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Effects of sleep problems and antidepressant use on the gut microbial community of Parkinson's disease patients

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SUMMARY Parkinson's disease (PD) patients display a modulation in microbial communities of the gut microbiota in comparison to that of healthy individuals. Parkinson's disease is commonly characterized by obstructive gait, rigidity, and bradykinesia where approximately 90% of Parkinson's patients experience sleep dysfunctions and are often diagnosed with depression. Although previous studies have demonstrated the potential effects of sleep problems and antidepressant (AD) use on the gut, the direct effect on the overall composition and relative abundance of gut microbial taxa remains unresolved. As a result, this study was conducted to characterize the effects of sleep problems and antidepressant use on the microbial composition of Parkinson's patients. Analysis of both sleep problems and antidepressant use showed no significant difference in the gut microbial composition of Parkinson's patients. However, phylogenetic diversity was significantly different based on antidepressant use. Moreover, family *Prevotellaceae* was decreased in Parkinson's patients with antidepressant use. Indicator taxa analysis identified 50 bacterial families that may potentially explain the differences in the microbial composition of antidepressant use. Overall, antidepressant use may affect microbial diversity of Parkinson's patients with decreased abundance in Prevotellaceae and identified indicator taxa.

INTRODUCTION

Parkinson's disease is a neurodegenerative disorder targeting the dopaminergic pathways in the brain that commonly affects geriatric populations worldwide (1). It is a progressive and chronic neurodegenerative disease known to be modulated by genetic and environmental elements (1). The years of healthy life lost on average attributed to Parkinson's disease is reportedly 17.7 years demonstrating the detrimental effects Parkinson's disease produces along with the expenses of rehabilitation and patient care (2). Parkinson's disease is distinguished by both motor and nonmotor features and is most commonly characterized by changes in motility including clinical symptoms of obstructive gait, rigidity, and bradykinesia which worsen with the progression of the disease (1, 3). Researchers have recently become more aware of nonmotor symptoms and their association with Parkinson's disease (2). Some non-motor symptoms are seen to manifest prior to disease diagnosis itself, where Parkinson's patients experience rapid eye movement (REM) sleep behavior disorder, fatigue, and depression (2). Parkinson's disease is also often distinguished by gastrointestinal comorbidities such as reduced colonic transit time, constipation, and alterations in the gut microbiota (5). Aside from Parkinson's disease, microbiota has also been associated in other neurological conditions such as autism spectrum disorder, depression, anxiety, and Alzheimer's disease (5). Recent studies have identified changes in the microbial communities of the gut microbiota in patients with Parkinson's compared to that of healthy individuals (6). More specifically, one study demonstrates a shift in the microbial composition where the Parkinson's microbiota has an increased abundance of the genera *Akkermansia*, *Lactobacillus,* and *Bifidobacterium* and a prominent depletion of short-chain fatty acid (SCFA) producing bacteria (7). Additionally, 16S sequencing of the microbiome of Parkinson's patients identified changes in the relative abundance of nine genera where shifts in gut microbial composition were indicative of an environment more prone to local inflammation (7).

In addition to changes in microbial composition and mobility deficits, approximately 90% of Parkinson's patients experience sleep dysfunctions such as insomnia, REM sleep behaviour disorder, and challenges with daytime alertness (8, 9). Sleep impairments are the most common non-motor symptom of Parkinson's disease (10). The relationship between the gut

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microbiome and lack of sleep remains unclear with conflicting results on whether diminished sleep leads to changes in gut microbial composition (11). In contrast, it has been found that total microbiome diversity is positively correlated with increased sleep efficiency and total sleep time (11). Moreover, certain phyla such as *Bacteroidetes* and *Firmicutes* have been positively correlated with sleep efficiency. It remains to be determined how sleep problems in Parkinson's patients translate into changes in the gut microbiota of Parkinson's patients.

Alongside sleep disorders, Parkinson's patients are often diagnosed with depression. Depression is a common comorbidity developed by the majority of Parkinson's patients where depressive episodes can worsen Parkinson's symptoms (12, 13). Depressive disorder has been correlated with changes in relative abundances of specific microbial taxa along with reduced microbial diversity and richness (14). Pharmacological interventions are common for the treatment of depression such as the use of selective serotonin reuptake inhibitors (SSRIs) (15). In *vitro* analysis demonstrates that antidepressants such as SSRIs display an ability to exert antimicrobial pressure and modulate community diversity (14). Antidepressants were seen to display greater antimicrobial activity on gram-positive bacteria compared to gram-negative (14). Elucidating the potential effects of antidepressant use on the gut microbiota of Parkinson's patients is required to better understand factors influencing the gut and their downstream effects on host disease.

Some studies have suggested that sleep quality may be influenced by the composition of the gut microbiome (16). While the mechanisms of the relationship remain unclear, sleep quality is found to be positively correlated with gut microbiome diversity (11). Because sleep impairments are the most common non-motor symptom in Parkinson's disease, the research team investigated whether there are differences in the diversity of the gut microbiome in Parkinson's patients with and without sleep problems. Because there is a positive correlation of sleep quality with gut microbiome diversity, it was hypothesized that sleep problems and lower sleep quality correlate to a decreased diversity of gut bacterial taxa in Parkinson's patients with sleep problems compared to patients without sleep problems.

As a result, this research set out to inspect a dataset of a study by Cirstea et al. which explored how metabolism and the microbiota are implicated in gut function of Parkinson's patients (5). A cohort of 300 people consisted of 197 being Parkinson's patients and 103 patients as a control category (5). 16S rRNA sequencing of fecal gut microbiota samples and metabolomics were performed in addition to collection of extensive categories of metadata (5). The metadata categories of 'Antidepressant use' (AD) with "Yes" (n = 45) and "No" (n = 118) categories were explored, along with 'Sleep problems' with "Yes" (n = 66) and "No" (n = 98) categories in Parkinson's patients only (n = 163). The combined categories of sleep problems and antidepressant use with respective combinations of "Yes_Yes" (n = 24), "Yes_No" (n = 52) , "No_Yes" (n = 32), and "No_No" (n = 79) were also explored.

In addition to sleep impairments, a higher prevalence of depression among patients suffering from Parkinson's disease has been well documented in the literature (13, 14, 15). In the treatment of depression in Parkinson's patients, pharmacological interventions are widely used despite the lack of conclusive evidence for their effectiveness (15). Although previous studies have demonstrated antimicrobial properties of antidepressants *in vivo,* the direct effect on the overall composition and relative abundance of gut microbial taxa remains unresolved (17). Thus, considering the possibility of antidepressant-induced gut dysbiosis, the gut microbiome diversity of Parkinson's patients with antidepressant use was compared to those without. Due to the intrinsic antimicrobial properties of antidepressants, a negative correlation was hypothesized to be likely between antidepressant use and microbial diversity in Parkinson's patients.

METHODS AND MATERIALS

Data import and quality control: The dataset used in this study was obtained from Cirstea et al (5), where the 16s rRNA V4 region was amplified using 515F/806R primers and sequenced on an Illumina MiSeq platform. This data consisted of 300 human participants aged 40 to 85 with 103 patients being part of the control category and 197 being Parkinson's patients (5). Using Quantitative Insights Into Microbial Ecology 2 (QIIME2) (18), a microbiome bioinformatics platform, raw sequences from the original study were imported and demultiplexed before undergoing a quality control step using the Divisive Amplicon

Denoising Algorithm 2 (19) algorithm. Prior to quality control, a truncation length of 251 base pairs was applied. The resulting output provided a features table containing the frequencies of Amplicon Sequence Variants (ASVs).

Metadata filtering and grouping: Taxonomy was assigned to the ASVs by training a Naive Bayes classifier using 16S rRNA reference sequences from the SILVA database (20, 21). The inquiry of this study only focused on the differences in the gut microbiota within Parkinson's Disease (PD) patients while examining the metadata categories of antidepressant use and sleep problems. A new metadata category was created using R by concatenating the antidepressant use column with the sleep problems column to examine both categories together. The resulting new column contained four possible categories ("Yes_Yes" (n = 24), "Yes_No" (n = 52), "No_Yes" (n = 32), "No_No" (n = 79)) reflecting the different combinations of sleep problems and antidepressant use. Using QIIME2, the data was filtered to exclude control patients resulting in a features table containing only Parkinson's patients. The Parkinson's patient filtered table was filtered again to generate a table with removed [sampleIDs] containing NA values for either sleep problems or antidepressant use.

Alpha and Beta diversity analysis: Using QIIME2, an alpha rarefaction curve was generated using the filtered dataset to determine rarefaction parameters. A sampling depth of 8836 was chosen because it was the saturation point where ASVs were most represented (1,440,268 features) while retaining a sufficient number of samples (163 samples, 83.59% retained) in each category. Using the SILVA 138 99% database, a Naive Bayes classifier was trained with 16S rRNA sequences and taxonomy was assigned to each sequence (22). In addition, rooted and unrooted phylogenetic trees were generated using representative sequences in order to create the core diversity metrics necessary for alpha and beta diversity analysis. Next, the metrics of Faith's phylogenetic distance (23) and Pielou's evenness (24) were used to evaluate alpha diversity for metadata categories of interest. Statistical significance was determined using the Kruskal-Wallis pairwise test. For assessing beta diversity, unweighted UniFrac and weighted UniFrac metrics were used with pairwise permutational analysis of variance (PERMANOVA) to determine significance (25, 26, 27). Plots for alpha and beta diversity were generated in R after importing in the necessary files from QIIME2.

Differential Abundance: Differential abundance analyses and plots were generated using R software (28). All analyses required tidyverse, vegan, ape, phyloseq, DESeq2 packages (29, 30, 31, 32, 33). Filtering of data to a sequencing depth of 8836 was performed in R (28). Filtering of data was performed when comparing gut microbial communities in Parkinson's patients with or without antidepressant use; control participant data were removed. After filtering, ASVs that were < 0.05% of the relative abundance were removed keeping only highly abundant taxa in the analysis. At the family taxonomic level, organisms that displayed a significant differential abundance with an FDR-corrected $P < 0.05$ were determined and visualized.

Relative Abundance Analysis: Relative abundance was determined and plotted using R software (28). First, the relative abundance of microbial taxa at different taxonomic levels was calculated. Following, bacterial genera that represented a relative abundance of < 0.05% were removed therefore, only abundant bacterial genera were retained for analysis. Finally, relative abundance plots were generated at varying taxonomic levels of family and genus levels using Tidyverse and ggplot2 packages (29) to visualize changes in relative abundance with different treatment groups.

Indicator Taxa Analysis: Indicator taxa analysis was performed in R (28) with the aid of the microeco package (34). The files generated from QIIME2 analysis (features table, phylogenetic tree, taxonomic data, metadata) were imported into R and the dataset was prepared for taxonomic abundance and indicator taxa analysis. The dataset was filtered for Parkinson's patients, rarefied to a depth of 8836 (identical parameters as diversity analysis), and filtered again to remove low abundant ASVs (<0.05%). The trans_diff class of the microeco package used the random forest method (35) - a machine learning classification

algorithm - to analyze taxonomic abundance and identify biomarkers (indicator taxa) at the family level. One plot was generated for relative abundance and another plot was generated to display the top 20 families (out of the 50 identified for antidepressant use) based on their indicator values (MeanDecreaseGini).

RESULTS

Combined sleep problems and antidepressant use do not alter the gut microbial composition of Parkinson's patients. Combined categories had no differences in alpha or beta diversity (Figure 1, Figure S1). Principal Coordinates Analysis (PCoA) plots appeared to show no distinct clustering between groups of sleep problems and antidepressant use in all four beta diversity metrics (Figure 1A, Figure S1), further supported lack of significant differences as assessed by PERMANOVA. Similarly, there were no differences in alpha diversity between the four groups (Figure S3). Thus, these results suggest that microbial compositions in Parkinson's patients did not significantly differ between combined categories of sleep problems and antidepressant use.

Antidepressant use alters the gut microbial composition of Parkinson's patients. Since combined categories had no difference, we investigated individual categories. Sleep was not an important factor for alpha diversity or beta diversity (Figure 1C, Figure S2). However, beta diversity for AD use demonstrated significance. The same diversity metrics were once again utilized to determine whether antidepressant use altered the gut microbial composition of Parkinson's patients. For alpha diversity (Faith's phylogenetic diversity and Pielou's evenness) Kruskal-Wallis pairwise testing found that there were no significant differences

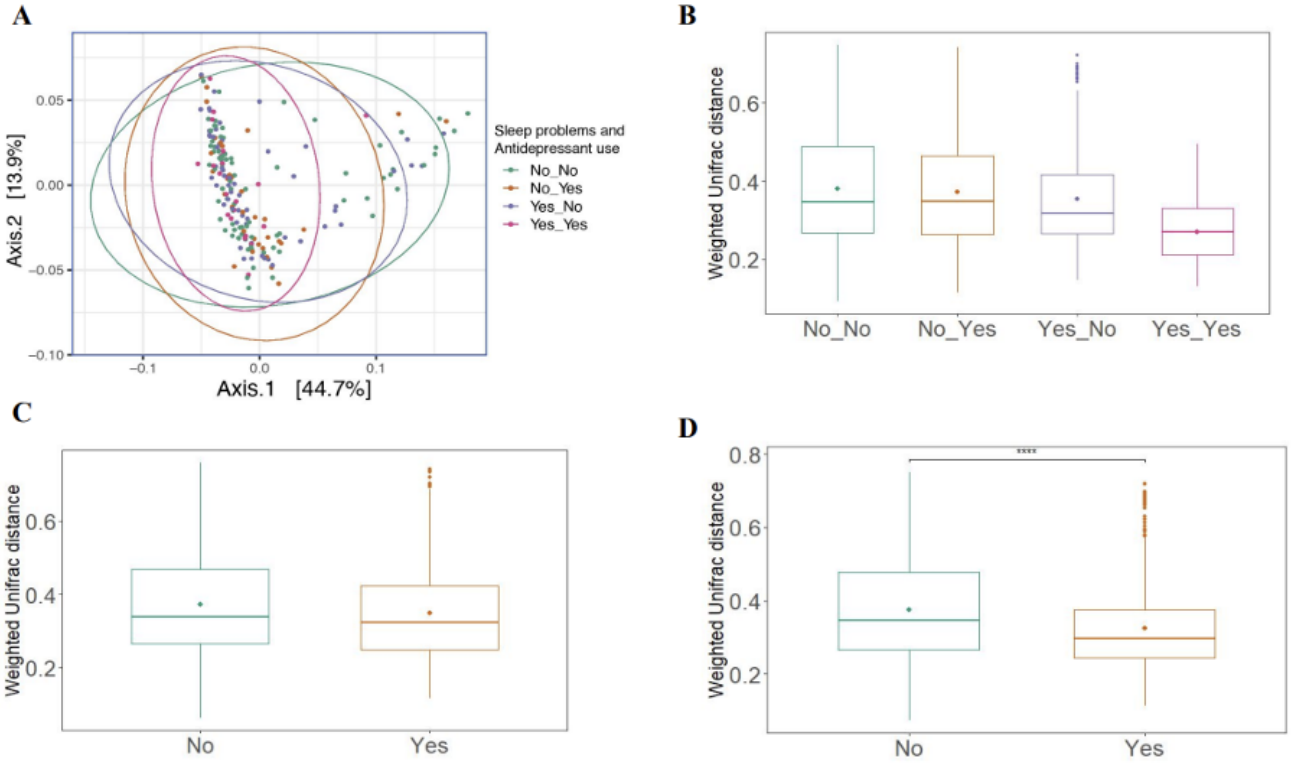

FIG. 1 Beta diversity analysis displays AD use significantly alters the gut microbial composition of Parkinson's patients, whereas the combined categories of sleep problems and AD use show no significant difference in microbial composition. Samples are grouped based on either having both sleep problems and AD use ("Yes_Yes"), sleep problems only ("Yes_No"), AD use only ("No_Yes"), or neither ("No_No"). **A)** Weighted UniFrac PCoA analysis **B)** Weighted UniFrac distance from all four groups displays no significant differences using Pairwise PERMANOVA ($p > 0.05$). **C)** Weighted UniFrac of sleep problems between ("Yes" vs "No") groups displays no significant differences using Pairwise PERMANOVA ($p > 0.05$). **D)** Weighted UniFrac of AD use between ("Yes" vs "No") groups displays a significant difference using Pairwise PERMANOVA ($p < 0.05$).

based on antidepressant use (Figure S5); however, the analysis of beta diversity metrics revealed significant differences in microbial composition between the two groups. There was a significant difference in diversity when considering both abundance and phylogenetic distance indicated by pairwise PERMANOVA results of weighted UniFrac (Figure 1D) Furthermore, the weighted UniFrac PCoA plot for antidepressant use displays a unique clustering of samples based on their similarity between the two groups. These observations are in accordance with previous studies which found that antidepressant use was associated with a change in microbial diversity in the gut (14).

Family Prevotellaceae is significantly altered in Parkinson's patients with antidepressant use**.** To characterize what microbes were influenced by antidepressant use and how they changed in response to it, differential abundance analysis was performed at the family taxonomic level (Figure 2A). An investigation of taxonomic composition at the family level revealed a large majority of bacterial families were shared between patients with antidepressant use (Figure 2A). Differential abundance analysis depicted no significant differences between the two groups of antidepressant use at the family level except for one microbial family, *Prevotellaceae where relative abundance was decreased* (Figure 2A, 2B). Relative and Differential abundance analysis confirmed a significant change in *Prevotellaceae* with FDR adjusted p-value metrics (Figure 2B, Figure S6). These results are additive to previous findings of diminished bacterial species within *Prevotellaceae* of Parkinson's patients compared to healthy controls (5). This study's analyses suggested that antidepressant use in Parkinson's patients modulates a decrease in the abundance and microbial diversity of gut microbes, specifically those belonging to the family *Prevotellaceae*.

Indicator taxa analysis reveals microbial families altered with change in antidepressant use status. Next, the study sought to determine whether there were any bacterial taxa that were indicators of the alterations in microbial diversity observed in Parkinson's patients who use antidepressants. To determine this, indicator taxa analysis was performed to elucidate the potential contribution of microbial families in explaining the changes found within the antidepressant use category (Figure 3A). The Mean Decrease Gini metric reported the contribution of each family to the observed changes between categories of antidepressant use. The higher the Mean Decrease Gini metric, the larger the potential role the microbe displayed in explaining the microbial alterations observed between antidepressant use categories. Indicator taxa analysis identified 50 microbial taxa at the family level thought to be responsible for shifts in microbial community composition (Figure 3A). To visualize the unique number of microbial taxonomic families between antidepressant use categories, the distribution of the top 20 unique microbial families, based on their indicator values, was visualized (Figure 3A). Samples in the "No" antidepressant group had more taxonomic families compared to the "Yes" group with a reduced families of bacteria present in Parkinson's patients with antidepressant use (Figure 3B).

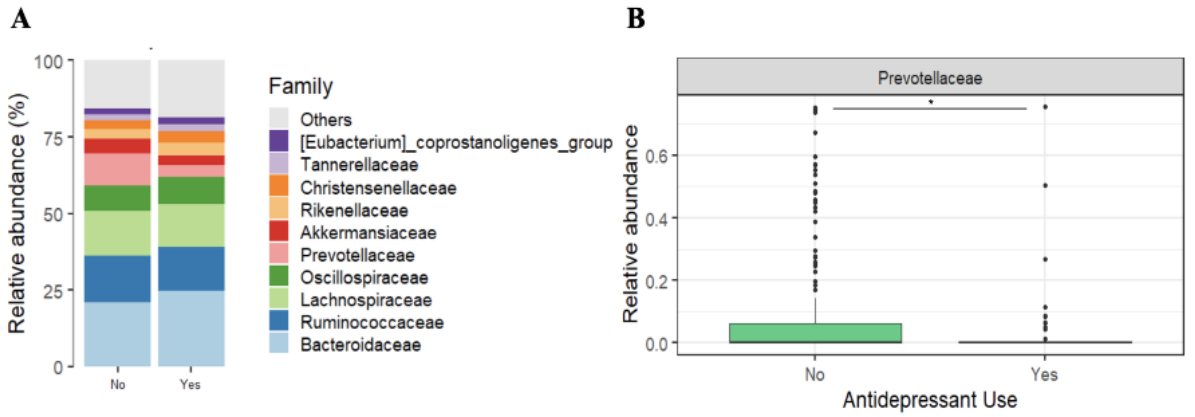

FIG. 2 *Prevotellaceae* is differentially abundant in Parkinson's patients with and without AD use. A) Relative Abundance at the family taxonomic level with or without AD use ("Yes" and "No" groups). **B)** Relative Abundance of *Prevotellaceae* family in "Yes" and "No" groups of AD use with FDR corrected analysis ($p < 0.05$).

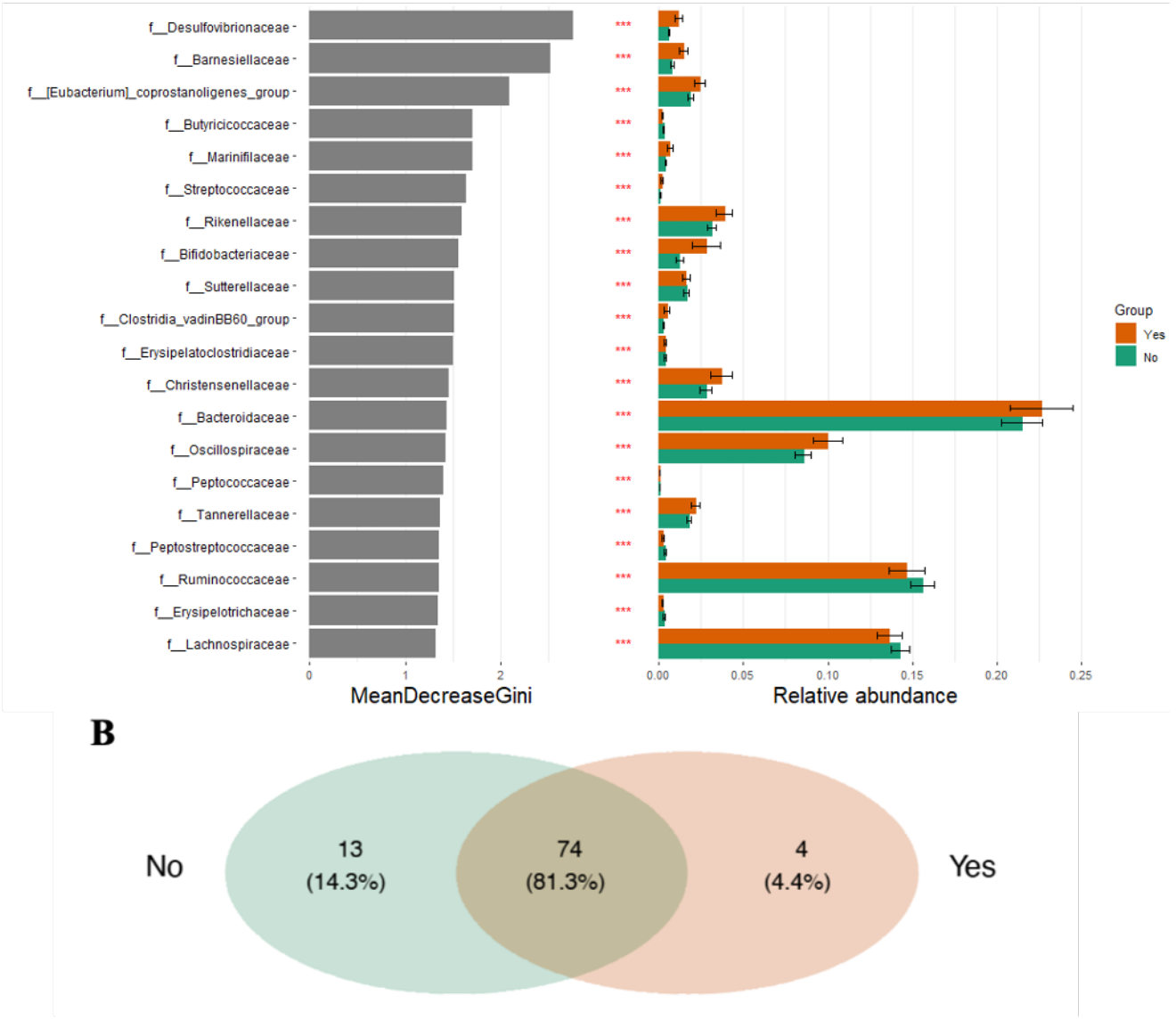

FIG. 3 AD use displays unique bacterial composition at the family taxonomic level and unique bacterial taxa at the family level in Parkinson's patient samples. A) Indicator taxa analysis displays the top 20 (based on the strength of the indicator value, Mean Decrease Gini) of the 50 identified significantly unique taxonomic family groups that are found in the gut of Parkinson's patients that use antidepressants compared to those that do not. **B)** Venn diagram of unique taxonomic families from Parkinson's patients with and without antidepressant use.

DISCUSSION

In comparison, antidepressant use alters gut microbial composition whereas sleep problems do not in Parkinson's patients. This study investigated the effects of sleep problems and antidepressant use on the gut microbiome composition of Parkinson's patients. Due to there being a positive correlation between sleep quality and gut microbiome diversity, sleep problems and lower sleep quality correlating to a decreased diversity of gut microbes in Parkinson's patients with sleep problems was hypothesized. Additionally, due to intrinsic antimicrobial properties of antidepressants, a negative correlation between antidepressant use and microbial diversity in Parkinson's patients was hypothesized. Both metadata categorieswere initially grouped together into four groups of "Yes_Yes," "Yes_No," "No_Yes," and "No_No" for QIIME 2 pipeline analysis using the dataset collected (5, 18). Further diversity analysis of combined categories of sleep problems and antidepressant use demonstrated that alpha and beta diversity between the four groups did not significantly

differ. This showed no significant correlation existed between Parkinson's patients who may have had both sleep problems and antidepressant use, either sleep problems or antidepressant use alone or neither. As a result, sleep problems and antidepressant use categories were individually explored in Parkinson's patients. Diversity metrics revealed that Parkinson's patients with or without sleep problems did not significantly differ dependent on either abundance or phylogenetic distance of samples. These results did not support the hypothesis of sleep problems and lower sleep quality correlating to a decreased diversity in the gut microbiota of Parkinson's patients. Despite past work mentioning a positive correlation between sleep quality and gut microbiome diversity, these trends were not observed within the dataset of this study (8). This demonstrates that there is no significant relationship between Parkinson's patients with or without sleep problems within our dataset. This may be attributed due to a lack of an appropriate sample size with samples being geographically confined to the Greater Vancouver region Although alpha and beta diversity metrics for sleep problems did not differ, beta diversity of antidepressant use in Parkinson's patients demonstrated a significant difference.

Changes in the microbiota of Parkinson's patients with antidepressant use. Measuring Weighted UniFrac distances of samples displayed that microbial taxa compositions in Parkinson's patients with antidepressant use were significantly different from patients who do not use antidepressants. All four beta diversity metrics (Jaccard, Weighted and Unweighted UniFrac and Bray Curtis), were applied to the antidepressant use category. Since no results were statistically significant except for the Weighted UniFrac distance metric, it can be concluded that differences in the microbial composition of samples were likely dependent on the phylogenetic relatedness of ASVs within patient samples. It was hypothesized that the antimicrobial properties of antidepressants negatively correlated between antidepressant use and microbial diversity in Parkinson's patients. Following the establishment of a difference in the microbial composition of Parkinson's patients with antidepressant use, this study sought to follow up on beta diversity differences by looking at specific contributing taxa. Differential abundance analysis demonstrated a significant decrease in the *Prevotellaceae. Prevotellaceae* has been noted in studies associating the gut microbiota and Parkinson's disease. Specifically, both *Bacteroides* and *Prevotellaceae* are less abundant in the gut microbiota of Parkinson's patients compared to healthy controls (36). Moreover, irritable bowel syndrome-like symptoms have been associated with a decrease in the abundance of microbes in the *Prevotellaceae* family (5). Since *Prevotellaceae* prevalence is reduced in Parkinson's patients, patients who also use antidepressants display an even lower abundance of *Prevotellaceae* when these variables of disease and treatment are combined. This can result in intensified symptoms of irritable bowel syndrome-like symptoms. *Prevotellaceae* is a family of gram-negative bacteria from the phylum of Bacteroidetes (37). The functions of this bacterial family are extensive such as degrading polysaccharides which produce SCFAs that are thought to participate in metabolic mediated gut-brain crosstalk (38). The systemic implications of reduced gastrointestinal motility, constipation, and irritable bowel-like symptoms of this bacterial taxon with decreased abundance are important to consider (5, Cirstea et al). These must be accounted for in future studies of how microbial composition is associated with Parkinson's disease and antidepressant use.

To further understand the observed microbial alterations, indicator taxa analysis was performed to determine which microbial taxa at the family level are implicated in the shifts observed in microbial composition. There were 50 significant families of bacteria identified by indicator taxa analysis in Parkinson's patients with antidepressant use. Among these families were *Desulfovibrionaceae*, *Christensenellaceae*, *Bacteroidaceae,* and *Lachnospiraceae* which potentially explain microbial shifts with variation in antidepressant usage. These four families are heavily associated with changes in PD patients and can potentially be responsible for the reflected changes in microbial composition, for the abundance of *Lachnospiraceae* is correlated with longer PD duration, cognitive decline, to worse motor symptoms (39). *Christensenellaceae* are thought to play a role in lipid metabolism and *Desulfovibrionaceae* is implicated in proteolytic metabolism in the gut which is considered detrimental (39, 5) Indicator taxa analysis reports the bacterial members of *Desulfovibrionaceae* and *Christensenellaceae* are found in higher abundances of Parkinson's

patients with antidepressant use demonstrating their increased potential ability to modulate gut composition through previously mentioned processes. . Moreover, *Bacteroidaceae* and *Lachnospiraceae* are important families responsible for multiple functions and features of the gut microbiota (40, 41). They are often found to be diminished in Parkinson's patients, but Bacteroidaceae displayed an increase in abundance in patients with antidepressant use (5). Previous studies have observed increased antimicrobial activity of some antidepressants on gram-negative bacteria which may be responsible for shifts noted in bacterial species (14). Nonetheless, a better understanding of these microbes concerning antidepressant use is required before a definite interpretation can be made. *Prevotellaceae* was not observed among the top 20 microbial families which were expected to explain the differences between antidepressant use. There is a disparity between the results observed with indicator taxa analysis and the results relative and differential abundance analysis presented. Since indicator taxa analysis uses a sophisticated machine learning classification algorithm to identify biomarkers (34), the absence of *Prevotellaceae* as an indicator taxa may be dependent on the modulation of gut composition via an identified indicator taxa rather than *Prevotellaceae* being responsible for the changes in microbial composition of antidepressant use itself.

Limitations A few limitations restrict the scope of this study. Firstly, the limitations of an imbalance for sex, associated observations only due to the cross-sectional nature of the study, and reduced metabolomics conduction capacity stated in *Cirstea et al* must be extended to the study. The existing metadata provided allows for correlational studies between varying categories within the dataset, but definite conclusions of cause and effect are unable to be drawn. Additionally, due to Parkinson's disease diagnoses disproportionately affecting men; the ratio of samples to males and females is not equivalent. As a result, the conclusions that are formulated within this study on Parkinson's patients may be more representative of male Parkinson's patients than females. This can be attributed to sex-based differences between male and female patients with their physiological and gut microbial responses to antidepressant use, sleep problems, and other components of Parkinson's disease (42).. In addition, a suspected limitation of this study is the disparity of sample sizes between groups within metadata categories of interest. This is apparent when metadata categories of antidepressant use and sleep problems were combined. The "Yes_Yes" group only contains 24 samples compared to 32 of the "Yes_No" category, 52 of the "No_Yes," and 79 patients in the "No_No" group. An unequal representation of samples, especially those in categories of interest such as "Yes_Yes" can mask potential significant differences due to a sample size that is not sufficiently representative of a population of Parkinson's patients with sleep problems and antidepressant use. Therefore, screening more Parkinson's patients with suspected antidepressant use and, or sleep problems allows for the analysis of a larger sample size which provides a better understanding of the true effects of the sleep problems and antidepressant use categories on gut microbial composition.

Moreover, another potential limitation is that the analysis of this study is conducted with a cohort of Parkinson's patients and control, healthy individuals, residing in the Greater Vancouver region during the time of the study. Previous literature demonstrates that gut microbiotas between geographical locations are seen to differ (43). One group argues that gut microbial composition differs according to diet and eating habits which are closely correlated to geographical location (44). As a result, these analyses and the associated conclusions may not be applicable to Parkinson's patients in other areas of the world where gut microbial compositions may display an inherently different gut microbial composition due to diet and eating habits. Therefore, the conclusions of this study are potentially limited to Parkinson's patients with a gut microbiota profile similar to that of the cohort patients found in the Greater Vancouver region.

Conclusions This computational analysis of the Parkinson's data from Cirstea et al., rendered curated outputs providing insight as to how sleep problems and antidepressant use modulate the microbial composition of the gut microbiota of Parkinson's patients. Although sleep deficits and antidepressant use are common in Parkinson's patients, results conclude that these factors combined did not alter the gut microbial composition of Parkinson's patients. Investigating each variable separately, antidepressant use but not sleep problems were

associated with changes in the gut microbiota of Parkinson's patients. These results were contrary to previous papers and indicate that sleep problems may not play a significant role in gut microbial composition. The relative abundance of the *Prevotellaceae* family significantly decreased in Parkinson's patients who use antidepressants. *Prevotellaceae* abundance is already decreased in Parkinson's patients and is associated with irritable bowel syndrome-like symptoms in Parkinson's patients. A decrease in the relative abundance of *Prevotellaceae* in antidepressant use suggests further exacerbation of symptoms. Indicator taxa analysis displayed the significant microbial taxa at the family taxonomic level implicated in alterations in the gut microbial composition of antidepressant use. Overall, this paper works towards identifying the role of sleep problems and antidepressant use in modulating microbial composition in Parkinson's patients.

Future Directions The interconnection of the microbiota and Parkinson's disease is an emerging topic subject to further investigation. This study provides foundational insight that can be expanded upon in future studies. In proceeding analyses, one should consider possible confounding factors stated in the literature that could be altering microbial composition such as mental health diagnosis. While sub-setting the data for antidepressant use and sleep problems mental health diagnoses were not considered in relation to these metadata categories. Therefore, changes in the microbial composition associated with mental health diagnoses, such as anxiety should be controlled for in future studies.

Additionally, as antidepressants do not all mechanistically act the same, it should not be assumed that all antidepressants interact with the gut microbiota uniformly. The varying chemical structures of antidepressants along with their varying mechanisms of action on the human body facilitate an uncertainty on whether each antidepressant uniformly modulates the same effect on the gut microbiota of Parkinson's patients. As a result, future steps should consider the types of commonly used antidepressants such as selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOs) and tricyclic antidepressants to investigate how these different treatments alter microbial diversity in Parkinson's disease patients. Specifying the type of antidepressants could allow for more precise studies to identify which pharmacological intervention not only alters the neurotransmitter pathways in the brain but also the bacterial composition in the gut.

Finally, changes in the microbial composition of healthy individuals with sleep disorders and antidepressant use can be studied. Since these are common features of both Parkinson's patients and pre-diagnosed Parkinson's, investigating the microbial composition of healthy individuals with sleep problems and antidepressant use could provide insight into the development of the neurodegenerative disease. In future studies, investigating the microbial composition of healthy patients with known predispositions could be used as a prescreening tool for the development of Parkinson's disease.

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CONTRIBUTIONS

This study is a result of the collective effect of all co-authors. The Data analysis in QIIME2 was performed by T.K and S.P. Data analysis in R was performed by C.C., and T.K. The abstract and introduction were written by C.C. and S.P. Methods were written by T.K., and S.P and results by all co-authors. C.C., and S.P. contributed to the discussion, limitations, conclusions and future directions. All co-authors edited the draft manuscript.

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