Journal of Clinical Periodontology

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Journal of Clinical Periodontology

ORIGINAL ARTICLE OPEN ACCESS

Periodontitis History Shapes the Early Peri-Implant Microbiome Formation: A Metagenomic Analysis

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Received: 29 October 2024 | **Revised:** 18 February 2025 | **Accepted:** 20 February 2025

Funding: This work was supported by National Natural Science Foundation of China (32270188 and 32070134), Key Research and Development Program of Shaanxi Province, China (2023-YBSF-162) and National Training Program of Innovation and Entrepreneurship for Undergraduates.

Keywords: dental implants | metagenomic analysis | peri-implant disease | periodontally compromised patient | periodontitis | periodontopathogens

ABSTRACT

Aim: This study aims to investigate the early alterations in microbiome construction and succession around dental implants in both periodontally healthy individuals and patients with a history of periodontitis during the first month after implant–crown placement.

Materials and Methods: Ninety-five subgingival plaque samples were collected from 10 periodontally compromised patients (PCP) and nine periodontally healthy patients (PHP) at four time points with a 1-week interval and analysed using dynamic metagenomic analysis. The study compared the formation and temporal change in the peri-implant microbiome in the PCP and PHP groups during the first month after the implant crown placement. A two-year follow-up examination was conducted to assess the clinical outcomes of early peri-implant dysbiosis.

Results: The results showed that PCP groups exhibited distinctively dysbiotic features in their peri-implant microbiome upon initial establishment, with an earlier and elevated emergence of periodontopathogens. This dysbiosis in the PCP group was associated with significantly higher modified sulcus bleeding index (mBI) scores compared with the PHP group. *Neisseria* was identified as a key driver of early peri-implant dysbiosis in patients with a periodontitis history.

Conclusions: This study established the first microbial link between periodontitis history and early peri-implant dysbiosis, highlighting the importance of early prevention strategies against peri-implant diseases in patients with a periodontitis history.

1 | Introduction

Tooth loss poses a great threat to public health worldwide due to its high prevalence and unneglectable adverse influence on overall health (Hugo et al. 2021). Untreated dental cavities (tooth decay) and severe periodontitis (gum disease with associated bone loss) are the two leading oral diseases that cause tooth loss (GBD 2017 Oral Disorders Collaborators et al. 2020; Wen et al. 2022). Implant therapy has currently become a popular and effective treatment for tooth loss (Howe et al. 2019; Buser et al. 2017). Beyond the implantation procedure itself, the long-term survival of the implant largely depends on the peri-implant health, which, nonetheless, is compromised by regional unresolved inflammation predominantly initiated

by microbial insults, namely peri-implant mucositis and peri-implantitis, with the latter being more severe, characterised by additional bone loss that eventually leads to implant failure (Renvert et al. 2018; Heitz-Mayfield and Salvi 2018; Schwarz et al. 2018).

Studies have shown that approximately one-third of patients who receive implant therapy and one-fifth of all implants inserted would experience peri-implantitis (Kordbacheh Changi et al. 2019; Lee et al. 2017). Compared with periodontitis, which affects natural teeth, peri-implantitis presents a greater challenge for treatment, primarily due to the technical complexity involved in effectively removing microbial plaque from the implant screw (Baima et al. 2022). Prevention of peri-implantitis is thus of particular importance to promote the overall long-term success rate of implant therapy.

Like periodontitis, peri-implantitis is microbial community-driven etiopathogenesis, despite the difference in composition and diversity of the microbial community in these two conditions (Carvalho et al. 2023; Zhuang et al. 2016; Retamal-Valdes et al. 2019). The same key pathogenic microorganisms responsible for periodontitis, such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (Yu et al. 2019; Kim et al. 2023), are also implicated in peri-implantitis (Belibasakis and Manoil 2021; Carra et al. 2022). Although the implantation operation is sterile, the peri-implant microbial community would be established shortly after implant insertion (Payne et al. 2017), whereas the detailed dynamics and contributing factors during this process are not fully understood. Recent studies have emphasised the pivotal influence of early colonisers on the properties and functions of the microbiota in different niches within the human body (Milani et al. 2017; Baker et al. 2024). A close insight into the initial establishment of peri-implant microbiota is critical to better our understanding and develop new strategies to prevent peri-implantitis.

Among the risky indicators of peri-implantitis, periodontitis history is unequivocally at the top (Renvert and Quirynen 2015; Serroni et al. 2024; Ravida et al. 2021). Periodontitis is a chronic gingival inflammatory condition initiated by dysbiosis of the subgingival microbial community, constituting a leading reason for bone absorption and eventual tooth loss, which takes up 30% of all cases of implant therapy (Slots 2017; Di Stefano et al. 2022; Eick et al. 2017). The current treatment for periodontitis is the mechanical removal (scaling and root planing) of the pathogenic microbial biofilm to reduce the amount of pathogenic microorganisms. However, while the treatment is generally effective to resolve inflammation in the short term, it is almost impossible to eradicate the disease permanently since the pathogenic microbiota would restore spontaneously, bringing back the symptoms. Regular periodontal intervention or supportive therapy after Steps 1–3 is required to control the disease, while the dysbiotic microbiota is hardly corrected (Schwarzberg et al. 2014; Nath et al. 2022). Despite a well-documented clinical correlation between periodontitis history and peri-implantitis, as well as similarities between the microbiomes associated with both conditions, there is still a lack of a direct microbial link between the dysbiotic periodontal microbiome prior to implantation and the establishment of the peri-implant microbiome thereafter.

Here we present a dynamic metagenomic study aiming to investigate the early alterations of microbiome construction and succession around implants in both orally healthy and periodontally compromised patients. Our hypothesis is that the early dynamics of peri-implant microbial community development differ between patients with a history of periodontitis and those with healthy periodontal conditions, which may pose a threat to the long-term success of dental implants. By examining these differences, our study provides novel insights into the early microbial formation associated with two distinct oral health statuses.

2 | Materials and Methods

2.1 | Subject Recruitment

This study was conducted following the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Xi'an Jiaotong University (Approval No: xjkqll[2020]NO.016; 2023-XJKQIEC-025-002). The present study conformed to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. Patients who will receive dental implants and regular maintenance at the Department of implant dentistry, Stomatological Hospital Affiliated to Xi'an Jiaotong University (China) were recruited for this study from January 2022 to March 2022. The study protocol was explained to each subject and informed consent was obtained. Inclusion criteria included good general health, as evidenced by medical history, age of at least 18 years, requiring one implant-supported single crown rehabilitation and willingness to participate in the study. Exclusion criteria included pregnancy or lactation, human immunodeficiency virus infection, smoking, use of immunosuppressant medications, bisphosphonates or steroids, use of chlorhexidine mouthwash or gel during the previous 2 weeks, and intake of systemic antibiotics or probiotics within the past 6 months.

Patients were assigned to one of two study groups based on the state of their periodontal health before the implant surgery: (a) periodontally healthy patients (PHP), comprising patients with healthy periodontal conditions and no signs of gingivitis or periodontitis, and (b) periodontally compromised patients (PCP), comprising patients diagnosed with periodontitis according to the 2018 AAP/EFP periodontal disease classification (Papapanou et al. 2018).

After enrolment, all patients received appropriate initial therapy, and the PCP group received comprehensive periodontal treatment according to the EFP clinical guidelines, including oral hygiene instruction, supragingival and subgingival instrumentation, and periodontal surgery when necessary (Sanz et al. 2020). After the periodontal condition had stabilised (probing pocket depth [PPD] $\leqq$ 4 mm; full mouth bleeding on probing [BOP] < 10%), single implant placement surgery was performed.

2.2 | Clinical Examination and Sample Collection

Two experienced dentists conducted all patient examinations using a standardised protocol for examination, data collection and measurement procedures. Demographic parameters,

including gender, age, and comprehensive medical and dental histories, were recorded. A full-mouth periodontal and implant clinical examination was performed at the six sites per tooth and implant, including oral hygiene index, plaque index (PI: 0/1/2/3), PPD and modified sulcus bleeding index (mBI: 0/1/2/3) at 2years after crown connection (Mombelli and Lang 1992; Mombelli et al. 1987).

Plaque samples were collected from a single implant and adjacent tooth after implant crown placement 30 min, weeks 1, 2, 3 and 4. Microbiome samples were collected using a non-invasive procedure based on a sampling protocol validated and adopted by the Human Microbiome Project (HMP) Consortium (Human Microbiome Project, C 2012). The selected sites were isolated using cotton rolls to prevent contamination with saliva and gently dried with an air syringe. Supragingival plaque was removed using sterile cotton pellets, and subgingival plaque samples were taken from six sites at each selected implant and tooth using sterile paper points and sterile titanium Gracey curettes. Technical replicates were sampled only after any potential bleeding had stopped to prevent contamination of the microbiological sample. After collection, samples were immediately placed in separate Eppendorf 1.5-mL microcentrifuge tubes (Eppendorf, Hamburg, Germany) containing sterile PBS buffer solution and frozen at −80°C for later analysis.

2.3 | DNA Sequencing and Data Analysis

Total genomic DNA was isolated using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek Inc., USA), following the manufacturer's protocol. The extracted DNA was quantified with Qubit 4 Fluorometer using Qubit 1× dsDNA HS Assay Kit (Thermo Fisher Scientific Inc., USA). Each metagenome was quantified initially, and if there was sufficient material (>1 ng), libraries were prepared using the Nextera-XT DNA Kit (Illumina Inc., San Diego, CA, USA) and the manufacturer's protocol. Thirty-minute samples, due to their low concentration, were not sequenced. The raw metagenomes produced were subjected to processing using FastqMcf. This entailed trimming positions with quality scores below 15, elimination of low-quality reads with mean quality scores below 25 and removal of reads shorter than 90 nt. BowTie was used to remove human and bacteriophage phiX174 DNA, which was included as Illumina spike-ins, by mapping the reads against the corresponding reference genomes. Kraken2 was employed to classify the clear sequences to taxonomy, using pre-built Refseq indexes. The relative abundance of species was estimated from the Kraken2 result through the use of Bracken.

2.4 | Statistical Analysis

A total of 95 subgingival samples were analysed, including 19 baseline samples collected from adjacent teeth at the time of implant placement and 76 follow-up samples collected from the implants at four time points (1, 2, 3 and 4 weeks post-implantation, with 19 samples per time point). Analysis was performed in the R environment, and plots were drawn with the R package *ggplot2* unless further specified. Alpha-diversity of each sample was analysed using the R package *phyloseq*. Beta-diversity was assessed by Bray–Curtis dissimilarity using the R package *vegan* and summarised by heatmap using the R package *pheatmap*. The dimension of bacterial community data was reduced by principal coordinate (PCoA) using the R package *ape*. The statistical difference between groups was tested by PERMANOVA using the R package *vegan*. The discriminant feature between groups was determined by Linear discriminant analysis effect size (LEfSe) using python scripts *lefse*. Pearson correlation was calculated by the R package *Hmisc*. The statistical difference of KEGG term was tested by Wilcoxon Rank Sum test on Monte Carlo resampling data using the R package *ALDEx2* (detailed methods were supplemented in Supporting Information).

3 | Result

3.1 | Description of Study Cohort

This cohort study included a total of 95 subgingival samples from 19 patients who underwent implant restoration and follow-up (Figure 1A). The participants were divided into two groups: a healthy group (PHP group) consisting of 9 participants (7 males and 2 females; mean age 30.6 ± 8.65 years) and a periodontitis history group (PCP group) consisting of 10 participants (7 males and 3 females; mean age 43.4 ± 10.6 years) (Table S1). The age difference between the two groups was statistically significant. Using the 2018 AAP/EFP periodontal disease classification (Papapanou et al. 2018), 10 participants in the PCP group were diagnosed with generalised Stage III Grade C periodontitis. After receiving active periodontal treatment, 10 patients with periodontitis achieved the stable status of periodontal condition (PPD ≦ 4 mm; BOP < 10%) which was considered to be qualified for the following implant therapy (Sanz et al. 2020). The periodontal symptoms remained controlled throughout the sampling and observation period, and none of the patients required prescription medication during this period.

3.2 | Dental Implants Have Distinct Early Subgingival Microbiota From Natural Teeth

To characterise the subgingival microbiota around newly placed dental implants, diversity and abundance analysis of the subgingival microbiome of dental implants was performed in all patients (consisting of both PCP and PHP groups), with adjacent natural teeth as a comparison. As shown in Figure 1B, compared with implants, adjacent natural teeth had a significantly higher alpha diversity, richness and rarity of species (weeks 1–4), as indicated by higher PPD, observed, and Chao1 indices at all the sampling time points. Beta diversity analysis confirmed that adjacent natural teeth had a significantly different subgingival microbial community from implants (Figure 1C).

The beta-diversity heatmap was then generated from the Bray–Curtis dissimilarity distance matrix to identify the relationship between the clinical parameters and the composition of the peri-implant microbiome. A cluster pattern was uncovered between different oral health statuses prior to implantation (PHP or PCP), but not with any other clinical parameter (Figure S2), emphasising the prominent influence of periodontitis history on the peri-implant microbiome.

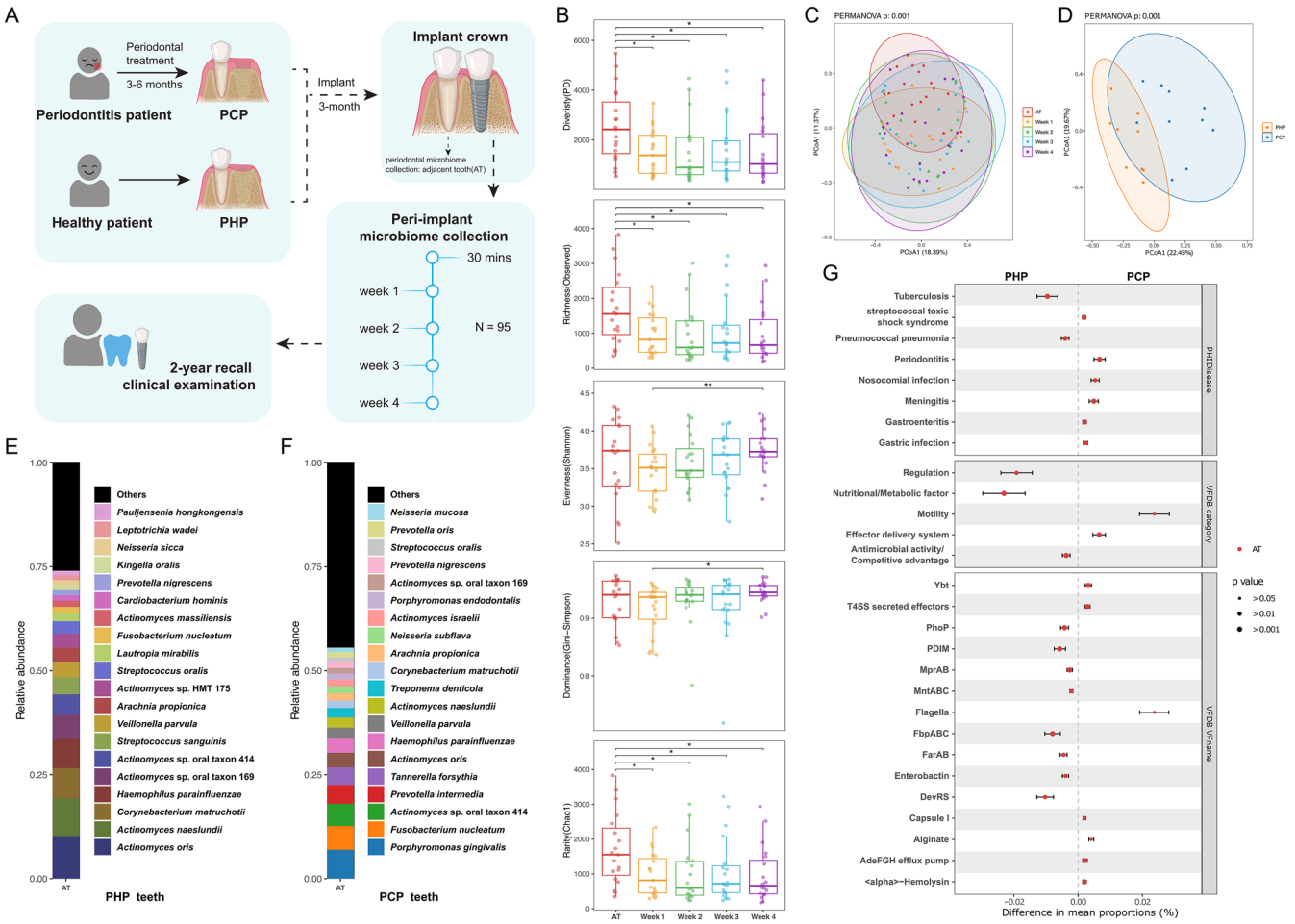

FIGURE 1 | Alpha- and beta-diversity of subgingival samples from natural teeth and dental implants. (A) The schematic diagram of the study design. N: Subgingival sample counts; PCP: Periodontally compromised patient; PHP: Periodontally healthy patient. (B) The alpha diversity indices of the number of phylogenetic diversity (PD), observed species, Shannon, Gini-Simpson, and Chao1 from periodontal samples, as well as peri-implant samples from week 1 to week 4. The box plot represents the medians, first and third quartiles. *p*-values were determined using Wilcoxon signed-rank tests. (C) Species-level Bray–Curtis distance principal coordinate analysis (PCoA) of the microbial composition of samples from adjacent teeth (AT) and different time point implant samples (week 1–week 4). The shaded area displays the confidence intervals. Statistical analysis was conducted using PERMANOVA (permutational multivariate analysis of variance based on distances). (D) Species-level Bray–Curtis distance PCoA of the subgingival microbial community of adjacent teeth (AT) samples from the PHP and PCP groups. (E, F) The top 20 most abundant species in adjacent teeth from the PCP (E) and PHP (F) groups. (G) Significant differences were found in natural teeth microbial communities of the PCP and PHP groups by comparing the functional terms from the Pathogens Host Interaction Database (upper panel) and Virulence Factor Database (middle and lower panel). The differences are reflected in the mean proportions between the two groups, with negative/positive values indicating down/up-regulation of these pathways in the PCP groups compared with the PHP.

3.3 | Peri-Implant Microbiome Differs Between PHP Group and PCP Group

Consistent with previous findings (Di Stefano et al. 2022; Iniesta et al. 2023), our analysis verified that the subgingival microbiome of natural teeth exhibited significant differences between the PHP and PCP groups as evidenced by significant dissimilarity ($p = 0.001$) in PERMANOVA analysis, even though all the samples from the PCP group were collected in a stable state after effective periodontal treatment and satisfactory maintenance of gingival health (Figure 1D). To further characterise these differences, the 'core' microbes in subgingival niches of natural teeth between the PHP and PCP groups were explored. As shown in Figure 1E,F, the 'core' periodontal microbiota of natural teeth in the PHP group primarily consisted of commensal-associated genera, including *Actinomyces*, *Streptococcus* and *Haemophilus* (Figure 1E). By contrast, the 'core' periodontal microbiota of natural teeth in the PCP group exhibited clear dysbiotic features, as evidenced by the presence of specific periodontitis-associated anaerobic genera (Perez-Chaparro et al. 2014), especially the keystone periodontopathogens such as *P. gingivalis*, *T. forsythia and T. denticola* (Figure 1F). Functional analysis using the Pathogens Host Interaction Database and Virulence Factor Database further reflected the pathogenic implications of the periodontal microbiome of natural teeth in the PCP group. Several infection and inflammation items were significantly up-regulated in the PCP microbiome, including periodontitis, streptococcal toxic shock syndrome, nosocomial infection, gastric infection, along with the elevated levels of critical virulence factors such as Ybt, T4SS secreted effectors, Flagella and Alginate (Figure 1G).

Regarding peri-implant microbiota, while several predominant species, such as *Haemophilus parainfluenzae*, *Neisseria sicca* and *Veillonella parvula*, were found to be most common between the PHP and PCP groups (Figure 2A,B), significant differences were detected in the abundance and diversity of other species, namely *Neisseria* subspecies, *Morococcus cerebrosus*, *Neisseria elongata*, *Prevotella denticola* and *Prevotella melaninogenica* (Figure 2C).

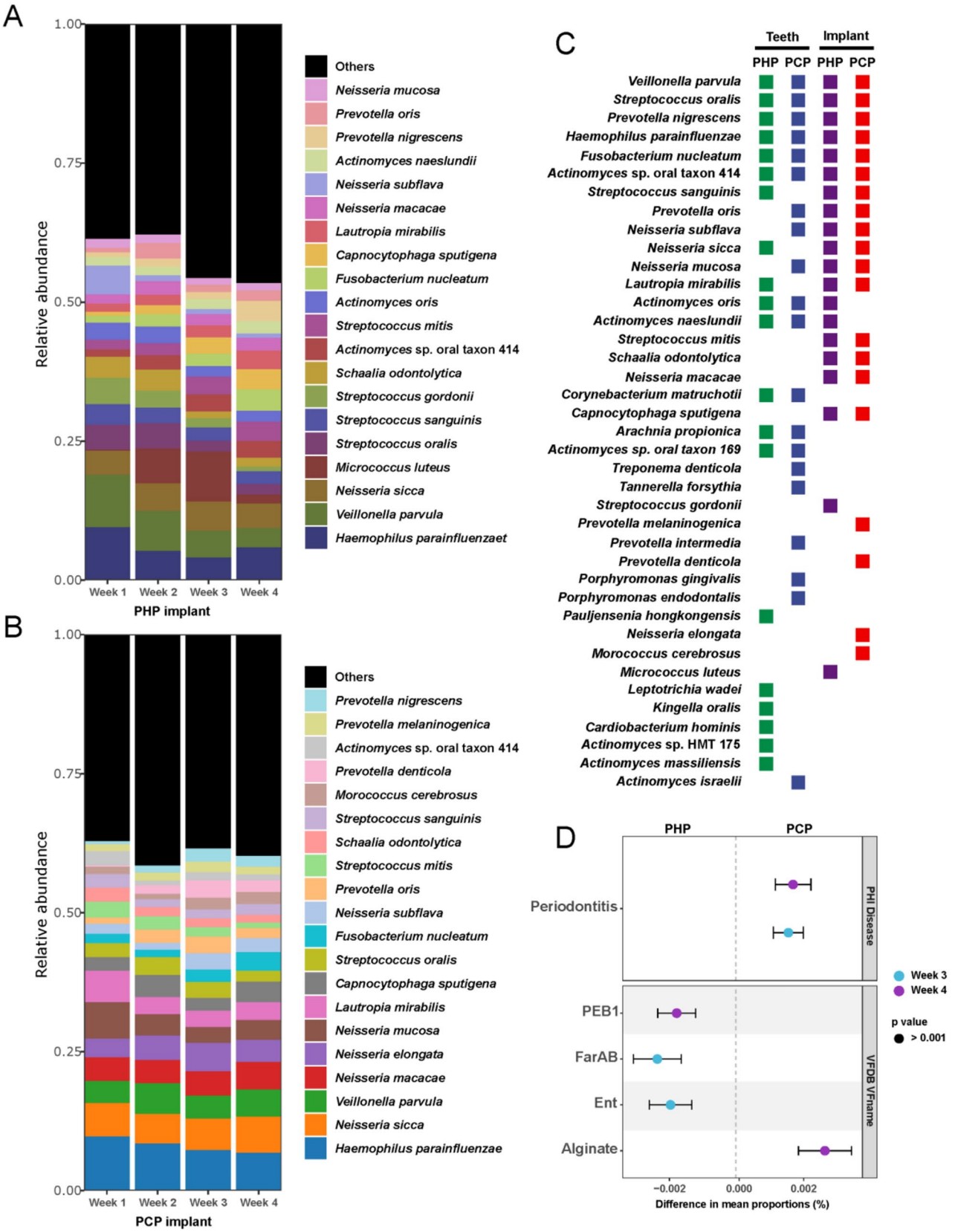

FIGURE 2 | Legend on next page.

FIGURE 2 | Dysbiosis of peri-implant subgingival microbial community in the PCP group. (A, B) The top 20 most abundant species in the peri-implant microbial community during week 1–week 4 from the PHP (A) and PCP (B) groups, with the rest of the species combined and grouped as others (black bar). (C) Plot showing the top 20 species in the teeth/implant subgingival microbial communities between PHP and PCP groups. (D) Significant differences were found in implant microbial communities of the PCP and PHP groups by comparing the functional terms from the Pathogens Host Interaction Database (upper panel) and Virulence Factor Database (lower panel). The differences are reflected in the mean proportions between the two groups, with negative/positive values indicating down/up-regulation of these pathways in the PCP groups compared with the PHP.

Further functional analysis revealed that the periodontitis item in the Pathogens Host Interaction Database was significantly up-regulated in the peri-implant microbial community of the PCP group at week 3 and week 4, indicative of the presence of various microbial factors involved in the initiation and progression of periodontitis. Further analysis of virulence factors using the Virulence Factor Database showed that during weeks 3 and 4 of the study, the peri-implant microbial community in the PCP group did not exhibit the same level of virulence factor expression as the adjacent teeth, with the only exception of Alginate (Figure 2D). These functional predictions hinted that the peri-implant microbial community in the PCP group is at higher risk for future infection and inflammation due to the presence of a potentially dysbiotic community, although the initial peri-implant microbial community appeared not as virulent as in adjacent natural teeth within the time frame of 1 month, providing a possible window for preventive interventions.

3.4 | Peri-Implant Microbiome of PCP Group Exhibits Distinct Temporal Dynamics From PHP Group

The endogenous oral microbiome, as 'microbial reservoirs', plays a crucial role in the establishment and maturation of the microbiome around a newly placed implant crown. To assess the influence of the adjacent teeth microbiome on the dental implant, alluvial figures were used to visually comprise the temporal dynamics of the top 20 species from the adjacent teeth in the implant niche (Figure 3A,B). Over 1 month, the abundance of certain bacterial species in the PHP group varied over time (Figure 3A). While commensal-associated species, including four *Actinomyces* species and *H. parainfluenzae*, remained stable, *Prevotella nigrescens*, *Capnocytophaga sputigena* and *Fusobacterium nucleatum* gradually increased from week 1 to week 4. The abundance of four *Streptococcus* species and *V. parvula* species declined over the same period. In the PCP group, the relative abundance of several periodontopathogenic bacteria, such as *P. gingivalis*, *T. forsythia*, *T. denticola* and others, gradually increases in the peri-implant microbial community over time (Figure 3B). Conversely, the commensal species *H. parainfluenzae* and *Actinomyces naeslundii* exhibit a decrease from week 1 to week 4 (Figure S3B). Compared with the PHP group, the peri-implant microbiome in the PCP group exhibited a general trend of a decline in commensal species and an increase in periodontopathogens within PCP communities.

To further illustrate the influence of the existing periodontal microbiome on the initial establishment of the peri-implant microbiome, we performed community correlation network construction at 1-week intervals within 1 month after implant-crown placement (Figure 3C). Adjacent natural teeth exhibited a different periodontal microbial correlation between the PCP and PHP groups, whereas the PCP group displayed a more complex correlation among pathogens at the phylum level. This is likely due to pathogens having a tendency to coexist and form complex microbial communities. As for the implant, the results showed that after 1 month, the peri-implant microbiome reached a similar level of complex correlation as the adjacent teeth. In contrast, the PHP group did not exhibit such a trend, as all commensal bacteria showed stable correlations regardless of whether they were in natural teeth or implants (Figure 3C).

3.5 | Early and Elevated Emergence of Periodontopathogens in Peri-Implant Microbiota in PCP Group Was Associated With Higher mBI Scores

The 'complex theory' classifies periodontal pathogens based on their association with periodontitis severity (Socransky et al. 1998). To get a deeper insight into the roles of periodontitis-associated dysbiosis in shaping the newly formed peri-implant microbial community, the microbiome in the peri-implant niche was grouped into different complexes accordingly (Figure 4C). Not surprisingly, while no red-complex species were detected in the subgingival microbiome of natural teeth of the PHP group, the abundance of all red and orange complex species in the PCP group was significantly higher, with the exceptions of *Prevotella intermedia* and *Campylobacter gracilis* (Figure 4D), reflecting the notion that even during clinical stability, the subgingival microbiome in periodontitis patients is still predominantly dysbiotic.

Time course analysis revealed that the presence of a high abundance of pathogenic bacteria around the adjacent teeth leads to an earlier emergence and higher abundance of periodontopathogens in peri-implant niches (Figure 4B). The red complex species, namely *P. gingivalis*, *T. forsythia* and *T. denticola*, emerged in the peri-implant subgingival community for the PCP group by week 1 and gradually increased over time. The orange complex also displayed a clear increase, while the green and yellow complexes decreased during the study period. The purple complex maintained a lower abundance and remained relatively stable. In contrast, in the PHP group, the appearance of the red complex in the implant subgingival community was delayed to week 4, in a much lower abundance than the PCP group at the same time point (Figure 4A).

Further comparison at the species level showed that all three red complex pathogens constitute a significantly higher proportion in the peri-implant microbiome of PCP than the PHP

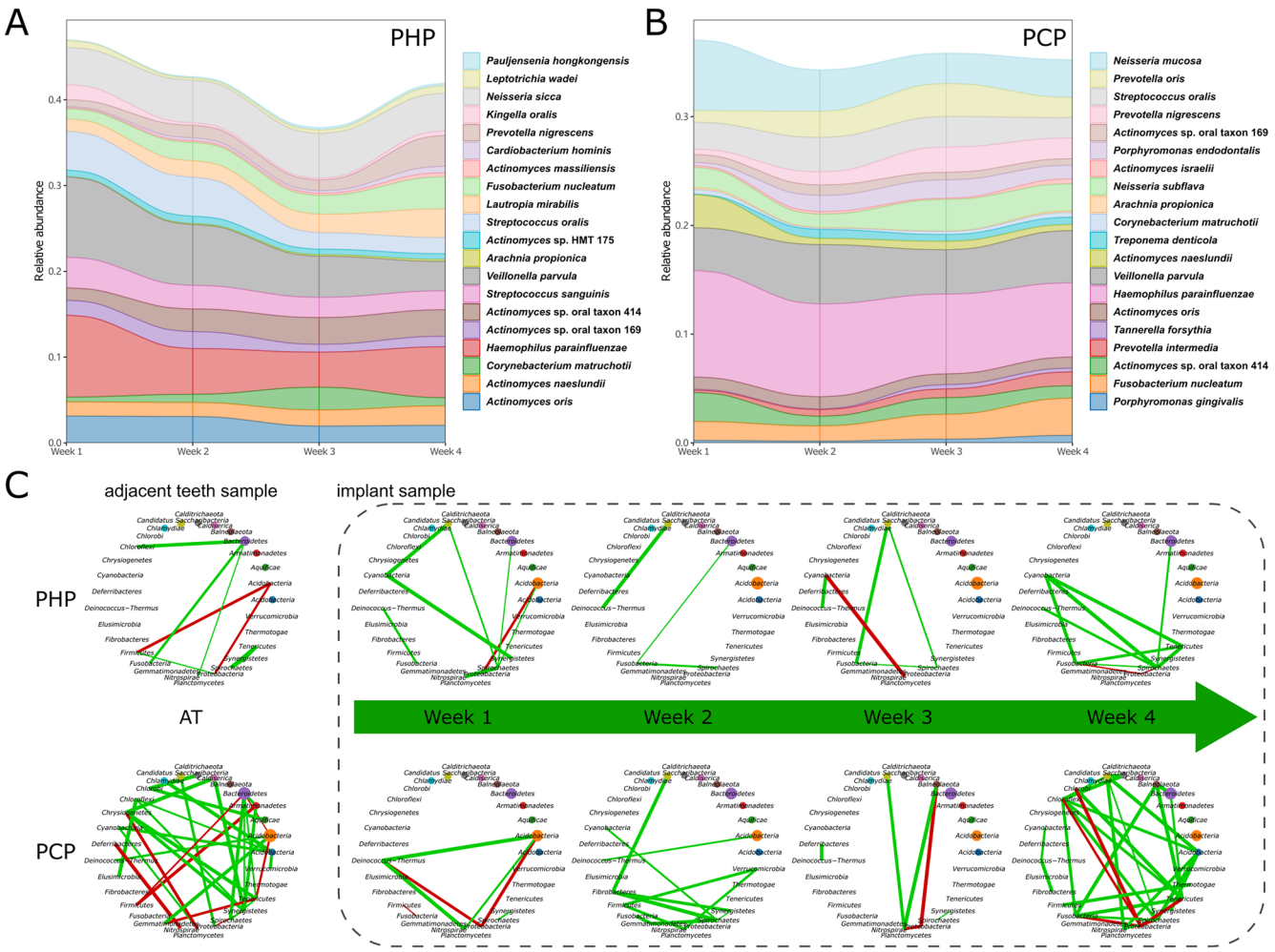

FIGURE 3 | Temporal microbial dynamics in the peri-implant subgingival microbial community between the PHP and PCP groups. Alluvia figures were plotted with relative abundance. Dynamic change of top 20 periodontal species from adjacent teeth in the subgingival microbial community between PHP group (A) and PCP group (B). Each microbial species is represented by a coloured band, with the width of the band corresponding to the relative abundance of the species at each time point. The bands are connected across time points to illustrate the changes in abundance over time. (C) The correlation network plots depict the strength of association (ordinary Pearson correlation) at the phylum level in the whole community at different groups. Correlations between phyla are displayed in green (positive) and red (negative) lines in various thicknesses indicative of the absolute *r* value (*rmcorr*'s *r*).

group at all observed time points. Moreover, within the orange complex, several species exhibited higher abundance in the peri-implant microbiota of the PCP group, including *Parvimonas micra*, *Campylobacter showae*, *Campylobacter rectus*, *C. gracilis*, *Fusobacterium periodonticum* and *P. intermedia* (Figure 4D).

Periodontopathogens have been proven to have a close correlation with clinical outcomes such as PPD and BOP/GI (Eick et al. 2017; Boyer et al. 2020; Chopra et al. 2020). To assess the impact of the earlier and higher presence of periodontopathogens on peri-implant tissue, clinical follow-up examinations were conducted 2 years after implant crown placement (Tables S2 and S3). The results showed that both PCP and PHP groups maintained good oral hygiene, as evidenced by no significant difference between the oral hygiene index (OHI) and the plaque index (PI). No implant showed any bone loss during the two-year study period, regardless of whether it was in the PCP or PHP group. The PPD showed a significant difference between PCP and PHP, but all of them were less than 4 mm, indicating a stable, compromised periodontal condition. However, compared with the PHP group, the PCP group exhibited significantly higher mBI scores (Table S2). Specifically, while 28.5% of sites in the PHP group showed isolated bleeding spots, 44% of sites in the PCP group demonstrated gingival bleeding on probing (Table S3). These results indicate a more pronounced level of gingival inflammation in the PCP group.

3.6 | *Neisseria* Is a Key Driver of Dysbiosis in the Peri-Implant Microbial Community of PCP Groups

We then attempted to identify whether there are biomarkers for the peri-implant dysbiosis in the early stages for better diagnosis and intervention. The Linear Discriminant Analysis Effect Size (LEfSe) algorithm was used to identify specific taxa that contribute to significant differences in abundance between PHP and PCP groups (Figure 5). The phylogenetic analysis summarised

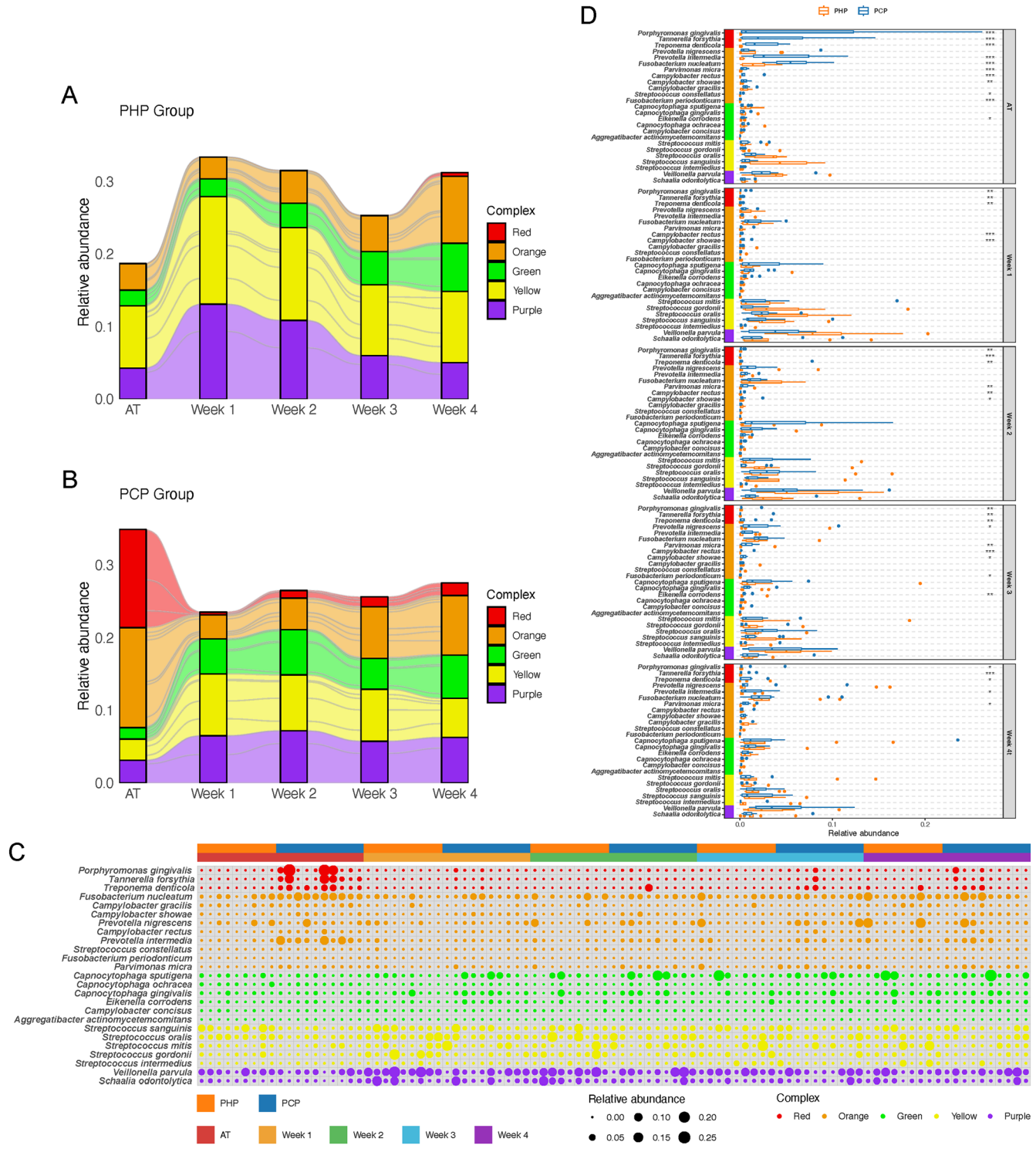

FIGURE 4 | Comparison of periodontopathogen complexes in the subgingival microbial community. (A, B) The different periodontopathogenic complexes are represented by their corresponding colour code (red complex, orange complex, yellow complex and purple complex species). (C) The detailed information for each sample, including the relative abundance (indicated by size) of each periodontopathogenic complex, is also represented by their corresponding colours. The top bar represents the different groups (PHP and PCP, adjacent teeth [AT] and different time point implants [week 1–week 4]). (D) Comparison of the different periodontopathogen complexes at the species level by relative abundance, and *p*-values were determined using Wilcoxon signed-rank tests. ***$p < 0.001$, ** $p < 0.01$, * $p < 0.1$.

the significant taxa and demonstrated that in the peri-implant subgingival community, *Porphyromonas*, *Parvimonas* and *Treponema* were significantly more abundant in PCP patients over week 1 to week 4, with a particularly higher prevalence of the *Neisseriaceae* family at week 4 (Figure 5C). Compared with the PHP group (Figure S4), *Rothia*, *Streptococcus*, *Lautropia*, *Morococcus* and *Neisseria* were more abundant in the PCP peri-implant niche than in the adjacent teeth. Over 4 weeks, only

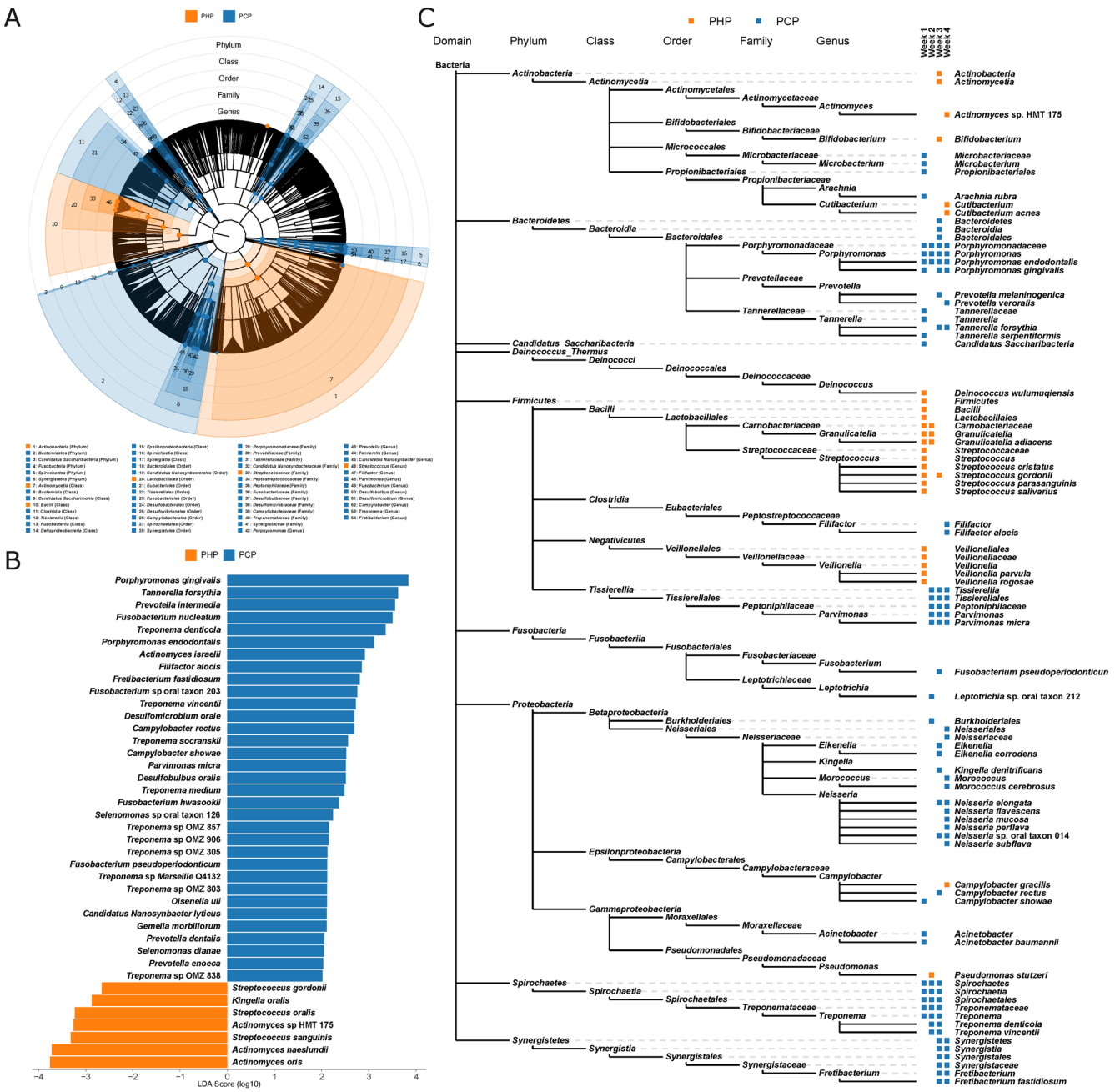

FIGURE 5 | The LEfSe and LDA analyses of significant taxa in healthy and periodontitis groups. Partial bacterial taxa differed significantly between the healthy and periodontitis groups in the subgingival microbial community, according to linear discriminant analysis coupled with effect size (LEfSe). (A) Shows bacterial clades that are differentially abundant in the healthy group (Orange) and periodontitis group (Blue). Clades in this graph were both statistically significant ($p < 0.05$) and had linear discriminant (LDA) scores ≥ 2.0 (B), which is considered a significant effect size. The number from the inside to the outside represents the significant classification level from phylum to genus listed in the lower panel. (C) The phylogenetic tree was constructed using the significant taxa identified at various taxonomic levels. The taxa that were found to be significant in the periodontitis group are shown in blue, while those in the healthy group are shown in orange.

Neisseria showed a significant presence from week 1 to week 4, indicating its potential role as a biomarker in the peri-implant microbiota of the PCP group (Figure S5).

Comparative analysis of *Neisseria* at the species level reveals significant increases in the peri-implant microbiome of the PCP group at week 3 and week 4, while the PHP group shows a decrease at these time points (Figure 6A). A total of 11 *Neisseria* species were identified in the peri-implant samples, all of which exhibited a significantly higher abundance in the PCP group compared with the PHP group at all the time points (Figure 6B). Further correlation network analysis highlights *Neisseria* as key connectors in the peri-implant microbial community in the PCP group, with species like *Neisseria* sp. oral taxon 014, *Neisseria mucosa*, and *Neisseria macacae* playing a crucial role in connecting the red and orange pathogen complex, especially *Neisseria* sp. oral taxon 014, which has a strong positive correlation with *P. gingivalis* and *T. forsythia* (Figure 6C).

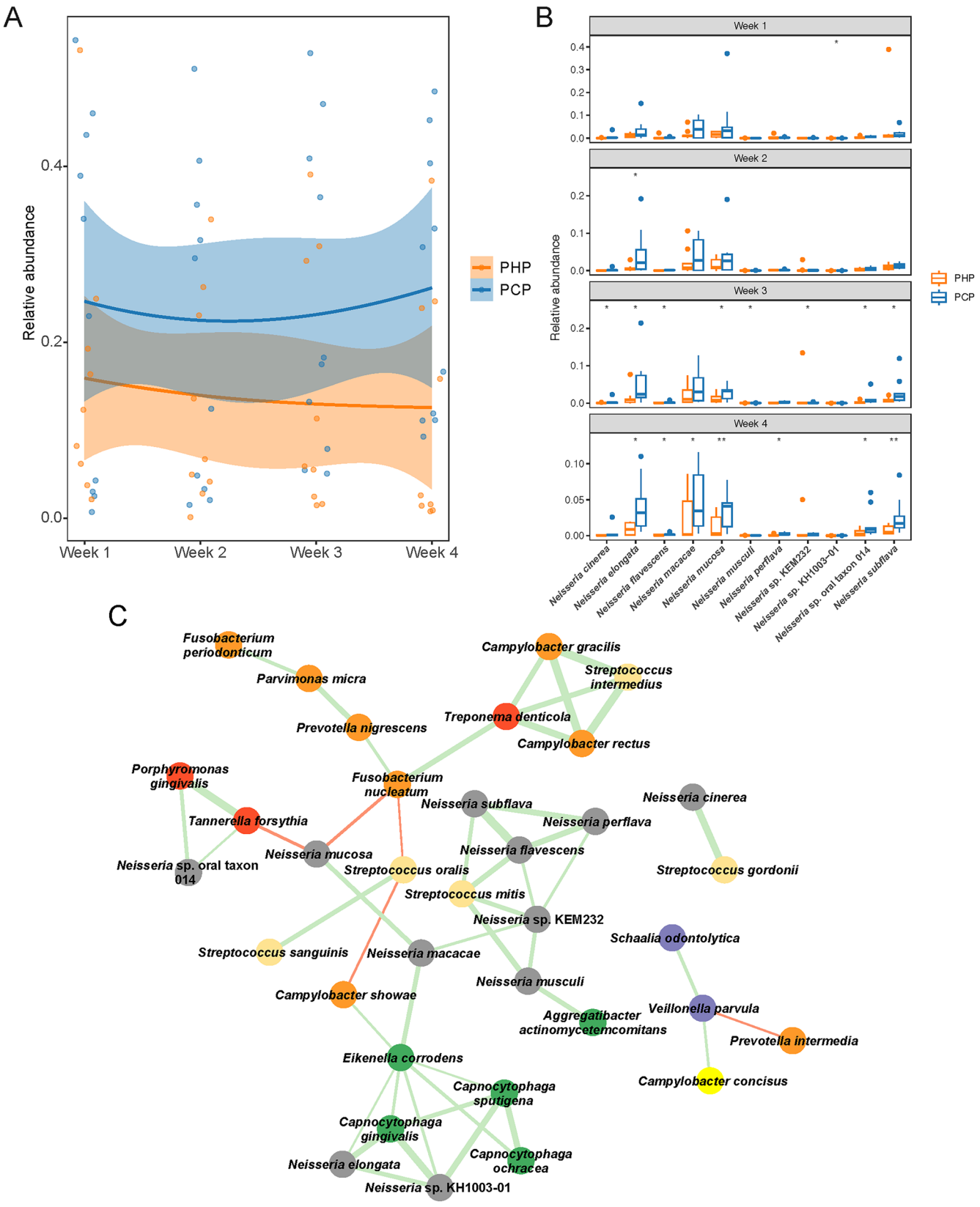

FIGURE 6 | Comparison of the *Neisseria* species in the implant subgingival microbial community between healthy and periodontitis groups. (A) Total relative abundance of *Neisseria* species between healthy and periodontitis groups, with the shading representing the error range. (B) The box plot showed 11 significant *Neisseria* species, comparing the implant subgingival microbial community between PHP and PCP groups. (C) The correlation network showed the relationship between the *Neisseria* species (grey circle) and periodontopathogen complex. Correlations between species are displayed in green (positive) and red (negative) lines in various thicknesses indicative of the absolute *r* value (*rmcorr*'s *r*).

4 | Discussion

It has been well recognised that the peri-implant microbiota plays a crucial role in implant health and peri-implant disease development, whereas its dynamic changes and correlation with overall oral health status are not well understood. By taking the periodontitis history into consideration, this metagenomic study revealed the early dynamics of the microbiome construction and succession around implants in both orally healthy and periodontally compromised patients.

The early peri-implant microbiome differs significantly from the adjacent periodontal microbiome in composition and diversity, regardless of the historical oral health status. Consistent with these previous findings (Yu et al. 2019; Kim et al. 2023), our study confirmed that, despite the considerable inter-individual variation, the early peri-implant microbiome (within 1 month) differs significantly from the adjacent periodontal microbiome in diversity and composition, regardless of the historical oral health status. This finding reinforces the premise that peri-implant and periodontal microbiota represent microbiologically distinct ecosystems, which has been proposed to be largely due to the differences in biofilm formation on implant Titanium surfaces compared with natural periodontal tissues (Bermejo et al. 2019). Thus, further studies are needed to understand the specific colonisation dynamics of these microbial communities around implants and their correlation with 'microbial reservoirs' in naturally periodontal sulci, especially given the background of periodontal diseases.

Periodontitis is one of the main causes of tooth loss, and its history is a significant risk factor for peri-implantitis. While the clinical correlation between periodontitis history and peri-implantitis has been widely recognised (Serroni et al. 2024; Ravida et al. 2021; Casado et al. 2013; Renvert and Persson 2009), the underlying mechanistic details, particularly microbial links, are still not fully understood. Although some known periodontitis-associated bacteria may also be implicated in peri-implant diseases, different microorganisms have been suggested to be involved in these two clinically distinct conditions (Kotsakis and Olmedo 2021). It is still not well understood how periodontitis-associated microbial reservoirs influence the formation of peri-implant microbiota. For periodontitis patients who need dental implants, surgery is often performed during the maintenance treatment phase following active periodontal treatment. However, our results revealed that even in periodontally stable conditions without clinical symptoms, periodontopathogens still persist in considerable abundance, resulting in a distinct dysbiosis in comparison with periodontally healthy individuals, echoing previous findings that bacterial colonisation does not necessarily correlate with clinical outcomes after full-mouth treatments for periodontitis stage III/IV (Schulz et al. 2022; Abdelbary et al. 2022). More importantly, although critical pathogenic microorganisms responsible for periodontitis, such as *P. gingivalis*, *T. forsythia* and *T. denticola* (Yu et al. 2019; Kim et al. 2023), have also been shown to be more abundant within diseased peri-implant niches compared with healthy implant sites, a significant separation of microbial profiles between peri-implantitis and periodontitis has also been documented.

Our study presents the first metagenomic investigation of the differences in peri-implant microbiome formation in two distinct microbial ecosystems: periodontally healthy and periodontally compromised patients. The peri-implant microbiome established in periodontally healthy individuals is mainly composed of commensal bacteria and matures throughout the study period. By contrast, in patients with a periodontitis history, there is a clear trend showing a decline in commensal species and an increase in periodontopathogens in the peri-implant microbiome within the first month after implant-crown placement. Keystone periodontal pathogens in the peri-implant microbiome of periodontally compromised patients were significantly more abundant than those in periodontally healthy patients from weeks 1–4. Our results implicated that the microbial communities that form around the implant in patients with a periodontitis history are more likely to be influenced by the existing dysbiotic periodontal microbiome, as evidenced by earlier emergence and higher abundance of 'red-complex' species.

Beyond the well-recognised oral pathogens, we are also very intrigued whether there are microbial biomarkers for initial peri-implant dysbiosis in periodontally compromised patients. Fortunately, LEfSe analysis revealed the possibility of the *Neisseria* genus to serve as an early peri-implant dysbiotic signature since all the 11 detected *Neisseria* species exhibited significantly higher abundance in the initial peri-implant microbiome in patients with historical periodontitis compared with periodontally healthy people. More importantly, the significance of *Neisseria* was further strengthened by its key roles in connecting several red-complex and orange-complex species, providing a new intervention target for peri-implant disease prophylaxis. One limitation of the current study is the relatively short follow-up time. This period offers insights into intermediate clinical status but may not fully reflect long-term peri-implantitis incidence and progression. Future studies with longer follow-up periods are needed for a better understanding of the microbial link between periodontitis history and elevated risk of peri-implant diseases. While the sample size was suitable for this study, it may restrict the generalisability of the findings. Larger, more diverse cohorts would strengthen the conclusions. In conclusion, this study has established a pivotal microbial link between periodontitis history and early peri-implant dysbiosis and identifies the key driver of dysbiosis in the early establishment of the peri-implant microbial community, providing valuable information to better our understanding of peri-implant microbiome dynamics and explore new therapies against peri-implant diseases.

Author Contributions

Miao Wang contributed to the conception and design, data acquisition, analysis and interpretation, drafted and critically revised the manuscript. Yi Bing Liu contributed to conception and design, data acquisition, interpretation, and critically revised the manuscript. Wai Man Tong contributed to design, data analysis and interpretation, drafted and critically revised the manuscript. Wai Keung Leung contributed to conception, data analysis and interpretation, critically revised the manuscript. Long Long He contributed to the conception, data interpretation and critically revised the manuscript. Xin Xu contributed to drafting and critically revised the manuscript. Dan Xu contributed to

the conception and design, data analysis and interpretation, drafted and critically revised the manuscript. Qin Zhou contributed to the conception and design, data analysis and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

Acknowledgements

This work was supported by National Natural Science Foundation of China grants (32270188 and 32070134), Key Research and Development Program of Shaanxi Province, China (2023-YBSF-162), and the National Training Program of Innovation and Entrepreneurship for Undergraduates. The authors also extend thanks to Prof. Chang Xiaofeng and Dr. Du Liangzhi for their valuable chairside assistance. Finally, the authors would like to thank all the patients who participated in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The MiSeq sequence reads and metadata were deposited in NCBI Short Reads Archive with Bioproject ID: PRJNA1148759 while other data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.