



# Microbiome differences in periodontal, peri-implant, and healthy sites: a cross-sectional pilot study

Giovanni Barbagallo<sup>1</sup> · Maria Santagati<sup>2</sup> · Alaa Guni<sup>3</sup> · Paolo Torrisi<sup>1</sup> · Ambra Spitale<sup>2</sup> · Stefania Stefani<sup>2</sup> · Sebastiano Ferlito<sup>1</sup> · Luigi Nibali<sup>3</sup>

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## Abstract

**Objectives** To explore microbial communities associated with health and disease status around teeth and dental implants.

**Materials and methods** A total of 10 healthy, 24 periodontitis, and 24 peri-implant sites from 24 patients were sequenced by next-generation sequencing. Microbial DNA was extracted and 16S rRNA gene was amplified. Bioinformatic analyses were performed using quantitative insights into microbial ecology (QIIME), linear discriminant analysis effect size (LEfSE), and STAMP.

**Results** Differences in microbial diversity across three types of sites were not statistically significant. Several genera and species were more prevalent in healthy compared with diseased sites, including *Lautropia*, *Rothia* and *Capnocytophaga* and *Kingella*. Among diseased sites, *Peptostreptococcaceae*, *Dialister*, *Mongibacterium*, *Atopobium*, and *Filifactor* were over-represented in peri-implantitis sites, while *Bacteroidales* was more abundant in periodontitis sites.

**Conclusions** Diseased periodontal and peri-implant sites and corresponding healthy sites have distinct microbiological profiles. These findings suggest that microbial analyses could identify biomarkers for periodontal health and disease and lead to the development of new strategies to improve periodontal health and treat peri-implant and periodontal diseases.

**Clinical relevance** The study contributes to improving our understanding of healthy, periodontally affected, and peri-implantitis sites which can improve our ability to diagnose, monitor, and manage these oral conditions.

**Keywords** Microbiome · Periodontitis · Peri-implantitis · Biomarkers · Polymerase chain reaction

## Introduction

The understanding of the role of risk factors involved in the onset and progression of periodontal diseases has changed only in the last few decades [1–3]. From the simplistic view

of the role of microbial pathogens causing signs and symptoms of periodontitis, the importance of the immune system and the inflammatory response of the host has emerged, leading to the concept of periodontitis as a multifactorial disease influenced by genetics and environmental risk factors [4–6].

Peri-implantitis is a pathological condition that occurs in peri-implant tissues and is characterized by inflammation in the peri-implant mucosa and progressive loss of supporting bone [7, 8]. Similar to the disease evolution process from gingivitis to periodontitis, it is assumed that peri-implant mucositis precedes peri-implantitis; in fact, the main aspect that differentiates peri-implantitis from peri-implant mucositis is bone loss. While inflammation can be primarily detected by bleeding on probing, progressive bone loss is assessed with radiographs [9, 10]. In all cases, the key role of inflammation in the response to bacterial challenges, seems to be responsible for the enhanced severity of the

Giovanni Barbagallo and Maria Santagati have contributed equally to this work.

✉ Maria Santagati  
m.santagati@unict.it

<sup>1</sup> Department of Surgery and Medical Specialties, Division of Dental Medicine, University of Catania, Catania, Italy

<sup>2</sup> Medical Molecular Microbiology and Antibiotic Resistance Laboratory (MMARLab), Department of Biomedical and Biotechnological Sciences (BIOMETEC), University of Catania, Catania, Italy

<sup>3</sup> Periodontology Unit, Centre for Host Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, UK

disease until the final consequence of the loss of teeth and dental implants.

Beyond poor plaque control and lack of regular maintenance therapy as risk factors for peri-implantitis, there is currently strong evidence from longitudinal and cross-sectional studies correlating the potential association between the history of periodontitis (chronic or aggressive) and peri-implantitis, reporting that patients suffering from periodontitis have higher odds of developing peri-implantitis compared to subjects without periodontitis [11, 12]. It is interesting to note that the onset and progression of peri-implantitis could be influenced by iatrogenic surgical and prosthetic factors [13]. In this context, it has been recognized by the Consensus report of the 7th European Workshop on Periodontology that iatrogenic factors such as inadequate restoration-abutment seating and the over contouring of restorations or implant-malpositioning may influence the development of peri-implantitis [7, 14]. Furthermore, the excess cement retained in the sulcus of the implant can also become the basis of colonization by oral microorganisms due to the rough surface structure of cement remnants may facilitate retention and biofilm formation, increasing the risk for peri-implantitis [15]. Several studies have investigated the potential microbial aetiology of peri-implant diseases, often analyzing the role of single bacterial species; more recently, studies have addressed the polymicrobial nature of the disease and pointed out the complex role of a dysbiotic peri-implant microbiome in the destructive tissue effects of the disease progression [16–19]. Bacterial colonization occurs within 30 min after implant placement. Fürst and co-authors examining subgingival plaque samples from implants and surrounding teeth with Checkboard DNA-DNA hybridization before surgery and at different times after surgery detected *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* at 12 weeks. These species were previously classified as red microbial complex of the subgingival plaque around the teeth [20, 21]. In the last few years, many papers have highlighted differences between dental and peri-implantitis microbial communities [22–24] even if these differences have not been identified. In a recent overview on the peri-implantitis microbiome focused on the identification of pathogens by advanced molecular techniques, Rakic and colleagues showed different microbial profiles between peri-implantitis, periodontitis and healthy tooth sites, also pointing out the quantitative rather than qualitative aspect of microbial composition as an important disease determinant [25].

The hypothesis behind this study is that differences in microbial communities exist between the periodontal and peri-implant environments. Therefore, the present study aimed to determine the composition of the microbiome healthy, periodontal and peri-implant sites using high throughput sequencing of the 16S rRNA gene amplicon to

determine a specific core microbiome and to identify, from a microbiological standpoint, which differences could contribute to periodontal and peri-implant diseases.

## Materials and methods

### Study population

Twenty-four subjects (15 females and 9 males) aged between 48 and 80 years were enrolled in this clinical study from 1 July to 10 August 2018. In this group, 7 subjects had a history of smoking, while the remaining subjects were classified as “non-smokers” (defined as smokers of < 5 cigarettes/day or ex-smokers for a minimum of 5 years or who had “never smoked”) (Table 1).

The recent classification of periodontal and peri-implant disease and condition was applied in this study [26]. All the individuals were diagnosed with at least 2 non-adjacent teeth affected by periodontitis and 1 implant by peri-implantitis. All implants involved in the study were fixed implant supported prosthesis. Written consent was obtained from all subjects enrolled and the study was approved by the Ethical Committee of Catania 2 (47/2018/CECT2).

**Inclusion criteria** (i) history of periodontitis (self-reported), (ii) at least 1 implant with peri-implantitis (according to definition above), (iii) 1 tooth with periodontitis and 1 healthy tooth, (iv) implants present with at least 1 year of loading, and (v) implants inserted in native bone.

**Exclusion criteria** (i) immediate post-extractive implant and/or past regenerative procedures, (ii) use of antibiotics and/or immune suppressants in the 3 months before enrollment, (iii) need for antibiotic prophylaxis before dental procedures, (iv) pregnancy and/or lactation and/or hormonal therapy, and (v) uncontrolled systemic diseases and conditions counter-indicating implant therapy.

### Sampling protocol

Subgingival plaque samples from healthy sites, periodontitis, and peri-implantitis implants from each subject included in this study were all collected on the same day.

Definition criteria for periodontitis teeth and peri-implantitis implants were chosen as follows:

- Periodontitis tooth (PA): Pocket Probing Depth (PPD)  $\geq 5$  mm, with evidence of radiographic bone loss  $> 33\%$  and with positive BOP
- Peri-implantitis implant (PI): implants with changes regarding increasing peri-implant probing depths (PPDi)

**Table 1** Demographic details and general information of the 24 patients

Patient	ID periodontal site1	ID peri-implant site2	ID healthy site3	Age (years)	Gender	Smoking status *	Abutment material	Number of teeth	Number of implants	Periodontal pockets > 4 mm	Peri-implant pockets > 4 mm
1	34 PA	33 PI	57 HE	62	F	0	CrCo	13	2	1	1
2	7 PA	10 PI	-	52	F	0	Titanium	19	7	1	15
3	46 PA	47 PI	56 HE	52	F	0	CrCo	26	3	1	1
4	44 PA	45 PI	60 HE	58	F	0	Titanium	24	3	3	1
5	8 PA	9 PI	58 HE	49	M	1	Titanium	21	3	8	6
6	25 PA	17.1 PI	-	55	F	0	Titanium	18	4	18	2
7	27 PA	28 PI	-	51	F	0	Titanium	11	2	1	2
8	47.1 PA	48 PI	-	80	F	0	Titanium	18	8	3	3
9	40 PA	41 PI	-	64	M	0	CrCo	20	5	4	1
10	31 PA	32 PI	-	59	F	0	CrCo	9	3	3	1
11	43 PA	42 PI	-	66	F	0	Titanium	23	1	1	1
12	18 PA	20 PI	-	65	M	0	Titanium	24	1	0	1
13	13 PA	14 PI	53 HE	63	F	1	Titanium	24	1	5	2
14	-	23 PI	-	70	M	1	Titanium	18	2	3	2
15	39 PA	38 PI	-	73	F	0	Titanium	8	8	1	1
16	-	15 PI	-	80	M	0	Titanium	16	2	20	1
17	29 PA	30 PI	54 HE	68	M	0	Titanium	16	2	16	0
18	12 PA	11 PI	-	58	F	1	Titanium	16	6	16	4
19	35 PA	36 PI	55 HE	66	M	0	Titanium	16	6	17	8
20	35.1 PA	21 PI	61 HE	58	F	1	Titanium	17	3	21	5
21	-	16 PI	-	57	F	1	Titanium	27	2	10	1
22	50 PA	49 PI	62 HE	62	F	0	Titanium	20	2	20	5
23	52 PA	51 PI	-	69	M	0	Titanium	18	4	25	1
24	-	5 A -PI	59HE	48	M	1	Titanium	10	8	8	9

\* smoker: 1, non-smoker: 0

<sup>1</sup>Lab code used for sequencing of samples from periodontal sites (PA)

<sup>2</sup>Lab codes used for sequencing of samples from peri-implant sites (PI)

<sup>3</sup>Laboratory codes used for sequencing of samples from healthy sites (HE)

- and RBL from baseline (1 year from prosthetic loading) and with positive peri-implant BOP
- Healthy tooth (HE): absence of clinical signs of inflammation, PPD  $\leq 3$  mm, CAL  $\leq 3$  mm, normal bone levels ranging from 1.0 to 3.0 mm apical to the CEJ [6].

Following a session of professional hygiene with rubber cups and brushes to remove the supragingival/supramucosal biofilm and plaque deposits, sampling was carried out by inserting 4 sterile endodontic paper cones (FKG®) with tip diameter 0.25 mm and 2% taper in the gingival/mucosal sulcus for each selected periodontitis, peri-implantitis, and healthy tooth site for 2 min. The sampling was isolated from saliva using cotton rolls to prevent contamination. Following collection, the paper cones were inserted in sterile Eppendorf tubes containing 2 ml saline solution of NaCl 0.9% and stored in a sealed refrigerated container and delivered to the BIOMETEC Department of the University of Catania within 2 h for microbiological analyses.

No formal sample size calculation was carried out for this study, choosing a convenience sample of 24 patients. This resulted in a total of 58 sites samples (10 HE, 24 PA, and 24 PI). Four of the 58 samples (all taken from PA sites) could not be included in the analysis, as the DNA yield was insufficient for further microbiome analysis (Fig. 1). When possible, 3 suitable sites per patient (as per definitions above) were sampled and analyzed (1 each for PA, PI, and HE), preferring implants with single crown restoration and non-restored teeth. However, this was possible only in 10

patients, while 14 patients had 2 samples analyzed (1 each from PI and PA sites).

## Microbiological protocol

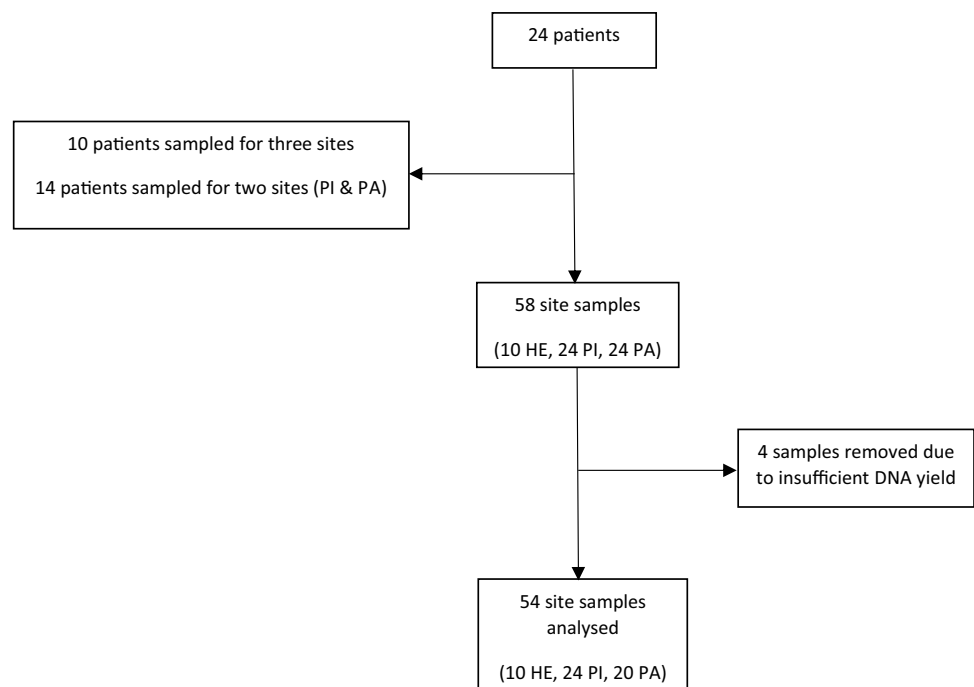
### DNA extraction, 16S rRNA gene library preparation, and sequencing

DNA from sterile paper cone samples was extracted with the PureLink® Genomic DNA Kit (Thermo Fisher Scientific, USA) [27]. Extracted DNA was checked for quality and quantity by a NanoDrop2000 Spectrophotometer and the Qubit 2.0 fluorometer (dsDNA HS assay, Invitrogen) and was amplified using the V3-V4 region of the 16S rRNA gene [28]. PCR products were purified by Agencourt AMPure XP magnetic beads (Beckman Coulter) and the quality of the products was assessed by Agilent2100 Bioanalyzer (Agilent Technologies, USA). Finally, 12 pM of the library mixture, spiked with 20% PhiX control, was paired-end ( $2 \times 300$ ) sequenced (MiSeqIllumina), at the B.R.I.T. unit (UNICT). The sequences are available in the NCBI BioProject database under accession number PRJNA548277.

### Processing of sequencing data, statistical and data analyses

QIIME pipeline (Quantitative Insights Into Microbial Ecology) version 1.9.1 was used to process the generated raw FASTQ files [29]. The paired-end sequences were assembled to a single read using FLASH [30] and quality-filtered  $\geq 80\%$  bases in a read above Q30 (Table S1). To focus only on

**Fig. 1** Flow-chart of sampling of patients. Ten patients had 3 sites sampled, while 14 patients had 2 sites sampled (1 each from PI and PA sites). This resulted in a total of 58 sites samples (10 healthy, 24 periodontitis, and 24 peri-implantitis sites). Four of the 58 samples (all taken from PA sites) could not be included in the analysis, as the DNA yield was insufficient for further microbiome analysis



the prominent taxa, a filtering step of 0.01% at the operational taxonomic unit (OTU) level was performed by running a workflow on QIIME (filter\_otus\_from\_otu\_table.py). The taxonomy of each 16S rRNA gene sequence was collapsed to OTUs using the open reference-based of Human Oral Microbiome Database (HOMD) at 97% of sequence similarity [31]. Chimeras were identified and removed by the UCHIME algorithm. Any reads that did not match the reference sequence collection were subsequently clustered de novo. Rarefaction curves were generated with QIIME and calculated using Explicet with a maximum depth of 74,469 sequences/sample [32]. The OTU tables were used for assessing  $\alpha$ -diversity indices (Chao-1, Shannon diversity) calculated from the taxonomic profiles and compared across the PA, PI, and HE groups. Independent Student's t-test and the Mann–Whitney U test were used to evaluate  $\alpha$ -diversity among the taxonomic profiles and compared across the PA, PI, and HE groups.  $\beta$ -diversity between PA, PI, and HE groups was performed by a weighted UniFrac distance matrix, and then visualized by principal coordinate analysis (PCoA) plot.

The core microbiome was determined by QIIME algorithms (*compute\_core\_microbiome.py*) and the diversity analysis was performed with the script *core\_diversity\_analyses.py* to detect the abundance of different taxonomic categories at the genus/species levels, defined as the OTUs that are present in at least 50% of the samples. The data obtained by *core microbiome* were used to evaluate the statistical diversity between PA, PI, and HE groups. OTU frequencies across sample groups (diseased/healthy) were compared using the Kruskal–Wallis test, a nonparametric ANOVA test. Statistical analysis of taxonomic profiles was performed using STAMP [33]. Extended error bar plots were computed (White's non-parametric t-test and  $p$ -value < 0.05) showing the bacterial taxa with a significant difference ( $p$ -value < 0.05). The linear discriminant analysis effect size (LEfSE) biomarker discovery tool [34] was used to identify discriminatory OTUs (LDA = 3.0).

## Results

A total of 54 sites from 24 patients with a mean age of 62 years old were included in this study. The average number of teeth and implants for the 24 patients were 18 teeth and 4 implants. The average number of periodontal pockets > 4 mm was 9 while an average of 3 sites present had peri-implant pockets > 4 mm (Table 1). Seven were smokers at the time of the study ( $\geq 5$  cigarettes/day) while the remaining were non-smokers ( $\leq 5$  cigarettes/day or ex-smokers for a minimum of 5 years). At the implant level, only 4 abutments were in chrome-cobalt while the rest were in titanium. The microbial profile was detected for all peri-implant sites

(PI), 20 periodontal sites (PA), and only 10 healthy teeth (HE). The average age of patients with healthy sites sampled ( $n = 10$ ) was 59 years, including 4 smokers while the average age of patients with periodontitis sites sampled ( $n = 20$ ) was 62 years, including 4 smokers.

## Microbial profile of healthy and diseased periodontal and peri-implant sites

A total of 24 PI, 20 PA, and 10 HE samples were sequenced using the MiSeq platform to determine the microbial diversity between diseased (PA and PI) and healthy samples. A total of 7,414,811 valid reads, with an average of 137,311 reads/participant, were generated (70,265–266,973 range), which were clustered in 376 OTUs, with at least 97% similarity level using the Human Oral Microbiome Database.

## Microbial profile

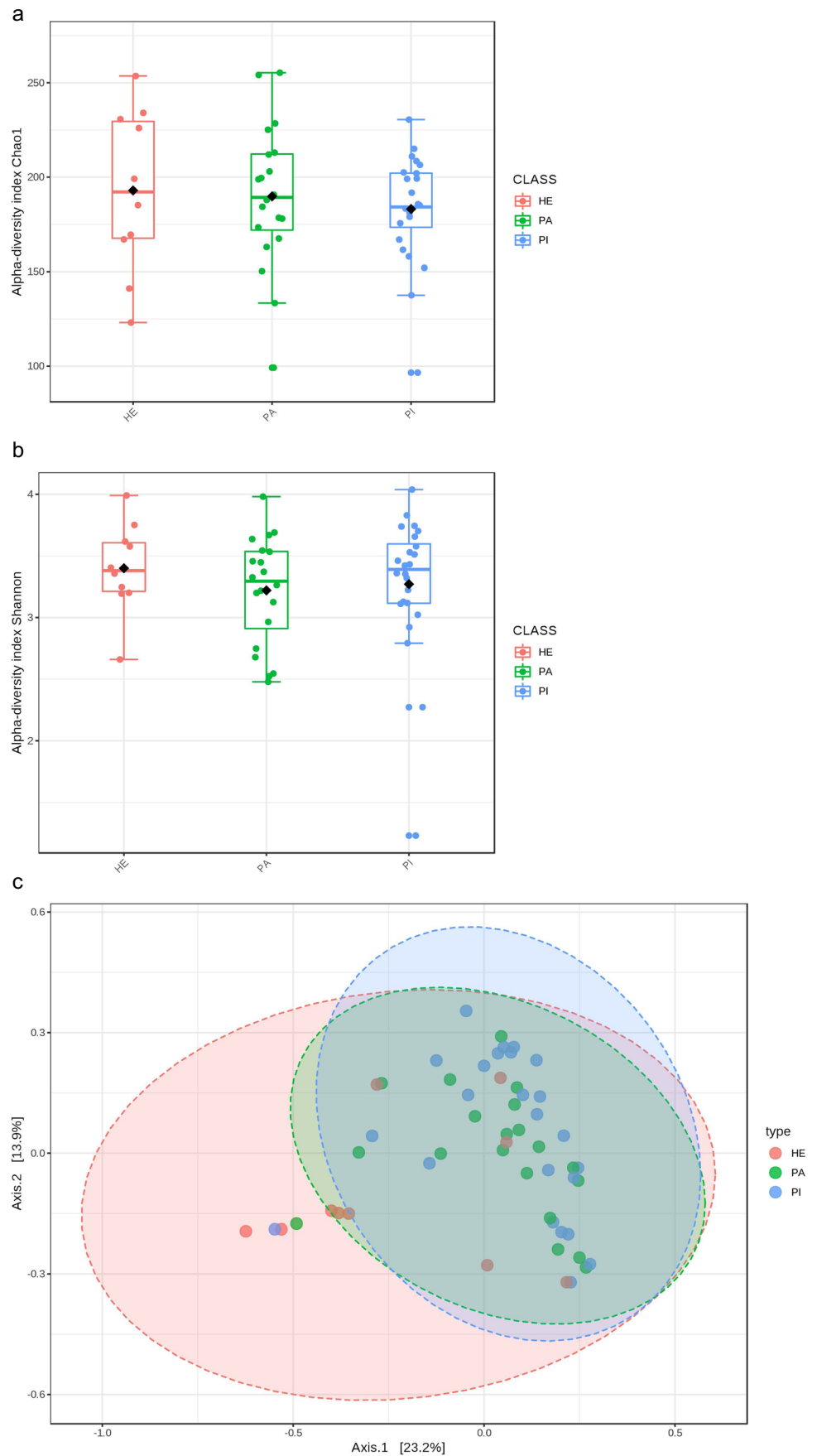
The microbial diversity within each group (alpha-diversity) was estimated by the Chao-1 index (community richness) and the Shannon H-index (diversity) (Fig. 2a, b). OTU richness was not significantly different in the HE group compared to the PA and PI groups (Chao-1 index,  $p = 0.705$ ; ANOVA). The Shannon diversity index showed that HE and PI had a slightly greater variety of microbial communities than PA sites, but the differences were not statistically significant ( $p = 0.443$ ; ANOVA). The microbial diversity between the three groups ( $\beta$ -diversity) revealed variation in both diseased and healthy groups. Most of the PI and PA samples clustered together and only two (one PA and one PI) were outliers (PERMANOVA,  $p = 0.001$ ) (Fig. 2c).

## Microbiome differences between healthy and diseased periodontal and peri-implant sites

Most taxa across all sites clustered predominantly in 10 phyla: *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, which accounted for approximately 85% of all isolates; other dominant taxa included *Absoconditabacteria* (SR1), *Actinobacteria*, *Fusobacteria*, *Saccharibacteria* (TM7), *Spirochaetes*, and *Synergistetes*. Among these, *Bacteroidetes* (33%) and *Firmicutes* (31.4%) were the most abundant in the PA and PI groups while *Proteobacteria* (29.1%) was the most abundant in the HE group (Figure S1).

Overall, a total of 109 genera and 187 species were identified. STAMP software analysis at taxonomic level showed that 22 genera had statistically significant difference in prevalence across the 3 study groups, namely *Lautropia* (the most prevalent), *Klebsiella*, *Escherichia*, *Cardiobacterium*, *Acidovorax*, *Burkholderiaceae*, *Alcaligenaceae*, *Rhodobacteraceae*, *Afipia*, *Bradyrhizobiaceae*, *Clostridiales*, *Bergeyella*, and *Corynebacterium* were more abundant in the

**Fig. 2**  $\alpha$ - and  $\beta$ -diversity between PA, PI and HE groups. **a** The  $\alpha$ -diversity associated with PA (green), PI (blue), and HE (red) samples was calculated by Chao-1 index (A, community richness) ( $p$ -value: 0.70468), and **b** Shannon H index (B, diversity) ( $p$ -value: 0.44315). **c** Principal coordinate analysis (PCoA) plot generated using weighted UniFrac distances based on the abundance of OTUs of PA (periodontitis  $n=20$ ), PI (peri-implantitis  $n=24$ ) and HE (healthy  $n=10$ ) samples (PERMANOVA,  $p$ -value: 0.001). Peri-implantitis (blue) and periodontitis (green) samples tended to cluster together with respect to healthy samples (red)



HE group, *Slackia*, *Bacteroidaceae* [G-1], *Eubacteriaceae* [XV], *Peptostreptococcaceae* [XI], *Selenomonas*, and *Lachnospiraceae* [G-7] were more abundant in PI samples, and *Mogibacterium*, *Olsenella*, and *Dialister* were showing increased abundance in PI and PA groups compared with healthy groups (Fig. 3; Table S1).

In pair-wise analyses:

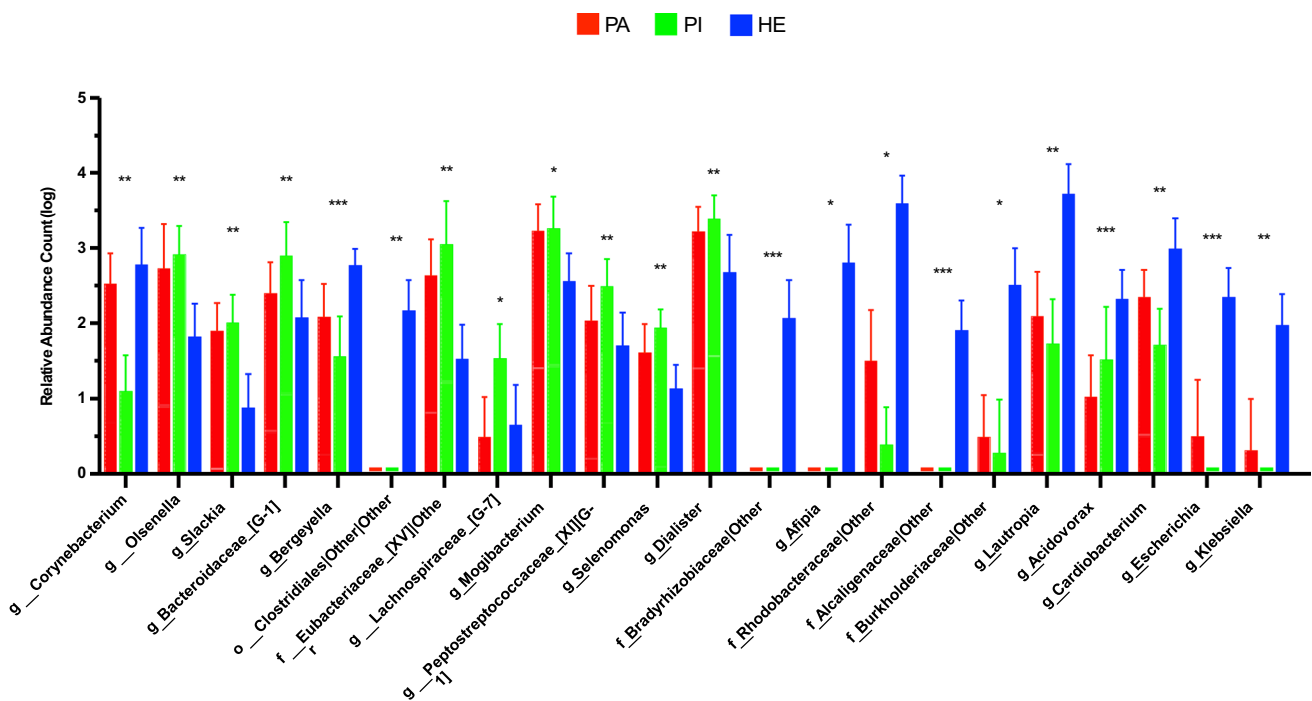
- 30 genera (Fig. 4a) and 36 species (Figure S2) had statistically significant difference in relative abundance between HE and PA groups. Among these species, *F. alocis*, *Parvimonas micra*, *Prevotella nigrescens*, and *Mogibacterium spp.* were the most abundant in PA, while *Lautropia mirabilis*, *Rhodobacteraceae*, *Bacillus*, and *Rhizobiales* were the most abundant in healthy sites.
- 46 genera (Fig. 4b) and 65 species (Figure S3) had statistically significant difference in relative abundance between HE and PI groups.
- 10 genera had statistically significant differences when comparing PA vs. PI sites, including *Atopobium*, *Bacteroidaceae* [G-1], *Peptostreptococcaceae* [XI], *Selenomonas*, *Peptoniphilaceae*, *Mollicutes*, and *Lachnospiraceae* are more abundant in PI sites and *Corynebacterium* and *Cardiobacterium* and *Lactobacillales* are more abundant in PA sites (Fig. 5). Fourteen

species were differentially abundant when comparing PA and PI sites (Figure S4).

Linear discriminant analysis (LDA) effect size was used ( $p$ -value < 0.05, LDA = 3.0) to evaluate the differences between the three groups at the genus level or higher taxonomic level, 45 OTUs were statistically discriminated between infected and healthy sites. Twenty-one OTUs were prevalent in healthy sites including *Lautropia*, *Rothia*, and *Capnocytophaga* and *Kingella*, suggesting their association with a healthy periodontium. Twenty-four OTUs were associated with PI (21 taxa) and PA (3 taxa) including *Peptostreptococcaceae* XI, *Dialister*, *Mogibacterium*, *Atopobium*, and *Filifactor* for PI and *Bacteroidales* for PA groups (Figure S5).

### Discussion

The present study investigated differences in the microbiota between periodontally healthy, periodontitis, and peri-implantitis sites in the same patients. It is still one of a few published studies employing 16 s rRNA analysis of the subgingival plaque for this aim. Overall, by comparing



**Fig. 3** Different bacterial abundance of the HE, PA, PI groups. **a** The microbial composition between HE and PA sites was explored in terms of the relative abundances at the genus level using STAMP software (White’s non-parametric  $t$ -test;  $p$ -value < 0.05) to determine statistically significant differences. **b** The microbial composition

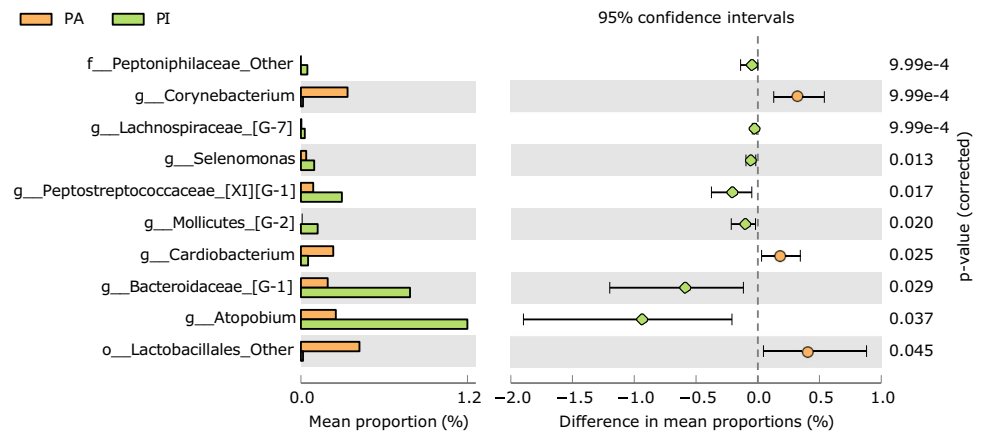
between HE and PI sites was explored in terms of the relative abundances at the genus level using STAMP software (White’s non-parametric  $t$ -test;  $p$ -value < 0.05) to determine statistically significant differences



**Fig. 4** Different bacterial abundance between PA and PI groups. The microbial composition between PA and PI sites was explored in terms of the relative abundances at the genus level using the STAMP soft-

ware (White's non-parametric *t*-test; *p*-value < 0.05) to determine statistically significant differences

**Fig. 5** Different bacterial abundance across the three groups, PA, PI, and HE. The significant differences in terms of abundant of genera in PA, PI, and HE samples were estimated by statistical analysis using ANOVA (one-way, *p*-value < 0.05)





the  $\alpha$ -diversity in each group of samples (HE, PA, and PI) and their clustering, PA and PI genera seem to overlap in many cases, while a slightly greater diversity (not statistically significant) was found in the healthy site. Significant differences at the genus level were found comparing healthy sites with diseased sites (periodontitis and peri-implantitis).

The greatest abundance of Proteobacteria in healthy sites was confirmed by statistical analysis at the genera level in which *Clostridiales*, *Bradyrhizobiaceae*, *Alcaligenaceae*, *Burkholderiaceae*, *Lautropia*, *Escherichia*, and *Klebsiella* were specifically, and almost exclusively, present, defining a microbial community associated with a healthy core microbiome [35]. Furthermore, *Actinomyces*, *Rothia*, *Streptococcus*, *Haemophilus*, and *Neisseria* were also among the most abundant taxa shared in the HE core. *Lautropia* and *Neisseria* has been shown to be significantly present in high abundance in subgingival sites associated with health [36]. *Streptococcus* and *Actinomyces* have also been shown to be more abundant in healthy individuals compared with periodontitis patients [37], which affirms the results obtained in this study. It is also important to note that 2 of the 10 healthy sites were prosthetically restored which may have affected the composition and quantity of the microbiota in this category. Interestingly, a recent study has also shown that successfully treated aggressive sites contained greater proportions of *Rothia*, *Lautropia*, and *Streptococcus* compared to persistent sites with aggressive disease [38].

On the other hand, in diseased sites (PA and PI), *Mogibacterium*, *Dialister*, *Prevotella*, *Filifactor*, *Alloprevotella*, and *Olsenella* were the most prevalent genera, with increased abundance compared with healthy sites. *F. alocis*, *Parvimonas micra*, *Prevotella nigrescens*, and *Mogibacterium spp.* were particularly in periodontal sites. This is consistent with previous studies, showing that *Filifactor*, as well as *Mogibacterium*, genera were associated with “persistent” aggressive periodontitis in a recent study [38] and with presence of periodontitis compared with periodontal health in another study [39]. Such species have unique properties in periodontal pathogenesis; for example, *Filifactor* is able to induce proinflammatory cytokines leading to apoptosis of gingival cells [40] and chronic inflammation [41]. Meanwhile, *Prevotella* can drive periodontal inflammation through recruitment of neutrophils via Th17 mediated immune response [41]. Among classic periodonto-pathogenic bacteria belonging to the “red complex” [25, 42, 43], *Treponema denticola*, *Tannerella spp.*, and *Porphyromonas gingivalis* were higher in disease groups in the present study, although not significantly more than in healthy sites. *Prevotella nigrescens*, part of the orange complex of periodontal pathogens showed equal abundance in periodontitis and peri-implantitis samples, while *Prevotella intermedia* and *Prevotella denticola* were significantly more abundant in peri-implantitis. Meanwhile, *Parvimonas micra*, *Filifactor*

*alocis*, and *Dialister invisus* were more abundant in the PI samples compared to the HE samples by STAMP, while *A. actinomycetem comitans* was missing in the disease conditions (PI and PA). Among other genera, *Atopobium*, *Bacteroidaceae* [G-1], *Peptostreptococcaceae* [XI], *Selenomonas*, *Peptoniphilaceae*, *Mollicutes*, and *Lachnospiraceae* were more abundant in PI sites and *Corynebacterium* and *Cardiobacterium* and *Lactobacillaceae* were more abundant in PA sites, lending support to the hypothesis in which distinct ecosystems were suggested in periodontitis vs. peri-implantitis [44, 45]. To our knowledge, this is the first study in which *Alloprevotella* (mainly *Alloprevotella tanneriae*) and *Atopobium* were detected as significantly more abundant in peri-implantitis.

Overall, the present study contributes to improving our understanding of a “disease core subgingival microbiome” (with some taxa shared between PI and PA and others more abundant in one or the other condition) and a “healthy core subgingival microbiome” (in which the genus *Lautropia* was particularly abundant).

The limitations of this study include the relatively small sample size and the different disease levels both in periodontal and peri-implant sites could influence microbiome composition. Furthermore, no sample size calculation was done prior to the study so the results which showed significant differences should be interpreted with caution. Lastly, bias can be introduced in the analysis as not all participating subjects had the same number of samples collected, potentially impacting the analysis due the hierarchical nature of the data (patient-tooth-site).

In conclusion, diseased sites (peri-implantitis and periodontitis) exhibit a distinct dysbiotic subgingival microbiological ecosystems compared with healthy sites in the same patients. Upon analysis of data of larger studies, specific treatments to manipulate the microbial profile of teeth and implants, directing them towards a more stable health-associated microbiome could be potentially be considered.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00784-021-04253-4>.

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**Author contribution** All authors contributed to the study conception and design. Material preparation and data collection were performed by Paolo Torrisi, Ambra Spitale, and Alaa Guni. Analysis was performed by Giovanni Barbagallo, Maria Santagati, and Sebastiano Ferlito. The original draft of the manuscript was written by Stefania Stefani,

Giovanni Barbagallo, Maria Santagati, and Luigi Nibali. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Declarations

**Ethics approval** The study was approved by the Ethical Committee of Catania 2 (47/2018/CECT2).

**Conflict of interest** The authors declare no competing interests.

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