Intra-oral single site comparisons of periodontal and peri-implant microbiota in health and disease

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(Running title: Periodontal versus peri-implant microbiomes)

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Key words: Oral microbiome, dental implant, oral disease, peri-implantitis, periodontitis, Illumina, etiology.

Author contributions

NPL, H-CL and L-FZ established the original cohort, and were responsible for performing and/or overseeing the subjects' recruitment and clinical examination. X-LY and YC performed the DNA sequencing and bioinformatic analysis. RMW, YC and X-LY conceived and designed this microbiome study, analyzed the data, and interpreted the results. RMW, YC, X-LY and WKL wrote the draft manuscript. All authors were involved in the preparation of the final version of the manuscript.

Abstract

Objective: Periodontitis and peri-implantitis are oral infectious-inflammatory diseases that share similarities in their pathology and etiology. Our objective was to characterize the single-site subgingival and submucosal microbiomes of implant-rehabilitated, partially dentate Chinese subjects (*n*=18) presenting with both periodontitis and peri-implantitis.

Materials and Methods:

Subgingival/submucosal plaque samples were collected from four clinically-distinct sites in each subject: peri-implantitis submucosa (DI), periodontal pocket (DT), clinically-healthy (unaffected) peri-implant submucosa (HI), and clinically-healthy (unaffected) subgingival sulcus (HT). The bacterial microbiota present was analysed using Illumina MiSeq sequencing.

Results: 26 phyla and 5,726 Operational Taxonomic Units (OTUs, 97% sequence similarity cut-off) were identified. Firmicutes, Proteobacteria, Fusobacteria, Bacteroidetes, Actinobacteria, Synergistetes, TM7 and Spirochaetes comprised 99.6% of the total reads detected. Bacterial communities within the DI, DT, HI and HT sites shared high levels of taxonomic similarity. 31 ‘core species’ were present in >90% sites; with *Streptococcus infantis/mitis/oralis* (HMT-070/HMT-071/HMT-638/HMT-677) and *Fusobacterium* sp. HMT-203/HMT-698 being particularly prevalent and abundant. Beta-diversity analyses (Permanova-test, weighted-UniFrac) revealed the largest variance in the microbiota was at the subject level (46%), followed by periodontal health status (4%). Differing sets of OTUs were associated with periodontitis and peri-implantitis sites, respectively. This included putative ‘periodontopathogens, such as *Prevotella*, *Porphyromonas*, *Tannerella*, Bacteroidetes [G-5] and *Treponema* spp. Interaction network analysis identified several putative patterns underlying dysbiosis in periodontitis/peri-implantitis sites.

Conclusions: Species (OTU) composition of the periodontal and peri-implant microbiota varied widely between subjects. The inter-subject variations in subgingival/submucosal microbiome composition outweighed differences observed between implant versus tooth sites, or between diseased versus healthy (unaffected) peri-implant/periodontal sites.

Introduction

The submucosal niches that surround osseointegrated titanium dental implants are notably different from the corresponding niches that surround natural teeth in terms of their anatomy, histology and physiology. Key differences include the lack of periodontal ligament tissues, the presence of a direct titanium-bone interface, the different arrangement of connective tissues surrounding the implant, a poorer blood circulation, and the absence of gingival crevicular fluid in peri-implant sites (Belibasakis, Charalampakis, Bostanci, & Stadlinger, 2015; Lang & Berglundh, 2011). Peri-implant diseases affecting dental implants are generally considered to be broadly equivalent to periodontal diseases affecting teeth in terms of their pathology and etiology. Peri-implantitis and periodontitis both represent infectious-inflammatory diseases that share a mixed-species bacterial etiology (Heitz-Mayfield & Lang, 2010; Lang & Berglundh, 2011). Analogous to periodontitis, peri-implantitis sites are characterized by a loss of supporting bone in addition to a profound inflammation of coronal soft tissue structures (Lang & Berglundh, 2011; Lindhe & Meyle, 2008; Mombelli, 1999).

Many of the bacterial species implicated in the etiology of periodontal diseases, such as *Porphyromonas gingivalis*, *Tannerella forsythia, Aggregatibacter actinomycetemcomitans*, *Treponema* spp. and *Prevotella* spp. have been shown to be more prevalent and/or more abundant within diseased peri-implant niches compared with healthy implant sites (Cortelli et al., 2013; Daubert, Weinstein, Bordin, Leroux, & Flemmig, 2015; Hultin et al., 2002; Kumar, Mason, Brooker, & O'Brien, 2012; Leonhardt, Bergstrom, & Lekholm, 2003; Mombelli, 1993; Mombelli & Décaillet, 2011; Persson & Renvert, 2014; Quirynen, Abarca, Van Assche, Nevins, & Van Steenberghe, 2007; Renvert, Roos-Jansåker, Lindahl, Renvert, & Rutger Persson, 2007). A history of periodontitis (Heitz-Mayfield, 2008; Karoussis et al., 2009) and the presence of residual periodontal pockets (Lee, Mattheos, Nixon, & Ivanovski, 2012; Ferreira, Silva, Cortelli, Costa, & Costa, 2006; Karoussis et al., 2004) have both been

established as risk factors for the (pathological) bacterial infection of implants. Such correlations also support the premise that peri-implantitis and periodontitis share etiological commonalities.

However, it has also been shown that (infected) implant sites may harbour bacterial taxa that are relatively rarely found in subgingival niches; e.g. staphylococci, peptostreptococci, enterobacteria, *Helicobacter* spp., (Maruyama et al., 2014; Mombelli & Décaillet, 2011; Persson & Renvert, 2014); which has been proposed to be largely due to the differences in biofilm formation on implant surfaces compared with natural periodontal tissues (Lang & Berglundh, 2011). Results from more recent studies employing high-throughput DNA sequencing-based analyses of 16S rRNA genes, which have analyzed bacterial composition with considerably greater sampling depth, have revealed significant dissimilarities between the taxa that were over-represented in peri-implantitis sites, compared to corresponding periodontitis sites (Dabdoub, Tsigarida, & Kumar, 2013; Ebadian, Kadkhodazadeh, Zarnegarnia, & Dahlén, 2012; Koyanagi et al., 2013; Kumar et al., 2012; Maruyama et al., 2014; Robitaille, Reed, Walters, & Kumar, 2016), although there are large variations in the respective differences reported. Some studies have indicated that periodontitis sites have greater taxonomic (species) diversity than peri-implantitis sites (Koyanagi et al., 2013), whilst other studied have noted the contrary (Dabdoub et al., 2013; Kumar et al., 2012), whereas other studies have noted no major differences (Aoki et al., 2012; Canullo et al., 2016).

The oral microbiome exhibits considerable inter-individual variation within healthy individuals (Bik et al., 2010; Nasidze, Li, Quinque, Tang, & Stoneking, 2009). There is also considerable site-to-site variation within analogous niches in the same oral cavity (Mira, Simón-Soro, & Curtis, 2017; Schwarzberg et al., 2014; Simón-Soro et al, 2013). Dabdoub *et al.* (Dabdoub et al., 2013) revealed that adjacent peri-implantitis and periodontitis sites shared

modest levels of taxonomic similarity, consistent with the premise that peri-implant and periodontal microbiota represented microbiologically distinct ecosystems. Thus, further studies are needed to understand the colonization dynamics of these respective microbial communities and their responses to different biological or environmental 'factors' affecting their resilience and resistance to oral disease (Robitaille et al., 2016).

Here, Illumina MiSeq sequencing was utilized to characterize and systematically compare the subgingival and submucosal microbiota of diseased and clinically-healthy (i.e. unaffected by disease) periodontal and peri-implant sites within a group of 18 Chinese subjects presenting with both periodontitis and peri-implantitis. The results paint a complex picture. Previously established 'periodontopathogens' may play important etiological roles in peri-implant and periodontal infections, but in general, inter-individual differences in subgingival/submucosal microbiome composition are considerably more prominent than intra-oral, site-to-site differences.

Materials and methods

Subject recruitment and sampling

The cohort described in this study has been previously reported (Zhuang et al., 2014; Yu et al., 2016). Clinical examinations were performed in the Department of Oral and Maxillofacial Implantology, Shanghai Ninth People's Hospital, affiliated to Shanghai Jiao Tong University, Shanghai, China. A detailed description of the subject recruitment, inclusion/exclusion criteria, and clinical examinations performed were reported (Zhuang et al., 2014; Yu et al., 2016). As previously noted, ethical approval was obtained from the University of Hong Kong/Hospital Authority Hong Kong West Cluster Institutional Review Board (HKU/HA HKW; UW 12-118) and from the Shanghai Ninth People's Hospital, affiliated to the School of Medicine, Shanghai Jiao Tong University (2012-67) (Zhuang et al., 2014). Briefly, the following clinical parameters were examined to characterize the status of periodontal and peri-implant lesions: Plaque Index (PI) (Silness & Löe, 1964) and modified Plaque Index (mPI) (Mombelli & Lang, 1994), modified Bleeding Index (mBI) (Mombelli & Lang, 1994), Gingival Index (GI) (Löe & Silness, 1963), bleeding on probing (BOP) scores (Lang et al., 1986) and probing pocket depth (PPD) (Glavind & Löe, 1967) as well as radiographic examination. Inclusion criteria included: (1) patients who were systemically healthy and willing to give the informed consent; (2) patients with a history of periodontitis; (3) upon examination, patients who had at least one tooth/implant site belonging to each of the four following clinically distinct categories: healthy (unaffected) implant tissue (HI), peri-implantitis (DI), periodontally healthy (unaffected) gingivae (HT), and periodontitis (DT). Exclusion criteria included: (1) fully edentulous patients; (2) patients who had used systemic antibiotics within 3 months prior to enrolment; (3) patients treated with systemic steroid medications or prophylactic antibiotics prior to clinical examination; and (4) patients taking any medication known to affect periodontal status within at least 2 weeks. The peri-

implantitis lesions (DI) were defined as having PPD ≥ 5 mm, with the presence of BOP and radiographic evidence of bone loss. The periodontitis lesions (DT) were defined as being positive for BOP, having PPD ≥ 4 mm, and having a loss of attachment ≥ 3 mm. The clinically healthy implant sites (HI) were defined as having probing depths ≤ 4 mm, being negative for BOP, with no radiographic evidence for bone loss. The clinically healthy tooth sites (HT) were defined as having probing depths ≤ 3 mm, being negative for BOP and having no clinical attachment loss. Subgingival and sub-mucosal plaque samples were respectively collected from the deepest periodontal pocket and deepest peri-implant site. Healthy subgingival and sub-mucosal plaque samples were respectively collected from randomly selected, tooth/implant sites, as has previously been reported (Zhuang et al., 2014). Briefly, prior to sampling, clinical sites were isolated and dried, and supragingival/supramucosal plaque and calculus were carefully removed. Subgingival/submucosal plaque was sampled by inserting 3 sterile paper points (No. 25) into the base of the sulcus or pocket for 10 s. The paper points were then placed in labelled Eppendorf tubes and frozen, for transportation to the laboratory for the subsequent extraction of DNA.

DNA sequencing and data analysis

Total bacterial genomic DNA was extracted using QIAamp DNA Mini kits (Qiagen, CA, USA) according to the manufacturer's instructions, as was previously described (Zhuang et al., 2014). PCR amplification of the hypervariable V3-V4 region of the 16S ribosomal RNA (rRNA) genes was performed using the universal bacterial primer pairs, 341F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR amplification and library construction were performed according to Illumina's standard protocol for 16S Metagenomic Sequencing Library Preparation (Part # 15044223 Rev. B).

Paired-end MiSeq sequencing was performed on the amplicon libraries using an Illumina MiSeq system (300PE) at the Beijing Genomics Institute (BGI Institute, Shenzhen, China). The paired-end MiSeq sequences were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME1) package (Caporaso et al., 2010) following the MiSeq SOP pipeline. Mothur (Schloss et al., 2009) was also used in the downstream sequence analysis. Details of the scripts used and the parameter settings are reported in the supplementary material.

Statistical analysis

Statistical analyses were conducted using various R packages (Team, 2015) and IBM SPSS Statistics software 23 (SPSS Inc., Chicago, IL, USA). Details of the packages used and the parameter settings are summarized in the supplementary methods section in the supplementary material.

Data depository

The sequences and metadata were deposited in NCBI Short Reads Achieve under the accession of biosamples: SAMN09464023 - SAMN09464094.

Results

Eighteen implant-rehabilitated, partially-dentate Chinese subjects (9 males, 9 females; mean age 54.8 ± 14.9 years) who had developed both periodontal and peri-implant infections were included in this study. Sixteen subjects were non-smokers, 2 were current smokers. Subgingival/submucosal plaque samples were collected from two implant sites and two tooth sites in each subject, as has previously been described (Yu et al., 2016; Zhuang et al., 2014). Specifically, these samples represented one clinically-healthy (unaffected) peri-implant site (HI); one peri-implantitis site (DI); iii) one clinically-healthy (unaffected) subgingival site (HT); and one periodontitis site (DT). The clinical parameters for the 4 respective sets of sampled sites are summarized in **Table 1** and **Table S1.**

All samples (n = 72) yielded DNA of sufficient quantity and quality to enable (bacterial) microbiome analysis via Illumina MiSeq sequencing of the V3-V4 hypervariable regions of 16S rRNA gene amplicons. A total of 4,425,705 assembled, quality-filtered sequencing reads were obtained at an average of 61,468 ± 21,183 reads per sample. Diverse bacterial taxa spanning 26 phyla were identified within the subject group (**Table 2**). Considered as a whole, the microbiota of this cohort were dominated by Firmicutes (40.33%), followed by Proteobacteria (17.45%), Fusobacteria (14.94%), Bacteroidetes (14.44%) and Actinobacteria (5.25%). In fact, with the addition of Synergistetes, TM7 (Saccharibacteria), and Spirochaetes; 99.6% of the total bacterial taxa detected belonged to these 8 phyla. It should be noted that the other 18 phyla were detected at low abundance; especially the Aquificae, Chlamydiae, Gemmatimonadetes, Nitrospirae, TM6, Verrucomicrobia and WPS-2 phyla; which were present at <0.01% of total reads obtained for each of the four respective clinical site categories; with some being undetectable in one or more of the respective niches (**Table 2**).

First, the bacterial phyla present within each of the four clinically-distinct sites were

examined. Specifically, all of the sequencing reads present in the DI sites (n = 18), the HI sites (n = 18), the DT sites (n = 18), and the HT sites (n = 18) were combined to generate four composite datasets representing each of the respective clinical sites. The respective distributions of the top eight phyla within the HT, DT, HI, and DI sites are shown in graphical form **Figure 1A**. It may be seen that the four different periodontal/peri-implant niches shared a reasonably similar overall community composition at the phylum level, with the top eight phyla being present at fairly similar proportions.

The respective taxa present within the clinically-diseased peri-implantitis/periodontitis sites (combined DI and DT sites; n = 36), the clinically-healthy (unaffected) peri-implant/periodontal sites (combined HI + HT sites; n = 36) were analogously analyzed; as were the submucosal implant sites (combined DI + HI sites) and the subgingival sites (combined DT + HT sites). This was done to determine if the respective distributions of phyla exhibited notable differences between healthy (unaffected) and diseased sites: regardless of whether this surrounded an implant or a tooth; or between implant versus tooth sites: regardless of clinical health status. The respective distributions of the top eight phyla within these four pairs of sites are shown in graphical form in **Figure 1B**.

Qualitatively-speaking, the relative abundance of Firmicutes was slightly higher in implant niches (HI + DI sites) than in periodontal niches (HT + DT sites) (44.4% versus 36.4%), whereas Fusobacteria showed a contrary trend (12.0% versus 15.7%.) (**Table 2**). Spirochaetes and TM7 (Saccharibacteria) were *ca*. 3-fold higher in peri-implantitis/periodontitis sites (DI + DT) compared to the clinically-healthy (or unaffected) peri-implant/periodontal sites (HI + HT). On the contrary, Proteobacteria were more abundant in the clinically-healthy or unaffected peri-implant/periodontal sites, than in peri-implantitis/periodontitis sites (**Figure 1B**, **Table 2**). Only the difference in the relative abundance of Spirochaetes taxa was found to be statistically significant (Kruskal-Wallis H

test, $p = 0.023$) but not after multiple comparison adjustment (BH-adjusted $p = 0.25$).

A heat-map showing the distributions of the top 8 bacterial phyla present in each subject is shown in **Figure 2**. There were considerable inter-individual differences in the respective composition of the microbiota across the 18 subjects, regardless of the health status or the type of clinical site sampled. For instance, TM7 was present at high abundance in all four clinical sites in subject P3, and Firmicutes dominated the microbiota found in all four clinical sites in subject P4. Subject P17 had relatively lower levels of Firmicutes in all sites, but had considerably higher levels of Proteobacteria.

Bacterial communities within the four respective clinical sites (HI, HT, DI, DT) had similar levels of taxonomic diversity

The quality filtered sequence reads were assigned to 5,726 operational taxonomic units (OTUs) using a sequence similarity cut-off of 97%. Coverage for each sample was estimated at 95.4% ± 2.3%, indicating sufficient sequencing depth to recover the majority of the bacterial taxa in each of the 72 plaque samples (**Figure S1**). The respective sets of HI, DI, HT and DT sites shared 2,901 OTUs in common (**Figure S2**). These OTUs accounted for 97.4 % of total reads, and approximately half of the total number of OTUs detected within the cohort.

Rarefaction curves calculated using three different diversity measures, i.e. the Chao 1 richness estimate, the number of observed OTUs, and the Phylogenetic Diversity (PD) measure, indicated that there were similar levels of taxonomic diversity in the respective HT, DT, HI and DI sites (**Figure S3**). Non-parametric comparisons of the microbiota detected within each of the four clinical site categories were conducted using Kruskal-Wallis H tests on the alpha-diversity Shannon entropy and Simpson indices (data not shown). Both the rarefaction curves and violin plots indicated higher diversities in the diseased sites (DT + DI), compared to the clinically-healthy (unaffected) sites (HT + HI). However, such differences

were not significant after adjustment for multiple comparisons using the Benjamini-Hochberg (BH) method (**Figure S4**).

Identifying the 'core' microbiota present in submucosal/subgingival niches

Since the bacterial communities within the four clinical sites had similar levels of taxonomic diversity and a high number of shared OTUs, we next sought to examine the 'core' microbiota that was present within the majority of samples (**Figure S5**). This was defined by setting the prevalence cut-off at 90% and the overall relative abundances at ≥ 0.1%. This resulted in the identification of 40 'core OTUs', which were agglomerated taxonomically to 31 'core species'. Descriptions for these 31 'core species' are summarized in **Table S2**, and their distributions within the respective subjects and sites are represented in a heat map shown in **Figure 3**. The dominant core species, which we named '*Streptococcus* sp. I mitis', corresponded to six very closely-related OTUs (OTU3, OTU1207, OTU4898, OTU912, OTU1594 and OTU328), with an overall abundance of 9.67% of total reads. These 6 OTUs spanned three formally-classified species belonging to the Mitis group (Jensen, Scholz, & Kilian, 2016) within the genus *Streptococcus* (*Streptococcus mitis, Streptococcus infantis* and *Streptococcus oralis* subsp. *tigurinus*, which corresponded to four different HMTs (HMT-070, HMT-071, HMT-638 and HMT-677).

The second most abundant core species, '*Fusobacterium* sp. I nucleatum' HMT-698/203 (4.0%) was highly prevalent and moderately abundant in the majority of samples. However significant variation amongst individuals were notable in the core microbiota. Furthermore this core microbiota only accounted for an average of 47.6% of reads per sample (range: 11.8–84%).

Beta-diversity comparisons confirm notable interpersonal variations in peri-implant/periodontal microbiota

To further compare the overall bacterial community compositions by different groupings, beta-diversity analyses based on the weighted UniFrac distance were computed, which takes into account both the abundance and the phylogenetic relationships amongst the taxa. The resultant distance matrices were visualized in nMDS ordinations (data not shown) and Permanova statistics were computed to test for the differences across different groups systematically at all taxonomic ranks (**Table S3**). The clustering of microbiota and test statistics were generally consistent at all ranks, hence only the results at the OTU level are reported here. There was no obvious clustering of the microbiota according to the four clinical sites (Permanova R^2=0.051, p = 0.224). The differences between individuals were prominent and contributed to 46% of the variance (p = 0.001). Further comparisons of the microbiota showed that periodontal/peri-implant health status (HI + HT versus DI + DT) and the (m)PI; but neither the PPD nor mBI/GI, contributed to the variance in the microbiota. Interestingly, the composition of the subgingival (HT + DT) and submucosal (HI + DI) microbiota were not statistically different (p = 0.728). Unweighted UniFrac metrics were also calculated to compare the microbiota at the OTU level, which considers the presence/absence of taxa and their phylogenetic relatedness. Only inter-personal factors (i.e. at the level of the individual, and according to gender) were found to be significant (**Table S3**).

Bacterial OTUs (species) associated with periodontal/peri-implant health and disease

Beta-diversity analyses showed that the largest variance in the microbiota was at the subject level (i.e. inter-individual differences; 46%), followed by the periodontal health status (3.6%), but not by the nature of the anatomical site (i.e. subgingival versus submucosal sites; 0.97%, p = 0.728). Next we looked for the bacterial taxa that exhibited significant differences

in their abundances across the groups when controlled for inter-personal variations. Differential abundance analyses were performed using DESeq2, to look for the average effects of health status and anatomical site of sampling across this cohort of patients; under the assumption that the microbial taxa actively involved in disease should be more prevalent and abundant within disease sites compared to corresponding healthy sites, when controlled for differences between individuals.

Thirty OTUs were significantly more abundant in periodontitis (DT) sites compared to the clinically-healthy (unaffected) subgingival (HT) sites within the same individual, in all samples (**Figure 4A**). These 'periodontitis-associated' taxa included several 'commonly-identified' putative periodontopathogens, as well as taxa less commonly associated with periodontal disease (summarised in **Table 3**). This included *Tannerella forsythia*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, four *Treponema* sp., *Filifactor* sp., *Capnocytophaga granulosa*, TM7 [G-1] sp., *Fusobacterium* sp. and *Veillonella* sp. In contrast, considerably fewer bacterial taxa (19 OTUs) were associated with peri-implantitis (**Figure 4B**). Levels of Bacteroidetes and *Prevotella* taxa (including *P. denticola*, *P. multiformis* and *P. fusca*) were notably higher in DI sites. However, there was still considerable overlap, with taxa associated with both peri-implantitis and periodontitis sites, such as *Johnsonella* sp. HMT-166, *Moraxella* sp. HMT-276 and unassigned *Prevotella* sp.

Some bacterial taxa were consistently more abundant in healthy periodontal niches (HT) such as the core taxa *Halomonas* sp. and *Actinomyces* sp. I. There were also *Streptococcus mutans* HMT-686, *Bacillus clausii*, *Bifidobacterium* sp., *Corynebacterium* sp., *Lachnoanaerobaculum orale*, *Lactobacillus* sp., *Prevotella* sp., and Veillonellaceae [G-1] sp. HMT-483. In healthy (unaffected) implant niches (HI), taxa including *Neisseria oralis*, unknown proteobacteria, unknown *Tannerella* sp., unknown *Porphyromonas* sp., *L. orale*, and *Prevotella* sp. were over-represented. Most notably, *L. orale* and *Prevotella* sp. were

associated with both healthy (unaffected) periodontal (HT) and peri-implant niches (HI).

Correlations between species and clinical parameters

Pearson correlation analyses were performed to identify species that showed significant changes in their respective abundances (in a positive or negative manner) that correlated with the BOP, PPD, mBI/GI or (m)PI clinical parameters in the subject group. The results were filtered to exclude the rare and low abundance taxa (i.e. abundance ≥ 0.01%, prevalence ≥ 30% in the cohort), so that the respective significant correlations may infer biological relevance. Results are shown in **Table S4**. *Eggerthia catenaformis* and an unclassified *Mycoplasma* sp. were both positively correlated to BOP and PPD. Two treponeme species, *Treponema parvum* (oral treponeme phylogroup 7) and *Treponema* sp. HMT-490 (oral treponeme phylogroup 4) also showed positive correlations with BOP and PPD, respectively. One unclassified Lachnospiraceae [G-7] sp. was negatively correlated to the (m)PI. There were no species correlated to GI using these criteria.

Correlation network among the microbial species

Network analysis was performed to describe the potential interactions among the microbiota via a SparCC modelled correlation based on their absolute abundances. For clarity, only significant correlations (p <0.05) with the strongest correlation coefficients (after 1000 bootstrap replicates) are shown in **Figures 5 and 6**. Strong positive correlations (i.e. cooperative interactions) were commonly observed among species from the same phylum. The most dominant core species *Streptococcus* HMT-070, *Fusobacterium* HMT-203 and *Veillonella dispar* formed distinctive clusters respectively with species from the same genera in all clinical sites. The strong positive correlation clusters between *Streptococcus* and *Veillonella*; and among the Proteobacterial species (*Halomonas*, *Sphingomonas*,

Pseudomonas, *Klebsiella*, *Staphylococcus*, Caulobacteraceae, Enterobacteriaceae) were well conserved in healthy (unaffected) and in diseased sites. Comparing the bacterial interaction networks between health and disease, several dysbiosis patterns were apparent in both periodontitis and peri-implantitis sites. These included reduced absolute abundance of *Streptococcus* species and enrichment of several 'periodontopathogens' such as *Porphyromonas*, *Tannerella*, *Fretibacterium* spp. and *Treponema* spp. In addition, *Filifactor alocis*, Dethiosulfovibrionaceae TG5 sp., *Fretibacterium* spp. and *Treponema* spp. formed more numerous, and more tightly clustered networks in DT and DI sites (**Figures 5** and **6**).

Discussion

Here, the subgingival and submucosal microbiota of clinically-healthy (unaffected) and diseased periodontal/peri-implant sites were systematically compared within a cohort of individuals (n = 18) presenting with both periodontitis and peri-implantitis. Specifically, the microbiota of clinically-diseased and clinically-healthy peri-implant sites, as well as clinically-diseased and clinically-healthy periodontal sites were characterized in each subject. Thus, we have intra-oral control sites for both periodontitis and peri-implantitis within each subject. To the best of our knowledge, this is the first time such a cohort has been subjected to high-throughput DNA sequencing-based microbiome investigation.

In general, the microbiota from the four distinct clinical sites (HT, HI, DT, DI) had similar alpha-diversities; i.e. they had similar levels of OTU (species) diversity at the site-level. In addition, the relative proportions of bacterial phyla detected were fairly similar within the four respective clinical sites; with taxa belonging to Firmicutes, Bacteroidetes, Fusobacteria and Proteobacteria dominating the communities. The 31 core species identified in this cohort (**Figure 3**) share considerable similarity with those previously identified as forming the 'core microbiome' in submucosal plaque sampled from both diseased (peri-implantitis) as well as clinically healthy implant niches in a Swiss cohort (Sanz-Martin et al., 2017). These were classified according to the HOMINGS methodology (Belstrøm et al., 2016). Matching our core OTUs with those identified by Sanz-Martin *et al*., this most notably includes *Streptococcus oralis* HMT 070 ('Streptococcus Genus Probe 4'), *Fusobacterium* HMT 203 ('Fusobacterium Genus Probe 4'), *Veillonella dispar*, and *Fusobacterium nucleatum* subsp. *vincentii* ('Fusobacterium Genus Probe 2'). Our data also correlates with that reported by Maruyama et al. (2014), who found that *F. nucleatum*, *S. oralis* and *Neisseria subflava* were abundant in both periodontitis and peri-implantitis lesions sampled from the same Japanese individuals. The respective compositions of the 'core' microbiota reported in

several other high throughput sequencing-based investigations of periodontal and peri-implant diseases also share high levels of overlap with that described here, e.g. (Zheng et al., 2015; Shiba et al., 2016; Tsigarida, Dabdoub, Nagaraja, & Kumar, 2015). We speculate that these similarities reflect the fundamental roles that these species play within subgingival/sub-mucosal biofilms, which may or may not be related to pathogenic processes.

In concordance with previous microbiome studies (Integrative HMP (iHMP) Research Network Consortium (2014); Lloyd-Price, Abu-Ali, & Huttenhower, 2016; Maruyama et al., 2014), we observed high levels of interpersonal variation in the subgingival/submucosal microbiota; i.e. the 18 subjects had notably distinct subgingival/submucosal community compositions. In fact, interpersonal variations made a larger contribution to the overall microbiota composition compared to the health status of the periodontal/peri-implant site. Furthermore, the nature of the anatomical niche (i.e. dental implant versus natural tooth) was not a major factor. This observation is in line with other studies that have examined intra-personal variations in microbiota present in periodontal and peri-implant niches (Payne et al., 2017; Schincaglia et al., 2017; Schwarzberg et al, 2014). It is conceivable that the distinct differences amongst individuals could result from a combination of behavioural, environmental, and biological factors, such as dietary and oral hygiene habits, anatomical characteristics, amount and content of saliva and crevicular fluid secretion, hormonal and immunological conditions (Marsh & Zaura, 2017; Mira et al., 2017). Further detailed studies are warranted to identify how these various factors/parameters respectively interplay with one another to affect the composition of subgingival and submucosal microbial communities within individual clinically-diseased and clinically-unaffected sites, in subjects with periodontitis and peri-implantitis.

The significant levels of inter-personal variation in oral microbial composition necessitate careful interpretation and robust testing, when clinical niches are compared

between individuals, especially for smaller cohorts. Therefore, in this study, equivalent pairs of diseased and healthy (unaffected) implant and tooth sites were compared within each individual, and were corrected for multiple comparisons, in order to examine the differences in the microbiota associated with periodontitis and peri-implantitis. Our findings revealed that a number of bacterial taxa were associated with diseased and healthy implant and tooth sites, respectively (**Table 3, Figure 4**). Some of these suspect 'bad-actors' have previously been identified as probable or putative 'periodontopathogens', implicated in the pathogenesis of both peri-implantitis and periodontitis. These include *P. gingivalis*, *P. endodontalis*, *Prevotella* sp., *Selenomonas* sp., *Filifactor* sp., *T. forsythia*, TM7 [G1], *Treponema* spp. (Lafaurie et al., 2017; Maruyama et al., 2014; Persson & Renvert, 2014; Zheng et al., 2015; Sanz-Martin et al., 2017). The identification of other periodontitis-associated taxa such as *C. granulosa* and peri-implantitis associated taxa such as *P. intermedia* are consistent with results from previous studies (Al-Radha, Pal, Pettemerides, & Jenkinson, 2012; Apatzidou et al., 2017; Zheng et al., 2015).

Several health-associated commensal taxa were also identified. These included *L. orale*, which was originally isolated from saliva of a healthy individual (Hedberg et al., 2012). This and related *Lachnoanaerobaculum* taxa have previously been positively associated with smokers (Duan et al, 2017), but also with healthy implant sites in smokers (Tsigarida et al, 2015). There were also 'probiotic' *B. clausii, Bifidobacterium* and *Lactobacillus* species, which have previously been shown to exhibit higher gene expression levels in periodontally-healthy individuals compared to those with periodontitis (Duran-Pinedo et al., 2014). Several gamma- and beta-proteobacteria were also positively associated with disease-free sites, consistent with other studies (Chen et al, 2018; Apatzidou et al., 2017). Network analysis revealed that many of these taxa, and many others, exhibited positive correlations with other putative health-associated taxa, and formed negative

correlations with putative disease-associated taxa (see below).

Results from weighted UniFrac analyses indicated significant differences in bacterial community composition were associated with the both disease status of the site as well as interpersonal factors (**Table S3**). Weighted UniFrac metrics take into account the abundance of each taxon and thus emphasize contributions made by the more abundant taxa. In contrast, when unweighted UniFrac metrics were used, only the interpersonal factors had significant effects. This suggests that dysbiosis in the periodontal/peri-implant niches analyzed involved significant changes in the abundance of predominant bacterial taxa, whereas the inter-personal variations in community composition were largely derived from the presence/absence of certain taxa.

E. catenaformis, and an unclassified *Mycoplasma* sp. were both positively correlated with BOP and PPD (**Table S4**). Little is currently known about the role of their role in oral health or disease. *E. catenaformis* has been identified in dental abscesses and has been associated with bacteremia (Kordjian, Schultz, Rosenvinge, Møller, & Pedersen, 2015). A draft genome sequence has been published recently (Rahman, Mullany, & Roberts, 2017), which carries genes known to be associated with both virulence and antibiotic resistance. Mycoplasmas have been positively associated with periodontitis, and with increased PPD (Kwek, Wilson, & Newman, 1990). Taxa from this genus have also been reported to form positive correlations with *Treponema* and *Fusobacteria* sp. in both healthy and diseased subgingival plaque samples (Chen et al, 2018), as well as with *Tannerella* and Synergistetes taxa in periodontitis sites (Shi et al, 2015). *Treponema parvum*, which was originally isolated from periodontitis and necrotizing ulcerative gingivitis lesions (Wyss et al, 2001), was associated with BOP here, and was recently associated with maximum pocket depth in peri-implantitis sites (Kröger et al., 2018).

For complex polymicrobial inflammatory diseases such as periodontitis and peri-

implantitis, it is difficult to define or differentiate commensals and pathobionts (indigenous commensals that exploit disruptions in host homeostasis, and flourish during disease onset and promote inflammation in disease development) (Hajishengallis & Lamont, 2016). The bacterial occurrence network analysis shown here (**Figures 5 and 6**) revealed a conserved pattern of dysbiosis within periodontitis and peri-implantitis niches that commonly involved higher abundances of, and/or positive co-correlations between: *Prevotella spp.*, *Treponema*, *Porphyromonas, Tannerella, Filifactor, Parvimonas, Desulfobulbus,* and Synergistetes taxa; consistent with findings from other microbiome studies (Apatzidou et al., 2017; Lafaurie et al., 2017; Park et al., 2015; Schincaglia et al., 2017; Shiba et al., 2016; Shi et al., 2015; Kröger et al, 2018; Chen et al., 2018). Several other taxa appear to play important etiological roles, including the as-yet uncultivated Peptostreptococcaceae [XI] and Bacteroidetes [G-5] phylotypes. Many of the correlations were conserved in health as well as disease, which may be indicative of biological (e.g. nutritional) co-dependencies (Vartoukian et al., 2016). These networks are consistent with known bacterial inter-species interactions, such as the well-established positive correlations of *Streptococcus* with *Veillonella* (Chalmers, Palmer, Cisar, & Kolenbrander, 2008; Egland, Palmer, & Kolenbrander, 2004; Kolenbrander et al., 2002).

Taxa within the Synergistetes phylum, including *Fretibacterium fastidiosum*, *Fretibacterium* sp. HMT 361 and 362, and Dethiosulfovibrionaceae TG5 sp., had an extensive range of positive correlations in periodontitis and peri-implantitis sites (**Figures 5B** and **6B**). There is a growing body of evidence associating certain Synergistetes taxa with periodontitis and peri-implantitis, especially *F. fastidiosum* and other 'cluster A' taxa (Vartoukian, Palmer, & Wade, 2009; Griffen et al, 2012; Belibasakis et al, 2013; Marchesan et al, 2015; Belibasakis, Öztürk, Emingil, & Bostanci, 2013; Belibasakis et al, 2016; Oliveira et al, 2016; Apidzidou et al, 2017). With the exception of *F. fastidiosum*, which is an asaccharolytic, obligate anaerobe that exhibits a nutritional dependence on *Fusobacterium*

nucleatum, very little is known about their cellular physiology (Vartoukian, Downes, Palmer, & Wade, 2013). We, speculate that the positive correlations between these oral Synergistetes taxa reflect the fact that they thrive within similar ecological environments, and may share similar metabolic co-dependencies.

The negative correlations apparent in **Figures 5** and **6**, as well as the results summarized in **Table 3** and **Figure 4**, support the hypothesis that certain taxa belonging to the genera *Rothia*, *Veillonella*, *Actinomyces*, *Corynebacterium*, *Streptococcus*, *Neisseria*, *Kingella*, *Leptotrichia*, and *Fusobacterium* (amongst others) may play active roles in maintaining periodontal and peri-implant health and homeostasis. This is consistent with the consensus findings previous reported in analogous investigations (Kröger et al, 2018; Chen et al., 2018; Shi et al., 2015; Sanz-Martin et al., 2017; Tsigarida et al., 2015; Zheng et al., 2015).

In the present study, it should be noted that the clinically-healthy sites refer to clinically asymptomatic sites within the oral cavities of patients that have both periodontal and peri-implant lesions at other non-adjacent locations. Thus, it is strongly suspected that a substantial proportion of these sites may not contain a resilient, 'health-associated' microbiota. It is speculated that some (possibly many) of these unaffected or clinically-healthy sites may contain microbial communities in a 'pre-dysbiotic' state (Hajishengallis & Lamont, 2016). Specifically, these niches may be depleted for beneficial community-stabilizing taxa, or may contain elevated levels of pathobionts that disproportionately disrupt normal homeostatic balance, promoting the development of periodontitis and/or peri-implantitis. Thus, the HI and HT sites should certainly not be viewed as being of 'pristine' periodontal health.

As noted in the introduction, this cohort has been studied previously (Zhuang et al., 2014; Yu et al., 2016). Consequently, this restricted the number of subjects that could be included in the present microbiome analysis (n = 18), which may be considered a notable

limitation of this investigation. Thus, the results and conclusions presented here should be viewed in light of our modest cohort size. In the first study, the quantity and prevalence of six (periodonto)-pathogens in the same cohort was examined using quantitative PCR (qPCR) targeting *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *F. nucleatum*, *P. intermedia*, and *Staphylococcus aureus* (Zhuang et al., 2014). The MiSeq sequencing of the microbiota described here revealed lower detection frequencies for all six taxa in the same cohort of samples when compared to qPCR assays. In addition, the MiSeq data reveals higher levels of interpersonal variation compared to the data previously obtained by qPCR quantification.

In the second study, a PCR-clone library sequencing-based approach targeting the 16S rRNA genes of Synergistetes taxa was employed to study their distributions within the cohort (Yu et al., 2016). Comparing the datasets, these two studies recovered many Synergistetes genotypes in common, including *F. fastidiosum*, *Fretibacterium* HMTs-360, 361 and 362; *Pyramidobacter piscolens* and *Jonquetella anthropi*. However, as would be expected, the higher sensitivity and broader coverage of the MiSeq analysis reported here identified several additional Synergistetes phylotypes such as Dethiosulfovibrionaceae TG5 sp. and Synergistaceae Candidatus *Tammella* sp.

In conclusion, the data of the present analysis suggest that within this clinically-distinctive cohort of periodontitis and peri-implantitis subjects, the composition of the submucosal and subgingival microbiota are reasonably similar. A common set of core oral microbiota was found consistently across individuals, which included *Streptococcus*, *Fusobacterium*, *Veillonella* and several other species. Whilst certain bacterial OTUs were correlated with clinical parameters related to peri-implant/periodontal health or disease, it should be carefully-noted that the bacterial communities resident within each of the respective subjects exhibited high levels of compositional variation.

Acknowledgements

Technical support from Mr. Raymond Tong of the Central Research Laboratories of the Faculty of Dentistry is gratefully acknowledged. Financial support from the Research Grants Council of Hong Kong, via General Research Fund Grant #17105115 is also acknowledged by RMW.

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Tables

Table 1. Summary of clinical parameters for each clinical site category within the subject group.

Clinically-healthy (unaffected) peri-implant site (HI); Peri-implantitis site (DI); Clinically-healthy (unaffected) subgingival tooth site (HT); Periodontitis site (DT).

Clinical parameters	**HI**	**DI**	**HT**	**DT**
Location of sampled site				
Maxillary anterior	3	1	8	2
Mandibular anterior	0	1	1	7
Maxillary posterior	8	4	4	7
Mandibular posterior	7	12	5	2
PPD (in mm, mean ± SD)	3.0 ± 0.7	8.1 ± 1.6	2.2 ± 0.7	5.4 ± 1.1
(m)PI (mean ± SD)	0.6 ± 0.8	2.1 ± 0.8	1.1 ± 0.9	2.6 ± 0.5
mBI/GI (mean ± SD)	0.2 ± 0.4	2.4 ± 0.5	0.6 ± 0.5	2.6 ± 0.5

Table 2. Relative abundances of bacterial phyla across the four clinical sites

Phylum	Total (%)	DI (%)	DT (%)	HI (%)	HT (%)
Acidobacteria	0.01	0.01	0.01	0.01	0.02
Actinobacteria	**5.18**	**2.87**	**6.44**	**5.92**	**5.5**
Aquificae	< 0.01	0	0	< 0.01	< 0.01
Bacteroidetes	**14.31**	**16.38**	**18.49**	**8.7**	**13.66**
Chlamydiae	< 0.01	< 0.01	0	0	< 0.01
Chloroflexi	0.07	0.14	0.07	0.06	0.02
Cyanobacteria	0.04	0.02	0.02	0.05	0.07
Elusimicrobia	0.03	0.07	0.06	< 0.01	0
Firmicutes	**40.37**	**41.93**	**34.54**	**49.31**	**35.71**
Fusobacteria	**14.95**	**13.56**	**16.47**	**12.6**	**17.17**
GN02 (Gracilibacteria)	0.01	< 0.01	0.02	0.02	0.01
Gemmatimonadetes	< 0.01	< 0.01	0	< 0.01	< 0.01
Nitrospirae	< 0.01	0	0	< 0.01	< 0.01
OD1 (Parcubacteria)	0.01	0.01	< 0.01	0.01	0.03
Planctomycetes	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Proteobacteria	**17.66**	**15.11**	**15.27**	**18.23**	**22.03**
SR1(Absconditabacteria)	0.05	0.12	0.06	0.01	0.02
Spirochaetes	**2.31**	**4.14**	**2.83**	**1.27**	**1.03**
Synergistetes	**2.47**	**2.26**	**2.1**	**2.16**	**3.37**
TM6 (Candidatus Dependentiae)	< 0.01	0	0	< 0.01	0
TM7 (Saccharibacteria)	**2.35**	**3.25**	**3.55**	**1.41**	**1.18**
Tenericutes	0.01	0.01	0.01	< 0.01	< 0.01
Verrucomicrobia	< 0.01	0	0	< 0.01	< 0.01
WPS-2	< 0.01	< 0.01	0	0	< 0.01
WS6 (Candidatus Dojkabacteria)	0.01	0.02	< 0.01	0.01	0.01
Thermi (Deinococcus-Thermus)	0.14	0.1	0.07	0.23	0.17

N.B. The 8 most abundant phyla are indicated with **bold text**

Table 3. Bacterial taxa present at higher abundances in the four respective clinical sites*

Clinically-healthy (unaffected) periodontal sites (HT)	Periodontitis sites (DT)
Halomonas sp. (OTU0) (core) *Escherichia coli* HMT-574 (OTU17) *Streptococcus mutans* HMT-686 (OTU27) *Veillonella dispar* HMT-160 (OTU1252) *Actinomyces* sp. I (OTU37) (core) *Klebsiella pneumoniae* (OTU25) *Kluyvera ascorbata* HMT-865 (OTU56) *Staphylococcus* sp. HMT-116/601/567 (OTU101) *Cutibacterium acnes* HMT-530 (OTU103) *Prevotella bivia* HMT-556 (OTU119) *Sphingomonas echinoides* HMT-003 (OTU152) *Ochrobactrum anthropi* HMT-544 (OTU142) *Pseudomonas aeruginosa* HMT-536 (OTU184) *Lactobacillus sp.* HMT-051/938/709/882 (OTU297) Bifidobacteriaceae [G-2] sp. HMT 407 (OTU313) *Paracoccus yeei* HMT-104 (OTU292) *Bacillus clausii* (OTU342) *Corynebacterium* sp. (OTU573) *Prevotella* sp. (OTU386) *Lachnoanaerobaculum orale* (OTU398) Veillonellaceae [G-1] sp. HMT-483 (OTU3298) *Prevotella* sp. (OTU2888)	*Tannerella forsythia* HMT-613 (OTU15) *Cardiobacterium valvalum* HMT-540 (OTU33) *Capnocytophaga granulosa* (OTU32) Bacteroidales [G-2] sp. HMT-274 (OTU43) *Johnsonella* sp. HMT-166 (OTU88) *Cardiobacterium hominis* HMT-633 (OTU125) Saccharibacteria (TM7) [G-1] HMT-488 (OTU94) Tissierellaceae ph2 sp. (OTU133, OTU1189) *Fusobacterium* sp. (OTU1278) *Selenomonas* sp. HMT-138/892/146 (OTU1368) Bacteroidales [G-2] sp. (OTU439) *Prevotella* sp. *dentalis* HMT-583 (OTU1857) *Treponema* sp. III HMT-254 (OTU245) *Treponema* sp. I HMT-242 (OTU3603, 1931, 3236) *Treponema* sp. III HMT-508 (OTU321) *Porphyromonas gingivalis* (OTU348) *Prevotella* sp. (OTU798) Bacteroidales BS11 sp. (OTU3132, OTU789) *Filifactor* sp. (OTU367) *Tannerella forsythia* (OTU611) *Porphyromonas endodontalis* (OTU434) *Selenomonas* sp. (OTU1240) *Moraxella* sp. HMT 276 (OTU597) Dethiosulfovibrionaceae TG5 sp. (OTU499) *Veillonella* sp. (OTU2105) Bacteroidetes [G-5] sp. (OTU799)
Clinically-healthy (unaffected) implant sites (HI)	**Peri-implantitis sites (DI)**
Streptococcus sp. III salivarius (OTU9) (core) *Leptotrichia* sp. HMT-219 (OTU113) *Prevotella* sp. (OTU386, OTU803) *Porphyromonas* sp. HMT 278/277 (OTU5580) *Tannerella* sp. (OTU1337) *Selenomonas* sp. (OTU433) *Actinobaculum* sp. (OTU561) *Lachnoanaerobaculum orale* (OTU398) Tissierellaceae ph2 sp. (OTU660) *Cardiobacterium* sp. (OTU2814) Unknown Proteobacteria sp. (OTU1746) Bacteroidales BS11 sp. (OTU3132) *Neisseria oralis* (OTU1564)	Bacteroidetes [G-5] sp. HMT-511 (OTU74) *Johnsonella* sp. HMT-166 (OTU88) Bacteroidetes [G-5] sp. (OTU222, 310, 382, 508) *Prevotella multiformis /denticola* HMT-685/291 (OTU1707) *Prevotella* sp. (OTU1675, 798, 369, 693, 426, 601) *Prevotella fusca* HMT-782 (OTU370) Peptostreptococcaceae [XI] sp. (OTU347) Bacteroidales [G-2] sp. (OTU395) Bacteroidales sp. (OTU558) *Moraxella* sp. HMT-276 (OTU597) *Mogibacterium* sp. (OTU615)

* Taxa are listed in order of their mean base relative abundance within each of the four

clinical sites (i.e. highest % read numbers at the top). 'Core' indicates the respective OTU corresponds to one of the 'core species' in the subject group.

Figure Legends

Figure 1. Relative abundances of bacterial phyla grouped by the four clinical site categories.

Panel A (upper) shows the proportions of the bacterial phyla present within the four clinical sites (DI, DT, HI HT); **Panel B** (lower) shows the proportions of phyla present within the clinically-diseased sites (DI + DT); clinically-healthy (unaffected) sites (HI + HT) dental implant sites (DI + HI) and the natural tooth sites (DT + HT) sampled. Graphs show the mean relative abundances of each phylum, which were calculated from all the samples within the respective categories. Only the top 8 phyla are shown (with >1% relative abundance).

Figure 2. Heatmap showing the relative abundances of the top 8 phyla within each subject.

Heatmap showing the relative abundance of the bacterial phyla identified within the 4 respective sites, in each of the 18 subjects. Subjects are numbered 1–18 on the *x*-axis, each divided into 4 columns representing the 4 tooth/implant sites (in the order DI, DT, HI, HT). The phyla are arranged in ascending order according to their respective abundances in all samples, on the *y*-axis. The total number of reads obtained for each phylum (in thousands), as well as the corresponding percentage of total reads obtained for the entire subject group, is shown in a bar chart format to the right of the heatmap. The relative abundance of the 8 phyla in each sample (reported as a percentage of the total number of reads for that sample) is shown above the heatmap.

Figure 3. Heatmap showing the distributions of the 31 'core' species within the subject group.

The heatmap summarizes the relative abundance of the 31 'core' bacterial species within each of the 72 submucosal/subgingival sites samples, within each site in each of the 18 subjects. The x-axis shows the samples arranged into 4 blocks that correspond to the 4 respective clinical sites (DI, DT, HI and HT). Each block contains the corresponding sample from each of the 18 subjects, in the order 1-18. The 31core species are arranged in ascending order on the *y*-axis, according to their respective relative abundances at the cohort level.

Figure 4. OTUs that have significantly-different abundances within diseased versus healthy periodontal and peri-implant sites.

Each plot summarizes the OTUs that have a statistically significant difference in their absolute abundances within healthy versus diseased periodontal and peri-implant sites [reported as a log2−fold (LFC) change; independent hypothesis weighting-filtered, $\alpha < 0.05$]. Each bar represents an individual OTU, which is identified at the species level and is colored according to its phylum. Positive LFC values refer to a higher abundance in the healthy site. **Panel A**: OTUs that have significantly different abundances within clinically-healthy (unaffected) periodontal sites (HT) versus periodontitis sites (DT). **Panel B**. OTUs that have significantly different abundances within clinically-healthy (unaffected) implant sites (HI) versus peri-implantitis sites (DI).

Figure 5. Bacterial interaction networks within the tooth sites (HT, DT).

Species correlation networks were constructed based on SparCC models. The sizes of the nodes (represented by filled circles) are proportional to the absolute abundance of the

bacterial species in question, which are colored according to phylum. A green line connecting two nodes represent a positive correlation (i.e. cooperation) between the respective pairs of species, whereas a red line represents a negative correlation (i.e. competition). Only the top 100 significant correlations are shown, which are sorted by absolute correlation coefficient values. **Panel A**: Interaction networks between species in the HT (clinically-healthy, or unaffected subgingival) sites. **Panel B**: Interaction networks between species in the DT (periodontitis) sites.

Figure 6. Bacterial interaction networks within the implant sites (HI, DI).

Panel A: Interaction networks between species in the HI (clinically-healthy, or unaffected implant) sites. **Panel B**: Interaction networks between species in the DI (peri-implantitis) sites. See Figure 5 legend for explanatory details.

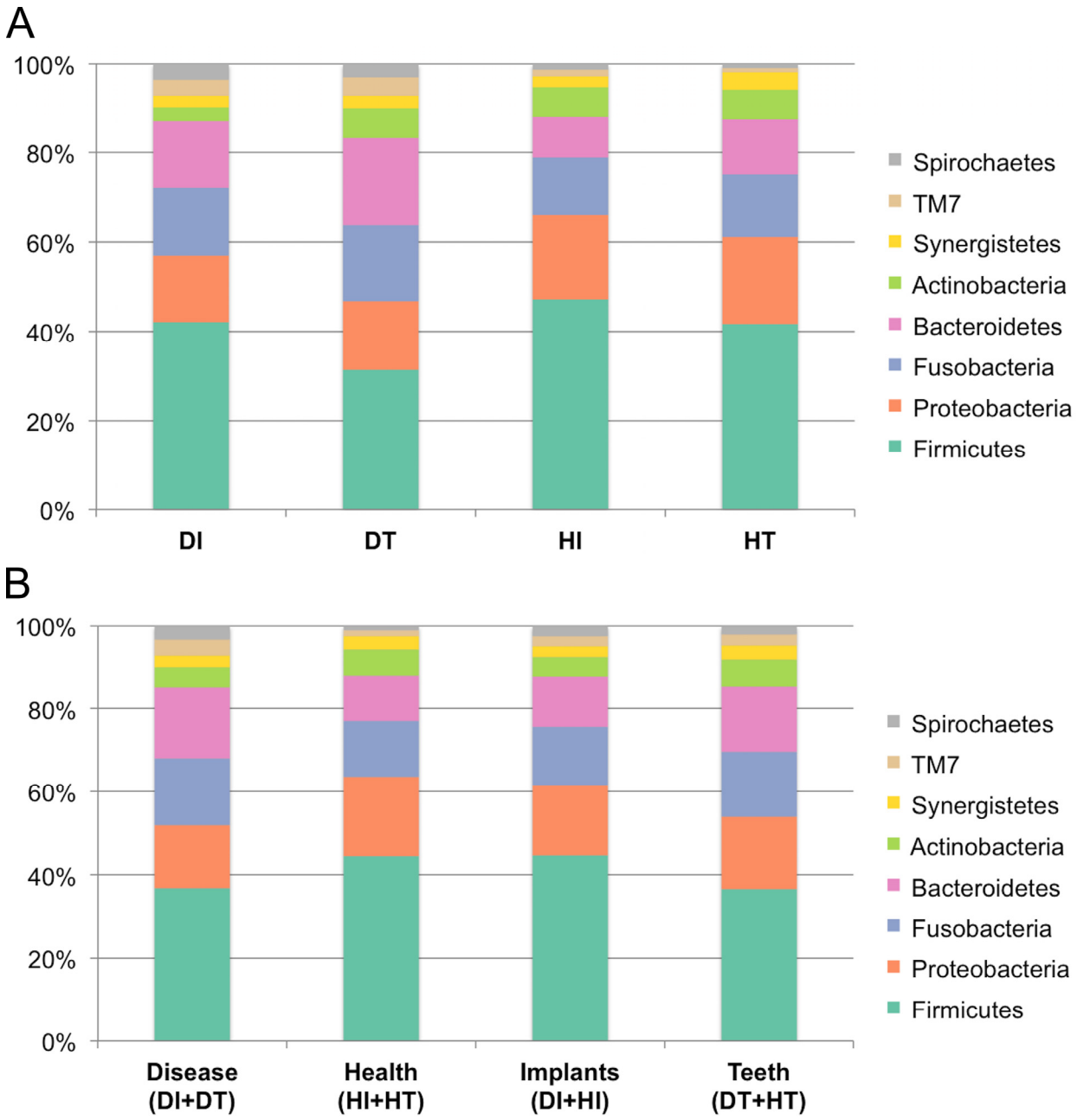


Figure 1. Relative abundances of bacterial phyla grouped by the four clinical site categories.

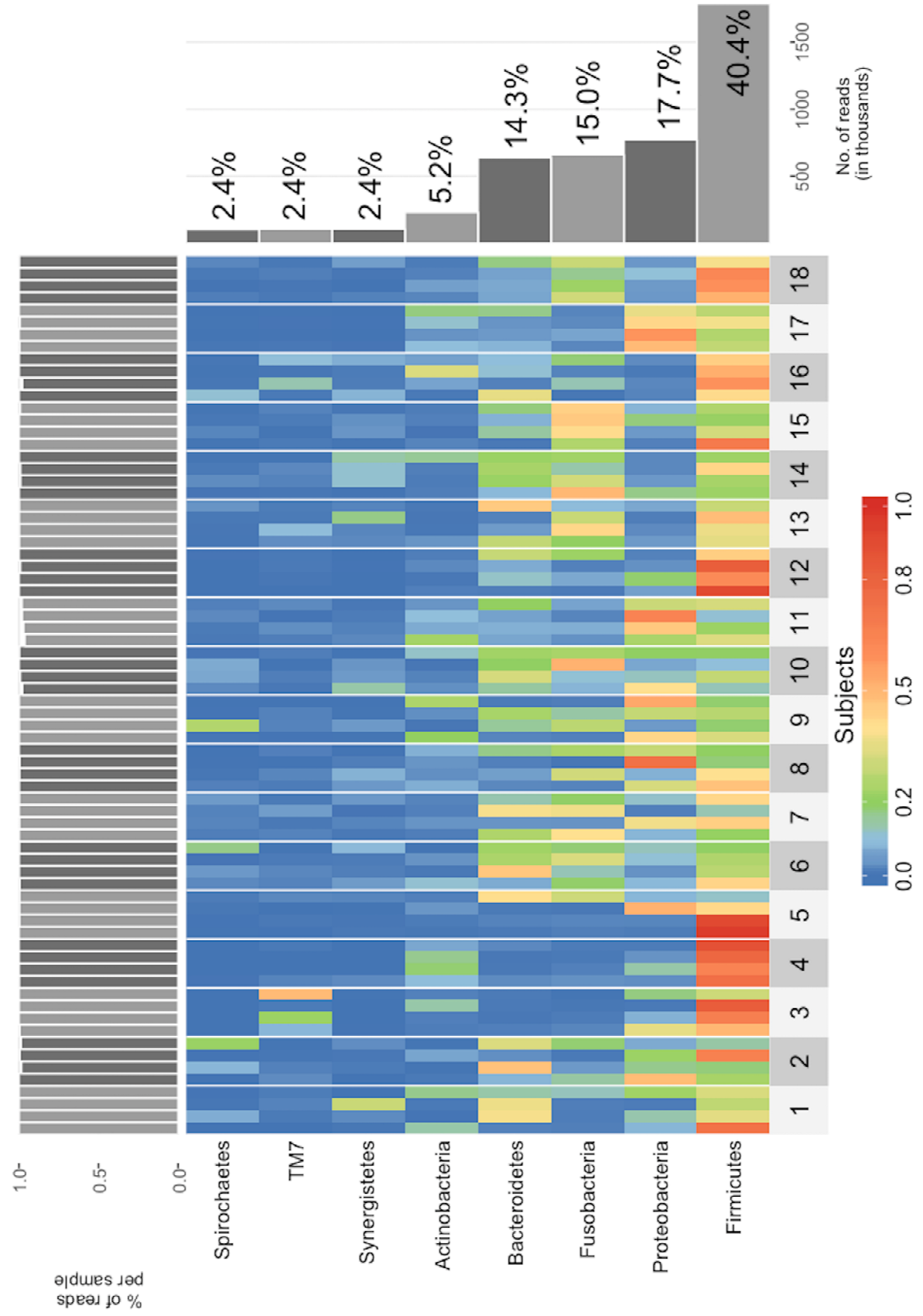


Figure 2. Heatmap showing the relative abundances of the top 8 phyla within each subject.

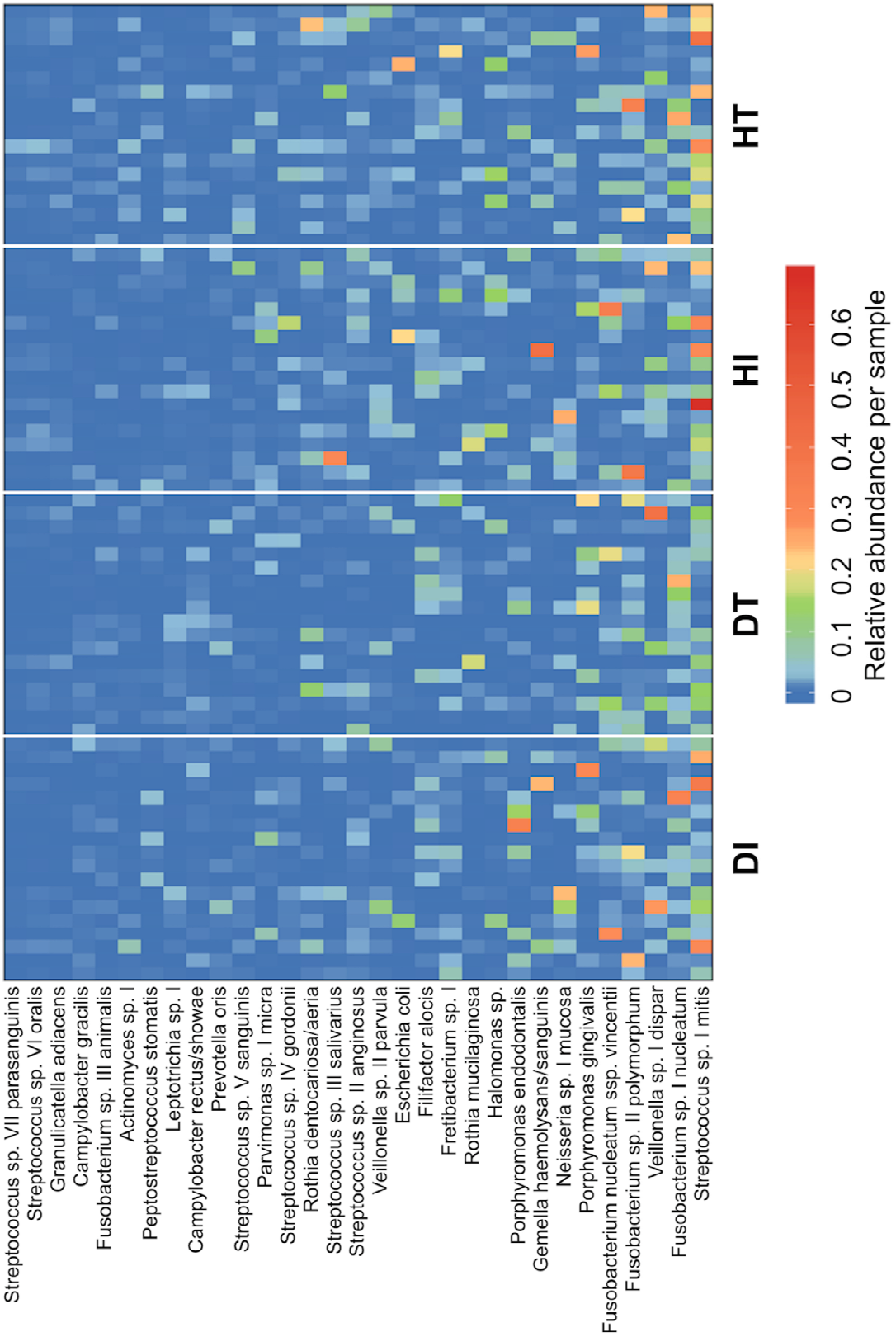


Figure 3. Heatmap showing the distributions of the 31 'core' species within the subject group.

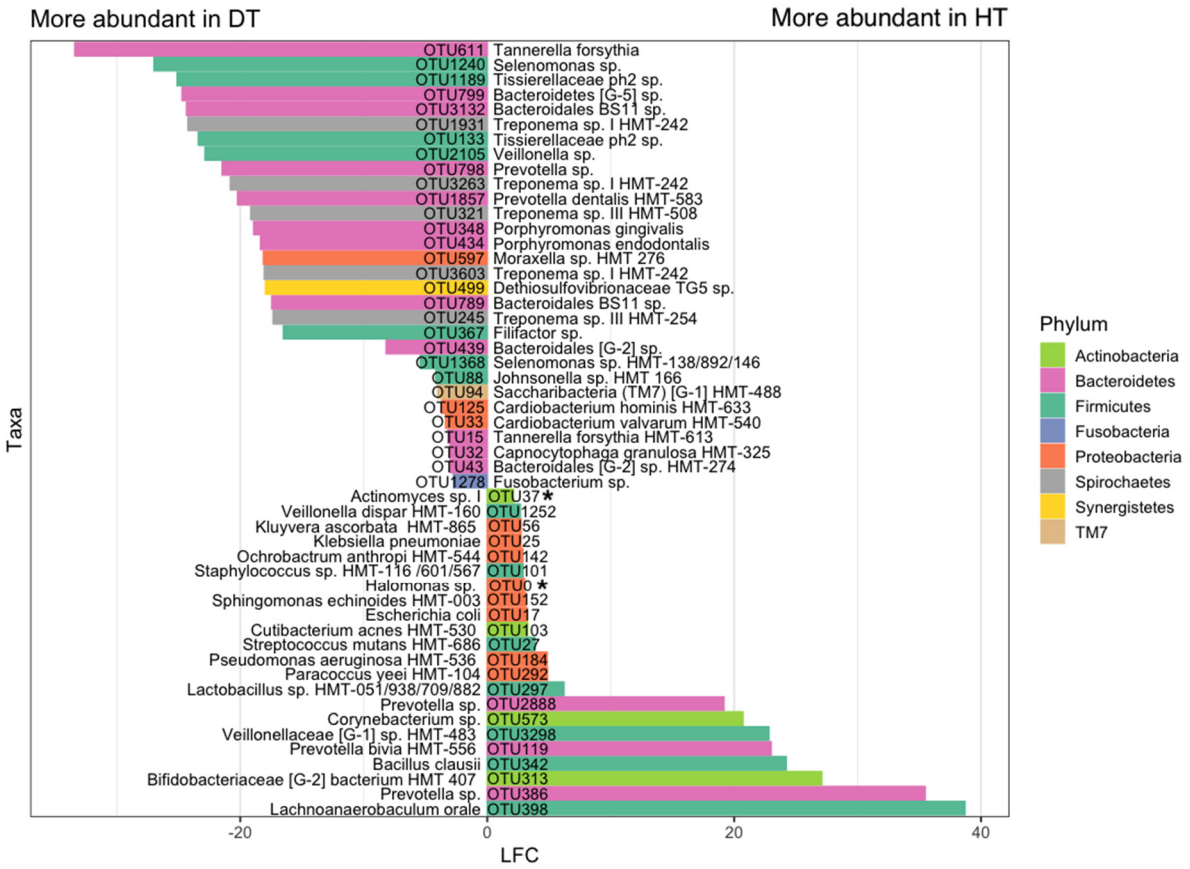
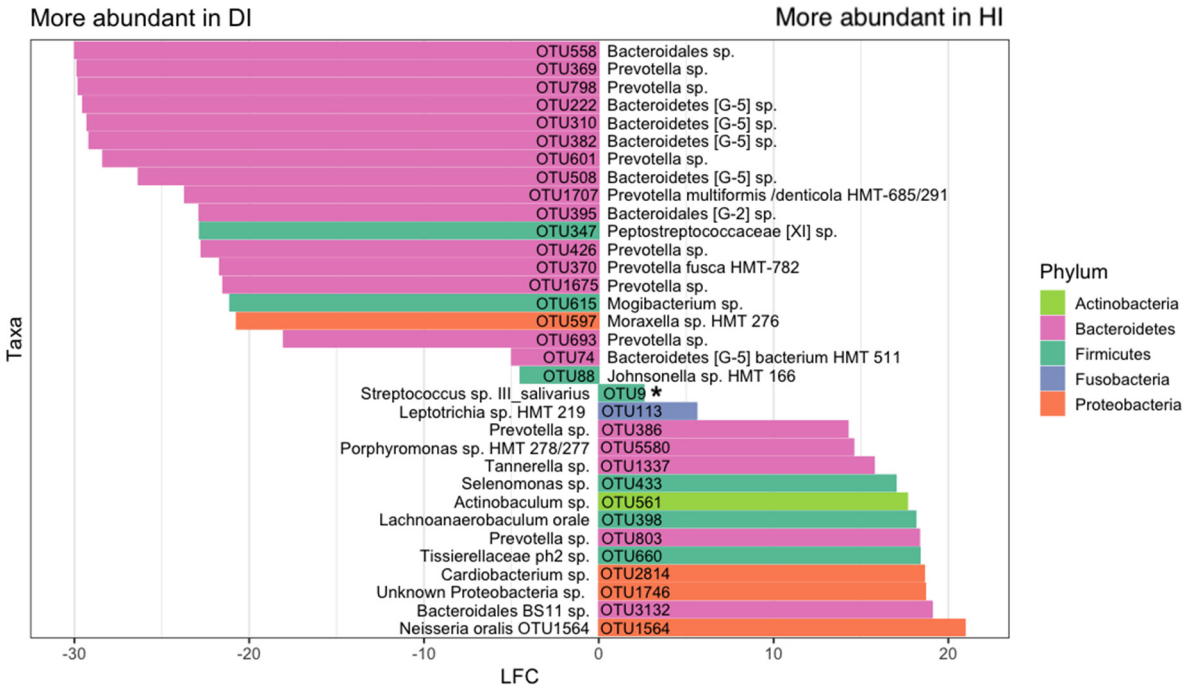


Figure 4. OTUs that have significantly-different abundances within diseased versus healthy periodontal and peri-implant sites.

Panel A: OTUs that have significantly different abundances within clinically-healthy (unaffected) periodontal sites (HT) versus periodontitis sites (DT).



Panel B. OTUs that have significantly different abundances within clinically-healthy (unaffected) implant sites (HI) versus peri-implantitis sites (DI).

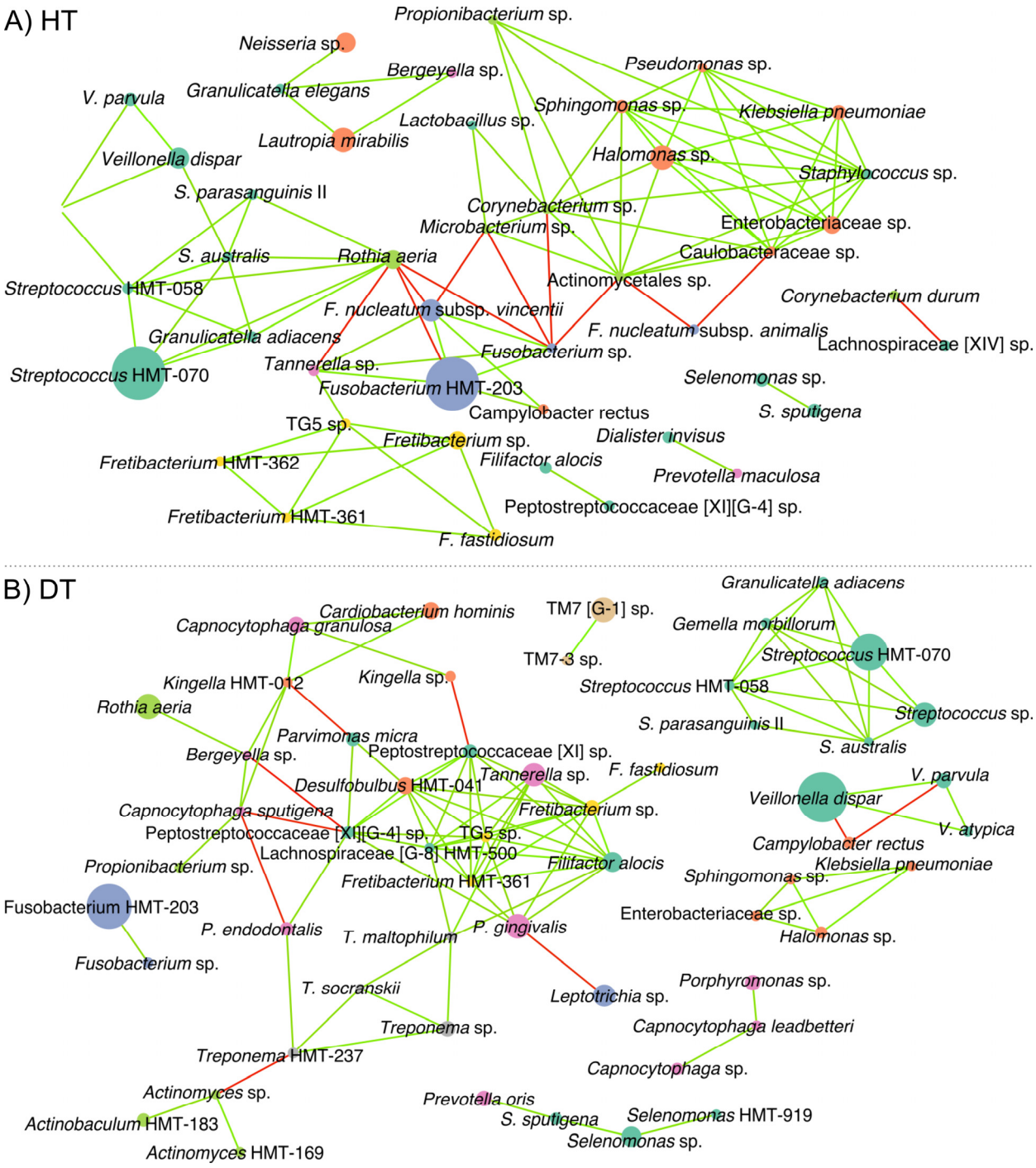


Figure 5. Bacterial interaction networks within the tooth sites (HT, DT).

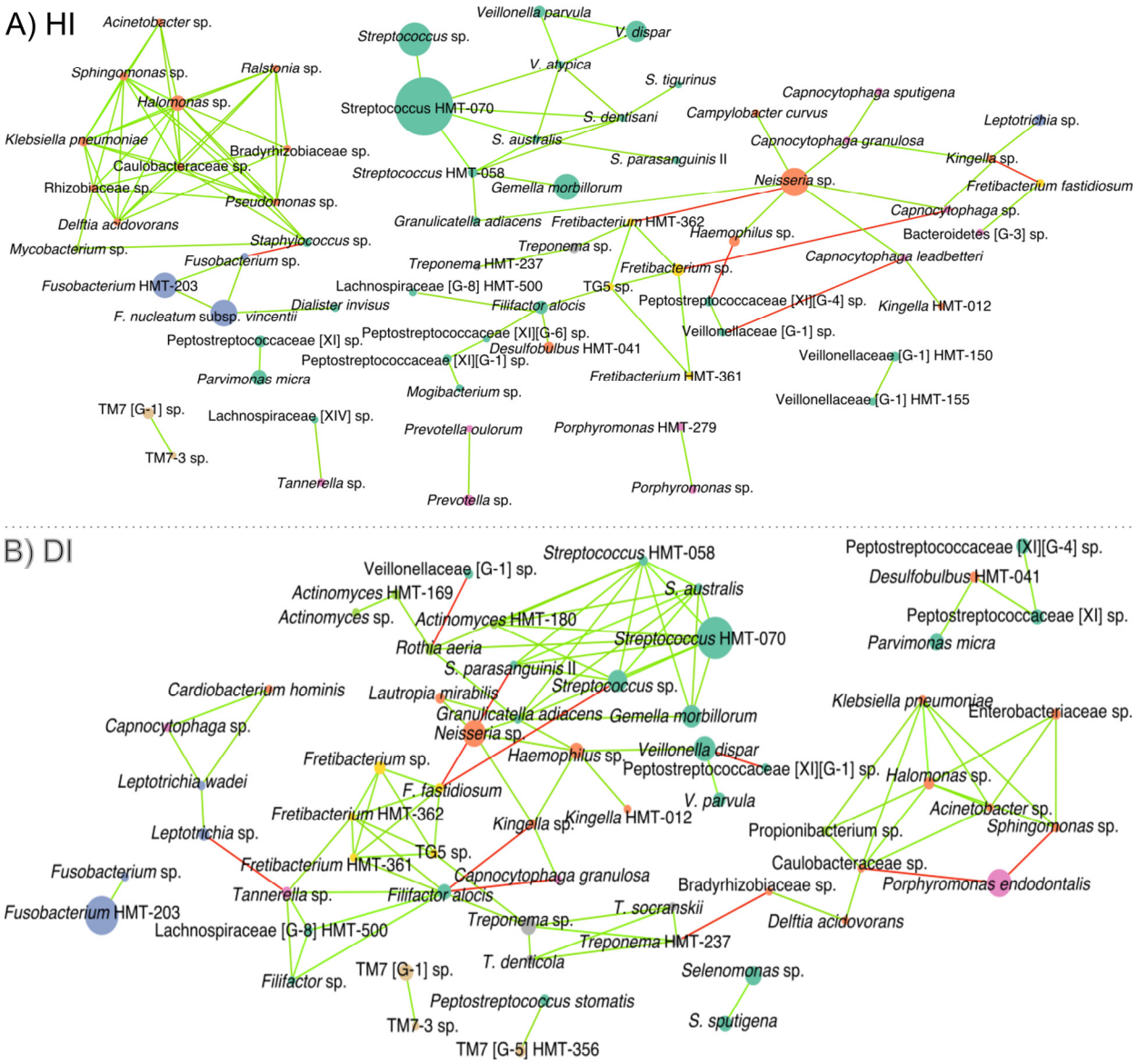


Figure 6. Bacterial interaction networks within the implant sites (HI, DI).