13 |
| Moderate/deep | 0.940 ± 0.013 | 0.939 ± 0.013 | 0.932 ± 0.013 | 0.934 ± 0.015 | 0.48 |

*Note:* Adjusted for total caloric intake, age, gender, race/ethnicity, education, smoking, body mass index, prediabetes and periodontitis as binary variables; among all sites, among shallow pocket sites and among moderate/deep pocket sites. $n = 651$ participants providing $n = 890$ plaque samples collected from 641 periodontal sites <3 mm and 249 periodontal sites ≥4 mm.
**p*-values for linear trend.

loss. The current results align with our previous findings in a few important ways. First, while we find a relationship between diet quality and subgingival microbiota, the findings are weak, as with diet–periodontal disease associations. Second, our prior findings only observed a relationship between diet quality and measures of current periodontal inflammation (probing depth and bleeding

**TABLE 4** | Association of components of the alternative healthy eating index score and Faith phylogenetic diversity, mean ± SE.

| | First quartile food type ($n = 223$) | Second quartile food type ($n = 222$) | Third quartile food type ($n = 222$) | Fourth quartile food type ($n = 223$) | *$p$-value |
|---|---|---|---|---|---|
| Vegetables | | | | | |
| Crude | 14.8 ± 0.22 | 13.9 ± 0.22 | 14.8 ± 0.22 | 14.4 ± 0.21 | 0.79 |
| Adjusted | 15.0 ± 0.32 | 14.3 ± 0.33 | 15.2 ± 0.32 | 15.0 ± 0.32 | 0.35 |
| a(No Perio) | 14.9 0.317 | 14.2 0.333 | 15.1 0.321 | 14.8 0.318 | 0.38 |
| Fruit | | | | | |
| Crude | 14.9 ± 0.22 | 14.2 ± 0.22 | 14.6 ± 0.22 | 14.2 ± 0.22 | 0.09 |
| Adjusted a | 15.2 ± 0.32 | 14.5 ± 0.33 | 15.3 ± 0.32 | 14.6 ± 0.33 | 0.43 |
| Adjusted b | 15.0 0.315 | 14.4 0.327 | 15.1 0.322 | 14.5 0.335 | 0.44 |
| Nuts/legumes | | | | | |
| Crude | 15.1 ± 0.22 | 14.7 ± 0.22 | 14.0 ± 0.21 | 14.1 ± 0.21 | 0.0001 |
| Adjusted a | 15.3 ± 0.34 | 15.1 ± 0.34 | 14.7 ± 0.32 | 14.7 ± 0.36 | 0.09 |
| Adjusted b | 15.2 0.344 | 15.0 0.337 | 14.5 0.318 | 14.5 0.356 | 0.04 |
| Whole grains | | | | | |
| Crude | 15.1 ± 0.22 | 14.0 ± 0.21 | 14.3 ± 0.22 | 14.4 ± 0.22 | 0.08 |
| Adjusted a | 15.5 ± 0.32 | 14.6 ± 0.32 | 14.8 ± 0.32 | 14.8 ± 0.32 | 0.06 |
| Adjusted b | 15.4 0.322 | 14.4 0.319 | 14.7 0.325 | 14.7 0.325 | 0.07 |
| Polyunsaturated fatty acids | | | | | |
| Crude | 14.5 ± 0.22 | 14.5 ± 0.22 | 14.6 ± 0.22 | 14.2 ± 0.22 | 0.59 |
| Adjusted a | 14.8 ± 0.32 | 15.0 ± 0.32 | 15.3 ± 0.33 | 14.8 ± 0.33 | 0.59 |
| Adjusted b | 14.6 0.321 | 14.9 0.324 | 15.1 0.327 | 14.7 0.334 | 0.63 |
| Omega-3 fatty acids | | | | | |
| Crude | 14.2 ± 0.22 | 14.5 ± 0.22 | 14.6 ± 0.22 | 14.5 ± 0.22 | 0.22 |
| Adjusted a | 14.6 ± 0.33 | 15.0 ± 0.32 | 15.1 ± 0.32 | 15.1 ± 0.32 | 0.14 |
| Adjusted b | 14.5 0.326 | 14.8 0.322 | 15.0 0.324 | 14.9 0.324 | 0.11 |
| Alcohol | | | | | |
| Crude | 14.5 ± 0.22 | 14.6 ± 0.22 | 14.5 ± 0.22 | 14.3 ± 0.21 | 0.57 |
| Adjusted a | 14.8 ± 0.34 | 15.0 ± 0.33 | 15.1 ± 0.31 | 14.9 ± 0.32 | 0.61 |
| Adjusted b | 14.6 0.344 | 14.8 0.327 | 15.0 0.315 | 14.8 0.326 | 0.49 |
| Trans fat | | | | | |
| Crude | 14.6 ± 0.22 | 14.2 ± 0.22 | 14.4 ± 0.22 | 14.6 ± 0.22 | 0.86 |
| Adjusted a | 15.1 ± 0.32 | 14.7 ± 0.31 | 15.1 ± 0.34 | 15.0 ± 0.33 | 0.93 |
| Adjusted b | 15.0 0.323 | 14.5 0.309 | 14.9 0.338 | 14.9 0.333 | 0.93 |
| Sodium | | | | | |
| Crude | 14.4 ± 0.22 | 14.6 ± 0.22 | 14.3 ± 0.22 | 14.6 ± 0.22 | 0.80 |
| Adjusted a | 14.9 ± 0.33 | 15.2 ± 0.32 | 14.8 ± 0.33 | 14.9 ± 0.32 | 0.86 |
| Adjusted b | 14.8 0.333 | 15.0 0.323 | 14.6 0.335 | 14.7 0.319 | 0.55 |

(Continues)

**TABLE 4** | (Continued)

| | First quartile food type ($n = 223$) | Second quartile food type ($n = 222$) | Third quartile food type ($n = 222$) | Fourth quartile food type ($n = 223$) | *$p$-value |
|---|---|---|---|---|---|
| Sugar-sweetened beverages + fruit juice | | | | | |
| Crude | 14.2 ± 0.21 | 14.2 ± 0.21 | 14.4 ± 0.22 | 15.1 ± 0.22 | 0.002 |
| Adjusted a | 15.0 ± 0.33 | 14.9 ± 0.33 | 14.6 ± 0.34 | 15.2 ± 0.33 | 0.86 |
| Adjusted b | 14.9 0.328 | 14.7 0.332 | 14.5 0.338 | 15.0 0.329 | 1.00 |
| Red and processed meat | | | | | |
| Crude | 14.1 ± 0.22 | 14.3 ± 0.22 | 14.4 ± 0.22 | 15.0 ± 0.22 | 0.002 |
| Adjusted a | 14.7 ± 0.33 | 15.0 ± 0.32 | 14.9 ± 0.32 | 15.2 ± 0.35 | 0.13 |
| Adjusted b | 14.5 0.332 | 14.8 0.318 | 14.8 0.319 | 15.1 0.351 | 0.09 |

*Note:* Adjusted a model includes age, gender, race/ethnicity, education, smoking, total caloric intake, body mass index, prediabetes, periodontal status, and total AHEI score. Adjusted b model is adjusted for everything listed above aside from periodontal status. $n = 890$ plaque samples among 651 participants.
*$p$-values for linear trend.

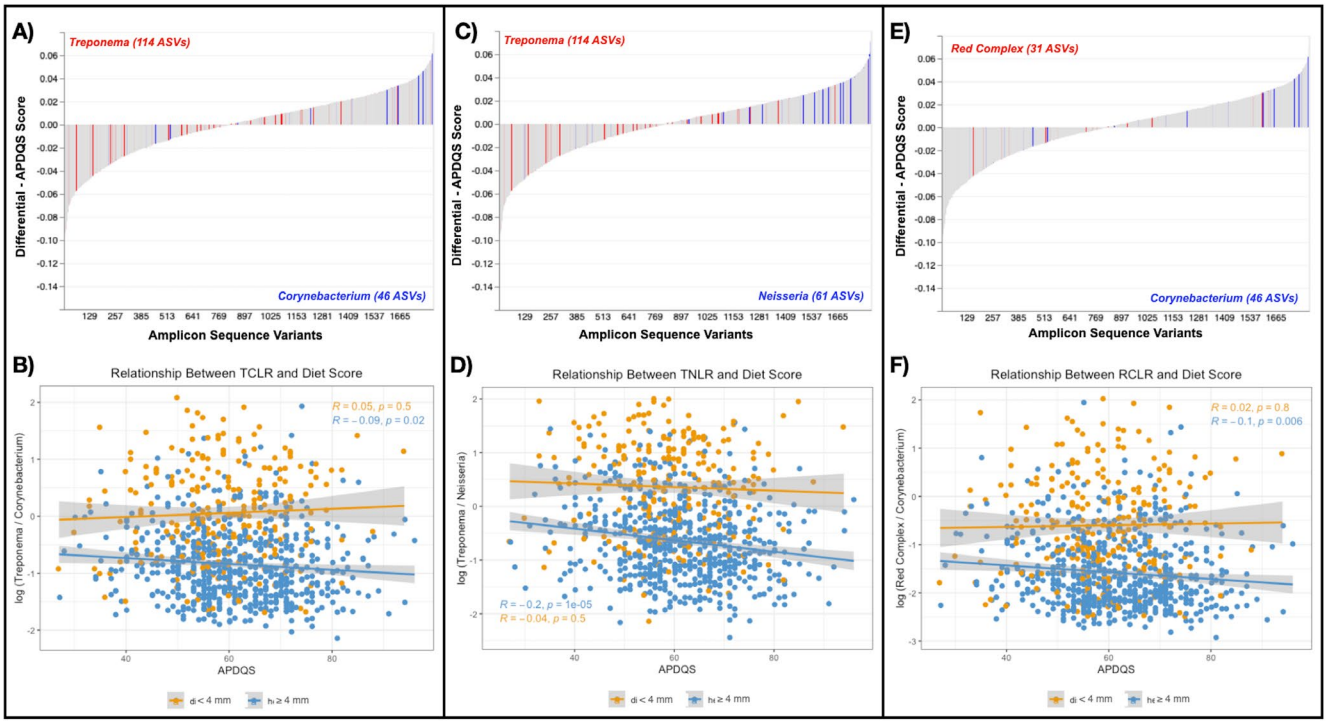


**FIGURE 1** | Log-ratio of taxa in relation to AHEI diet score. (A) Songbird-based differential ranking of microbes with respect to their association with AHEI score. Taxa in the genera *Treponema* are highlighted in red and taxa in the genera *Corynebacterium* are highlighted in blue. (B) Scatter plot of the log-ratio of *Treponema*:*Corynebacterium* in relation to AHEI score among shallow and moderate/deep sites. (C) Songbird-based differential ranking of microbes with respect to their association with AHEI score. Taxa in the genera *Treponema* are highlighted in red and taxa in the genera *Nesseria* are highlighted in blue. (D) Scatter plot of the log-ratio of *Treponema*:*Nesseria* in relation to AHEI score among shallow and moderate/deep sites. Songbird-based differential ranking of microbes with respect to their association with AHEI score. (E) Taxa in the genera *Red Complex* are highlighted in red and taxa in the genera *Corynebacterium* are highlighted in blue. (F) Scatter plot of the log-ratio of *Red Complex*:*Corynebacterium* in relation to AHEI score among shallow and moderate/deep sites.

on probing) but not historical periodontal disease (attachment loss and tooth loss), suggesting that diet might be most relevant for acute inflammatory outcomes occurring in the early natural history of periodontal disease. In ORIGINS, subgingival microbiota were strongly related to current probing depth and bleeding on probing (DeMayo et al. 2021; Marotz et al. 2022): thus, we expected to see diet associated with microbial biomarkers of periodontal inflammation in the current analyses.

Higher diet quality was related to decreased diversity of Faith, Observed and Shannon but not Simpson when restricting the analysis to <4 mm probing depth sites. These differences may arise from the distinct nature of the various diversity metrics.

**TABLE 5** | Association between diet scores (alternative healthy eating index and a priori diet quality score) and taxa ratios, mean ± SE.

| | First quartile AHEI ($n = 223$) | Second quartile AHEI ($n = 222$) | Third quartile AHEI ($n = 222$) | Fourth quartile AHEI ($n = 223$) | $p$-value* |
|---|---|---|---|---|---|
| RCLR | | | | | |
| Model 1 | −1.15 ± 0.066 | −1.20 ± 0.066 | −1.37 ± 0.065 | −1.58 ± 0.064 | <0.0001 |
| Model 2 | −1.13 ± 0.066 | −1.15 ± 0.068 | −1.30 ± 0.069 | −1.45 ± 0.072 | 0.0003 |
| Model 3 | −1.08 ± 0.082 | −1.09 ± 0.086 | −1.24 ± 0.086 | −1.39 ± 0.088 | 0.0004 |
| Model 4 | −1.09 ± 0.093 | −1.12 ± 0.100 | −1.24 ± 0.098 | −1.38 ± 0.099 | 0.001 |
| Model 5 | −1.06 ± 0.093 | −1.08 ± 0.100 | −1.22 ± 0.098 | −1.34 ± 0.099 | 0.002 |
| TCLR | | | | | |
| Model 1 | −0.47 ± 0.054 | −0.49 ± 0.054 | −0.64 ± 0.054 | −0.79 ± 0.054 | <0.0001 |
| Model 2 | −0.46 ± 0.055 | −0.45 ± 0.057 | −0.60 ± 0.057 | −0.70 ± 0.060 | 0.001 |
| Model 3 | −0.43 ± 0.069 | −0.43 ± 0.072 | −0.57 ± 0.072 | −0.68 ± 0.074 | 0.001 |
| Model 4 | −0.45 ± 0.078 | −0.45 ± 0.083 | −0.57 ± 0.082 | −0.67 ± 0.083 | 0.002 |
| Model 5 | −0.43 ± 0.077 | −0.42 ± 0.083 | −0.55 ± 0.081 | −0.64 ± 0.083 | 0.003 |
| TNLR | | | | | |
| Model 1 | −0.18 ± 0.060 | −0.19 ± 0.060 | −0.49 ± 0.060 | −0.64 ± 0.059 | <0.0001 |
| Model 2 | −0.18 ± 0.059 | −0.16 ± 0.061 | −0.44 ± 0.062 | −0.52 ± 0.065 | <0.0001 |
| Model 3 | −0.10 ± 0.074 | −0.08 ± 0.077 | −0.35 ± 0.077 | −0.43 ± 0.079 | <0.0001 |
| Model 4 | −0.11 ± 0.084 | −0.09 ± 0.089 | −0.35 ± 0.088 | −0.42 ± 0.089 | <0.0001 |
| Model 5 | −0.09 ± 0.083 | −0.05 ± 0.089 | −0.32 ± 0.088 | −0.38 ± 0.089 | <0.0001 |
| | **First quartile APDQS ($n = 246$)** | **Second quartile APDQS ($n = 214$)** | **Third quartile APDQS ($n = 230$)** | **Fourth quartile APDQS ($n = 200$)** | **$p$-value*** |
| RCLR | | | | | |
| Model 1 | −1.14 ± 0.063 | −1.44 ± 0.067 | −1.32 ± 0.065 | −1.45 ± 0.069 | 0.01 |
| Model 2 | −1.13 ± 0.063 | −1.38 ± 0.069 | −1.20 ± 0.070 | −1.32 ± 0.073 | 0.14 |
| Model 3 | −1.05 ± 0.083 | −1.32 ± 0.085 | −1.13 ± 0.087 | −1.25 ± 0.088 | 0.13 |
| Model 4 | −1.07 ± 0.094 | −1.33 ± 0.097 | −1.14 ± 0.098 | −1.27 ± 0.101 | 0.15 |
| Model 5 | −1.05 ± 0.093 | −1.30 ± 0.097 | −1.12 ± 0.098 | −1.23 ± 0.101 | 0.19 |
| TCLR | | | | | |
| Model 1 | −0.47 ± 0.052 | −0.68 ± 0.056 | −0.58 ± 0.054 | −0.70 ± 0.057 | 0.01 |
| Model 2 | −0.47 ± 0.052 | −0.63 ± 0.058 | −0.49 ± 0.058 | −0.61 ± 0.062 | 0.22 |
| Model 3 | −0.43 ± 0.070 | −0.60 ± 0.071 | −0.45 ± 0.073 | −0.58 ± 0.074 | 0.21 |
| Model 4 | −0.45 ± 0.078 | −0.63 ± 0.081 | −0.47 ± 0.082 | −0.59 ± 0.084 | 0.26 |
| Model 5 | −0.43 ± 0.078 | −0.60 ± 0.081 | −0.44 ± 0.081 | −0.56 ± 0.084 | 0.33 |
| TNLR | | | | | |
| Model 1 | −0.18 ± 0.058 | −0.37 ± 0.062 | −0.44 ± 0.060 | −0.56 ± 0.064 | <0.0001 |
| Model 2 | −0.19 ± 0.057 | −0.33 ± 0.063 | −0.33 ± 0.063 | −0.46 ± 0.067 | 0.003 |
| Model 3 | −0.08 ± 0.075 | −0.25 ± 0.077 | −0.23 ± 0.079 | −0.36 ± 0.079 | 0.003 |
| Model 4 | −0.11 ± 0.085 | −0.27 ± 0.088 | −0.25 ± 0.089 | −0.38 ± 0.091 | 0.003 |
| Model 5 | −0.08 ± 0.085 | −0.24 ± 0.088 | −0.22 ± 0.088 | −0.34 ± 0.091 | 0.01 |

*Note:* Model 1: adjusted total caloric intake; Model 2: Model 1 + age, gender, race/ethnicity, education; Model 3: Model 2 + smoking; Model 4: Model 3 + body mass index, prediabetes; Model 5: Model 4 + periodontal status. Mixed models were used to account for multiple plaque samples per person. $n = 890$ plaque samples among 651 participants.

*$p$-values for linear trend.

The Faith index is an explicit measure of phylogenetic diversity going beyond measures of richness, evenness or dominance, which are concepts better reflected by Observed, Simpson and Shannon indices. Our results suggest that higher quality diet might lead to a less phylogenetic diversity of oral microbiota.

The relationship between subgingival microbial diversity and periodontal disease has been inconsistent in prior studies, with some studies reporting reduced diversity (Huang et al. 2021; Ai et al. 2017; Farina et al. 2019) as periodontal disease develops and becomes more severe (vs. health controls), while others have found increased diversity (Griffen et al. 2012; Abusleme et al. 2013; Marotz et al. 2022) in the presence of periodontal disease. Similarly, the biological and phylogenetic diversity of oral microbial communities in patients with diabetes and prediabetes were also significantly reduced compared to patients with normoglycaemia (Huang et al. 2021). These conflicting prior findings make it challenging to make firm statements about the role of reduced diversity in relation to oral and systemic health. However, in the context of ORIGINS, our prior findings that increased diversity is adversely associated with periodontal and systemic outcomes (Marotz et al. 2022) are consistent with our current findings in which increased diversity is related to lower quality diet. Interestingly, a priori defined taxa ratios (RCLR, TCLR, TNLR), which were previously shown to be strongly associated with periodontal pocket depth in this cohort (Marotz et al. 2022), were generally more strongly related to diet quality than diversity metrics, even though these measures do not incorporate information about the overall microbial community, suggesting that these ratios might better capture ecological shifts early in the natural history of periodontal disease. For all three taxa ratios, as AHEI and APDQS scores increased, taxa ratios decreased significantly, suggesting a more favourable oral microbial ecology. We were unable to detect a relationship between taxa ratios and specific food components of AHEI (Table S1), and it is unclear which food groups lead to a more salutary oral microbiome. Future studies that can mechanistically explain these patterns of diet–microbiome associations will be important for understanding whether dietary patterns are a causal factor influencing the oral microbiome as opposed to a non-causal correlate confounded by other health behaviours and/or phenotypic characteristics.

While diet has been linked to a wide range of chronic disease outcomes, including periodontal disease (DeMayo et al. 2021; Woelber and Tennert 2020), existing literature investigating the association between diet and the oral microbiome is limited and generally the findings are weak and inconsistent. One prior study found that total carbohydrate, glucose load and sucrose were inversely associated with subgingival bacterial α-diversity (Millen et al. 2022), while a prior randomized trial found that randomization to an anti-inflammatory diet did not change the microbial composition of the subgingival plaque despite reducing gingival inflammation (Woelber et al. 2019). A comparison of subgingival microbiota between vegetarians and non-vegetarians found modest differences in select taxa but no difference in α-diversity between the groups (Khocht et al. 2021). Similarly, previous research has found modest (Tennert et al. 2020; Kato et al. 2017) or no (Claesson et al. 2012) associations between diet and salivary or supragingival microbial composition. Similarly, short-term studies in animal models found no difference in growth rates of oral bacteria in the presence or absence of food (Beckers and van der Hoeven 1982), nor any difference in the total number of bacteria in saliva in animals after 18 h of fasting compared with fed animals (Vach et al. 2022).

Knowledge about the role of diet in the composition of the oral microbiome is important for addressing a major limitation in previous studies linking the oral microbiome to a variety of chronic diseases. Specifically, a lack of diet data in most prior studies precludes the ability to assess the role of confounding by diet (Demmer et al. 2015, 2017, 2019; Desvarieux et al. 2005, 2010; Tonelli, Lumngwena, and Ntusi 2023). It is possible that different and more precisely defined dietary patterns are related to the oral microbiome, or, perhaps, it is the diet's influence on the metabolome (and not the microbiome) that is most relevant to periodontal disease. Nevertheless, the current findings suggest that any residual confounding related to diet is likely to be very modest.

Some important limitations should be noted in the current study. First, the analysis is cross-sectional and did not assess whether dietary patterns (or changing diet patterns) are related to longitudinal changes in the oral microbiota. Since our study only collected dietary data at one point in time, it does not reflect dietary history or change in diet among participants. Future studies that can assess patterns regarding diet and oral microbial diversity over time can inform the potential for diet interventions to prevent shifts in subgingival ecology that promote disease. In addition, the use of an FFQ asking about diet patterns in the preceding 12 months prevents more nuanced analyses related to the short-term influence of diet on the subgingival microbiota. It is also important to note that FFQs may underreport because of the imprecision with recording consumed foods portions as well as demographic and psychosocial factors, but it is a validated tool to measure long-term diet and the correlations that have been identified for a number of foods and nutrients when comparing between FFQ and 24-h recalls or biochemical measures (Kipnis et al. 2003; Subar et al. 2001). Presently, we only have 16S data available informing taxonomy but not community functionality. ORIGINS participants are relatively young and healthy compared to the general population. Although this gives us greater insight into a population not often studied, these results may not be generalizable to an older and sicker population. Lastly, by following participants over time, we can better understand how diet changes and even disease incidence affect oral microbial compositions.

We have found that a healthier dietary pattern is cross-sectionally modestly associated with decreased subgingival microbial diversity among a diverse sample of generally healthy young adults. Future intervention studies that can assess the role of specific diets on subgingival microbiome composition using metagenomic approaches will be important for better understanding causality.

## Author Contributions

**Rebecca L. Molinsky:** substantial contributions to the conception and design of the study, acquisition and analysis of the data, drafting the article and revising it critically for important intellectual content, final approval of the version to be published, agreement to

be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Abigail J. Johnson:** substantial contributions to the conception of the study and interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Lisa Marotz:** substantial contributions to the acquisition and analysis of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Sumith Roy:** substantial contributions to the acquisition of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Bruno Bohn:** substantial contributions to the acquisition of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Charlene E. Goh:** substantial contributions to the interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Ching-Yuan Chen:** substantial contributions to the interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Bruce Paster:** substantial contributions to the interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Rob Knight:** substantial contributions to analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Jeanine Genkinger:** substantial contributions to the analysis, and interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Panos N. Papapanou:** substantial contributions to the acquisition, analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **David R. Jacobs:** substantial contributions to the interpretation of data, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Ryan T. Demmer:** substantial contributions to conception and design, acquisition, and interpretation of data, drafting the article and revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.