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Dietary fiber consumption is associated with the selection of key microbial species but does not affect overall microbial diversity in a Colombian cohort

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SUMMARY The gut microbiome is known to be affected by factors in the host diet. In particular, dietary fiber intake is associated with changes to gut microbial diversity, as one of the key functions of the microbiota is to degrade non-digestible fibers into nutrients and beneficial compounds such as short chain fatty acids (SCFAs). These changes have been characterized in populations at fiber intake extremes, such as populations following Westernized or hunter-gatherer (non-Westernized) lifestyles. However, this relationship has not been well-studied in populations undergoing a shift from a hunter-gatherer to a Westernized lifestyle, a phenomenon currently underway in Colombia. To understand the impact of dietary fiber intake on the host gut microbiota, we analyzed an existing dataset from a Colombian cross-sectional study using a bioinformatics approach. We found that overall microbial diversity does not seem to be impacted by fiber intake. However, there was evidence of over-representation of certain anti-inflammatory SCFA-producing microbial species in individuals with adequate fiber intake. Indicator species analysis also revealed that the enrichment of several SCFA-producing microbes were indicative of adequate fiber consumption. Overall, our findings indicate that dietary fiber does not influence microbial diversity but does enrich specific beneficial microbial taxa in the Colombian population. These results appear to support a beneficial role of fiber consumption and could ultimately aid in our understanding of the gut microbiome in semi-Westernized populations such as the Colombian people.

INTRODUCTION

Diet is widely acknowledged to have a significant impact on the composition of the gut microbiota (1). However, the bulk of our understanding of this relationship is derived from previous comparative microbiota studies that have mainly focused on comparing heavily Westernized versus hunter-gatherer diets (2). The Westernized diet is common in North America and Europe, while hunter-gatherer diets are more frequently observed in less industrialized areas, such as the Hadza tribe in Tanzania (2). These diets represent two different extremes and are distinct from each other due to differences in the total quantity of carbohydrates consumed, with Western populations consuming greater proportions of easily digestible starches and sugars, and hunter-gatherers consuming mainly unrefined, complex carbohydrates (2). While the traditional Colombian diet is similarly composed of complex carbohydrates, with recent urbanization and economic growth, Colombians increasingly experience a Westernized lifestyle (2). Aspects of this Western lifestyle seen in the Colombian population include a shift in population from rural to urban settings, rising rates of physical inactivity, and increasing prevalence of diseases such as obesity and

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cardiovascular disease (2). However, the composition of the traditional Colombian diet coupled with the observation that Colombians possess gut microbiotas distinguishable from Westernized countries suggest that this Westernization process has not extended to the diet and is not complete (2). These less-studied populations in the midst of Westernization present an intriguing area of research to determine whether the microbiota evolves gradually from a non-Westernized to Westernized composition.

In a Colombian cross-sectional study, de la Cuesta-Zuluaga et al. focused on understanding the effect of the microbiota on several health parameters and the relative abundances of microbial taxa used to distinguish between Westernized versus non-Westernized populations (2). They found that in the Colombian cohort, taxa normally characteristic of non-Westernized hunter-gatherer diets, such as *Prevotella* and *Treponema*, were present (2). Interestingly, taxa such as *Bacteroides* and *Bifidobacterium,* which are characteristic of industrialized countries, were also detected (2). This indicates a gut microbiota phenotype characteristic of both extremes, yet unique in the finding that the abundances of these species were different compared to what is seen in hunter-gatherer and industrialized populations (2). They also found that the gut microbiota composition is associated with conditions such as obesity and cardiovascular disease. Decreased risk of developing these diseases was observed in individuals with gut microbiotas dominated by microbes correlated with *Ruminococcaceae* and *Akkermansia-Bacteroidales* bacterial families (2). However, the specific links between key microbial taxa, microbiome diversity, and dietary fiber intake have yet to be fully characterized in this cohort (2).

Highlighting the importance of diet in microbiome composition, Rothschild et al (2018) demonstrated that over 20% of inter-person microbiome variability is attributed to diet-related factors (3). This variation in host microbiome caused by diet is particularly important because many studies over the years have demonstrated the relationship between microbial diversity, functionality, and host health (4–8). In a mutualistic relationship, commensal microbes are known to feed on nutrients derived from the host diet, producing metabolites that can in turn benefit and alter the host environment (5). Since these microbes play a crucial role in the metabolism of nutrients, immune function, and pathogen defense, changes in the gut microbiota composition have thus been linked with various inflammatory and autoimmune disorders such as inflammatory bowel disease (IBD) (4).

Short-chain fatty acids (SCFAs) are one well-studied example of a microbial metabolite with implications on host health (5). Indeed, SCFA-producing microbes present a highly relevant subset of microbes to study in relation to diet because of their role in metabolizing dietary fiber. Importantly, complex polysaccharides such as fibers cannot be broken down by host enzymes (5). These fibers can only be degraded through anaerobic fermentation by certain members of the gut microbiota such as SCFA-producing species (4, 5). SCFAs such as butyrate, acetate, and propionate are involved in maintaining the integrity of the intestinal barrier, increasing mucus production, decreasing inflammation, and are used as a metabolic energy source by colonocytes in the large intestine (4, 5, 9). Healthy individuals with high fiber intake are known to possess microbiomes not only enriched in these SCFA-producers, but also with increased microbial diversity, a feature that is said to contribute to positive host health outcomes (10). Analysis of gut microbiomes derived from individuals consuming a Westernized diet low in fibers unable to be digested by the host has also been associated with decreased abundance of SCFA-producing microbes, which has been linked to higher rates of inflammatory and cardiometabolic diseases such as IBD and diabetes (2). As such, gut dysbiosis as a result of inadequate fiber intake could potentially lead to a decrease in SCFA-producing microbes and thus a decrease in anti-inflammatory effects that could negatively impact host health.

Overall, the links between fiber consumption in the Colombian population and gut microbiota composition remain an interesting area to explore as they are a population in the midst of Westernization. In this study, we aimed to investigate the differences in gut microbiota diversity and abundance of known SCFA-producing microbes between Colombians with adequate versus inadequate fiber consumption. In order to eliminate potential confounding factors on gut microbiota composition such as age and sex, we also replicated our analyses on a subset of older Colombian females within the cohort. Using bioinformatics analyses, we demonstrated that although there is no evidence of overall

changes in diversity, fiber consumption results in changes to the relative abundances of specific species of microbes. These results may help characterize the links between dietary fiber intake and the gut microbiota. This will ultimately aid in our understanding of the gut microbiome in semi-Westernized populations such as the Colombian people.

METHODS AND MATERIALS

Sourcing gut microbiome sequencing data. The 16S rRNA gene sequencing data from 441 Colombian adults' gut microbiomes was obtained from a study performed by de la Cuesta-Zuluaga et al., which examined the effects of Westernization on the gut microbiota (2). Raw sequences can be found at SRA-NCBI under Bioproject PRJNA417579 (2).

Grouping of samples based on fiber intake in the whole Colombian cohort. In addition to the gut microbiome sequencing data, the metadata provided by the original paper also included information on daily dietary fiber intake and caloric consumption (2). To account for differences in caloric intake, fiber consumption (recorded in total grams of fiber) was divided by total calories consumed per day. Samples were then grouped into individuals with "adequate" and "inadequate" daily fiber consumption. The clinically relevant cut-off utilized in this study was determined to be 14 grams of fiber per kilocalories of food consumed as recommended by the Government of Colombia (11). After grouping, it was revealed that sample sizes were clearly disproportionate, with the vast majority of samples clustering in the "inadequate" fiber intake group. In order to prevent this large difference in sample size from potentially impacting our analyses, we further subset samples in the "inadequate" category into groups of individuals with "high inadequate", "mid inadequate", and "low inadequate" fiber intake by evenly dividing the number of samples in the inadequate group by 3.

Subsetting of samples based on sex and age and subsequent grouping based on fiber intake. In consideration of previous research where it was found that the gut microbiome changes with sex and age (12, 13), the Colombian adults were subset by sex (male/female) and age group. The age groupings utilized in this study were the categories previously assigned by de la Cuesta-Zuluaga et al. where "younger adults" were aged 18 - 40 and "older adults" were aged 41 - 62. The older female cohort was chosen for further analyses because the majority of individuals in our sample group of interest (individuals with adequate fiber intake) belonged to this group. As before, the subset older female samples were then grouped into "adequate" and "inadequate" fiber intake groupings based on the Colombian government's recommended daily fiber intake (normalized by caloric intake) (11). Samples in the "inadequate" category were then further subset into groups of "high", "mid", and "low" inadequate fiber intake, with each grouping composed of relatively even sample numbers.

QIIME2 processing of metadata. Microbiome analysis was performed in QIIME2 v2021.11 (14). Amplicon sequence data from the gut microbiomes of 441 Colombian adults were demultiplexed, quality filtered, and denoised using the q2-demux and q2-dada2 plugins, the latter of which is based on DADA2 (15). Forward (GTGCCAGCMGCCGCGGTAA) and reverse (GGACTACHVGGGTWTCTAAT) primer sequences provided by de la Cuesta-Zuluaga et al. were trimmed to extract the V4 hypervariable region of interest, and the sequences were truncated at the 250th nucleotide to maintain mean quality scores >Q30. The QIIME2 classification plugin (q2-feature-classifier) was used to classify amplicon sequence variances (ASVs) via a naive Bayes machine-learning taxonomic classifier trained against the SILVA 138 99% identity reference sequence database (16). MAFFT (via q2-alignment) and FastTree2 (via q2-phylogeny) were used to perform multiple sequence alignment and generate a phylogenetic tree respectively (17,18). After filtering out mitochondria and chloroplast sequences, an alpha rarefaction curve was generated to determine optimal sampling depth for diversity analyses. Rarefaction depth was chosen to maximize the number of samples retained in the "adequate" fiber consumption group while also maximizing the number of features analyzed. For analysis of microbial diversity, samples were randomly subsampled to a sampling depth of 22513 for the whole cohort analyses, and 9438 for the subset older female cohort. All QIIME2 processing is outlined in Script #1.

Alpha and beta diversity analyses. Alpha and beta diversity analyses were performed on the whole cohort and the subset older female cohort in R v 4.2.2 using the phyloseq package (19, 20). All alpha diversity metrics (observed features, Chao1, ACE, Shannon's, Simpson's, Inverse Simpson's, and Fisher indices) were calculated for each of the groupings based on data rarefied to the indicated sampling depths. Similarly, beta diversity metrics (Bray-curtis, Jaccard, weighted UniFrac, and unweighted UniFrac) were calculated for each of the groupings using the vegan package in R v 4.2.2 (21). All steps are outlined in Script #2.

Differential abundance of microbial genera using DESeq2. Analysis of differentially abundant microbial genera was conducted using the DESeq2 package in R v 4.2.2 (22). Log2fold-change was calculated using samples with inadequate fiber intake as a reference. Volcano plots comparing log10padj to log2fold-change were generated and annotated using ggplot2 and ggrepel respectively (23, 24). For the total cohort, we labeled only significantly differentially abundant genera with $p_{adj} < 1 \times 10^{-7}$. Likewise, for the older female cohort, we chose to label only significantly differentially abundant genera with $p_{adj} < 1 \times 10^{-4}$. All steps are outlined in Script #3.

Determination of core microbiome associated with adequate versus inadequate fiber intake. Core microbiome analysis was performed using the microbiome package in R v 4.2.2 (25). A Venn diagram of the number of shared core microbiome members was subsequently generated using the ggVennDiagram package (26). All steps are outlined in Script #3.

Identification of indicator species associated with fiber intake. Finally, we performed an indicator species analysis on both the whole cohort and older female cohort using the indicspecies package in R v 4.2.2 (27). All steps are outlined in Script #3.

Statistical analyses. Alpha diversity statistical analyses were performed using pairwise Kruskal-Wallis tests in R v 4.2.2. Beta diversity statistical analyses were performed using PERMANOVA in R v 4.2.2 (28). For all statistical testing, a p-value of less than 0.05 was considered significant.

RESULTS

Fiber intake does not affect alpha diversity of the gut microbiome at a whole-cohort level. Due to the well-established connection between diet and gut microbiome diversity, we first set out to investigate differences in alpha diversity between individuals with adequate and inadequate dietary fiber consumption (1). To accomplish this, we categorized individuals from the whole cohort ($n = 441$) based on the Colombian government's recommended daily fiber intake value of 14 g/kcal (11). After categorization, 419 individuals were designated as having "inadequate" fiber intake, and 22 individuals as having "adequate" fiber intake. To account for the noticeable differences in sample size between "inadequate" and "adequate" fiber groupings, we further subsetted the "inadequate" samples into three smaller subgroupings: "high inadequate" ($n = 140$), "mid inadequate" ($n = 139$), and "low inadequate" ($n = 139$) (Fig. 1A). However, we found no significant differences between fiber groupings across all alpha diversity metrics tested (Fig. 1C). For measures concerning species richness, we compared differences in the number of observed features and Chao1 indices with fiber intake. We also investigated differences in alpha diversity using measures incorporating species richness, abundance, and evenness, such as the Shannon's, Fisher's, Simpson's, and Inverse Simpson's and abundance-based coverage estimator (ACE) indices. However, across all diversity metrics tested, statistical analysis via Kruskal-Wallis testing revealed that there were no significant differences in microbiome diversity with dietary fiber intake. This finding was also supported by beta diversity analysis of weighted UniFrac ordination, which showed no visible clustering on Principal Coordinate Analysis (PCoA) plots (Fig. S1). These results suggest that fiber consumption has minimal to no effects on gut microbiome diversity in de la Cuesta-Zuluaga et al.'s Colombian cohort.

Fiber intake does not affect alpha diversity of the gut microbiome in older females. We also decided to investigate a smaller subset of the cohort in order to account for potential

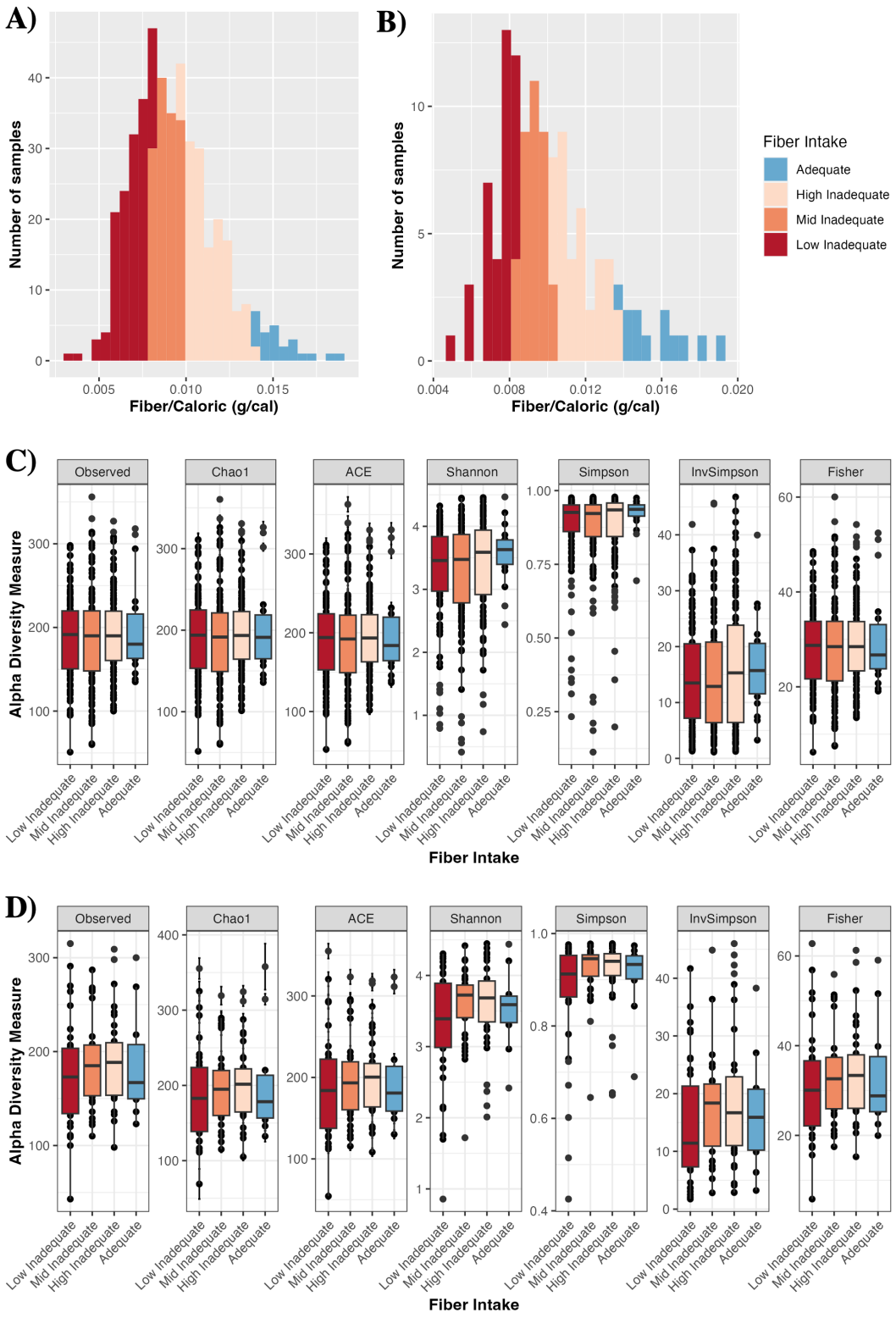

FIG. 1 Alpha diversity of the gut microbiome is unaffected by fiber intake at both a whole-cohort level and in an older female subset. **A)** Categorization of whole-cohort data (n = 441) into "adequate" (n = 22), "high inadequate" (n = 140), "mid inadequate" (n = 139), and "low inadequate" (n = 139) fiber groupings. B) Categorization of subset older female individual data (n = 120) into "adequate" (n = 11), "high inadequate" (n = 37), "mid inadequate" (n = 36), and "low inadequate" (n = 36) fiber groupings. C**)** Alpha diversity metrics calculated at a whole-cohort level based on fiber groupings. **D**) Alpha diversity metrics calculated for older female individuals based on fiber intake. Statistical analysis was performed using Kruskal-Wallis testing, $p > 0.05$ for all tests performed.

confounding variables. From previous studies, age and sex are known to contribute to diversity of the gut microbiome (12, 13). Therefore, we subset the total cohort into females aged 41-62 years old (n = 120). As before, we categorized the samples based on their reported daily fiber intake, assigning them into "adequate" (n = 11), "high inadequate" (n = 37), "mid inadequate" (n = 36), and "low inadequate" (n = 36) fiber intake groups (Fig. 1B). Despite removing the potential confounding variables of sex and age, we observed no significant differences in alpha diversity with fiber intake across each of the previously mentioned diversity metrics (Fig. 1D). This further highlights that fiber consumption appears to have minimal effect on gut microbial diversity, even after accounting for confounding variables such as age and sex.

Adequate fiber intake in both the total and older female cohort is associated with shifts in select bacterial species. Since we did not observe significant differences in diversity from our alpha diversity analyses, we sought to determine if any microbial genera were differentially abundant across samples with varying fiber intake. To accomplish this, we employed the DESeq2 package in R and generated volcano plots to visualize significantly up- and downregulated genera in individuals with "adequate" and "inadequate" fiber intake (Fig. 2A-B) (22). The identities of these differentially abundant genera are also shown in bar plots corresponding to analyses of the total cohort (Fig. 2C) and older female cohort (Fig. 2D). In the whole cohort analysis, we identified *Ruminococcaceae* UCG-010 and *Bacteroides* to be significantly over-represented in individuals with adequate fiber intake (Fig. 2A, 2C). We also observed that there were significantly more species over-represented with inadequate fiber consumption, which included *Prevotella*, *Megasphaera*, *Alloprevotella*, and *Clostridia* UCG-014 (Fig. 2A, 2C). With the subset older female cohort, we identified *Gastraanaerophilales*, *Paraprevotella*, *Corynebacterium*, *Eubacterium*, and *Roseburia* as being significantly over-represented in individuals with adequate fiber intake (Fig. 2B, 2D). Genera that were under-represented with adequate fiber consumption included *Clostridia* UCG-014, *Megasphaera*, *Dialister*, and *Prevotella* (Fig. 2B, 2D). Interestingly, we did not find any significantly over-represented genera in common between total and older female cohorts, though we identified *Megasphaera*, *Clostridia* UCG-014, and *Prevotella* as being similarly under-represented in individuals with adequate fiber consumption across both cohorts. These results suggest that although overall microbial diversity is unaffected by fiber consumption in the Colombian population, several bacterial species experience shifts in relative abundance.

A core microbiome of 8 species comprises all samples regardless of fiber consumption. Since our DESeq2 analysis showed that several genera were differentially abundant between individuals with adequate versus inadequate fiber consumption, we decided to investigate the core microbiome composition of the different groups. We defined members of the core microbiome to be species that are present in over 25% of samples in the group at relative abundances of greater than 1%. Our analysis showed that there were few distinct microbial taxa unique to the core microbiomes of individuals with adequate fiber consumption in the total and older female cohort. There were 3 (12% of identified core microbes) associated with adequate fiber intake in the total cohort and 4 (17%) in the older female cohort (Fig. 3). Among individuals with adequate fiber consumption in both cohorts, there were 3 shared core microbes (12% of identified core microbiome members). The core microbiome shared among all groups tested contained 8 species (33% of identified core microbiome members). These results demonstrate that the microbiomes of each of the four groups are similar since they have a large proportion of shared core taxa.

Individuals with adequate fiber intake can be identified via three indicator genera. The lack of significant differences in alpha diversity prompted further analysis into the unique species present in the gut microbiomes of individuals with adequate versus inadequate fiber intake. To this end, we performed indicator species analysis on both the total cohort and the subset older female cohort. For individuals with adequate fiber intake in the total cohort, we identified 15 indicator species, including *Lachnospiraceae* UCG-001, *Turicibacter*, *Ruminococcaceae* CAG-352, *Eubacterium ventriosum* group, *Gastraanaerophilales*, and *Prevotellaceae* Ga6A1 group (Table 1). In contrast, individuals with inadequate fiber consumption possessed a single indicator species, confirmed to be *Megasphaera* (Table 1). In the subset older female cohort, individuals with adequate fiber intake can be identified

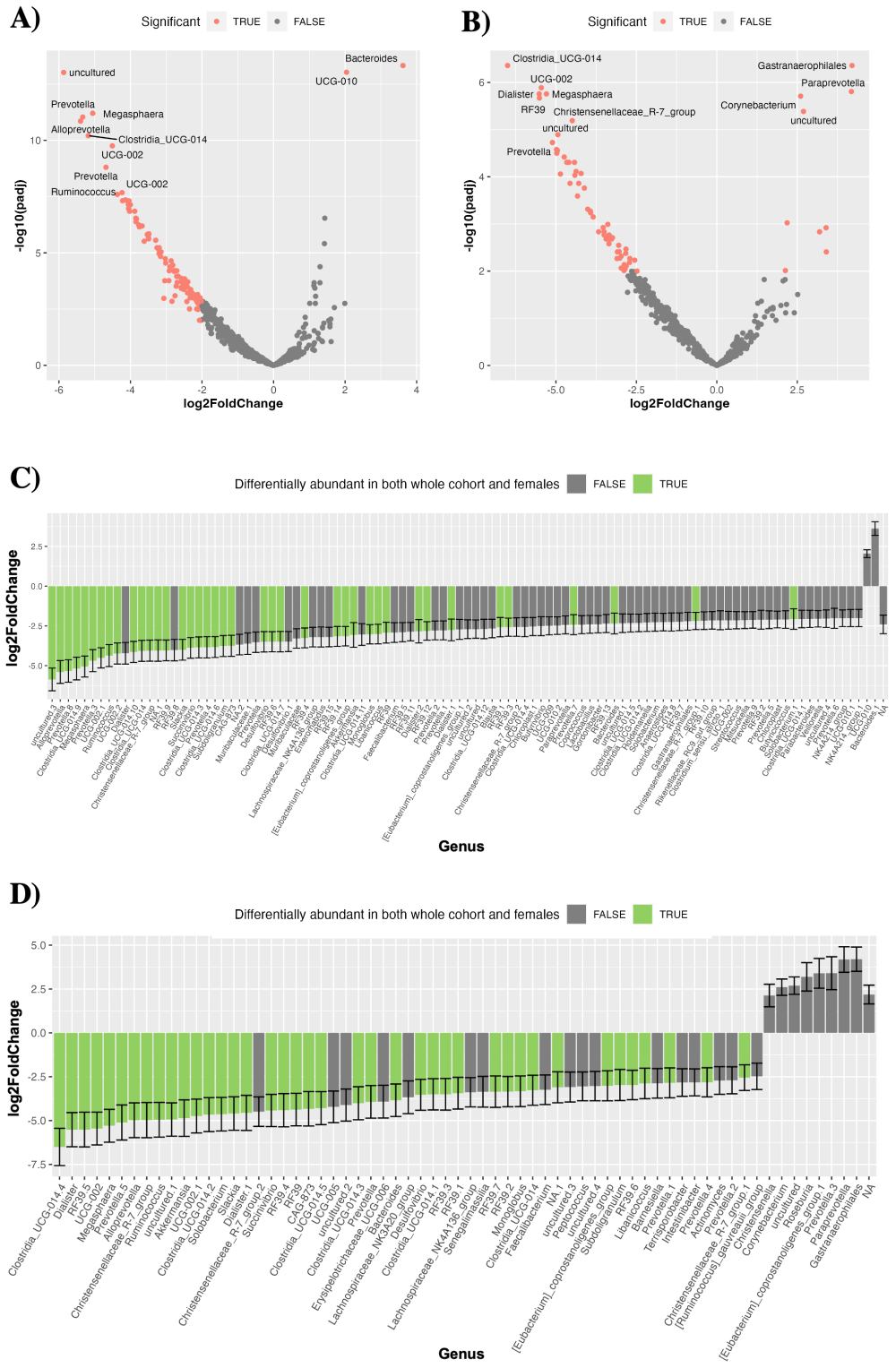

FIG. 2 Adequate fiber intake in both the total and older female cohort is associated with shifts in select bacterial species. **A)** Volcano plot indicating significantly over- and under-represented genera in individuals with adequate fiber consumption across the whole Colombian cohort. Only genera found to be significantly differentially abundant with $p_{adj} < 0.0000001$ are labelled. B) Volcano plot indicating significantly over- and under-represented genera with adequate fiber consumption in the older female cohort. Only genera found to be significantly differentially abundant with padj < 0.0001 are labelled. C**)** Bar plot comparing log2 fold change of genera associated adequate versus inadequate fiber intake across the whole cohort. **D)** Bar plot comparing log2 fold change of genera associated with adequate versus inadequate fiber intake in the older female subset of the cohort.

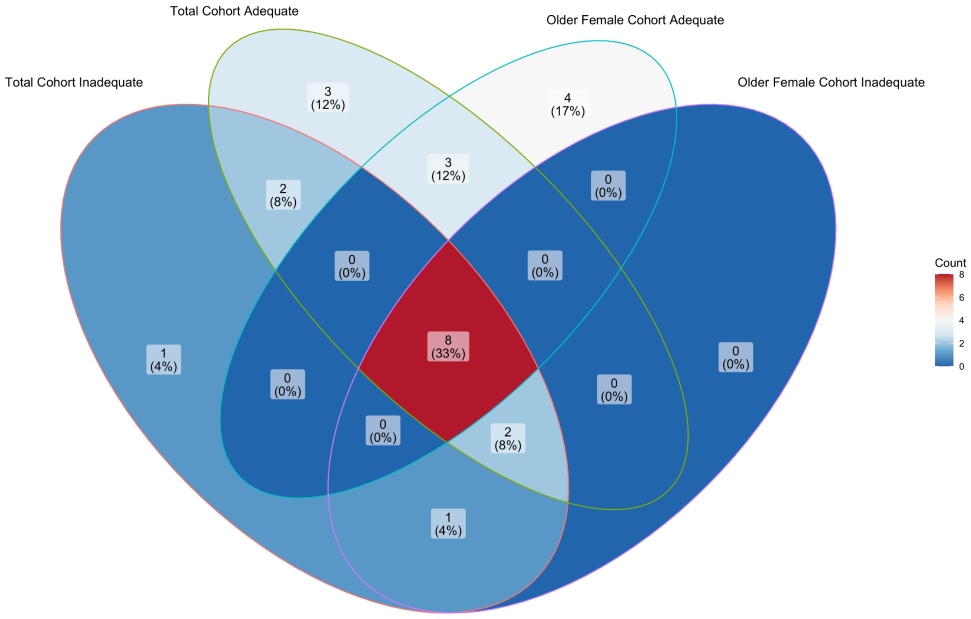

FIG. 3 A core microbiome of 8 species comprises the microbiomes of all samples regardless of fiber consumption. The four-circle Venn diagram shows the core microbiomes as determined by abundant taxa (>1% abundance) present in 25% of individuals in each indicated cohort. 8 species (33% of all core microbes identified) are shared amongst all groups and cohorts tested. 12% and 17% of core microbes are uniquely found in individuals with adequate fiber consumption in the total cohort and older female cohort respectively. Individuals with adequate fiber consumption in both the total and older female cohorts share 3 species comprising their core microbiomes.

via 6 indicator species: *Shuttleworthia*, *Gastranaerophilales*, *Neisseria*, *Staphylococcus*, *Ruminococcaceae* CAG-352, and *Eubacterium eligens* group (Table 1). No indicator species were found to be associated with inadequate fiber intake in this cohort. Interestingly, three genera were found to be common amongst individuals with adequate fiber consumption

TABLE. 1 Microbiomes from individuals with adequate fiber consumption in the total and older female cohort can be identified via three indicator genera. The value "1" denotes that the indicator species is associated with the group in the designated column, while "0" denotes that the indicator species is not present in the respective group. Bolded species represent the ones in common between the whole cohort and older female cohort. Indicator species analysis *p*-value significance threshold was $p < 0.05$. NA or blank values under the species column denote missing species identity.

Genus	***Species***	***Adequate fiber group***	***Inadequate fiber group***	***Indicator species stat.***	***p-value***
Total cohort					
Lachnospiraceae UCG-001	NA	1	0	0.679	0.010
Uncultured	uncultured bacterium	1	0	0.682	0.005
Gastranaerophilales	**NA**	**1**	**0**	**0.734**	**0.005**
Scardovia	*Scardovia wiggsiae*	1	0	0.517	0.030
Neisseria	**NA**	**1**	**0**	**0.527**	**0.010**
Chromohalobacter	NA	1	0	0.229	0.035
[Eubacterium] saphenum group	*Eubacterium saphenum*	1	0	0.319	0.005
Turicibacter	NA	1	0	0.613	0.045
Anoxybacilus	NA	1	0	0.228	0.035
CAG-352	**uncultured bacterium**	**1**	**0**	**0.691**	**0.010**
Oscillospiraceae	uncultured rumen	1	0	0.225	0.035
Lactonifactor	NA	1	0	0.229	0.035
Anaerosporobacter		1	0	0.568	0.015
[Eubacterium] ventriosum	uncultured *Lachnospiraceae*	1	0	0.727	0.005
Megasphaera	*Veillonellaceae*	0	1	0.659	0.045
Older female cohort					
Shuttleworthia	uncultured bacterium	1	0	0.394	0.030
Gastranaerophilales	**NA**	**1**	**0**	**0.819**	**0.005**
Neisseria		**1**	**0**	**0.497**	**0.045**
Maihella	*Desulfovibrio* sp.	1	0	0.376	0.040
Staphylococcus		1	0	0.551	0.035
CAG-352	**uncultured bacterium**	**1**	**0**	**0.735**	**0.040**
[Eubacterium] eligens group		1	0	0.862	0.005

across both the total and the older female cohort. These were *Gastanaerophilales*, *Ruminococcaceae* CAG-352, and *Neisseria* (bolded in Table 1). Overall, these results demonstrate that adequate fiber intake is associated with more unique microbial species compared to individuals with inadequate fiber intake.

DISCUSSION

This study aimed to explore the effect of dietary fiber intake on gut microbial diversity in a Colombian cohort using previously published data collected by de la Cuesta-Zuluaga et al (2). Alpha and beta diversity analyses showed that there were no significant differences in diversity in the whole cohort nor the subset older female cohort. However, both cohorts showed differences in specific microbial taxa associated with inadequate and adequate fiber intake groups based on the DESeq2 and indicator species analyses.

Alpha and beta diversity are unaffected by fiber intake. Common metrics that are examined in gut microbiota studies are metrics of diversity. Alpha diversity metrics describe species diversity within groups, while beta diversity metrics describe species diversity between groups (27). We found that there were no significant differences in alpha and beta diversity metrics in both the whole cohort and sex and age subset group, thus indicating that fiber does not appear to affect microbiome diversity (Fig. 1, Fig. S1). This is inconsistent with much of the previously reported literature, which have demonstrated correlations between dietary fiber consumption and increased gut microbial diversity (29). A possible reason for why we did not see the same trend could be that our sample sizes were not large enough to magnify these differences, or that there were simply no drastic changes to the diversity of the microbiota as measured by alpha and beta diversity metrics. It could also be that within our population of interest, variables other than fiber consumption may be driving these microbiota changes. Based on our DESeq analyses, we did observe evidence of over- and under-representation of specific taxa, which indicates that changes in the microbial population are occurring, but they do not appear to be significant enough to alter measurements of overall diversity **(**Fig. 2). One factor that might influence these changes is that dietary fiber can be consumed in a variety of different forms. For example, studies have shown that soluble, partially soluble, and insoluble forms of dietary fiber can differentially influence the up- and downregulation of specific microbial species (30). Additionally, a host of additional confounding factors, such as cardiometabolic status, obesity, and medication may also influence the observed results (31, 32).

Similar species were observed to be under-represented in individuals with adequate fiber consumption across both the whole cohort and the older females group. Since we did not observe significant differences in alpha and beta diversity, we investigated whether there are any specific differentially abundant taxa with fiber intake. Alpha diversity measures the variation of microbes within a single group, and beta diversity compares the microbial variation between groups. In contrast, the indicator taxa method analyzes the abundance and prevalence of microbes in the gut to identify the taxa characteristic of the specific niche and its environmental conditions (27). In our analysis, *Ruminococcaceae* UCG-010 and *Bacteroides* were found to be over-represented in adequate fiber-consuming individuals within the whole cohort (Fig. 2A, 2C). In other studies, *Bacteroides fragilis* was similarly over-represented in diets rich in soluble fiber, which is in line with the trend seen in this study (33). Comparatively, in our study we found that there were a greater number of species over-represented with inadequate fiber consumption. Interestingly, some of these species are correlated with increased fiber intake including *Megasphaera* (34)*, Prevotella* (35)*,* and *Alloprevotella* (36)*,* since they are thought to be capable of fermenting fiber. It is known that these species are capable of switching their metabolism to be able to utilize host-derived glycans for energy instead of dietary fiber, which may explain their enrichment (37). In the gut, glycans can be found decorating host mucin in the intestinal mucus layer, therefore microbes which are capable of degrading mucin may potentially contribute to altered intestinal barrier function (37). Therefore, increased prevalence of these organisms in individuals with inadequate fiber consumption may be detrimental. Another possibility might be that upregulation of these taxa is in fact linked to other factors such as host protein or fat intake, which can also influence microbial metabolism and induce variations in the microbiome (38).

In the older female cohort, we did not see any species that were also over-represented in the whole cohort. Some genera that were over-represented with adequate fiber consumption in the older female cohort specifically included *Gastranaerophilales*, *Paraprevotella*, *Corynebacterium*, *Eubacterium*, and *Roseburia* **(**Fig. 2B, 2D). *Paraprevotella* is known to be a SCFA producer (39). It produces succinic and acetic acids as the end product of glucose metabolism and is also known to produce butyrate (39, 40). *Roseburia* is also known to be a prominent SCFA producer, mainly producing the SCFA butyrate, which is known to have anti-inflammatory properties in the gut (41). Certain species of *Eubacterium*, such as *Eubacterium rectale,* are also known to be one of the main butyrate-producing gut microbes (42). These results are consistent with our analysis of the whole cohort in this study, and in other studies that have found SCFA-producing microbes to be over-represented in individuals with high fiber diets (29). *Clostridia* UCG-014, *Megasphaera*, *Dialister*, and *Prevotella* were found to be under-represented with adequate fiber consumption in older females. Again, many of these are normally associated with high fiber diets but may be over-represented with inadequate fiber consumption as seen here for the reasons listed previously (37, 38). Interestingly, we did find that *Megasphaera*, *Clostridia* UCG-014, and *Prevotella* were under-represented in both cohorts in individuals with adequate fiber consumption despite all of these being known SCFA producers (34, 43).

The whole cohort and older female cohort have a similar core microbiome. After establishing that certain genera are differentially abundant with adequate versus inadequate fiber intakes for both cohorts, we were interested to see the number of indicator taxa shared between the whole cohort and subset older female group. We filtered the samples to only retain those that are considered biologically abundant (1%) and present in at least 25% of the samples within each group. Our results showed that the adequate fiber groups in both the total cohort and older female subset have more similar core microbiomes (3 taxa) compared to the inadequate fiber groups for both cohorts (1 taxon) (Fig. 3). However, each of the four groups (adequate and inadequate fiber intake for both cohorts) seem to be similar since 8 core taxa (33%) are shared among them. This is consistent with our alpha and beta diversity analyses, which also suggest there are minimal differences between the microbiomes of individuals with adequate and inadequate fiber intake.

Specific indicator taxa are associated with dietary fiber intake groupings and several of these are common between the whole cohort and older female group. Finally, we performed an indicator taxa analysis for our fiber consumption groupings to determine if any particular taxa are associated with adequate or inadequate fiber intake (Table 1). Despite only 22 individuals from the whole cohort of 441 samples being grouped into the adequate fiber consumption category, 15 species were identified as indicator species for this group, compared to only a single species identified from all of the inadequate fiber microbiomes (Table 1). The reason for this might be that indicator species analysis places greater weight on species that are unique to a group, with the relative abundance of the species being less important. Of the indicator species identified for the adequate fiber group, *Lachnospiraceae*, *Turicibacter*, *Ruminococcaceae* CAG-352, and *Eubacterium ventriosum* group are all SCFA-producing microbes (44-47). This finding aligns with the literature, which states that since SCFA-producing microbes metabolize fiber, they should theoretically be more abundant in the adequate fiber intake group (29). In particular, several of these taxa are associated with improved health outcomes, with Lachnospiraceae recognized as a core gut microbe, Ruminococcaceae associated with decreased risk of depression, and the Eubacterium ventriosum group associated with decreased risk of colorectal cancer (44, 46-47).

Interestingly, in individuals with inadequate fiber consumption, the sole indicator species, *Megasphaera elsdenii,* has been shown to produce SCFAs (34). A possible reason for this microbe being associated with inadequate fiber diets is that in inadequate fiber intake conditions, low abundance microbes – such as *Megasphaera* – may possess a survival advantage within the gut due to reduced biological competition. They could also potentially be able to utilize alternative energy sources such as glycans. Although we did not observe a statistically significant change in microbial diversity in this group, inadequate fiber intake may have resulted in decreased abundance of key competitors of Megasphaera that fill similar niches within the gut. Interestingly, this genus has been associated with depression in diabetic patients, though its overall function in the human gut remains largely unclear (48-49).

In the sex- and age-restricted cohort, there were fewer taxa identified as indicator species for the adequate fiber intake group, and none were identified for the inadequate fiber group (Table 1). Of the fiber-associated indicator species, three genera were found to have been previously identified with adequate fiber intake in the whole cohort analysis — *Gastranaerophilales*, *Ruminococcaceae* CAG-352, and *Neisseria* (Table 1). Although the exact roles of *Neisseria* and *Gastranaerophilales* in the gut are unknown, *Gastranaerophilales* is thought to aid in digestion and produce vitamins B and K (50). *Ruminococcaceae* CAG-352 is well-known to have anti-inflammatory roles, likely owing to the fact that it is a SCFA producer (46). This is in line with previous studies which show that high fiber diets are correlated with an increase in SCFA-producing microbes (9).

Limitations There are several reasons why our findings may differ from the previous literature on fiber consumption and gut microbiome diversity. This could be due to dataset-specific confounding factors, such as the variety of ages, sexes, cardiometabolic status, and caloric intake - all of which have been previously shown to influence gut microbial composition (2). Additionally, various public health agencies report recommended daily fiber intakes in sex-specific values (51). Therefore, we controlled for differences in calories consumed by normalizing the fiber values by caloric intake. We also used a non-sex-specific cut-off for recommended daily fiber provided by the Colombian government (11). To account for the potential confounding variables of age and sex, part of our study focused on a specific age and sex group (older females, aged 41 - 62). However, the biggest limitation is still likely the effect of other confounding variables such as BMI, medications, cardiometabolic status, and city of residency on our results (2). These variables could also contribute to variations in microbial diversity and composition, which could obscure the differences that we were expecting to see in individuals with different fiber intakes. One limitation that arose when we subsetted the samples to account for age and sex as confounding variables was decreased sample size. Since we decided to focus on the older female group, this greatly reduced the sample size for individuals with adequate fiber intake, thereby resulting in decreased statistical power. Additionally, we were unable to account for sources and types of dietary fiber for each sample due to limited data availability from de la Cuesta-Zuluaga et al.'s study. Different types of dietary fiber can impact the gut microbiota in various ways, which could potentially affect our ability to make conclusions about dietary fiber intake as a whole (30). Additionally, we found that even individuals designated as having "adequate" fiber consumption in our study did not have clearly different intakes compared to those having "inadequate" fiber diets. This could have led to the lack of differences seen in our study. Finally, although our study focused on the effects of dietary fiber in the diet, we were unable to account for other components of the diet, such as proteins and lipids. These other dietary macromolecules can also affect the composition of the gut microbiome in ways that were not accounted for in our study.

Conclusions Overall, our findings indicate that dietary fiber intake does not appear to significantly affect microbial diversity in the gut in this semi-Westernized population of Colombian people. However, we demonstrated that several species of microbes, including certain species and genera of SCFA-producing microbes, were over-represented. Although these findings do not corroborate the conclusions of most existing literature, this could be due to the limitations of our study and the previously-unexplored effects of a semi-Westernized diet on the gut microbiota. Regardless, these findings lay a foundation for future studies that could investigate the roles of the specific microbes, their corresponding metabolic pathways, and if there are correlations to other factors such as systemic inflammation and various disease outcomes.

Future Directions Future studies can build off of our findings and also explore related areas of interest. For instance, instead of using the two broad ranges of age groupings provided in the metadata, a narrower range of age groups can be compared to see if the differences are more pronounced. The same bioinformatics analyses could be performed to examine whether there are changes in diversity and relative abundances of particular species of microbes. These relationships could be further explored by examining specific pathways using PICRUSt2

analysis and linking the findings to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (52). Another suggestion would be to explore the correlation between fiber intake and inflammation. The links between specific SCFA-producing microbes can be examined to observe if they are linked to lower systemic inflammation levels as measured by the C-reactive protein (CRP) marker. This could be done by plotting the relative abundance of known SCFA-producers and performing correlation analysis with CRP levels. Additionally, this metadata could potentially be combined with other studies that have gathered metadata on the Colombian population to generate a larger sample size and improve statistical significance. Future studies could also expand beyond just looking at fiber and investigate the links between other dietary components such as carbohydrates and proteins to see whether these have an effect on health-related variables, including systemic inflammation.

As mentioned previously, de la Cuesta-Zuluaga et al. did not specify the different sources of dietary fiber consumed by each individual. If we were to perform an additional study to further characterize the Colombian microbiome, we would recommend more in-depth characterization of the dietary sources of not only fiber, but also proteins, lipids, and other carbohydrates. This would allow for a multifactorial analysis of the effects of dietary nutrients on the gut microbiome, and would avoid some of the limitations that we have highlighted in our study. In the future, these findings would ultimately be used as an asset in the development of diet-related personalized medicine treatments that mitigate adverse disease outcomes and progression.

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CONTRIBUTIONS

JC: Methods, Figures, Results, Discussion.
VL: Abstract, Introduction, Methods, Discussion, References.
CS: Abstract, Introduction, Methods, Figures, Results.
JY: Introduction, Methods, Figures, Results, References.
All members contributed equally to bioinformatics analyses and extensive proofreading and editing of the manuscript.

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