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RESEARCH ARTICLE

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Characterization of the salivary microbiome of adults with inflammatory bowel disease

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ABSTRACT

Background: Perturbations of the gut microbiota in patients with inflammatory bowel disease (IBD) have been extensively characterised, but changes to the oral microbiome remain understudied. This study aimed to evaluate the oral microbiome of adults with IBD and of matched controls.

Methods: Saliva samples and data were obtained from a Canadian population cohort ($n = 320$). The salivary microbiome was characterised using 16S rRNA gene sequencing and examined for differences between control participants and those with IBD, as well as disease subcategories (Crohn's Disease and Ulcerative Colitis).

Results: Alpha diversity was significantly lower in participants with IBD than controls in unadjusted models and many remained significant after adjusting for covariates. Significant differences in some beta diversity metrics between participants with IBD and controls were found, although these did not remain significant when adjusted for covariates. Ten genera were significantly differentially abundant between cases and controls. *Veillonella* and *Streptococcus* were both increased in abundance in IBD cases vs controls (25% vs 22% and 14% vs 12%, respectively).

Conclusion: These results showcase changes in oral microbial diversity and composition in those living with IBD and highlight the potential of using the salivary microbiome as a biomarker for screening or monitoring IBD.

KEY MESSAGES

- Alpha diversity is significantly lower in participants with IBD, especially in richness measures, persisting even after adjusting for covariates.
- The reduction in alpha diversity was mainly observed in female participants, emphasizing the need to consider sex-specific differences in microbiome research.
- Several genera, including *Veillonella* and *Streptococcus*, were more abundant in participants with IBD, suggesting potential microbial markers for this condition.

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Oral microbiome; inflammatory bowel disease (IBD); bioinformatics; Crohn's disease; ulcerative colitis; saliva

Introduction

With the increasing rates and societal burdens associated with inflammatory bowel disease (IBD) [1], there is an urgent need for better ways to manage the illness. Globally, the number of people living with IBD continues to increase [1] and is close to 5 million, with Canada suffering some of the highest burdens of IBD in the world [2]. People with IBD experience multiple symptoms due to inflammation within their gastrointestinal tract and disruptions in digestion and absorption, costing healthcare systems billions of dollars per year [3,4]. Due to compounding prevalence, there will be more people living with IBD and a greater need for effective management strategies [5].

IBD is an inflammatory condition of the intestines and includes Crohn's disease (CD) and ulcerative colitis

(UC). The pathology of IBD is multifactorial, involving genetics, the immune system, the environment and the gut microbiome [6]. The composition of the gut microbiome is altered in individuals with IBD compared to healthy individuals [7–9]. In particular, the shift in gut bacteria appears to be characterized by a reduction in alpha diversity, changes in specific taxa such as a reduction in Firmicutes and increases in species from the family Ruminococcaceae [7,8,10]. Recent data from the Genetic Environmental Microbial (GEM) project indicate that microbiome changes precede the onset of inflammation in siblings of patients with IBD, who go on to develop IBD subsequently [11,12]. The gut microbiome is not the only body site that has been explored in relation to IBD. There is growing evidence that the oral microbiome may also play a role in IBD [13–19].

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The oral microbiome is a rich microbial environment containing hundreds of species [20,21] that play an important role in both local and systemic health [17,22–24]. A few studies conducted over the past decade have demonstrated an association between the salivary microbiome and IBD in adults and children, demonstrating significant differences in overall community composition and differential abundance of specific taxa [13–17,19,25]. The majority of these studies were conducted in unstimulated saliva samples from small groups of young adults, most from Asian countries using amplicon sequencing. Most recently, Kang et al. [15] aimed to identify unique microbiome patterns in saliva from over 300 mostly male IBD patients and explore potential oral microbial markers for differentiating CD and UC [15]. They showed a significant reduction in alpha diversity with IBD, shifts in taxonomic composition, and through machine learning models, the ability to distinguish between patients with IBD and healthy controls, as well as between patients with CD compared to UC [15]. While early research suggests the oral microbiome as a potential diagnostic tool or therapeutic target, further testing and validation in additional datasets that include diverse locations and populations should be conducted before adopting oral microbiome markers into clinical practice.

This study aimed to characterize the oral microbiome of adults living with IBD in Atlantic Canada. The Atlantic region of Canada not only has the highest incidence in the country but some of the highest in the world [2,26]. We hypothesized that participants with IBD will have reduced microbial diversity and altered composition compared to those without IBD.

Material and methods

Study population

This is a nested case-control study of Atlantic Canadians living with IBD. Samples and data for this study were acquired from the Atlantic Partnership for Tomorrow's Health (PATH) cohort (<https://www.atlanticpath.ca/>). All participants provided written informed consent prior to data/sample collection and Research Ethics Boards in each Atlantic province (New Brunswick: Horizon Health Network and Vitalite Health Network; Nova Scotia: Nova Scotia Health Authority Research Ethics Board and IWK Research Ethics Board; Newfoundland and Labrador: Health Research Ethics Board Newfoundland; Prince Edward Island: Health Prince Edward Island) approved the original data and sample collection procedures. The secondary analysis of data and biological samples was approved by the Dalhousie University Research Ethics Board (#2018–4420). Information on recruitment, data collected at baseline and cohort demographics have been previously published [27]. The baseline questionnaire

included sociodemographic information, anthropometric data, lifestyle behaviours and health history as previously described [27]. A portion of participants also had anthropometric measures and biological samples collected.

To address the aim of the study, the current project involves a sub-sample of Atlantic PATH participants, who had self-reported being diagnosed with IBD and provided a saliva sample at baseline. Participants that had not been diagnosed with IBD (controls) were matched 1:1 to IBD participants for sex (male/female), age (± 3 years), and smoking status (current, never/former). This resulted in a total of 320 participants, *i.e.* 160 matched case: control pairs. These case: control pairs were maintained for the IBD subtype (CD, UC) analysis. Of the participants that had IBD, 65, 64 and 31 participants self-reported that they had previously received CD, UC, or both diagnoses, respectively. Note that participants who indicated that they had been diagnosed with both CD and UC were included in the overall IBD group but not in either of the CD or UC groups.

Co-variables from questionnaires and physical measures

Based on our previous analysis of the oral microbiome in the Atlantic PATH cohort [28], select demographics, health behaviours, and physical measures were included as covariates in the statistical analysis. Participants self-reported sex and age at questionnaire completion as well as several health behaviours (smoking, dental visits, light exposure during sleep, and diet). For smoking, participants were classified as current smokers or non-smokers. Participant's last visit to a dental professional was classified as <6 months ago, 6 to <12 months ago, 1 to <2 years ago, or 3 or more years ago (all participants had reported visiting a dental professional at some point in their life). The average quantity of light entering the room of the participant when sleeping was reported as virtually no light or some light. Dietary components were reported as servings per day of vegetables, fruit-vegetable juice, refined grains, nuts/seeds and frequency of added salt (never, rarely, sometimes, most times). Physical measures were collected by trained research staff and included body composition (fat-free mass, kg) and anthropometric measures such as weight (kg), height (cm), BMI (kg/m²), waist (cm), and waist-to-hip ratio. Medication data provided by participants was coded according to the Anatomical Therapeutic Chemical (ATC) Classification System. ATC code level 2, the Pharmacological or Therapeutic subgroup, was used to determine the most reported medication classes.

Microbiome sequencing and bioinformatics

16S rRNA gene sequencing of the oral microbiome was conducted at the Integrated Microbiome Resource at Dalhousie University. Briefly, DNA was extracted from saliva samples using QIAamp 96 PowerFecal QIAcube HT kit, and then underwent PCR amplification of the V4-V5 16S rRNA gene region and was sequenced using an Illumina MiSeq as previously described [28,29].

Initial sample processing was carried out using QIIME2 (version 2020.8) [30] following the Microbiome Helper Amplicon Standard Operating Procedure v2 [31]. Briefly, raw reads were imported into QIIME2 and primers were trimmed using Cutadapt [32]. Paired-end reads were denoised using the DADA2 denoising algorithm [33] with trim lengths of 260 and 160 bp for forward and reverse reads, respectively, and 2 errors allowed in each. Amplicon Sequence Variants (ASVs) were initially classified taxonomically using the scikit-learn [34] naive Bayes classifier trained on the full-length 16S rRNA gene SILVA v138 database [35] available on the QIIME2 website [36]. ASVs that were classified as mitochondria or chloroplasts or that were unclassified at the domain level were removed from further analyses. ASVs were then re-classified using a scikit-learn [34] naive Bayes classifier trained on the full-length 16S rRNA gene Human Oral Microbiome Database (HOMD; version 15.22) [20] in QIIME2 (version 2022.2), as this was previously found to be better for classifying ASVs within the oral microbiome [37].

ASVs not present in 10% or more of samples were removed, and samples with <2500 reads were removed.

Statistical analysis

All statistical analysis was conducted using Python Version 3.12.4 and R version 4.1.2. Demographic, health behaviours and physical measures were assessed for normality using the normaltest function within the Scipy package (version 1.14.0) [38]. Differences between cases and controls were tested using t-tests (ttest_ind function within Scipy) for variables that were normally distributed ($p < 0.05$) or Mann-Whitney U tests (Mann-Whitney U function within Scipy) for variables that were not. Six metrics were calculated (Scikit-Bio version 0.6.2) [39] at the ASV level to assess alpha diversity: Chao1, Faith's phylogenetic diversity, Observed features, Shannon diversity, Simpson's index of diversity, and Simpson's evenness. Analysis of Variance models (ANOVA) (the anova_lm function within version 0.14.2 of the statsmodels package [40] were used to test for statistical differences in alpha

diversity between cases and controls. Six metrics were used to assess beta diversity at the ASV level: Bray-Curtis dissimilarity, Aitchison's distance, Robust Aitchison's distance (Scikit-Bio), Weighted UniFrac distance, Unweighted UniFrac distance (phyloseq version 1.38.0 [41], and Phylogenetic Robust Aitchison's distance (Gemelli plugin [42] within QIIME2 version 2022.11). Permutational multivariate analysis of variance PERMANOVA (the adonis2 function within version 2.6–4 of the vegan package [43]) with 999 permutations was used to test for significance with beta diversity metrics. All statistical tests for the alpha and beta diversity analysis were run with three models: (i) Cases vs controls, unadjusted for other metadata variables ($n = 320$), (ii) Cases vs controls, unadjusted for other metadata variables ($n = 238$, *i.e.* only those participants with full metadata information available), and (iii), Cases vs controls, adjusted for all variables previously found significant by either Bray-Curtis or Weighted UniFrac in the Atlantic PATH cohort ($n = 238$): smoking, age, sex, dental visit, BMI, height, weight, waist, waist-to-hip ratio, fat-free mass, sleeping light exposure, vegetable servings, juice servings, refined grain servings, nuts/seed servings, and frequency of additional salt [28]. The anova_lm and adonis2 functions were used as described above to test for statistical differences in alpha and beta diversity, respectively, between participants with IBD that were or were not taking several commonly used medications (A07, N02, N06, C09 and C10).

Following our previous recommendations to determine differentially abundant taxa [44], five different differential abundance tools were used at the genus level: (i) ALDEx2 within the aldex2 R package [45] (version 1.26.0); (ii) ANCOM-BC2 within the ANCOMBC R package [46] (version 1.4.0); (iii) ANCOM-BC within the ANCOMBC R package [47] (version 1.4.0); (iv) MaAsLin2 with the Maaslin2 R package [48] (version 1.8.0); and (v) radEmu within the radEmu R package [49] (version 1.2.0). Genera were considered to be significantly differentially abundant if two or more of these tests found them to be significant (False Discovery Rate adjusted p -value ≤ 0.1).

All plotting and visualisation were carried out using custom scripts within Python Version 3.12.4, using the additional packages matplotlib (version 3.9.1) [50], pandas (version 2.2.2) [51] and NumPy (version 1.26.4) [52]. Phylogenetic trees were visualised using a modified version of the draw function within Biopython [53]. All code used for analysis is on Github: https://github.com/R-Wright-1/ibd_oral_microbiome. Raw sequencing data is available in the NCBI SRA under project PRJEB70783.

Results

Demographic, medication use, and lifestyle behaviours of participants

The demographic and lifestyle characteristics of the 320 participants analyzed are detailed in Table 1 and Supplementary Table S1. Medications reported by participants with and without IBD are summarized in Supplementary Table S2. There were 160 participants with any IBD disease (including individuals who self-reported as having both CD and UC), 65 with CD, and 64 with UC. The age of the participants ranged from 36–69 years with a median age of 55.6 years for cases and 54.7 for controls. Compared to controls, a smaller proportion of participants with IBD and UC had visited a dental professional in the past 6 months. Despite matching on BMI, participants with IBD had a significantly larger waist circumference and consumed more servings of refined grains and fewer servings of vegetables compared to controls. Compared to controls, participants with CD consumed significantly fewer daily servings of vegetables while participants with UC consumed significantly more daily servings of refined grains. All other demographic and lifestyle characteristics examined were similar between cases and controls. The most commonly reported medications by control participants (taken by at least 5% of participants) were lipid-modifying agents, psychoanaleptics, antivirals, drugs for acid-related disorders and sex hormones. Most of these medications were also frequently reported by participants with IBD (Supplementary Table S3). The most frequently reported medication class in IBD participants was Antidiarrheals, intestinal anti-inflammatory/anti-infective agents, which included 5-aminosalicylates (5-ASAs) such as sulfasalazine (A07EC01), mesalazine (A07EC02), olsalazine (A07EC03), and the corticosteroid budesonide (A07EA06).

Alpha diversity

Figure 1 and Supplementary Figure S1 show alpha diversity at the ASV level in participants with IBD, CD or UC. All alpha diversity metrics except Simpson's evenness were significantly ($p \leq 0.05$) lower in participants with IBD compared to those without IBD at the ASV level in the unadjusted models (models 1 and 2) and remain significant in the adjusted model (except Faith's phylogenetic diversity, $p = 0.056$; Supplementary Table S4A). Participants with CD compared to those without CD had significantly lower observed taxa and Shannon diversity (models 1 and 2) at the ASV level (Figure 1 and Supplementary Figure S1). Participants with UC compared to those without

UC had significantly lower observed taxa (model 1 and 3; model 2 $p = 0.051$), Shannon diversity (models 1 and 3), Faith's phylogenetic diversity (model 1), and Chao1 (model 3) at the ASV level (Figure 1 and Supplementary Figure S1).

In the sex-stratified analysis, male participants with IBD, CD or UC compared to those without had no significant differences in alpha diversity metrics (Supplementary Table S4B). In female participants, all alpha diversity metrics except Simpson's evenness were significantly lower in participants with IBD compared to those without IBD in all models (excluding Faith's phylogenetic diversity in model 3; Supplementary Table S4C). For female participants with CD, alpha diversity was significantly lower than controls for Chao1 richness and observed taxa in models 1 and 2 and Shannon and Simpson's Index of diversity in all models (Table S4C). For UC, only Shannon diversity was significantly lower than controls for model 1.

In acknowledgment of the larger sample size of females and potential for more statistical power, a separate analysis was conducted in a subset of females that were down sampled to the same sample size as the male group ($n = 100$). In the reduced model, Shannon diversity is significantly lower in female participants with IBD compared to those without IBD at the ASV level (model 1; Supplementary Table S4D).

Our previous work demonstrated a minimal influence of medications on the microbial diversity of individuals living without major chronic conditions [54]. However, since IBD participants in the current study reported taking medications commonly prescribed for IBD, the influence of the top medication classes (ATC level 2 classes reported by at least 10% of IBD participants, $n = 22$) on microbial diversity and composition was assessed. Neither alpha nor beta diversity metrics were influenced by the most commonly reported medications (Supplementary Table S5). We chose to characterise the medications taken by participants at ATC Level 2 because the data for the numbers of other ATC 2nd level medication classes or more detailed chemical subgroups (i.e. ATC level 5) was too sparse to perform statistical analysis.

Beta diversity

Phylogenetic Robust Aitchison PCA (Figure 2), Bray-Curtis dissimilarity, Aitchison's distance, Robust Aitchison's distance, Weighted UniFrac distance and Unweighted UniFrac distance at the ASV level were used to assess beta diversity (Supplementary Table S6). The effect sizes for all beta diversity comparisons between cases and controls were small (R^2 0.005–0.036 for significant

Table 1. Participant characteristics.

	Normality test			IBD			CD			UC		
	Overall	Cases	Controls	Test	Cases	Controls	Test	Cases	Controls	Test	Cases	Controls
Proportion female												
Age (years)	Stat = 2.97, $p = 0.2267$	55.56 (STD: 8.01, Range: 36–69) [160]	54.71 (STD: 7.76, Range: 36–69) [160]	Stat = 0.95, $p = 0.3411$	55.51 (STD: 8.31, Range: 37–69) [65]	54.82 (STD: 8.05, Range: 36–67) [65]	Stat = 0.48, $p = 0.6331$	55.92 (STD: 7.58, Range: 36–69) [64]	54.89 (STD: 7.27, Range: 36–69) [64]	Stat = 0.78, $p = 0.4371$	55.92 (STD: 7.58, Range: 36–69) [64]	54.89 (STD: 7.27, Range: 36–69) [64]
Proportion that are current smokers	Stat = 35.2, $p = 0.0$	0.04 [157]	0.04 [157]	NA	0.05 [65]	0.05 [65]	NA	0.02 [61]	0.02 [61]	NA	0.02 [61]	0.02 [61]
Time since last dental visit	Stat = 13.06, $p = 0.0015$	<6 m: 0.61; 6 m to <1 yr: 0.24; 1 hr to <2 yr: 0.08; 2 yr to <3 yr: 0.01; ≥3 yr: 0.06 [159]	<6 m: 0.73; 6 m to <1 yr: 0.18; 1 yr to <2 yr: 0.06; 2 yr to <3 yr: 0.01; ≥3 yr: 0.02 [159]	Stat = 14264.0, $p = 0.0171$	<6 m: 0.68; 6 m to <1 yr: 0.25; 1 yr to <2 yr: 0.05; 2 yr to <3 yr: 0.03; ≥3 yr: 0.03 [65]	<6 m: 0.75; 6 m to <1 yr: 0.14; 1 yr to <2 yr: 0.05; 2 yr to <3 yr: 0.03; ≥3 yr: 0.03 [65]	Stat = 2240.5, $p = 0.4533$	<6 m: 0.57; 6 m to <1 yr: 0.21; 1 yr to <2 yr: 0.11; 2 yr to <3 yr: 0.03; ≥3 yr: 0.08 [63]	<6 m: 0.71; 6 m to <1 yr: 0.21; 1 yr to <2 yr: 0.06; 2 yr to <3 yr: 0.03; ≥3 yr: 0.02 [63]	Stat = 2336.0, $p = 0.0443$	<6 m: 0.57; 6 m to <1 yr: 0.21; 1 yr to <2 yr: 0.11; 2 yr to <3 yr: 0.03; ≥3 yr: 0.08 [63]	<6 m: 0.71; 6 m to <1 yr: 0.21; 1 yr to <2 yr: 0.06; 2 yr to <3 yr: 0.03; ≥3 yr: 0.02 [63]
BMI (kg/m ²)	Stat = 2.66, $p = 0.2651$	27.59 (STD: 4.91, Range: 18.0–43.0) [160]	26.77 (STD: 4.52, Range: 19.0–44.0) [160]	Stat = 1.55, $p = 0.1213$	27.27 (STD: 5.23, Range: 19.0–43.0) [65]	26.55 (STD: 4.9, Range: 19.0–44.0) [65]	Stat = 0.8, $p = 0.4248$	27.57 (STD: 4.26, Range: 18.0–36.0) [64]	26.71 (STD: 3.89, Range: 19.0–38.0) [64]	Stat = 1.18, $p = 0.2402$	27.57 (STD: 4.26, Range: 18.0–36.0) [64]	26.71 (STD: 3.89, Range: 19.0–38.0) [64]
Body Weight (kg)	Stat = 3.97, $p = 0.1375$	76.66 (STD: 15.15, Range: 41.9–130.2) [160]	74.71 (STD: 14.32, Range: 46.4–121.8) [160]	Stat = 1.18, $p = 0.2383$	75.72 (STD: 15.31, Range: 49.5–130.2) [65]	73.36 (STD: 14.26, Range: 49.7–121.8) [65]	Stat = 0.91, $p = 0.3667$	76.89 (STD: 14.27, Range: 41.9–111.3) [64]	75.9 (STD: 13.04, Range: 47.7–107.3) [64]	Stat = 0.41, $p = 0.6849$	76.89 (STD: 14.27, Range: 41.9–111.3) [64]	75.9 (STD: 13.04, Range: 47.7–107.3) [64]
Waist Circumference (cm)	Stat = 1.64, $p = 0.4402$	92.66 (STD: 13.75, Range: 59.0–134.5) [152]	89.39 (STD: 12.6, Range: 63.5–122.5) [152]	Stat = 2.16, $p = 0.0317$	92.33 (STD: 12.49, Range: 73.0–124.0) [62]	88.11 (STD: 12.41, Range: 63.5–122.5) [61]	Stat = 1.86, $p = 0.0648$	92.4 (STD: 12.52, Range: 59.0–121.0) [60]	89.79 (STD: 11.9, Range: 65.3–118.3) [60]	Stat = 1.16, $p = 0.2473$	92.4 (STD: 12.52, Range: 59.0–121.0) [60]	89.79 (STD: 11.9, Range: 65.3–118.3) [60]
Waist-to-Hip Ratio	Stat = 0.34, $p = 0.8449$	0.88 (STD: 0.08, Range: 0.68–1.12) [159]	0.87 (STD: 0.08, Range: 0.63–1.07) [160]	Stat = 1.71, $p = 0.0888$	0.88 (STD: 0.07, Range: 0.73–1.05) [65]	0.86 (STD: 0.08, Range: 0.69–1.01) [65]	Stat = 1.03, $p = 0.3045$	0.89 (STD: 0.08, Range: 0.72–1.07) [64]	0.88 (STD: 0.09, Range: 0.63–1.07) [64]	Stat = 1.18, $p = 0.2408$	0.89 (STD: 0.08, Range: 0.72–1.07) [64]	0.88 (STD: 0.09, Range: 0.63–1.07) [64]
Height (cm)	Stat = 0.48, $p = 0.7876$	166.66 (STD: 8.46, Range: 146.4–195.2) [160]	166.82 (STD: 8.92, Range: 147.6–187.5) [160]	Stat = –0.17, $p = 0.8678$	166.95 (STD: 8.97, Range: 150.0–195.2) [65]	166.3 (STD: 8.91, Range: 152.0–187.4) [65]	Stat = 0.41, $p = 0.6834$	166.71 (STD: 8.53, Range: 146.4–182.0) [64]	168.22 (STD: 8.56, Range: 153.0–187.5) [64]	Stat = –0.99, $p = 0.3228$	166.71 (STD: 8.53, Range: 146.4–182.0) [64]	168.22 (STD: 8.56, Range: 153.0–187.5) [64]
Exposure to light at night	Stat = 12.2, $p = 0.0022$	Virtually none: 0.43; Some light: 0.53; A lot of light: 0.03 [159]	Virtually none: 0.43; Some light: 0.53; A lot of light: 0.04 [159]	Stat = 12518.5, $p = 0.8656$	Virtually none: 0.39; Some light: 0.59; A lot of light: 0.02 [64]	Virtually none: 0.46; Some light: 0.51; A lot of light: 0.03 [65]	Stat = 2206.0, $p = 0.4965$	Virtually none: 0.48; Some light: 0.5; A lot of light: 0.02 [64]	Virtually none: 0.51; Some light: 0.43; A lot of light: 0.06 [63]	Stat = 2013.0, $p = 0.9891$	Virtually none: 0.48; Some light: 0.5; A lot of light: 0.02 [64]	Virtually none: 0.51; Some light: 0.43; A lot of light: 0.06 [63]
Vegetables (servings/day)	Stat = 2.2, $p = 0.3331$	2.06 (STD: 1.51, Range: 0.0–8.0) [159]	2.46 (STD: 1.43, Range: 0.0–7.0) [159]	Stat = –2.44, $p = 0.0154$	1.8 (STD: 1.48, Range: 0.0–6.0) [65]	2.35 (STD: 1.53, Range: 0.0–7.0) [65]	Stat = –2.08, $p = 0.0396$	2.49 (STD: 1.48, Range: 0.0–8.0) [63]	2.59 (STD: 1.28, Range: 0.0–6.0) [63]	Stat = –0.38, $p = 0.7021$	2.49 (STD: 1.48, Range: 0.0–8.0) [63]	2.59 (STD: 1.28, Range: 0.0–6.0) [63]
Juice (servings/day)	Stat = 4.78, $p = 0.0917$	0.66 (STD: 1.01, Range: 0.0–6.0) [156]	0.62 (STD: 0.96, Range: 0.0–7.0) [159]	Stat = 0.34, $p = 0.7352$	0.59 (STD: 1.07, Range: 0.0–6.0) [64]	0.52 (STD: 0.77, Range: 0.0–3.0) [65]	Stat = 0.43, $p = 0.6694$	0.71 (STD: 0.93, Range: 0.0–4.0) [63]	0.73 (STD: 1.18, Range: 0.0–7.0) [63]	Stat = –0.08, $p = 0.9341$	0.71 (STD: 0.93, Range: 0.0–4.0) [63]	0.73 (STD: 1.18, Range: 0.0–7.0) [63]
Refined Grains (servings/day)	Stat = 6.83, $p = 0.033$	0.4 (IQR: 1.0, Range: 0.0–5.0) [135]	0.3 (IQR: 1.0, Range: 0.0–4.0) [156]	Stat = 11988.5, $p = 0.0366$	0.4 (IQR: 1.0, Range: 0.0–5.0) [60]	0.3 (IQR: 1.0, Range: 0.0–2.0) [63]	Stat = 2069.0, $p = 0.356$	1.0 (IQR: 1.25, Range: 0.0–3.0) [48]	0.3 (IQR: 1.0, Range: 0.0–4.0) [63]	Stat = 1880.0, $p = 0.0229$	1.0 (IQR: 1.25, Range: 0.0–3.0) [48]	0.3 (IQR: 1.0, Range: 0.0–4.0) [63]
Fat Free Mass (kg)	Stat = 5.62, $p = 0.0602$	50.47 (STD: 10.9, Range: 20.3–77.9) [148]	50.33 (STD: 10.23, Range: 32.9–77.9) [151]	Stat = 0.11, $p = 0.9094$	49.18 (STD: 10.78, Range: 20.3–75.2) [60]	48.75 (STD: 9.07, Range: 32.9–67.4) [61]	Stat = 0.24, $p = 0.8144$	51.94 (STD: 10.91, Range: 34.0–77.9) [58]	52.64 (STD: 10.49, Range: 37.7–77.9) [59]	Stat = –0.35, $p = 0.7286$	51.94 (STD: 10.91, Range: 34.0–77.9) [58]	52.64 (STD: 10.49, Range: 37.7–77.9) [59]

(Continued)

Table 1. (Continued).

	Normality test			IBD			CD			UC		
	Overall	Cases	Controls	Test	Cases	Controls	Test	Cases	Controls	Test	Cases	Controls
Frequency of salt added to food	Stat = 5.3, $p = 0.0705$	Never: 0.24; Rarely: 0.43; Sometimes: 0.18; Almost always: 0.16 [140]	Never: 0.3; Rarely: 0.31; Sometimes: 0.23; Almost always: 0.16 [152]	Stat = -0.05, $p = 0.9602$	Never: 0.2; Rarely: 0.48; Sometimes: 0.16; Almost always: 0.16 [61]	Never: 0.25; Rarely: 0.34; Sometimes: 0.26; Almost always: 0.15 [61]	Stat = -0.09, $p = 0.9273$	Never: 0.29; Rarely: 0.45; Sometimes: 0.12; Almost always: 0.14 [49]	Never: 0.33; Rarely: 0.26; Sometimes: 0.25; Almost always: 0.16 [61]	Stat = -0.61, $p = 0.5404$		
Nuts or Seeds (servings/day)	Stat = 1.6, $p = 0.4497$	0.62 (STD: 0.73, Range: 0.0–4.0) [140]	0.66 (STD: 0.62, Range: 0.0–3.0) [156]	Stat = -0.56, $p = 0.574$	0.48 (STD: 0.5, Range: 0.0–2.0) [61]	0.63 (STD: 0.58, Range: 0.0–2.0) [63]	Stat = -1.56, $p = 0.1225$	0.87 (STD: 0.91, Range: 0.0–4.0) [49]	0.69 (STD: 0.68, Range: 0.0–3.0) [63]	Stat = 1.16, $p = 0.2468$		

Continuous variables are presented as the mean or median (standard deviation or interquartile range) depending on whether the normality test was significant, as well as the range of values.

Categorical variables show the proportion of participants in each group.

Numbers in square brackets show the total number of participants with available information for this metadata variable.

The test column shows results of tests for significant differences between cases and controls. Where the normality test showed that data were normally distributed, a t-test was used. Where the normality test showed data were not normally distributed, a Mann-Whitney U test was used.

Significant differences are indicated with bold lettering.

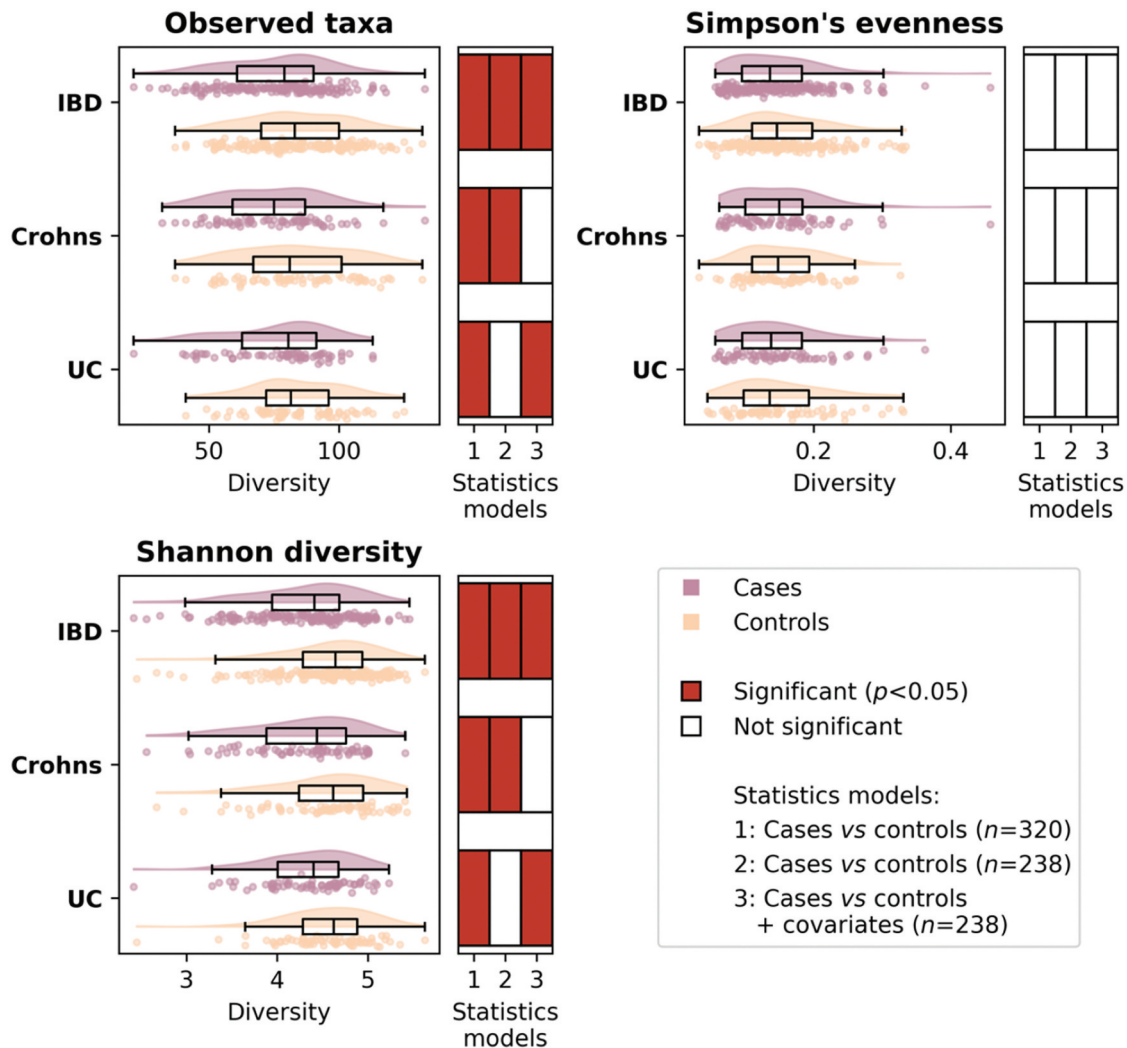


Figure 1. Alpha diversity at the ASV level in participants with IBD, CD or UC. Panels show observed taxa, Simpson's evenness and Shannon diversity. Results for these alpha diversity metrics in addition to Chao1 richness, faith's phylogenetic diversity and Simpson's index of diversity are shown in supplementary figure S4. In each panel, results for individual participants are shown with points with boxplots showing the median, upper and lower quartiles while whiskers show the range of the data (1.5 times the interquartile range). Statistical significance for ANOVA tests is indicated in red ($p \leq 0.05$). Full results for all ANOVA tests are shown in supplementary table S4.

associations and R^2 0.000–0.068 for non-significant associations; Supplementary Table S6). Significant ($p \leq 0.05$) associations with beta diversity were observed in participants with IBD compared to matched controls using Bray-Curtis dissimilarity, Aitchison's distance (both models 1 and 2), Phylogenetic Robust Aitchison PCA and Unweighted UniFrac distance (both model 1 only). In participants with CD compared to matched controls, significant associations were observed for Aitchison's and Unweighted UniFrac distance (models 1 and 2). None of these associations remained significant after adjusting for covariates (model 3) and no significant associations were identified for participants with UC compared to matched controls (Supplementary Table S6A).

In the sex-stratified analysis, none of the beta diversity metrics were significant in males (Supplementary

Table 6B), whereas similar overall patterns to the whole cohort were observed in females. More specifically, in female participants with IBD compared to controls, significant associations were observed for Bray-Curtis dissimilarity (model 1), Aitchison's distance and Phylogenetic Robust Aitchison PCA (both models 1 and 2) and in female participants with CD compared to controls, significant associations were observed for Aitchison's distance (models 1 and 2), Phylogenetic Robust Aitchison PCA (model 1) and Unweighted UniFrac distance (models 1 and 2). Again, none of the associations remained significant after being adjusted for covariates (model 3) and no significant associations were observed for female participants with UC compared to matched controls (Supplementary Table S6C). In the female subset with the same number of females as males, all significance was lost (Supplementary Table S6D).

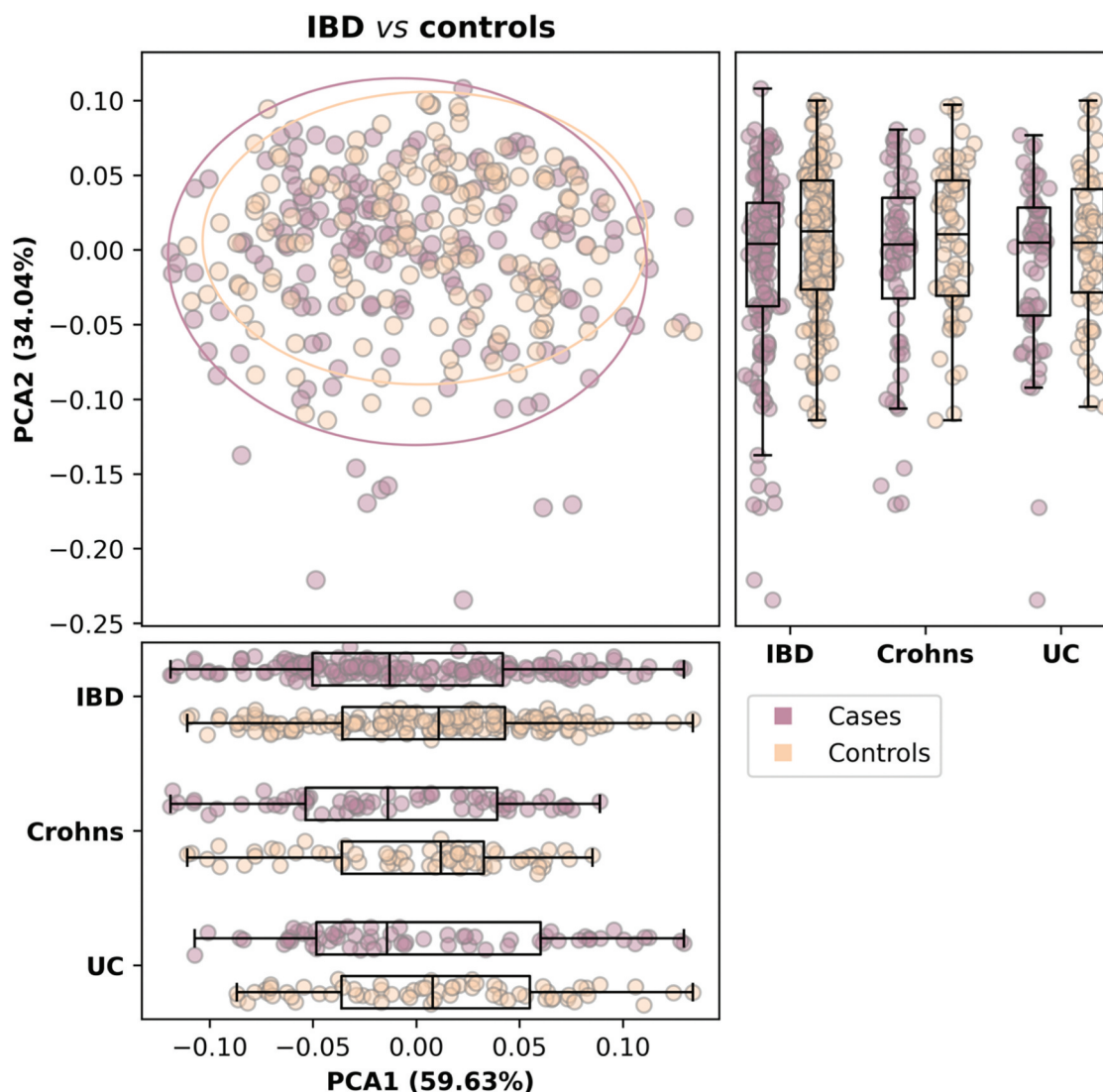


Figure 2. Ordination plot showing phylogenetic robust Aitchison PCA at the ASV level for participants with IBD. Each point shown represents a sample for a participant, with ellipses showing the confidence interval (3 standard deviations) for IBD and matched controls. Panels to the bottom and right show boxplots showing the median, upper and lower quartiles and whiskers representing the range of the data (1.5 times the interquartile range) for each axes separately. Values shown on each axis label indicate the proportion of within-sample variation represented by that axis.

Abundance and differential abundance at the genus level

The oral microbiome has already been characterised in healthy participants within the Atlantic PATH cohort [28], and we therefore focused on the differences between participants with IBD, CD or UC and matched healthy controls. We carried out differential abundance tests at the genus level using five tools (ALDEx2, ANCOM-BC2, ANCOM-BC, MaAsLin2 and radEmu) and consider a genus to be significantly differentially abundant (DA) if ≥ 2 tests find the genus significant with a False Discovery Rate (FDR) corrected $p \leq 0.1$ (Supplementary Table S7). Seven and four genera were identified as DA in IBD and CD cases *vs* controls, respectively (Supplementary Table S7F). No genera were identified as DA between UC and matched controls. The

DA genera were visualised using relative abundance (Supplementary Figure S2) and Centered Log Ratio (CLR) abundance (Supplementary Figure S3). Relative abundance is more intuitive, while CLR abundance accounts for the compositional nature of microbiome data [55]. For the CLR abundance, a value of zero indicates that the abundance of that genus is equal to the mean $\log_2$ abundance of all genera, while positive or negative values indicate higher or lower abundances, respectively, than the mean $\log_2$ relative abundance.

Of the seven genera that were DA between IBD cases and controls, four were higher in cases than controls (*Cutibacterium*, *Staphylococcus*, *Streptococcus* and *Veillonella*) and three were higher in controls than cases (*Parvimonas*, unidentified Ruminococcaceae and unidentified Veillonellaceae). All four genera that were

DA between CD cases and controls were higher in controls than cases (unclassified Firmicutes, *Ottowia*, unclassified Ruminococcaceae and *Tannerella*). Only unclassified Ruminococcaceae was identified as significantly DA between cases and matched controls for both IBD and CD; in both groups it was higher in abundance in controls than cases (Supplementary Figures S2 and S3). Within the top 20 most abundant genera, only two were DA, *Veillonella* and *Streptococcus*, both of which were increased in abundance in IBD cases vs controls (25% vs 22% and 14% vs 12%, respectively; Figure 3).

Due to the small number of genera that were DA when testing only using model 1, we did not perform these tests using models 2 and 3 or for the sex-stratified groups.

Discussion

This study expands the current literature by including a large cohort of adults aged 36–69 years living in a region with particularly high rates of IBD [2,26]. This study demonstrates that participants with IBD compared to matched controls have lower alpha diversity in their salivary microbiome using various measures, particularly in measures of richness (Figure 1). This finding is driven by the signal among female participants within this study. Separate analyses of CD and UC show similar patterns, although not statistically significant across all

models. Beta diversity analysis demonstrated dissimilarity in salivary community composition between participants with IBD and controls in several models and some with CD but showed a high degree of similarity in participants with UC compared to controls (Figure 2).

The oral microbiome has distinct niches [21,56], and changes in microbial communities observed in individuals with IBD may be site- and taxon-specific [18]. Somineni et al used a random forest classifier to determine whether oral microbiota could be used to distinguish participants with IBD from healthy control patients. The study showed that samples from 4 different oral sites as well as stool could be used to distinguish patients with IBD from healthy controls with saliva samples performing the best, followed by stool samples [18].

Compared to the current study, much of the research on the salivary microbiome of individuals with IBD has been conducted in relatively small sample sizes in younger populations from predominately Asian countries with a large proportion of male participants [13,14,17,19,57,58]. Nevertheless, the current study finds several similar patterns in a large, Canadian cohort composed of approximately 68% females aged 36–69 years. For example, nearly all studies, including the current one (Figure 2), reported a significant association with multiple metrics of beta diversity in participants with IBD, including specific subtypes, compared to controls [13–15,17,19].

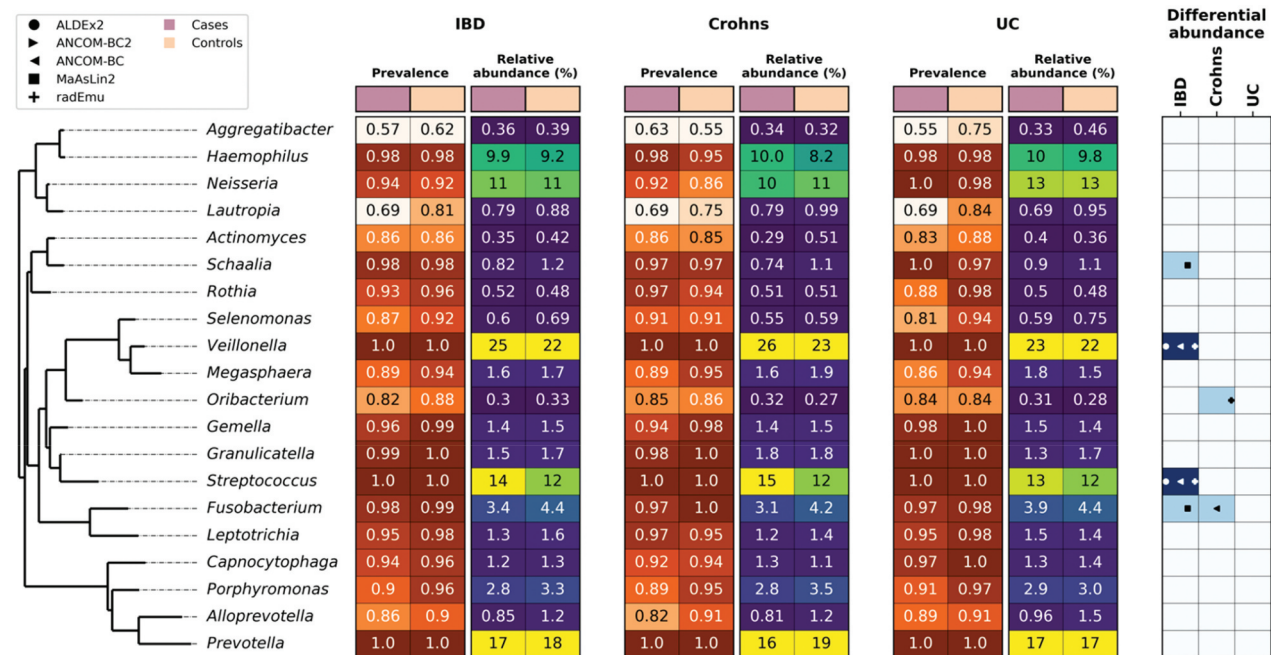


Figure 3. Prevalence, abundance and differential abundance of the 20 most abundant genera (relative abundance). Plots show (from left) phylogenetic tree of the genera, prevalence and mean relative abundance within IBD, CD and UC participants and differential abundance tests finding the genera significantly differentially abundant (false discovery rate corrected $p \leq 0.05$). Markers show which of the tests identified the genus as differentially abundant and the intensity of the blue colour shows the number of tests finding a genus differentially abundant. Supplementary figures S2 and S3 show relative abundance and CLR abundance, respectively, for all genera found to be significant by 2+ differential abundance tests.

Interestingly, a recent larger study with both CD and UC participants (over 300 IBD participants total) reported significance in the overall PERMANOVA analysis but showed that some individuals with CD or UC displayed a profile closer to that of controls, while others displayed a more discrete microbial pattern. A possible reason for this observation may be the phase of disease activity. Zhang et al showed a significant association with beta diversity with control participants and those in the remission phase clustering together, and the IBD participants within the active phase clustering separately [58].

Zhang et al also showed significant influence of disease activity with alpha diversity, characterized by lower microbial richness in participants with active disease compared to control participants or those in remission [58]. Unfortunately, the population cohort used in the current study did not capture information on disease activity. This may account for some of the discrepancies in the literature as some studies on the salivary microbiome showed reduced diversity with multiple different metrics [13–15] (Figure 2), but not all [14,17,19].

We were able to identify several genera that were differentially abundant between participants with IBD or CD and controls (Supplementary Figure S2 & S3). Although differences in sequencing methods and bioinformatic approaches across studies may further influence interpretation of sequencing results [59,60], some similar taxa were identified in other studies. In agreement with our findings in the salivary microbiome (Figure 3), enrichment of the genera *Veillonella* [13,17] and *Streptococcus* [19] in the saliva has been previously reported in individuals with IBD compared with controls. Likewise, Elmaghawry et al reported similar increases in the abundance of *Streptococcus* in the dorsum tongue microbiome of those with IBD compared to those without [61]. The study also showed reduced *Veillonella* which contrasts with the current study and other literature [13,17] however, as discussed above compositional changes between control and IBD participants may be site- and taxon-specific [18].

There is evidence that oral microbiome can be translocated along the gastrointestinal tract and can alter the gut microbiota and influence disease [62–65]. Translocation may occur via enteral transmission due to the swallowing of saliva along with microbiota or via hematological transmission due to injuries in oral tissues that allow the microbiota or metabolites to enter systemic circulation [63]. There is disruption of oral microbiota in individuals with dental caries, gingivitis and periodontitis [66–68], and patients with IBD have a high prevalence of dental caries and periodontitis [69–71]. It is possible that these microbes found in higher abundance in patients with dental caries or periodontal disease may translocate

from the oral cavity and colonize the intestine. Interestingly, Mottawea et al demonstrated that the resident oral microbiota *Atopobium parvulum* was increased in colon samples of patients with CD and positively correlated with disease severity [64]. Thus, further emphasizing the role of oral microbiota in the colon and its connection to IBD activity.

Within the intestine, oral bacteria may compete for nutrients or displace commensal gut microbiota that play an important role in intestinal mucosal integrity, protection against pathogens, and immunoregulation. The possible role of specific oral microbiota modulating immune pathways and disrupting normal intestinal homeostasis has been reviewed previously [72]. Furthermore, accumulating evidence suggests that immune responses in individuals with IBD may be further influenced by genetics and microbial interactions with environmental exposures. Valuable insights into genetic and environmental risk factors and the etiology of CD have been gained from the GEM project, a prospective cohort of approximately 5,000 healthy asymptomatic first-degree relatives of patients with CD [73]. This includes the identification of intestinal microbial signatures in healthy first-degree relatives that are associated with CD risk up to 5 years prior to onset [12]. Additionally, the GEM project identified early life exposures (family size, pet ownership) that were associated with changes in the gut microbiome composition and risk of CD in first degree relatives [74]. The GEM study also demonstrated that individuals who develop CD later have abnormal barrier function several years before the onset of CD [75] and that healthy first-degree relatives of patients with CD may improve their intestinal microbiome and barrier function by following specific dietary patterns [76]. Given the connection between the oral and gut microbiome, future oral microbiome studies may consider exploring the genetic and environmental risk factors that were identified in the intestinal microbiome by the GEM project.

This study is among the largest salivary microbiome studies in individuals with IBD to date and provides new information on the salivary microbiome in a unique population that has some of the highest rates of IBD globally. However, limitations of our study include the self-reporting of IBD diagnosis (potentially explaining the group of participants who reported both CD/UC diagnoses) and the inability to link oral microbiome changes to disease course (e.g. information was lacking regarding the presence of active disease symptoms, subclinical inflammation such as fecal calprotectin and IBD-treatment) in a large region-based cohort. Another limitation of self-reported data is the potential for bias, social desirability, or memory inaccuracies. The agreement (Cohen's Kappa) between self-reported data on

chronic diseases and medical records can range from slight ($\kappa \leq 0.29$) to excellent ($\kappa > 0.8$) [77–80] depending on the disease. However, studies show that self-reported diagnosis of IBD and specific subtypes show moderate (κ 0.52) to near perfect alignment (κ 0.99) with medical records [81,82]. The use of self-reported questionnaire data allowed us to include several additional covariates that are not normally captured in clinical records. Much of the existing literature does not account for or fails to report whether analyses include covariates, yet several additional factors may influence the oral microbiota, including demographics, host health, lifestyle behaviours, diet, and anthropometric measures [28,57,83]. Another limitation of the cohort is the lack of information from oral examinations or other self-reported oral health information related to flossing, caries, dentate, or disease. Previous research has shown that oral health factors such as last dental visit, frequency of flossing, presence of natural teeth, periodontal disease, and caries contribute to oral microbial variation [28,84–87]; and should be considered in future studies. Finally, the generalizability of the findings are limited. Participants are aged 36–69 years and are from a region of Canada with particularly high rates of IBD, with the majority of participants being female and Canadian-born. Future research should examine diverse populations with IBD while incorporating disease activity and oral health, as well as longitudinal studies to understand how the oral microbiome changes over time in individuals with IBD.

Conclusions

Our research, along with others, demonstrates a relationship between the oral microbiome and IBD [13–17,19]. Given the existing literature and ease of sample collection (quick, non-invasive), the oral microbiome has the potential to be developed further as a prevention strategy at the population level or used as a diagnostic tool or therapeutic target for patients with IBD. At the population level, future research is necessary to expand our understanding of how environmental factors that are associated with the future risk of IBD may influence the oral microbiome and help direct population-level strategies for prevention. Modification of the oral microbiome for the management of IBD warrants further investigation and could include possible intervention with probiotics or treatment with targeted antibiotics to eliminate specific pathogenic bacteria. Furthermore, multi-omic approaches could be used to further understand strain-level changes and the complex interaction between the microbiota and their metabolites on host immune response. Finally, longitudinal tracking of the oral

microbiome in individuals with IBD over time is necessary to understand the association between disease activity and the best time for therapeutic intervention in those living with IBD.

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Disclosure statement

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

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Author contributions

J.V.L., V.D., and M.G.I.L. proposed the project; M.G.I.L. supervised the project; R.J.W. processed and prepared datasets, analyzed datasets and generated figures; V.D. and R.J.W. wrote the manuscript. J.V.L. and M.G.I.L. provided constructive feedback on the manuscript. All authors reviewed and approved the final manuscript.

Data availability statement

All sequencing data have been uploaded to the European Nucleotide Archive and are available under the accession number PRJEB70783. Code used to analyze all data is available at https://github.com/R-Wright-1/ibd_oral_microbiome. Metadata used in this project cannot be shared publicly because participant consent and ethical restrictions do not permit public sharing of the data. Deidentified data and biosamples from Atlantic PATH are available to researchers through a data access process. Additional information can be obtained by contacting info@atlanticpath.ca.

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