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Distinct Profiles of Fecal Volatile Organic Compounds Discriminate Ulcerative Colitis Patients With an Ileoanal Pouch From Those With an Intact Colon

Chu K. Yao[1] | Kraig Green[2] | Alice S. Day[3,4,5] | Zaid M. Ardalan[1] | Rachael Slater[2] | Robert V. Bryant[3,4,5] | Peter R. Gibson[1] | Chris Probert[2]

[1]Department of Gastroenterology, School of Translational Medicine, Monash University and Alfred Health, Melbourne, Victoria, Australia | [2]Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, UK | [3]Inflammatory Bowel Disease Service, Department of Gastroenterology, The Queen Elizabeth Hospital, South Australia, Australia | [4]Basil Hetzel Research Institute, South Australia, Australia | [5]School of Medicine and Health Sciences, University of Adelaide, North Terrace, Adelaide, South Australia, Australia

Correspondence: Chu K. Yao (chu.yao@monash.edu)

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ABSTRACT

Fecal volatile organic compounds (VOCs) offer insights into gut microbiota function that may drive the pathogenesis of ulcerative colitis (UC). This cross-sectional study aimed to compare dietary intake and VOC patterns in UC patients with an ileoanal pouch compared to those with an intact colon. Seven-day food records and fecal samples were collected from UC patients with an intact colon ($n = 28$) or an ileoanal pouch ($n = 11$). Fecal VOC profiles were analyzed using gas chromatography-mass spectrometry. Dietary intake in both groups was largely similar. The mean Jaccard similarity index of VOC was 0.55 (95% CI:0.53, 0.56) in the pouch compared with 0.48 (0.47, 0.49) in the colon group ($p < 0.01$). A lower proportion of VOC classes was detected in the pouch, including sulfide (9% vs. 57%; $p < 0.01$), branched-chain fatty acids (BCFAs; 45%–64% vs. 93%–96%; $p < 0.01$), and ketones (45%–64% vs. 93%–96%; $p < 0.01$), along with a higher proportion of butyric acid (91% vs. 29%; $p < 0.001$). Unrelated to diet, VOC profiles show less functional diversity, reduced protein and greater carbohydrate fermentation, and altered production of secondary metabolites in the UC-pouch compared with the intact colon. These differences in the metabolic environment of the gut microbiota provide insights into pathogenesis and suggest that microbial-targeted interventions should be tailored accordingly.

1 | Introduction

Ulcerative colitis (UC) is a relapsing and remitting inflammatory disease affecting the large bowel. The pathogenesis of UC remains unclear, but abnormal patterns of microbial fermentation and associated production of metabolites have been identified as catalysts or perpetuators of inflammation [1, 2]. Despite conventional therapies targeting immune-mediated inflammatory pathways, approximately 30% of individuals with UC develop medically refractory colitis and require surgery, with total proctocolectomy

Abbreviations: BCFA, branched-chain fatty acids; CI, confidence intervals; ELISA, enzyme linked immunosorbent assay; PCA, principal component analysis; PDAI, pouch disease activity index; SCFA, short-chain fatty acids; UC, ulcerative colitis; VOC, volatile organic compounds.

Chu K. Yao, Kraig Green, and Alice S. Day are joint first authors.

Peter R. Gibson and Chris Probert are joint senior authors.

and an ileal pouch-anal anastomosis being the preferred surgery [3]. This involves the creation of a fecal reservoir from the terminal ileum to replace the anorectum. However, the ileoanal pouch remains susceptible to inflammatory changes similar to those that occurred in the intact colon [4].

As the pouch gradually adapts to mimic a colon-like environment, there are clues suggesting functional activities of the colonic microbiota, including carbohydrate and protein fermentation, may be acquired by the reconstructed ileoanal pouch [5], albeit to a lesser extent than in the colon [6]. Carbohydrate fermentation by colonic microbiota appears to be defective in UC [7], but this is poorly studied along with other metabolic activities in both UC pouches and those with an intact colon. Altered carbohydrate fermentation with reduced production or utilization of fecal short-chain fatty acids (SCFA) has been shown in active pouchitis [8], together with signals of abnormal protein fermentation with increased fecal hydrogen sulfide production, similar to that occurring in the colon of patients with UC [9]. This suggests the same microbial pathways are active in both the intact colon and pouch, but this has not yet been adequately explored.

Metabolomic technology has paved the way for understanding more about the colonic intraluminal environment and the role of microbial activities in the pathogenesis of gastrointestinal diseases. Fecal volatile organic compounds (VOCs) are intermediary end products generated in the colon and pouch through microbial metabolic activities and/or as a result of dietary intake and physiological processes [6, 10, 11]. In UC, fecal VOC profiles differ between active and inactive disease, but this remains poorly characterized in association with a healthy or inflamed pouch [6, 11]. Differing VOC profiles in inflammatory and non-inflammatory states provide clues that functional microbial activities are involved in luminal disease and, perhaps, its pathogenesis. VOC profiles have the potential to serve as predictors of response to microbial-modulating therapies such as diet, as recently observed with irritable bowel syndrome [12, 13].

Given the strong interest in targeting the gut microbiota as a therapeutic strategy for UC and pouchitis, characterization of colonic VOC profiles may provide mechanistic insight into the pathways involved. The study aimed to identify patterns of VOC production in a cohort of well-characterized patients with UC who have an intact colon in comparison to those with an ileoanal pouch in order to obtain insights into the metabolic activities of the colonic and pouch microbiota.

2 | Experimental Section

This is a cross-sectional study where baseline fecal samples and clinical and dietary intake data from two cohorts of patients with UC, those with an ileoanal pouch and those with an intact colon enrolled in two separate dietary studies [14, 15] were analyzed for VOC profiles. The detailed methodology of the studies is published elsewhere [14, 15].

Patients with UC with an intact colon and those with a pouch were recruited from tertiary institutions in South Australia and Victoria, respectively, between 2018 and 2020. Inclusion criteria included patients with an intact colon with mild to moderate disease activity (defined as a total Mayo score of 3–10 and a Mayo endoscopic sub-score ≥ 1) and patients with an ileoanal pouch constructed for UC with bowel continuity for ≥ 12 months. All patients were on stable medical therapy. The exclusion criteria are published in association with the dietary trials [14, 15]. Briefly, patients were excluded if they had another inflammatory bowel disease or celiac disease, were pregnant or breastfeeding, had used fiber supplements in the last 2 weeks, or consumed a habitual vegan or lacto-vegetarian diet. Disease activity was assessed using the total Mayo score in patients with an intact colon and the total Pouch Disease Activity Index (PDAI) in those with a pouch at study commencement by a gastroenterologist (RVB, ZMA). Current pouchitis was defined as a total PDAI score ≥ 7.

The studies received their respective ethics approval from the Central Adelaide Local Health Network Human Research Ethics Committee (HREC/16/RAH/24, R20160202) or the Alfred HREC (58716) and were registered with the Australian New Zealand Clinical Trials Registry (ACTRN12621000374864 & ACTRN12619000063112). Written informed consent was obtained from all participants prior to study enrollment.

2.1 | Clinical and Dietary Assessment

Demographic and clinical information, including current medical therapies, UC disease distribution based on the Montreal classification, and pouch phenotype were recorded. Fecal calprotectin (measured by ELISA) was also assessed. Habitual dietary intake of participants was assessed using 7-day food diaries for energy, macronutrients, dietary fiber, fermentable, oligo-, mono-, disaccharides, and polyols (FODMAP), sulfur amino acids, and inorganic sulfur as outlined in the Supporting Information: Methods.

2.2 | Fecal VOC Analysis

Detailed methodology regarding fecal sample collection is described in the Supporting Information: Methods. Frozen fecal samples were sent to the University of Liverpool on dry ice and stored at −80°C. After defrosting samples at room temperature, an aliquot of 400–500 mg was stored in 10 mL headspace vials at −80°C until analysis of fecal VOCs. Samples were analyzed in two batches, the UC samples with intact colon in 2020 and the UC-pouch in 2022 by gas chromatography-mass spectrometry according to previously published methods [16]. Each peak generated represented an individual VOC, and these were matched using Automated Mass Spectral Deconvolution Identification Software (version 2.73, 2017) and the National Institute of Standards and Technology mass spectral library (version 2.3, 2017). For identification of VOCs, the criteria of a match factor of ≥ 800 and a probability of $\geq 60\%$ were utilized. All VOCs were reported using IUPAC names, but for readability purposes, common names derived from the Human Metabolome Database [17] were also reported. Data were aligned using the MATLAB package for R (version 1.28.0) using R Statistical Software (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria).

2.3 | Statistical Analyses

Statistical analyses of clinical and dietary data were performed using SPSS v27 statistical analysis software (IBM; New York, USA), whereas fecal VOCs were analyzed using MetaboAnalyst version 5.0 (www.metaboanalyst.ca.org) and GraphPad Prism version 8.4.3 (GraphPad Software, Massachusetts, USA). After assessing for normality, comparisons between UC groups were computed using unpaired *t*-test or Mann–Whitney test for non-parametric data. Descriptive data were presented as mean (95% confidence interval [CI]) or median [IQR] for non-parametric data. Due to potential batch effects, analysis was carried out on a qualitative basis, where present compounds were given a value of 1 and absent a value of 0. By analyzing the data in such a way, the risk of batch effects is greatly minimized in comparison to a quantitative approach. Differences in VOC profiles between patient cohorts were analyzed based on absolute values using principal component analysis (PCA) and Chi-square analysis. Intra-group similarity was computed by calculating the mean Jaccard index for each group. A p value ≤ 0.05 was used to denote statistically significant differences in VOC presence in the chi-square analysis. Due to the exploratory nature of the study, a false discovery rate was not applied to adjust for multiple comparisons. Pearson correlations between dietary variables, fecal calprotectin, and quantitative VOC abundances were also performed using the Corrplot package (version 0.92) in the R statistical software and significant correlations were determined by $p \leq 0.05$. Data used for the correlations were normalized by the median, log-transformed and scaled by mean centering, and missing values were replaced with 1/5th of the minimum positive value.

3 | Results

3.1 | Participants

A total of 40 UC patients were recruited. One patient with an ileoanal pouch withdrew due to the COVID-19 pandemic. Consequently, 11 patients with an ileoanal pouch and 28 patients with an intact colon completed the 7-day food diaries and fecal assessments. Table 1 shows the patient characteristics. The ileoanal pouch patients were older than the intact colon group ($p = 0.02$; Fisher's exact test), but both cohorts were otherwise well matched for sex and smoking status. Five of the UC-pouch patients (45%) had current pouchitis, whereas all the patients with an intact colon had active disease.

3.2 | Habitual Dietary Intake

Habitual dietary intakes are displayed in Table 2 for the two patient groups. Median intake of lactose ($p = 0.02$; Mann–Whitney test) as well as mean intake of methionine ($p = 0.03$; unpaired *t*-test) were 2.3- and 1.5-fold higher in patients with an ileoanal pouch, whereas patients with an intact colon consumed higher amounts of mannitol (by 2.5-fold; $p = 0.03$; Mann–Whitney test). No other differences, including for protein ($p = 0.58$), inorganic sulfur ($p = 0.35$), dietary fiber ($p = 0.99$), resistant starch ($p = 0.67$), or total FODMAPs ($p = 0.69$) were observed.

3.3 | VOCs

Similar numbers of VOCs were detected in individual fecal samples from the two cohorts (a full list is provided in Supporting Information). Median numbers of VOCs in the pouch were 58 (range: 45 to 80; coefficient of variation: 16.8%) compared with those with intact colons, 55 (36 to 71; 14.6%; $p = 0.33$, Mann–Whitney test; Figure 1A). When all VOCs detected were considered, a high degree of overlap was demonstrated between each cohort (Figure 1B), demonstrating consistency in the variety of compounds detected when comparing cohorts overall. The mean Jaccard similarity index shown in Figure 1C was significantly higher in the pouch group compared to in the intact colon group ($p < 0.0001$, unpaired *t*-test).

A comparison of the percentage occurrence of individual VOCs revealed 32 VOCs differed between the two groups ($p < 0.05$; Chi-square analysis) and these are outlined in Table 3. A complete list of individual VOCs is also provided in Table S1. While there is a minimal difference in the range of compounds detected at least once in either group, the differences are considerable when considering the frequency with which these compounds are detected. Specifically, classes of VOCs that occurred at a lower percentage in the pouch group were sulfide ($n = 1$), medium- or branched-chain fatty acids (BCFA) ($n = 3$), methyl ester ($n = 1$), and ketones ($n = 5$). Additionally, no other sulfide compounds were detected in pouch patients. In contrast, the percentage occurrence of the SCFA, butanoic (butyric) acid, secondary alcohol ($n = 1$), aldehydes ($n = 3$), and methyl esters ($n = 2$) was significantly greater in the pouch compared with those with an intact colon.

PCA of the two groups based on the presence or absence of individual VOC compounds showed that the two cohorts can be clearly distinguished from each another in terms of VOC profile (Figure 2A). Furthermore, patterns of VOC in patients with current pouchitis ($n = 5$) were not different from those without ($n = 6$) (Figure 2B).

3.4 | Correlations Between Dietary Intake, Fecal Calprotectin, and VOC Profiles

The relationships between fecal calprotectin and specific dietary components on the one hand, and individual VOC concentrations on the other, were examined by correlation analysis (Figure 3). In patients with a pouch (Figure 3A), positive correlations were found between fecal calprotectin on the one hand and hexanal, propyl- and methyl-butanoate on the other (all $p \leq 0.05$). With regards to dietary intake, positive correlations were observed between total protein and ethyl-2-propanoate ($p \leq 0.05$). Methionine intake was inversely correlated with levels of (E)-hex-2-enal. Lactose intake was strongly and positively associated with 2-methylbutanoic acid ($p \leq 0.05$) and 3-methylbutanoic acid ($p \leq 0.05$).

In patients with an intact colon (Figure 3B), fecal calprotectin was positively correlated ($p \leq 0.05$) with hexanal and with (E)-hex-2-enal and negatively correlated ($p \leq 0.05$) with pentanoic acid. Total protein and sulfur protein intake were positively correlated ($p \leq 0.05$) with 3,4-hexanedione and hexanal, and total

TABLE 1 | Baseline characteristics of adults with ulcerative colitis with an ileoanal pouch ($n = 11$) and those with an intact colon ($n = 28$).

	Characteristics	Ileoanal pouch ($n = 11$)	Intact colon ($n = 28$)	p value
Demographics	Age, median [IQR], years	56 [40–61]	42 [32–54]	0.02[a]
	Female, *n* (%)	4 (36)	15 (54)	0.48[b]
	Caucasian, *n* (%)	11 (100)	19 (68)	0.04[b]
	Active smoker, *n* (%)	1 (9)	0 (0)	0.99[b]
Disease duration, median [IQR], years	Ulcerative colitis	—	8 [3–13]	N/A
	Age of pouch	10 [7–20]	—	N/A
Disease distribution, *n* (%)	Proctitis (E1)	—	3 (11)	N/A
	Left sided (E2)	—	14 (50)	
	Extensive (E3)	—	11 (39)	
Pouch phenotype[c], *n* (%)	Healthy pouch with no history of pouchitis	2 (18)	—	N/A
	No current pouchitis	4 (37)	—	
	Current pouchitis	5 (45)	—	
Disease activity, median [IQR]	Total Mayo score	—	7 [6–8]	N/A
	Total Pouch Disease Activity Index	3 [2–3]	—	
Inflammatory biomarker, median [IQR]	Fecal calprotectin, µg/g	292 [176–527]	392 [187–897]	0.74[a]
Current drug therapies, *n* (%)[d]	None	3 (27)	4 (14)	0.11[b]
	Antibiotics	1 (9)	0 (0)	
	Corticosteroids	1 (9)	3 (11)	
	5-aminosalicylic acid	0 (0)	20 (71)[e]	
	Immunomodulators	3 (27)	4 (14)	
	Biologics/ advanced therapies	2 (18)	6 (21)	

[a]Mann–Whitney test.
[b]Fisher's exact test.
[c]All patients had J-pouches.
[d]One patient with UC and ileoanal pouch had probiotics as sole therapy.
[e]Oral 5-aminosalciylic acid only in 11, oral and topical in 9.

sulfur protein intake positively correlated with (E)-hex-2-enal. In the intact colon group, lactose did not show a statistically significant correlation with either 3-methylbutanoic acid or 2-methyl butanoic acid, as was observed in the pouch group, but was positively correlated ($p \leq 0.05$) with pentane-2,3-dione, 3,4-hexanedione, hexanal, and (E)-hex-2-enal.

4 | Discussion

Current understanding of the metabolic capacity of the colonic and pouch microbial communities in UC is limited, with most studies assessing microbiota composition rather than function. In both cohorts, a reduction in microbial diversity has been reported [18, 19]. In UC patients with an intact colon, a reduction in microbes known to produce butyrate has been observed [18, 20]. However, the adaptive changes to the microbial structure and function following proctocolectomy and ileal pouch anastomosis remain unclear. The literature is divided on whether the pouch microenvironment does fully adapt to become a "colonic" environment [19, 21, 22]. This gap in knowledge sets the premise for this study, to gain insights into the functional activities of the gut microbiota in UC patients with an intact colon compared with those with an ileoanal pouch in order to inform future designs of therapeutic interventions or the development of pathogenic biomarkers.

TABLE 2 | Habitual daily dietary intake of adults with ulcerative colitis with an ileoanal pouch ($n = 11$) compared to those with an intact colon ($n = 28$).

Daily intake		Ulcerative colitis ileoanal pouch ($n = 11$)	Ulcerative colitis intact colon ($n = 28$)	p value
Energy	kJ	8397.3 (7579.3, 9215.2)	8854.2 (7757.6, 9952.9)	0.62
	kcal	1999.4 (1804.6, 2194.1)	2108.2 (1846.8, 2369.5)	0.66
Protein, g	Total	96.7 (82.1, 115.2)	90.8 (78.7, 102.9)	0.58
	Sulfur amino acids	2.2 (0.4, 0.7)	1.7 (1.4, 2.0)	0.05
	Methionine	1.6 (1.1, 2.0)	1.1 (0.9, 1.3)	0.03*
	Cysteine	0.7 (0.5, 0.9)	0.5 (0.4, 0.6)	0.15
Inorganic sulfur, mg		157.5(114.3, 200.7)	133.7 (105.4, 161.9)	0.35
Fat, g	Total	93.0 (77.8, 108.9)	95.0 (82.0, 107.0)	0.88
Carbohydrates, g	Total	176.2 (152.3, 200.0)	202 (174, 229)	0.26
Total fibre, g	Resistant starch	2.5 (2.1, 3.0)	2.8 (2.0, 3.7)	0.67
	Dietary fibre	20.2 (16.7, 23.6)	21.5 (18.2, 24.8)	0.99
FODMAPs, g[a]	Total[b]	5.6 [4.8–6.8]	6.0 [3.9–8.5]	0.69
	Oligosaccharides	3.8 [2.7–4.3]	3.6 [2.7–5.0]	0.71
	• Fructans	3.2 [2.1–3.5]	2.6[1.9–3.8]	0.85
	• GOS	0.6 [0.4–0.9]	0.9 [0.6–1.3]	0.15
	Lactose	17.3 [9.4–36.6]	7.4 [1.4–14.3]	0.02*
	Excess fructose	1.1 [0.6–2.0]	0.9 [0.5–1.5]	0.76
	Polyols	0.7 [0.4–1.4]	1.1 [0.7–1.6]	0.11
	• Sorbitol	0.5 [0.3–0.7]	0.5 [0.2–0.8]	0.62
	• Mannitol	0.2 [0–0.6]	0.5 [0.2–0.8]	0.03*

Note: Data are presented as mean (95% confidence intervals) and compared with unpaired t-test unless stated.

[a]Median [IQR] and data analyzed using Mann–Whitney test.

[b]Total FODMAPs is calculated as a sum of oligosaccharides, excess fructose, and polyols.

*Denotes significant p values ($p \leq 0.05$).

Several findings were prominent. First, a distinct separation of VOCs was observed between the patient groups. Second, specific metabolite differences were confined to a reduction in metabolites associated with protein fermentation as well as ketones in patients with an UC pouch. Third, the discordant VOC patterns occurred despite similarities in demographic characteristics and minimal differences in dietary intake of these two cohorts, highlighting that metabolic processes are altered likely in response to the removal of a colon. Furthermore, the luminal microenvironment is different in a pouch compared with that in an intact colon, and, therefore, microbial-targeted interventions to modify those environments, whether dietary or other therapies, would require tailoring to the anatomy. These findings also provide key insights into potential pathogenic mechanisms, such as the microbial alterations underpinning the pathogenesis of UC, and a role of potentially toxic metabolites of protein fermentation being injurious in the development of pouchitis.

Our observations of distinct VOC profiles highlighting the differences in the luminal microenvironment in these two cohorts agree with earlier findings, in which distinct microbial structure and functional pathways were enriched in patients with UC compared with this in UC pouchitis [23]. In our study, there was considerable overlap of VOCs produced in both a UC colon and pouch, indicating that a large degree of functional activities are retained. However, there was a much lower intra-group diversity in VOC profiles detected in the pouch. While the diversity index values do not suggest a particularly strong level of similarity or dissimilarity in themselves, the mean similarity index was nonetheless statistically significantly higher in the pouch group. This suggests that the range of functional activities occurring in the pouch were more alike compared with those in an intact colon. The reduced diversity of metabolic activities of the pouch compared with other subtypes of inflammatory bowel disease has previously been reported [6]. Analysis of the microbiota composition in the current study, although not performed, may generate insights into the microbiota communities that may contribute to these functional differences.

This is the first study that has compared protein fermentative activities between patients with an intact colon and those with a pouch. Of importance is the finding that the frequencies of BCFAs, isovaleric acid, and ethylmethylacetic acid, were lower by 30% to nearly half in the pouch patients compared to those with an intact colon. Two main factors influence the rate of protein fermentation: substrate availability and the vigor of carbohydrate

TABLE 3 | VOC present in patients and ulcerative colitis who had an intact colon or an ileoanal pouch.

Compound, IUPAC name	Common name	Frequency of occurrence (%)			*p* value[1]
		Ulcerative colitis Ileoanal pouch ($n = 11$)	Ulcerative colitis intact colon ($n = 28$)	Difference	
Metabolites detected at lower frequency in ileoanal pouch					
Acids					
• 3-Methylbutanoic acid	Isovaleric acid	63.6	96.4	32.7	< 0.01
• 2-Methylbutanoic acid	Ethylmethylacetic acid	45.5	92.8	47.4	< 0.001
• Pentanoic acid	Valeric acid	9.1	89.2	80.2	< 0.001
Ketones					
• Pentane-2,3-dione	2,3-Pentanedione	36.4	82.1	45.8	< 0.01
• 5-Methylhexan-2-one	5-Methyl-2-hexanone	18.1	96.4	78.3	< 0.001
• Butan-2-one	Butanone	9.1	64.2	55.2	< 0.001
• Heptan-4-one	4-Heptanone	9.1	53.5	44.5	< 0.05
• Hexan-2-one	2-Oxohexane	0.0	50.0	50.0	< 0.01
Methyl ester					
• 2-Methylpropyl butanoate	2-Methylpropyl butanoate	0.0	57.1	57.1	< 0.01
Sulfides					
• (Methyldisulfanyl)methane	Dimethyldisulfide	9.1	57.1	48.1	< 0.01
Metabolites detected at lower frequency in UC patients with an intact colon					
Acids					
• Butanoic acid	Buytric acid	90.9	28.6	−62.3	< 0.001
Alcohol					
• Butan-2-ol	2-Butanol	45.5	0.0	−45.5	< 0.001
Aldehydes					
• Hexanal	Hexanal	100.0	60.7	−39.3	< 0.05
• Furan-2-carbaldehyde	2-Furancarboxaldehyde	45.5	14.3	−31.2	< 0.05
• (E)-hex-2-enal	2-Hexenal	72.7	7.1	−65.6	< 0.001
Ketones					
• 3-Methylbut-3-en-2-one	3-Methyl-3-buten-2-one	90.9	46.4	−44.5	< 0.05
• 3,4-Hexanedione	3,4-Hexanedione	45.5	7.1	−38.3	< 0.01
• Heptan-2-one	4-Heptanone	100.0	3.6	−96.4	< 0.001
Methyl esters					
• Methyl butanoate	Methyl butyrate	90.9	42.9	−48.1	< 0.01
• Methyl 3-methylbutanoate	Methyl 3-methylbutanoate	18.2	0.0	−18.2	< 0.05
• Methyl propanoate	Methyl propionate	81.8	0.0	−81.8	< 0.001
Other esters					
• Ethyl acetate	Ethyl acetate	90.9	21.4	−69.5	< 0.001
• Ethyl propanoate	Ethyl propionate	100.0	21.4	−78.6	< 0.001
• Propyl acetate	Propyl acetate	100.0	35.7	−64.3	< 0.001
• Ethyl 2-methylpropanoate	Ethyl 2-methylpropanoate	36.4	0.0	−36.4	< 0.001
• Propyl propanoate	Propyl propionate	90.9	39.2	−51.6	< 0.001

(Continues)

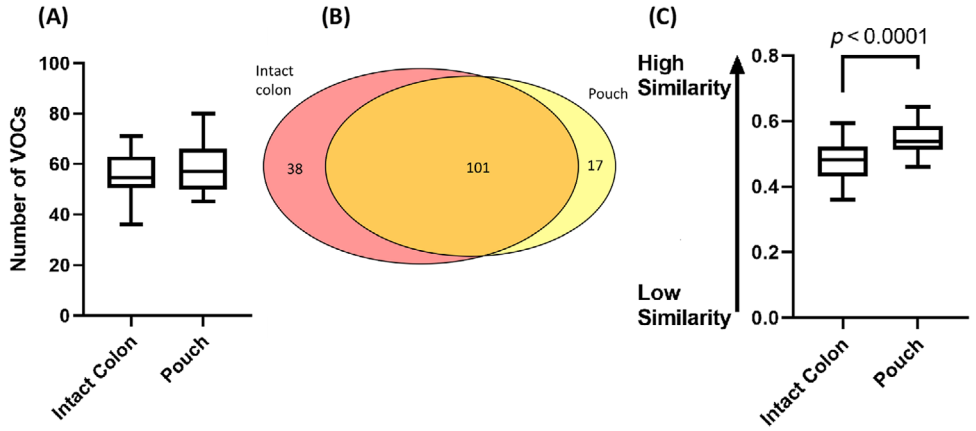

FIGURE 1 | (A) Box and whisker plot displaying the median number of VOCs detected in each of the two patient cohorts. (B) Venn diagram showing the number of VOCs detected in ulcerative colitis with an intact colon and those with an ileoanal pouch. (C) Graph showing the Jaccard similarity index as a measure of dissimilarity of volatile organic compounds. Bars and error bars represent mean (95% confidence intervals).

TABLE 3 | (Continued)

Compound, IUPAC name	Common name	Frequency of occurrence (%)			*p* value[1]
		Ulcerative colitis Ileoanal pouch (*n* = 11)	Ulcerative colitis intact colon (*n* = 28)	Difference	
• Ethyl 2-methylbutanoate	Ethyl 2-methylbutyrate	36.4	0.0	−36.4	< 0.001
• Butyl acetate	n-Butyl acetate	45.5	7.1	−38.3	< 0.01
• Propan-2-yl butanoate	Isopropyl butyrate	27.27	3.57	−23.7	< 0.05
• Propyl butanoate	Propyl butyrate	90.91	28.57	−62.3	< 0.001
• Propyl 3-methylbutanoate	Propyl 3-methylbutanoate	54.55	3.57	−51.0	< 0.001
Other					
• 2-Pyrrolidin-2-ylpyrrolidine	2,2'-Bipyrrolidine	36.36	3.57	−32.8	< 0.01

Note: Volatile organic compounds (VOC) are named according to the IUPAC system but to improve readability, common names obtained from the Human Metabolome Database16 are also listed. Values are shown as proportion, *n* (%) and analyzed using Chi-square analysis.
*Only VOC which differed significantly following chi-square analysis ($p < 0.05$) are shown here but a full list of VOC are attached to the Supporting Information.

fermentation. First, the amount of substrate (proteins) present will vary according to the amount of the undigested dietary protein that is delivered to the colon, this increasing with increased overall protein intake and/or reduced digestibility of the protein, and the endogenous source of protein that will increase in the presence of exudation from an inflamed colon [24]. In the current study, differences in protein intake were minimal between patient cohorts, suggesting that dietary intake alone could not account for such differences. Additionally, despite the 1.6-fold higher mean intake of methionine and a trend for higher total sulfur-amino acid intake in the UC pouch compared to those with an intact UC colon, dimethyl disulfide, a volatile sulfur compound produced from fermentation of sulfur-containing protein [24], was only detectable in 1/11 of these pouch patients. No other sulfur compounds were detected. These findings are surprising given volatile sulfur compounds (dimethyl disulfide, dimethyl trisulfide) were reported to be detected in more than 3 in 4 of patients with pouchitis in another study [6] and proposed the pathogenic role of hydrogen sulfide, a major by-product of protein fermentation in pouchitis. Our findings, while in a small sample of ileoanal pouch patients, suggest that protein fermentation may be a minor metabolic activity in the majority of patients with an ileoanal pouch.

The second factor that influences the rate of protein fermentation is that of carbohydrate fermentation, since the latter is the preferred energy source for bacteria in the anaerobic environment of the colon. Increasing the rate of carbohydrate fermentation by increasing fermentable carbohydrate supply inhibits protein fermentation, as demonstrated in fecal slurries ex vivo [24]. The metabolic capabilities of the microbiota also play a role. Carbohydrate fermentation is deficient in the distal colon of patients with UC. For example, fecal concentrations of SCFA were low in patients with quiescent UC, even when fermentable carbohydrate delivery to the distal colon was increased through diet.[5] In the current study of patients with an intact colon and active UC,

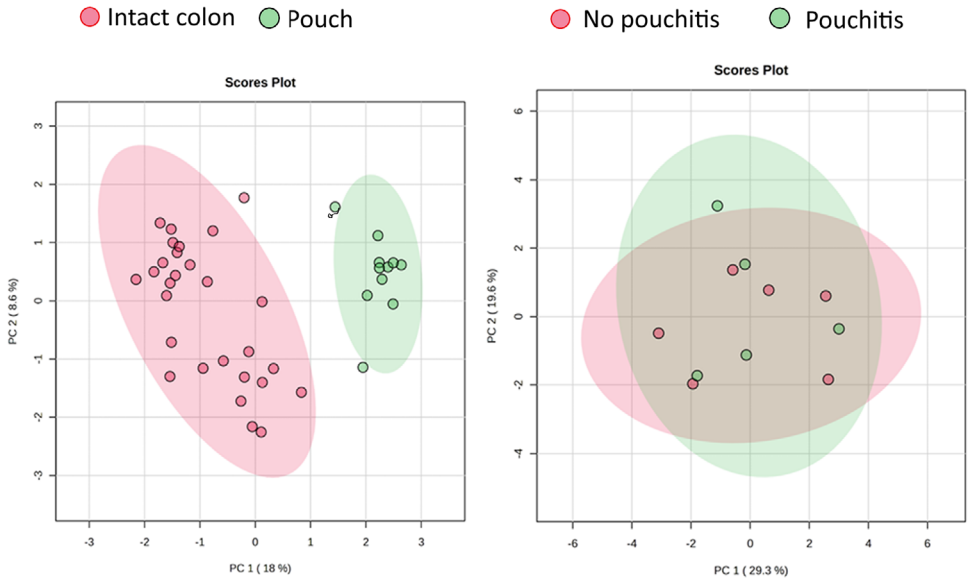

FIGURE 2 | Principal component analysis of the presence or absence of volatile organic compounds in the two groups—shaded area shows 95% CI. Absolute values were used for the analysis, such that a present compound was represented by a one and an absent compound was represented by a nought. (A) Principal component analysis of patients with either an intact colon ($n = 28$) or an ileoanal pouch ($n = 11$). (B) Principal component analysis of patients with an ileoanal pouch, separated by current pouchitis status defined by total Pouch Disease Activity Index (PDAI) > 7 ($n = 5$ with current pouchitis, $n = 6$ without pouchitis).

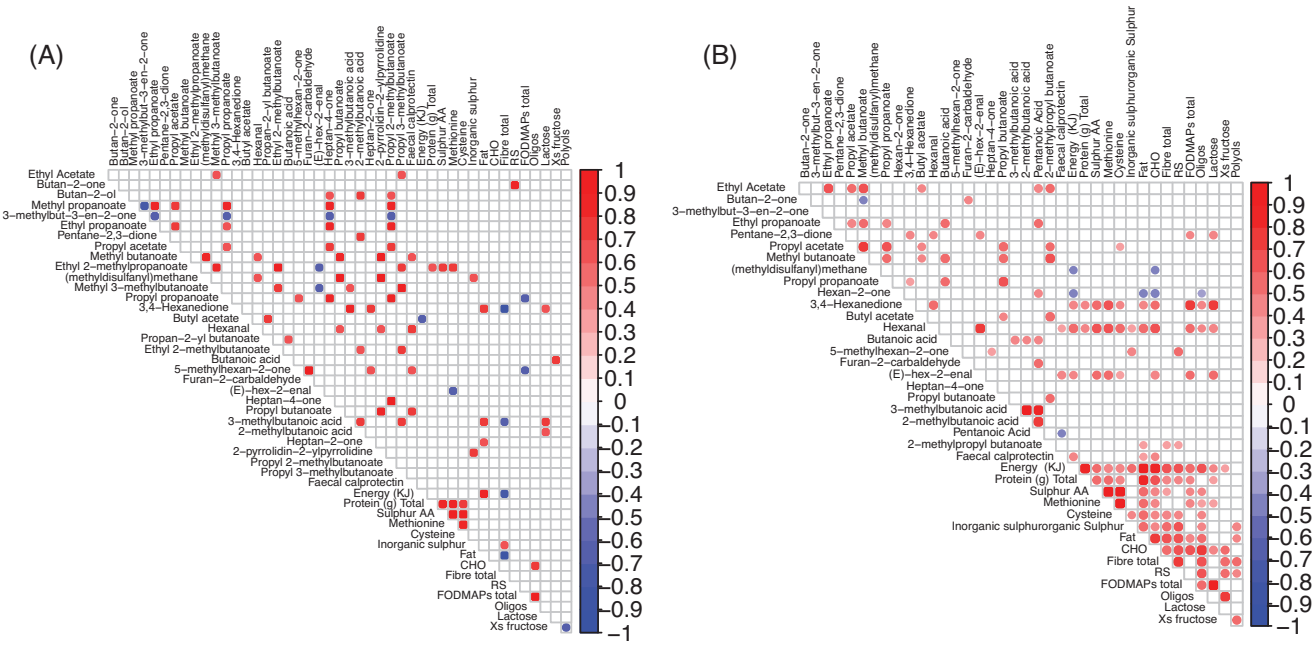

FIGURE 3 | Correlation matrix of dietary intake, fecal calprotectin and individual volatile organic compounds for ulcerative colitis patients with (A) an ileoanal pouch ($n = 11$) and (B) with an intact colon ($n = 28$). Positive correlations are shown in blue and negative correlations in red. The size of the circle correlates with the strength of the p value ($p \leq 0.05$). Only significant correlations (analyzed using Pearson's correlations) are shown. sulfur AA, sulfur amino acids; CHO, carbohydrates; kJ, kilojoules; Oligos, oligosaccharides; RS, resistant starch; Xs fructose, excess fructose.

it was not surprising that butyrate was detected in a minority, considering the background diet contained low amounts of total and fermentable fiber and the likelihood that fermentable substrate was likely to be exhausted in the proximal colonic regions. This is in addition to the reduced microbial fermentative activities in a proportion of individuals with UC. In contrast, there was a high occurrence of butyrate in UC pouch patients. Despite them having similarly low intakes of fermentable fiber, substrate delivery to the pouch from dietary and endogenous sources is likely to be substantial. Furthermore, the microbial community in the pouch is likely to be rich in carbohydrate-fermenting microbes, akin to the microenvironment of the proximal colon,

and this may represent an adaptive process following colectomy. The cross-sectional design of this study, however, limited further assessment of microbial changes occurring in the pouch. All in all, these findings suggest that carbohydrate fermentation may be a potential therapeutic target in UC, but not in pouchitis. Examination of the microbial communities and their metabolic capabilities is warranted to be able to fully elucidate relevance to disease pathogenesis.

The other discriminatory VOCs between the cohorts are secondary metabolites. Specifically, a low occurrence of ketones has been demonstrated in patients with a pouch. Little is known regarding the role of ketones that are downregulated in the gut including butanone, 2-3 pentadione, 2-oxohexane, 4-heptanone, and ethyl isobutyl ketone, except that they are found in a range of foods, and their reduced presence in luminal contents may act as a biomarker for lower intake of these foods. 2-oxohexane, which was detected in 50% of patients with UC and an intact colon and not in pouch effluent, has been described as potentially toxic and associated with active UC [17].

That the metabolic environments are very different in the pouch and intact colon in patients with UC is not surprising. The duration of exposure of contents from the small bowel to the microbial-rich environment of the colon is at least tenfold longer than the few hours spent in the pouch [25]. There are inherent differences between colonic and ileal epithelium, including how metabolites produced by the gut microbiota are handled [26]. For example, the uptake of SCFA by the colonic epithelium is highly efficient compared to the ileal epithelium, where its absorption can reach saturation [27]. Microbial communities in the pouch also have limited functional diversity compared with those in the colon [28]. These aspects have implications for the design of microbial-targeted interventions such as diet. For example, increasing carbohydrate fermentation in patients with an intact colon may be reasonable to address its possible pathogenic role, but a similar attack in patients with pouchitis may lead to excessive luminal SCFA levels, with potential toxic effects to the pouch mucosa and adverse effects on pouch function [29].

A major strength of our observations is that current (habitual) dietary intake was carefully measured with prospective food intake and that this indeed was very similar between the two groups. Hence, we can confidently say that none of the differences in VOC characteristics have dietary explanations and are more likely related to physiological or microbial differences. Our study had several limitations. First, quantitative analysis of metabolites was not performed in this study as an attempt to minimize potential batch differences in VOC assessments between the two patient cohorts analyzed at different timepoints. Second, the number of patients, particularly with pouches, was small, and disease activity was heterogeneous amongst the intact colon and pouch cohorts. The patterns of VOCs in the PCA analysis of the six pouch patients without inflammation (according to standard histopathological criteria) were not obviously different from the five with pouchitis; this aspect requires specific investigation. Third, a healthy control cohort to provide a reference for VOC profiles was not studied, limiting the interpretation of the data generated from the intact colon. Fourth, the large increase in fecal esters may be artefactual as storage conditions of fecal samples was inconsistent between the two cohorts. Increased formation of fecal esters is specific to samples being stored at −20°C before processing [30]. However, these storage conditions did not affect SCFA, BCFA, and sulfide concentrations. Fifth, this is a cross-sectional study examining presence of gaseous metabolites within the microenvironment, and the structure of the microbial community was not examined. Therefore, inferences cannot be made between presence or absence of microbes and differences in metabolites produced through fermentation in the two cohorts. Finally, age, ethnicity, and the use of medications, including 5-ASA that may exert luminal effects were different in the intact colon and pouch groups. These aspects deserve further investigation in a large cohort where participants are better matched for these characteristics.

In conclusion, this exploratory study has demonstrated differences in the metabolic milieu and, hence, microbial function in patients with UC with an intact colon and with an ileoanal pouch, independently of dietary intake. The differences were characterized by lower diversity of VOCs across individuals with a pouch, lower predominance of protein fermentative capacity, and altered production of secondary metabolites. Moreover, altered carbohydrate fermentation pathways are a key feature of functional alterations of the microbiota in patients with an intact colon. These findings have pertinent ramifications, particularly with the design of therapeutic interventions, including dietary manipulation targeting the gut microbial function in UC. Future, larger studies, including an analysis of VOCs from a reference cohort of healthy individuals are much needed to confirm these preliminary findings.

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Conflicts of Interest

RVB has received grant/research support/speaker fees from AbbVie, Ferring, Janssen, Shire, Takeda, GSK, and Emerge Health; and is a shareholder in Biomebank. PRG has served as consultant or advisory board member for Anatara, Atmo Biosciences, Topas, and Comvita. He has received research grants for investigator-driven studies from Atmo Biosciences and Mindset Health and speaker honoraria from Dr Falk Pharma and Mindset Health. He holds shares in Atmo Biosciences. CKY has received research grants for investigator-driven studies from Atmo Biosciences and honoraria from Viatris, Yakult Australia. Alice S. Day has received research support for investigator-led studies from ECCO, The Hospital Research Foundation and GUTSY Group. Alice S. Day has also received consulting fees or honoraria from BiomeBank, AbbVie, Ferring, and Janssen. KG, ZMA, and CP have no disclosures to declare in relation to this work. The Department of Gastroenterology, which CKY, PRG, ZMA are from, financially benefits from the sales of a digital application, booklets, and online courses on the FODMAP diet.

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.