# UNIVERSITY<sup>OF</sup> BIRMINGHAM University of Birmingham Research at Birmingham

# The severity of human peri-implantitis lesions correlates with the level of submucosal microbial dysbiosis

Kroeger, Annika; Hülsmann, Claudia; Fickl, Stefan; Spinell, Thomas; Huttig, Fabian; Kaufmann, Frederic; Heimbach, Andre; Hoffmann, Per; Enkling, Norbert; Renvert, Stefan; Schwarz, Frank; Demmer, Ryan T; Papapanou, Panos N; Jepsen, Soren; Kebschull, Moritz DOI:

10.1111/jcpe.13023

License: None: All rights reserved

Document Version Peer reviewed version

Citation for published version (Harvard):

Kroeger, A, Hülsmann, C, Fickl, S, Spinell, T, Huttig, F, Kaufmann, F, Heimbach, A, Hoffmann, P, Enkling, N, Renvert, S, Schwarz, F, Demmer, RT, Papapanou, PN, Jepsen, S & Kebschull, M 2018, 'The severity of human peri-implantitis lesions correlates with the level of submucosal microbial dysbiosis', *Journal of Clinical Periodontology*, vol. 45, no. 12, pp. 1498-1509. https://doi.org/10.1111/jcpe.13023

Link to publication on Research at Birmingham portal

#### Publisher Rights Statement: Checked for eligibility 3/12/2018

"This is the peer reviewed version of the following article: Kroger et al, Journal of Clinical Periodontology, 2018, which has been published in final form at https://doi.org/10.1111/jcpe.13023. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."

#### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.

•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.

•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?) •Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

#### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

# The Severity of Human Peri-Implantitis Lesions Correlates with the Level of Submucosal Microbial Dysbiosis

A. Kröger<sup>1,14</sup>, C. Hülsmann<sup>1</sup>, S. Fickl<sup>2</sup>, T. Spinell<sup>3,4</sup>, F. Hüttig<sup>5</sup>, F. Kaufmann<sup>2</sup>, A. Heimbach<sup>6</sup>, P. Hoffmann<sup>6,7</sup>, N. Enkling<sup>8</sup>, S. Renvert<sup>9</sup>, F. Schwarz<sup>10,11</sup>, Ryan T. Demmer<sup>12</sup>, P. N. Papapanou<sup>13</sup>, S. Jepsen<sup>1</sup>, and M. Kebschull<sup>1,13,15</sup>

<sup>1</sup> Department of Periodontology, Operative and Preventive Dentistry, University of Bonn, Bonn, Germany

<sup>2</sup> Department of Periodontology, University of Würzburg, Würzburg, Germany

<sup>3</sup> Department of Operative Dentistry and Periodontology, LMU Munich, Munich, Germany

<sup>4</sup> Private Practice, Bolzano, Italy

<sup>5</sup> Department of Prosthodontics, University of Tübingen, Tübingen, Germany

<sup>6</sup> Department of Human Genetics, University of Bonn, Bonn, Germany

<sup>7</sup> Department of Biomedicine, University of Basel, Basel, Switzerland

<sup>8</sup> Department of Prosthodontics, University of Bonn, Bonn, Germany

<sup>9</sup> Department of Health Sciences, Kristianstad University, Kristianstad, Sweden

<sup>10</sup> Department of Oral Surgery and Implantology, Carolinum, Goethe University, Frankfurt, Germany

<sup>11</sup> Department of Oral Surgery, Universitätsklinikum Düsseldorf, Düsseldorf, Germany

<sup>12</sup> Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, USA

<sup>13</sup> Division of Periodontics, Section of Oral, Diagnostic and Rehabilitation Sciences, Columbia University College of Dental Medicine, New York, USA

<sup>14</sup> Department of Oral Surgery, School of Dentistry, University of Birmingham, Birmingham, UK

<sup>15</sup> The School of Dentistry, University of Birmingham, Birmingham, UK

#### Keywords

microbiome; peri-implantitis; peri-implant disease; NGS; 16s; next generation sequencing; dysbiosis

#### Correspondence address

Moritz Kebschull,

The School of Dentistry University of Birmingham Phone: +44-121-466-5059 Email: moritz@kebschull.me

#### Conflict of Interest and Sources of Funding Statement

This study was supported with funds from the DG PARO Innovation Award.

The authors declare that there are no conflicts of interest in this study.

### Abstract

**Aim:** To cross-sectionally analyze the submucosal microbiome of peri-implantitis (PI) lesions at different severity levels.

**Materials and Methods:** Microbial signatures of 45 submucosal plaque samples from untreated periimplantitis lesions obtained from 30 non-smoking, systemically healthy subjects were assessed by 16s sequencing. Linear mixed models were used to identify taxa with differential abundance by probing depth, after correction for age, gender, and multiple samples per subject. Network analyses were performed to identify groups of taxa with mutual occurrence or exclusion. Subsequently, the effects of peri-implant probing depth on submucosal microbial dysbiosis was calculated using the microbial dysbiosis index.

**Results:** In total, we identified 337 different taxa in the submucosal microbiome of peri-implantitis. Total abundance of 12 taxa correlated significantly with increasing probing depth; a significant relationship with lower probing depth was found for 16 taxa. Network analysis identified two mutually exclusive complexes associated with shallow pockets and deeper pockets, respectively. Deeper peri-implant pockets were associated with significantly increased dysbiosis.

**Conclusion:** Increases in peri-implant pocket depth are associated with substantial changes in the submucosal microbiome and increasing levels of dysbiosis.

### **Clinical Relevance**

**Scientific rationale for the study:** Peri-implantitis is increasingly prevalent and poses a significant clinical challenge. Thus far, the microbial signatures of peri-implantitis sites of different severity have not been characterized. Specifically, it is unknown whether deeper peri-implant pockets feature an increased level of dysbiosis.

**Principle findings:** The microbiome of peri-implantitis lesions consists of both common periodontal bacteria and novel species. Peri-implant probing depth correlates with distinct microbial profiles, and deeper pockets show higher levels of dysbiosis.

**Practical implications:** Peri-implant pockets contain disease-associated biofilms that become increasingly dysbiotic with increasing probing depth.

### Introduction

Peri-implantitis is an increasingly prevalent chronic inflammatory disease, with up to 22 % of patients being affected (Derks and Tomasi, 2015). Similar to periodontitis, the chronic inflammatory condition affecting teeth and caused by dysbiotic biofilms in susceptible hosts. Principal clinical signs of peri-implantitis include pocketing, bleeding-on-probing and/or suppuration. Both diseases lead to destruction of supporting tissues and, in terminal stages, to the loss of the implant or the tooth (Lindhe et al., 2008). Both diseases share common risk factors, including environmental exposures, such as smoking and poor oral hygiene, and systemic predispositions such as diabetes. Interestingly, a history of periodontitis has been shown to increase the risk for subsequent peri-implantitis, suggesting commonalities in their etiology (Sousa et al., 2016, Stacchi et al., 2016, Renvert and Quirynen, 2015). Still, peri-implantitis differs from periodontitis with respect to anatomic features and cellular composition of the lesion (Carcuac and Berglundh, 2014, Berglundh et al., 2011) and, most notably, to disease progression rate which is significantly higher in peri-implantitis than in periodontitis (Salvi et al., 2017).

Thus far, the pathophysiological underpinnings accounting for the faster progression rate in periimplantitis are poorly understood. Distinctions in key features of the lesion-associated microbiomes of the two diseases can conceivably play a role in this respect. Specifically, peri-implantitis lesions of increasing clinical severity may be associated with specific microbial signatures that may result in accelerated progression.

Thus far, no studies have investigated the relationship between peri-implantitis severity and the associated submucosal microbial patterns. Previous work has mainly focused on identifying microbial differences between healthy and diseased peri-implant sites (Al-Ahmad et al., 2018, Apatzidou et al., 2017, Sanz-Martin et al., 2017, Shiba et al., 2016, Zheng et al., 2015, Maruyama et al., 2014, Tamura et al., 2013, Kumar et al., 2012, Koyanagi et al., 2010). Importantly, confounders including general health status, smoking, age, gender, and prior treatment have generally not been accounted for in the existing literature, hampering inferences on the microbial features of peri-implantitis at different levels of disease severity.

In this work, we therefore sought to characterize the submucosal microbiome of peri-implantitis (PI) lesions at different severity levels. To do so, we used 16s rRNA sequencing to analyze of previously untreated peri-implantitis lesions from systemically healthy, non-smoking subjects. We identified taxa with significant associations with deeper or shallower peri-implant pockets and demonstrate a link between deeper probing depths and increasing levels of microbial dysbiosis.

### Material and Methods

### Study population and clinical procedures

The protocol for the study was approved by the institutional review boards / ethics committees at the Universities of Bonn, Würzburg, Tübingen and Düsseldorf, Germany, and the regional ethics committee of the Jönköping district, Sweden. The study was conducted in accordance with the guidelines of the World Medical Association Declaration of Helsinki. Each participant was informed of potential risks and benefits of this study and gave written informed consent.

Study participants were recruited at the dental clinics of the universities of Bonn, Würzburg, Tübingen, Düsseldorf and the Dental Hospital Jönköping. Participants had to be at least 18 years old, non-diabetic, non-smokers or former smokers (> 6 months) who did not receive any antibiotics or anti-inflammatory drugs within the past six months prior to the examination. Subjects that qualified for the diagnosis of periodontitis as a manifestation of systemic diseases, and subjects that were pregnant or lactating were excluded. All participants had at least one dental implant with peri-implantitis that had not received any therapeutic intervention in the preceding 6 months, except for occasional supragingival prophylaxis. Sites had to qualify for Cumulative Interceptive Supportive Therapy C.I.S.T., (Lang et al., 2000) scheme grade D, i.e., probing depth (PD)  $\geq$  5mm, presence of bleeding on probing (BoP), and bone loss  $\geq$  3mm. All participants underwent a full-mouth periodontal examination, each implant was probed at six sites and the maximum PD (PDMax). BoP/suppuration, implant/surface type, reconstruction method and the width of keratinized mucosa were recorded.

### Sample harvesting and sequencing preparation

Submucosal plaque samples were obtained from each peri-implantitis affected implant before surgical intervention, using one sterile paper points at each of the mesial, distal, buccal and lingual aspects of the implant, and were subsequently pooled (Jervoe-Storm et al., 2007). Sample tubes were stored immediately at -20°C or -80°C until further processing. DNA was extracted and purified using spin columns (Sigma-Aldrich GenElute Bacterial Genomic DNA Kit, Sigma-Aldrich, Munich, Germany). Quality and quantity of DNA was assessed spectrophotometrically using a Nanodrop spectrophotometer.

#### Sequence Processing

Sequencing data of the V3-V4 hypervariable region of the 16s rRNA gene were obtained using the Illumina MiSeq platform (Illumina, San Diego, California, United States) and a paired-end approach with 2x300 bp reads (Caporaso et al., 2011) providing substantial depth and breadth of coverage (Frey et al., 2014, Nelson et al., 2014). Library preparation was performed following the Illumina 16S rRNA Sequencing library preparation guide (Illumina, 2013) using the Nextera XT Index Kit (Illumina, San Diego, California, United States), PhiX Control Kit (Illumina, San Diego, California, United States) and MiSeq V3 reagent kit (Illumina, San Diego, California, United States) and MiSeq V3 reagent kit (Illumina, San Diego, California, United States) and equantity was measured on a Nanodrop spectrophotometer. Subsequently, a PCR amplification was performed using the Kapa Hifi Hotstart ReadyMix (KAPABIOSYSTEMS, Boston, Massachusetts, United States) and the primer pair 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC (Klindworth et al., 2013). Sequencing of the obtained libraries followed the manufacturer's instructions. The mean paired-end reads/sample were 109,945 ± 35,169.

Quality control of the obtained reads was performed using FastQC (Andrews, 2010). Data processing, including demultiplexing, quality filtering to a minimum Phred score of 20 and joining of paired end reads, was carried out using QIIME 1.9.1 (Caporaso et al., 2010b). In the next step, the open reference picking strategy was selected. Sequences were clustered with a similarity threshold of 97 % via UCLUST (Edgar, 2010) to operating taxonomic units (OTUs). Assignment of representative sequences, picked by QIIME, was done by the RDP classifier v2.2 (Wang et al., 2007), and alignment

to the reference database, the Human Oral Microbiome Database v15.1 (Chen et al., 2010) was conducted via PyNAST (Caporaso et al., 2010a). OTU data were normalized using the DESeq2 approach in QIIME (Love et al., 2014, McMurdie and Holmes, 2014).  $\alpha$ -diversity through whole tree PD (Moritz and Faith, 1998) - a measure of abundance-based richness, was assessed using QIIME. The same platform was used to assess  $\beta$ -diversity by means of unweighted UniFrac (Lozupone and Knight, 2005).

### Statistical Analysis

The relationship of the deepest PD per implant (PDMax) with the abundance of each OTU was assessed through regression analysis using linear mixed models in R 3.3.3 (R Development Core Team, 2008), utilizing the lme4 1.1-15 package (Bates et al., 2015) in conjunction with ImerTest 2.0-36 to test for significance (Kuznetsova et al., 2017). The higher within-subject correlation was taken into account by modeling the subjects as a random effect. Furthermore, gender and age distribution were added to the model as fixed factors. Results were corrected for multiple testing through FDR-adjustment (Benjamini et al., 2001).

Taxa significantly associated with probing depth were considered in the network analysis. Taxa cooccurrence and/or co-exclusion were determined via renormalization and permutation with the R package ccrepe ('Compositionality Corrected by Renormalization and Permutation') 1.10.0, designed to detect correlations in microbial data using the nc.score as the similarity measure.

To assess the extent of dysbiosis of the biofilm associated with each implant site, we utilized the microbial dysbiosis index (MD-index) (Gevers et al., 2014), calculated as follows:

 $MDI = \log(\frac{[\text{total abundance of OTUs positively associated with PDmax}]}{[\text{total abundance of OTUs negatively associated with PDmax}]})$ 

Regression analysis was run with mixed effects linear models correcting for gender, age and within subject correlation to detect possible associations of MDI and PD, as outlined above.

Analysis of microbiome characteristics of deep and shallow pocket groups was conducted via Chi-Square-Test (Chernoff and Lehmann, 1954).

### Results

### Study population

A total of 45 implants with peri-implantitis, obtained from 30 subjects, were included in the present analyses. As shown in Table 1, 70.0% of the participants were female with a mean age of  $64.7\pm11.5$  years [range 32-82 years]. On average, participants had  $15.85 \pm 8.04$  teeth and  $3.46 \pm 1.61$  dental implants. Mean PDmax was  $8.00\pm2.04$ mm [range 5-11mm]. All implants showed BoP, and suppuration was observed at 73% of the sites.

### Structure and Diversity of the Bacterial Community

All samples provided sufficient DNA quality and quantity for analysis. OTUs were classified in 14 phyla, 169 genera and 337 species-level phylotypes. A complete list of observed taxonomic units can be viewed in the supplement material (Supplemental Table S1).

### Dysbiosis

A highly significant ( $p = 1.32 \times 10^{-7}$ ) positive association of the MD-index with increasing PDmax was observed (Figure 1).

### Characteristics of the Microbiome in Relation to Disease Severity

To characterize the microbial shifts in relation to PDmax leading to the observed increase in dysbiosis, we performed linear mixed model analyses of the normalized relative abundance of observed phylotypes on the species-level in relation to PDmax. We identified 28 significantly differentially abundant taxa after correction for age and gender (Table 2). Abundance of the taxa *Eubacteriaceae [XV], Fretibacterium sp. HMT 362, Fretibacterium fastidiosum, Peptostreptococcaceae [XI][G-6], Alloprevotella sp. HMT 473, Fastidiosipila sanguinis, Filifactor alocis, Peptostreptococcaceae [XI][G-4], Bacteriodetes [G-3] bacterium HMT 365, Treponema parvum, Clostridiales [F-1][G-1] bacterium HMT 093, and Orobacterium was significantly (FDR-corrected p<0.05) higher with increasing PDmax; whereas the taxa Selemonas sp. HMT 136, Granulicatella elegans, Rothia aeria, Corynebacterium durum, Veillonella dispar, Acinetobacter, Streptococcus oralis subsp. dentisani clade 398, Veillonella parvula, Bifidobacterium, Bergeyella, Veillonella rogosae, Neisseria, Veillonella atypica, Rhodobacteriaceae, Lautropia mirabilis, Oribacterium asaccacrolyticum were significantly (FDR-corrected p < 0.05) decreased in samples with increasing PDmax. Virtually identical results were obtained when using alternative methodology. Results on different taxonomic levels can be found in the Supplemental Material (Table S2).* 

Taxa associated with increasing PDmax were exclusively anaerobes, whilst taxa associated with lower PDmax showed significantly different (p < 0.03), more variable oxygen requirements (Table 3).  $\alpha$ -diversity was significantly (p < 0.033) decreased in groups with deeper pockets (> 7 mm) (Figure 2) demonstrating higher species richness in samples from shallower pockets (< 7 mm) and vice versa. Collectively, the principle coordinate analysis plots PCoA (Figure 3) suggest that the second principle coordinate (PC2) layers the samples by PDmax (A), which is positively associated with MD-index (C), representing similar species diversity of samples with similar probing depths.  $\alpha$ -diversity, on the other hand, tends to be distributed on PC1. The scatter density plot of the samples of whole tree PD versus MD-index suggests that samples with higher MD-index (> 0.1) are characterized by markedly reduced species richness, as compared to the samples with lower MD-index.

### **Correlation Network**

Correlation analysis using the ccrepe package showed a clear division into two groups of cooperation units. Lautropia mirabilis, Neisseria, Granulicatella elegans, Bergeyella, Rothia aeria,

Corynebacterium durum, Oribacterium asaccharolyticum, Selemonas sp. HMT 136, Veillonella rogosae, Veillonella parvula, Veillonella atypica, Veillonella dispar, Streptococcus oralis subsp. dentisani clade 398 and Bifidobacterium formed one complex with strong co-occurrence of taxa that negatively associated with PDmax. A second, smaller complex negatively associated with PDmax was formed by the taxa Acinetobacter and Rhodobacteraceae. These two complexes showed mutual exclusion with the three complexes comprised of taxa positively associated with PDmax: (i) Filifactor alocis, Bacteroidetes bacterium HMT 365 and Clostridiales [F-1][G-1] bacterium HMT 093; (ii) Peptostreptococcaceae [XI][G-6], Peptostreptococcaceae [XI][G-4], Fretibacterium sp. HMT 362 and Fretibacterium fastidiosum; and (iii) Eubacteriaceae [XV] . Figure 4 shows a visualization of the network, detailed results are captured in table 4. Additionally, findings from genus-level analysis are presented in the Supplemental Material (Supplemental Table S3, Supplemental Figure SF1).

### Discussion

Our findings reveal significant associations of specific features of the submucosal microbiome from peri-implantitis lesions with peri-implantitis severity, expressed through the depth of the peri-implant pocket. Our analyses cover multiple levels of the submucosa bacterial ecology and aspects of co-occurrence and co-exclusion. To the best of our knowledge, this is the first study investigating these associations on a whole-microbiome level, and the first study that included microbial samples from a well-defined patient cohort specifically enrolled to minimize bias introduced by subject-related factors (including smoking, diabetes, or medications) and local factors (such as prior therapeutic interventions at the site). Possible remaining confounders were addressed using multivariate analyses.

In analyses examining the effects of ecological shifts on disease severity, we observed that the MDindex, a measure of submucosal bacterial dysbiosis, is positively associated with increasing periimplant pocket depths, suggesting that potential pathogens outcompete health-associated taxa (Marsh, 2015), similar to earlier observations derived from several other human diseases – including diabetes, autism, obesity, and periodontitis (Boulange et al., 2016, Buffington et al., 2016, Carding et al., 2015). To the best of our knowledge, our study is the first to show such an association in the context of human peri-implantitis.

When analyzing the microbial profiles underlying the observed dysbiosis, our findings indicate that peri-implantitis lesions are primarily inhabited by anaerobic gram-negative taxa, corroborating previous studies by other investigators (Sanz-Martin et al., 2017, Koyanagi et al., 2010, Tamura et al., 2013, Al-Ahmad et al., 2018, Apatzidou et al., 2017, Kumar et al., 2012). All aforementioned studies compared peri-implant health and peri-implantitis, whereas our study focused on peri-implantitis lesions with differing disease severity. Interestingly, the taxa identified in our analyses as associated with shallower peri-implant pocket depth do overlap with those identified as associated with health in case-control studies, e.g., *Streptococcus* and *Veillonella*.

In addition, we identified several taxa not previously reported to occur at peri-implantitis sites, including *Oribacterium asaccharolyticum, Veillonella dispar, Granulicatella elegans* and *Corynebacterium durum*, i.e., natural inhabitants of the human aerodigestive tract (Chen et al., 2018, Kumar et al., 2006, Sizova et al., 2014, Ohara-Nemoto et al., 2005).

A number of the taxa identified to be associated with deeper peri-implant pockets have been associated with severe periodontal disease, e.g., *Filifactor alocis* (Aruni et al., 2015, Aruni et al., 2014), *Fretibacterium fastidiosum* (Vartoukian et al., 2013, Correa et al., 2017, Moon et al., 2015), *Alloprevotella, Clostridiales* (Chen et al., 2018) or *Treponema* (Socransky et al., 1998) – especially *Treponema parvum*, which was also found in sites originating from acute necrotizing ulcerative gingivitis lesions (Wyss et al., 2001). In addition, we also identified significant positive associations with taxa not previously linked to peri-implantitis or periodontitis, e.g., *Fastidiosipila sanguinis*, a species recently discovered in human blood samples (Falsen et al., 2005) but hitherto not related to human nosology.

In addition, the dysbiotic shift observed in the transition from shallower to deeper pockets is characterized by decreasing species richness: The group consisting of exclusively anaerobic, mostly gram negative taxa, some of which are known periodontal pathogens, outcompeted the health-associated taxa that were prevalent in samples with shallower probing depths.

Network analysis facilitated the study of inter-related taxa and further identified two groups of mutually exclusive complexes. Interestingly, we found the taxa in the first, larger group to be associated with shallow pockets, whilst the taxa in the second group were significantly more numerous in deeper pockets. These findings indicate that peri-implantitis is clearly not a mono-infection by a specific pathogen, but rather a multifactorial, dysbiosis-associated process. Specifically,

within the limits of a study only including 'diseased' implants, our data point to the importance of a balanced peri-implant ecosystem, with an equilibrium between health-associated bacteria and pathogens (Socransky and Haffajee, 2005).

Strengths of our study include stringent inclusion criteria, a sizeable patient sample, and use multivariate analyses to account for possible residual confounders. Limitations include the cross-sectional nature of the study and the absence of a control group of implants without peri-implantitis that obviously preclude inferences regarding the temporality of the observed associations and cannot prove a cause and effect relationship between submucosal microbial dysbiosis and peri-implantitis. Longitudinal studies are needed to establish such relationships, but their implementation is challenging for both logistical and ethical reasons. Although our findings partly corroborate a number of earlier reports, discrepancies can conceivably be explained by differing analytic and data processing approaches, differences in sequencing platforms, length and sensitivities, and different 16s target regions (Kozich et al., 2013).

Taken together, this is the first study to demonstrate an association of both previously recognized and novel taxa with the severity of human peri-implantitis. Within the peri-implantitis microbiome, we identified groups with significant co-occurrence and co-exclusion, suggesting that alterations of ecological scale, rather than single taxa, underlie the progression of a lesion. Furthermore, we demonstrated a significant positive association between the level of microbial dysbiosis and the probing depth of the peri-implantitis lesion. Our findings provide a basis for further mechanistic work of the role of microbial ecological shifts and host responses in dysbiosis-associated peri-implant tissue destruction.

## Acknowledgements

This study was supported by the DG PARO Innovation Award.

We thank the Next-Generation-Sequencing core-facility of the University of Bonn Medical Center for providing their sequencing service.

The authors report no conflicts of interest.

### References

- Al-Ahmad, A., Muzafferiy, F., Anderson, A. C., Wölber, J. P., Ratka-Krüger, P., Fretwurst, T., Nelson, K., Vach, K. & Hellwig, E. (2018) Shift of microbial composition of peri-implantitis-associated oral biofilm as revealed by 16S rRNA gene cloning. *Journal of Medical Microbiology*. doi:doi:10.1099/jmm.0.000682.
- Andrews, S. (2010) FastQC: A Quality Control Tool for High Throughput Sequence Data.
- Apatzidou, D., Lappin, D. F., Hamilton, G., Papadopoulos, C. A., Konstantinidis, A. & Riggio, M. P. (2017) Microbiome of peri-implantitis affected and healthy dental sites in patients with a history of chronic periodontitis. *Arch Oral Biol* 83, 145-152. doi:10.1016/j.archoralbio.2017.07.007.
- Aruni, A. W., Mishra, A., Dou, Y., Chioma, O., Hamilton, B. N. & Fletcher, H. M. (2015) Filifactor alocis--a new emerging periodontal pathogen. *Microbes Infect* 17, 517-530. doi:10.1016/j.micinf.2015.03.011.
- Aruni, W., Chioma, O. & Fletcher, H. M. (2014) Filifactor alocis: The Newly Discovered Kid on the Block with Special Talents. *J Dent Res* **93**, 725-732. doi:10.1177/0022034514538283.
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting Linear Mixed-Effects Models Using Ime4. 2015 67, 48. doi:10.18637/jss.v067.i01.
- Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N. & Golani, I. (2001) Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* **125**, 279-284.
- Berglundh, T., Zitzmann, N. U. & Donati, M. (2011) Are peri-implantitis lesions different from periodontitis lesions? *Journal of Clinical Periodontology* **38 Suppl 11**, 188-202. doi:10.1111/j.1600-051X.2010.01672.x.
- Bitrian, M., Gonzalez, R. H., Paris, G., Hellingwerf, K. J. & Nudel, C. B. (2013) Blue-light-dependent inhibition of twitching motility in Acinetobacter baylyi ADP1: additive involvement of three BLUF-domain-containing proteins. *Microbiology* 159, 1828-1841. doi:10.1099/mic.0.069153-0.
- Boulange, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K. & Dumas, M. E. (2016) Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Med* **8**, 42. doi:10.1186/s13073-016-0303-2.
- Buffington, S. A., Di Prisco, G. V., Auchtung, T. A., Ajami, N. J., Petrosino, J. F. & Costa-Mattioli, M.
   (2016) Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* 165, 1762-1775. doi:10.1016/j.cell.2016.06.001.
- Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L. & Knight, R. (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**, 266-267. doi:10.1093/bioinformatics/btp636.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. (2010b) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7, 335-336. doi:10.1038/nmeth.f.303.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N. & Knight, R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* **108 Suppl 1**, 4516-4522. doi:10.1073/pnas.1000080107.
- Carcuac, O. & Berglundh, T. (2014) Composition of human peri-implantitis and periodontitis lesions. *J Dent Res* **93**, 1083-1088. doi:10.1177/0022034514551754.
- Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M. & Owen, L. J. (2015) Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis* **26**, 26191. doi:10.3402/mehd.v26.26191.

- Chen, C., Hemme, C., Beleno, J., Shi, Z. J., Ning, D., Qin, Y., Tu, Q., Jorgensen, M., He, Z., Wu, L. & Zhou, J. (2018) Oral microbiota of periodontal health and disease and their changes after nonsurgical periodontal therapy. *ISME J* **12**, 1210-1224. doi:10.1038/s41396-017-0037-1.
- Chen, T., Yu, W. H., Izard, J., Baranova, O. V., Lakshmanan, A. & Dewhirst, F. E. (2010) The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database (Oxford)* **2010**, baq013. doi:10.1093/database/baq013.
- Chernoff, H. & Lehmann, E. L. (1954) The Use of Maximum Likelihood Estimates in \$\chi^2\$ Tests for Goodness of Fit. Ann. Math. Statist. **25,** 579-586. doi:10.1214/aoms/1177728726.
- Correa, J. D., Calderaro, D. C., Ferreira, G. A., Mendonca, S. M., Fernandes, G. R., Xiao, E., Teixeira, A. L., Leys, E. J., Graves, D. T. & Silva, T. A. (2017) Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status. *Microbiome* 5, 34. doi:10.1186/s40168-017-0252-z.
- Derks, J. & Tomasi, C. (2015) Peri-implant health and disease. A systematic review of current epidemiology. *J Clin Periodontol* **42 Suppl 16**, S158-171. doi:10.1111/jcpe.12334.
- Edgar, R. C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460-2461. doi:10.1093/bioinformatics/btq461.
- Falsen, E., Collins, M. D., Welinder-Olsson, C., Song, Y., Finegold, S. M. & Lawson, P. A. (2005)
   Fastidiosipila sanguinis gen. nov., sp. nov., a new Gram-positive, coccus-shaped organism from human blood. *Int J Syst Evol Microbiol* 55, 853-858. doi:10.1099/ijs.0.63327-0.
- Frey, K. G., Herrera-Galeano, J. E., Redden, C. L., Luu, T. V., Servetas, S. L., Mateczun, A. J., Mokashi, V. P. & Bishop-Lilly, K. A. (2014) Comparison of three next-generation sequencing platforms for metagenomic sequencing and identification of pathogens in blood. *BMC Genomics* 15, 96. doi:10.1186/1471-2164-15-96.
- Gevers, D., Kugathasan, S., Denson, L. A., Vazquez-Baeza, Y., Van Treuren, W., Ren, B., Schwager, E., Knights, D., Song, S. J., Yassour, M., Morgan, X. C., Kostic, A. D., Luo, C., Gonzalez, A., McDonald, D., Haberman, Y., Walters, T., Baker, S., Rosh, J., Stephens, M., Heyman, M., Markowitz, J., Baldassano, R., Griffiths, A., Sylvester, F., Mack, D., Kim, S., Crandall, W., Hyams, J., Huttenhower, C., Knight, R. & Xavier, R. J. (2014) The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 15, 382-392. doi:10.1016/j.chom.2014.02.005.
- Illumina, T. (2013) 16S Metagenomic Sequencing Library Preparation.
- Jalava, J. & Eerola, E. (1999) Phylogenetic analysis of Fusobacterium alocis and Fusobacterium sulci based on 16S rRNA gene sequences: proposal of Filifactor alocis (Cato, Moore and Moore) comb. nov. and Eubacterium sulci (Cato, Moore and Moore) comb. nov. Int J Syst Bacteriol 49 Pt 4, 1375-1379. doi:10.1099/00207713-49-4-1375.
- Jervoe-Storm, P. M., Alahdab, H., Koltzscher, M., Fimmers, R. & Jepsen, S. (2007) Comparison of curet and paper point sampling of subgingival bacteria as analyzed by real-time polymerase chain reaction. *J Periodontol* **78**, 909-917. doi:10.1902/jop.2007.060218.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. & Glockner, F. O. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* **41**, e1. doi:10.1093/nar/gks808.
- Koyanagi, T., Sakamoto, M., Takeuchi, Y., Ohkuma, M. & Izumi, Y. (2010) Analysis of microbiota associated with peri-implantitis using 16S rRNA gene clone library. *J Oral Microbiol* **2**. doi:10.3402/jom.v2i0.5104.
- Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**, 5112-5120. doi:10.1128/AEM.01043-13.
- Kumar, P. S., Leys, E. J., Bryk, J. M., Martinez, F. J., Moeschberger, M. L. & Griffen, A. L. (2006) Changes in periodontal health status are associated with bacterial community shifts as

assessed by quantitative 16S cloning and sequencing. *J Clin Microbiol* **44**, 3665-3673. doi:10.1128/JCM.00317-06.

- Kumar, P. S., Mason, M. R., Brooker, M. R. & O'Brien, K. (2012) Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. *J Clin Periodontol* **39**, 425-433. doi:10.1111/j.1600-051X.2012.01856.x.
- Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. (2017) ImerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*. doi:10.18637/jss.v082.i13.
- Lang, N. P., Wilson, T. G. & Corbet, E. F. (2000) Biological complications with dental implants: their prevention, diagnosis and treatment. *Clin Oral Implants Res* **11 Suppl 1**, 146-155.
- Lindhe, J., Meyle, J. & Group, D. o. E. W. o. P. (2008) Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* **35**, 282-285. doi:10.1111/j.1600-051X.2008.01283.x.
- Love, M. I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**, 550. doi:10.1186/s13059-014-0550-8.
- Lozupone, C. & Knight, R. (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* **71**, 8228-8235. doi:10.1128/AEM.71.12.8228-8235.2005.
- Marsh, P. D. (2015) The commensal microbiota and the development of human disease an introduction. *J Oral Microbiol* **7**, 29128. doi:10.3402/jom.v7.29128.
- Maruyama, N., Maruyama, F., Takeuchi, Y., Aikawa, C., Izumi, Y. & Nakagawa, I. (2014) Intraindividual variation in core microbiota in peri-implantitis and periodontitis. *Sci Rep* **4**, 6602. doi:10.1038/srep06602.
- McMurdie, P. J. & Holmes, S. (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* **10**, e1003531. doi:10.1371/journal.pcbi.1003531.
- Moon, J. H., Lee, J. H. & Lee, J. Y. (2015) Subgingival microbiome in smokers and non-smokers in Korean chronic periodontitis patients. *Mol Oral Microbiol* **30**, 227-241. doi:10.1111/omi.12086.
- Moritz, C. & Faith, D. P. (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* **7**, 419-429. doi:doi:10.1046/j.1365-294x.1998.00317.x.
- Nelson, M. C., Morrison, H. G., Benjamino, J., Grim, S. L. & Graf, J. (2014) Analysis, optimization and verification of Illumina-generated 16S rRNA gene amplicon surveys. *PLoS One* **9**, e94249. doi:10.1371/journal.pone.0094249.
- Ohara-Nemoto, Y., Kishi, K., Satho, M., Tajika, S., Sasaki, M., Namioka, A. & Kimura, S. (2005) Infective endocarditis caused by Granulicatella elegans originating in the oral cavity. *J Clin Microbiol* 43, 1405-1407. doi:10.1128/JCM.43.3.1405-1407.2005.
- R Development Core Team (2008) R: A Language and Environment for Statistical Computing.
- Renvert, S. & Quirynen, M. (2015) Risk indicators for peri-implantitis. A narrative review. *Clin Oral Implants Res* **26 Suppl 11,** 15-44. doi:10.1111/clr.12636.
- Salvi, G. E., Cosgarea, R. & Sculean, A. (2017) Prevalence and Mechanisms of Peri-implant Diseases. J Dent Res **96**, 31-37. doi:10.1177/0022034516667484.
- Sanz-Martin, I., Doolittle-Hall, J., Teles, R. P., Patel, M., Belibasakis, G. N., Hammerle, C. H. F., Jung, R.
   E. & Teles, F. R. F. (2017) Exploring the microbiome of healthy and diseased peri-implant sites using Illumina sequencing. *J Clin Periodontol* 44, 1274-1284. doi:10.1111/jcpe.12788.
- Schell, M. A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., Zwahlen, M. C., Desiere, F., Bork, P., Delley, M., Pridmore, R. D. & Arigoni, F. (2002) The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract. *Proc Natl* Acad Sci U S A 99, 14422-14427. doi:10.1073/pnas.212527599.
- Shiba, T., Watanabe, T., Kachi, H., Koyanagi, T., Maruyama, N., Murase, K., Takeuchi, Y., Maruyama, F., Izumi, Y. & Nakagawa, I. (2016) Distinct interacting core taxa in co-occurrence networks enable discrimination of polymicrobial oral diseases with similar symptoms. *Sci Rep* 6, 30997. doi:10.1038/srep30997.

- Shukla, S. K., Paustian, D. L., Stockwell, P. J., Morey, R. E., Jordan, J. G., Levett, P. N., Frank, D. N. & Reed, K. D. (2004) Isolation of a fastidious Bergeyella species associated with cellulitis after a cat bite and a phylogenetic comparison with Bergeyella zoohelcum strains. *J Clin Microbiol* 42, 290-293.
- Simon, M., Scheuner, C., Meier-Kolthoff, J. P., Brinkhoff, T., Wagner-Dobler, I., Ulbrich, M., Klenk, H. P., Schomburg, D., Petersen, J. & Goker, M. (2017) Phylogenomics of Rhodobacteraceae reveals evolutionary adaptation to marine and non-marine habitats. *ISME J* 11, 1483-1499. doi:10.1038/ismej.2016.198.
- Sizova, M. V., Muller, P. A., Stancyk, D., Panikov, N. S., Mandalakis, M., Hazen, A., Hohmann, T., Doerfert, S. N., Fowle, W., Earl, A. M., Nelson, K. E. & Epstein, S. S. (2014) Oribacterium parvum sp. nov. and Oribacterium asaccharolyticum sp. nov., obligately anaerobic bacteria from the human oral cavity, and emended description of the genus Oribacterium. *Int J Syst Evol Microbiol* 64, 2642-2649. doi:10.1099/ijs.0.060988-0.
- Socransky, S. S. & Haffajee, A. D. (2005) Periodontal microbial ecology. *Periodontol 2000* **38**, 135-187. doi:10.1111/j.1600-0757.2005.00107.x.
- Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent, R. L., Jr. (1998) Microbial complexes in subgingival plaque. *J Clin Periodontol* **25**, 134-144.
- Sohngen, C., Podstawka, A., Bunk, B., Gleim, D., Vetcininova, A., Reimer, L. C., Ebeling, C., Pendarovski, C. & Overmann, J. (2016) BacDive--The Bacterial Diversity Metadatabase in 2016. Nucleic Acids Res 44, D581-585. doi:10.1093/nar/gkv983.
- Sousa, V., Mardas, N., Farias, B., Petrie, A., Needleman, I., Spratt, D. & Donos, N. (2016) A systematic review of implant outcomes in treated periodontitis patients. *Clin Oral Implants Res* **27**, 787-844. doi:10.1111/clr.12684.
- Stacchi, C., Berton, F., Perinetti, G., Frassetto, A., Lombardi, T., Khoury, A., Andolsek, F. & Di Lenarda, R. (2016) Risk Factors for Peri-Implantitis: Effect of History of Periodontal Disease and Smoking Habits. A Systematic Review and Meta-Analysis. *Journal of Oral & Maxillofacial Research* 7, e3. doi:10.5037/jomr.2016.7303.
- Tamura, N., Ochi, M., Miyakawa, H. & Nakazawa, F. (2013) Analysis of bacterial flora associated with peri-implantitis using obligate anaerobic culture technique and 16S rDNA gene sequence. *Int J Oral Maxillofac Implants* 28, 1521-1529. doi:10.11607/jomi.2570.
- Vartoukian, S. R., Downes, J., Palmer, R. M. & Wade, W. G. (2013) Fretibacterium fastidiosum gen. nov., sp. nov., isolated from the human oral cavity. *Int J Syst Evol Microbiol* **63**, 458-463. doi:10.1099/ijs.0.041038-0.
- Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73, 5261-5267. doi:10.1128/AEM.00062-07.
- Wyss, C., Dewhirst, F. E., Gmur, R., Thurnheer, T., Xue, Y., Schupbach, P., Guggenheim, B. & Paster, B.
   J. (2001) Treponema parvum sp. nov., a small, glucoronic or galacturonic acid-dependent oral spirochaete from lesions of human periodontitis and acute necrotizing ulcerative gingivitis. *Int J Syst Evol Microbiol* 51, 955-962. doi:10.1099/00207713-51-3-955.
- Yutin, N. & Galperin, M. Y. (2013) A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. *Environ Microbiol* 15, 2631-2641. doi:10.1111/1462-2920.12173.
- Zheng, H., Xu, L., Wang, Z., Li, L., Zhang, J., Zhang, Q., Chen, T., Lin, J. & Chen, F. (2015) Subgingival microbiome in patients with healthy and ailing dental implants. *Sci Rep* 5, 10948. doi:10.1038/srep10948.

### Figure Legends

#### Figure 1

Mean Dysbiosis Index (MDI) of each sample plotted against probing depth (PD [mm]). The slope of the line is statistically significant ( $p = 1.32 \times 10^{-7}$ ). MDI increases with growing PD, indicating gain of taxa abundance associated with disease severity, whilst taxa with opposite association show decreased abundance.

### Figure 2

Results of  $\alpha$ -diversity-analysis: (A) Rarefaction plot: samples with shallow pockets (blue) show larger whole tree PD measures. Samples of deeper pockets (PDmax > 7 mm) on the other hand demonstrate lower species richness. (B) Boxplot diagram of whole tree PD measure of shallower and deeper pocket sample groups.  $\alpha$ -diversity is significantly (p < 0.033) decreased in latter group.

### Figure 3

Principle coordinate plots, whereas each axis exemplifies one of the three top principle coordinates seizing the major part of the diversity. Shown in percent, the fraction of diversity is described. (A) shows the samples colored by grouping of deeper (PDmax > 7 mm) and shallower pockets (PDmax  $\leq$  7 mm), (B) by the scope of whole tree PD and (C) reflecting the gradient of MDI. The scatter plot in (D) of alpha diversity versus MDI: samples with increased MDI (> 0.1) show a sudden reduction of species richness.

#### Figure 4

Network of taxa significantly associated with disease severity. Red lines indicate co-exclusion while green lines indicate co-occurrence. The results were filtered for better visualization: Correlations with fdr-corrected p-values <0.1. Weights vary between -0.424 and 0.673. The width of the lines mirrors the weight strengths. Taxa with positive association with pocket depth appear above the broken line without co-occurring taxa associated with shallower pockets which cluster below the broken line. Results can be viewed in Table 2 (unfiltered version in Supplement Material Table S3).

## Tables

### Table 1

List of selected study characteristics.

Study Characteristics	
Population-specific	30 patients
Female	70% (21 individuals)
Male	30% (9 individuals)
Mean age	64.7±11.5 years (range: 32y-82y)
Mean number of teeth	15.85 ± 8.04 (range: 0-27 teeth)
Mean number of dental implants	3.46 ± 1.6 (range: 1-≥8 implants)
Site-specific	<ul> <li>45 implants with peri-implantitis</li> <li>Inclusion criteria: <ul> <li>Implant sites qualified for CIST (Lang et al., 2000) therapy scheme D</li> <li>PD ≥ 5mm</li> <li>BoP +</li> <li>Bone loss ≥ 3mm</li> </ul> </li> </ul>
Mean PDmax	8.00±2.04 mm (range: 5mm-11mm)
Suppuration	Observed in 73% of sites

### Table 2

Abundance of taxa associated with PDmax. Positive estimates stand for positive correlation and vice versa. Only taxa with an FDR-corrected p-value < 0.05 are shown. Residual taxa and analyses on other taxa-level can be viewed in supplement material (Table S2).

Taxon	Estimate	Std	p-	FDR-
		Error	value	corr.
Eubacteriaceae [XV]	0.7598	0.1414	0.0000	0.0014
Fretibacterium sp. HMT 362	0.4660	0.0938	0.0001	0.0088
Selenomonas sp. HMT 136	-0.7589	0.1695	0.0001	0.0095
Granulicatella elegans	-0.6547	0.1450	0.0002	0.0135
Fretibacterium fastidiosum	0.4069	0.0916	0.0002	0.0135
Rothia aeria	-0.7634	0.1916	0.0003	0.0167
Corynebacterium durum	-0.3884	0.1046	0.0006	0.0206
Peptostreptococcaceae [XI][G-6]	0.5954	0.1561	0.0005	0.0206
Veillonella dispar	-0.5955	0.1539	0.0005	0.0206
Acinetobacter	-0.6184	0.1526	0.0006	0.0206
Streptococcus oralis subsp. dentisani clade 398	-0.4994	0.1313	0.0009	0.0267
Alloprevotella sp. HMT 473	0.4511	0.1222	0.0010	0.0286
Veillonella parvula	-0.4540	0.1233	0.0012	0.0310
Fastidiosipila sanguinis	0.3670	0.1065	0.0014	0.0326
Bifidobacterium	-0.5306	0.1563	0.0019	0.0332
Bergeyella	-0.4549	0.1326	0.0018	0.0332
Filifactor alocis	0.4915	0.1312	0.0017	0.0332
Veillonella rogosae	-0.4570	0.1367	0.0018	0.0332
Neisseria	-0.6571	0.1906	0.0016	0.0332
Veillonella atypica	-0.6161	0.1904	0.0025	0.0414
Rhodobacteraceae	-0.4294	0.1328	0.0026	0.0416
Peptostreptococcaceae [XI][G-4]	0.4873	0.1424	0.0028	0.0424
Bacteroidetes [G-3] bacterium HMT 365	0.4473	0.1322	0.0034	0.0468
Lautropia mirabilis	-0.5693	0.1793	0.0032	0.0468
Treponema parvum	0.5249	0.1628	0.0035	0.0468
Clostridiales [F-1][G-1] bacterium HMT 093	0.4993	0.1516	0.0037	0.0473
Oribacterium	0.4296	0.1377	0.0041	0.0496
Oribacterium asaccharolyticum	-0.3888	0.1272	0.0040	0.0496

### Table 3

### Characteristics of the taxa significantly associated with PDmax

\* Information were compiled from the American Type Culture Collection database

### \*\* Information were compiled from *BacDive* (Sohngen et al., 2016)

Таха	Gram	Anaerobic/
	+/-	aerobic
positive association with PDmax		
Eubacteriaceae [XV]***	+	NA
Fretibacterium sp. HMT 362 **	-	anaerobic
Fretibacterium fastidiosum **	-	anaerobic
Peptostreptococcaceae [XI][G-6] *	+	anaerobic
Bacteroidetes [G-3] bacterium HMT 365 **	NA	anaerobic
Peptostreptococcaceae [XI][G-4] *	+	anaerobic
Oribacterium**	NA	anaerobic
Treponema parvum **	-	anaerobic
Filifactor alocis (Jalava and Eerola, 1999)	+	anaerobic
Alloprevotella sp. HMT 473 **	NA	anaerobic
Fastidiosipila sanguinis**	+	anaerobic
Clostridiales [F-1][G-1] bacterium HMT 093 (Yutin and Galperin, 2013)	+	anaerobic
negative association with PDmax		
Bifidobacterium (Schell et al., 2002)	+	anaerobic
Selenomonas sp. HMT 136**	-	anaerobic
Granulicatella elegans**	+	anaerobic
Rothia aeria **	+	aerobic
Acinetobacter (Bitrian et al., 2013)	-	aerobic
Streptococcus oralis subsp. dentisani clade 398 **	+	anaerobic
Neisseria **	-	aerobic
Veillonella parvula **	-	anaerobic
Rhodobacteraceae (Simon et al., 2017)	-	NA
Bergeyella (Shukla et al., 2004)	-	aerobic
Veillonella rogosae **	-	anaerobic
Lautropia mirabilis **	-	anaerobic
Veillonella atypica **	-	anaerobic
Veillonella dispar **	-	anaerobic
Oribacterium asaccharolyticum **	+	anaerobic
Corynebacterium durum **	+	aerobic

From	То	Weight	FDR-	Interaction
			corr.	Туре

### Table 4

Edgelist of the network analysis. It displays interaction of two taxa, whereas each line represents one single interaction. Negative values in the weight column mean co-exclusion, whilst positive values indicate co-occurrences. Only edges with significant FDR-corrected p-values <0.1. The complete list can be displayed in the supplement material (Table S3).

Selemonas sp. HMT 136	Bifidobacterium	0.6610	0.0000	co-occurrence
Fretibacterium sp. HMT				
362	Fretibacterium fastidiosum	0.5888	0.0000	co-occurrence
Veillonella rogosae	Veillonella dispar	0.6734	0.0000	co-occurrence
Veillonella dispar	Selemonas sp. HMT 136	0.5609	0.0000	co-occurrence
Neisseria	Bergeyella	0.5491	0.0000	co-occurrence
Veillonella parvula	Veillonella dispar	0.5849	0.0000	co-occurrence
Veillonella dispar	Bifidobacterium	0.5262	0.0001	co-occurrence
Acinetobacter	Rhodobacteraceae	0.4874	0.0002	co-occurrence
Neisseria	Rothia aeria	0.4863	0.0003	co-occurrence
Neisseria	Lautropia mirabilis	0.4999	0.0003	co-occurrence
Veillonella rogosae	Veillonella parvula	0.5269	0.0014	co-occurrence
	Streptococcus oralis subsp.			
Veillonella dispar	dentisani clade 398	0.4422	0.0036	co-occurrence
Neisseria	Granulicatella elegans	0.4429	0.0037	co-occurrence
Selemonas sp. HMT 136	Oribacterium asaccharolyticum	0.4953	0.0038	co-occurrence
Corynebacterium durum	Rothia aeria	0.4692	0.0047	co-occurrence
Veillonella parvula	Selemonas sp. HMT 136	0.4817	0.0055	co-occurrence
Veillonella rogosae	Selemonas sp. HMT 136	0.5026	0.0059	co-occurrence
Veillonella dispar	Veillonella atypica	0.4132	0.0065	co-occurrence
Filifactor alocis	Bacteroidetes bacterium HMT 365	0.4879	0.0073	co-occurrence
Eubacteriaceae [XV]	Rothia aeria	-0.4064	0.0082	co-exclusion
	Streptococcus oralis subsp.			
Veillonella rogosae	dentisani clade 398	0.4195	0.0106	co-occurrence
	Clostridiales [F-1][G-1] bacterium			
Filifactor alocis	НМТ 093	0.4911	0.0110	co-occurrence
Bergeyella	Rothia aeria	0.4292	0.0114	co-occurrence
Peptostreptococcaceae		0 4226	0 0 0 0 0 0	
[XI][G-4]	Oribacterium asaccharolyticum	-0.4226	0.0208	co-exclusion
	Bergeyella	0.3834	0.0423	co-occurrence
fretidiosum	Dantastrantasassasaa [VIIIC 6]	0 4005	0 0425	
Clostridiales [E_1][G_1]		0.4005	0.0455	co-occurrence
hacterium HMT 093	Bacteroidetes bacterium HMT 365	0 4320	0 0450	co-occurrence
Veillonella parvula	Veillonella atvnica	0 4449	0.0567	co-occurrence
Veillonella rogosge	Bifidobacterium	0 4224	0.0656	
vemonena rogosae	Clostridiales [F-1][G-1] bacterium	0.1221	0.0000	
Acinetobacter	HMT 093	-0.4243	0.0673	co-exclusion
Fretibacterium				
fastidiosum	Peptostreptococcaceae [XI][G-4]	0.3254	0.0749	co-occurrence
	Streptococcus oralis subsp.			
Veillonella parvula	dentisani clade 398	0.3807	0.0940	co-occurrence
Selemonas sp. HMT 136	Corynebacterium durum	0.4102	0.0943	co-occurrence

### Supplemental Material

### Table S1

Overview of all relative abundances of observed taxa split up into taxa level phylum, genus and species. Data are normalized and display the mean of each PD-group.

### Table S2

Complete regression analysis results of phylum-, class-, order-, family-, genus- and species-level.

### Table S3

Complete edgelist of the network analysis on species-level.

### Table S4

Complete edgelist of the network analysis on genus-level.

### Supplemental Figure SF1

Network of taxa significantly associated with disease severity on genus level. Red lines indicate coexclusion while green lines indicate co-occurrence. The results were filtered for better visualization: Correlations with FDR-corrected p-values <0.1. The width of the lines mirrors the weight strengths. Taxa with positive association with pocket depth appear above the broken line without co-occurring taxa associated with shallower pockets which cluster below the line.