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**Composition of airway bacterial community correlates with chest HRCT in adults with bronchiectasis**

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**Summary at a Glance**

This study reports on the relationship between airway bacterial community composition as measured by high throughput sequencing of the 16S rRNA marker-gene, chest HRCT and clinical measures in BE. The association between loss of diversity and chest HRCT severity suggests bacterial dominance with pathogenic bacteria may contribute to disease pathology.

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**Abstract**

**Background and objective**

In Bronchiectasis not caused by Cystic Fibrosis (BE), chronic, polymicrobial airway infection contributes to the underlying pathogenesis of disease. There is little information on whether bacterial community composition relates to clinical status. We determined the relationship between bacterial community composition, chest high resolution computed tomography (HRCT) scores and clinical markers in BE.

**Methods**

A sub-group of BE patients from a previous cross-sectional study were analysed. Spontaneously expectorated sputum was analysed using culture-independent sequencing on the Roche 454-FLX platform covering the V1-V3 region of the 16S rRNA marker-gene. Chest HRCT scans, multiple breath washout, spirometry and blood inflammatory markers were collected. Pearson (r) correlation coefficient was used to assess relationships.

**Results**

Data from 21 patients were analysed (mean [SD] age 64.0 [7.7]; female:male 14:7; mean [SD] $FEV_1$ 76.5 [17.2]). All bacterial community composition metrics (bacterial richness, diversity, evenness, dominance) correlated with percentage BE score, with more severe HRCT abnormality relating to lower bacterial richness, evenness and diversity (range r= -0.47 to -0.66; $p<0.05$). Inflammation (C-reactive protein and white cell count) was greater in patients with lower diversity and richness (range r=-0.44 to -0.47; $p<0.05$). Bacterial community characteristics did not correlate with lung function.

**Conclusion**

This is the first study to indicate a relationship between bacterial community characteristics by 16S rRNA marker-gene sequencing, structural damage as determined by chest HRCT and clinical measures in BE. The association between loss of diversity and chest HRCT severity suggests bacterial dominance with pathogenic bacteria may contribute to disease pathology.

**Key Words:** bronchiectasis, bacterial community composition, airway microbiome, lung function

**Short title:** Airway bacterial community and HRCT

## Introduction

Bronchiectasis (BE) not caused by cystic fibrosis (CF) is a debilitating illness with symptoms of recurrent cough, daily sputum production, recurrent chest infections, and poor health-related quality of life (HRQoL) [1, 2]. Patients are frequently chronically infected with bacterial pathogens [3]. Chronic and polymicrobial airway infection contributes to the underlying pathogenesis of the disease, with progressive lung damage resulting from recurrent bacterial infections and inflammatory responses [4]. Traditional culture-based methods have identified *Haemophilus influenzae, Pseudomonas aeruginosa* and *Streptococcus pneumoniae* as commonly detected bacterial pathogens [1]. The application of culture-independent techniques has detected a much greater variety of microbes and can provide a more comprehensive description of the composition of the microbial community [5]. A number of studies have investigated the microbial community composition in BE, however there is less information on how community profiles relate to clinical outcomes in BE.

The aim of this study was to explore the relationship between bacterial community composition in sputum (bacterial richness, diversity, evenness and dominance), and chest high resolution computed tomography (HRCT) scores, $FEV_1$% predicted, $FEF_{25-75}$% predicted, lung clearance index (LCI) and blood inflammatory markers (white cell count [WCC] and C-reactive protein [CRP]). We hypothesised that quantitative measurements from HRCT were related to bacterial diversity.

## Methods

### Recruitment

A sub-group of patients from a previously published study (conducted between September 2011 and September 2012) was analysed [6]. In brief, adults with a radiological diagnosis of non-CF BE by HRCT were recruited from the regional BE clinic at Belfast City Hospital, Belfast Health and Social Care Trust. Patients were either non-smokers or ex-smokers. No current

smokers were included. Sputum and blood sample were collected and spirometry and LCI measurements undertaken at a single visit, with a HRCT scan performed within 24 hours of each study visit. Full inclusion and exclusion criteria are provided in the supplementary appendix S1. Written informed consent was obtained from all patients. The study was approved by the Office of Research Ethics Committee for Northern Ireland (ORECNI) reference: 10/ NIR03/44.

**Clinical data**

Demographic data (gender, age, smoking status), a full medical history (including the number of pulmonary exacerbations requiring intravenous antibiotic treatment in the previous 12 months) and current medication was recorded.

**Sputum processing**

*(i) Routine culture*

Routine culture results from the patients most recent expectorated sputum sample, carried out by the Belfast Health and Social Care Trust (BHSCT) clinical microbiology laboratory, and the presence or absence of target pathogens was recorded. If no pathogens were detected but the clinical specimen grew normal commensal flora, a report was issued detailing “no significant growth". The status of *P. aeruginosa* infection as defined by the Leeds criteria [7]. Routine culture for the BE patient population did not assess for non-tuberculosis mycobacterium NTM or fungi.

**(ii)** *Culture independent analysis*

During the scheduled study visit, a spontaneously expectorated sputum sample was collected. Samples were observed for adequate volume (>200mg) and for samples containing a mixture of sputum plugs and liquid phase, the sputum plugs were separated and used for all downstream applications. Processing of sputum samples for culture-independent analysis; extraction of bacterial DNA, library preparation for sequencing on the Roche 454-FLX platform

and processing of raw sequence output were conducted as previously described [5]. After normalisation (via rarefaction), based on the sample with the fewest reads (1800), a total of 37,800 reads were included in all downstream analysis.

**HRCT**

Inspiratory and expiratory HRCT scans of the chest were performed within 24 hours of the study visit, on a 64-slice CT scanner (Siemens AG, Munich, Germany) in the supine position. Two thoracic radiologists (JL, TL) reviewed each scan independently, using a standardized scoring method [8]. This result gives a global CT score of BE and, in addition, scores each lobe on type of BE, extent, mucus plugging, air trapping and emphysema. The complete scoring system can be seen in the supplementary appendix S2. A score for each lobe was recorded, which was then converted to a total score for each of the parameters assessed: total BE score, average bronchiectatic bronchus size, degree of mucus plugging, degree of peribronchial thickening, parenchymal score, and degree of air trapping (hyperinflation score). The higher the number in the scoring method, the more severe the abnormality. Scores were converted into a percent score for comparison.

**Spirometry**

Spirometry was performed during the study visit in accordance with American Thoracic Society/European Respiratory Society guidelines on a MicroLab 3500 spirometer (CareFusion, Basingstoke, UK) [9]. Percent predicted values for $FEV_1$ and $FEF_{25-75}$ were calculated from the all age's reference data published by Stanojevic and colleagues [10].

**LCI**

A MBW test was performed during the study visit using the Innocor gas analyzer (Innovision A/S, Odense, Denmark), modified as previously described to use an open circuit wash-in protocol [11, 12]. The MBW test was carried out before spirometry. During test, patients performed

tidal breathing in the seated position with a nose clip on. Three MBW trials were performed with the mean result of at least two acceptable trials recorded. A test was excluded if functional residual capacity differed by more than 10% from the median of recordings or there was evidence of a leak or irregular breathing pattern. Analysis of washout data was performed with custom software (Simple Washout program).

**Blood inflammatory markers**

A blood sample was collected during the study visit and processed in the routine hospital laboratory for WCC ($10^9$ cells/L) and CRP (mg/L).

**Statistical analysis**

Statistical analysis was performed in SPSS (version 21 PASW Statistics 21; IBM, Armonk, NY), Prism (version 5.01; GraphPad Software Inc., La Jolla, CA) and in the R statistical environment using the vegan package (version 2.4-6) [13]. Ecological indices for the bacterial community composition (alpha diversity), i.e. taxonomic richness [S], Shannon-Wiener diversity index [H], evenness [$e^{H/S}$] and dominance [D] were calculated for each sample. The relationship between clinical variables and ecological community indices were assessed using Pearson correlation coefficient and linear regression [14, 15]. Difference between sub-groups were assessed using Kruskal Wallis test. A p value of $<0.05$ was considered significant. Details of statistical packages are included in supplementary appendix S3.

## RESULTS

Twenty-one subjects from a cohort of 60 subjects (35%) (study 2 in a project to assess whether the lung clearance index is a repeatable and more sensitive indicator of CT scan abnormalities than spirometry in BE; recruited between September 2011 and September 2012) provided a sputum sample and therefore had data available for analysis [6]. Only 35% of subjects provided a sample during the study visit as they were also instructed to collect a 24 hour sputum samples (ahead of the study visit) and not always able to produce an additional fresh sample.

There was a female predominance with the majority idiopathic in aetiology. The full subject characteristics of the 21 subjects are detailed in Table 1.

Using Roche 454-FLX sequencing of the V1V3 region of the 16S rRNA marker-gene, the most common organisms identified (top 10 primary taxa by total read number) included *Haemophilus* spp. (28.2%), *Streptococcus* spp. (23.8%), *Veillonella* spp. (6.7%), *Moraxella* spp. (5.0%), *Corynebacterium* spp. (4.1%), *Prevotella* spp. (4.0%), *Pseudomonas* spp. (3.8%), *Stenotrophomonas* spp. (2.4%), Unclassified family Gamellaceae (2.1%) and *Neisseria* spp. (2.0%). Further information on the relative abundance, defined as the proportion of taxa in each of the samples, is shown in Supplementary Table S2.

All metrics of bacterial community composition correlated with chest HRCT percentage bronchiectasis score (Table 2). The relationship indicated that the more severe the HRCT abnormality the lower the bacterial richness, evenness and diversity. The strongest correlations were seen between chest HRCT percent bronchiectasis score and the Shannon Wiener diversity index ($r=-0.66$; $p=0.001$) and between the chest HRCT percent bronchiectasis score and dominance ($r=0.65$; $p=0.001$) (Supplementary figures S1-6).

There was a significant relationship between blood CRP and both the Shannon Wiener diversity index (r=-0.47; p=0.03) and dominance (r=0.50; p=0.02) (Table 2 and supplementary figure S7). There was also a significant relationship between blood WCC and richness (r=-0.44; p=0.04) (Table 2 and supplementary figure S8).

There was no relationship between any measure of bacterial community composition and $FEV_1$, $FEF_{25-75}$ or LCI (range r=0.01 to 0.21; p>0.05) (Table 2 and supplementary figures S9-11).

On inspection of the relationship between the relative abundance (proportion of taxa in each sample) of the top 10 primary taxa identified, and the HRCT and clinical measures, positive relationships were seen between the abundance of recognised pathogens *Haemophilus* spp.*, Stenotrophomonas* spp. and *Pseudomonas* spp. and a number of clinical measures (i.e. greater relative abundance correlated to a worse score in the clinical measure) (Table 3). Conversely, negative relationships were seen between the abundance of anaerobic organisms belonging to genus *Veillonella* and *Prevotella* and some clinical measures (i.e. greater relative abundance correlated to a better score in the clinical measure).

Considering *P. aeruginosa* colonisation status as measured by routine laboratory culture (never, intermittent, chronic colonisation), only $FEV_1$ (p=0.04) and LCI (p=0.04) could significantly discriminate between groups (never, intermittent and chronically colonised). None of the chest HRCT markers (BE, airway thickening, mucus plugging, parenchymal, air trapping and overall total score) showed a significant difference between these groups.

Eleven out of 21 subjects were taking oral antibiotics at the time of sampling (9/21 macrolides; 2/21 other oral antibiotics). There was a significant negative relationship between those taking oral antibiotics and community richness (r=-0.58; p=0.006) and Shannon Wiener diversity index (r=-0.44; p=0.047) indicating that those taking antibiotics were more likely to have less

bacterial community richness and diversity in the airway. There was no difference in any of the ecological community measures in those taking inhaled antibiotics or inhaled corticosteroids ($p>0.05$).

## DISCUSSION

This is the first study to suggest a relationship between bacterial community characteristics as measured by 16S rRNA marker-gene sequencing, structural abnormality as measured by chest HRCT and a range of clinical measures in patients with BE. In this study, we found that some bacterial community characteristics correlated with structural abnormalities as measured by total chest HRCT score, percentage BE, airway thickening and percentage parenchymal score, with a greater abnormality as measured by HRCT relating to a lower bacterial richness, evenness and diversity. The relationship between blood inflammatory markers, CRP and WCC, and some of the bacterial community composition markers support these findings, with greater inflammation relating to lower bacterial diversity. The relative abundance of some recognised pathogens showed positive relationships with some HRCT and clinical measures, whereas negative relationships were seen between the abundance of anaerobic organisms, HRCT and clinical measures. Such organisms have previously been associated with milder lung disease in CF [16]. Results from this study in BE suggest a similar picture. However, overall bacterial community characteristics did not correlate with lung function.

We have previously demonstrated significant relationships between CT and LCI in BE, which we postulated reflected early lung changes before changes in $FEV_1$ were observed [6]. The relationships seen in this study between HRCT and bacterial community characteristics further

add to the clinical picture, indicating that the microbiome may also be important to consider in the early detection of lung disease.

In this study, significant differences in lung function were seen only with regard to *P. aeruginosa* colonisation status as per routine laboratory culture. It is well established that chronic colonisation with *P. aeruginosa* is associated with poorer lung function in BE [17, 18]. It is less clear how the wider microbiome community composition impacts lung function in BE. HRCT markers are the most sensitive modality in detecting lung disease, particularly changes early in the disease trajectory and/or small degrees of change. Changes in chest HRCT may be evident before lung volumes, flow (spirometry), gas mixing efficiency (LCI) and symptoms are apparent as has been shown in CF [19]. Decreased bacterial diversity in this study was associated with changes detected by chest HRCT, indicating that a lack of diversity may be associated with more severe disease. Therefore, analysis of the airway microbiota in this study may provide a better understanding of the disease pathophysiology in BE.

The relationship observed between greater severity of disease (as measured by HRCT) and lower bacterial richness, evenness and diversity cannot imply causation in this study, and there may be other contributors to disease processes in the airway that are not reflected in the sputum microbiota such as the potential contribution of fungi and/or viruses. Furthermore, the lack of available samples from healthy controls for comparison with samples from BE is a potential limitation to the current study. Although our cohort consisted of individuals with mild to moderate BE, the omission of individuals without marked disease prevents determination of whether people with BE were significantly different from healthy age-matched individuals. However, in a previous study, we observed differences between individuals diagnosed with mild to moderate COPD, smokers and healthy non-smokers, where both community diversity and evenness were significantly lower in individuals with COPD when compared to both of the non-COPD groups [20]. Furthermore, a number of studies have demonstrated a narrowing in airway bacterial taxa and community diversity with disease progression, severity, antibiotic use and age [21-27].

The results from this study add to those reported by Rogers et al. 2013 who reported a relationship between bacterial diversity and inflammation (sputum neutrophil count) in 41 patients with BE. In contrast to our study, Rogers et al. did find that bacterial diversity was significantly positively correlated with $FEV_1$ [28]. The reason for the absence of a relationship between bacterial diversity and lung function in our study may be partly explained by the patient group characteristics. Patients in our study had a milder disease phenotype with a mean [SD] number of pulmonary exacerbations in the previous year 0.2 [0.5] compared with 5.0 [2.8] in the study by Rogers et al. Changes in lung function may have been less apparent in the present study. Furthermore, the smaller cohort studied (n=21) may not have been large enough to show these relationships. In a prospective six-month study, Cox and colleagues examined samples from 85 subjects to assess the associations between bacterial diversity and clinical outcomes [23]. In agreement with our findings, the authors found that the structure of the bacterial community related only weakly to underlying disease as measured by $FEV_1$. Weak relationships between the microbiome composition and clinical status have also been reported in CF by Zhao et al [26]. A further possible explanation for the weak relationship observed between bacterial diversity and clinical outcomes in this study may be the complexity of disease processes which could impact the microbiome e.g. presence of co-morbidities, viral and fungal infections and antibiotic use, as well as the heterogeneity of the patient group. These factors may have introduced significant variability. This study included a number of different underlying aetiologies of BE, which has been previously reported to account for some variance in bacterial communities [23]. Sub-group analysis to stratify patients according to factors which likely impact significantly (disease aetiology, pulmonary exacerbation frequency, antibiotic use) was not appropriate in this small cohort, but is important in future larger studies. The importance of the predominant organism in relation to clinical status has been highlighted by Rogers et al. who found that *P. aeruginosa* and *H. influenzae* dominant communities were associated with poorer clinical outcomes, compared to other taxa dominated communities in BE [29]. In this study, culture of *P. aeruginosa* was associated with poorer $FEV_1$ and LCI but no other individual organism was associated with poorer outcomes.

The significant relationship observed in those patients taking oral antibiotics (macrolides and others) and some of the airway microbiota community characteristics is important to consider as other studies have highlighted antibiotic treatment to be a primary driver for changes in microbiota community structure in CF [30]. The cross-sectional nature of the current study did not allow for the assessment of the response to antibiotic treatment following pulmonary exacerbations, which is the period where most significant changes in the airway microbiota occur. Our findings needs to be replicated in a larger group, including regression analysis to assess the contribution of potential confounding factors in predicting microbiota community structure in BE patients.

The small sample size is a clear limitation and the analysis performed was exploratory. Replication of these correlation analyses and subsequent findings in a larger sample is required with sub-group study analysis considering dominant taxa. More comprehensive characterisation of the clinical status of the patient group in this study (e.g. use of the bronchiectasis severity index score [BSI]) would have useful, given the heterogeneity of the BE patient population. However, this study pre-dated the publication of the BSI score and a component of this composite measure (MRC dyspnoea score) was not collected. Collection of validated composite measure data alongside microbiota data in future studies would facilitate this analysis. This study used spontaneously expectorated sputum samples whereas it could be argued that induced sputum may be more representative by providing greater coverage of the airway. Previous studies including spontaneously expectorated sputum, induced sputum and BAL have shown that although bacterial diversity reported across each method differed in sensitivity, the content was not significantly different [28, 31]. Studies based on marker-gene sequencing of short amplicons have an inherent lack of taxonomic resolution to the level of species. Hence, limitations associated with using genus-level bacterial data, as described in this study, may mask any potential differences in how different bacterial species and/or strains within the same genera interact with their human host.

In conclusion, we report a significant correlation between bacterial community composition in subjects with BE, markers of chest HRCT and blood inflammatory markers. The association between loss of diversity and chest HRCT severity suggests bacterial dominance with pathogenic bacteria may contribute to disease pathology.

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**Author contributions:**

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**Table 1: Characteristics of study subjects (n=21)**

| | Subjects (n=21) |
|---|---|
| Mean (SD) age in years | 64.0 (7.7) |
| Females: males (n) | 14:7 |
| Ethnicity<br>n (%) Caucasian | <br>21 (100) |
| Smoking status<br>n (%) Non-smoker<br>n (%) Ex-smoker | <br>17 (81)<br>4 (19) |
| Disease aetiology<br>n (%) Post infectious<br>n (%) No cause identified<br>n (%) Inflammatory bowel disease<br>n (%) Connective tissue disease<br>n (%) Immune defect | <br>9 (43)<br>9 (43)<br>1 (5)<br>1 (5)<br>1 (5) |
| Medication use<br>Oral antibiotics<br>Mucoactives<br>Inhaled antibiotics<br>Macrolides<br>Inhaled corticosteroids<br>Inhaled bronchodilators | <br>2 (9.5)<br>2 (9.5)<br>3 (14.3)<br>9 (42.9)<br>18 (85.7)<br>19 (90.5) |
| Routine lab culture<br>n (%) *H. influenzae*<br>n (%) no significant growth<br>n (%) *P. aeruginosa*<br>n (%) *S. pneumoniae* | <br>9 (43)<br>8 (38)<br>3 (14)<br>1 (5) |
| n (%) chronic *P. aeruginosa* infection* [7] | 2 (10) |
| Number of PEx in last year | 0.2 (0.5) [0-2] |
| Mean (range) bacterial community richness (no. species) | 27 (9-45) |
| Mean (range) bacterial community evenness | 0.19 (0.07-0.29) |
| Mean (range) bacterial community dominance | 0.41 (0.17-0.94) |
| Mean (range) Shannon Wiener Index | 1.47 (0.17-2.41) |
| Mean (SD) $FEV_1$ % predicted | 76.5 (17.2) [40-110] |
| Mean (SD) $FEF_{25-75}$ % predicted | 61.4 (30.8) [22-168] |

| | |
|---|---|
| Mean (SD) LCI no. turnovers [range] | 9.5 (1.7) [7.3 to 12.9] |
| Mean (SD) CRP mg/L | 4.1 (4.2) [1-20] |
| Mean (SD) WCC x $10^9$/L | 7.0 (1.8) [4-10.8] |

*PEx: pulmonary exacerbation*

*WCC: white cell count*

*CRP: C-reactive protein*

*Routine culture

**Table 2: Univariate correlations (Spearman's rank correlation coefficient) for clinical variables and the main ecological indices of the bacterial community composition (n = 21). P-value <0.05 denote significance.**

| | Richness [S] | Shannon [H] | Evenness [$e^{H/S}$] | Dominance [D] |
|---|---|---|---|---|
| **HRCT Total** | **-0.456 (0.038)** | **-0.544 (0.011)** | -0.429 (0.052) | **0.546 (0.010)** |
| **Bronchiectasis** | **-0.567 (0.007)** | **-0.655 (0.001)** | **-0.474 (0.030)** | **0.654 (0.001)** |
| **Airway Thickening** | -0.306 (0.178) | -0.415 (0.061) | **-0.443 (0.044)** | 0.389 (0.082) |
| **Mucus Plugging** | -0.273 (0.232) | -0.347 (0.123) | -0.192 (0.405) | 0.309 (0.172) |
| **Parenchymal** | **-0.453 (0.039)** | **-0.489 (0.024)** | -0.323 (0.153) | **0.453 (0.039)** |
| **Air Trapping** | -0.242 (0.291) | -0.286 (0.209) | -0.219 (0.340) | 0.289 (0.204) |
| **CRP mg/L** | -0.394 (0.077) | **-0.467 (0.033)** | -0.338 (0.134) | **0.494 (0.023)** |
| **WCC x $10^9$/L** | **-0.442 (0.045)** | -0.422 (0.056) | -0.121 (0.601) | 0.408 (0.067) |
| **$FEV_1$ % predicted** | -0.106 (0.646) | -0.01 (0.964) | -0.01 (0.966) | 0.048 (0.836) |
| **$FEF_{25-75}$ % predicted** | -0.144 (0.533) | -0.023 (0.920) | 0.007 (0.975) | 0.012 (0.958) |
| **LCI (no. of turnovers)** | -0.194 (0.400) | -0.214 (0.351) | -0.087 (0.707) | 0.161 (0.485) |

**Table 3: Univariate correlations between (Spearman's rank correlation coefficient) for clinical variables and the relative abundance of the 10 top taxa (n = 21). P-value <0.05 denote significance.**

| | HRCT Total | Bronchiectasis | Airway Thickening | Mucus Plugging | Parenchymal | Air Trapping | CRP (mg/L) | WCC x $10^9$/L | $FEV_1$ % predicted | $FEF_{25-75}$ % predicted | LCI (no. turnovers) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Haemophilus** | 0.18 (0.45) | 0.28 (0.22) | 0.24 (0.30) | 0.12 (0.61) | 0.13 (0.57) | -0.03 (0.91) | **0.48 (0.03)** | 0.39 (0.08) | 0.27 (0.24) | 0.14 (0.53) | -0.13 (0.59) |
| **Streptococcus** | -0.36 (0.11) | **-0.44 (0.05)** | -0.39 (0.08) | -0.19 (0.42) | -0.22 (0.33) | -0.15 (0.53) | **-0.44 (0.05)** | **-0.54 (0.01)** | -0.12 (0.59) | -0.12 (0.61) | -0.22 (0.33) |
| **Veillonella** | **-0.50 (0.02)** | **-0.59 (0.01)** | -0.26 (0.26) | **-0.48 (0.03)** | **-0.61 (0.001)** | -0.33 (0.14) | **-0.45 (0.04)** | **-0.53 (0.01)** | 0.06 (0.81) | 0.1 (0.67) | -0.36 (0.11) |
| **Moraxella** | 0.19 (0.42) | -0.18 (0.45) | 0.22 (0.33) | 0.21 (0.36) | 0.37 (0.10) | **0.47 (0.03)** | 0.19 (0.41) | -0.11 (0.64) | -0.04 (0.86) | -0.19 (0.42) | 0.10 (0.67) |
| **Corynebacterium** | 0.04 (0.87) | 0.00 (0.99) | -0.15 (0.53) | 0.15 (0.52) | -0.26 (0.25) | 0.02 (0.92) | **-0.48 (0.03)** | 0.09 (0.70) | 0.13 (0.57) | 0.11 (0.63) | -0.14 (0.53) |
| **Prevotella** | **-0.51 (0.02)** | **-0.56 (0.01)** | -0.38 (0.09) | **-0.43 (0.05**) | **-0.45 (0.04)** | -0.36 (0.11) | -0.27 (0.23) | **-0.47 (0.03**) | 0.04 (0.88) | 0.02 (0.92) | -0.39 (0.08) |
| **Pseudomonas** | **0.53 (0.01)** | **0.49 (0.02)** | 0.34 (0.13) | **0.53 (0.01)** | 0.05 (0.82) | 0.22 (0.33) | -0.05 (0.81) | **0.56 (0.01**) | -0.24 (0.29) | -0.14 (0.55) | 0.06 (0.79) |
| **Stenotrophomonas** | -0.02 (0.93) | 0.02 (0.92) | -0.27 (0.24) | 0.03 (0.91) | **0.44 (0.05)** | 0.05 (0.83) | -0.04 (0.88) | -0.18 (0.43) | **-0.51 (0.02)** | **-0.51 (0.02)** | 0.37 (0.10) |
| **f_Gemellaceae_unclassified_2** | -0.36 (0.11) | **-0.56 (0.01)** | -0.06 (0.81) | -0.12 (0.61) | -0.19 (0.40) | -0.09 (0.69 | -0.27 (0.24) | -0.41 (0.07) | -0.13 (0.57) | -0.1 (0.67) | -0.26 (0.26) |
| **Neisseria** | -0.05 (0.83) | -0.27 (0.24) | 0.05 (0.82) | 0.13 (0.58) | 0.05 (0.83) | 0.04 (0.85) | 0.01 (0.97) | 0.13 (0.58) | 0.2 (0.39) | -0.02 (0.93) | -0.14 (0.56) |

**Supplementary Figure legends**

**Figure S1** HRCT Air Trapping Score (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) scatterplot and univariate linear regression analysis

**Figure S2** HRCT Airway Thickening Score (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S3** HRCT Bronchiectasis Score (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S4** HRCT Mucus Plugging Score (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S5** HRCT Parenchymal Score (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S6** HRCT Total Score (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S7** C-reactive protein (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S8** White Cell Count (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S9** $FEV_1$% predicted (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S10** $FEF_{25-75}$% predicted (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis

**Figure S11** LCI (vertical axis; dependent variable) and ecological measurements for bacterial community composition (horizontal axis) *s*catterplot and univariate linear regression analysis