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Microbial diversity of smokers is not influenced by dietary fiber intake although smoking alters functional pathway abundances

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SUMMARY The gut microbiome is crucial for maintaining human health and is influenced by many environmental factors such as age, ethnicity, lifestyle practices, and dietary habits. While studies have shown that smoking can alter the microbiomes of various body sites, such as the upper GI tract (esophagus and stomach), the impact of smoking on the gut microbiome remains understudied. Dietary fiber intake has been linked to increased gut microbiota diversity and reduced weight gain, but it is unclear whether dietary fiber intake can counteract the negative effects of unhealthy lifestyle practices such as smoking. In this study, we investigated the impact of smoking on the microbial and functional composition of the gut microbiome and the potential mitigative effects of fiber on these possible changes. Here, we showed that smoking status did not change gut microbiome alpha and beta diversity of 441 Colombian adults. However, our functional pathway analysis identified several differentially abundant pathways between smoking and non-smoking individuals. Examination of the interactive effects of fiber intake on the microbial composition of smoking and non-smoking individuals revealed that fiber intake has no significant effect on microbiome alpha diversity. Overall, our study highlights that single-factor predictors like smoking status may not significantly impact gut microbiomes.

INTRODUCTION

The gut microbiome, defined as the microorganisms (bacteria, archaea, fungi, and viruses) that live along the gastrointestinal tract, is thought to play a pivotal role in health and disease pathogenesis (1). Studies have shown that it can provide many benefits for humans, such as energy recovery from metabolism of nondigestible components of foods, protection of the host from pathogenic invasion, and modulation of the immune system (2, 3). However, the gut microbiota's composition is highly variable (4). These variations can harbor significant implications in terms of intestinal and extraintestinal health conditions (4). Consequently, it is becoming increasingly apparent that the etiologies of various diseases and conditions are associated with different gut microbiome compositions, although direct causal relationships have not been fully established.

De la Cuesta-Zuluaga *et al.* aimed to determine the relationship between the gut microbiome and various demographic, health-related, and dietary parameters (5). To this end, the 16S rRNA sequences of 441 Colombian adults were sequenced to investigate changes in microbiome diversity in response to lifestyle changes and disease-associated dysbiosis (5). The authors showed that various environmental factors shape the composition of the gut microbiome and have a potential role in disease development. As such, we used this dataset to explore if smoking status and dietary fiber intake contribute to gut microbiome and functional pathway differences.

Research shows that smoking can have an impact on the composition of the periodontal, upper GI (esophagus and stomach), and respiratory microbiomes, with some studies indicating that smokers have increased abundances of anaerobic bacteria in the upper GI microbiome compared to non-smokers (6, 7). For example, a study conducted by Vogtmann *et al.* showed that the immunosuppressive nature of tobacco may be responsible for changes in the composition of the upper GI microbiome (8). Moreover, smokers have also been found

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to possess increased levels of taxa associated with pathways involved in the breakdown of smoking-associated toxins and the production of reactive oxygen species compared to those who have never smoked (9). Despite this evidence, the influence of smoking on the gut microbiome has been understudied. Thus, we hypothesized that the practice of smoking has deleterious effects on the gut microbiota, resulting in a reduction in microbial diversity, as well as alterations to key metabolic pathways.

In addition to smoking, dietary fiber intake has been linked to increased gut microbiota diversity and lower long-term weight gain (10, 11). Studies have shown that a high dietary fiber intake is associated with a decreased risk of chronic inflammatory diseases such as cardiovascular disease and inflammatory bowel disease (12, 13). However, it is unclear whether fiber intake can offset the negative effects of unhealthy lifestyle practices like smoking by promoting the growth of metabolically beneficial bacteria. Thus, we sought to investigate the impact of smoking on the microbial diversity and functional potential of the gut microbiome and whether increased fiber intake can mitigate the negative effects of smoking.

METHODS AND MATERIALS

Dataset and metadata. The dataset used in this investigation was generated from a study performed by de la Cuesta-Zuluga *et al.*, in which they performed 16S rRNA sequencing on fecal samples collected from 441 men and women, between 18 and 62 years old, residing in the five largest urban centers of Colombia, namely Bogota, Medellin, Cali, Barranquilla, and Bucaramanga (5). In addition to fecal samples, participants underwent 24-hour dietary recall interviews to determine their caloric and macronutrient intake. The raw DNA reads have been deposited in the SRA-NCBI under BioProject PRJNA417579. This paper focuses on two metadata categories, namely, smoking status, and fiber intake.

Data processing using the QIIME2 pipeline. We used the Quantitative Insights Into Microbial Ecology2 (QIIME2) software to import the 16S rRNA sequences from our dataset (14). First, we demultiplexed data using the "demux emp-paired" command. Then, we used the Divisive Amplicon Denoising Algorithm 2 (DADA2) algorithm to perform denoising by executing the "dada2 denoise-paired" command (15). We set a truncation length of 150 nucleotides to retain sufficient sequence quality while removing low-quality regions. After denoising our dataset, we clustered similar sequences into OTUs using the "vsearch cluster-features-closed-reference" command, which applied closed-reference clustering against a reference database, generating an ASV table. This table was used for downstream analysis, including alpha and beta diversity analysis, taxonomic classification, and functional analysis. To determine the appropriate sequencing depth for our analysis, we generated an alpha rarefaction plot using QIIME2 (14). We chose a sequencing depth of 24,132 reads as the optimal threshold to maximize sample richness while maintaining a sufficient number of samples for statistical analysis. We performed the filtering and rarefaction steps using "subset_taxa", "filter_taxa" and "rarefy_even_depth" functions from the "phyloseq" package in R version 4.2.2 (2022-10-31) (16).

Data processing using R Studio. From QIIME2, we found a sequencing depth of 24,132 reads to be optimal. We used R to perform additional filtering and rarefaction of the data to remove mitochondrial and chloroplast DNA sequences using "subset_taxa" and "rarefy_even_depth" from the "phyloseq" package (16).

Alpha diversity analysis of Columbian cohort samples based on smoking status. We compared the alpha diversity of smokers and non-smokers using three metrics (Chao1, Shannon diversity and the Observed features metrics) in R. The Wilcoxon ranked-sum test was used to test for significance. We used the "wilcox.test" command from the base R "stats" package to assess the significance of differences in alpha diversity between smokers and non-smokers (17).

Beta diversity analysis of Columbian cohort samples based on smoking status. We compared the beta diversity of smokers and non-smokers in R to better reveal any differences in microbial community composition. We used the "phyloseq" package in R to perform our beta diversity analysis that was visualized with principal coordinate analysis (PCoA) plots, generated by using the "phyloseq" package and the "ggplot2" package, to allow for further customization of the plots (16). We tested various beta diversity metrics, namely, Bray-Curtis dissimilarity, Jaccard dissimilarity and the unweighted Unifrac distance metric. Each of these metrics were subsequently assessed for significance via PERMANOVA analysis using the "adonis2" command from the "vegan" package in R (18).

PICRUSt2.0 analysis. Using the export tool on QIIME2, the following files were converted to PICRUSt2 compatible file formats: (i) the rarefied feature table (ii) ASV sequences. We then used the PICRUSt2 pipeline to generate a matrix of pathway abundances for each sample (19).

General trends in pathway abundance for smokers/non-smokers were visualized with a heatmap. To generate this heatmap, the matrix of pathway abundances and metadata was imported into R and was pre-processed as follows: (i) rare pathways (<20% representation in samples) were discarded, (ii) extreme values (pathways with values above the 99th percentile) were also discarded, and (iii) pathway abundance was converted into relative abundance for each sample. Using this filtered table, a heatmap with the smoker metadata was generated. To identify individual pathways between non-smokers and smokers that were differentially abundant, we used STAMP (20) to generate a list of differentially abundant pathways using corrected *p*-values (generated by using Welch's t-test).

Linear Model Analysis on alpha diversity based on smoking status. Linear regression was conducted with the base "stats" package included with R (17). We used the "lm" function in R to fit a linear model with the Shannon diversity metric as the response variable, with smoking status and fiber as predictor variables. To visualize overall diversity trends across varying fiber intake ranges, we generated a faceted plot, separating smokers and non-smokers, using the "ggplot2" package (21).

RESULTS

No significant differences in alpha diversity exist between smokers and non-smokers. To investigate the potential impact of smoking status on microbial diversity we first sought to determine whether alpha diversity differed between smokers and non-smokers among the adult population in Colombia. To achieve this aim, we performed an alpha diversity analysis using three alpha diversity metrics: Chao1, Observed richness, and the Shannon diversity index.

We determined that there were no significant differences in alpha diversity between smokers and non-smokers using either Shannon (Wilcoxon rank-sum, $p = 0.0674$), Chao1 (Wilcoxon rank-sum, $p=0.1514$), or observed features (Wilcoxon rank-sum, $p=0.1254$) alpha diversity metrics (Fig. 1).

Beta-diversity composition does not change between smokers and non-smokers. In order to investigate potential differences in microbial composition between smokers and non-smokers in the Colombian adult cohort, we utilized three beta-diversity metrics: the Bray-Curtis dissimilarity, Unweighted Unifrac, and Jaccard dissimilarity metrics (Supplemental Fig. S1). The Bray-Curtis metric measures dissimilarity based on the abundance of shared taxa, while the Unweighted Unifrac distance assesses microbial dissimilarity based on evolutionary relationships between taxa. The Jaccard dissimilarity metric measures dissimilarity based on the presence or absence of taxa between samples. These metrics allowed us to evaluate the distances between microbial diversities and gain a better understanding of potential differences between smokers and non-smokers.

Despite our initial hypothesis that smoking status would be associated with significant differences in microbial community diversity, our beta-diversity analyses did not show any notable differences between smokers and non-smokers as there were no significant clustering

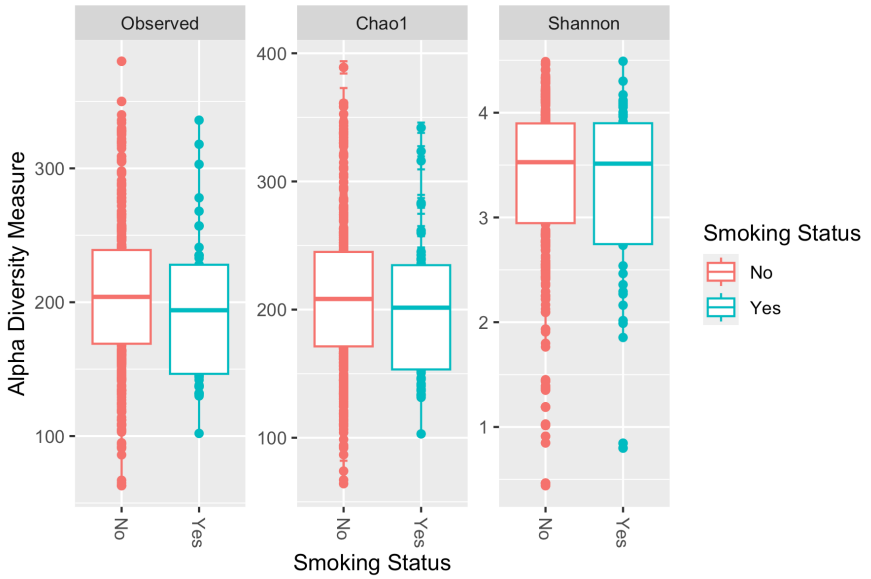

FIG. 1 No significant difference in alpha diversity between smokers and non-smokers. Observed, Chao1, and Shannon indices of smokers (teal, n = 47) and non-smokers (red, n = 338). All alpha-diversity comparisons were not statistically different as per the Wilcoxon rank-sum test (see **Table 1** for *p*-values).

TABLE. 1 Wilxocon ranked-sum test is non-significant for alpha-diversity metrics comparing smokers and non-smokers.

Diversity Metric	Statistical Test	P-value
Observed	Wilcoxon ranked sum test	0.1254
Chao1	Wilcoxon ranked sum test	0.1514
Shannon	Wilcoxon ranked sum test	0.6742

patterns observed between the two populations as demonstrated by the Bray-Curtis PCoA analysis (PERMANOVA, p=0.084) (Fig. 2).

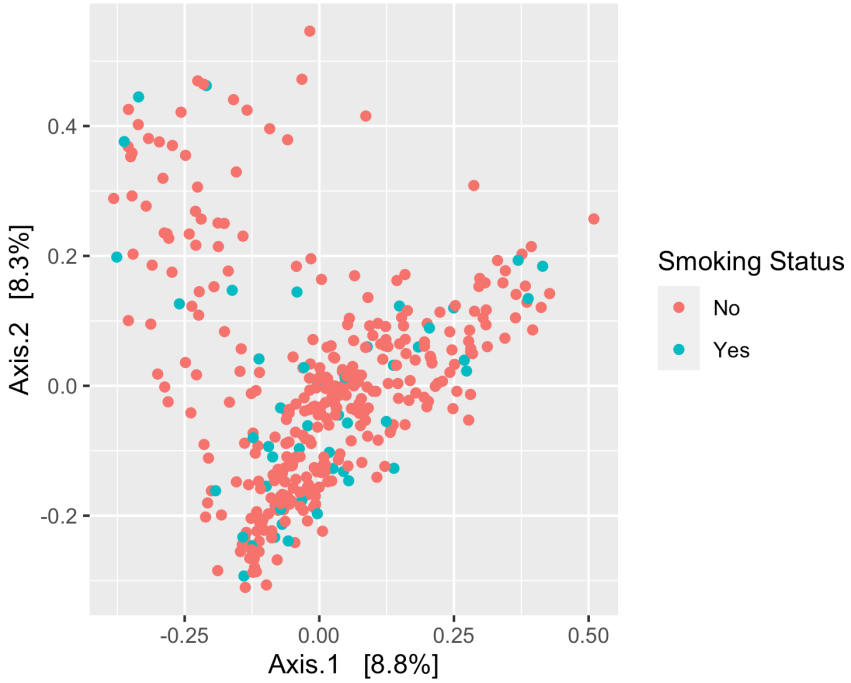

FIG. 2 Bray-Curtis-based PCoA reveals microbial composition does not change between smokers and non-smokers. Smokers (teal, n = 47) and non-smokers (red, n = 338) did not have significantly different microbial community composition (PERMANOVA, p = 0.084).

Metabolism, Biosynthesis, Degradation, and Assimilation Pathways are Differentially Abundant between Smokers and Non-smokers. To better understand the functional pathway differences between smokers and non-smokers, we first sought to identify general trends in pathway abundance related to smoking status in the Colombia adult cohort

(Fig. 3). We used PICRUSt2 (21), a bioinformatics tool to predict the functional abundance of a given sample from marker gene (*e.g.*, 16S rRNA) sequencing data, and to infer which pathways were enriched in smoking and non-smoking groups. Our analysis reveals that some pathways were differentially abundant (Fig. 4). Interestingly, we identified a cluster of samples with differentially abundant pathways, but these samples did not correspond to any single categorical variable (*e.g.*, sex, cardiometabolic status, age range) (Supplemental Fig. S2-S5).

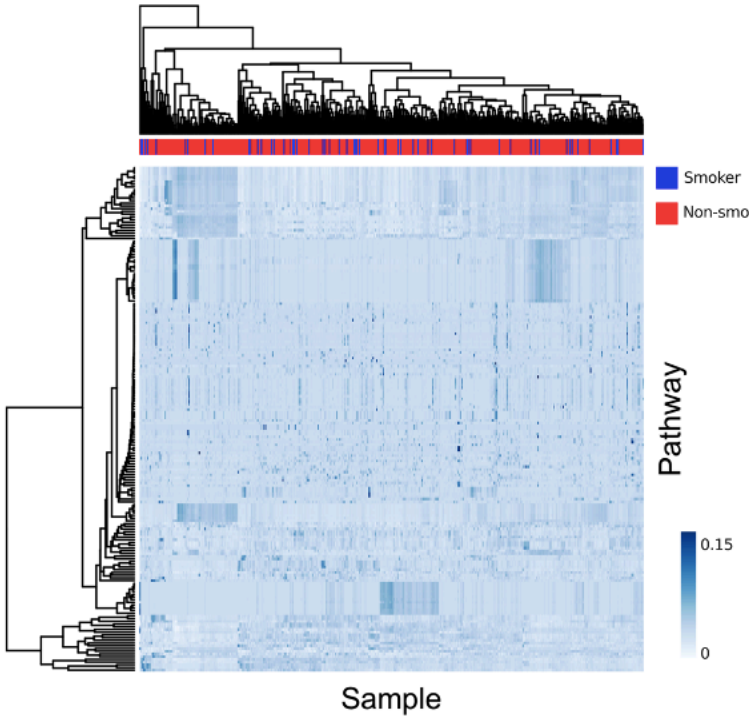

FIG. 3 Heatmap of clustered pathways shows no general association between differentially abundant pathways and smoking status. Dendrograms represent hierarchical clustering by the Euclidean distance metric and the complete method. Above the heatmap, smokers and non-smokers are represented in blue and red, respectively. Sample n = 385. Pathway n = 419.

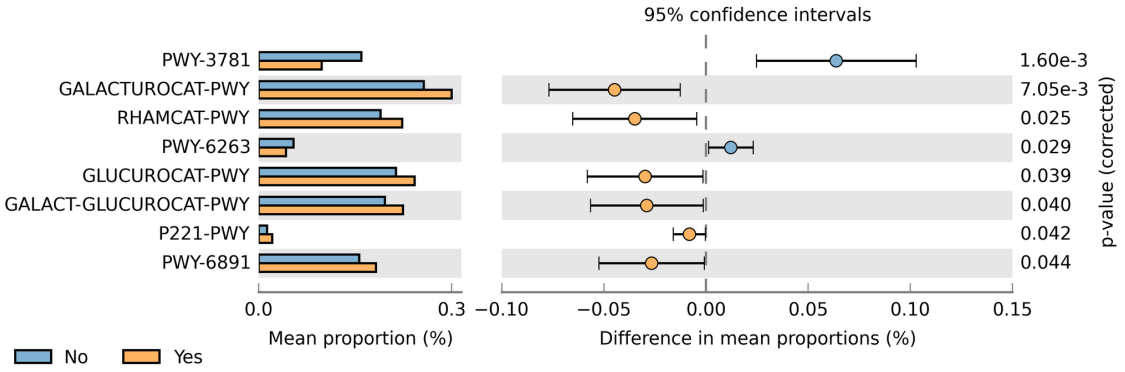

FIG. 4 Eight differentially abundant pathways reveal functional differences between smokers and non-smokers. Non-smokers are represented by blue, and smokers are represented by yellow. Corrected *p*-values from Welch's t-test between smokers and non-smokers.

Functional analysis revealed eight differentially abundant pathways between smokers and non-smokers (Fig. 4). These pathways include PWY-3781, GALACTUROCAT-PWY, RHAMCAT-PWY, PWY-6263, GLUCUROCAT-PWY, GALACT-GLUCUROCAT-PWY, P221-PWY, PWY-6891. With the exception of PWY-3781 and PWY-6263, the pathways found are more abundant in smokers than in non-smokers. The superclasses of these eight pathways are related to either energy generation, biosynthesis, degradation, utilization, and assimilation (22). These findings suggest that smoking decreases overall energy generation which leads to the increased need for alternate degradation and assimilation pathways to make energy.

Fiber intake has no significant effect on microbiome alpha-diversity. To measure the effects of dietary fiber on the diversity of smokers and non-smokers, we ran a linear model with smoking status and fiber as predictors and Shannon diversity as the response variable (Fig. 5). Our analysis reveals that there were no statistically significant differences in Shannon diversity of smokers across varying fiber levels (linear regression, $p = 0.2518$).

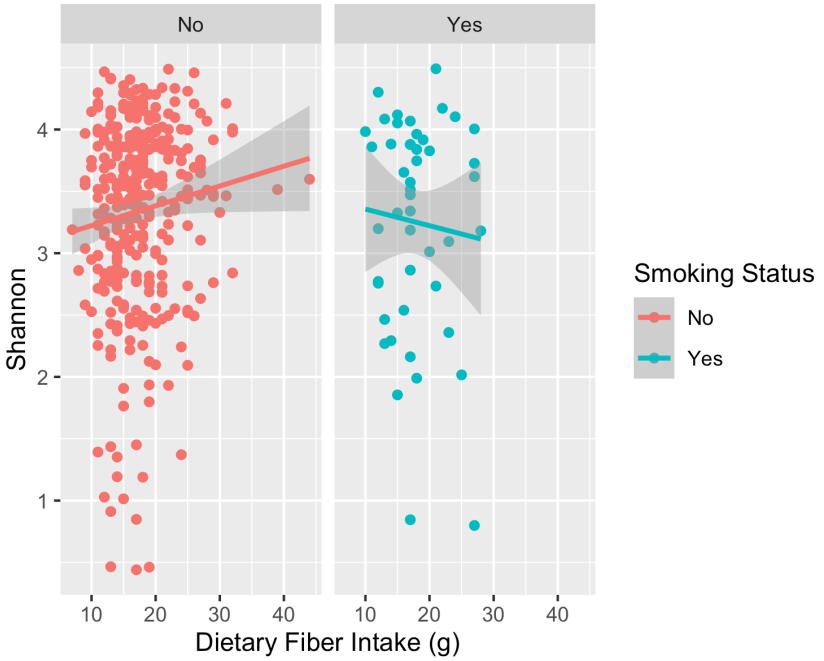

FIG. 5 The interactive effects of dietary fiber and smoking on diversity are non-significant. Plot is faceted, separating non-smokers (left) and smokers (right). The fitted line shows the relationship between Shannon diversity and dietary fiber intake for smokers (teal, n = 47) and non-smokers (red, n = 338). Linear regression $p = 0.2518$ for the interactive effects of fiber with smoking status on Shannon Diversity.

DISCUSSION

In this investigation, we sought to examine the influence of environmental factors on the gut microbiota's diversity by looking at possible compensatory effects of dietary fiber on the microbial diversity of smokers. To do this, we utilized pre-existing metadata from De la Cuesta-Zuluaga *et al.* and tested for the interactive effects of smoking and dietary fiber, specifically (5).

Microbial diversity. Our diversity analysis revealed no significant difference in alpha diversity between smoking and non-smoking populations in the examined dataset (Fig. 1). This finding is consistent with previous studies, such as those conducted by Prakash *et al.* and Lee *et al.* (22, 23). This result could be attributed to several reasons, including the fact that smoking may not have a direct impact on alpha diversity, or that other confounding lifestyle and dietary factors may have a stronger influence on alpha diversity.

Moreover, our beta diversity analysis, which employed three metrics for measuring beta diversity, revealed no significant clustering patterns between smoking and non-smoking populations (Fig. 2). This finding is in contrast with previous studies that suggest that smoking is linked to significant compositional differences in gut microbial beta diversity of “never” and “former” smokers (22, 23). Notably, Prakash *et al.* found significant differences between “former” and “never” smokers, whereas Lee *et al.* found no difference (pairwise PERMANOVA) between the two groups (22, 23). The discrepancy between our results and those of previous studies could be attributed to the relatively small number of smokers (n = 47) compared to non-smokers (n = 338), as well as the lack of separation between “former” and “never smokers” in the dataset used for our study. Therefore, it is possible that the small sample size of smokers might have resulted in an underpowered statistical analysis. Additionally, the percentage of variance captured in our Bray-Curtis-based PCoA plot was 17.1%, which is quite low and likely due to the high inter-individual variability of our cohort.

Additionally, it is worth noting that other factors, such as age, cardiometabolic health, and medication use, can influence our results. For instance, de la Cuesta-Zuluega *et al.* suggested that poor cardiometabolic health can influence the gut microbiome and decrease alpha diversity between healthy and unhealthy individuals (5). However, we did not account for cardiometabolic health status in our study, which could have confounded our findings.

Therefore, future studies with larger sample sizes and more detailed health and lifestyle data are necessary to confirm our findings and better understand the complex relationship between smoking and gut microbiome diversity.

Functional abundance. We discovered that six pathways were significantly more abundant in smokers, whereas two pathways were more abundant in non-smokers. These pathways were diverse in function (*e.g.*, generation of precursor metabolites and energy, biosynthesis, or degradation, utilization, and/or assimilation).

PWY-3781 (aerobic respiration I - cytochrome c) is related to the generation of precursor metabolites and energy (24). Our results show PWY-3781 is more abundant in non-smokers than smokers. This is consistent with literature as studies show smoke exposure mediates dysfunction of electron transport chain components, such as cytochrome C, which subsequently results in decreased respiration (25). This suggests that smoking creates an environment that favors strict or facultative anaerobes over strict aerobes.

PWY-6263 (superpathway of menaquinol-8 biosynthesis II) and PWY-6891 (thiazole component of thiamine diphosphate biosynthesis II) are pathways involved in the biosynthesis of molecules and cell structure components (25). Specifically, PWY-6263 is involved with the biosynthesis of vitamin K2 (menaquinones) (24). The main dietary source of Vitamin K2 is meat and fermented foods; therefore, the dietary intake derived from these sources is directly related to a healthy diet (26). Studies have shown that smoking is highly associated with an unhealthy diet as it reduces appetite and increased satiety (27). This evidence supports our findings as the PWY-6263 pathway was found to be less abundant in smoking individuals. PWY-6891 is involved in the biosynthesis of Vitamin B1 (thiamin diphosphate), which is crucial in energy metabolism (24). Smoking, specifically chronic nicotine exposure, has been linked to lower levels of Vitamin B1 uptake in mouse models; thus, the increased abundance of PWY-6891 seen among smoking individuals in our study may be indicative of a compensatory adaptation to increase Vitamin B, as it is important for cellular functions (28).

GLUCUROCAT-PWY (superpathway of β-D-glucuronosides degradation), GALACTUROCAT-PWY (D-galacturonate degradation I), GALACT-GLUCUROCAT-PWY (superpathway of hexuronide and hexuronate degradation), RHAMCAT-PWY (L-rhamnose degradation I), and P221-PWY (octane oxidation) are all pathways involved with degrading substrates to serve as sources of nutrients and energy, utilizing exogenous sources of metabolites, or assimilating certain sources of essential bioelements (24). Of these pathways, GLUCUROCAT-PWY, GALACTUROCAT-PWY, and GALACT-GLUCUROCAT-PWY are the most relevant to smoking. A previous study exposing mice to third-hand smoke revealed significant increases in degradation pathways such as GALACTUROCAT-PWY, GLUCUROCAT-PWY, and GALACT-GLUCUROCAT-PWY (29). These findings are consistent with our data. The GALACTUROCAT-PWY is involved with the degradation of plant biomasses, specifically pectin, into products that can enter the central metabolism system (30). In agreement with our findings that smoking increases the abundance of GALACTUROCAT-PWY, pectin is a component of tobacco (31). Moreover, glucuronidation, a process of detoxification that produces glucuronosides, is upregulated in the presence of cigarette smoke (31, 32). Glucuronosides are inputs of the GLUCUROCAT-PWY and GALACT-GLUCUROCAT-PWY (24). Therefore, the increase in abundance of these two pathways matches the increase in toxin intake induced by smoking. Overall, these degradation pathways found are important as they provide biosynthetic precursors and interconvert detoxification products or less common sugars into ones that can enter the central metabolism system (24, 28, 33).

Fibre intake. To investigate the potential compensatory effects of dietary fiber intake on the gut microbiome diversity of smokers, we conducted a linear regression, with Shannon diversity indices as the response variable, and smoking status and fiber intake as the predictor variables. Our linear model analysis reveals no effect of dietary fiber intake on the Shannon diversity of smokers and non-smokers. Interestingly, we observed a negative trend between smokers' Shannon diversity indices and increased fiber intake, whereas the opposite trend was seen in non-smokers. Several studies have shown that dietary fiber intake is associated with increased gut microbial diversity and a more beneficial gut microbial profile (1–3). Our results suggest that smoking hinders the beneficial effects of an increased dietary fiber intake.

Our linear regression was limited due to discrepancies in fiber intake ranges between the two populations being analyzed. Specifically, the dataset we examined had a wider range of fiber intake values for the non-smoking individuals, ranging from 7 to 44 grams, compared to a narrower range of 10 to 28 grams for the smoking individuals. This discrepancy in fiber intake ranges could have influenced our results and potentially confounded our findings. A smaller range of fiber intake values in the smoking group may lead to a smaller effect size, reducing the power of the statistical analysis and making it more difficult to detect a significant effect of fiber intake on beta diversity in this group. Further, since the fiber intake ranges were different for the smoking and non-smoking populations in this study, it is possible that the observed differences in beta diversity between the groups were due to variations in fiber intake rather than smoking status alone. Therefore, it may be difficult to determine whether fiber intake or smoking status is the true driver of the observed effects on beta diversity.

Additionally, there could be other factors contributing to this result, such as differences in participant demographics, and smoking habits. One potential reason for the lack of significant effect of fiber intake on the gut microbiome in smokers could be that smoking-induced perturbations to the gut microbiome are more severe and cannot be fully compensated by increased dietary fiber intake alone. These findings are consistent with studies that have found smoking to be associated with an increase in anaerobic bacteria and a decrease in beneficial bacteria, leading to an imbalance in the gut microbiome (7). Overall, these alpha-diversity findings suggest that smoking may have a more profound impact on the gut microbiome than dietary fiber intake alone and highlight the need for further research on the potential compensatory effects of dietary fiber intake on the gut microbiome in the context of smoking-induced dysbiosis.

However, the possible negative trend observed in the linear model showing the interactive effect of smoking and increasing fiber intake was unexpected (Fig. 5). This trend not only refutes our hypothesis that fiber has compensatory effects on the microbiome diversity of smokers, but also suggests higher fiber negatively impacts microbial diversity in smokers. One possible explanation for this result is that in conjunction with smoking, increased fiber may provide the proper nutrients for smoking-associated taxa, decreasing overall gut microbial species evenness (22).

We conducted beta diversity analysis with both Bray-Curtis and unweighted UniFrac distances, the latter of which considers the phylogenetic distance between samples. We observed that clustering was based on fiber intake, rather than smoking status, indicating that fiber intake may have a greater impact on beta diversity than smoking status, even when considering the phylogenetic distance between samples.

Limitations Our study had several limitations that may have affected the results. One major limitation was the small sample sizes for the smoking populations that were examined. Small sample sizes can affect the statistical power and limit the generalizability of the findings, thereby affecting our ability to accurately detect differences in microbial diversity. Future studies are encouraged to conduct a more in-depth analysis of possible microbial diversity differences among smoking and non-smoking populations using balanced sample sizes between smokers and non-smokers alike to overcome this limitation.

Overall, these limitations suggest that future studies should consider carefully controlling for potential confounding factors, such as differences in fiber intake, to more precisely identify the effects of smoking on the gut microbiota.

Conclusions In conclusion, our findings indicate that smoking does not affect gut microbial diversity and that there was no effect of dietary fiber on diversity of either smoking or non-smoking groups. However, our functional analysis revealed several differentially abundant pathways between smokers and non-smokers such as the PWY-3781 (aerobic respiration I - cytochrome c) and GALACT-GLUCUROCAT-PWY (superpathway of hexuronide and hexuronate degradation). This suggests that smoking decreases overall energy generation which leads to the increased need for alternate degradation and assimilation pathways to make energy. This knowledge could help inform the development of new preventative and

treatment strategies for smoking-related diseases that incorporate the role of the gut microbiota.

Future Directions There are various avenues we suggest exploring to better elucidate the relationship between dietary intake of macronutrients on microbial functional diversity, especially in the context of microbiome-related disease.

Within the current dataset by de la Cuesta-Zuluaga *et al.*, there are several other dietary macronutrients other than fiber, such as carbohydrates, proteins, and fats, that may affect the microbial functional diversity in smokers. For instance, De Filippo *et al.* found a typical Western diet high in animal protein, sugar, and fat resulted in lower microbial richness and biodiversity compared to high fiber diets (34, 35).

Additionally, rather than looking at how each individual dietary component affects the gut microbiome, it is worth investigating how a well-rounded diet can impact GI microbiome. The Institute of Medicine of the National Academies recommends individuals obtain the following breakdown based on % of total calories: 45-65% from carbohydrates, 20-35% from fats, and 10-35% from proteins (36). Prior studies show a well-rounded diet increases microbiome diversity and enables adaptations to perturbations, and perhaps such perturbations include smoking-induced dysbiosis (37).

As an extension of our study, performing indicator species analysis can reveal which microbial species are more or less abundant based on smoking status in the Columbian cohort, as previous studies indicate that taxa *Lachnospira* and phylum Firmicutes, and Proteobacteria were depleted while taxa *Prevotella, Veillonellaceae,* and phylum Bacteroidetes were enriched (22, 23).

A dataset that separates smoking status into “never”, “former” and “current” smokers, and tracks if “current” smokers are casual or heavy smokers may provide better resolution on how smoking affects the microbiome (22, 23, 38).

Moreover, our gut microbiota has been closely linked with many diseases like intestinal bowel diseases, like Crohn’s disease which is linked to both the microbiome and smoking (39–41) Therefore, finding similar datasets that track other microbiota-related diseases can allow further investigation into whether fiber has any compensatory effects on microbial dysbiosis inherent diseases, as older studies have shown a lack of non-infectious colonic diseases in populations consuming high fiber (42).

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CONTRIBUTIONS

Ashley T: Performed all QIIME2 processing and analysis, PICRUSt2 abundant pathway results and discussion sections, and references.

Dongkyu L: Troubleshooted and ran script for PICRUSt2. Generated all figures and data for PICRUSt2 using R and STAMP.

Lijia Z: Performed all R processing for diversity analysis, generated figures and future directions.

Maximilian T: Contributed to abstract, introduction, materials and methods, discussion, limitations, and figure captions.

All authors contributed equally to the editing and writing of all other sections.

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