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# Metagenomics indicates an interplay of the microbiome and functional pathways in Parkinson's disease

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Previous studies suggest there are distinct gut microbial and functional variations in patients with Parkinson's disease (PwPD) that may reveal potential microbiome signatures or biomarkers to aid in early detection of the disease. In this case-control study, we used whole genome sequencing to compare the stool samples of 55 PwPD to 42 healthy controls (HC) from a public database (BioProject Accession PRJEB39223). For bacterial phyla, we observed a greater relative abundance in *Firmicutes* and *Actinobacteria* among PwPD, while that of *Bacteroidetes* was lower. For phages, PwPD had a greater relative abundance of *Siphoviridae*, *Tectiviridae*, and *Podoviridae*, while *Microviridae* was lower. Moreover, we described 10 functional pathways that most significantly differed between PwPD and HC (all  $P < 0.0001$ ). In conclusion, significant differences were observed in gut bacteria, phages, and functional pathways between PwPD and HC that both support and conflict with previous case-control studies and warrant further validation.

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of nigrostriatal dopaminergic innervation and the aggregation of misfolded  $\alpha$ -synuclein<sup>1</sup>. Gastrointestinal dysfunction—one of the earliest and most common nonmotor manifestations of PD—often precedes or parallels the onset of motor symptoms<sup>2–4</sup>. PD has also been positively associated with several gastrointestinal conditions, including chronic constipation<sup>5,6</sup>, irritable bowel syndrome<sup>7,8</sup>, inflammatory bowel disease<sup>9</sup>, and colonic diverticular disease<sup>10</sup>. As such, it has been postulated PD pathophysiology is mediated, in part, through the gut microbiota<sup>11,12</sup>. Gut dysbiosis can promote inflammation, intestinal permeability, and aberrant immune responses, all of which have been implicated in  $\alpha$ -synuclein misfolding and downstream neurodegenerative processes<sup>11,13,14</sup>. Animal studies demonstrate that microbial changes precede motor dysfunction in PD<sup>12,15</sup> and that these shifts are required to induce  $\alpha$ -synucleinopathy, neuroinflammation, and motor deficits<sup>13,15</sup>. These findings have intensified interest in identifying potential gut microbial signatures or biomarkers for PD.

Whole genome sequencing (WGS) enables a comprehensive analysis of genetic content, including bacteria, viruses, fungi, protists, and functional metabolic pathways. An increasing number of WGS-based observational studies have been conducted in patients with PD (PwPD) in Germany<sup>16</sup>, China<sup>17–20</sup>, South Korea<sup>21</sup>, Taiwan<sup>22,23</sup>, Japan<sup>24</sup>, Italy<sup>25</sup>, London<sup>26</sup>, Canada<sup>27,28</sup>,

and the US<sup>29,30</sup>. However, findings have varied across cohorts, and further research is needed to validate these findings. To build on this growing body of work, we conducted a case-control study using WGS to characterize gut microbial composition and functional pathways in PwPD and healthy controls (HCs) in a North American cohort.

## Results

Baseline characteristics for PwPD and HC are presented in Table 1. In the PD group, the mean age was 66.0 years, and sex was evenly distributed. Participants were primarily based in the US (95.0%), representing 17 states, and most identified as white (90.9%). Over half reported an annual household income exceeding \$150,000 USD (50.9%), and having obtained a graduate or professional degree (50.9%). In terms of clinical characteristics, nearly all participants had idiopathic PD (98.2%), with an average of 7.0 years since diagnosis; over half were classified as Hoehn and Yahr stage 1 (56.4%). In the control group, the mean age was 54.8 years; 76.2% were female; and all were based in Massachusetts, USA.

## Bacteria

At the phylum level, PwPD exhibited greater relative abundance of *Firmicutes* and *Actinobacteria* and lower abundance of *Bacteroidetes* and an

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**Table 1 | . Sociodemographic and clinical characteristics of patients with Parkinson's disease compared to healthy controls<sup>a</sup>**

|                                                     | Parkinson's disease (n = 55) | Healthy controls (n = 42) |
|-----------------------------------------------------|------------------------------|---------------------------|
| Age, years – mean ± SD                              | 66 ± 7.7                     | 54.8 (± 5.2)              |
| Sex – no. (%)                                       |                              |                           |
| Male                                                | 27 (45.7%)                   | 10 (23.8%)                |
| Female                                              | 28 (54.2%)                   | 32 (76.2%)                |
| Geolocation – no. (%)                               |                              |                           |
| United States                                       | 52 (95.0%)                   | 42 (100.0%)               |
| Canada                                              | 3 (5.0%)                     | 0 (0.0%)                  |
| Race – no. (%)                                      |                              | N/A                       |
| White                                               | 50 (90.9%)                   |                           |
| Non-white                                           | 5 (9.1%)                     |                           |
| Annual household income, USD – no. (%)              |                              | N/A                       |
| < \$60,000                                          | 8 (14.5%)                    |                           |
| \$60,000 to < \$80,000                              | 4 (7.2%)                     |                           |
| \$80,000 to < \$100,000                             | 7 (12.7%)                    |                           |
| \$100,000 to \$150,000                              | 6 (10.9%)                    |                           |
| ≥ \$150,000                                         | 30 (50.9%)                   |                           |
| Education level – no. (%)                           |                              | N/A                       |
| Less than college degree                            | 13 (23.6%)                   |                           |
| College degree                                      | 14 (25.5%)                   |                           |
| Graduate/professional degree                        | 28 (50.9%)                   |                           |
| Type of Parkinsonism – no. (%)                      |                              | N/A                       |
| Idiopathic Parkinson's disease                      | 54 (98.2%)                   |                           |
| Other Parkinsonism                                  | 1 (1.8%)                     |                           |
| Years since Parkinson diagnosis – mean ± SD         | 7.0 ± 11.5                   | N/A                       |
| Estimated Hoehn & Yahr stage – no. (%)              |                              | N/A                       |
| 1 (unilateral involvement only, minimal disability) | 31 (56.4%)                   |                           |
| 2 (both sides affected, balance is stable)          | 13 (23.6%)                   |                           |
| 3 (mild to moderate disability, balance affected)   | 11 (20.0%)                   |                           |

<sup>a</sup>Data for healthy controls were obtained from a public database (BioProject Accession PRJEB39223). Available sociodemographic information was limited. Clinical characteristics related to Parkinsonism were not applicable.

unclassified phylum compared with HCs (all  $P < 0.05$ ; Fig. 1). PwPD demonstrated significantly higher bacterial  $\alpha$ -diversity by both the Shannon (Wilcoxon Rank Sum  $P = 0.004$ ; Fig. 2A) and Simpson indices ( $P = 0.0002$ ; Fig. 2B), reflecting greater community evenness and dominance relative to HCs. In contrast, Chao1 was significantly lower in PwPD ( $P < 0.0001$ ; Fig. 2C), indicating reduced bacterial taxonomic richness. Bray-Curtis  $\beta$ -diversity revealed significant differences in overall community composition between groups (permutational multivariate analysis of variance [PERMANOVA]  $P = 0.001$ ; Fig. 2D). While heat map visualization did not demonstrate clear group separation (Fig. S1), linear discriminant analysis effect size (LEfSe) identified 56 bacterial taxa within the *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and unclassified phyla that were differentially enriched between groups (Fig. 3).

## Phages and viruses

Phage community composition differed between PwPD and HC at the family level. On visual inspection of group-level differences, PwPD exhibited lower abundance of *Microviridae* and *Podoviridae* and higher abundance of *Myoviridae*, *Siphoviridae*, and *Tectiviridae* compared with HCs (Fig. S2A). Phage  $\alpha$ -diversity differed between groups, with PwPD demonstrating both greater evenness by Simpson's index ( $P = 0.01$ ; Fig. 4A) and taxonomic richness by Chao1 index ( $P = 0.001$ ; Fig. 4B), while Shannon diversity did not differ significantly (data not shown). Bray-Curtis  $\beta$ -diversity analysis revealed borderline-significant dissimilarity between phage community structure between groups ( $P = 0.047$ ; Fig. 4C). Heat map visualization suggested potential differences in phage family-level relative abundance patterns between PD and HC samples, with some clustering by groups observed, although substantial inter-individual variability was also present (Fig. S2B).

Bray-Curtis  $\beta$ -diversity analysis of DNA viruses demonstrated significant dissimilarity between PwPD and HCs (Fig. S3;  $P = 0.001$ ). In the heat map (Fig. S4), we observed some HC samples with relatively high abundance of the *Adenoviridae* but little detection of viral families in PwPD (Fig. S4).

## Protists and fungi

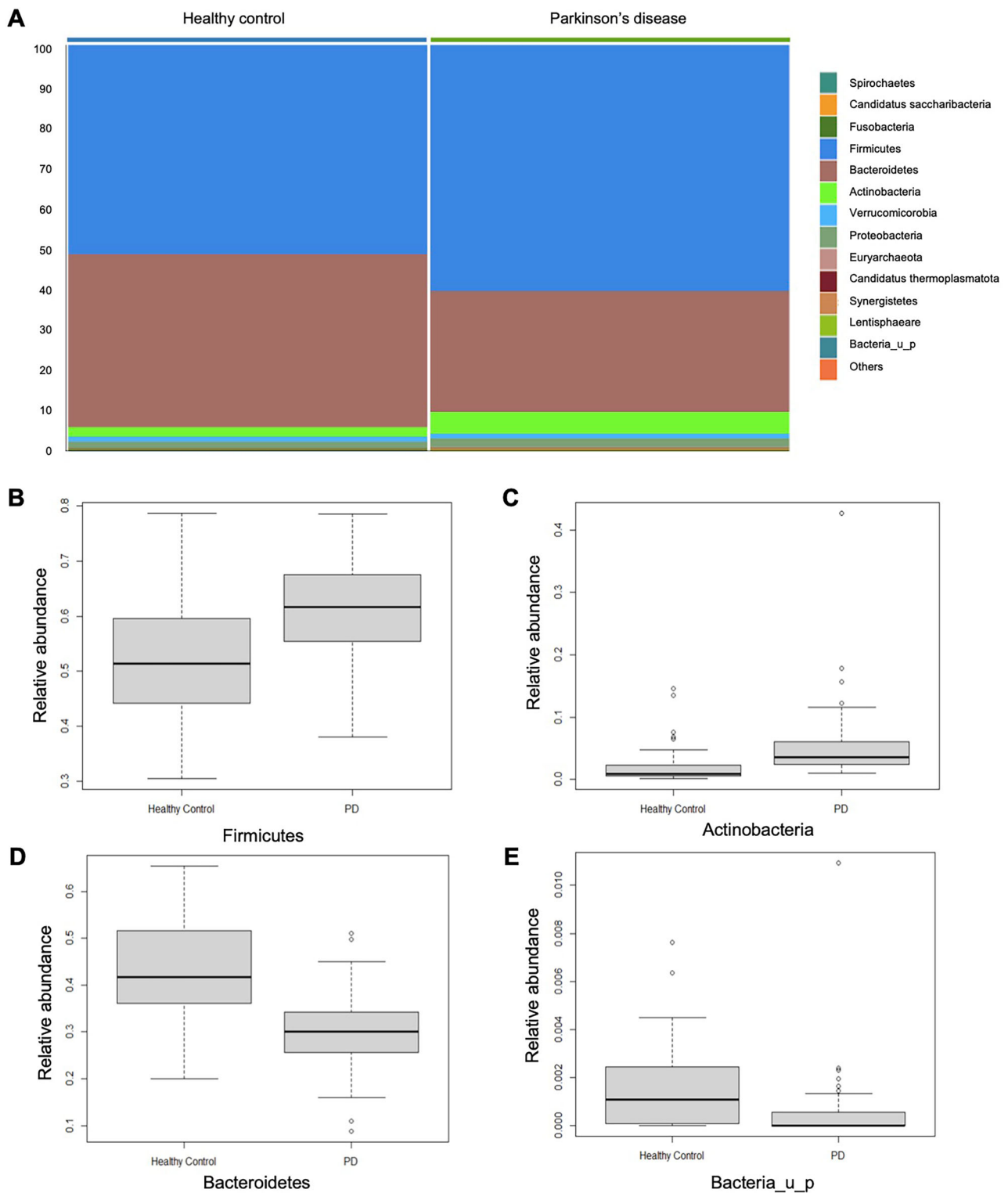
Similar to findings of DNA viruses, Bray-Curtis  $\beta$ -diversity analysis indicated overall compositional differences between PwPD and HCs ( $P = 0.001$ ; Fig. S5). However, visual inspection of the heat map showed low overall abundance of fungal species and high inter-individual variability across samples, resulting in limited statistical power to detect group-level differences (Fig. S6). In contrast, protist communities did not differ significantly between groups by  $\beta$ -diversity ( $P = 0.091$ ; Fig. S7), indicating broadly similar community composition in PwPD and HCs. Heat map visualization revealed low overall abundance of detected protists and sporadic presence of taxa across samples (Fig. S8). Nevertheless, *Blastocystis* species were detected more frequently among PwPD than HCs, although this observation was descriptive and not supported by formal statistical testing.

## Functional pathways

Lastly, functional profiling revealed marked differences in predicted metabolic potential between PwPD and HC. PwPD demonstrated significantly greater functional  $\alpha$ -diversity, with higher Simpson ( $P < 0.001$ ; Fig. 5A) and Chao1 indices ( $P < 0.001$ ; Fig. 5B), indicating both greater evenness and a larger number of detectable MetaCyc pathways. Bray-Curtis  $\beta$ -diversity showed clear separation between groups ( $P = 0.001$ ; Fig. 5C), suggesting distinct functional pathway compositions differences in PwPD. We then examined individual MetaCyc pathway terms that significantly differed in mean abundance between groups. The top 10 most significantly different pathways included: pentose phosphate pathway, fatty acid and  $\beta$ -oxidation I, inosine-5-phosphate biosynthesis III, anhydromuropeptide recycling, trichloroacetic acid (TCA) cycle VI, colanic acid building blocks biosynthesis, transfer RNA (tRNA) processing, phosphoenolpyruvate carboxykinase (PEPCK)-type C4 photosynthetic carbon assimilation cycle, guanosine nucleotide degradation III, and glutaryl CoA degradation (all  $P < 0.0001$ ; Fig. 6).

## Discussion

In this North American case-control study, we observed significant gut microbial and functional differences between PwPD and HC using WGS. PwPD exhibited distinct bacterial community structure characterized by altered phylum-level composition and differences in  $\alpha$ - and  $\beta$ -diversities, alongside shifts in the relative abundance of phage families.  $\beta$ -diversity analyses further demonstrated significant differences in overall community composition of phages, viruses, and fungi between PwPD and HCs, suggesting broad multi-kingdom alterations in the gut microbiome associated with PD. Functional profiling also revealed significant differences in predicted microbial metabolic potential, with enrichment of multiple MetaCyc pathways in PwPD.

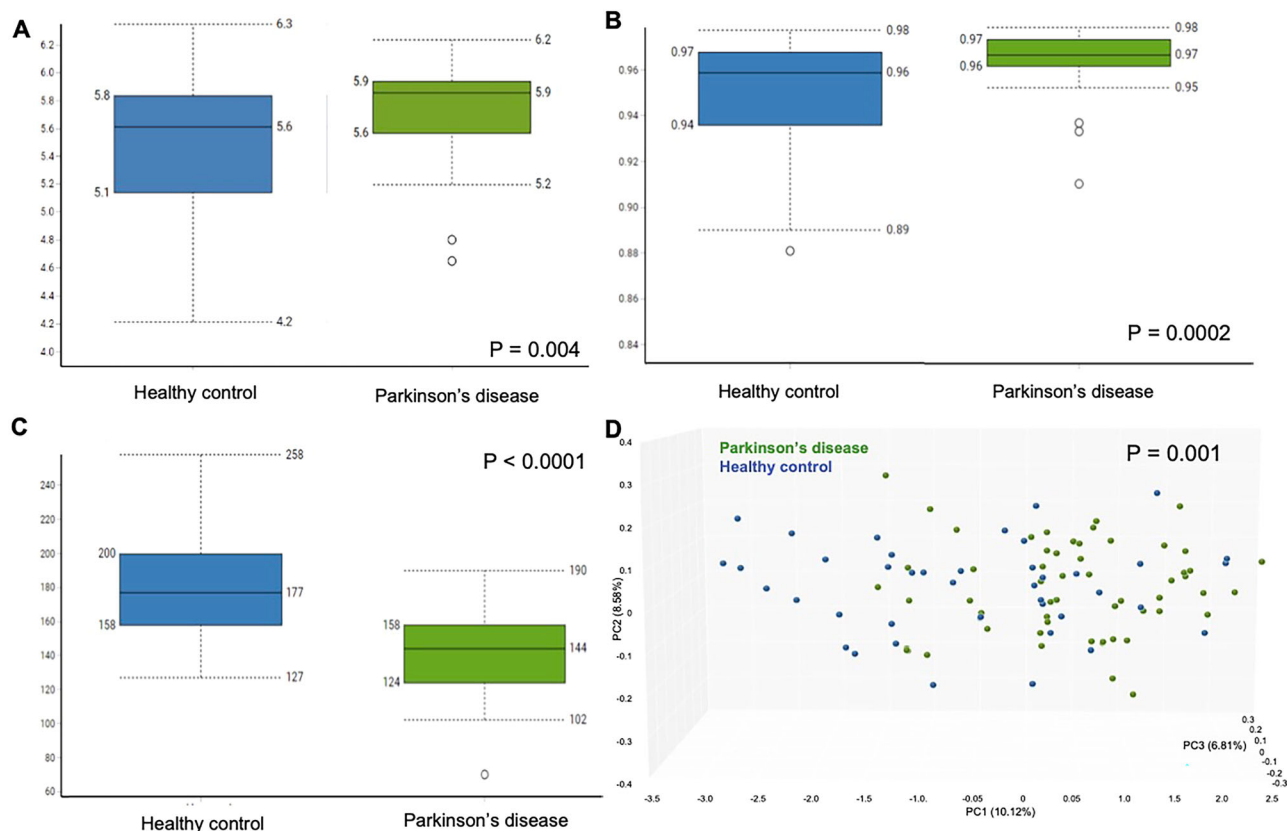


**Fig. 1 | Phylum-level bacterial community composition in patients with Parkinson's disease and healthy controls.** **A** Stacked bar plot comparing mean relative abundance of gut bacterial phyla between groups. Boxplots comparing relative abundance of *Firmicutes* (**B**), *Actinobacteria* (**C**), *Bacteroidetes* (**D**), and unidentified

phyla (**E**) between groups (all  $P < 0.05$ ). Boxes indicate the median and interquartile range; points represent outliers.  $P$  value was calculated using Wilcoxon rank sum tests. Data are based on non-subsampled bacterial profiles generated using the CosmosID-HUB platform.

*Firmicutes* and *Bacteroidetes* represent the two dominant bacterial phyla in the human gut microbiome, and changes in their composition have been implicated in metabolic and inflammatory conditions<sup>31,32</sup>. An increased *Firmicutes* to *Bacteroidetes* (F/B) ratio has been associated with obesity<sup>31,33–35</sup>, while a decreased ratio has been reported during weight loss<sup>31</sup> and in

inflammatory bowel disease<sup>35–37</sup>. Given the increased prevalence of weight loss and gastrointestinal inflammation in PwPD, a reduced F/B ratio might be expected in PD<sup>9,38</sup>. However, our observation of an increased F/B ratio aligns with several prior WGS-based studies<sup>16,17,20,29,30</sup>, suggesting this finding may characterize PD-associated gut dysbiosis despite differing clinical phenotypes.



**Fig. 2 | Bacterial  $\alpha$ - and  $\beta$ -diversities in patients with Parkinson's disease and healthy controls.** Boxplots comparing Shannon (A), Simpson (B), and Chao1 (C)  $\alpha$ -diversity indices between groups. Boxes indicate the median and interquartile range; points represent outliers.  $P$  values were calculated using Wilcoxon rank sum tests. **D** Principal coordinates analysis (PCoA) plot of Bray-Curtis  $\beta$ -diversity. Each point represents an individual subject, with Parkinson's disease samples shown in green

and healthy controls in blue. Distances between points reflect differences in phage community composition based on Bray-Curtis dissimilarity. Percent variance explained by each principal coordinate is shown on the axes.  $P$  value was calculated via PERMANOVA. Data are based on non-sampled bacterial profiles generated using the CosmosID-HUB platform.

Lower concentrations of fecal short chain fatty acids (SCFAs) have been observed in PwPD<sup>17,39–42</sup> and linked to worse cognitive and motor outcomes compared with HC<sup>17</sup>. However, previous studies have reported both a lower<sup>29,30</sup> and greater abundance<sup>43</sup> of SCFA-producing bacteria among PwPD. Although some taxa within the *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* phyla include SCFA producers<sup>44</sup>, phylum-level shifts do not necessarily reflect changes in specific SCFA-producing taxa. In the present study, we did not observe enrichment of well-characterized SCFA-producing genera and therefore cannot infer altered SCFA production capacity, particularly in the absence of metabolite measurements. Further research integrating fecal and plasma SCFA measurement with metagenomic data is needed to clarify whether changes in microbial composition correspond to alterations in SCFA production and how these may relate to PD outcomes.

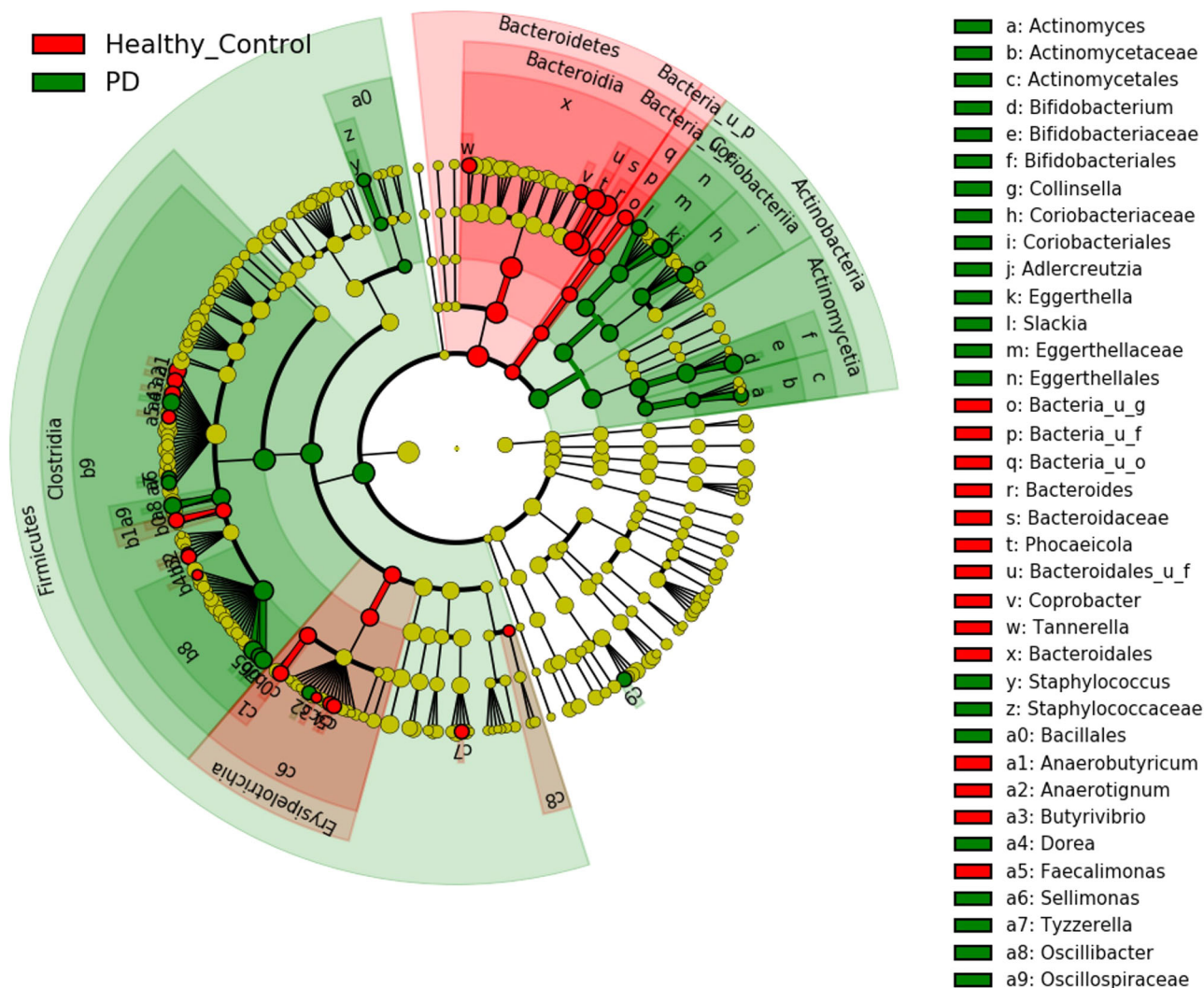
We observed notable differences in the phageome between PwPD and HCs, with changes consistent with observations in inflammatory bowel disease<sup>45</sup>. Tetz et al.<sup>46</sup> reported similar findings and proposed that shifts toward lytic phage populations may reflect dysregulation of bacteriophage-bacteria interactions in PD, potentially contributing to bacterial community instability. However, because phage-host relationships were not examined in the present study, our findings should be interpreted descriptively. Previous PD studies have both supported<sup>16</sup> and conflicted<sup>17,46</sup> our results for phage  $\alpha$ - and  $\beta$ -diversities, and additional work is needed to determine whether phage alterations represent disease-specific signatures or non-specific features of gut dysbiosis.

Recently, a study from Taiwan<sup>47</sup> also showed that hepatitis C virus infection is associated with PD risk, and the influenza virus, Coxsackie virus, Japanese encephalitis virus, and HIV have also been associated with

secondary PD<sup>48,49</sup>. In the present study, we identified reduced detection of DNA viral families in PwPD, consistent with findings reported by Bedarf et al.<sup>16</sup>. In contrast, Qian et al.<sup>18</sup> reported viral enrichment in PD and suggested these discrepancies could be due to differences in viral databases and extraction methods. Study location and population may also contribute to differences in virome profiles, as our findings were concordant with German<sup>16</sup> but not Chinese PD cohorts<sup>18</sup>. Future studies with harmonized viral reference databases and diverse cohorts are needed to clarify whether virome alterations are consistently associated with PD.

Lastly, we observed significant differences in several functional pathways between PwPD and HC. Current literature and WGS limitations constrain inferences about the pathways identified; however, mechanistic evidence on byproducts of these pathways may suggest linkages to PD pathogenesis. For instance, tRNA processing is disrupted under stress conditions, leading to the accumulation of tRNA-derived fragments (tRFs)<sup>50,51</sup>. tRFs have been proposed as potential biomarkers in PD and other neurodegenerative disorders<sup>52–54</sup>. Unique tRF signatures have been observed in serum, cerebrospinal fluid, and the prefrontal cortex of PwPD<sup>50,51</sup>, but whether microbiome-associated tRNA processing contributes to peripheral or neuronal tRF pools remains unknown.

The guanosine nucleotide degradation III pathway was also enriched in PwPD. Metcalfe-Roach et al.<sup>28</sup> similarly reported perturbations in purine nucleotide metabolism in PD. As degradation of guanine nucleotides contributes to uric acid production<sup>55</sup>, and lower uric acid levels have been associated with PD risk and progression<sup>56–60</sup>, these findings may reflect altered purine metabolism in PwPD. However, whether enrichment of this pathway represents compensatory microbial adaptation or reduced metabolic throughput cannot be determined without corresponding uric acid



**Fig. 3 | Differentially abundant bacteria in patients with Parkinson's disease and healthy controls.** Cladogram generated using linear discriminant analysis effect size (LEfSe) depicting bacterial taxa significantly enriched in patients with Parkinson's disease (green) or healthy controls (red), organized by phylogenetic hierarchy from

phylum to genus. LEfSe was calculated with a Kruskal-Wallis  $\alpha$ -value of 0.05, a Wilcoxon  $\alpha$ -value of 0.05, and a linear discriminant analysis (LDA) score threshold of 2.0. Data are based on non-subsampled bacterial profiles generated using the CosmosID-HUB platform.

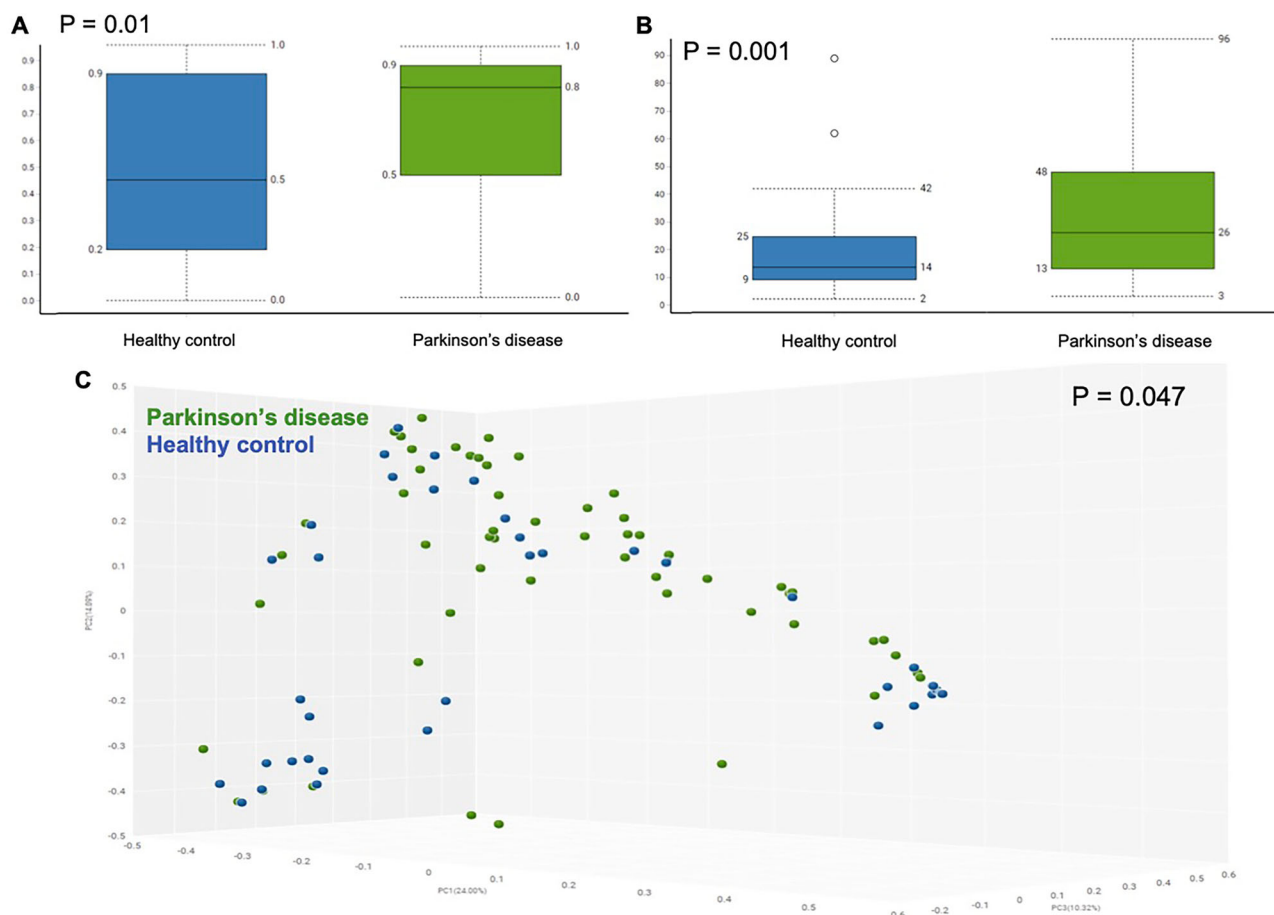
measurements. Further research is needed to elucidate whether these functional pathways may induce differences in metabolite concentration with linkages to PD symptoms.

Another functional pathway enriched in PwPD was the colanic acid biosynthesis pathway. Colanic acid is a stress-induced exopolysaccharide that promotes bacterial adhesion to the intestinal mucosa and has been associated with mucosal inflammation in conditions such as Crohn's disease<sup>61</sup>. Similar enrichment of exopolysaccharide- and capsule-associated pathways has been reported in an independent PD cohort<sup>62</sup>, suggesting that microbial cell-envelope stress responses may be a recurring finding across PD metagenomic studies. Although speculative, prior work showing that intestinal inflammation correlates with  $\alpha$ -synuclein expression in enteric neurites<sup>29</sup> raises the possibility that such microbial changes may occur within a pro-inflammatory gut environment.

The pentose phosphate pathway was also enriched in PwPD, a finding of interest because this pathway generates nicotinamide adenine dinucleotide phosphate (NADPH), a critical cofactor for antioxidant defense. Dysregulation of the pentose phosphate pathway has been implicated in oxidative stress and neuroinflammation in PD, and its enrichment in the microbiome may reflect bacterial adaptation to increased oxidative stress in

the gut microenvironment<sup>63</sup>. Finally, we additionally observed differences in pathways related to fatty acid  $\beta$ -oxidation, glutaryl-CoA degradation, anhydromuropeptide recycling, and alternative TCA cycle variants. Although there is limited evidence suggesting their relevance in PD, these pathways may be consistent with microbial metabolic responses to physiological stress, nutrient fluctuations, or other patterns observed in inflammatory gastrointestinal conditions<sup>64,65</sup>. Future studies integrating metagenomics with metabolomic and host inflammatory markers will be important for clarifying the biological and clinical significance of these pathways.

This study has several important limitations. First, we used historical controls to characterize our HC group, which introduces the possibility of technical confounding, as samples were processed using different library preparation kits by different people, in different labs, and with different protocols and instruments. These methodological differences can introduce batch effects that influence taxonomic and functional comparisons, making it difficult to determine whether some observed differences reflect true biological variation or differences arising from sample processing. In addition, because the historical controls lacked harmonized socio-demographic, clinical, and lifestyle data, we were unable to adjust for factors that meaningfully shape dietary habits and the gut microbiota. As a result,



**Fig. 4 | Phage  $\alpha$ - and  $\beta$ -diversities in patients with Parkinson's disease and healthy controls.** Boxplots comparing Shannon (A) and Chao1 (B)  $\alpha$ -diversity indices between groups. Boxes indicate the median and interquartile range; points represent outliers.  $P$  values were calculated using Wilcoxon rank sum tests. C Principal coordinates analysis (PCoA) plot of Bray-Curtis  $\beta$ -diversity. Each point represents an individual subject, with Parkinson's disease samples shown in green and healthy

controls in blue. Distances between points reflect differences in phage community composition based on Bray-Curtis dissimilarity. Percent variance explained by each principal coordinate is shown on the axes.  $P$  value was calculated via PERMANOVA. Data are based on subsampled phage profiling at a depth of 10 million reads per sample using the CosmosID-HUB platform.

our findings warrant further validation and should be considered hypothesis-generating.

Second, potential changes in the concentrations of byproducts or their associations with functional pathways could not be determined, as we did not collect metabolite data. Gene copy number variations also prevented a clear correlation between bacterial abundance and pathway gene expression. As a result, functional pathway differences identified in this study should be interpreted as reflecting predicted metabolic potential rather than actual metabolic activity. Without corresponding metabolomic measurements, we cannot determine whether the observed pathway enrichment results in meaningful shifts in metabolite production, host exposure, or biological relevance to PD. Integrating metagenomics with metabolomic and transcriptomic data in future studies will be critical for validating these pathway-level findings and clarifying their physiological impact. Third, detection of DNA viruses, fungi, and protists was sparse, limiting statistical power and interpretation in these domains. Finally, the PD group was predominantly white, affluent, well-educated, and based in North America, limiting the generalizability of the findings to the broader PD population.

Nevertheless, this case-control study contributes to the growing body of metagenomic research in PD. These findings should be viewed as hypothesis-generating and underscore the need for prospective, multi-omics studies in diverse populations to identify potential microbiome signatures or biomarkers that could enhance the prediction, diagnosis, and treatment of PD.

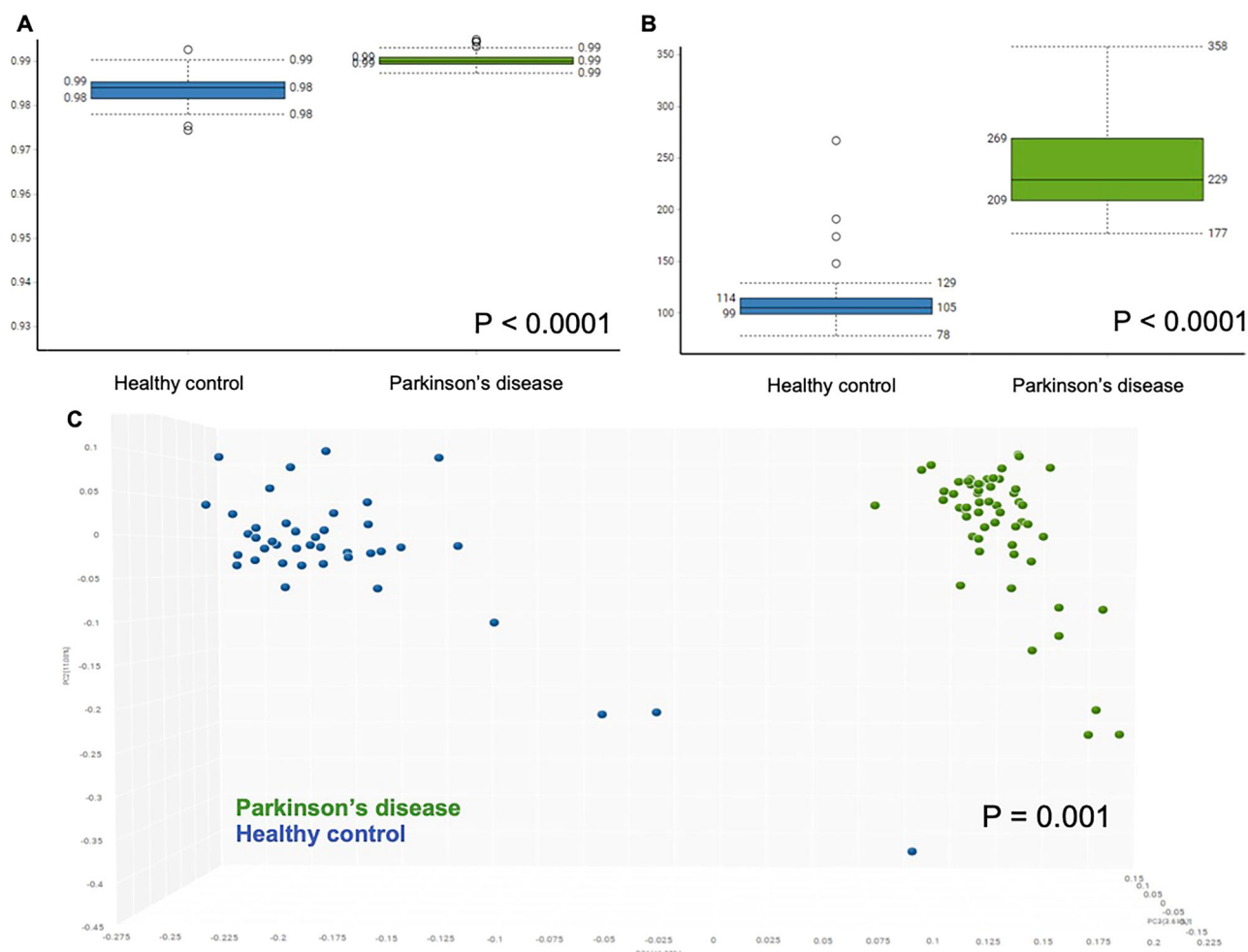
## Methods

### Sample population

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Bastyr University (IRB #21-1698; approved 1 December 2021), and informed consent was obtained for all participants. In August 2019, 60 individuals with PD from across the US and Canada attended the Bastyr University Parkinson Summer School, an annual intensive five-day retreat in Washington state. Prior to the retreat, stool sample collection kits were mailed to participants' homes, and 57 participants provided and mailed samples directly to the CosmosID laboratory. Two samples were excluded from analysis because one was from outside of North America and the other arrived more than six months after the others, resulting in a total of 55 PD cases. Data for 42 HCs were obtained from a publicly available dataset (BioProject Accession PRJEB39223; PREDICT1)<sup>66</sup> and were selected to match the PD group by country (US) and age distribution.

### Whole genome sequencing

PD stool samples were stored at  $-80^{\circ}\text{C}$  until extraction and processed within one month of receipt at the CosmosID laboratory. Microbial DNA from PD samples was extracted using the Qiagen Powersoil Pro Kit. DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina) with IDT Unique Dual Indexes (1 ng DNA input) and purified using Ampure magnetic beads (Beckman Coulter). Libraries were quantified using a Qubit 4 fluorometer with the Qubit™ double-stranded DNA



**Fig. 5 | Functional pathway  $\alpha$ - and  $\beta$ -diversities in patients with Parkinson's disease and healthy controls.** Boxplots comparing Shannon (A) and Chao1 (B)  $\alpha$ -diversity indices between groups. Boxes indicate the median and interquartile range; points represent outliers.  $P$  values were calculated using Wilcoxon rank sum tests. C Principal coordinates analysis (PCoA) plot of Bray-Curtis  $\beta$ -diversity. Each point represents an individual subject, with Parkinson's disease samples shown in green

and healthy controls in blue. Distances between points reflect differences in functional pathways based on Bray-Curtis dissimilarity. Percent variance explained by each principal coordinate is shown on the axes.  $P$  value was calculated via PERMANOVA. Data are based on subsampled functional profiling at a depth of 8 million reads per sample using the CosmosID-HUB platform.

High-Sensitivity Assay Kit and sequenced on the Illumina NextSeq 2000 platform with paired-end 150 base-pair reads. As described previously<sup>66</sup>, HC samples were extracted using the Qiagen Dneasy 96 PowerSoil Pro Kit, and DNA libraries were prepared using the NEBNext Ultra II Kit and sequenced on the Illumina NovaSeq 6000 platform.

### Taxonomic profiling

Taxonomic classification and relative abundance profiling were performed using the CosmosID-HUB platform, which applies a  $k$ -mer reference-matching algorithm against curated microbial reference databases. Taxonomic calls were filtered using CosmosID default quality thresholds. Bacterial analyses were conducted using non-subsampled data due to consistently high sequencing depth, whereas viral, phage, fungal, and protist analyses were performed using profiles subsampled to 10 million reads per sample.

### Functional profiling

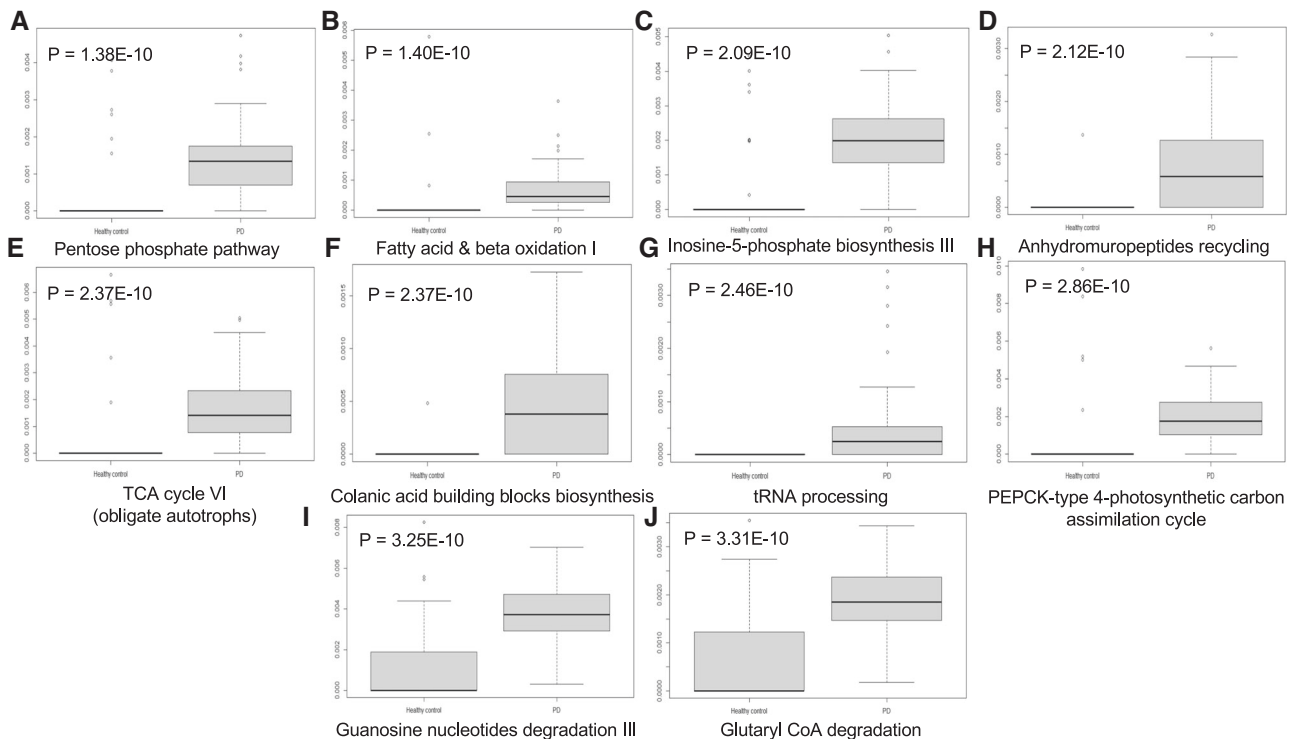
Initial quality control, adapter trimming and preprocessing of metagenomic sequencing reads were done using BBduk<sup>67</sup>. The quality-controlled reads were then subjected to a translated search against a comprehensive and non-redundant protein sequence database, UniRef\_90. The UniRef90 database, provided by UniProt<sup>68</sup>, represents a clustering of all non-redundant protein sequences in UniProt, such that each sequence in a

cluster aligns with 90% identity and 80% coverage of the longest sequence in the cluster. The mapping of metagenomic reads to gene sequences were weighted by mapping quality, coverage, and gene sequence length to estimate community-wide weighted gene family abundances. Gene families were then annotated to MetaCyc<sup>69</sup> reactions (metabolic enzymes) to reconstruct and quantify MetaCyc metabolic pathways in the community<sup>68</sup>. To facilitate comparisons across multiple samples with different sequencing depths, the abundance values were normalized using total-sum scaling normalization to produce "copies per million," analogous to transcripts per million (TPMs) in RNA sequencing (RNA-Seq) units. Functional pathway profiling was conducted using reads subsampled to 8 million per sample.

### Data analyses

Relative abundance stacked bar plots were generated from CosmosID-HUB using phylum-, family-, and species-level filtered matrices. Heat maps were used to visualize cross-sample patterns in relative abundance and clustering of taxa within microbial domains, and were interpreted descriptively. Descriptive observations based on visual inspection of stacked bar plots and heat maps were identified as such and were not treated as quantitative comparisons.

For  $\alpha$ -diversity, Shannon, Simpson, and Chao1 indices were visualized in boxplots, and group-level differences were evaluated using Wilcoxon



**Fig. 6 | Functional pathways enriched in patients with Parkinson's disease.** Boxplots of top 10 MetaCyc pathway terms that had significantly greater mean abundance in patients with Parkinson's disease compared to healthy controls, including pentose phosphate pathway (A), fatty acid and  $\beta$ -oxidation I (B), inosine-5-phosphate biosynthesis III (C), anhydromuropeptides recycling (D), tri-chloroacetic acid (TCA) cycle VI (E), colanic acid building blocks biosynthesis (F),

transfer RNA (tRNA) processing (G), PEPCK-type C4 photosynthetic carbon assimilation cycle (H), guanosine nucleotide degradation III (I), and glutaryl CoA degradation (J). Boxes indicate the median and interquartile range; points represent outliers. P values were calculated using Wilcoxon rank sum tests. Data are based on subsampled functional profiling at a depth of 8 million reads per sample using the CosmosID-HUB platform.

Rank-Sum tests.  $\beta$ -diversity was visualized via principal coordinates analysis (PCoA), with dissimilarity computed using the Bray-Curtis index. Group-level differences were assessed via PERMANOVA. For bacteria, LefSe was calculated based on phylum-, genus-, species-, and strain-level matrices with a Kruskal-Wallis  $\alpha$ -value of 0.05, a Wilcoxon  $\alpha$ -value of 0.05, and a linear discriminant analysis (LDA) score threshold of 2.0<sup>70,71</sup>.

For all analyses,  $P < 0.05$  were considered statistically significant. P values were not adjusted for multiple comparisons, and results were interpreted as hypothesis-generating. All analyses were performed within the CosmosID-HUB comparative analysis module.

## Data availability

The datasets generated and/or analyzed during the current study are not publicly available because the processed outputs incorporate proprietary algorithms and reference databases owned by CosmosID but are available from the corresponding author on reasonable request.

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## Author contributions

L.K.M., B.E.O., and D.M.W. contributed to the conception of the study and design of the study. B.E.O., D.M.W., and K.M. performed the statistical analysis, data curation, and data visualization. L.K.M. provided supervision and D.J.F. provided management of the project. L.K.M., S.E.E., and J.F. were involved in data collection. S.J.P. and B.E.O. wrote the first draft of the manuscript. All authors: provided critical edits to the manuscript and read and approved the submitted version.

## Competing interests

B.E.O., D.M.W., and K.M. were employed by CosmosID Inc at the time of the study. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Additional information

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