**ABSTRACT**

High-protein diets may be linked to gut inflammation due to increased production of hydrogen sulfide (H₂S), a potential toxin, as an end product of microbial fermentation in the colon by sulfidogenic sulfate-reducing bacteria (SRB). We hypothesized that dietary content of sulfur-containing amino acids (SAA) leads to variation in the relative abundances of intestinal SRB, which include Desulfovibrio and Bilophila taxa. To test this hypothesis we performed a pilot crossover study in four healthy volunteers, who consumed two interventional diets for 10–14 days, containing high or low SAA content. The total energy intake was similar between the two dietary extremes. Microbial communities were characterized by 16S rRNA gene amplicon and shotgun next-generation D_{1}NA sequencing. While the relative abundance of Desulfovibrio differed among participants (ANOVA P= 0.001), we could not detect a change with dietary treatments. Similarly, no differences in Bilophila abundance were observed among individuals or dietary arms. Inter-personal differences in microbial community composition and functional gene categories differed between subjects and these differences were maintained over the course of the study. These observations are consistent with re-analysis of two previously published dietary intervention studies. Finally, we found that inter-personal differences in the taxonomic composition of fecal microbiota, including the relative abundances of SRB, were maintained over time in 19 healthy individuals in our stool donor program. These results suggest that the use of dietary interventions alone may be insufficient for rapid therapeutic targeting of SRB. Nevertheless, these pilot data provide a foundation to inform future, statistically powered, studies.

**INTRODUCTION**

Diseases associated with chronic gut inflammation, such as Crohn’s disease and ulcerative colitis (UC), have increased in incidence since World War II in Western countries, and are emerging rapidly in countries that are becoming more westernized.¹ Dietary changes that include increased content of animal protein and saturated fat are associated with westernization. One possible mechanism that may link increased dietary protein and fat content with gut inflammation is increased production of hydrogen sulfide (H₂S), which is an end product of microbial fermentation and a potential toxin.²,³ Sulfate-reducing bacteria (SRB) constitute one of the main producers of H₂S in the gut, and it is hypothesized that increased content of dietary sulfur may enhance SRB activity.⁴

There is increasing clinical interest in the role of diet in the management of inflammatory bowel diseases. One mechanistic premise of dietary therapy is its capacity to change the composition and activity of intestinal microbiota. However, the potency and efficiency of dietary interventions, a critical issue in clinical practice, in inducing such changes is unclear. David et al. reported rapid changes in fecal microbial community structure in individuals alternating between diets composed entirely of animal or plant products, which overwhelmed inter-individual differences in...
microbial gene expression. In contrast, in a highly controlled short-term feeding experiment, Wu et al. observed that switching between high-fat/low-fiber and low-fat/high-fiber diets over 10 days did not overcome individual variation, although some changes in microbiome composition were detectable within 24 h. These investigations did not focus their analyses on diet-induced responsiveness of microbial groups competing for hydrogen, which include the SRB, methanogens, and acetogenic bacteria.

Here we performed a preliminary study using healthy volunteers to probe responsiveness of SRB abundance in response to low- (Low-S) and high-sulfur (High-S) diets. These data were compared to previous dietary intervention studies. We also measured the abundance of SRB in longitudinal samples from a population of healthy stool donors participating in our fecal microbiota transplant (FMT) program, as it could be a criterion for rational donor selection in the treatment of intestinal inflammation.

### Results

**Participants and dietary intake**

Three males and one female participant were consented, enrolled, and completed the intervention study. Mean age was 41.3 ± 12.5 years; mean body mass index was 30.8 ± 4.82 kg/m², range: 26.6–37.8 kg/m². The baseline measurement of H₂S production was 0.003, 0.018, 0.020, and 0.006 mL H₂S per gram of dry weight feces, for study participants 01 through 04, respectively. Mean dietary intake for each study interval is presented in Table 1. Total energy intake (kcal) was not significantly different between High-S and Low-S diet interventions. However, intake of carbohydrates, fiber, and vegetable protein were higher in the Low-S intervention as compared to High-S intervention. Intakes of total protein, animal protein, methionine, and cysteine were higher in the High-S intervention as compared to the Low-S intervention.

| Variable                  | Baseline* | High-S | Washout | Low-S   | P-value (High-S vs. Low-S) |
|---------------------------|-----------|--------|---------|---------|---------------------------|
| Total energy (kcal)       | 1850 ± 274| 1810 ± 659| 2390 ± 729| 1820 ± 519| 0.81                      |
| Energy from carbohydrate (%) | 45.5 ± 8.57| 28.6 ± 12.3| 41.8 ± 7.31| 56.0 ± 7.82| <0.001                    |
| Energy from fat (%)       | 32.1 ± 4.75| 44.4 ± 10.1| 38.1 ± 6.74| 33.1 ± 7.90| 0.014                     |
| Energy from protein (%)   | 19.6 ± 6.82| 24.9 ± 6.97| 18.4 ± 6.75| 9.47 ± 1.61| <0.001                    |
| Total carbohydrate (g)    | 215 ± 55.9| 133 ± 71.7| 253 ± 81.4| 279 ± 116| <0.001                    |
| Total sugars (g)          | 58.0 ± 33.7| 59.1 ± 41.8| 67.9 ± 35.7| 68.0 ± 29.3| <0.001                    |
| Added sugars (g)          | 21.4 ± 23.01| 17.9 ± 16.8| 32.0 ± 24.4| 24.9 ± 19.2| 0.067                     |
| Total fiber (g)           | 23.2 ± 7.24| 19.0 ± 8.93| 26.2 ± 7.85| 36.9 ± 12.7| <0.001                    |
| Total fat (g)             | 68.5 ± 16.6| 92.9 ± 37.9| 105 ± 43.9| 684 ± 24.5| 0.069                     |
| Total protein (g)         | 90.5 ± 26.0| 110 ± 40.4| 108 ± 40.1| 479 ± 15.8| <0.001                    |
| Animal protein (g)        | 53.8 ± 34.2| 84.4 ± 36.2| 72.9 ± 38.2| 465 ± 5.1| <0.001                    |
| Vegetable protein (g)     | 36.7 ± 16.3| 26.1 ± 25.0| 35.4 ± 9.6| 43.3 ± 15.1| 0.031                     |
| Methionine (g)            | 1.95 ± 0.812| 2.56 ± 0.954| 2.47 ± 1.07| 0.761 ± 0.278| <0.001                    |
| Cystine (g)               | 1.19 ± 0.346| 1.37 ± 0.544| 1.42 ± 0.519| 0.748 ± 0.229| <0.001                    |

**Changes in SRB and bacterial community composition associated with dietary sulfur intervention**

Amplicon sequencing of the 16S rRNA genes allows detection and quantification of the relative abundances of members of the genus Desulfovibrio, which is thought to be the dominant SRB in the human microbiome, as well as the members of the genus Bilophila, which contains sulfite-reducing bacteria previously found at increased abundances following animal-based dietary intervention. The relative abundances of Desulfovibrio were found to differ significantly among participants of the dietary sulfur intervention study (ANOVA P = 0.001; Figure 1). In fact, two of the participants (01 and 02) had barely detectable or undetectable levels of Desulfovibrio. There was no correlation between diet and relative abundance of Desulfovibrio in any of the individuals (P = 0.677). Similarly, no differences in relative abundances of Bilophila were observed.
among any of the individuals or dietary arms ($P = 0.850$). The relative abundances of *Desulfovibrio* and *Bilophila* were significantly and positively correlated in this cohort ($\rho = 0.347$, $P = 0.031$).

The dominant genera in fecal microbial communities of participants were *Bacteroides*, *Prevotella*, and *Faecalibacterium* (Figure 1). Only a few OTUs showed significant differences in relative abundance between the High-S and Low-S dietary interventions (Figure 2(a)). Similarly, no significant differences in the Shannon or Chao1 indices were observed among the dietary arms (i.e., High-S, Low-S, or washout, Supplementary Table S1).

Ordination of Bray-Curtis dissimilarities among samples by principal coordinate analysis revealed

![Distributions of abundant genera in fecal samples from individual participants throughout the course of the diet intervention study.](Image)

Figure 1. Distributions of abundant genera in fecal samples from individual participants throughout the course of the diet intervention study. Numbers in parentheses reflect numbers of samples represented in each stacked bar. Relative abundances of *Desulfovibrio* (●) and *Bilophila* (●) taxa are shown on the right y-axis. Error bars reflect the standard error. Although the mean relative abundances of *Desulfovibrio* in subject 03 appear to correlate with dietary sulfur content, it is notable that on day 3 into the High-S diet the relative abundance of *Desulfovibrio* in this participant’s stool increased from 0.3% to 1.3%, but it dropped to 0.04% on day 7 into the High-S diet.

![Genus-level classification of OTUs that differed significantly between the dietary interventions in A) the sulfur dietary intervention and B) the animal-/plant-based dietary intervention. Significant differences were determined by LEfSe, comparing only the samples reflecting only the communities in samples collected during the dietary intervention (not washout or baseline). For simplicity, only samples with LDA scores ≥3.0 are shown in panel B.](Image)

Figure 2. Genus-level classification of OTUs that differed significantly between the dietary interventions in A) the sulfur dietary intervention and B) the animal-/plant-based dietary intervention. Significant differences were determined by LEfSe, comparing only the samples reflecting only the communities in samples collected during the dietary intervention (not washout or baseline). For simplicity, only samples with LDA scores ≥3.0 are shown in panel B.
a distinct separation of the communities from different subjects (Figure 3(a)). Furthermore, differences in community composition were significantly different among subjects (ANOSIM \( r = 0.997, P < 0.001 \)), but community compositions were not significantly differentiated on the basis of dietary intervention \( (i.e., \text{washout, High-S, or Low-S diet}; r = -0.031, P = 0.743) \). Moreover, when subjects were analyzed individually (Figure S1), differences in community composition also did not differ based on dietary intervention (ANOSIM \( P \geq 0.093 \)).

Shotgun sequencing revealed a similar taxonomic distribution to that characterized by amplicon sequencing (Figure 4), although members of the genera *Desulfovibrio* and *Bilophila* were not detected in the shotgun sequencing dataset. Microbial communities in individual subjects were predominantly comprised of species within the *Bacteroides, Prevotella*, and *Faecalibacterium*, although fewer members of less abundant genera were characterized (Figure 4). Notably, ~45% of the fecal bacterial community in participant 03 was comprised of *Prevotella copri*. While abundances of this bacterium were greater in communities from samples collected during the washout or on the Low-S diet (Figure 4), differences in relative abundances of the predominant species did not differ significantly by diet (ANOVA \( P \geq 0.111 \)), among all four subjects together. Ordination of Bray-Curtis distances similarly revealed the separation of communities by an individual (Figure S2, ANOSIM \( r = 0.622, \text{pairwise } P \leq 0.006; \text{Bonferroni-corrected } \alpha = 0.008 \)), but not by diet \( (r = -0.028, P = 0.754) \).

Similarly, functional annotations revealed the separation of communities by an individual (ANOSIM \( r = 0.389, P < 0.001; \text{Figure S3} \)), but not due to dietary intervention \( (r = 0.025, P = 0.217) \). No functional gene categories (characterized using level 4 EC nomenclature) or pathway abundances, normalized as percentages, differed significantly as a result of diet among all participants or individuals, as determined by LEfSe (data not shown).

To assess the functional capacity for sulfate reduction among individual participants, the relative abundances of sulfate adenylyltransferase (EC 2.7.7.4) and adenylylsulfate reductase (EC 1.8.99.2) were specifically investigated among individual participants. While the relative abundance of sulfate adenylyltransferase was significantly greater in participant 02 \( (\text{post-hoc } P \leq 0.020) \) than all other participants, no other significant differences among individuals or dietary arm were noted, and other genes known to be involved in dissimilatory sulfate reduction could not be identified. Ordination of gene abundances by Bray-Curtis dissimilarity showed some overlap in functional
capacity among individuals, with statistically similar distributions of functional genes between Subjects 01 and 02, 02 and 03, and 03 and 04 ($P \geq 0.044$, Bonferroni-corrected $\alpha = 0.008$).

Distribution of SRB in healthy stool donors

Results of this small dietary intervention study suggested that the abundances of Desulfovibrio and Bilophila in fecal microbiota of healthy individuals are not altered by short-term dietary interventions. Given our hypothesized importance of the relative abundance of SRB in the selection of stool donors for FMT treatment of patients with IBD, we evaluated the distribution of members of the sulfate-reducing genus Desulfovibrio and the sulfite-reducing Bilophila in 19 pre-screened, healthy stool donors who have participated in our FMT donor program. We performed 16S rRNA gene profiling of bacterial communities in 350 fecal samples collected over time from the individual donors (Figure S4). Different numbers of samples per donor reflect differences in the frequency of donation for each donor, and samples were evaluated without respect to time between donations. While Desulfovibrio was not detected in samples from five donors (numbers 3, 6, 13, 47, and 51, with 13, 1, 11, 3, and 18 samples, respectively), Bilophila was almost always present and was undetectable in only Donor 16. The majority of healthy individuals had bacterial communities comprised of only a small (<0.1%) proportion of Desulfovibrio. Five donors had greater proportions of this genus, which was consistently present at $\geq 0.1\%$ of the community, but never exceeded 1.0%. In contrast, Bilophila comprised, on average, $\leq 0.2\%$ of communities. Moreover, abundances of Desulfovibrio and Bilophila were weakly negatively correlated with each other (Spearman’s $\rho = -0.213$, $P < 0.0001$). Ordination by PCoA revealed independent clustering of communities by an individual (Figure S5), and ANOSIM revealed significant differences in community compositions among all individuals ($r = 0.978$, $P < 0.001$).

Animal- and plant-based dietary changes in SRB and bacterial community composition

We also analyzed published sequence data from the animal- and plant-based dietary intervention study in 10 subjects reported by David and colleagues.\(^5\)
Similar to what was seen in our dietary intervention cohort, the abundances of *Desulfovibrio* differed significantly among individuals (ANOVA < 0.0001; Figure S6A), but not as a result of a change in diet (P = 0.126; Figure S6B). In contrast, the abundances of *Bilophila* differed significantly among individuals and dietary treatment arms (P < 0.0001) and were present at significantly greater abundances in samples collected while on the animal-based diet (P < 0.0001). The abundances of *Desulfovibrio* and *Bilophila* were weakly negatively correlated in this cohort (r = -0.307, P < 0.0001), in contrast to data obtained from our intervention cohort.

Bacterial communities characterized from the prior dietary intervention studies were comprised of much greater abundances of members of the *Bacteroides*, with very low abundances of *Prevotella*, relative to our dietary intervention cohort (Figures S6 and S7). David and colleagues also observed significantly greater abundances of several OTUs within the genus *Bacteroides* in samples obtained during the plant-based diet, relative to that seen during the animal-based diet in the study. However, similar to what was found our dietary intervention, few significant differences in alpha diversity were observed among dietary arms (i.e., baseline, diet, or washout; Table S1). Despite this, the Shannon indices were lower when on an animal-based diet (4.03 ± 0.09), relative to that seen at baseline or washout from either diet (means 4.27–4.30; post-hoc P ≤ 0.019). More importantly, no significant differences (r = 0.015, P = 0.272) in community composition related to diet were observed among all samples, and microbial communities from the same individuals did not differ between the animal- and plant-based dietary arms (r = -0.029 to 0.244, P ≥ 0.001; Bonferroni-corrected α < 0.001), except in one case (r = 0.277, P < 0.001).

The detection of some sulfate reducers among intestinal microbiota has been shown to be problematic. For examples, *Desulfovibrio* could not be identified in the CAFE study of Wu et al., which tested the stability of the microbiome in 10 subjects randomized to high-fat/low fiber or low-fat/high fiber diets in a 10-day controlled feeding study. Similarly, *Bilophila* was rarely detected in data from the CAFE study and was sporadically present in only three subjects at abundances ≤0.15% of the total microbial community. Information regarding which subject received which diet was not available from this study, but the only significant difference in alpha diversity was observed between samples collected from the 2nd and 9th collection days, where communities on the 9th collection day had greater Shannon indices (2.50 ± 0.14 vs. 2.21 ± 0.19; P = 0.026; Table S1). Ordination of Bray-Curtis dissimilarity matrices in both the animal/plant and CAFE dietary interventions showed significant clustering of communities by subject (Figure 3(b, c), ANOSIM r = 0.922 and 0.811, respectively, P < 0.001).

**Discussion**

This study was initiated to test whether the relative abundance of SRB in the intestinal microbiota is rapidly responsive to dietary sulfur content. The dominant SRB in the human colon is thought to be members of the genus *Desulfovibrio*, in the δ subdivision of the class Proteobacteria. However, we found no changes in relative abundances of *Desulfovibrio* with our dietary interventions, which tested reasonable extremes of sulfur intake. Similarly, we saw no impact on the relative abundances of *Desulfovibrio* on our re-analysis of data from the David, et al. study of microbiome responses to animal- versus plant-based dietary extremes. Similar to what was seen in our prior study by David, et al., we noted an increased relative abundance of *Bilophila* in the animal-based diet, but these short-term dietary changes did not lead to major changes in the overall fecal microbial community structure. Rather, bacterial community compositions remained distinct among individuals by taxonomic and gene content analysis. These results are consistent with the conclusions reached by Wu and colleagues in the CAFE study.

The obvious limitation of our study is its small size. However, the data allow a power analysis to determine the necessary number of human participants to detect differences in the SRB genera *Desulfovibrio* and *Bilophila* using amplicon-based sequencing of the V5-V6 hypervariable regions of the 16S rRNA gene. Changes in the abundance of *Desulfovibrio* between high- and low-S diets among all participants had a moderate effect size of 0.40, while the effect size for *Bilophila* was smaller (0.14). As a result, a sample size of 49
individuals was calculated to provide statistical power ($\beta = 0.80$, $\alpha = 0.05$) to detect significant differences in *Desulfovibrio* abundances, while $n = 374$ is necessary for powered analysis of changes in *Bilophila* abundances.

In contrast to results presented in the study by David et al. we did not find an increase in *Bilophila wadsworthia* during a High-S diet that had high protein and fat content. However, our *ad libitum* diet had relatively lower content of fat, reaching a peak at 44.4% in the High-S intervention as compared to 69.5% of kcal in the dietary intervention used by David *et al*. Therefore, it is possible that participants in the study done by David, *et al.* experienced greater induction of taurocholate synthesis, which provided more taurine to *B. wadsworthia*. This, however, was not measured. Fiber intake was greater, on average, in our intervention cohort, declining an average of just 4 g per day in the High-S diet, as compared to baseline and reaching a peak of 36.9 g/day in the Low-S diet.

The resilience of the overall intestinal microbial community structure to dietary extremes, as measured by the taxonomic composition of bacteria and their gene content in different individuals, may appear somewhat surprising. The current literature generally emphasizes rapid responsiveness of microbiota to the amount and composition of the intestinal nutrient flow. However, given the redundancy in the functional potential of different microbial taxa and a high degree of compositional variation among individuals, taxonomic and metagenomic analyses tend to accentuate inter-individual differences due to the unique microbial assemblages comprising each person’s microbiota. Similar to many studies, we did see some OTUs that segregated by High-S and Low-S dietary interventions. However, the biological significance of these changes in our study is unclear due to an under-powered sample size. All such findings require confirmation in much larger validation cohorts. It is also likely that functional activity of microbial communities, which may be measured by transcriptomics and metabolomics, is more likely to reflect short-term dietary changes than strictly taxonomic analyses, which provided insufficient sequence depth to characterize most genes likely involved in sulfate reduction pathways.

Since our study had a number of limitations, no definitive conclusions can be drawn from the pilot data generated here. Moreover, the diets were self-administered and sulfur content was estimated from self-reported intake of protein. Other dietary sources of sulfur, including food modifiers and additives, such as sulfiting agents, carrageenan, and non-protein sources of sulfur in some plant-based foods, none of which are quantifiable by the methodology we employed. However, these are relatively minor sources of dietary sulfur compared to protein. Methodological weaknesses also included the variability in baseline diets, lack of randomization with respect to the initial diet, and failure of consumption during the washout period to match the baseline. All of these factors could have contributed to negative results.

The lack of detection of SRB in some samples may reflect relative abundances near the sequencing limit of detection, rather than true absence. As a result, *Desulfovibrio* and *Bilophila* were not detected in the shotgun dataset, which likely reflects a lower sensitivity of this method to detect rare taxa. Importantly, one of the goals of the current work was to investigate SRB as a potential target for rational donor selection for FMT treatments in conditions associated with gut inflammation. We did observe differences in baseline SRB relative abundances among healthy stool donors in our program; however, extrapolation of these data to FMT outcomes will require further investigations. The responsiveness of the relative abundances of SRB was the main focus of the study. While we could not detect an effect of dietary extremes on the abundance of SRB or the individuality of microbiomes in participants, fluctuation among different taxa was noted in the course of the study and samples during the wash-out were not identical to baseline. It is possible that other taxa or functional microbial groups are more responsive to changes coincident with sulfur content extremes, such as fiber content or the sulfur compounds present, but could not be identified given the small size of the study.

In summary, this small, pilot study revealed very low abundances of two groups of SRB that were somewhat consistent within individuals, but varied with low-to-moderate effect sizes, as a result of the dietary intervention. We did observe
considerable heterogeneity among healthy individuals with respect to the relative abundances of SRB examined. Future, large cohort studies will be necessary to more accurately characterize shifts in these low-abundance members of the community in response to dietary changes. Moreover, it may be that other SRB are involved in intestinal sulfur metabolism that needs to be defined by multi-omics and newer culture-based studies.

**Materials and methods**

**Dietary intervention study**

A crossover pilot study was conducted in four healthy volunteers. Each participant consumed two 10- to 14-day intervention diets: 1) one low in sulfur-containing amino acids (Low-S diet), emphasizing plant-based foods and fat sources; and 2) another high in sulfur-containing amino acids (High-S diet), emphasizing animal protein and fat sources, soy products, nuts, seeds, and cruciferous vegetables. Interventions were separated by a 10- to 14-day washout period, in which participants resumed their typical diets. Participants meeting inclusion criteria were consented at the screening clinic visit. Routine clinical examinations, assessments, laboratory evaluations, and sample collections were completed at 10 clinic visits over approximately 50 days.

Participants were recruited from among biomedical researchers who were interested in dietary effects on the intestinal microbiome. No monetary reimbursement was provided and diets were self-administered. Three of the individuals chose to begin the intervention with the high-sulfur diet, and one chose to begin the intervention with a low-sulfur diet. Participants were assessed at baseline to determine demographics and characteristics, dietary habits, and measurements of endpoints of interest. Frequent communication between participants, the registered dietitian, and other study investigators were maintained throughout the trial to ensure compliance with dietary intake and data collection.

The specifics of recruitment, participants, and study procedures are detailed in the Supplementary Materials. The dietary intervention study and investigations within the stool donor program were approved by the Institutional Review Board of the University of Minnesota.

**Diet intervention design**

Two intervention diets were designed by a registered dietitian trained in nutrient analysis, who provided detailed instructions for each intervention diet, based on the information in Supplementary Table 1. High-sulfur foods were defined as having an SAA content >0.5 g/100 g food item. Sulfur content (g) was derived from total methionine and cysteine per 100 g of food or beverage, as specified by the USDA National Nutrient Database for Standard Reference, Release 28. Dairy products were included in the High-S diet due to their milk fat content. During the Low-S diet intervention, participants were advised to completely avoid foods high in SAAs, strictly eating foods included on the “Low-Sulfur Foods” list. During the High-S diet intervention, participants were instructed to eat foods strictly from the “High-Sulfur Foods” list. Participants recorded time of food intake and any deviations from the prescribed diet in the provided study log.

**Dietary intake assessment**

Dietary intake data were collected at baseline and during the washout and intervention periods. Three-day diet records were completed by participants prior to the intervention start date, twice during each intervention, twice during the washout period, and one week after the conclusion of the second intervention diet to determine independent, ad libitum dietary habits. A registered dietitian trained and certified in the Nutrient Data System for Research (NDSR) dietary interviewing and assessment protocols developed by the Nutrition Coordinating Center (NCC) at the University of Minnesota instructed participants on methods for accurate quantification and recording of dietary intake. Analysis of 3-day diet records was done using NDSR, version 2016.

**Bioinformatics**

Sequence data were processed using mothur (version 1.35.1). Amplicon sequence data were trimmed to 150 nt to remove low quality
3′-regions and paired-end joined using fastq-join,\textsuperscript{15} with a mean overlap of approximately 20 nt. Quality trimming was performed to remove sequences with homopolymers >8 nt, ambiguous bases, >2 nt mismatches to primer sequences or mean quality scores <35 over a sliding window of 50 nt. High-quality sequences were aligned against the SILVA database (version 128),\textsuperscript{16} further screened to match the V5-V6 target region in the alignment, and subjected to a 2% pre-cluster to remove likely sequence errors.\textsuperscript{17} Chimeras were identified and removed using UCHIME (version 4.2.40),\textsuperscript{18} and samples were rarefied by random subsampling to 23,000 reads per sample,\textsuperscript{19} providing a mean estimated Good’s coverage of 99.3 ± 0.00%. The healthy donor dataset was rarefied to 25,000 reads per sample. Operational taxonomic units were classified at 97% similarity by complete-linkage clustering, and taxonomic classification was performed against the Ribosomal Database Project release (version 16).\textsuperscript{20}

Additional datasets from two dietary intervention studies that also utilized next-generation sequencing to characterize the microbiome were obtained from previously published studies archived under SRA BioProject SRP002424 (22) and MG-RAST project number 6248.\textsuperscript{5} Data were processed as described above, except neither data set was screened for matches to primers, which could not be identified in the data, and fastq files were not available for the MG-RAST dataset to screen for quality. The V1–2 hypervariable regions were amplified from samples from the CAFE study (SRP002424) and sequence reads were rarefied to 2,700 reads per sample, in the present analysis. The animal/plant diet intervention study sequenced the V4 hypervariable region and samples were rarefied to 10,000 reads per sample, in the present analysis. The mean Good’s coverage of all samples in the previous dietary intervention datasets were 99.3 ± 0.0, and 91.1 ± 0.2%, respectively. Grouping of samples from David, et al.\textsuperscript{5} was done as described in the original manuscript based on transit time – the dietary intervention for the animal-based diet was taken as sample collections from days 2–5 and days 2–4 for the plant-based diet.

Taxonomic information was obtained from shotgun sequencing data using MetaPhlAn2 (version 2.6.0),\textsuperscript{21} and functional annotations were performed using HUMAnN2 (version 0.11.1).\textsuperscript{22} Relative abundances of species were interpreted as a percentage of 10,000 reads per sample for downstream analyses. Gene family annotations were made against the Uniref 90 database and analyzed based on enzyme commission (EC) level 4 assignments. Taxonomic and functional data were rarefied such that each sample had the same number of total annotations.

**Statistical analyses**

Coverage, alpha, and beta diversity indices; ordination plots; and beta diversity statistics were calculated using mothur. Taxa (OTUs) that were differentially abundant among groups were identified by linear discriminant analysis (LDA) of effect sizes (LEfSe).\textsuperscript{23} Ordination was performed by principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities.\textsuperscript{24,25} Differences in community composition were evaluated by analysis of similarity (ANOSIM) using Bray-Curtis distances.\textsuperscript{26} Differences among groups were evaluated by ANOVA with Tukey’s post-hoc test, and Spearman correlations were performed to relate taxa abundances with gas production. Correlations and ANOVA were done using XLSTAT ver. 17.06 (Addinsoft, Belmont, MA), and all statistics were evaluated at \( \alpha = 0.05 \) with Bonferroni corrections for multiple comparisons.

**Abbreviations**

- FMT: fecal microbiota transplant
- High-S: high-sulfur
- H\(_2\)S: hydrogen sulfide
- IBD: inflammatory bowel disease
- Low-S: low-sulfur
- SAA: sulfur-containing amino acids
- SRB: sulfate reducing bacteria
- UC: ulcerative colitis

**Disclosure of Potential Conflicts of Interest**

A. Dostal Webster is an employee of the International Food Information Council (IFIC) and the IFIC Foundation, which are primarily supported by the broad-based food, beverage, and agricultural industries. This research was completed prior to her employment at IFIC. None of the other authors have any conflicts to declare.
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