Inflammatory Bowel Diseases, 2025, **00**, 1–12
https://doi.org/10.1093/ibd/izaf177
Original Research Article - Basic Science

Functional Profiling Demonstrates That a Sulfide-Reducing Diet Achieves Microenvironmental Targets in Ulcerative Colitis

Alice S. Day, PhD[1,2,*], Rachael Slater, PhD[3], Remy B. Young, PhD[4,5], Reuben Z. Wheeler, BSc (hons)[6], Vanessa R. Marcelino, PhD[7], Natasha K. Maddigan, PhD[2], Samuel C. Forster, PhD[4,5], Samuel P. Costello, PhD[1,2], Wendy Uylaki, BSc (hons)[1,2], Chris S.J. Probert, MBChB MD[3], Jane M. Andrews, PhD[8,9], Chu K. Yao, PhD[10], Peter R. Gibson, MB BS (Hons) MD[10], Robert V. Bryant, PhD[1,2]

[1]Inflammatory Bowel Disease Service, Department of Gastroenterology and Hepatology, Queen Elizabeth Hospital, Adelaide, SA, Australia
[2]Basil Hetzel Research Institute, School of Medicine, Faculty of Health Sciences, University of Adelaide and University of South Australia, Adelaide, SA, Australia
[3]Department of Molecular and Clinical Cancer, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom
[4]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia
[5]Department of Molecular and Translational Sciences, Monash University, Clayton, VIC, Australia
[6]College of Science and Engineering, Flinders University, Bedford Park, SA 5042, Australia
[7]Melbourne Integrative Genomics, Department of Microbiology and Immunology, Peter Doherty Institute, School of BioSciences, University of Melbourne, Melbourne, VIC 3010, Australia
[8]School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, SA, Australia
[9]Inflammatory Bowel Disease Service, Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, SA, Australia
[10]Department of Gastroenterology, School of Translational Research, Monash University and Alfred Health, Melbourne, VIC, Australia

*Corresponding author: Alice Day, Basil Hetzel Institute, 37 Woodville Road, Woodville South 5011, South Australia, Australia (alice.day@adelaide.edu.au).

Abstract

Background: As a dietary approach to reducing inflammation in ulcerative colitis, the 4-SURE (4 Strategies to Sulfide Reduction) diet was designed to correct pathogenic alterations of excessive protein fermentation and hydrogen sulfide (H_2S) production in the distal colon. We aimed to perform a deep functional analysis (microbial and metabolomic) of the feces of 28 adults with mild-moderately active ulcerative colitis who adhered to the 4-SURE diet over 8 weeks to explore whether the 4-SURE diet could modulate the intraluminal environment as intended.

Methods: Fecal samples were collected at week 0 and 8 of dietary intervention, processed and aliquoted. Metagenomic sequencing was undertaken to identify changes in H_2S-metabolizing genes, while gas chromatography–mass spectrometry was used to analyze fecal volatile organic compounds and H_2S production.

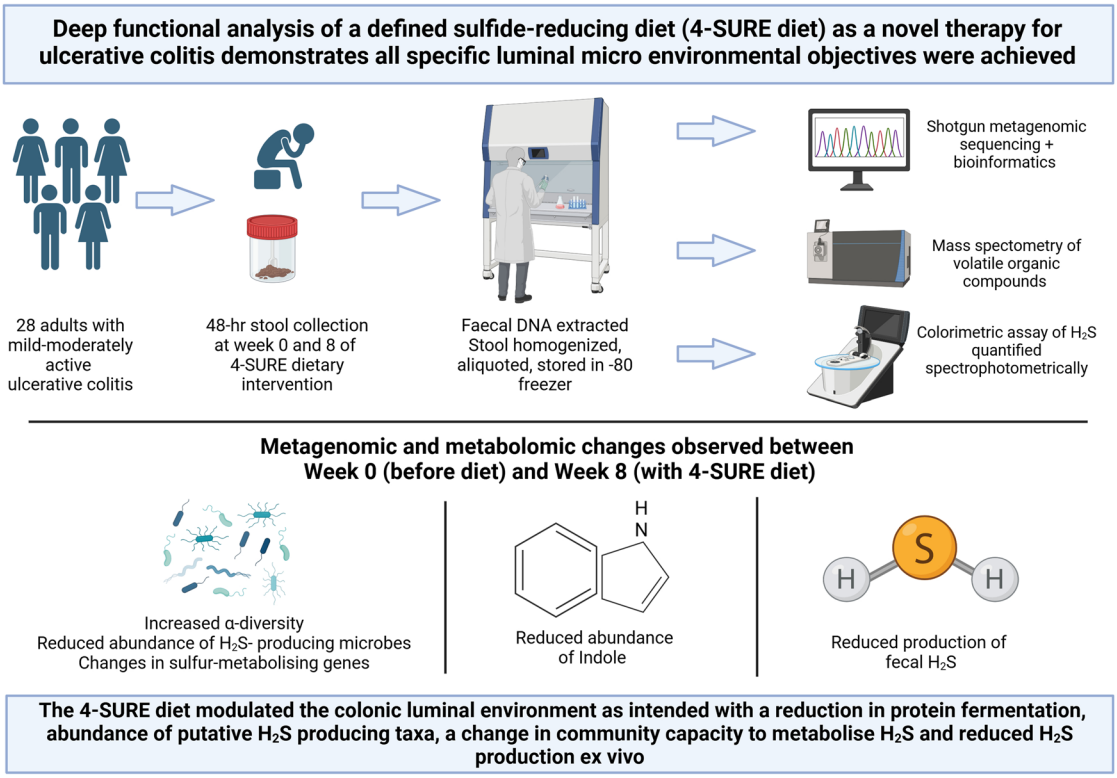
Results: The 4-SURE diet significantly increased alpha diversity between weeks 0 and 8. By random forest plot classifier, the abundance of taxonomic groups comprising known H_2S-producing genera were markedly lower at week 8, specifically *Odoribacter* and Peptostreptococcaceae, and were of highest importance in discriminating between before- and after-diet samples. The capacity for bacterial H_2S metabolism was altered with diet, with differences in 12 of 67 analyzed sulfur-metabolizing genes identified. H_2S production and indole, a specific marker of protein fermentation, were significantly decreased due to the diet.

Conclusions: Here, we demonstrate that the objectives of the 4-SURE diet were fulfilled. This application of deep functional analysis to a dietary intervention study is novel and highlights an exemplar framework for including microbial and metabolomic biomarkers of pathogenic relevance in the analysis of therapeutic diet strategies. (Australian New Zealand Clinical Trials Registry, Number: ACTRN12619000063112).

Received for publication: May 11, 2025. Editorial Decision: July 17, 2025.
© The Author(s) 2025. Published by Oxford University Press on behalf of Crohn's & Colitis Foundation.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

Graphical abstract



Key words: ulcerative colitis, fermentation, microbiota, hydrogen sulfide

Lay Summary

The 4-SURE diet reduced protein fermentation and microbial hydrogen sulfide production in adults with ulcerative colitis, demonstrating its intended intraluminal effects. This study proposes a successful framework for evaluating therapeutic diets using integrated microbial and metabolomic analysis.

Key Messages

What is already known?

The 4-SURE (4 Strategies to Sulfide Reduction) diet induced clinical and endoscopic response in an open-label trial in active ulcerative colitis (UC). The proposed mechanism was modulation of microbial fermentation and hydrogen sulfide (H_2S) production, but these effects have not been fully characterized.

What is new here?

This study provides the first functional evidence that the 4-SURE diet alters H_2S-producing taxa and microbial sulfur metabolism and lowers protein fermentation and ex vivo fecal H_2S, confirming diet-induced modulation of key pathogenic pathways of interest.

How can this study help patient care?

Diet is a viable adjunct therapy for UC. These findings support biomarker-driven frameworks for evaluating mechanistic efficacy of therapeutic diets in future UC trials.

Introduction

Ulcerative colitis (UC) is a chronic relapsing disease characterized by continuous mucosal inflammation of the large bowel that always involves the rectum, extending proximally. The pathogenesis of UC is largely unknown, although most hypotheses have focused on aberrations of the colonic epithelium. The influence of luminal factors are often overlooked, particularly the impact of microbial dysbiosis, fermentation, and noxious metabolite production. An evolving pathogenic hypothesis relates to colonic concentration-dependent hydrogen sulfide (H_2S) production and ensuing epithelial injury, associated with distal microbial-driven proteolytic fermentation.[1,2]

Epidemiology shows Westernization is associated with an increased incidence and prevalence of UC and diet is a key environmental risk factor.[3] Westernized habitual UC dietary patterns favor high animal protein and lower carbohydrate intakes.[4] The compositional and functional taxonomic differences observed in the fecal microbiome of those with active UC compared with healthy controls might reflect these dietary patterns. Reduced alpha diversity, a higher abundance of proteolytic and sulfate-reducing bacteria with capacity to produce H_2S, and a lower abundance of saccharolytic-degrading species and butyrate production are observed.[5–8]Preclinical and in vitro models demonstrate colonic H_2S production increases with availability of protein for fermentation, while inversely, carbohydrate is preferentially fermented relative to protein and attenuates H_2S production.[9,10] In humans, proximal anaerobic microbial communities favor carbohydrate fermentation when substrate is available, whereas protein fermentation increases

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

distally, dissimilating proteolytic metabolites, including H_2S, other gases, and volatile organic compounds (VOCs), into the luminal environment.[11–14] Although associations have been made between higher intakes of animal protein and increased risk of developing UC, and reduced time to relapse, a causal relationship between microbial-generated H_2S and UC disease course remains uncertain.[15,16] Plausibly, this is for 3 reasons. First, H_2S is highly volatile and challenging to accurately and reproducibly measure in vivo or indirectly in freshly passed feces in the absence of intraluminal gas-sensing technology.[9,10,17] Second, diet therapy trials are notoriously complex to design, control, and blind.[18] Third, UC diet trials lack microbial and metabolomic biomarkers and exploration of how diet may mechanistically exert an intraluminal effect.[19–21]

A novel diet, designated the 4-SURE (4 Strategies to Sulfide Reduction) diet, was designed to modulate proteolytic and saccharolytic fermentation, H_2S production and short-chain fatty acid (SCFA) delivery in the colon.[9] In a proof-of-concept study in patients with active UC, dietician-led teaching of the diet achieved the dietary aims, was well tolerated and improved disease activity.[22] Furthermore, the effects on short and branched-chain fatty acids in the feces suggested enhanced saccharolytic relative to protein fermentation was achieved. The aim of the present study was to further explore the mechanistic action of the 4-SURE diet from the pilot study by a deeper assessment of the theoretical framework of novel microbial and metabolomic endpoints. Specifically, the objectives of this study were to (1) describe and compare microbial composition and function, (2) measure production of colonic microbiota–derived VOCs before and during application of the 4-SURE diet, (3) measure change in fecal H_2S production ex vivo, and (4) compare the microbial and metabolomic differences between patients whose mucosal inflammation responded to the diet as against those whose did not.

Methods

Study design

This was a prospective, observational before-and-after study examining compositional and functional microbial changes in adults with active UC who followed the 4-SURE diet for 8 weeks. Each participant was their own control. Comprehensive details of this study and protocol are described elsewhere (Figure S1).[22]

Briefly, adults with mild-to-moderately active UC on stable therapy were recruited.[22] Eligible participants completed a 14-day run-in period to collect prospective endoscopic, clinical and dietary data, including a 48-hour stool collection while consuming usual habitual diet. Participants then followed 4-SURE dietary advice for 8 weeks. Further stool samples were collected at weeks 4 and 8. The primary outcome measure was dietary tolerability. Adherence was assessed using 7-day weighed food diaries and predefined self-reported compliance categories.[22] Responders were defined as those with an overall improvement in disease activity and total Mayo score ≤3 including a reduction in Mayo endoscopic score ≥1.

The study was approved by Central Adelaide Local Health Network Human Research Ethics Committee (HREC/16/RAH/24, R20160202). The study protocol was registered with Australian New Zealand Clinical Trials Registry (ACTRN12619000063112).

4-SURE diet

The 4 central strategies of the 4-SURE intervention diet were to modify the habitual diet to achieve (1) prescribed targets of resistant starch and slowly fermentable nonstarch polysaccharide, (2) reduction of total protein intake to a prescribed target range, (3) restriction of sulfur-containing amino acids, and (4) avoidance of specific food additives.[22]

Fecal collection and processing

Patients completed 48-hour stool collections with specific advice to avoid urine contamination. Freshly passed stools were placed and sealed in sterile fecal collection bags, which were immediately stored at −21 °C in order to inhibit further metabolic activity of the microbiota and minimize loss of VOCs.[23] The samples remained frozen for up to 5 days before being transferred for storage at −80 °C.[22]

Frozen stool samples were processed and aliquoted in 2 ways. For metagenomic and H_2S analysis, the first stool collected at each time point was aliquoted in a semi-thawed state and immediately re-frozen and stored at −80 °C to minimize anaerobic loss of bacteria and to fix the community state. For VOCs and other analysis of metabolites, all remaining stool from the 48-hour collection was defrosted and homogenized in a sterile beaker under a fume hood. Multiple aliquots were then taken and refrozen at −80 °C for storage.

Fecal metagenome sequencing

Fecal DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals) according to the manufacturer's instructions, using the recommended FastPrep96 instrument (MP Biomedicals) for the homogenization step. DNA quality and quantity were measured on a NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific), and DNA integrity was assessed by 1% agarose gel electrophoresis. DNA was shipped to BGI Tech Solutions for paired-end metagenomic sequencing as per the BGI protocol.

Quality control was performed with Trimmomatic v.0.38[24] to remove any remaining low-quality sequence reads. Human sequences were removed by mapping quality-controlled reads against the human CRCh38 reference genome assembly with bowtie2 v2.3.5.[25] Taxonomic profiling was performed with CCMetagen v.1.4,[26] which indicated that the bowtie2 step did not remove all human sequences. Therefore, additional filtering was performed to remove all eukaryotic sequences from taxonomic profiling results apart from known gut-associated microeukaryotes (fungi, Platyhelminthes, Apicomplexa, Bacillariophyta, Apicomplexa, Evosea, Fornicata, Parabasalia, and Blastocystidae). Finally, samples with <500 000 microbial reads for either baseline or endpoint were removed, resulting in a final dataset of 38 high-quality samples from 19 individuals.

Alpha diversity was assessed with the vegan R package[27] and statistically tested using a Wilcoxon signed rank test. Beta diversity was assessed via principal component analysis (pca) after centred log-ratio (clr) transformation with mixOmics.[28] To understand what drives differences in the microbiome composition before and after 4-SURE diet, a random forest classifier was used, splitting the data into training (70%) and test (30%) sets, and using a 5-fold cross-validation.

To investigate potential shifts in the abundance of genes involved in sulfur cycling in the microbiome, a database of 67

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

genes was generated (Table S1).[29–32] Reference protein sequences were downloaded from UniProt,[33] and a custom database was built with DIAMOND v2.1.9.[34] Forward reads of 38 quality-filtered metagenomes were further processed for the removal of human sequences by removing reads mapping to *Homo sapiens* identified by Kraken2 v2.1.3[35] using KrakenTools v1.2. The resulting metagenomes were screened using DIAMOND v2.1.9[34] blastx against the custom sulfur cycling database using a maximum of 1 hit per sequence read. Gene hits were filtered for a minimum length of 40 amino acids, 60% identity, and 80% query coverage. Gene counts were normalized by sample read counts using counts per million (cpm), and significant differences were determined using a Wilcoxon signed rank test between time points and Mann-Whitney *U* test between responders and non-responders. Data were visualized with GraphPad Prism v10.2.0 (GraphPad Software) and ggplot2 v3.4.4.

Fecal VOCs

Homogenized stool aliquots were shipped on dry ice to University of Liverpool for VOC analysis. This was performed by gas chromatography–mass spectrometry as described previously.[36] Samples were analyzed in 450 mg aliquots in 10 mL headspace vials. Prior to analysis, samples were heated for 30 minutes at 60 °C. A DVB-CAR-PDMS–coated solid-phase microextraction fiber was used to extract VOCs from the fecal headspace for the duration of 20 minutes.

VOC data were processed using AMDIS (v2.71, 2012) and the National Institute of Standards and Technology mass spectral library (v2.3, 2017) and the R package metab.[37] The Shapiro-Wilk test was used to check for normality before performing either parametric or nonparametric statistics for paired or non-paired data where appropriate. Statistical analysis was performed in the online software MetaboAnalyst 5.0 (https://www.metaboanalyst.ca). For comparison of VOC abundance, data were filtered to retain VOCs that were present in at least 50% of samples of at least 1 group. Missing values were replaced with a half minimum value for the VOCs and the data were log-transformed (glog) and Pareto scaled prior to statistical analysis. Unadjusted and adjusted *P* values by Benjamini-Hochberg with correction for multiple comparisons were reported.

Fecal sulfide

Production and quantification of sulfide in fecal slurries were carried out using a modified assay based on established methodologies for sulfide determination in water.[38] This colorimetric method utilizes the reaction between sulfide and specific reagents to produce methylene blue, which can be quantified spectrophotometrically. Fecal samples were processed into slurries and incubated in triplicate under controlled anaerobic conditions using nutrient media designed to simulate sulfide production from protein sources. Sulfide concentrations were then determined spectrophotometrically.

Results

Twenty-eight patients with UC were enrolled and completed the 8 week 4-SURE diet intervention study. Baseline demographic characteristics, disease distribution and clinical disease activity are summarized in Table S2. Detailed clinical results are reported elsewhere.[22] Briefly, the dietary intervention was well tolerated with excellent self-reported dietary adherence. Dietary targets, assessed via weighed food diaries, were wholly achieved by all participants with an overall 20% reduction in total protein intake to median 72.4 g/d (interquartile range [IQR] 63.5-81.2 g/d) at week 8 ($P = .007$), a 60% increase in total fiber intake to 34.4 g/d (IQR 30.2-38.6 g/d) at week 8 ($P < .0001$), and 342% increase in resistant starch to 12.6 g/d (IQR 10.7-14.4 g/d) ($P < .0001$). Clinical response occurred in 13 (46%) of 28 patients and endoscopic improvement in 10 (36%) of 28 patients, with overall improvement in disease activity (responders) according to Mayo criteria occurring in 8 (29%) of 28 patients.[22]

Fecal microbiota composition

Metagenome analysis of 38 high-quality microbiome samples identified 223 microbial genera, including bacteria, viruses, and microbial eukaryotes (Table S3). The majority of microbiome members before and after dietary intervention belonged to the Bacteroidota and Bacillota phyla (Figure 1). Median Shannon diversity was 1.65 (IQR 1.26-1.86) at week 0 and 1.80 (IQR 1.46-2.05) at week 8 ($P = .012$) (Figure 2A). However, beta-diversity analysis did not reveal community-wide changes in the taxonomic composition of the microbiota (Figure 2B).

The accuracy of the random forest classifier on the test data was 57.1%, suggesting a weak prediction potential of specific microbial genera affected by the diet intervention, likely to due to the small sampling size. The most important feature pointed to a reduction in *Peptostreptococcus* and *Odoribacter* genera at the end of the diet intervention (Figure 3).

A total of 12 putative sulfur cycling genes were significantly different between weeks 0 and 8 (Figure 4). Following the 4-SURE diet, there were 8 genes with a significant increase in abundance (*sseA* [$P = .006$], *CBS* [$P = .011$], *luxS* [$P = .018$], *metC* [$P = .049$], *asrC* [$P = .011$], *cysK* [$P = .04$], *metE* [$P = .023$], *iscS* [$P = .014$]) and 4 genes with a significant decrease in abundance (cpm) (*aspB* [$P = .012$], *yhaM* [$P = .04$], *dcyD* [$P = .023$], *phsC* [$P = .045$]) (Figure 4). The *aspB* gene, aspartate aminotransferase, had the greatest mean reduction (25.1 cpm) in abundance, with majority of patients (n = 13 of 19) having decreased counts. The *iscS* gene showed the greatest increase in mean abundance change, increasing by 78.4 cpm, with 15 of 19 patients having increased *iscS*.

Fecal VOCs

A total of 173 VOCs were identified across all samples (Table S4). No difference in the number of VOCs before and after diet were observed respectively (medians of 62 VOCs [IQR 47-77 VOCs] at week 0 and 62 VOCs [IQR 49-75 VOCs] at week 8; $P = .13$). A total of 65 VOCs were present in at least 50% of samples at either time point, of which 6 altered significantly in abundance between weeks 0 and 8 (Figure 5; Table S5). One VOC, indole, a metabolite of protein fermentation, decreased in abundance at week 8 in majority of participants and remained significant postcorrection for multiple comparisons (n = 20 of 28) ($P = .007$).

Fecal H_2S

Production of fecal H_2S ex vivo for 25 participants measured before dietary intervention concentrations was 2.61 µmol/g stool (IQR 2-4.96 µmol/g stool) (wet weight). By 8 weeks of the 4-SURE diet, fecal H_2S concentrations were reduced by 45% to 1.41 µmol/g stool (IQR 0.81–2.27 µmol/g stool) ($P = .002$) (Figure 6).

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

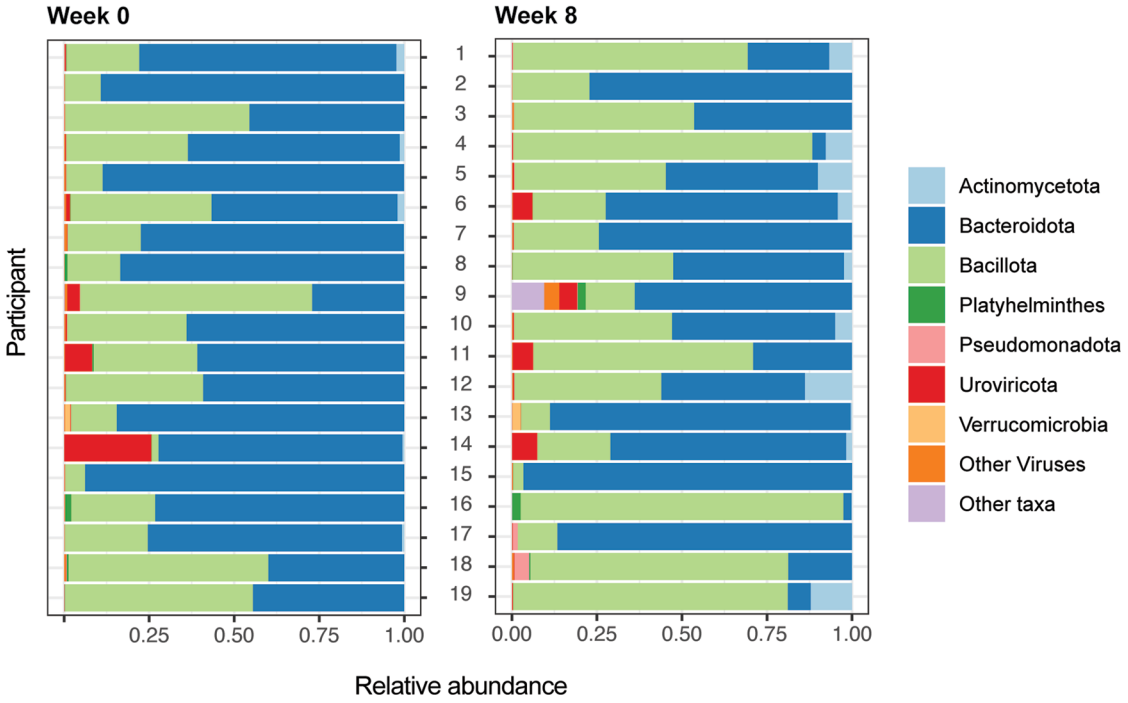


Figure 1. Relative abundance of phylum-level diversity at baseline and end of study. Data shown for 19 participants.

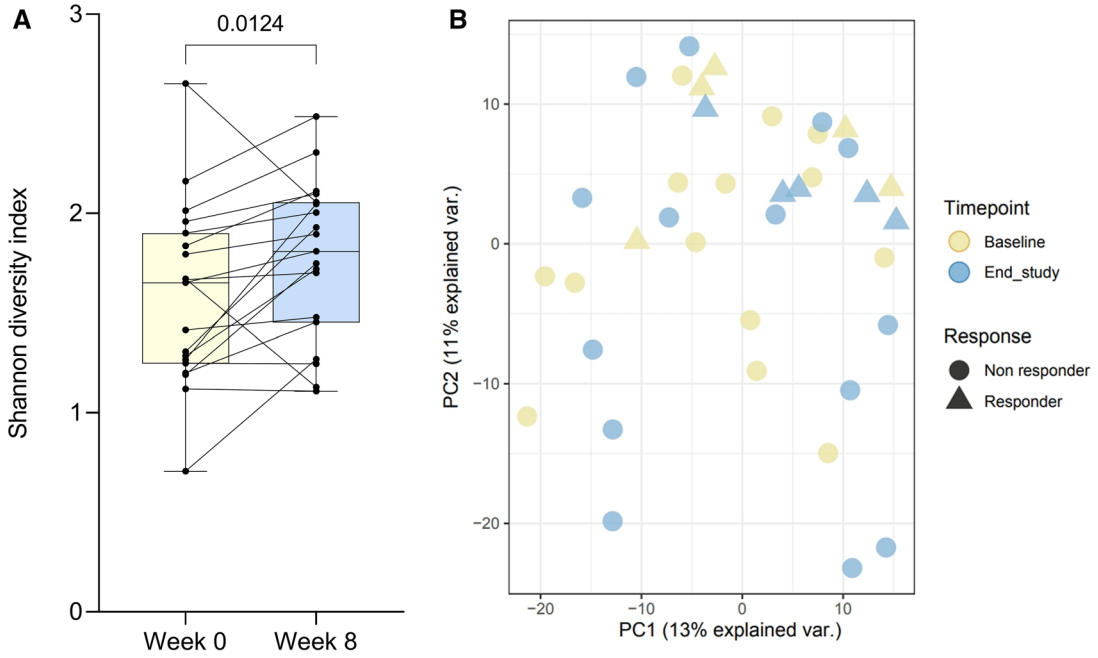


Figure 2. (A) Alpha-diversity (Shannon diversity index) at weeks 0 and 8, performed at genus taxonomic rank. A significant difference was observed between weeks 0 and 8 ($P = .012$; Wilcoxon signed rank test). (B) Beta-diversity plot of the microbiome community composition (Centered log-ratio (clr)-transformed) between weeks 0 and 8, performed at genus taxonomic rank. The first and second principal components (PCs) (explaining 13% and 11% of the variance, respectively) are shown.

Association of Overall Endoscopic and Clinical Response with Metagenomic, VOC, and Fecal H_2S Analyses

Comparisons were made between the results of the 8 participants who fulfilled criteria as responders at 8 weeks with those of the 20 who did not, designated nonresponders. Microbiome comparisons were only made for 38 quality-controlled reads, 5 responders, and 14 nonresponders.

No significant differences in the overall microbial composition between the responders and nonresponders at baseline or week 8 ($P = .31$, permutational analysis of variance) were observed. However, comparison of the 67 putative sulfur cycling genes at week 8 revealed significantly different gene abundances between responders and nonresponders in 2 genes, *sseA* ($P = .03$) and *sqr* ($P = .03$). The *sseA* gene was higher in abundance in nonresponders (mean 4.9 cpm) than

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

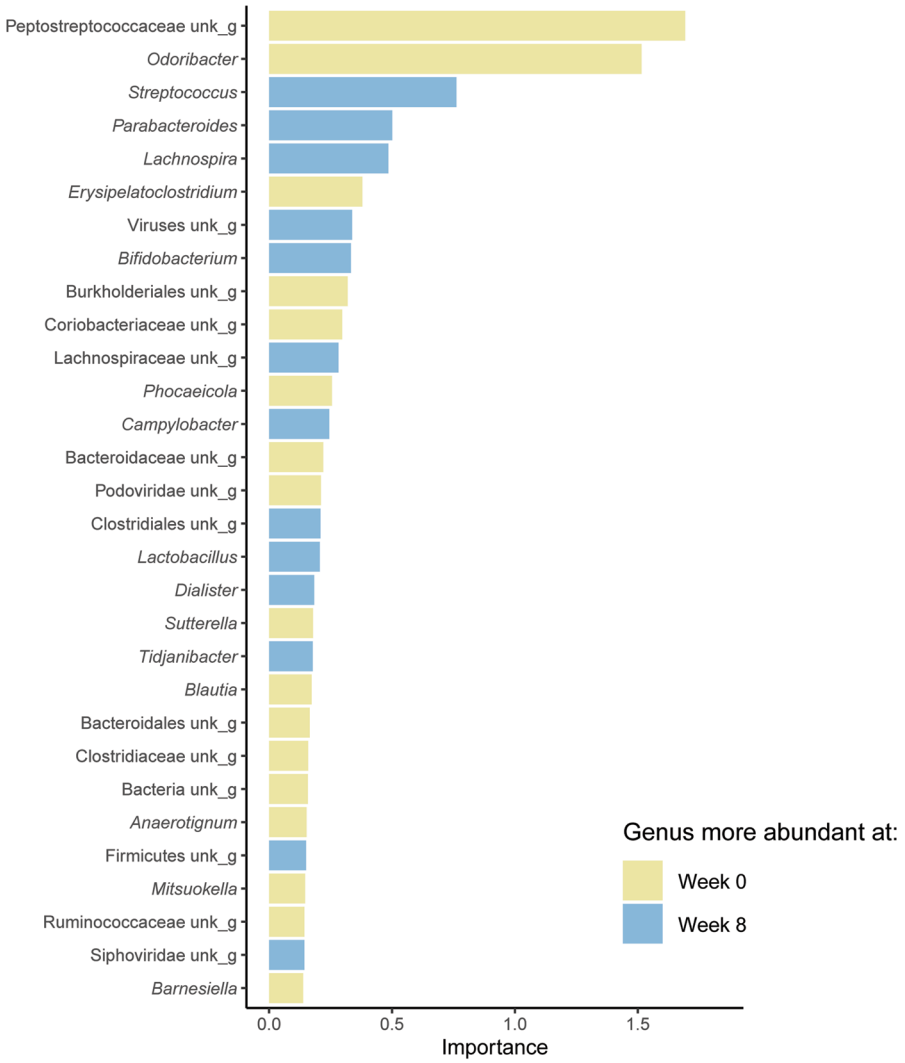


Figure 3. Random forest variable importance highlighting the microbial genera that contributed most for microbiome differences before and after the 8-week 4-SURE (4 Strategies to Sulfide Reduction) diet intervention for 19 participants.

responders (1.2 cpm), whereas *sqr* was higher in abundance in responders (9.0 cpm) than nonresponders (3.7 cpm) (Figure 7A, B).

The number of VOCs in responders at baseline was a mean of 69 (95% confidence interval [CI], 51-87) compared with 63 (95% CI, 43-83) in nonresponders ($P = .13$), with statistically significant differences in the abundance of butyl 2-methylbutanoate and butyl pentanoate (Figure S2A, B). The abundance of butyl 2-methylbutanoate remained significant postcorrection for multiple comparisons ($P = .05$). In responders, the median number of VOCs before diet was 73 (IQR 61-78) compared with 62 (IQR 60-64) at week 8 ($P = .03$; paired t test), whereas nonresponders had no change (mean 63 [95% CI, 45-81] compared with 62 [95% CI, 44-80]; $P = .67$]. After 8 weeks of the 4-SURE diet, the number of VOCs was similar between responders 62 (50-74) and nonresponders 62 (44-80) ($P = .95$).

In responders (n = 7), fecal H_2S reduced by a 40% from a median 2.19 µmol/g (IQR 1.49-4.51 µmol/g) stool (wet weight) at week 0 compared with 1.31 µmol/g (IQR 0.63-1.77 µmol/g) at week 8 ($P = .18$). In nonresponders (n = 18), fecal H_2S concentrations reduced by 42% from 2.44 µmol/g (IQR 1.81-4.93 µmol/g) to 1.41 µmol/g (IQR 0.98-2.40 µmol/g), respectively ($P = .006$).

Discussion

The 4-SURE diet was designed to modulate colonic H_2S production and optimize SCFA delivery to ameliorate epithelial inflammation.[22] A deep microbial, functional, and metabolomic analysis of the feces before and during the 4-SURE diet was undertaken to explore these hypotheses, and pleasingly demonstrated that the dietary objectives were fulfilled. Specifically, reduction in abundance of putative H_2S-producing taxa and change in community capacity to metabolize H_2S and reduction in protein fermentation were achieved, together with reduced production of H_2S ex vivo in the feces. This application of a compositional and functional analysis to a diet study is novel and highlights exemplar framework for analyzing the proposed mechanistic action of therapeutic diet strategies.

The gut microbial ecosystem in active UC is known to lack diversity and richness compared with healthy individuals, but

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

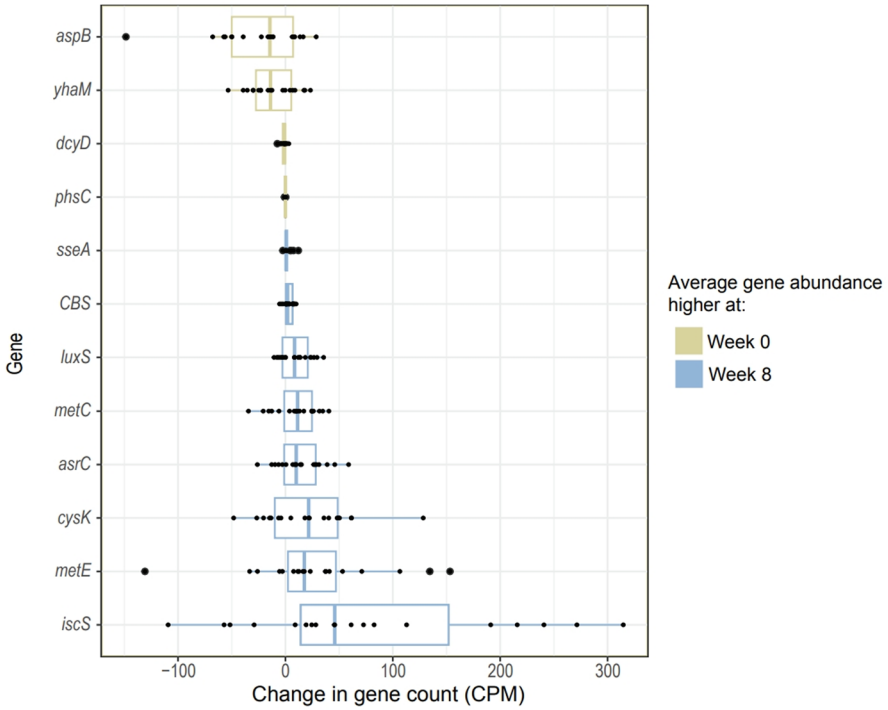


Figure 4. Change in gene abundance from weeks 0 to 8 for the 12 genes with significantly different abundances after the 4-SURE (4 Strategies to Sulfide Reduction) diet. Significance determined with Wilcoxon signed rank test ($P < .05$) after adjusting for multiple comparisons with Benjamini-Hochberg method. Data shown as difference between week 8 and week 0 counts per million (CPM) for each gene for 19 participants.

the pathogenic relevance remains unclear.[39] Microbial diversity of Bacteroidota and Bacillota phyla increased with the 4-SURE diet suggesting a community shift toward a more diverse microbiome with capacity to degrade complex carbohydrates was achieved. At a functional level, these data corroborate the increased ratio of carbohydrate-to-protein fermentation previously reported. Structural and functional changes in the microbial community after 8 weeks of the 4-SURE diet are illuminated, suggesting that carbohydrate fermentation was "switched on" with a greater production of SCFAs, as reported in the initial study of this cohort.[22]

An abundance of cysteine-degrading bacteria is known to be higher in the feces of patients with IBD compared with those in healthy individuals.[12] In this study, abundance of known H_2S-producing taxa *Odoribacter*[12] and taxa associated with active UC, Peptostreptococcaceae,[40,41] were markedly lower after 8 weeks of diet, corroborating with the clinical and endoscopic response observed.[22] Abundance of 12 genes known to be involved in global sulfur cycling were also significantly altered with the 4-SURE diet, indicating a shift in microbiome sulfur-cycling potential. Of relevance, the *aspB* gene had the greatest mean reduction in majority of patients. The *aspB* gene encodes aspartate aminotransferase, a key enzyme linking amino acid metabolism to cellular respiration in bacteria through the production of the Krebs cycle intermediates, oxaloacetate and glutamate, from aspartate.[42] The observed decrease in *aspB* abundance may result from reduced dietary protein intake, which limits amino acid availability to diminish protein fermentation pathways.[43] Notably, increased *aspB* abundance has been associated with elevated H_2S production from L-cysteine in anaerobic systems, suggesting that the dietary intervention may also alter sulfur metabolism.[44] However, the diverse and poorly characterized functions of aspartate aminotransferases paired with the lack of post-transcriptional and phenotypic data make the functional implications of these changes challenging to interpret without further investigation.[42]

When comparing putative sulfur-cycling genes at week 8 between responders and nonresponders, 2 genes had significantly different abundances. First, the gene *sseA* that encodes 3-mercaptopyruvate sulfurtransferase, an enzyme directly involved in H_2S production through the degradation of cysteine,[12,45] was higher in abundance in nonresponders. This observation suggests that the microbiome in nonresponders was enriched in cysteine-degrading microbes with an elevated capacity for H_2S production. Notably, *sseA* is prevalent in Pseudomonadota, particularly within genera implicated in UC, including *Citrobacter*, *Enterobacter*, and *Escherichia*.[12,46,47] This enrichment may indicate that nonresponders harbor microbial communities resistant to dietary modulation, maintaining pathways that support sulfur metabolism and H_2S production.

Second, the *sqr* gene that encodes SQR (sulfide-quinone reductase), an enzyme involved in detoxifying H_2S by oxidizing it to elemental sulfur or thiosulfate,[48] was significantly more abundant in the fecal metagenomes of responders compared with nonresponders. This increase supports the change in sulfur metabolism pathways observed with concurrent reduction in H_2S production, aligning with the goal of the dietary intervention to alter the capacity of the gut microbiome to metabolize H_2S.[49,50] These findings position *sqr* as a potential marker of microbial adaptation to dietary changes. Statistically significant differences in sulfur-metabolizing genes were identified between responders and nonresponders, but further research is required to confirm these changes in a larger sample and elucidate mechanistic implications for UC.

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

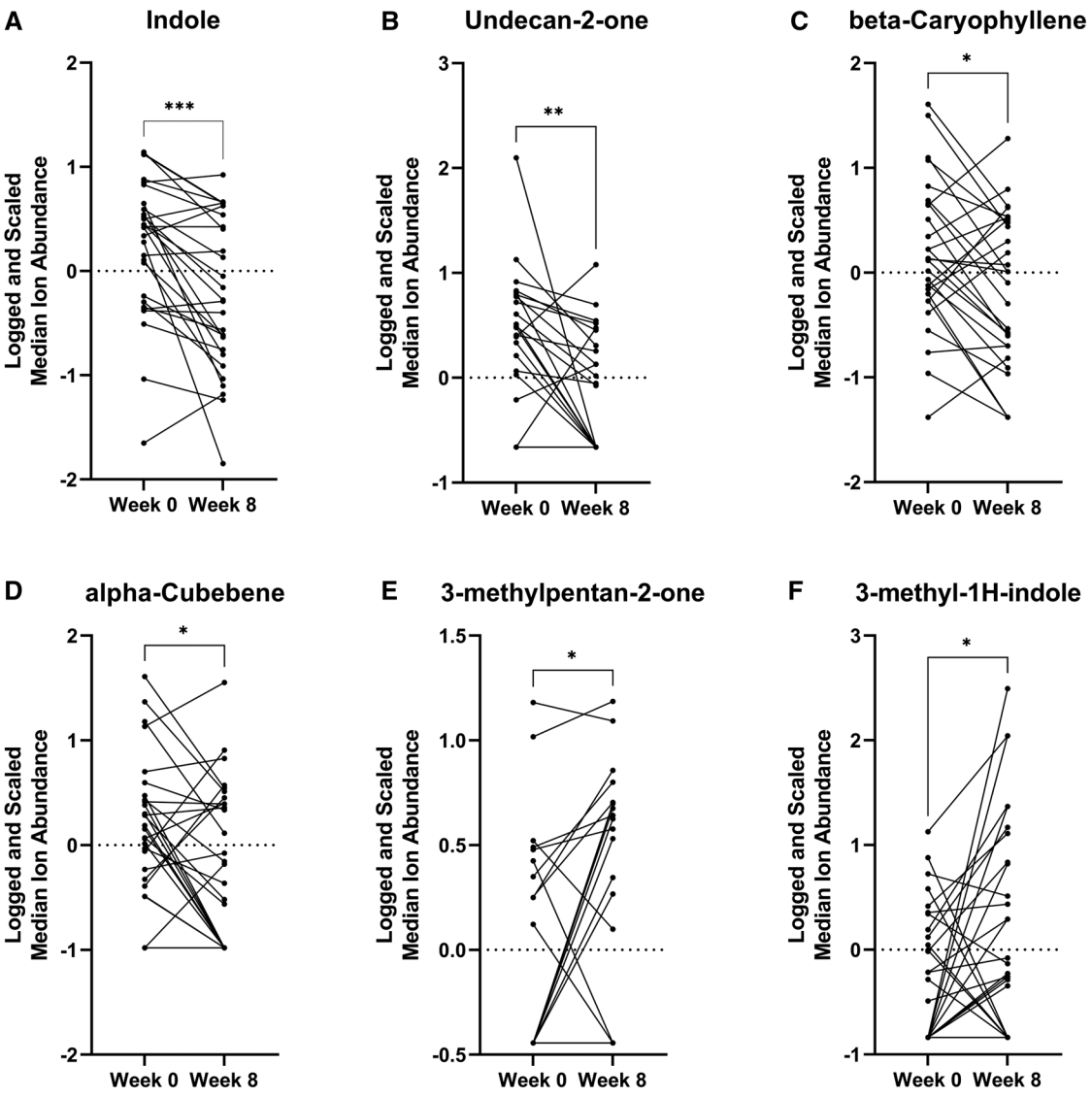


Figure 5. (A-F) Volatile organic compounds that altered in abundance in 28 adults with mild-to-moderately active ulcerative colitis who followed the 4-SURE (4 Strategies to Sulfide Reduction) diet for 8-weeks presented as a log2 fold change (Wilcoxon signed rank test without false discovery rate correction). (A) Indole, (B) undecan-2-one, (C) beta-caryophyllene, (D) alpha-cubebene, (E) 3-methylpentan-2-one, and (F) 3-methyl-1H-indole. A log2 value of below −1 or above 1 represents a 2-fold difference in these volatile organic compounds.

Many microbiota-generated VOCs in feces are metabolites of fermentable dietary substrate yet have not been examined as a metabolomic biomarker in a dietary strategy in UC.[11] Overall, similar to the stable abundance of taxa observed, VOCs did not markedly change before and with diet, but VOCs of relevance to the intended mechanistic action of the 4-SURE diet did. Specifically indole, a marker of protein fermentation, was less abundant.[51] This could reflect the overall reduction in protein intake achieved through adherence to the 4-SURE diet with subsequent reduction in fermentation. Alternatively, these data may demonstrate protein fermentation was "switched off" due to concurrent increased ingestion of fermentable fibers. Either way, these data support the aforementioned change in ratio of carbohydrate-to-protein fermentation and suggest microbial shifts in community toward increased capacity for fermentation of complex carbohydrates in preference to protein with concurrent modulation of metabolic pathways.[9,22,51–53]

Last, in keeping with the H_2S hypothesis and central intent of the 4-SURE diet, H_2S production was suppressed with dietary change. Bacterial production of H_2S in the colonic lumen has paradoxical effects. At low levels, H_2S has an anti-inflammatory, protective effect on the epithelial lining, but when H_2S concentrations exceed the capacity of colonocytes for oxidation, in the presence of excess nitric oxide, it becomes toxic.[54,55] Excess production and prolonged exposure to H_2S and nitric oxide are proposed to damage the epithelial lining and impair SCFA metabolism, perpetuating mucosal injury with ensuing inflammation.[55] Distal colonic protein fermentation is proposed in the pathogenic mechanisms of a toxic H_2S gradient. Fermentable fibers have previously been shown in vitro to suppress excess H_2S production.[9] In the present study, following the 4-SURE diet led to a marked suppression of H_2S production, demonstrating that the central, intended mechanistic action of the diet was achieved in this cohort.

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

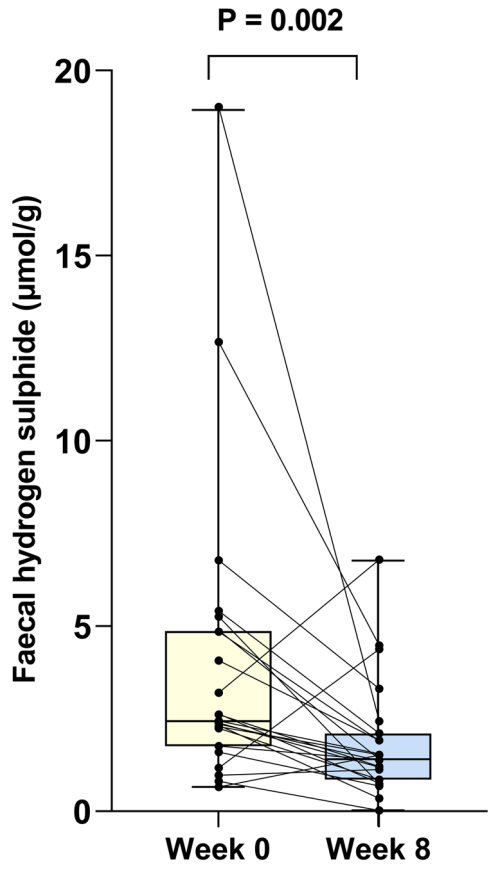


Figure 6. Change in ex vivo production of fecal hydrogen sulfide in the fecal samples of 25 adults with mild-moderately active ulcerative colitis before and after dietary intervention (Wilcoxon signed rank test; $P = .002$).

There are 2 major strengths of this deep mechanistic analysis. First, meticulous examination of the different steps in the metabolic pathways within the colonic luminal environment thought to be involved in the pathogenesis of UC was performed in an attempt to examine the hypothesized mechanistic action of diet. Precision methodology was used for stool collection and processing to minimize degradation of fecal DNA and loss of gaseous metabolites. Use of shotgun metagenomic sequencing with bioinformatics is exemplar.[56] Though more costly, it is advantageous to use in diet trials for whole genome analysis of microbial composition, identification of disease-specific genes and microbial functional potential before and after application of diet.[56] This also allows for this greater degree of accuracy in taxonomic profiling compared with 16S rRNA sequencing of a genome fragment.[57] Integration of VOCs as a biomarker of fermentation is novel and provides a more in-depth insight into existing analyses, also hypothesis generating for future UC trials. Last, rigorous methodology was undertaken to develop and validate a H_2S colorimetric assay that was reliable and reproducible as H_2S is notoriously challenging to measure.[54] The second strength of the study was examination of the link between mechanisms and mucosal inflammation in the context of an applied dietary therapeutic intervention. To do this, strict criteria, not restricted to symptoms and including endoscopic mucosal inflammation, were used to define response at 8 weeks. This overall approach has not been applied to a new dietary therapy targeting specific pathogenic mechanisms in patients with UC. It may provide a benchmark for deep functional analysis in future diet intervention studies and also provide a deeper insight into the optimal duration of a dietary intervention required to induce changes to the metabolome and pattern of inflammation.

There were limitations. First, there was not a control group to compare whether the 4-SURE diet could induce a similar finding in healthy control subjects. Second, the sample size was small, particularly when the differences between responders and nonresponders were being addressed, which was further compounded by the smaller number of responders. The use of purely clinical response (defined as a reduction in partial Mayo ≥2 and a 48% clinical response was achieved) would have generated near equal numbers in each group.[22] However, inflammatory response is the desired endpoint and symptoms can be unreliable in defining this. The small sample size constrained the ability to identify microbiome signatures, as exhibited in the low predictive power of the random forest model. This may have also reduced the power to detect biologically relevant significant differences in sulfur cycling genes. Therefore, future studies in a larger sample will allow for a more comprehensive characterization of microbiome changes induced by the 4-SURE diet. Third, although intended microbial and metabolomic changes were observed, 8 weeks is a short time frame for an IBD therapy to exert its effect, and the optimal time frame for a defined therapeutic diet to exert its effect remains unknown. Fourth, there were limitations of the methods used. For example, the analysis of sulfur cycling genes with shotgun metagenomics does not indicate if these genes were active in the community. Thus, to further hypothesize the mechanism of the 4-SURE diet, metatranscriptomics may be employed to identify which genes are active in these communities.

Conclusions

Diet is emerging in the therapeutic armamentarium in UC, intended to correct and restore microbial composition and function. These exploratory data suggest the 4-SURE diet modulated key biomarkers in the colonic luminal environment as intended. Application of a compositional and functional analysis to a diet study is novel and highlights exemplar framework for including microbial and metabolomic biomarkers of pathogenic relevance in the analysis of therapeutic diet strategies, with recommendations for refinement in future trials.

Acknowledgments

The authors thank Bronwyn Hutchens, The Queen Elizabeth Hospital Inflammatory Bowel Disease Service, Dr James Fon, Dr Karmen Telfer, Dr Bron Lett, and Dakota Rhys-Jones. The graphical abstract created using BioRender.

Author Contributions

Study design and conception (A.S.D., R.V.B., S.P.C., P.R.G., C.K.Y., J.M.A.). Data collection (A.S.D, W.U.). Data analysis (A.S.D, R.S, R.Z.W., R.B.Y., N.K.M., W.U.). Interpretation of results (A.S.D., R.V.B., P.R.G., R.S., R.Z.W., R.B.Y., N.K.M., S.C.F., S.P.C., C.S.P.). Writing of manuscript (A.S.D., R.S., R.Z.W., R.B.Y., N.K.M.) Critical review and revision (A.S.D., R.V.B., P.R.G., R.S., R.Z.W., R.B.Y., N.K.M., S.C.F., S.P.C., C.K.Y., J.M.A., C.S.P., W.U.).

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

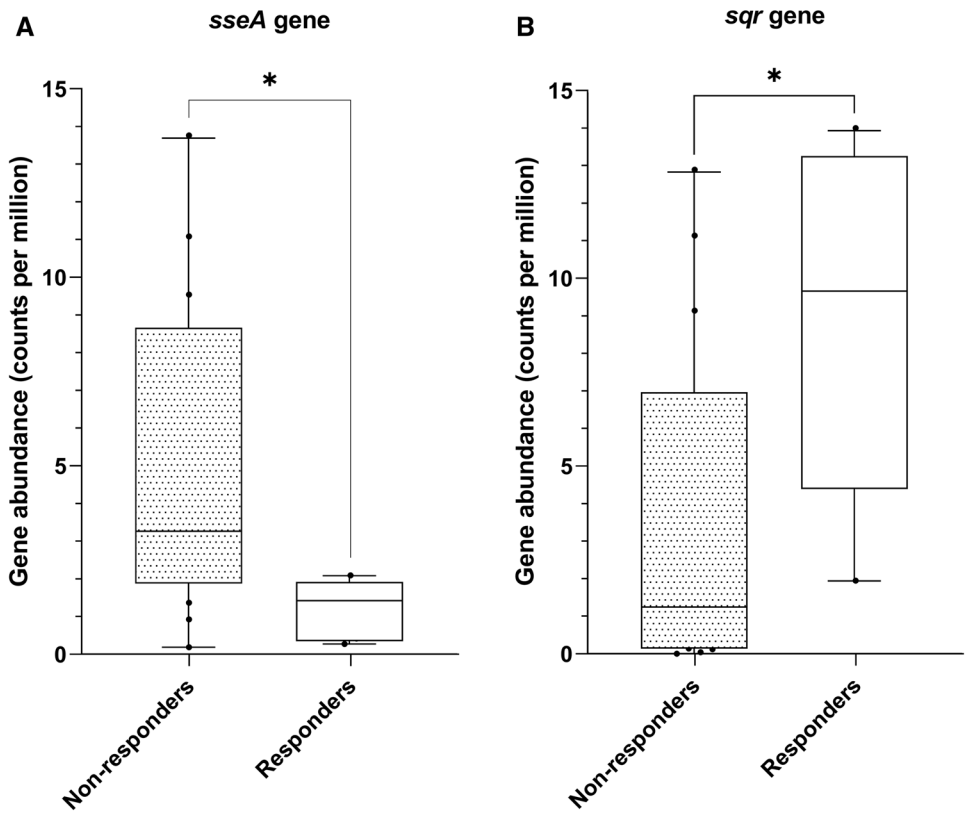


Figure 7. (A) Gene abundances for genes significantly different between responders (n = 5) and nonresponders (n = 14) for the *sseA* gene. (B) Gene abundances for genes significantly different between responders (n = 5) and nonresponders (n = 14) for the *sqr* gene. *Significance determined with Mann-Whitney *U* test, $P = .034$ (*sseA*) and $P = .034$ (*sqr*).

Supplementary Data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

Funding

This work was partly supported by a Gastrointestinal Society of Australia FICE Grant, a Hospital Research Foundation Project Grant and Michelle McGrath Fellowship, GUTSY Group Project Grant, a European Crohn's Colitis Project Grant, and a Commonwealth Research Stipend administered through the University of Adelaide.

Conflicts of Interest

ASD has no competing interests to declare. RS reports there are no competing interests to declare. RZW is an option holder in BiomeBank. RBY is an option holder in BiomeBank. WU has no competing interests to declare. NKM has no competing interests to declare. VRM reports there are no competing interests to declare. SCF owns shares in BiomeBank SPC owns shares in BiomeBank. SPC has received advisory, speaking fees or research support from Ferring, Falk, Microbiotica, Janssen. CJP has no competing interests to declare. JMA has served as a speaker, a consultant and an advisory board member for, and has received research funding from, Abbott, AbbVie, Allergan, Anatara, AstraZeneca, Bayer, Celgene, Falk, Ferring, Gilead, Hospira, Immunic, ImmunsanT, Janssen, MSD, Nestle, Progenity, Pfizer, Sandoz, Shire, Takeda, Vifor, RAH Research Fund, The Hospital Research Fund with all monies received by her department for research support. CKY has received research funding from Atmo Biosciences, Ferring Pharmaceuticals, Danone and Yakult Australia. Her Department financially benefits from the sales of a digital application, booklets and on-line courses on the FODMAP diet. PRG has served as a consultant or advisory board member for Anatara, Atmo Biosciences, Immunic Therapeutics, Novoviah, Novozymes, Intrinsic Medicine, Topas and Comvita. He has received research grants for investigator-driven studies from Atmo Biosciences. He holds shares in Atmo Biosciences. His department financially benefits from the sales of a digital application, booklets and online courses on the FODMAP diet RVB has served as a speaker, a consultant and an advisory board member for (all fees paid to employer for research support), and has received research funding from AbbVie, Ferring, Janssen, Shire, Takeda, Dr Falk, Emerge Health. RVB owns shares in BiomeBank.

Data Availability

Data associated with this paper can be found and accessed via 10.1093/jn/nxac093 and upon contacting the corresponding author. Data will be deposited in a recognized data repository at University of Adelaide.

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

References

1. Teigen LM, Geng Z, Sadowsky MJ, Vaughn BP, Hamilton MJ, Khoruts A. Dietary factors in sulfur metabolism and pathogenesis of ulcerative colitis. *Nutrients*. 2019;11:931.
2. Pitcher MC, Cummings JH. Hydrogen sulphide: a bacterial toxin in ulcerative colitis?*Gut*. 1996;39:1-4.
3. Ananthakrishnan AN. Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol*. 2015;12:205-217.
4. Lambert K, Pappas D, Miglioretto C, et al. Systematic review with meta-analysis: dietary intake in adults with inflammatory bowel disease. *Aliment Pharmacol Ther.* 2021;54:742-754.
5. Barberio B, Facchin S, Patuzzi I, et al. A specific microbiota signature is associated to various degrees of ulcerative colitis as assessed by a machine learning approach. *Gut Microbes*. 2022;14:2028366.
6. Kushkevych I, Dordević D, Vítězová M. Possible synergy effect of hydrogen sulfide and acetate produced by sulfate-reducing bacteria on inflammatory bowel disease development. *J Adv Res*. 2021; 27:71-78.
7. Machiels K, Joossens M, Sabino J, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut*. 2014;63:1275-1283.
8. Galipeau HJ, Caminero A, Turpin W, et al. ; CCC Genetics, Environmental, Microbial Project Research Consortium. Novel fecal biomarkers that precede clinical diagnosis of ulcerative colitis. *Gastroenterology*. 2021;160:1532-1545.
9. Yao CK, Rotbart A, Ou JZ, Kalantar-Zadeh K, Muir JG, Gibson PR. Modulation of colonic hydrogen sulfide production by diet and mesalazine utilizing a novel gas-profiling technology. *Gut Microbes*. 2018;9:510-522.
10. Magee EA, Richardson CJ, Hughes R, Cummings JH. Contribution of dietary protein to sulfide production in the large intestine: an in vitro and a controlled feeding study in humans. *The Am J Clin Nutr.* 2000;72:1488-1494.
11. Hamer HM, De Preter V, Windey K, Verbeke K. Functional analysis of colonic bacterial metabolism: relevant to health?*Am J Physiol Gastrointest Liver Physiol*. 2012;302:G1-G9.
12. Braccia DJ, Jiang X, Pop M, Hall AB. The capacity to produce hydrogen sulfide (H(2)S) via cysteine degradation is ubiquitous in the human gut microbiome. *Front Microbiol*. 2021;12:705583.
13. Pierre JB, ed. *Metabolism of Nutrients by Gut Microbiota*. 1st ed. Royal Society of Chemistry; 2022.
14. Korpela K. Diet, microbiota, and metabolic health: trade-off between saccharolytic and proteolytic fermentation. *Annl Rev Food Sci Technol*. 2018;9:65-84.
15. Dong C, Chan SSM, Jantchou P, et al. Meat intake is associated with a higher risk of ulcerative colitis in a large european prospective cohort studyø. *J Crohns Colitis*. 2022;16:1187-1196.
16. Jowett SL, Seal CJ, Pearce MS, et al. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. *Gut*. 2004;53:1479-1484.
17. Teigen L, Mathai PP, Lopez S, et al. Differential hydrogen sulfide production by a human cohort in response to animal- and plant-based diet interventions. *Clin Nutr.* 2022;41:1153-1162.
18. Day AS, Ballard TM, Yao CK, Gibson PR, Bryant RV. Food-based interventions as therapy for inflammatory bowel disease: important steps in diet trial design and reporting of outcomes. *Inflamm Bowel Dis*. 2025;31:1121-1137.
19. Fritsch J, Garces L, Quintero MA, et al. Low-fat, high-fiber diet reduces markers of inflammation and dysbiosis and improves quality of life in patients with ulcerative colitis. *Clin Gastroenterol Hepatol*. 2021;19:1189-1199.e30.
20. Olendzki B, Bucci V, Cawley C, et al. Dietary manipulation of the gut microbiome in inflammatory bowel disease patients: pilot study. *Gut Microbes*. 2022;14:2046244.
21. Sarbagili-Shabat C, Albenberg L, Van Limbergen J, et al. A novel UC exclusion diet and antibiotics for treatment of mild to moderate pediatric ulcerative colitis: a prospective open-label pilot study. *Nutrients*. 2021;13:e3736.
22. Day AS, Yao CK, Costello SP, et al. Therapeutic potential of the 4 strategies to SUlfide-REduction (4-SURE) diet in adults with mild to moderately active ulcerative colitis: an open-label feasibility study. *J Nutr.* 2022;152:1690-1701.
23. Ahmed I, Greenwood R, Costello B, Ratcliffe N, Probert CS. Investigation of faecal volatile organic metabolites as novel diagnostic biomarkers in inflammatory bowel disease. *Aliment Pharmacol Ther.* 2016;43:596-611.
24. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30:2114-2120.
25. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9:357-359.
26. Marcelino VR, Clausen P, Buchmann JP, et al. CCMetagen: comprehensive and accurate identification of eukaryotes and prokaryotes in metagenomic data. *Genome Biol*. 2020;21:103.
27. Dixon P. VEGAN, a package of R functions for community ecology. *J Veg Sci.* 2003;14:927-930.
28. Rohart F, Gautier B, Singh A, Lê Cao K-A, KA LC. mixOmics: an R package for 'omics feature selection and multiple data integration. *PLoS Comput Biol*. 2017;13:e1005752.
29. Wolf PG, Cowley ES, Breister A, et al. Diversity and distribution of sulfur metabolic genes in the human gut microbiome and their association with colorectal cancer. *Microbiome*. 2022;10:64.
30. Agren D, Schnell R, Oehlmann W, Singh M, Schneider G. Cysteine synthase (CysM) of Mycobacterium tuberculosis is an O-phosphoserine sulfhydrylase: evidence for an alternative cysteine biosynthesis pathway in mycobacteria. *J Biol Chem*. 2008;283:31567-31574.
31. Walker A, Schmitt-Kopplin P. The role of fecal sulfur metabolome in inflammatory bowel diseases. *Int J Med Microbiol*. 2021;311: 151513.
32. Nonaka G, Takumi K. Cysteine degradation gene yhaM, encoding cysteine desulfidase, serves as a genetic engineering target to improve cysteine production in Escherichia coli. *AMB Express*. 2017;7:90.
33. UniProt Consortium. UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res*. 2021;49:D480-D489.
34. Buchfink B, Reuter K, Drost HG. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat Methods*. 2021;18: 366-368.
35. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol*. 2019;20:257.
36. Reade S, Mayor A, Aggio R., et al. Optimisation of sample preparation for direct SPME-GC-MS analysis of murine and human faecal volatile organic compounds for metabolomic studies. *J Anal Bioanal Tech* 2014;5:184.
37. Aggio R, Villas-Bôas SG, Ruggiero K. Metab: an R package for high-throughput analysis of metabolomics data generated by GC-MS. *Bioinformatics*. 2011;27:2316-2318.
38. Cline JD. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanography*. 1969;14:454-458.
39. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol.* 2018;11:1-10.
40. Ankersen DV, Weimers P, Marker D, et al. eHealth: Disease activity measures are related to the faecal gut microbiota in adult patients with ulcerative colitis. *Scand J Gastroenterol.* 2020;55:1291-1300.
41. Nguyen LH, Ma W, Wang DD, et al. Association between sulfur-metabolizing bacterial communities in stool and risk of distal colorectal cancer in men. *Gastroenterology*. 2020;158:1313-1325.
42. Jansen RS, Mandyoli L, Hughes R, et al. Aspartate aminotransferase Rv3722c governs aspartate-dependent nitrogen metabolism in Mycobacterium tuberculosis. *Nat Commun.* 2020;11:1960.

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025

43. Diether NE, Willing BP. Microbial fermentation of dietary protein: an important factor in diet-microbe-host interaction. *Microorganisms*. 2019;7:19.
44. Fu Q, Li C, Liu Z, et al. The impact of bisphenol a on the anaerobic sulfur transformation: promoting sulfur flow and toxic H(2)S production. *Environ Sci Technol*. 2024;58:8043-8052.
45. Hayes JA, Lunger AW, Sharma AS, et al. Engineered bacteria titrate hydrogen sulfide and induce concentration-dependent effects on the host in a gut microphysiological system. *Cell Reports*. 2023;42: 113481.
46. De Cruz P, Prideaux L, Wagner J, et al. Characterization of the gastrointestinal microbiota in health and inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18:372-390.
47. Khorsand B, Asadzadeh Aghdaei H, Nazemalhosseini-Mojarad E, Nadalian B, Nadalian B, Houri H. Overrepresentation of Enterobacteriaceae and Escherichia coli is the major gut microbiome signature in Crohn's disease and ulcerative colitis; a comprehensive metagenomic analysis of IBDMDB datasets. *Front Cell Infect Microbiol*. 2022;12:1015890.
48. Fike DAB, Leavitt AS, William D. Geomicrobiology of sulfur. In: Newman D, Kappler A, eds. *Ehrlich's Geomicrobiology*. 6th ed. Taylor & Francis Group, LLC; 2015:38.
49. Wang T, Ran M, Li X, et al. The pathway of sulfide oxidation to octasulfur globules in the cytoplasm of aerobic bacteria. *Appl Environ Microbiol*. 2022;88:e0194121.
50. Mendes SS, Miranda V, Saraiva LM. Hydrogen sulfide and carbon monoxide tolerance in bacteria.*Antioxidants (Basel)*. 2021;10:729.
51. Tennoune N, Andriamihaja M, Blachier F. Production of indole and indole-related compounds by the intestinal microbiota and consequences for the host: the good, the bad, and the ugly. *Microorganisms*. 2022;10:930.
52. Blachier F, Beaumont M, Kim E. Cysteine-derived hydrogen sulfide and gut health: a matter of endogenous or bacterial origin. *Curr Opin Clin Nutr Metab Care*. 2019;22:68-75.
53. Le Leu RK, Young GP, Hu Y, Winter J, Conlon MA. Dietary red meat aggravates dextran sulfate sodium-induced colitis in mice whereas resistant starch attenuates inflammation. *Dig Dis Sci*. 2013;58:3475-3482.
54. Blachier F, Andriamihaja M, Larraufie P, Ahn E, Lan A, Kim E. Production of hydrogen sulfide by the intestinal microbiota and epithelial cells and consequences for the colonic and rectal mucosa. *Am J Physiol Gastrointest Liver Physiol*. 2021;320:G125-G135.
55. Roediger W. Review article: nitric oxide from dysbiotic bacterial respiration of nitrate in the pathogenesis and as a target for therapy of ulcerative colitis. *Aliment Pharmacol Ther.* 2008;27:531-541.
56. Simpson HL, Campbell BJ. Review article: dietary fibre-microbiota interactions. *Aliment Pharmacol Ther.* 2015;42:158-179.
57. Durazzi F, Sala C, Castellani G, Manfreda G, Remondini D, De Cesare A. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Sci Rep*. 2021;11:3030.

Downloaded from https://academic.oup.com/ibdjournal/advance-article/doi/10.1093/ibd/izaf177/8246827 by University of Liverpool user on 03 November 2025