Research Article

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# Association of Coffee and Tea Intake with the Oral Microbiome: Results from a Large Cross-Sectional Study

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## Abstract

**Background:** The oral microbiota play a central role in oral health, and possibly in carcinogenesis. Research suggests that coffee and tea consumption may have beneficial health effects. We examined the associations of these common beverages with the oral ecosystem in a large cross-sectional study.

**Methods:** We assessed oral microbiota in mouthwash samples from 938 participants in two U.S. cohorts using 16S rRNA gene sequencing. Coffee and tea intake were assessed from food frequency questionnaires. We examined associations of coffee and tea intake with overall oral microbiota diversity and composition using linear regression and permutational MANOVA, respectively, and with taxon abundance using negative binomial generalized linear models; all models adjusted for age, sex, cohort, body mass index, smoking, ethanol intake, and energy intake.

**Results:** Higher tea intake was associated with greater oral microbiota richness ($P = 0.05$) and diversity ($P = 0.006$), and shifts in overall community composition ($P = 0.002$); coffee was not associated with these microbiome parameters. Tea intake was associated with altered abundance of several oral taxa; these included Fusobacteriales, Clostridiales, and *Shuttleworthia satelles* (higher with increasing tea) and Bifidobacteriaceae, *Bergeyella*, Lactobacillales, and *Kingella oralis* (lower with increasing tea). Higher coffee intake was only associated with greater abundance of *Granulicatella* and Synergistetes.

**Conclusions:** In the largest study to date of tea and coffee consumption in relation to the oral microbiota, the microbiota of tea drinkers differed in several ways from nondrinkers.

**Impact:** Tea-driven changes to the oral microbiome may contribute to previously observed associations between tea and oral and systemic diseases, including cancers. *Cancer Epidemiol Biomarkers Prev; 27(7); 814–21. ©2018 AACR.*

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## Introduction

The oral microbiome, comprising more than 600 bacterial species (1), plays a central role in the maintenance of oral health (2). Consequently, dysbiosis of microbiota in dental plaques can cause the oral diseases of periodontitis and caries (3). Additionally, oral dysbiosis has been associated with systemic cancers, including head and neck cancer (4), pancreatic cancer (5), and esophageal cancer (6). While the importance of the oral microbiome in human health is becoming increasingly clear, little is known regarding factors that influence oral microbiome composition. The human oral microbiota comes into direct contact with orally ingested dietary factors, undoubtedly contributing to food metabolic pathways (7); at the same time, dietary exposures lead to ecological adaptation and selection of the microbial community (7).

Coffee and tea are commonly consumed beverages among Americans (8, 9), and both have received attention for purported health benefits. Reports from large cohort studies indicate a robust inverse association of coffee consumption with total mortality and cause-specific mortality from cancer (10–12). Similar findings have been reported for tea consumption (13–15), though effects may differ for green versus black tea (14). Coffee and tea have also been inversely associated with head and neck cancer risk (16–18), while tea may also prevent dental caries, periodontitis, and tooth loss (19–21). Both coffee and tea are complex mixtures containing many biologically active compounds, including caffeine and polyphenols, which may have antioxidant, antimutagenic, antiproliferative, and/or anti-inflammatory effects (22–24); a wide variety of mechanisms may contribute to disease protection at different systemic sites.

Some evidence suggests that coffee and tea drinking may affect the oral microbiome (25–27), which could be a further mechanism for the effects of these beverages on oral and/or systemic health, including their chemopreventive properties. However, the associations of coffee and tea drinking with oral microbiome composition have not been comprehensively examined in a large study. We evaluated these associations in a large cross-sectional analysis of American adults from two well-characterized cohort studies, the American Cancer Society (ACS) Cancer Prevention

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Study II (CPS-II) and the National Cancer Institute (NCI) Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). Oral microbiota were assessed via 16S rRNA gene sequencing of microbial DNA from oral wash samples, and oral microbiome diversity and composition were evaluated in relation to frequency of coffee and tea intake.

## Materials and Methods

### Study population

Participants were drawn from the NCI PLCO cohort (28) and the ACS CPS-II cohort (29), which are described in detail in the above-cited references. Both cohorts included U.S. adult men and women, collected demographic, medical and lifestyle information, and followed participants prospectively for cancer incidence. Oral wash samples were collected from a subset of each cohort.

All subjects included in the present cross-sectional analysis were originally selected from the CPS-II and PLCO cohorts as cases or controls for collaborative nested case–control studies of the oral microbiome in relation to head/neck cancer (4) and pancreatic cancer (5). Participants are organized into 4 study groups: CPS-IIa, CPS-II participants in the head and neck study; CPS-IIb, CPS-II participants in the pancreas study; PLCOa, PLCO participants in the head and neck study; and PLCOb, PLCO participants in the pancreas study. Cases were participants who developed one of these two types of cancers at any point after collection of the oral wash samples. Age and sex-matched controls were selected by incidence density sampling among cohort members who provided an oral wash sample and had no cancer prior to selection.

From the original 1,215 participants selected for inclusion in the case–control studies (CPS-II $n = 543$ and PLCO $n = 672$), we excluded participants missing smoking status or food frequency questionnaire (FFQ) data, participants for whom sequencing failed, participants with implausible daily energy intakes based on FFQ responses (<500 or >4,000 kcal/day), and one participant with low library depth (1,516 sequence reads), leaving 938 participants remaining (CPS-II $n = 457$ and PLCO $n = 481$). All participants provided written informed consent and all protocols were conducted in accordance with the U.S. Common Rule and approved by the New York University School of Medicine Institutional Review Board.

### Coffee, tea, and covariate assessment

Coffee and tea intake and information on other covariates were extracted from questionnaires preceding oral wash sample collection for each participant. Frequencies of coffee and tea intake (cups per day) were assessed by validated FFQs in both cohorts to ascertain usual consumption over the past year (30). We evaluated coffee and tea intake as continuous variables and as categorical variables, by categorizing participants into 4 categories as follows: 0 cups/day (no intake), <1 cup/day, ≥1 and <3 cups/day, and ≥3 cups/day.

### Oral wash sample collection

Participants were asked to swish with 10 mL Scope mouthwash (P&G) for 30 seconds and expectorate into a tube (28, 29). Samples were shipped to each cohort's biorepository and stored at −80°C until use. We have shown that oral microbiome composition via this collection method is comparable with that of fresh-frozen saliva (31).

### Microbiome assay

Bacterial genomic DNA was extracted from oral wash samples using the Mo Bio PowerSoil DNA Isolation Kit. As reported previously (32), 16S rRNA gene sequencing on the extracted DNA was performed. 16S rRNA gene amplicon libraries were generated using primers incorporating FLX Titanium adapters and a sample barcode sequence, allowing unidirectional sequencing covering variable regions V3 to V4 (Primers: 347F- 5′GGAGGCAGCA-GTAAGGAAT-3′ and 803R- 5′CTACCGGGTATCTAATCC-3′). Five nanograms genomic DNA was used as the template in 25 μL PCR reaction buffer for 16S rRNA gene amplicon preparation. Cycling conditions were one cycle of 94°C for 3 minutes, followed by 25 cycles of 94°C for 15 seconds, 52°C for 45 seconds and 72°C for 1 minute followed by a final extension of 72°C for 8 minutes. The generated amplicons were then purified using Agencourt AMPure XP kit (Beckman Coulter). Purified amplicons were quantified by fluorometry using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). Equimolar amounts ($10^7$ molecules/μL) of purified amplicons were pooled for sequencing. Pyrosequencing (Roche 454 GS FLX Titanium) was carried out according to the manufacturer's instructions.

### Sequence data processing

Sequence reads were demultiplexed, and poor-quality reads excluded, using default parameters in QIIME (33). Quality-filtered reads were clustered into operational taxonomic units (OTU) against the Human Oral Microbiome Database (HOMD) reference sequence collection (version 14.5; ref. 34), and assigned HOMD taxonomy, using QIIME script *pick_closed_reference_otus.py* (33). The final dataset for this analysis of $n = 938$ participants contained 9,921,097 reads [mean ± SD = 10,577 ± 2,819; range = (3,084–33,784)] and 681 OTUs. We generated a phylogenetic tree from aligned HOMD reference sequences using FastTree (35). Quality control data showing good reproducibility between replicates have been published previously for this dataset (36).

### Statistical analysis

α-Diversity (within-subject diversity) was assessed by richness, the Shannon diversity index, and community evenness, calculated in 100 iterations of rarefied OTU tables of 3,000 sequence reads per sample using the *alpha_rarefaction.py* script in QIIME (33). This depth was chosen based on the minimum sequencing depth among the samples (min = 3,084). We examined whether coffee and tea intake were associated with α-diversity using linear regression models adjusting for age, sex, study (CPS-IIa, CPS-IIb, PLCOa, PLCOb), current smoking, body mass index (BMI; kg/m$^2$), energy intake (kcal/day), ethanol intake (grams/day), and coffee or tea intake (cups/day; in tea and coffee models, respectively). Coffee and tea intake were modeled as categorical and continuous variables in separate models.

β-diversity (between-subject diversity) in relation to coffee and tea intake was assessed at OTU level using permutational multivariate analysis of variance (PERMANOVA; ref. 37) of the weighted UniFrac distance (38). PERMANOVA models ('adonis' function, vegan package, R) were adjusted for age, sex, study (CPS-IIa, CPS-IIb, PLCOa, PLCOb), current smoking, BMI (kg/m$^2$), energy intake (kcal/day), ethanol intake (grams/day), and coffee or tea intake (cups/day; in tea and coffee models, respectively). Coffee and tea intake were modeled as categorical and continuous variables in separate models.

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We used negative binomial generalized linear models, as implemented in DESeq2 (39), to test the associations of coffee and tea intake with microbial taxa abundance at different taxonomic levels. The raw counts of 681 OTUs were agglomerated to 12 phyla, 26 classes, 41 orders, 71 families, 156 genera, and 555 species. Prior to this analysis, we filtered the data to include only taxa with $\geq$2 sequence reads in $\geq$5% of participants (47 participants), to exclude rare taxa and thereby minimize the number of statistical tests conducted (8 phyla, 17 classes, 24 orders, 42 families, 79 genera, and 295 species). DESeq2 default outlier replacement, independent filtering of low-count taxa, and filtering of count outliers were turned off. Models were adjusted for age, sex, study (CPS-IIa, CPS-IIb, PLCOa, PLCOb), current smoking, BMI (kg/m$^2$), energy intake (kcal/day), ethanol intake (grams/day), and coffee or tea intake (cups/day; in tea and coffee models, respectively). Coffee and tea intakes were modeled as categorical and continuous variables in separate models. *P* values at each taxonomic level were adjusted for the false-discovery rate (FDR), after removal of models with maximum Cook's distance > 10.

We conducted sensitivity analyses, including analyses stratified by cohort and smoking status, analyses of regular and decaffeinated coffee or tea separately, analyses using those drinking no coffee and no tea as the referent group, and analyses excluding participants who reported adding sugar to their coffee or tea (the latter information available in PLCO cohort only). For findings based on low taxon counts, we tested whether coffee or tea was associated with presence/absence (carriage) of the taxon. All statistical tests were two sided. A *P* value <0.05 was considered of nominal significance, and an FDR-adjusted *P* value (*q* value) <0.05 was considered significant after multiple comparisons adjustment. Analyses were conducted using R 3.4.0.

## Results

In this study population, 86% of participants consumed coffee (71% $\geq$1 cups per day) and 75% consumed tea (22% $\geq$1 cups per day). Those with the highest coffee intakes were more likely to currently smoke and have higher mean alcohol intakes in both the CPS-II and PLCO cohorts (Table 1). Those with the highest tea intakes in the CPS-II cohort were more likely to be female and nonsmokers (Table 1).

Tea intake was positively associated with higher oral microbial richness, Shannon diversity, and evenness, in multivariable-adjusted linear regression models (Table 2). An increased intake of one cup of tea per day related to 1.47 (95% CI: 0.02–2.93) more OTUs present in the oral cavity on average ($P = 0.05$), as well as higher Shannon diversity ($\beta = 0.04$, $P = 0.006$) and community evenness ($\beta = 0.004$, $P = 0.009$). Similarly, tea intake was positively associated with shifts in overall oral microbial composition ($P = 0.002$); this was most apparent when comparing those who consumed $\geq$3 cups/day to those who consumed none ($P = 0.02$; Table 3). In contrast, coffee intake was not associated with oral microbial richness, diversity (Table 2) nor overall composition (Table 3).

When stratifying by cohort, tea intake was positively associated with Shannon diversity in both the CPS-II and PLCO cohorts ($P = 0.01$ and $P = 0.07$, respectively), and with altered overall microbial composition in PLCO only ($P = 0.003$; Supplementary Table S1). Additionally, sensitivity analyses using those with no coffee and no tea consumption as the referent group

**Table 1.** Demographic characteristics of participants by daily coffee and tea intake in the CPS-II and PLCO cohorts

| | CPS-II | | | | | PLCO | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cups per day | | | | | Cups per day | | | | |
| **Coffee** | **None** | **<1** | **[1–3)** | **≥3** | ***P*-trend[a]** | **None** | **<1** | **[1–3)** | **≥3** | ***P*-trend[a]** |
| *N* | 53 | 78 | 273 | 53 | | 76 | 61 | 81 | 263 | |
| Age (y; mean ± SD) | 72.8 ± 6.4 | 72.5 ± 6.6 | 73.1 ± 5.9 | 71.5 ± 5.6 | 0.53 | 63.4 ± 5.1 | 63.7 ± 5.5 | 63.5 ± 5.3 | 63.9 ± 5.1 | 0.41 |
| Male (%) | 49.1 | 62.8 | 56.8 | 75.5 | 0.06 | 73.7 | 59 | 63 | 67.3 | 0.72 |
| White (%) | 94.3 | 96.2 | 98.9 | 98.1 | 0.04 | 96.1 | 93.4 | 95.1 | 96.2 | 0.67 |
| BMI[b] (kg/m$^2$; mean ± SD) | 25.5 ± 5.3 | 26.8 ± 4.6 | 26.2 ± 4.2 | 26 ± 3.6 | 0.97 | 27.6 ± 5.2 | 26.9 ± 4.8 | 27.1 ± 5 | 27.3 ± 3.7 | 0.22 |
| Current smoker (%) | 5.7 | 2.6 | 5.5 | 15.1 | 0.06 | 6.6 | 1.6 | 3.7 | 13.3 | 0.007 |
| Alcohol (g/day; mean ± SD) | 3.5 ± 7.9 | 8.9 ± 14.8 | 10.4 ± 14.3 | 14.4 ± 17.2 | <0.0001 | 12.7 ± 57.4 | 14.2 ± 62.9 | 9.8 ± 20 | 16.8 ± 39.2 | <0.0001 |
| Tea (cups/day; mean ± SD) | 0.8 ± 1.7 | 0.6 ± 1.0 | 0.5 ± 0.8 | 0.4 ± 1.1 | 0.21 | 1.5 ± 2.7 | 1 ± 1.6 | 1.3 ± 1.9 | 0.9 ± 1.6 | 0.89 |
| **Tea** | **None** | **<1** | **[1–3)** | **≥3** | ***P*-trend[a]** | **None** | **<1** | **[1–3)** | **≥3** | ***P*-trend[a]** |
| *N* | 113 | 262 | 70 | 12 | | 118 | 236 | 70 | 57 | |
| Age (y; mean ± SD) | 71.9 ± 6.5 | 73.1 ± 5.8 | 72.7 ± 6.1 | 74.8 ± 7.0 | 0.20 | 63.7 ± 5.2 | 63.8 ± 5.4 | 64.0 ± 4.6 | 63.2 ± 4.9 | 0.87 |
| Male (%) | 81.4 | 52.3 | 47.1 | 66.7 | <0.0001 | 76.3 | 63.1 | 61.4 | 66.7 | 0.12 |
| White (%) | 98.2 | 98.5 | 95.7 | 91.7 | 0.12 | 93.2 | 95.8 | 97.1 | 98.2 | 0.10 |
| BMI[b] (kg/m$^2$; mean ± SD) | 25.9 ± 4.3 | 26.3 ± 4.1 | 26.1 ± 4.6 | 26.1 ± 7.8 | 0.75 | 26.9 ± 4.1 | 27.1 ± 4.2 | 27.3 ± 5.3 | 28.1 ± 4.3 | 0.10 |
| Current smoker (%) | 14.2 | 4.2 | 1.4 | 0.0 | 0.0001 | 13.6 | 7.2 | 4.3 | 14.0 | 0.60 |
| Alcohol (g/day; mean ± SD) | 11.7 ± 17.1 | 9.8 ± 13.8 | 7.5 ± 12.4 | 5.1 ± 7.9 | 0.24 | 13.6 ± 26.5 | 17.2 ± 50.0 | 6.1 ± 9.8 | 16.3 ± 65.6 | 0.95 |
| Coffee (cups/day; mean ± SD) | 2.1 ± 1.8 | 1.7 ± 1.3 | 1.6 ± 1.4 | 1.1 ± 1.3 | 0.11 | 4.2 ± 4.5 | 4.0 ± 3.2 | 3.8 ± 3.6 | 3.0 ± 3.5 | 0.27 |

[a]*P* values are from Spearman correlations for continuous variables or $\chi^2$ test for trend in proportions for categorical variables.
[b]Participants missing BMI ($n = 28$) imputed with cohort-specific medians.

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**Table 2.** Association of α-diversity metrics with coffee and tea intake as categorical or continuous variables

| | Categorical | | | | | Continuous | |
|---|---|---|---|---|---|---|---|
| | β (95% CI)[a] | | | | *P*-trend[b] | β (95% CI)[a] | *P* |
| **Coffee** | **None ($n = 129$)** | **<1 c/day ($n = 139$)** | **[1–3) c/day ($n = 354$)** | **≥3 c/day ($n = 316$)** | | **Per cup per day** | |
| Richness | Ref. | −0.72 (−9.042–7.602) | −3.752 (−10.982–3.477) | −3.732 (−10.985–3.521) | 0.218 | −0.216 (−1.038–0.606) | 0.606 |
| Shannon index | Ref. | 0.021 (−0.138–0.18) | −0.04 (−0.178–0.099) | −0.029 (−0.168–0.11) | 0.483 | 0.002 (−0.013–0.018) | 0.777 |
| Evenness | Ref. | 0.005 (−0.012–0.022) | −0.001 (−0.016–0.014) | 0 (−0.015–0.015) | 0.744 | 0.001 (−0.001–0.002) | 0.528 |
| **Tea** | **None ($n = 231$)** | **<1 c/day ($n = 498$)** | **[1–3) c/day ($n = 140$)** | **≥3 c/day ($n = 69$)** | | **Per cup per day** | |
| Richness | Ref. | −0.883 (−6.408–4.641) | 9.465 (2.052–16.878) | 2.717 (−6.756–12.189) | 0.065 | 1.473 (0.015–2.931) | 0.048 |
| Shannon index | Ref. | −0.042 (−0.148–0.064) | 0.166 (0.024–0.308) | 0.143 (−0.038–0.324) | 0.013 | 0.039 (0.011–0.067) | 0.006 |
| Evenness | Ref. | −0.005 (−0.017–0.006) | 0.011 (−0.004–0.026) | 0.018 (−0.001–0.037) | 0.024 | 0.004 (0.001–0.007) | 0.009 |

[a]Parameters are from linear regression models with specified α-diversity metric (averaged over 100 iterations of rarefied OTU table at 3,000 sequence reads/sample) as outcome. All models were adjusted for age, sex, study (CPS-IIa, CPS-IIb, PLCOa, PLCOb), current smoking, BMI (kg/m$^2$), energy intake (kcal/day), ethanol intake (grams/day), and coffee or tea intake (cups/day; in tea and coffee models, respectively).
[b]Trend tests across groups were done by entering the categorical variables into the models as continuous terms.

(Supplementary Table S2) and excluding participants who reported adding sugar to their coffee or tea (Supplementary Table S3) did not materially change any of the associations for diversity and overall composition. Associations for diversity and overall composition were similar among regular and decaffeinated coffee and tea intakes (Supplementary Table S4), though only the association of regular tea with overall oral microbiome composition was statistically significant ($P = 0.008$ for regular tea; $P = 0.054$ for decaffeinated tea). Finally, we stratified by smoking status (never, former, or current smoker) as those with highest coffee intake were more likely to smoke, and smoking is known to influence the oral microbiome; however, associations of coffee intake with diversity and overall composition were similar among never, former, and current smokers (Supplementary Table S5).

We next examined associations of coffee and tea intake with microbial taxa abundance using negative binomial generalized linear models. Coffee intake was associated with greater abundance of family Carnobacteriaceae and its genus *Granulicatella* (phylum Firmicutes), and phylum Synergistetes (Table 4; Fig. 1A). The findings for these taxa appear to be driven by the highest category of coffee consumption. For low-count phylum Synergistetes, the trend was also apparent for carriage of the phylum (Supplementary Table S6). These associations were consistent for regular, but not decaffeinated, coffee intake (Supplementary Table S7). When stratified by cohort, we observed that family Carnobacteriaceae and genus *Granulicatella* were only associated with coffee intake in the CPS-II cohort (*P*-heterogeneity = 0.002 and 0.02, respectively; Supplementary Table S8).

Tea intake was associated with greater abundance of several microbial taxa, including class Clostridia, order Clostridiales, genus *Shuttleworthia* and species *Shuttleworthia satelles* (phylum Firmicutes); and class Fusobacteriia and order Fusobacteriales (phylum Fusobacteria; Table 4; Fig. 1B). For low-count species *Shuttleworthia satelles*, the trend was also apparent for carriage of the species (Supplementary Table S6). Tea intake was also associated with lower abundance of order Bifidobacteriales and family Bifidobacteriaceae (phylum Actinobacteria); class Flavobacteriia, order Flavobacteriales, family Flavobacteriaceae and genus *Bergeyella* (phylum Bacteroidetes); class Bacilli, order Bacillales, family Gemellaceae, and order Lactobacillales (phylum Firmicutes); and species *Kingella oralis* (phylum Proteobacteria; Table 4; Fig. 1B). Some of the trends appear to be driven by the highest category of tea drinking, particularly for Fusobacteriales, *Bergeyella*, and *Kingella oralis*. When analyzing these associations by regular and decaffeinated tea intake, they were generally more apparent for regular than decaffeinated tea intake (Supplementary Table S7). Associations remained statistically significant in the CPS-II cohort, though cohort heterogeneity was only significant for classes Flavobacteriia and Bacilli (*P*-heterogeneity = 0.04 and 0.01, respectively; Supplementary Table S8).

## Discussion

In this large cross-sectional study of American adults, tea, but not coffee, drinking was associated with significant differences in the diversity and composition of the oral microbiota. More specifically, increased tea intake was associated with higher oral microbiota diversity, and altered abundance of several taxonomic groups, including lower abundance of Bifidobacteriaceae, *Bergeyella*, Lactobacillales, and *Kingella oralis*, and higher abundance of Fusobacteriales, Clostridiales, and *Shuttleworthia satelles*. Findings were generally more apparent for regular tea intake than decaffeinated tea intake, perhaps because regular tea was more commonly consumed in this study population. Additionally, significant findings were often restricted to those drinking 1 or more cups of tea per day compared with no consumption, indicating that occasional tea drinking may not affect the oral microbiome. As only 22% of participants reported drinking 1 or more cups of tea per day, power was limited for these tea

**Table 3.** Association of β-diversity with coffee and tea intake as categorical or continuous variables

| | Categorical | | | | | Continuous |
|---|---|---|---|---|---|---|
| | *P*[a] | | | | | *P*[a] |
| | **None** | **<1 c/day** | **[1–3) c/day** | **≥3 c/day** | ***P*-trend[a,b]** | **Per cup per day** |
| **Coffee** | Ref. | 0.68 | 0.69 | 0.49 | 0.43 | 0.90 |
| **Tea** | Ref. | 0.75 | 0.12 | 0.02 | 0.003 | 0.002 |

[a]*P* values from PERMANOVA of weighted UniFrac distance, adjusting for age, sex, study (CPS-IIa, CPS-IIb, PLCOa, PLCOb), current smoking, BMI (kg/m$^2$), energy intake (kcal/day), ethanol intake (grams/day), and coffee or tea intake (cups/day; in tea and coffee models, respectively).
[b]Trend tests across groups were done by entering the categorical variables into the models as continuous terms.

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**Table 4.** Association of coffee and tea intake as categorical or continuous variables with abundance of oral microbial taxa[a]

| | | Categorical | | | | | | Continuous | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean normalized count | Fold change (95% CI)[b] | | | *P*-trend[c] | q-trend[c] | Fold change (95% CI)[b] | *P* | *Q* value |
| **Coffee** | | **None** | **<1 c/day** | **[1–3) c/day** | **>3 c/day** | | | **Per cup per day** | | |
| Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae (Family) | 187.76 | Ref. | 1.03 (0.87–1.21) | 1 (0.86–1.15) | 1.26 (1.09–1.45) | 0.0018 | 0.067 | 1.03 (1.01–1.05) | 0.00016 | 0.006 |
| Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae; Granulicatella (Genus) | 175.3 | Ref. | 1.01 (0.86–1.19) | 0.98 (0.85–1.13) | 1.22 (1.06–1.41) | 0.0075 | 0.13 | 1.03 (1.01–1.05) | 0.00026 | 0.018 |
| Synergistetes (Phylum) | 1.27 | Ref. | 0.97 (0.72–1.31) | 1.01 (0.75–1.36) | 1.54 (1.14–2.07) | 0.0029 | 0.02 | 1.03 (1–1.06) | 0.042 | 0.24 |
| **Tea** | | **None** | **<1 c/day** | **(1–3) c/day** | **>3 c/day** | | | **Per cup per day** | | |
| Actinobacteria; Actinobacteria; Bifidobacteriales (Order) | 30.27 | Ref. | 0.55 (0.43–0.7) | 0.55 (0.4–0.76) | 0.45 (0.31–0.65) | NA[d] | NA[d] | 0.93 (0.88–0.98) | 0.0081 | 0.039 |
| Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae (Family) | 28.45 | Ref. | 0.51 (0.4–0.66) | 0.5 (0.36–0.7) | 0.42 (0.28–0.61) | NA[d] | NA[d] | 0.92 (0.87–0.97) | 0.0034 | 0.042 |
| Bacteroidetes; Flavobacteriia (Class) | 38.28 | Ref. | 0.9 (0.78–1.04) | 0.89 (0.75–1.06) | 0.8 (0.66–0.97) | 0.033 | 0.15 | 0.96 (0.93–0.99) | 0.008 | 0.042 |
| Bacteroidetes; Flavobacteriia; Flavobacteriales (Order) | 34.77 | Ref. | 0.92 (0.79–1.06) | 0.91 (0.75–1.11) | 0.67 (0.53–0.86) | 0.0063 | 0.063 | 0.94 (0.91–0.98) | 0.0014 | 0.015 |
| Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae (Family) | 33.66 | Ref. | 0.9 (0.78–1.05) | 0.93 (0.76–1.13) | 0.66 (0.52–0.85) | 0.0091 | 0.16 | 0.94 (0.91–0.98) | 0.0018 | 0.034 |
| Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Bergeyella (Genus) | 3.95 | Ref. | 1.15 (0.97–1.36) | 0.92 (0.74–1.15) | 0.82 (0.62–1.08) | 0.095 | 0.52 | 0.93 (0.9–0.97) | 0.0014 | 0.048 |
| Firmicutes; Bacilli (Class) | 6990.38 | Ref. | 1.12 (0.99–1.25) | 0.82 (0.71–0.95) | 1.01 (0.85–1.19) | 0.13 | 0.29 | 0.96 (0.94–0.99) | 0.0058 | 0.042 |
| Firmicutes; Bacilli; Bacillales (Order) | 281.53 | Ref. | 1.33 (1.15–1.54) | 0.97 (0.8–1.18) | 0.96 (0.75–1.21) | 0.37 | 0.61 | 0.95 (0.92–0.99) | 0.0073 | 0.039 |
| Firmicutes; Bacilli; Bacillales; Gemellaceae (Family) | 270.2 | Ref. | 1.36 (1.17–1.58) | 0.97 (0.8–1.18) | 0.93 (0.72–1.18) | 0.27 | 0.5 | 0.94 (0.91–0.98) | 0.0018 | 0.034 |
| Firmicutes; Bacilli; Lactobacillales (Order) | 5789.13 | Ref. | 1.03 (0.92–1.15) | 0.76 (0.66–0.89) | 0.92 (0.76–1.11) | 0.01 | 0.069 | 0.96 (0.94–0.99) | 0.011 | 0.04 |
| Firmicutes; Clostridia (Class) | 204.05 | Ref. | 0.98 (0.89–1.09) | 1.09 (0.96–1.25) | 1.27 (1.08–1.48) | 0.0028 | 0.044 | 1.03 (1.01–1.06) | 0.01 | 0.042 |
| Firmicutes; Clostridia; Clostridiales (Order) | 203.33 | Ref. | 1 (0.89–1.12) | 1.12 (0.96–1.31) | 1.31 (1.08–1.59) | 0.0038 | 0.063 | 1.04 (1.01–1.07) | 0.0093 | 0.039 |
| Firmicutes; Clostridia; Clostridiales; Lachnospiraceae_[XIV]; Shuttleworthia (Genus) | 0.9 | Ref. | 0.88 (0.63–1.22) | 1.58 (1.06–2.35) | 2.33 (1.5–3.62) | 2.60E−05 | 0.0018 | 1.16 (1.09–1.24) | 1.80E−06 | 0.00012 |
| Firmicutes; Clostridia; Clostridiales; Lachnospiraceae_[XIV]; Shuttleworthia; satelles (Species) | 0.82 | Ref. | 0.87 (0.62–1.23) | 1.68 (1.1–2.56) | 2.52 (1.55–4.09) | 2.00E−05 | 0.0051 | 1.19 (1.11–1.28) | 1.00E−06 | 0.00026 |
| Fusobacteria; Fusobacteriia (Class) | 240.24 | Ref. | 0.93 (0.84–1.03) | 1.04 (0.91–1.19) | 1.16 (0.99–1.36) | 0.069 | 0.22 | 1.03 (1.01–1.06) | 0.0086 | 0.042 |
| Fusobacteria; Fusobacteriia; Fusobacteriales (Order) | 234.42 | Ref. | 0.95 (0.85–1.06) | 1.07 (0.92–1.24) | 1.22 (1.01–1.48) | 0.038 | 0.13 | 1.05 (1.02–1.08) | 0.0012 | 0.015 |
| Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella; oralis (Species) | 5.06 | Ref. | 1.42 (1.11–1.81) | 1.18 (0.86–1.62) | 0.57 (0.39–0.85) | 0.14 | 0.81 | 0.89 (0.84–0.95) | 0.00016 | 0.02 |

[a]Taxa included in the table were associated with coffee or tea intake at $q$ trend <0.05 for categorical variable or $q$ value < 0.05 for continuous variable.

[b]Parameters are from DESeq2 models adjusted for age, sex, study (CPS-IIa, CPS-IIb, PLCOa, PLCOb), current smoking, BMI (kg/m$^2$), energy intake (kcal/day), ethanol intake (grams/day), and coffee or tea intake (cups/day; in tea and coffee models, respectively).

[c]Trend tests across groups were done by entering the categorical variables into the models as continuous terms.

[d]*P* not calculated due to heavy outlier influence on model (maximum Cook's distance > 10).



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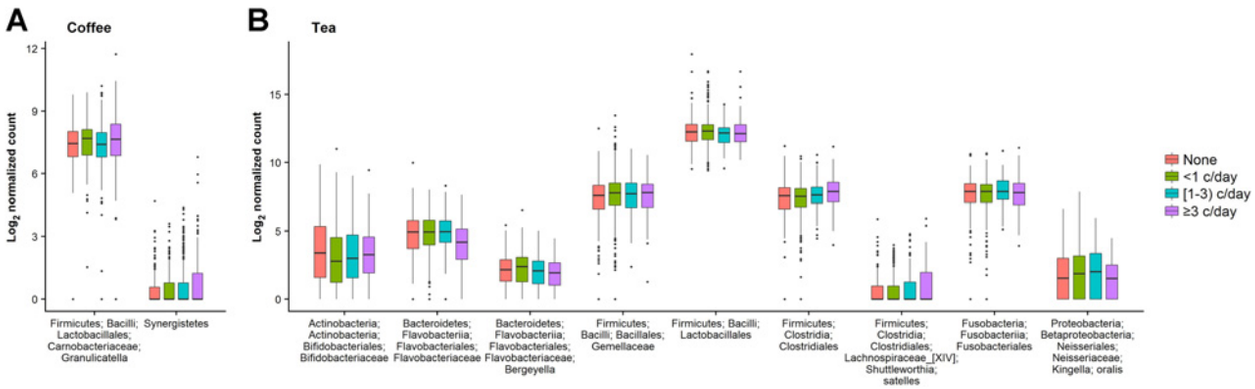


**Figure 1.**

Coffee and tea intake and abundance of oral microbial taxa. Taxon counts were normalized for DESeq2 size factors and $\log_2$ transformed after adding a pseudocount of 1. **A,** Taxa associated with coffee. **B,** Taxa associated with tea.

drinking categories, further underlining the strength of the significant findings.

Tea and coffee beverages each are composed of many varied bioactive chemical compounds which could affect the oral microbiota. Green tea polyphenolic catechins, including epigallocatechin gallate and epicatechin gallate, possess moderate antimicrobial activity and can inhibit the growth and adherence of a wide range of bacteria (40, 41). Black tea, the fermented form of green tea, contains these catechins at lower concentrations, in addition to containing oxidation products (e.g., theaflavins) not found in green tea (40). Roasted coffee α-dicarbonyl compounds (42) and melanoidins (43) may result in antimicrobial activity of brewed coffee. Caffeine is present in both coffee and tea and may also contribute to antibacterial activity (42).

Tea may protect against pathogenic oral diseases, namely dental caries (21, 40) and periodontitis (19, 20), lending further support to its effects on the oral microbiota. Though we did not observe significant associations of tea intakes with known cariogenic or periodontal pathogens (i.e., *Streptococcus mutans*, *Porphyromonas gingivalis*), tea drinking was associated with significantly greater diversity and altered composition of the oral microbiota. Greater diversity of a microbial community is generally thought to be beneficial, as it bestows functional versatility on the community and indicates nondominance by pathogenic bacteria. Specifically in regards to the oral microbiota, oral disease states (caries, periodontal disease) have been associated with reduced oral microbiome diversity (44, 45), suggesting that tea promotes a healthy oral microbiome. Only a few studies have examined the relationship between tea intake and oral microbiota in humans. Similar to our findings, a study of 21 healthy adults in Austria found that the oral gingival microbiota composition was significantly associated with tea consumption, but not coffee (27); however, that study did not present results on the associations of tea with oral microbiome diversity or specific taxa. Another study of 93 subjects in Italy observed that tea drinkers had lower counts of total bacteria, *Streptococcus mutans*, and *Lactobacillus* in saliva samples than nondrinkers (26). Similarly, we observed that higher tea intakes were associated with lower abundance of the order Lactobacillales, though not its genus *Lactobacillus*, which is more specifically related to dental caries (46). In our study, tea intake was associated with a greatest increase in abundance of the species *Shuttleworthia satelles*. This bacterium was first isolated from the human oral cavity in 2002 and described as obligately anaerobic and saccharolytic (i.e., carrying out sugar fermentation; ref. 47). While little is known regarding the role of *S. satelles* in human health, one study showed higher abundance of *S. satelles* in the saliva of caries-free compared with caries-affected 11- to 12-year-old children (48), suggesting its association with improved oral health.

The impact of coffee on oral health is mixed—while coffee is associated with reduced risk for oral cancers (16, 17), its typical consumption with sugar additives (49) may prevent it from exhibiting an overall protective effect on oral disease (caries, periodontal disease; refs. 20, 50, 51). We did not observe associations of coffee intake and overall oral microbiome diversity or composition in our study, even after exclusion of participants adding sugar to their coffee (though this sensitivity analysis was limited by small sample size). This result is inconsistent with two Italian studies, one ($n = 93$) reporting that coffee was associated with lower counts of total bacteria, *Streptococcus mutans*, and *Lactobacillus* in saliva (26), and the other ($n = 75$) reporting that coffee was associated with reduced oral microbiome diversity and altered composition (25). The study of 21 healthy adults in Austria previously mentioned reported no association between coffee intake and overall oral microbiome composition, consistent with our findings. Though many factors can contribute to inconsistencies among studies, including different sample types and laboratory methodologies, it should also be noted that Mediterranean populations (e.g., Italy) prepare coffee differently than North American and Northern European populations; this could also contribute to differences in results between our studies (52).

Our study has several strengths: the large sample size ($n = 938$), the comprehensive assessment of the oral bacterial community using 16S rRNA gene sequencing, and the control of confounders known to affect the oral microbiome such as smoking and alcohol drinking. Our study also has several limitations. The cross-sectional design does not allow us to infer causality of associations, though reverse causation is unlikely in the case of a dietary exposure. We did not have information on the oral health status of the study participants, which may be an important confounder in our analysis. Residual confounding may also contribute to our findings, as tea drinking may be associated with other diet and socioeconomic factors which could also influence oral

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microbiota. Measurement error inherent in FFQs (53) may lead to misclassification of coffee and tea drinking level, though coffee and tea are among the best of the dietary exposures that can be assessed by FFQs (54, 55). We lacked information on type of tea (green or black) and whether tea was drunk iced or hot. Green tea contains higher levels of antimicrobial catechins than black tea (40), while hot or cold beverages transiently change oral temperature (56); these distinctions may lead to different effects on the oral microbiota and thus on oral health. Finally, as our study population was mostly white, results may not be generalizable to other racial or ethnic groups.

In summary, to our knowledge, this is the largest study of tea and coffee consumption in association with the oral microbiota, and our findings suggest higher tea consumption may increase diversity and alter overall composition of the oral microbial community. These findings are consistent with a potential beneficial effect of tea on oral diseases, but, as our study was cross-sectional, future studies are required to determine whether these oral microbiome changes contribute to oral and other diseases. Tea and coffee have both been associated with reduced risk for head and neck cancer (16–18), a cancer type with possibly bacterial etiology (4, 57). Though beyond the scope of the current study, further research may elucidate whether tea-driven changes to the oral microbiome contribute to tea's chemopreventive action. Additionally, studies are needed to confirm our findings, particularly due to inconsistencies observed between the two cohorts in our analysis, CPS-II and PLCO. As the oral microbiota are vital players in oral and systemic health, it will be important to further analyze how common drinking habits influence this dynamic ecosystem.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** B.A. Peters, R.B. Hayes, J. Ahn
**Development of methodology:** M.P. Purdue, R.B. Hayes
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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** B.A. Peters, N.D. Freedman, R.B. Hayes, J. Ahn
**Writing, review, and/or revision of the manuscript:** B.A. Peters, M.L. McCullough, M.P. Purdue, N.D. Freedman, C.Y. Um, S.M. Gapstur, R.B. Hayes, J. Ahn
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** N.D. Freedman
**Study supervision:** N.D. Freedman, J. Ahn

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