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Nutrients May Play a Complex Role in Influencing Microbiota Composition and Function in Parkinson's Disease Patients

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SUMMARY Parkinson's disease is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons, leading to symptoms such as tremors and gastrointestinal dysfunction. Previous studies have shown reduced gut microbial diversity and imbalances in Parkinson's disease patients including increases in inflammatory microbes and decreases in commensals. However, the impact of dietary macronutrients and micronutrients on gut microbiota composition in Parkinson's disease patients remains unclear. By analyzing published microbiota data containing nutrient intakes of Parkinson's disease patients and non-Parkinson's disease controls, we aimed to identify nutrients that are associated with gut microbiota composition in Parkinson's disease patients. We observed correlations between certain nutrients and changes in beta diversity among Parkinson's disease patients. We then segregated the Parkinson's disease and control data based on consumption of each of these identified nutrients and performed taxonomic analyses and functional pathways analysis. These analyses compared the upper and lower nutrient consumption groups for Parkinson's disease and control populations. The core microbiome and indicator species analyses revealed that microbial composition is more responsive to nutrient intake in the control group than in Parkinson's disease patients, with a greater number of core ASVs and indicator taxa identified in control groups. Differential abundance analysis further showed that high nutrient intake generally led to the upregulation of more ASVs, with some exceptions, such as coffee in PD patients and NSP in non-PD controls, where downregulation was more prominent. The functional pathway analysis identified that nutrients have a greater metabolic effect on Parkinson's disease patients than control. Ultimately, our survey into how nutrients drive changes in microbial diversity in Parkinson's disease patients may help guide future research into nutrient intake catered to the prevention and management of Parkinson's disease.

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INTRODUCTION

Parkinson's disease (PD) is a multi-system, multi-symptomatic disorder and is the second most common age-related neurodegenerative condition diagnosed in North America (1). PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra (2). PD presents in patients as both motor and non-motor symptoms (3, 4). Motor symptoms include bradykinesia, rigidity, rest tremors, and gait disorder (3). Non-motor symptoms include sensory disturbances, cognitive impairment, and gastrointestinal dysfunction (4). In particular, gastrointestinal dysfunction and gut microbiome research in PD have been gaining momentum in recent years (5). Previous studies have shown that reduced gut microbial diversity in PD patients correlates with an increase in gastrointestinal

symptoms (5). Furthermore, PD gut microbiota composition is characterized by enriched inflammatory microbes such as *Akkermensia* and depleted short-chain fatty acid (SCFA) producing bacteria such as *Roseburia* and *Faecalibacterium* (6). Since SCFAs possess anti-inflammatory properties and aid in maintaining intestinal wall health, a decrease in SCFA can lead to increased inflammation and exacerbate gastrointestinal disorders in PD (7).

A high intake of animal fats, primarily saturated fatty acids, is associated with PD whereas the intake of polyunsaturated fatty acids (PUFAs) may reduce PD risk and offer neuroprotective effects (8). Previous studies have found that protein-restricted diets result in lower levodopa doses needed and fewer PD motor symptom fluctuations (9). Additionally, high carbohydrate intake was found to be positively associated with PD risk (10). PD patients also tend to consume high amounts of sugars and carbohydrates which can potentially exacerbate symptoms (11). Furthermore, diets rich in refined carbohydrates, such as added sugars, can lead to gut dysbiosis and are implicated in PD pathology (12).

Vitamins are grouped into water-soluble (vitamins B and C) and fat-soluble (vitamins A, D, E, and K) compounds where they act as enzymatic cofactors or regulate gene expression, respectively (13). Specific deficiencies of vitamins B6, B12, D, and E have been linked with the development of PD (14). Inadequate mineral consumption of zinc, magnesium, manganese, and phosphorus is linked with PD while high intake of these minerals is correlated with a reduction of PD severity and motor dysfunction (15). Additionally, PD is associated with inflammation in the gut which may be caused by inadequate vitamin and mineral intake increasing the abundance of inflammatory microorganisms (16). Therefore, understanding the consequences of inadequate vitamin and mineral intake on the composition of the gut microbiome may provide new insight into proper nutritional intake for the protection and cessation of PD.

This study will analyze a dataset published by Cirstea et al. (17). To investigate the relationship between gut microbiota and PD, Cirstea et al. collected fecal samples, clinical symptoms, and dietary information from 197 PD patients and 103 non-PD controls between the ages of 40 and 80 (17). The authors characterized the microbiota using 16S sequencing (17). Increases in *Akkermansia* and *Bifidobacterium* in the PD samples were observed, along with a decrease in SCFA-producing *Faecalibacterium* and Lachnospiraceae (17). Furthermore, previous studies using this dataset have analyzed individual nutrients such as dietary fiber and vitamin B on microbial composition in PD patients (11, 18). While an association between PD and gut microbiota composition was established, the authors did not evaluate the impact of different nutrients on the microbiota composition in PD patients.

Insufficient vitamin and mineral intake and excess intake of carbohydrates, protein, and fat may lead to gut dysbiosis and decrease beta diversity. However, the mechanisms underlying gut dysbiosis are poorly understood and it is unclear how nutrient consumption is linked to PD microbiota composition. Thus, we aim to explore the impact of dietary nutrient and food group intakes on gut microbiome composition and functionality in PD patients.

To investigate how intake levels of different nutrients and food groups affect the diversity and functionality of the gut microbiome, we first determined what nutrients play a role in driving beta diversity. Next, we identified distinct taxa and differences in variability in gut microbial composition in PD patients vs control participants associated with intake of the identified significant nutrients. Finally, we analyzed differences in the regulation of functional pathways in the gut microbiome in PD vs control participants. The identified nutrient-correlated taxa and metabolic pathways reveal a complex relationship between nutrient consumption and PD status that suggests the importance of several nutrients and taxa, but the involvement of numerous additional factors is not included in our analysis. These results may help guide future research into diets and nutrient intake catered to the prevention and management of PD while also determining key microbial species responsible for driving PD symptoms.

METHODS AND MATERIALS

Sourcing Dataset. The dataset originated from Cirstea et al.'s "Microbiota Composition and Metabolism Are Associated With Gut Function in Parkinson's Disease." (17). 300 participants, including 197 PD patients and 103 non-PD controls were recruited at the Pacific Parkinson's Research Centre at the University of British Columbia (17). Participants filled

out the 130 food item EPIC-Norfolk Food Frequency Questionnaire (FFQ) and then intake levels of 46 nutrients and 14 food groups were calculated using the FFQ EPIC Tool for Analysis (FETA) (17). For fecal microbiome analysis, the bacterial 16S rDNA V4 region was amplified and sequenced using the Illumina MiSeq platform (17).

QIIME2 Processing. QIIME2 (v2023.7) (19) was used for demultiplexing the amplicon sequence data, then DADA2 (20) was used as the denoising tool. The maximum read length was 251 nucleotides, with more than 98% of the samples having the same read length. Reads were truncated at the maximum read length of 251 nucleotides to maintain read quality above Phred quality score of 30 (Figure S1A). Taxonomic classification for samples was generated using the QIIME2 q2-feature-classifier, and SILVA 138-99 database (21). Phylogenetic tree was then created using MAFFT (22) for sequence alignment, and FastTree2 (23) for the actual construction of the tree.

Rarefaction. To determine rarefaction depth, rarefaction plots were generated in QIIME2 viewer and RStudio (19). Based on the rarefaction curve, rarefaction depth was established at 5000, with sampling depth retaining around 40% of the features and 295 (98.33%) samples at the specified sampling depth (Figure S1B).

Alpha and Beta Diversity Metrics: Using the rarefied QIIME2 processed dataset, the adonis2.0 function in the vegan (v2.6-4) (22) package in R (v4.3.1) was used to do PERMANOVA analysis (24) to assess the effect of each nutrient on the Bray-Curtis dissimilarity and Jaccard distance. Results were filtered to include only those with a p-value < 0.05 and the results were then plotted using cowplot with ggplot2 (v3.4.4) (25). Alpha diversity metrics were produced with the following R packages in R: tidyverse (v2.0.0) (26), phyloseq (v1.46.0) (27), ggplot2 (v3.4.4) (25). For each nutrient or food group, Chao1, Simpson Index, Shannon Index, ACE, and Fisher's alpha were calculated using multivariate linear regression, and statistical significance ($p < 0.05$) was determined with Student's T-test.

Classification of Nutrient Levels: Following the outcomes of PERMANOVA analysis, specific categories were chosen for further investigation: non-starch polysaccharides (NSP), total folate, vitamin C, vitamin B2, fructose, and coffee drinker. Fruits and vegetables were omitted from this labeling. Using the non-rarified QIIME2 processed dataset, concentration labeling was applied to each category, except for coffee drinker, whereby the upper quartile was designated as "high", and the lower quartile as "low" (PD n = 46, control n = 26). For coffee drinkers, individuals reporting "Daily Multiple Cups" were categorized as "high" (PD n = 38, control n = 28), while those reporting "No" were classified as "low" (PD n = 53, control n = 25).

Core Microbiome Analysis. Core microbiome R package (v 1.24.0) (28) was used on the classified phyloseq object, with relative abundance and prevalence thresholds set as indicated by figures. Results were resolved to the species level and visualized with four-way Venn diagrams using the ggVennDiagram R package (v 1.5.2) (29). For a complete list of core microbiome members for each group, refer to the GitHub file complete_asv_table.csv in the aim_3 folder.

Indicator Species Analysis. In the phyloseq object, amplicon sequence variants were agglomerated atthe genus level and transformed into relative abundance. Indicator species analysis was conducted using the 'zoo' (v 1.8.12) (30), 'tidyverse' (v 2.0.0) (26), 'tidyr' (v 1.3.1) (31), 'phyloseq' (v 1.46.0) (27), 'indispecies' (v 1.17.14) (32). Consumption of each significant nutrient was separated into four quartiles. Indicator species were identified for the highest quartile in consumption for PD and control patients, the lowest quartile in consumption for PD and control, and the second or third quartile in consumption for PD or control patients. Present indicator species were defined as identified in only one category while absent indicator species were defined as identified in all categories except one. For a complete overview of indicator taxa for each group, refer to the GitHub folder Aim_4.

Differential Abundance Analysis. Differential abundance analysis was conducted in R for high and low nutrient classifications for both PD and non-PD control groups using the R packages DESeq2 (v1.42.0) (33), tidyverse (v2.0.0) (26), phyloseq (v1.46.0) (27), and ggplot2 (v3.4.4) (25). The classified phyloseq object was transformed to remove nonzero values by the addition of one read pseudo count. ASVs were linked to their taxonomic genus and bar plots of significant ASVs with a log 2-fold change +/- 2, adjusted p-value < 0.01, and mapped to respective phyla were generated for each nutrient and disease status group. Total upregulated and downregulated genera were tabulated for each nutrient and disease status group.

PICRUSt2 Functional Pathway Analysis. PICRUSt2 (v2023.2) (34) was used in QIIME2 (35) for functional gene annotation of the 16S rRNA amplicon sequences. MataCyc metabolic pathway (36) differential abundance was then predicted using DESeq (33) to determine the impact each nutrient had on the functional capacity of the microbiome. The pathways that were up and downregulated in PD and non-PD patients were filtered for an adjusted p-value < 0.05 and a log 2-fold change of +/- 2. PCA plots (Figure S2, S3) and heat maps (Figure S4, S5) were created to visualize the results using ggpicrust2, and bar plots of the pathways were created with ggplot2 (25).

Data Repository. Corresponding data and code are stored in the GitHub repository located at https://github.com/LH3311/team8.git

RESULTS

Eight nutrients and food groups have a significant effect on the PD gut microbiome. To determine the specific nutrients that induce changes to the beta-diversity of PD patients, permutational multivariate analysis of variance (PERMNOVA) analysis (24) was used to assess the effect of each nutrient on the Bray-Curtis dissimilarity and Jaccard distance using the adonis2.0 function in the *vegan* R package (37). There were 8 nutrients and food groups found to have a significant (p-value < 0.05) effect on Bray-Curtis dissimilarity, while 6 had a significant (p-value < 0.05) effect on Jaccard distance within the PD patients (Figure 1). Coffee, NSP, folate, fruit, vitamin C, and vitamin B2 had effects on both measures, while fructose and vegetables only had a significant effect on Bray-Curtis dissimilarity (Figure 1).

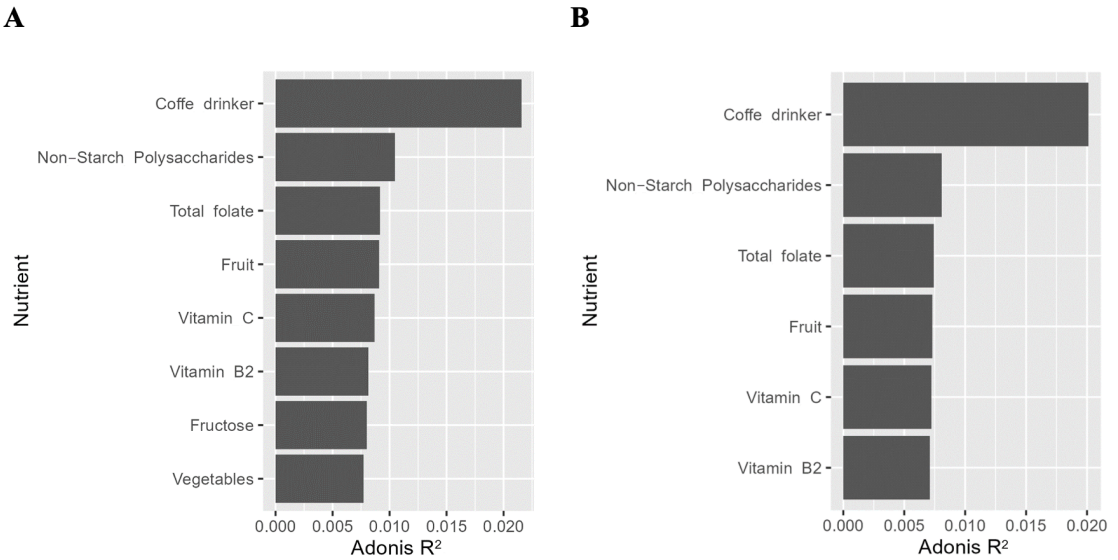

FIG. 1 Nutrients found to drive beta-diversity in PD patients. PERMANOVA analysis was used to assess the effect of each nutrient on the Bray-Curtis dissimilarity and Jaccard distance. Bar plots showing the nutrients that have a significant (P < 0.05) effect on **(A)** Bray-Cutris dissimilarity and **(B)** Jaccard distance in PD patients.

Nutritional effects correlated significantly to gut microbiome alpha diversity in control but not in PD patients. To explore the effect of different nutrients and food groups on the gut microbiome diversity of PD patients, we conducted multivariate linear regression with Chao1, Shannon Index, Simpson Index, ACE, and Fisher's alpha. Alpha diversity analysis did not identify any significant nutrients as potential modulators for the PD gut microbiota, indicating that nutrition did not impact gut microbiome abundance or evenness (Figure 2). In the non-PD control population, beta-carotene was significant according to the Simpson Index (Figure 2A). Furthermore, non-alcoholic beverages were significant according to Fisher's alpha, Chao1, and ACE indices in the control population (Figure 2BCD). Since no significant nutrients were identified following alpha diversity analysis, downstream investigations used the most significant nutrients determined by beta diversity analysis.

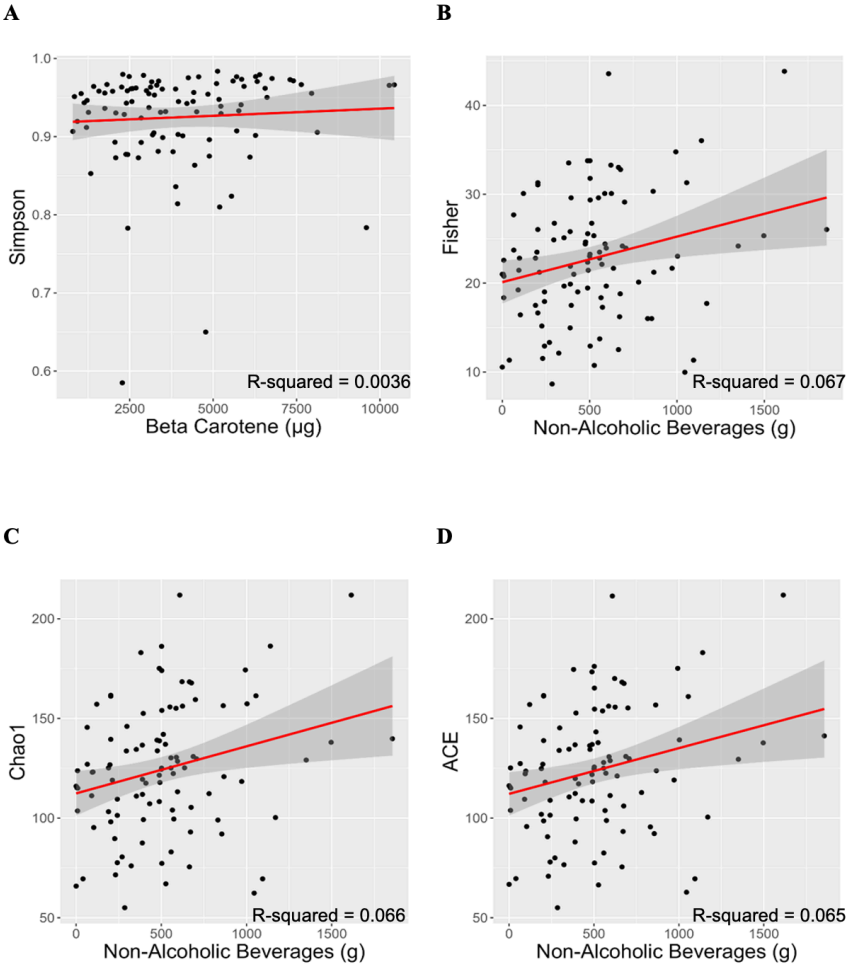

FIG. 2 Nutritional effects correlated significantly to gut microbiome alpha diversity in non-PD patients but not in PD patients. Multivariate linear analysis was used to assess the effect of each nutrient on the Simpson Index, Fisher's alpha, Chao1, ACE, and Shannon Index. (**A**) Beta-carotene (µg) and Simpson Index in non-PD patients (R-squared = 0.0036). Non-alcoholic beverages (g) against (**B**) Fisher's alpha (R-squared = 0.067), (**C**) Chao1 (R-squared = 0.066), and (**D**) ACE (R-squared = 0.065) in non-PD patients. All linear regressions shown are significant as determined by Student's T-test ($P < 0.05$).

Microbial composition may be more sensitive to nutrient consumption in non-PD participants compared to PD. To investigate if there were particular taxonomic groups associated with high/low nutrient intake, core microbiome analysis was applied to the classified dataset (Figure 3). The majority of core microbiome analysis resulted in one ASV belonging strictly to one particular group (PD high intake, PD low intake, control high intake, control low intake), except for the fructose low intake group which contained three distinct ASVs (Figure 3B). The largest core microbiome was between all the groups and consisted of three ASVs across all nutrients. Decreasing the relative abundance threshold to 0, the largest core microbiome was still between all groups across all nutrients, however, the core microbiome for control groups increased in ASVs compared to PD groups (Figure S6).

An indicator species analysis was conducted to determine if the presence or absence of specific taxa could be predictive of the nutrient consumption level and disease status of study participants. The analysis showed that indicator taxa were more frequently associated with control participants than with PD patients across the different categories (Table 1).

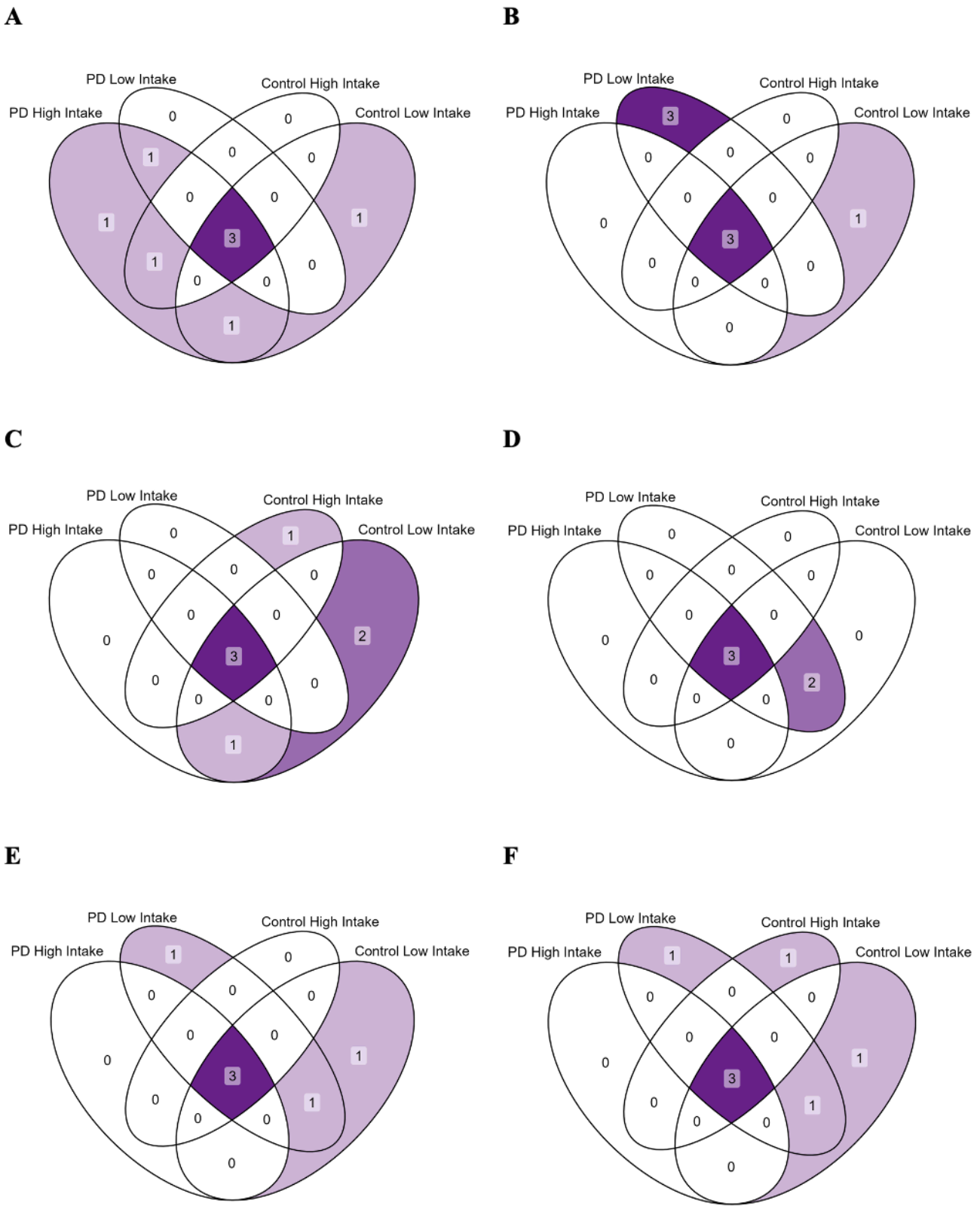

FIG. 3 Core microbial gut composition is not significantly affected by nutrient intake. Four-way Venn diagrams representing the number of unique species detected across high and low nutrient intake in PD (n =46), and control groups (n= 26) for Coffee (**A**), Fructose (**B**), NSP (**C**), Folate (**D**), Vitamin B2 (**E**), and Vitamin C (**F**). Core microbiome determined by abundant taxa (> 1% relative abundance), present in 50% of individuals.

TABLE. 1 Indicator Species Analysis. Indicator species analysis was conducted for each nutrient with participants categorized by consumption level and PD status and the number of indicator species for each category is indicated. Present taxa are only indicator taxa of the indicated group while absent taxa are indicator taxa of all groups except in the indicated group. The second and third-quartile nutrient consumption group is not included.

Nutrient	PD High *Present (Absent)*	PD Low *Present (Absent)*	Control High *Present (Absent)*	Control Low *Present (Absent)*
Coffee	1 (0)	0 (0)	0 (0)	1 (3)
Fructose	2 (0)	0 (0)	2 (0)	1 (0)
NSP	0 (0)	0 (1)	2 (2)	0 (1)
Folate	2 (0)	0 (0)	5 (1)	0 (0)
Vitamin B2	1 (0)	1 (0)	1 (0)	0 (0)
Vitamin C	1 (1)	0 (1)	0 (0)	1 (1)

Furthermore, control participants with any high nutrient consumption had the most indicator taxa across the six nutrients with ten present and three absent. However, by nutrient, folate consumption had the largest number of associated indicator taxa. The taxonomic analysis identified Lachnospiraceae as an indicator family for three different genera of control high folate, one genus of control high fructose, one genus of control low fructose, and one genus of control low coffee and absent indicator family of PD low in vitamin C. Only three indicator taxa at the genus level were identified in multiple categories of nutrient consumption and PD status. UCG-008 from the family Butyricicoccaceae was identified as an indicator of control high in fructose, NSP, and folate, GCA-900066575 from Lachnospiraceae was identified as an indicator of control high in NSP and folate, and *pectinophilus* from the genus *Bacteroides* was identified as an indicator of control high in fructose and folate.

Nutrients drive the overall upregulation of differentially abundant genera in both PD and non-PD participants. To assess the effect of nutrient intake on the number of differentially abundant genera in PD and non-PD controls, we performed a differential abundance analysis (DESeq2) by comparing the high vs low nutrient intake groups in both PD and non-PD controls. Our analysis revealed a general trend that high nutrient intake drives an overall greater number of upregulated ASVs relative to downregulated ASVs regardless of nutrient and disease status (Table 2). Particularly, NSP consumption in PD patients had a

TABLE. 2 Differential Abundance Analysis of High vs Low Nutrient Intake in PD and non-PD Controls. Total number of significantly upregulated and downregulated genera (ASVs) with log2FoldChange > 2 and adjusted p-value < 0.01 in high vs low nutrient intake in PD and non-PD controls. Low nutrient intake group was set as the reference for DESeq analysis.

Nutrient	Disease Status	Downregulated ASVs	Upregulated ASVs
Coffee	PD	18	9
Coffee	Control	9	18
Fructose	PD	13	31
Fructose	Control	13	21
NSP	PD	14	37
NSP	Control	23	21
Folate	PD	16	28
Folate	Control	12	31
Vitamin B2	PD	9	23
Vitamin B2	Control	12	31
Vitamin C	PD	12	20
Vitamin C	Control	12	21

dramatic effect on the number of upregulated ASVs with 37 upregulated genera. Interestingly, both coffee consumption in PD and NSP consumption in non-PD controls had more downregulated ASVs compared to upregulated ones (Figure S7, S8). In particular, the coffee PD group exhibited downregulation of Firmicute ASVs and an upregulation of Bacteroidota ASVs (Figure S7). In the coffee non-PD group, the opposite was seen. In addition, all PD

groups had a downregulation of *Akkermansia* besides in the high vitamin B2 PD group (Figure S7). Altogether, the intake of most nutrients contributed to an overall upregulation of ASVs with complex taxonomic diversity in both PD and controls.

Nutrients generally have a greater metabolic effect on PD patients than non-PD participants. To investigate the functional changes of the microbiome due to low vs high intake of nutrients, PICRUSt2 (34) was used to determine which MetaCyc metabolic pathways were up and down-regulated. In PD patients, 63 unique functional pathways showed significant differences in abundance between low and high intake of fructose, NSP, folate, vitamin B2, and vitamin C (Figure S2, S4). Furthermore, in non-PD patients, 31 unique functional pathways showed significant differences in abundance between low and high intake of coffee, folate, and vitamin B2 (Figure S3, S5). After significance (adjusted p-value < 0.05) MetaCyc pathways were filtered for a log 2-fold change of +/- 2, and there were a total of 19 pathways affected by 4 of the intake of nutrients (fructose, NSP, folate, and vitamin C) in PD patients, while 12 pathways were affected by 3 nutrients in non-PD patients (coffee drinking, folate, vitamin B2), with 9 of these being downregulated by vitamin B2 (Table 3, Figure S9). This indicates that overall, nutrients have a greater metabolic influence in PD patients. Folate was the only nutrient that had a significant effect on functional pathways in both PD and non-PD patients, indicating that it influences the metabolism of the microbiome regardless of disease status (Table 3, Figure S9). The pathway affected by the most nutrients in PD patients was the nicotinate degradation pathway, which was found to be downregulated by low intakes of NSP, folate, and vitamin C (Table S1). Another pathway that was affected by multiple nutrients in PD patients was sulfoglycolysis, which was upregulated by low intakes of fructose, NSP, and vitamin C (Table S1).

TABLE. 3 PICRUSt2 Functional Pathway Analysis. Total number of significant (p-adjust < 0.05) MetaCyc metabolic pathways filtered for a log two-fold change of +/- 2 showed up or down-regulated pathways in low vs high intake of nutrients.

Low vs High Intake	Downregulated Pathways in PD	Upregulated Pathways in PD	Downregulated Pathways in Control	Upregulated Pathways in Control
Coffee	0	0	1	0
Fructose	0	1	0	0
NSP	1	5	0	0
Folate	3	1	0	2
Vitamin B2	0	0	9	0
Vitamin C	4	4	0	0

DISCUSSION

In this study, we investigated the effects of different nutrient intake levels on the gut microbiome in PD patients and control participants. Beta diversity has also been shown to be significantly altered in PD patients, with PD patients showing widespread dysbiosis in their gut microbiota (39). Diet plays a significant role in the composition of the gut microbiota (40). PERMANOVA analysis of Bray-Curtis dissimilarity found that eight nutrients may play a significant role in driving beta diversity in PD patients. Among these, six nutrients, coffee, fructose, NSP, folate, vitamin B2, and vitamin C were investigated further.

Multivariate linear regression did not identify any significant nutrients with regard to alpha diversity metrics in PD patients (Figure 2). For non-PD controls beta-carotene and non-alcoholic beverages were significant contributors to gut microbiome abundance and evenness (Figure 2). Thus, from the alpha diversity analysis, PD pathophysiology seems to overshadow nutritional effects on the gut microbiome abundance and evenness (Figure 2). The PD gut microbiome is typically characterized by increased *Akkermansia* and *Bifidobacterium* and decreased *Faecalibacterium* and Lachnospiraceae (17). While certain nutrients and diets such as a low carbohydrate, high-fat diet in healthy subjects have been associated with higher abundances of *Akkermansia* and lower abundances of *Bifidobacteria*, these nutritive effects were not seen in our alpha diversity analysis of PD patients nor in the control population (41).

Thus, PD pathophysiology appears to overshadow the effect of nutrition on gut microbiome alpha diversity.

Core microbiome analysis overall did not identify a robust core microbiome for specific nutrient intake groups in PD patients (< 3 species per high/low intake group). Additionally, indicator species analysis showed low quantities of taxa identified. In PD patients with low fructose intake, the number of species observed was equivalent to the core microbiome shared across all groups. Subsequent examination within this group identified two *Bacteroides* species and one *Agathobacter* species. The abundance of *Bacteroides* has been correlated with heightened levels of pro-inflammatory cytokines in non-tremor PD patients (42). It is plausible that low fructose intake might influence inflammatory pathways in PD individuals, potentially exacerbating inflammatory processes associated with the disease.

In an indicator species analysis at the genus level, three taxa were identified in multiple categories of PD status and nutrient consumption level. Some similarities were identified in the indicator families and genera in our analysis with those identified by Cirstea et al. to be differentially abundant in PD (17). Cirstea et al. identified decreased abundance of the family Lachnospiraceae in PD patients (17). Our analysis revealed Lachnospiraceae as a present indicator of multiple control categories and absent indicator in one PD category. This may suggest a role in consumption levels of multiple nutrients impacting the abundance of Lachnospiraceae which may protect against PD (43). Conversely, Cirstea et al. identified increased abundance of the family Desulfovibrionaceae in PD patients while the family was identified as an indicator taxon of control high in vitamin B2 (17). Core microbiome and indicator species analysis of these nutrients did not identify a consistent species in PD patient groups, suggesting that nutrient intake in this specific dataset may have a limited influence on shaping gut microbe profiles for PD patients. However, both analyses revealed that compared to PD groups, control groups overall had more core microbiome members and indicator species, suggesting decreased sensitivity to nutrient consumption in the gut microbiome of PD patients.

From DESeq analysis, we observed an overall trend that high nutrient intake caused a greater number of upregulated ASVs compared to downregulated ASVs in most nutrient and disease status groups (Table 2). However, coffee intake in PD and NSP intake in control displayed an inverse trend. Interestingly, the coffee PD group had a mass downregulation of ASVs belonging to the Firmicutes family and an upregulation of ASVs from Bacteroidota (Figure S7). The opposite was observed in coffee non-PD control where more Firmicute ASVs were upregulated and more Bacteroidota were downregulated. These findings support previous research linked with a decrease in the number of Firmicutes and dysregulated Firmicutes/Bacteroidota ratio in PD patients (44). From our analysis, the differential abundance of Firmicutes and Bacteroidota remained fairly constant across PD and non-PD controls (Figures S7 and S8). These results may potentially highlight a dysbiotic effect of coffee consumption in PD. Our results also identified downregulation of the taxa *Akkermansia* in all PD high and low nutrient groups besides vitamin B2 where the taxa were upregulated (Figure S7). *Akkermansia* is positively associated with pathologies involving the gut-brain axis such as Parkinson's disease and may play a role in promoting intestinal inflammation damaging the mucosal layer (50). Interestingly, Cristea et al. identified an increase in the abundance of *Akkermansia* in PD gut microbiomes (17). Our results perhaps point towards a possible factor protecting against an upregulation of *Akkermansia* as high nutrient intake may drive a less inflammatory gut associated with PD.

With our investigation showing only weak correlations between high and low intake of nutrients and gut microbial diversity, we investigated if intake levels affected the functional capacity of the microbiome. Our functional pathway analysis revealed that nutrients have a differential and generally greater metabolic effect on PD patients, with 19 functional pathways being affected by four nutrients in PD patients, while only 12 pathways were affected by three nutrients in the control participants. Only folate intake affected both PD patients and control participants. We found that the sulfoglycolysis pathway was upregulated by low intakes of NSP, fructose, and vitamin C in PD patients. Sulfoglycolysis is a catabolic process where sulfoquinovose produced by photosynthetic organisms is metabolized to produce energy and dihydroxyacetone phosphate and dihydroxypropanesulfonate (DHPS) (45). DHPS is an intermediate to hydrogen sulfide (H2S) by sulfate-reducing bacteria (46).

Murros found the microbiome of Parkinson's disease patients have increased amounts of H2S producing bacteria (47). Excess produced H2S can enter the cell interior, increasing the amount of reactive oxygen species which can induce formation of alpha-synuclein oligomers in nervous system cells (47). These alpha-synuclein oligomers disrupt synaptic function and are linked neuropathologically to PD (48). Thus, the upregulation of the sulfoglycolysis pathway by low intakes of NSP, fructose, and vitamin C in PD patients may provide increased flux of H2S precursors resulting in excess H2S, potentially contributing to PD pathogenesis. We also found that pathways related to protein degradation were upregulated in PD patients, but not control participants with 3 pathways related to L-arginine degradation upregulated by low intake on NSP. Interestingly, low intake of NSP, folate, and vitamin C were all associated with a downregulation of the nicotinate degradation I pathway. Nicotinate mononucleotide (NMN), one of the metabolites produced by this pathway, is a precursor for NAD+, which has been shown to play a role in brain aging and neurodegenerative diseases, including PD (38). A recent phase 1 clinical trial investigating NAD+ replenishment therapy, reported functional improvements in PD patients, indicating the therapeutic potential of increasing NAD+ (50). The mean vitamin C intake, 60.7 mg/day for males and 57.6 mg/day for females, among the PD low group was below the estimated average requirement (EAR), which is the intake estimated to meet the needs of 50% of the population (75 and 60 mg/day for males and female respectively) (50) (Table S2). Furthermore, the low-intake group of both NSP and folate was not even 50% of the EAR, indicating that the low-intake groups for these nutrients were approaching deficiency levels (50) (Table S2). Taken together, our results indicate that ensuring adequate intake of fructose, NSP, folate, and vitamin C may be beneficial for PD patients.

Limitations As mentioned by Cirstea et al., all results are correlational and cannot be described as causative. Other limitations of our study include the mechanisms of the Food Frequency Questionnaire (FFQ) and dataset collection. Diet is reported by the participants and only recorded once. Therefore, we cannot track how changes in nutrient consumption influence gut microbiota composition. Furthermore, specific nutritional consumption levels may not be accurate and are estimated based on the (FFQ). Dietary assessment questionnaires are also known to have several sources of error in them, including the participant's ability to accurately remember what their intake of particular foods was (51, 52). Participant weight was not included in the source publication metadata and nutrient consumption level cannot be controlled for weight. Therefore, for the core microbiome, indicator species, differential abundance, and functional pathway analysis were separated into four quartiles of nutrient consumption which may not be biologically relevant without controlling for other factors. PICRUSt2 predicts functional pathways based on the relative abundance of the taxa within the community using the 16S sequencing data, however, this method is highly dependent on the reference genomes and their annotation and does not consider actual protein expression, so it can only be taken as a rough approximation (53). Additionally, the significance of the PERMANOVA analysis was filtered based on p-values and not the p-adjusted value which could introduce false positives into our results and inflate the significance of the calculated p-values. As p-values were used, some of the observed significant differences might be due to random chance rather than true effects, potentially compromising the reliability and validity of our conclusions.

Conclusions Our results indicate that nutrients may play a complex role in the composition and functionality of the microbiota in PD patients. We identified six nutrients (fructose, NSP, folate, vitamin B2, vitamin C, and coffee) that may correlate with gut microbiota beta diversity and used these for downstream investigations. PD patients may be less sensitive to nutrient-induced changes to their microbiota composition compared to non-PD individuals. From a microbiota functional perspective, we discovered that nutrients possibly had a greater effect on the metabolic pathways of the microbiome in PD compared to non-PD controls. Our investigation highlighted the complex relationship between specific nutrient intake and changes to the gut microbiome, which is further regulated by disease status. Future investigations are necessary to fully elucidate the mechanisms involved to determine the importance of nutrient consumption in PD.

Future Directions Since nutritional intake levels were only recorded once in the Cirstea et al. dataset, it is unknown whether participants' nutritional intake varied significantly temporally and if this single nutritional data snapshot accurately captured long-term dietary patterns (17). Future studies can employ a longitudinal design that can record long-term nutritional intake to determine potential PD gut microbiome compositional changes. Furthermore, future investigations can use metatranscriptomics, shotgun metagenomics, metabolomics, or shotgun proteomics to confirm the observed functional changes that were inferred from 16s amplicon data. Another extension to these findings could incorporate a targeted approach where taxa known to be affected by certain nutrients are examined to determine potential effects on this dataset or other datasets.

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CONTRIBUTIONS

RC conducted QIIME2 processing, beta diversity analysis, and functional pathway analysis. KZ conducted alpha-diversity analysis. LH conducted core microbiome analysis, coded nutrient classification, and assisted others in R scripts. AV conducted indicator taxa analysis. GN conducted differential abundance analysis. All authors contributed equally to writing and editing the manuscript.

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