Title: Cross-kingdom analysis of microbial communities in Cystic Fibrosis and Bronchiectasis

Short title: Cross-kingdom analysis of airway microbial communities

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Abstract

Background

Cystic fibrosis (CF) and non-CF bronchiectasis (BX) are characterised by severe chronic infections. Fungal and bacterial components of infection are both recognized. Little however is known about how fungal and bacterial organisms interact and whether these interactions impact on disease outcomes.

Methods

Quantitative PCR and next-generation sequencing of ITS2 and 16S rRNA gene was carried out on 107 patients with CF or BX with clinically defined fungal infection status for all patients. The relationship between fungal and bacterial community composition was extensively explored using: random forest modelling, correlation network analysis, multi-omics factor analysis, and sample-wise clustering, to understand associations both within and between the microbial communities and their relationship to respiratory disease.

Results

Random forest modelling demonstrated distinct fungal and bacterial communities within CF and BX patients. The inclusion of both kingdoms in the models did not improve discrimination between the two diseases. Within the CF patients, bacterial community composition was independent of clinical fungal disease status. Bacterial and fungal communities did not relate to the presence of CF pulmonary exacerbations (CFPE). Correlation network analysis found intra-kingdom interactions were predominant in the data. Multi-omics factor analysis (MOFA) revealed latent factors corresponding to single kingdoms. Thus, in the bacterial community we identified two distinct clusters characterised by the presence or absence of *Pseudomonas*-domination. This was independent of

fungal community which was characterised by a second set of independent clusters dominated by *Saccharomycetes*.

Conclusions

In this study we were unable to detect clear evidence of clinically significant inter-kingdom interactions between the bacterial and fungal communities. While further work is required to fully understand microbial interaction within the lung, our data suggests that interkingdom interactions may not be the primary driver of patient outcomes, particularly in the context of fungal infection.

Keywords: (10 words)

Mycobiome, microbiome, machine learning, random forest, cystic fibrosis, bronchiectasis

Introduction

Chronic respiratory infections are the leading causes of morbidity and mortality for the chronic suppurative lung diseases (CSLD) cystic fibrosis (CF) and non-CF bronchiectasis (BX) (1, 2). Bacterial infections are a major pathophysiological factor in disease progression in these patients (3, 4). The impact of fungal infections is increasingly being recognised and fungal infections have been associated with higher disease burdens, increased exacerbation rates, and accelerated clinical decline (5, 6).

The development of robust microbial sequencing protocols has revealed complex fungal communities within the lungs of patients with CF and BX, exhibiting a range of clinical disease manifestations (7, 8). The recognition of these fungal communities has led to the investigation of bacterial and fungal interactions and their roles in disease progression. Insights into these complex associations and interactions are helped through evolving statistical methodology (8).

Machine learning techniques have proven to be effective for host phenotype prediction from microbiome profiles with random forests exhibiting the strongest predictive performance in host-trait prediction tasks (9). This superior performance is driven in part by their ability to model non-linear interactions between variables. This property is especially useful in microbiome studies, in which the covariates represent a dynamic and interacting ecological system of microbes. Random forests are therefore often preferred over more interpretable linear models, with a recent systematic review finding that random forest was the most popular machine learning model for differential abundance testing in microbiome studies (10).

100 In this study we aimed to explore fungal and bacterial interactions within the
101 lung of patients with chronic respiratory diseases (CF and BX) and understand
102 their relationship to underlying disease, fungal disease diagnosis and CF
103 pulmonary exacerbations (CFPE). Using random forest analysis we explored the
104 predictive power of bacterial and fungal community composition and the
105 presence of CF (n=83) or BX (n=24). This pipeline was subsequently applied to
106 two fungal disease sub-groups within the CF disease group (see Table 1), defined
107 by the presence (n=20) or absence (n=39) of clinical diagnoses of fungal
108 bronchitis (FB). Due to the small number of patients with BX (n=24), we
109 confined this sub-group analysis to CF patients only. Motivated by recent work
110 by Soret *et al.* (8), we also investigated CFPEs.
111 The random forest analyses were complemented by unsupervised approaches
112 investigating both intra- and inter-kingdom associations between taxa
113 (correlation network analysis and multi-omics factor analysis (11)) and samples
114 (Dirichlet multinomial mixtures (12)). The focus of both sets of the analyses was
115 to find evidence of interactions between the two kingdoms.
116
117 **Table 1. Patient demographics**. Data for all samples included in this
118 manuscript. Only samples with paired fungal and bacterial sequencing that
119 passed all quality control steps (see Supplementary Material) were taken
120 forward for analysis. Differences in continuous variables were calculated using a
121 one-way t-test, while a chi-square test was used for categorical variables.
122

	BX (n = 24)	**CF** (n = 83)	*P*
Age/yrs (mean [SD])	59.79 (11.06)	32.00 (11.90)	<0.001
Male (%)	10 (41.7)	47 (56.6)	0.288

BMI (mean [SD])	26.51 (8.73)	22.51 (6.50)	0.029
FEV^1 % predicted (mean [SD])	58.24 (25.23)	48.81 (17.79)	0.047
Disease Group (%)			<0.001
ABPA	8 (33.3)	14 (16.9)	
CNPA	5 (20.8)	0 (0.0)	
FB	0 (0.0)	20 (24.1)	
NAFD	8 (33.3)	39 (47.0)	
NTM	3 (12.5)	10 (12.0)	
Exacerbation* (%)	1 (4.2)	36 (43.4)	0.001

CF: Cystic fibrosis, BX: Non-CF bronchiectasis, BMI: Body mass index, FEV^1: Forced expiratory volume in 1 second, ABPA: Allergic bronchopulmonary aspergillosis, CNPA: Chronic necrotising pulmonary aspergillosis, FB: Fungal bronchitis, NAFD: No active fungal disease, NTM: Non-tuberculosis mycobacteria. * Patients were defined as exacerbating by a change in clinical status from baseline that resulted in a new pulmonary antibiotic treatment.

Methods

Study Design

A prospective, cross-sectional study of spontaneously expectorated sputum from adults with CF and BX was carried out at the Royal Brompton NHS trust between April 2013 and July 2014 (7). Ethical approval was obtained from the Royal Brompton and Harefield Hospital Biomedical Research Unit Ethics Committee (Advanced Lung Disease Biobank study number: 10/H0504/9). Written informed consent was obtained from all study participants prior to sample collection. This study was conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice and the guiding principles of the Declaration of Helsinki and the Research Governance

142 Framework for Health and Social Care.

143 Patient details, including treatments, were collected and subjects were
144 partitioned into defined clinical subgroups according to the diagnostic criteria
145 defined in Cuthbertson *et al.* 2020 (7) (Table 1). Participants within the study
146 cohort were classified into four clinically defined fungal disease groups (see
147 Table 1): (i) fungal bronchitis (FB), (ii) Allergic bronchopulmonary aspergillosis
148 (ABPA), (iii) chronic necrotising pulmonary aspergillosis (CNPA, BX only); and
149 (iv) non-tuberculous mycobacteria (NTM).

150 Sputum samples were collected and processed as previously described, with half
151 the sample sent for routine clinical microbiological culture and the other half
152 stored at -80°C for DNA analysis (7). DNA extraction was performed using the
153 DNA fast spin kit for soil (MPBio, California, USA) according to the
154 manufacturer's instructions. Extraction controls were blinded and processed
155 along with patient samples (7).

156

157 ***Quantitative PCR***

158 Bacterial biomass was quantified by SYBR green quantitative PCR (qPCR) (13).
159 Fungal biomass was estimated using a modified Taqman based qPCR assay as
160 previously described (7). All qPCR reactions were performed in triplicate.

161

162 ***DNA sequencing***

163 16S rRNA gene and ITS2 sequencing were performed on the Illumina MiSeq
164 platform using dual barcode fusion primers. Bacterial sequencing was performed
165 on the V4 region of the 16S rRNA gene as previously described (13). ITS2
166 sequencing was performed using the primers, ITS2F (5'-CAR CAA YGG ATC TCT

167 TGG-3') and ITS2R (5'-GAT ATG CTT AAG TTC AGC GGG T-3') with ligated
168 adaptors (7). Extraction controls, PCR negative and mock communities were
169 included on all sequencing runs.
170 Sequence processing of 16S rRNA gene and ITS2 data was carried out using
171 QIIME 1.9 as described previously in Cuthbertson *et al.* 2017 (13) and
172 Cuthbertson *et al.* 2020 (7) respectively.
173 All sequences were submitted to the European nucleotide database. Bacterial
174 data can be accessed under project number PRJEB33064 with the fungal data
175 accessible under project number PRJEB33434.
176
177 ***Statistical analysis***
178 Statistical analysis was carried out in R version 3.5.1. Data was analysed in
179 phyloseq version 1.24.2 (14). Decontamination of the data was carried out using
180 decontam version 1.1.2 (15); full details are available in Supplementary Material.
181 Differences between categorical variables were calculated using Wilcoxon rank
182 sum test and Kruskal-Wallis. Pearson correlation was used for tests between
183 continuous variables. Differences in microbial community composition were
184 tested with PERMANOVA using the Adonis function from vegan version 2.5-6
185 [23]. Random forests models were fitted using the caret (version 6.0) and ranger
186 (version 0.12) packages 4. The DirichletMultinomial package (version 1.36) was
187 used for sample-wise clustering (16). MOFA analysis used the MOFA2 package
188 (version 1.7) (11). *P*-values were adjusted for multiple corrections using false
189 discovery rate (FDR) throughout.
190
191 ***Random forest-based two-sample testing***

192 Random forest (RF) binary classifiers were used to investigate associations
193 between microbial community composition and the three groups described in
194 Table 2. We agglomerated the OTU counts to each rank in Class, Order, Family,
195 Genus and Species. For each rank we trained three models with the following
196 covariates:

197 1. the agglomerated counts of the bacterial OTUs;

198 2. the agglomerated counts of the fungal OTUs; or

199 3. the agglomerated counts of both the bacterial and fungal OTUs.

200 The five different agglomeration ranks were used in order to investigate the
201 differences between groups at different levels of the taxonomic hierarchy. For all
202 models, any agglomerated taxa present in fewer than 20% of samples were
203 removed. Removal of rare samples has been shown to improve stability and
204 reproducibility of random forest analyses while still retaining discriminative taxa
205 and leaving predictive performance unchanged (17).

206

207 **Table 2. Patient groups investigated using random forest.** These groups
208 were chosen to maximise sample sizes and power of the analyses. The Group
209 phenotype was limited to the FB (n = 20) and NAFD (n = 39) groups within the
210 CF population. BX samples were removed from the group analysis due to the low
211 numbers.

212

Group	Description	Classes	n
Disease	Whether a patient has CF or BX	CF or BX	83 CF, 24 BX (107 total)
Fungal disease (CF only)	Whether a CF patient has been diagnosed with FB or NAFD	FB or NAFD	20 FB, 39 NAFD (59 total)

CFPE (CF only)	Whether a CF patient was experiencing pulmonary exacerbation when the sample was collected (clinician defined).	Yes or No	36 Yes, 47 No (83 total)

CF: cystic fibrosis, BX: non-cystic fibrosis bronchiectasis, FB: fungal bronchitis, NAFD: no active fungal disease.

The predictive performance of each RF model was estimated using 5-fold nested cross-validation with 5 folds in the inner loop. The inner loop performed model selection using a random hyperparameter search of ten combinations of mtry and splitrule. Both inner and outer folds were sampled in a stratified manner meaning that the class proportions in each fold reflected the proportions of the entire dataset. The predictive performance of the models was evaluated using area under receiver-operating characteristic (ROC) and precision-recall (PR) curves. Ninety-five % confidence intervals on the cross-validated area under the curves (AUCs) were computed using the precrec package (18). The statistical significance of AUC values were computed using a label permutation test, where observed AUCs were compared to AUCs from random forest models trained on permuted labels. The dependence of the random forest results to the number of outer-loop folds was investigated by repeating all analyses using 10 outer folds (Supplementary Table S1).

Similar results for this dataset showing the robustness of the predictive metrics (area under ROC and PR curves) and the increased stability of variable importance scores under different transformations are included in the Supplementary Material (Table S1 and Figure S1-5). For the results in the main

text, taxa reads were converted to relative abundances by dividing by the total per-sample reads. The random forest AUC and variable importance results were robust under four additional transformations (identity, centre log-ratio (CLR), log(x+1), and division by the sum of dataset reads). Please see Supplementary Table S1 for AUC results for the random forest models under the different transformations.

Random forest variable importance and differential abundance analysis

One of the primary benefits of random forest modelling is the ability to perform a variable importance analysis. Here, we use both of the two most popular variable importance measures for random forest - Mean Decrease Gini and Mean Decrease Accuracy. In addition, we include the de-biased Mean Decrease Gini scores proposed by Nembrini *et al.* (19).

Each random forest was grown using 1,000 trees to achieve stability in the variable importance scores. The statistical significance of MDA and de-biased MDG scores were assessed using *P*-values calculated using the permutation method of Altmann *et al.* with 1,000 permutations (20). Random forest variable importance scores are unsigned (do not give an effect direction), although an approximate effect direction can be obtained using partial dependence plots. Such plots visualise the marginal effect of a variable on the predictions of a model and are used here to gain further insight into the dependence of model prediction on the relative abundance of different taxa (21).

Correlation analysis, MOFA and sample-wise clustering

259 We analysed the correlation structure amongst the taxa included in the random
260 forest analysis. Samples were centred log-ratio (CLR) transformed prior to
261 calculating correlations to account for the compositional nature of 16S rRNA
262 gene and ITS2 sequencing data. The CLR transformation was applied separately
263 to the 16S rRNA gene and ITS2 samples, as has been previously applied to find
264 accurate cross-omic interactions (22). This pre-transformation requires that the
265 resulting correlations are interpreted as the log-ratios abundance relative to the
266 sample geometric mean rather than in absolute terms. MOFA was run using the
267 same pre-processing as Haak *et al.* (23) (agglomeration to Genus and
268 transformation using CLR prior to analysis). Sample-wise clustering was
269 performed using Dirichlet Multinomial mixtures (12) on the un-transformed
270 reads of each kingdom separately. The number of clusters was selected by
271 comparing the Akaike and Bayesian information criteria and the Laplace
272 approximation of the model evidence for 1 to 15 clusters.
273
274
275 **Results**
276 ***Description of data***
277 After decontamination and removal of samples with less than 2,000 reads (n =
278 2), 107 samples were retained for downstream analyses (for demographics see
279 Table 1). For the ecological analyses, bacterial reads were rarefied to 2,357 while
280 fungal reads were rarefied to 2,542. All other analyses used un-rarefied reads.
281
282 ***Ecological analysis***
283 *Differences between diseases*

284 We performed an ecological analysis on the rarefied data (full details are
285 available in Supplementary Material). In short, Wilcoxon rank sum tests revealed
286 both bacterial and fungal diversity were significantly higher in patients with BX
287 than CF ($P < 0.001$). Similarly, bacterial biomass was significantly higher in the
288 BX group (W = 1,100, effect size = 0.241, $P = 0.018$) but no significant difference
289 in fungal biomass was observed. PERMANOVA revealed significant but small
290 differences in community composition between CF and BX (bacterial, $R^2 = 0.066$,
291 $P < 0.001$; fungal $R^2 = 0.028$, $P = 0.004$).
292
293 *Differences between fungal disease groups in cystic fibrosis*
294 Within the CF group, we compared the two largest fungal disease groups, FB
295 (n=20) and NAFD (n=39). We observed significant differences in fungal alpha
296 diversity between the NAFD and FB groups (Wilcoxon rank sum test, $P < 0.05$).
297 There were no significant differences, however, in bacterial biomass or diversity.
298
299 *CFPE*
300 There was no significant difference in bacterial or fungal, biomass or alpha
301 diversity measures (Wilcoxon rank sum test, $P > 0.1$) between CFPE subjects and
302 those that were stable.
303
304 ***Random forest analysis***
305 *Discriminative power of bacterial and fungal communities*
306 We further investigated differences in fungal and bacterial community
307 composition between groups of patients using random forest modelling (Figure

308 1). For each set of group labels, we trained a random forest binary classifier with
309 covariates being OTU reads agglomerated to Class, Order, Family, Genus, or
310 Species level. Differences between groups were quantified using the area under
311 ROC or PR curve (AU-ROC and AU-PRC). The null hypothesis (no difference
312 between the groups) implies an AU-ROC=0.5 and an AU-PR equal to the
313 proportion of the positive class. The baseline AU-PRC therefore varies between
314 the different sets of group definitions.

315

316

317 **Figure 1:** Discriminative power (quantified using area under ROC and PR curve)
318 of random forest models predicting (a,b): disease status; (c,d): fungal disease
319 status (CF only); and (e,f) and CFPE (CF only). The expected values for a random
320 classifier (indicating no difference between groups) is denoted by a black dotted
321 line. For AU-ROC (plots a,c,e) this is 0.5, while for AU-PRC (plots b,d,f) while for
322 AU-PRC the value is the proportion of samples in the positive class. Error bars
323 are 95% confidence intervals. *: $P < 0.10$, **: $P < 0.05$ for 100 replicates of a label
324 permutation test. P-values adjusted using false discovery rate.

325

326

327 Statistically significant differences between the CF and BX groups were evident
328 at every taxonomic rank. These differences were detected using both the AU-ROC
329 (Figure 1a) or AU-PRC (Figure 1b) metrics. We also found that the differences
330 between CF and BX did not differ significantly between the two kingdoms (label

331 permutation test, $P > 0.10$), meaning that both kingdoms have equally distinct
332 communities between CF and BX. In addition, including both communities in a
333 random forest does not increase the predictive power (label permutation test, P
334 > 0.10).

335 Figures 1c and 1d show that fungal disease group within the CF patients is
336 independent of bacterial community composition. As expected, fungal
337 community composition is a good predictor of fungal disease status within the
338 CF group. Adding the bacteria to the random forest model decreased predictive
339 power at all ranks. The bacterial covariates represent additional noise in the
340 context of fungal disease group however, the models still have better than-
341 random performance due to the inclusion of the fungal taxa (label permutation
342 test, $P < 0.05$).

343 Finally, CFPE was found to be independent of both bacterial and fungal
344 community composition (Figures 1e and 1f), none of the random forest models
345 had predictive power significantly better than random (label permutation test, P
346 > 0.10).

347

348 *Differential abundance analysis*

349 Random forest models that detected a significant difference between their two
350 classes were then analysed using three variable importance measures: mean
351 decrease accuracy (MDA); mean decrease Gini (MDG); and de-biased mean
352 decrease Gini (corrected MDG, (19)). Statistical significance can only be assessed
353 for the MDA and de-biased MDG scores. Only Genus-level agglomeration was

354 included as it is the highest reliable taxonomic resolution for both 16S rRNA
355 gene and ITS2 sequencing (24). The four random forest models that detected a
356 difference between their respective groups were:

357 1. distinguishing CF/BX using bacterial and fungal genera;

358 2. distinguishing CF/BX using bacterial genera;

359 3. distinguishing CF/BX using fungal genera; and

360 4. distinguishing FB/NAFD using fungal genera (CF group only).

361 The most highly-ranked taxa for these four models are shown in Figure 2(a-d).
362 For Model 1, *Penicillium* (direction BX) is the most highly-ranked genus
363 according to all three of the variable importance methods. *Pseudomonas*
364 (direction CF), *Malassezia* and *Neisseria* (direction BX) are also highly-ranked. All
365 four of these associations are significant ($p<0.05$) when using the de-biased MDG
366 scores, but not for MDA scores.

367

368

369 **Figure 2:** Each row shows the four top-ranked taxa and partial dependence for a
370 random forest model distinguishing (a,b): BX/CF from bacterial and fungal
371 genera; (c,d): Bx/CF from bacterial genera; (e,f): Bx/CF from bacterial genera ;
372 and (g,h): FB/NAFD from fungal genera (CF group only). **: P < 0.10, **: P < 0.05*
373 for 100 replicates of a label permutation test. *P*-values adjusted using false
374 discovery rate.

375

376

377 Importance rankings can be augmented by partial dependence plots (Figure 2
378 b,d,f,h) which visualise the marginal effect of a single variable on the prediction
379 of the model. These provide effect directions for the unsigned variable
380 importance scores as well as insight into the type of dependence. For example,
381 the majority of the effect of the important taxa for Model 1 (Figure 2b) occurs
382 when the relative abundance increases from zero to non-zero relative
383 abundance. This is the case for *Pseudomonas*, where the likelihood of a BX
384 prediction quickly decreases as its relative abundance increases, before the rate
385 of decrease slows.

386 Model 2 ranks *Treponema*, *Neisseria* (direction BX), *Pseudomonas* and *Tanerella*
387 (direction CF) highly (Figure 2c). Overlap is observed with Model 1 as the two
388 models share bacterial covariates. The partial dependence (Figure 2d) shows
389 that increasing *Pseudomonas* relative abundance does not increase the likelihood
390 of CF until it increases beyond 50%.

391 Model 3 (Figure 2e), clearly indicates that *Penicillium* is the most important taxa
392 and is significantly associated with an increased probability of BX in this cohort.
393 Once again, there is overlap with Model 1 due to shared fungal covariates.
394 Compared to Model 1, however, this fungal-only model places higher importance
395 on *Penicillium*, which can be seen from the much steeper increase in the partial
396 dependence (Figure 2f) at small relative abundance.

397 Model 4 (Figure 2g) ranks *Candida* (direction NAFD) as the most important taxa,
398 followed by *Exophiala*, *Aspergillus* and *Scedosporium* (direction FB). *Candida* is an
399 opportunistic pathogen, but these partial dependence plots (Figure 1h) show a

400 lower likelihood of FB prediction from the model as a sample becomes
401 increasingly *Candida*-dominated.
402
403 **Correlation analysis and clustering**
404 Correlation network analysis is a useful tool to explore microbial associations (8,
405 25, 26). Strong correlations are commonly observed in microbiome studies and
406 correlations can be positive; associated with microbes inhabiting common
407 ecological niches, or negative; indicating competition. In this dataset we observe
408 clear structure at the genus level (Figure 3a), with blocks of positively correlated
409 bacterial (*Steptococcus*, *Veillonella*, *Rothia*, *Selemonas*, *Prevotella*, *Fusobacterium,*
410 *Atopobium, Neisseria, Haemophilus* and others) and fungal genera (*Serratia*,
411 *Talaromyces*, *Filobasildea*, *Fusarium* and *Trichosporon*). The genera in these
412 blocks come from a single kingdom and so do not indicate prominent cross-
413 kingdom dependencies in the community structure. In addition, there are no
414 significant correlations between members of these two blocks of taxa ($P > 0.01$),
415 suggesting that the two blocks are largely independent of one another. This may
416 be because they occupy separate niches in the respiratory tract or due to
417 sampling bias.
418
419
420 **Figure 3:** (a) Pearson correlation analysis shows that taxa form correlated
421 clusters with members of the same kingdom. Bacterial and fungal abundances
422 are agglomerated to Genus level and transformed (separately) using centred log-
423 ratio prior to calculating correlations. Genera accounting for more than 0.1% of
424 reads in their respective kingdom are shown. * indicates pairwise correlations

425 for which $P < 0.01$. (b): The P-values for the correlations in panel (a) are smaller
426 for correlations within each kingdom than between the two kingdoms (c): Multi-
427 omics factor analysis (MOFA) identifies latent factors that describe the variance
428 in the composition of both kingdoms. However, these latent factors each describe
429 variance in a single kingdom.
430
431
432 The relative importance of intra- and inter-kingdom correlations was further
433 explored by considering the P-values from the pairwise correlations (Figure 3b),
434 which indicated that significant correlations occur within each kingdom more
435 than between. Figure 3b also shows that, while the most extreme correlations
436 are between pairs of fungal genera, the correlation patterns within the bacterial
437 genera are overall more significant.
438 A dedicated multi-omics integration approach was used to further investigate
439 the underlying drivers of cross kingdom community structure by applying Multi-
440 omics factor analysis (MOFA, (11)). MOFA is an unsupervised method that finds
441 latent factors that explain the variance across different "views" of the same
442 samples: in this analysis, bacterial and fungal abundances only identify latent
443 factors that explain variance in a single kingdom (Figure 3c). This is true at five
444 different taxonomic levels of agglomeration (Class, Order, Family, Genus,
445 Species). This provides further evidence on the lack of detectable cross-kingdom
446 dependencies in this dataset.
447 Community structure within the microbial kingdoms across samples was further
448 analysed with Dirichlet mixture components, grouping samples into distinct
449 clusters with similar composition (12). This unsupervised approach provides

450 insight into community-level structure across samples, which may or may not
451 correspond to the pre-defined clinical labels.
452 All 107 samples were clustered using Dirichlet Multinomial Mixture models
453 using raw count values agglomerated to one of the taxonomic ranks. This was
454 performed separately on the two kingdoms and resulted in two sets of cluster
455 labels for each agglomeration rank. Using information-theoretic goodness of fit
456 measures (Figure S6), two distinct bacterial clusters were found at Genus level
457 and two fungal clusters were found at Class level. Both the bacterial (Figure 4a,
458 top) and fungal (Figure 4a, bottom) clusters are separable in Bray-Curtis
459 principal coordinate space.
460 The clusters for bacterial genera are defined by *Pseudomonas* domination (Figure
461 4d) while the fungal class clusters are defined by *Saccharomycetes* domination
462 (Figure 4e).
463
464
465 **Figure 4:** (a, left) Clustering of samples based on bacterial genera abundances
466 identifies two clusters that are separable in Bray-Curtis principle co-ordinate
467 analysis (PCoA) space. (a, right) Clustering using fungal class abundance also
468 finds two clusters that are separable in Bray-Curtis space. The two sets of cluster
469 labels do not correspond to one another nor to clinical labels (see Table 3). (b):
470 Random forest two-sample testing shows that the bacterial cluster assignments
471 are independent of fungal community composition. (c) The fungal cluster
472 assignments show a weak association with bacterial community composition,
473 with only the PR-curves suggesting an association. (d) The bacterial composition
474 of the samples when ordered by cluster clearly shows that they correspond to

475 presence or absence of domination by *Pseudomonas* species. (e) The two fungal
476 clusters are defined by presence or absence of *Saccharomycetes* domination. *: P
477 < 0.10, **: P < 0.05 for 100 replicates of a label permutation test. P-values
478 adjusted using false discovery rate.

479

480

481 Neither the bacterial nor the fungal clusters agree (Adjusted Rand index=-0.01).
482 There is also very low similarity between the cluster labels and clinical labels
483 (Table 3). The ARI values are close to zero, other than for fungal Class cluster and
484 fungal disease status within the CF group (ARI=0.26), however, values still show
485 low levels of agreement. The random forest two-sample testing procedure
486 showed that fungal class is independent of the bacterial community at all levels
487 of agglomeration (Figure 4b). A weak (ROC curves not significantly better than
488 random) association between *Saccharomycetes* domination and bacterial
489 Species, Genus and Family abundance was, however, observed (Figure 4c).

490

491 **Table 3. Adjusted Rand Index (ARI).** ARI between bacterial/fungal cluster
492 assignments and clinical labels show that there is low similarity between the
493 either set of cluster labels and other clinical labels used in this study. The ARI
494 between the fungal and bacterial clustering labels was -0.01.

	Disease	Group (CF only)	CFPE (CF only)
ARI(bacterial cluster, clinical label)	-0.02	-0.02	-0.01
ARI(fungal cluster, clinical label)	0.06	0.26	-0.01

495

496

497 **Discussion**

498 Identifying factors predicting the prevalence and severity of chronic respiratory
499 infections may be crucial for improving clinical outcomes in CSLDs. To date the
500 majority of research has been focused on bacterial pathogens. An increasing
501 number of recent studies however, are showing that fungal infection plays a key
502 role in chronic disease progression both independently of and in concert with
503 the bacterial airway community (8, 27, 28). As such, understanding the inter-
504 kingdom association present within the lungs is an essential step towards
505 effective antimicrobial treatments.

506 A primary motivation of this study was to explore inter-kingdom interactions.
507 Such interactions have been reported previously in both CF and BX (8, 28), as
508 well as in many other settings (29-31). Despite using a range of statistical
509 approaches, we did not however in this present study find strong evidence of
510 such interactions in our dataset (either in general or in relation to CF or BX).
511 Including both kingdoms in the random forest models did not increase the
512 discriminative power of any of the random forest models, while fungal disease
513 status of the CF group was independent of bacterial community composition.
514 Both the correlation and MOFA analysis failed to find evidence of cross-kingdom
515 interactions and instead identified sets of kingdom-specific features that were
516 largely independent of one another. Finally, the sample-wise clustering found
517 that a characteristic feature of fungal community composition (domination by
518 *Saccharomycetes*) was distinct from *Pseudomonas* domination.

519 Taken together, these results suggest no important cross-kingdom interactions
520 present in this dataset. This is surprising given that both the fungal and bacterial
521 communities are sharing the same niche and so must compete for resources, as

522 well as being affected by common environmental changes. Given the results of
523 previous studies, it is unclear whether such interactions biologically exist but are
524 simply not detectable in this dataset.

525 A recent study carried out by Hughes *et al*. established that using culture-based
526 methodologies, the known CF pathogens, *Pseudomonas aeruginosa* and
527 *Aspergillus fumigatus* are rarely cultured from the same sample (32). Despite
528 this, our culture independent techniques clearly show the common presence of
529 both *Pseudomonas aeruginosa* and *Aspergillus fumigatus* reads in the samples. It
530 is possible that microbial interactions within the lungs may not be detected by
531 DNA-based methods. Future work may require functional analyses to explore
532 relative microbial gene expression within the lung.

533 Despite many similarities in the symptoms and treatments of CF and BX, we
534 identified fundamental differences in their microbial communities. Using random
535 forest modelling we found that CF/BX status depends on both fungal and
536 bacterial community composition in this cohort. Furthermore, we found that
537 both communities are equally discriminative of CF/BX status, but the inclusion of
538 both communities in the models does not increase predictive power. This is
539 further evidence that the fungal and bacterial communities are independently
540 distinct between CF and BX and does not provide any evidence of clinically
541 relevant cross-kingdom interactions.

542 These observed differences between the CF and BX groups are likely to be driven
543 by the physiological differences underlying the individual diseases and their
544 effect on the host environment (33). These differences may also be influenced by
545 age, which is a perfect confounder for disease status in this cohort. It is not

546 possible to correct for this confounding effect as CF is a disease affecting
547 individuals from childhood and BX affects older age groups.
548 Variable importance analysis with these random forest models identified a set of
549 genera from both kingdoms that are associated with increased likelihood of CF
550 (*Pseudomonas* and *Scedosporium*) and BX (*Penicillium*, *Neisseria*, *Campylobacter*,
551 *Trichocomaceae*, *Malassezia*, *Enterobacteriaceae* and *Talaromyces*).
552 These results are consistent with the known role of *Pseudomonas aeruginosa* as
553 one of the most common pathogens associated with CF lung disease. In non-CF
554 bronchiectasis, *Pseudomonas* infection may be associated with more severe
555 disease (34) but it was not a prominent factor in our BX patients.
556 Members of the *Neisseria* genus are commonly isolated in the upper respiratory
557 tract with some species being known pathogens (35). Our results may suggest
558 that a pathogenic role for *Neisseria* spp. could be considered for BX and warrants
559 further investigation. Fungal species associated with BX were primarily part of
560 the *Penicillium* genus. Symptomatic infections with *Penicillium* spp. are rare (36)
561 and *Penicillum* spp. are widely present in the air making it a logical part of the
562 normal respiratory flora.
563 The microbiota between patients with and without a clinical fungal infection
564 using the random forest pipeline found that the fungal disease status of the CF
565 group was independent of bacterial community composition, but not fungal
566 community composition. The analysis identified several drivers of fungal
567 bronchitis (*Trichocomaceae*, *Scedosporium*, *Exophiala* and *Aspergillus*) while also
568 finding that increasing *Candida* decreases the likelihood of a fungal bronchitis
569 diagnosis, consistent with previous findings (7).

570 Despite the association with the NAFD group, members of the *Candida* genus
571 (including *Candida albicans* and *Candida parapsilosis*) are well-known
572 opportunistic human pathogens, particularly in immunocompromised patients
573 (6). In adult CF patients, *Candida spp.* colonization has been shown to be
574 associated with use of inhaled steroids, diabetes mellitus and antibiotic
575 treatment. Despite these observations the virulence potential of *C. albicans* in CF
576 is still being explored (6). In the current study *Candida spp*. were present with a
577 lower relative abundance in the FB group suggesting that dominance of
578 filamentous fungi may out-compete *Candida spp.* in these patients. More work is
579 therefore needed to understand the role members of the *Candida* genus play in
580 CF disease progression.
581 Pulmonary exacerbations are major clinical events in patients with CF resulting
582 in lung function decline and clinical disease progression (37). The presence of
583 bacteria and viruses is commonly associated with poor outcomes during CFPE
584 but defining their exact role is challenging. Recent evidence has suggested fungal
585 infections are also associated with increased CFPE although to date few studies
586 have explored this area. A recent publication by Soret *et al*. investigated CFPE
587 using an adapted penalised linear model and cross-sectional data and identified
588 two fungal genera, *Aspergillus* and *Malassezia*, associated with CFPE (8). Our
589 analyses however found that CFPE status was independent of both bacterial and
590 fungal community composition.
591 The importance of viruses has been shown by the sharp reduction in the
592 incidence of CFPE during the COVID pandemic (38). Future studies should
593 include assays for respiratory viruses, and longitudinal measurements may be

594 used to test if intra-patient variation within the bacterial and fungal communities
595 is a contributing factor.
596 We further explored the bacterial and fungal communities and their cross-
597 kingdom dependencies using a series of unsupervised statistical analyses.
598 Correlation network analysis identified two blocks of positively co-correlated
599 genera, where each block contained taxa from a single kingdom. Positive
600 correlations are often interpreted to imply mutualistic relationships between
601 organisms and are often observed between phylogenetically related microbes
602 (39). Negative correlations may imply competition within a niche due to
603 competition for resources. These correlations have previously been observed in
604 multi-omic analyses of CFPE (8). Both positive and negative correlations
605 however are often due to unmeasured factors affecting the host environment
606 and so do not necessarily imply a direct relationship between taxa.
607 Multi-omics analysis using MOFA also found no evidence of cross-kingdom
608 interactions, as the analysis identified a set of kingdom-specific latent factors.
609 The lack of strong cross-kingdom correlation patterns and the results of the
610 MOFA analysis indicates a surprising degree of independence between the two
611 kingdoms although this is inconsistent with previous studies that have indicated
612 a number of inter-kingdom interactions existing within the lung (8, 28).
613 Unsupervised sample-wise clustering analysis identified characteristic features
614 of the dataset identifying two bacterial clusters at the Genus level and two fungal
615 clusters at the Class level. These two sets of cluster labels had low similarity with
616 one another and with the clinical labels from the random forest analyses,
617 suggesting that the relevant structure of the communities may be primarily due
618 to other (possibly environmental) factors. The bacterial clusters were driven by

619 dominance of *Pseudomonas* within individual samples. *Pseudomonas* was also
620 identified by the random forest analysis as being associated with CF, but these
621 clustering results suggest that *Pseudomonas*-dominance is not the only predictor
622 of CF in this cohort. The bacterial cluster label was independent from fungal
623 community composition, providing additional evidence of independence
624 between the bacterial and fungal communities.

625 Inter-kingdom correlations were generally weaker than those within either
626 kingdom (measured by proportion of significant correlations at different
627 significance thresholds). This further indicates that intra-kingdom interactions
628 may play a minor role in these subjects. In addition, correlation patterns
629 between bacterial genera were stronger than those between fungal genera.

630 Our analysis has several limitations that should be considered when interpreting
631 the results. Machine learning is a powerful tool for exploring microbial
632 interactions and drivers of disease, but understanding the limitations of the
633 models is vital for interpretation. Most importantly, associations identified by
634 machine learning models such as random forest do not imply causal links.
635 Furthermore, the importance scores from random forests should be interpreted
636 with care. Using multiple random forest variable importance scores and
637 transformations in the differential abundance analysis reduces the danger of
638 spurious associations but does not provide a framework that allows quantitative
639 statements to be made.

640 A further limitation of this study is the use of 16S rRNA gene sequencing and
641 ITS2 sequencing for the exploration of these communities. This technology
642 allows us to understand the microbial community present within the lung but
643 provides no information on their activity or function.

644

645

646 **Conclusions**

647 Our study suggests that the role the fungal microbiota play in chronic respiratory
648 disease is independent of that played by the bacterial microbiota. Longitudinal
649 studies are required to understand the full impact of fungal infection in CF and
650 BX. Importantly improvements in clinical diagnosis of fungal infections, whether
651 by sequence analysis, transcriptomics, or advanced cultures, could underpin the
652 improvement of patient outcomes. While further work is required to fully
653 understand microbial interaction within the lung, our data suggests that inter-
654 kingdom interactions may not be a major driver of patient outcomes particularly
655 those associated with fungal infection.

656

657 **Declarations**

658 **Availability of data and material**

659 Sequencing data is freely assessable through the European nucleotide database,
660 bacterial data can be accessed under project number PRJEB33064, while the
661 fungal data can be accessed under project number PRJEB33434.
662 Data analysis scripts are freely available on figshare, under the project "Machine
663 learning for exploring microbial inter-kingdom associations in Cystic Fibrosis
664 and Bronchiectasis" DOIs: https://doi.org/10.6084/m9.figshare.17897708 and
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667

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676 manuscript.
677

678 **Authors' contributions**

679 All authors listed provided substantial contributions to this manuscript. Study
680 concept and design; MFM, WOCC, SF, LC, NJS, MRL, MJC. Acquisition of data; IF,
681 PJ, LC, MJC. Statistical analysis and interpretation of the data; LC, JIH. Drafting of
682 the manuscript; LC, JIH, WOCC, SF. Critical revision of the manuscript was carried
683 out by all authors.
684
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686 **References**

687 1. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet.
688 2009;373(9678):1891-904.
689 2. King PT. The pathophysiology of bronchiectasis. Int J Chron Obstruct
690 Pulmon Dis. 2009;4:411-9.
691 3. Zemanick ET, Hoffman LR. Cystic Fibrosis: Microbiology and Host
692 Response. Pediatr Clin North Am. 2016;63(4):617-36.
693 4. Maselli DJ, Amalakuhan B, Keyt H, Diaz AA. Suspecting non-cystic fibrosis
694 bronchiectasis: What the busy primary care clinician needs to know. Int J Clin
695 Pract. 2017;71(2).
696 5. Chotirmall SH, O'Donoghue E, Bennett K, Gunaratnam C, O'Neill SJ,
697 McElvaney NG. Sputum Candida albicans presages FEV(1) decline and hospital-
698 treated exacerbations in cystic fibrosis. Chest. 2010;138(5):1186-95.

699 6. Chotirmall SH, Greene CM, McElvaney NG. Candida species in cystic
700 fibrosis: A road less travelled. Med Mycol. 2010;48 Suppl 1:S114-24.
701 7. Cuthbertson L, Felton I, James P, Cox MJ, Bilton D, Schelenz S, et al. The
702 fungal airway microbiome in cystic fibrosis and non-cystic fibrosis
703 bronchiectasis. Journal of Cystic Fibrosis. 2021;20(2):295-302.
704 8. Soret P, Vandenborght LE, Francis F, Coron N, Enaud R, Avalos M, et al.
705 Respiratory mycobiome and suggestion of inter-kingdom network during acute
706 pulmonary exacerbation in cystic fibrosis. Sci Rep. 2020;10(1):3589.
707 9. Statnikov A, Henaff M, Narendra V, Konganti K, Li Z, Yang L, et al. A
708 comprehensive evaluation of multicategory classification methods for
709 microbiomic data. Microbiome. 2013;1(1):11.
710 10. Kleine Bardenhorst S, Berger T, Klawonn F, Vital M, Karch A, Rubsamen N.
711 Data Analysis Strategies for Microbiome Studies in Human Populations-a
712 Systematic Review of Current Practice. mSystems. 2021;6(1).
713 11. Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, et al. Multi-
714 Omics Factor Analysis-a framework for unsupervised integration of multi-omics
715 data sets. Mol Syst Biol. 2018;14(6):e8124.
716 12. Holmes I, Harris K, Quince C. Dirichlet Multinomial Mixtures: Generative
717 Models for Microbial Metagenomics. Plos One. 2012;7(2).
718 13. Cuthbertson L, Craven V, Bingle L, Cookson W, Everard ML, Moffatt MF.
719 The impact of persistent bacterial bronchitis on the pulmonary microbiome of
720 children. PLoS One. 2017;12(12):e0190075.
721 14. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible
722 interactive analysis and graphics of microbiome census data. PLoS One.
723 2013;8(4):e61217.
724 15. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple
725 statistical identification and removal of contaminant sequences in marker-gene
726 and metagenomics data. Microbiome. 2018;6(1):226.
727 16. Morgan M. DirichletMultinomial: Dirichlet-Multinomial Mixture Model
728 Machine Learning for Microbiome Data. R package version 1340. 2021.
729 17. Cao Q, Sun X, Rajesh K, Chalasani N, Gelow K, Katz B, et al. Effects of Rare
730 Microbiome Taxa Filtering on Statistical Analysis. Front Microbiol.
731 2020;11:607325.
732 18. Saito T, Rehmsmeier M. Precrec: fast and accurate precision-recall and
733 ROC curve calculations in R. Bioinformatics. 2017;33(1):145-7.
734 19. Nembrini S, Konig IR, Wright MN. The revival of the Gini importance ?
735 Bioinformatics. 2018;34(21):3711-8.
736 20. Altmann A, Tolosi L, Sander O, Lengauer T. Permutation importance: a
737 corrected feature importance measure. Bioinformatics. 2010;26(10):1340-7.
738 21. Friedman JH. Greedy function approximation: A gradient boosting
739 machine. The Annals of Statistics. 2001;29(5):1189:232.
740 22. Quinn TP, Erb I. Another look at microbe–metabolite interactions: how
741 scale invariant correlations can outperform a neural network. bioRxiv. 2019.
742 23. Haak BW, Argelaguet R, Kinsella CM, Kullberg RFJ, Lankelma JM, Deijs M,
743 et al. Integrative Transkingdom Analysis of the Gut Microbiome in Antibiotic
744 Perturbation and Critical Illness. mSystems. 2021;6(2).
745 24. Johnson JS, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, et
746 al. Evaluation of 16S rRNA gene sequencing for species and strain-level
747 microbiome analysis. Nat Commun. 2019;10(1):5029.

748 25. Matchado MS, Lauber M, Reitmeier S, Kacprowski T, Baumbach J, Haller D,
749 et al. Network analysis methods for studying microbial communities: A mini
750 review. Comput Struct Biotechnol J. 2021;19:2687-98.
751 26. Gupta S, Hjelmso MH, Lehtimaki J, Li X, Mortensen MS, Russel J, et al.
752 Environmental shaping of the bacterial and fungal community in infant bed dust
753 and correlations with the airway microbiota. Microbiome. 2020;8(1):115.
754 27. Nguyen LDN, Deschaght P, Merlin S, Loywick A, Audebert C, Van Daele S,
755 et al. Effects of Propidium Monoazide (PMA) Treatment on Mycobiome and
756 Bacteriome Analysis of Cystic Fibrosis Airways during Exacerbation. Plos One.
757 2016;11(12).
758 28. Mac Aogain M, Narayana JK, Tiew PY, Ali N, Yong VFL, Jaggi TK, et al.
759 Integrative microbiomics in bronchiectasis exacerbations. Nat Med.
760 2021;27(4):688-99.
761 29. Liu NN, Jiao N, Tan JC, Wang Z, Wu D, Wang AJ, et al. Multi-kingdom
762 microbiota analyses identify bacterial-fungal interactions and biomarkers of
763 colorectal cancer across cohorts. Nat Microbiol. 2022;7(2):238-50.
764 30. Heisel T, Montassier E, Johnson A, Al-Ghalith G, Lin YW, Wei LN, et al.
765 High-Fat Diet Changes Fungal Microbiomes and Interkingdom Relationships in
766 the Murine Gut. mSphere. 2017;2(5).
767 31. Sokol H, Leducq V, Aschard H, Pham HP, Jegou S, Landman C, et al. Fungal
768 microbiota dysbiosis in IBD. Gut. 2017;66(6):1039-48.
769 32. Hughes DA, Rosenthal M, Cuthbertson L, Ramadan N, Felton I, Simmonds
770 NJ, et al. An invisible threat? Aspergillus positive cultures and co-infecting
771 bacteria in airway samples. J Cyst Fibros. 2022.
772 33. Schafer J, Griese M, Chandrasekaran R, Chotirmall SH, Hartl D.
773 Pathogenesis, imaging and clinical characteristics of CF and non-CF
774 bronchiectasis. Bmc Pulmonary Medicine. 2018;18.
775 34. Davies G, Wells AU, Doffman S, Watanabe S, Wilson R. The effect of
776 Pseudomonas aeruginosa on pulmonary function in patients with bronchiectasis.
777 Eur Respir J. 2006;28(5):974-9.
778 35. Weyand NJ. Neisseria models of infection and persistence in the upper
779 respiratory tract. Pathog Dis. 2017;75(3).
780 36. Lyratzopoulos G, Ellis M, Nerringer R, Denning DW. Invasive infection due
781 to penicillium species other than P. marneffei. J Infect. 2002;45(3):184-95.
782 37. de Boer K, Vandemheen KL, Tullis E, Doucette S, Fergusson D, Freitag A, et
783 al. Exacerbation frequency and clinical outcomes in adult patients with cystic
784 fibrosis. Thorax. 2011;66(8):680-5.
785 38. Cookson W, Moffatt M, Rapeport G, Quint J. A Pandemic Lesson for Global
786 Lung Diseases: Exacerbations Are Preventable. Am J Resp Crit Care.
787 2022;205(11):1271-80.
788 39. Weiss S, Van Treuren W, Lozupone C, Faust K, Friedman J, Deng Y, et al.
789 Correlation detection strategies in microbial data sets vary widely in sensitivity
790 and precision. Isme J. 2016;10(7):1669-81.
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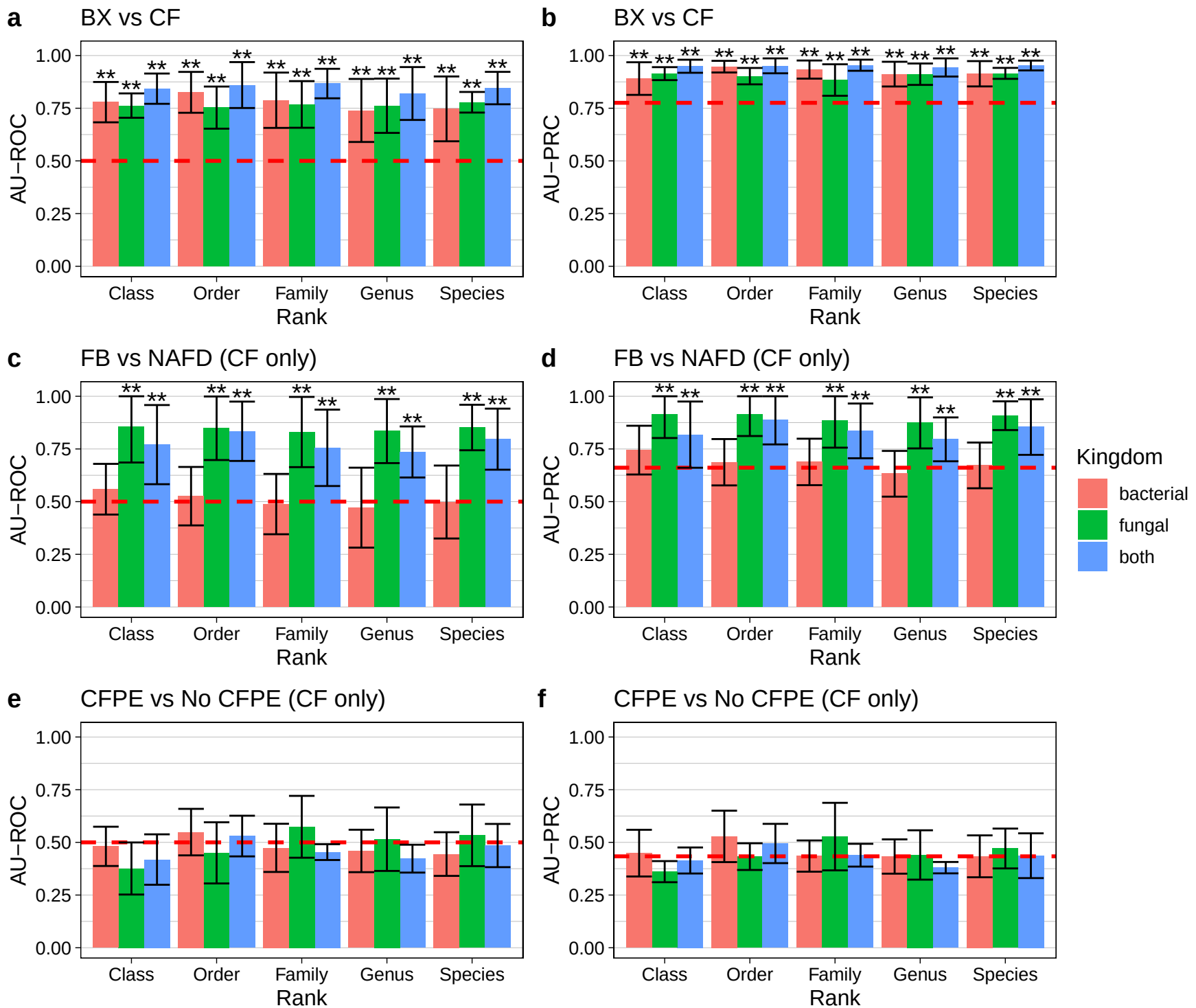
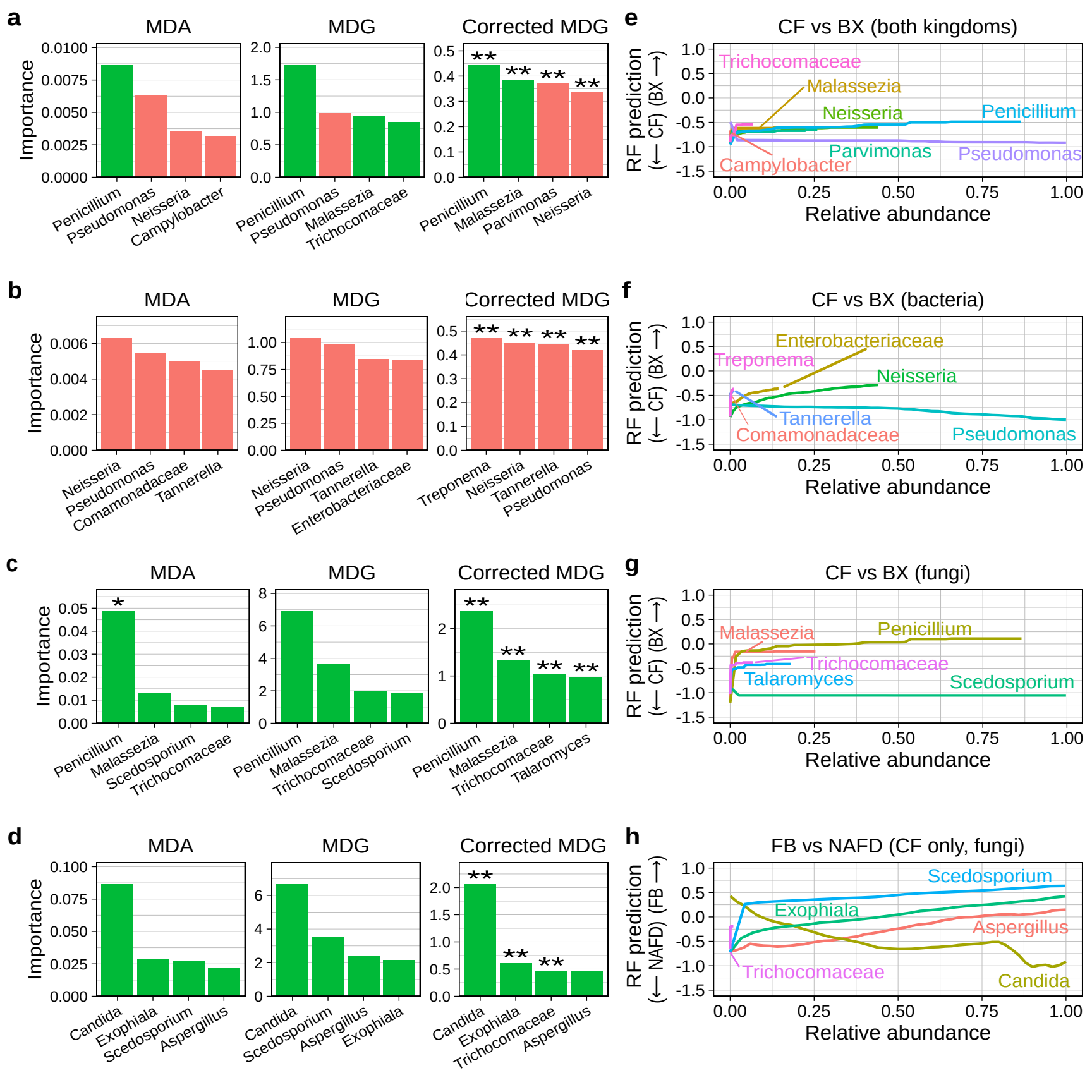
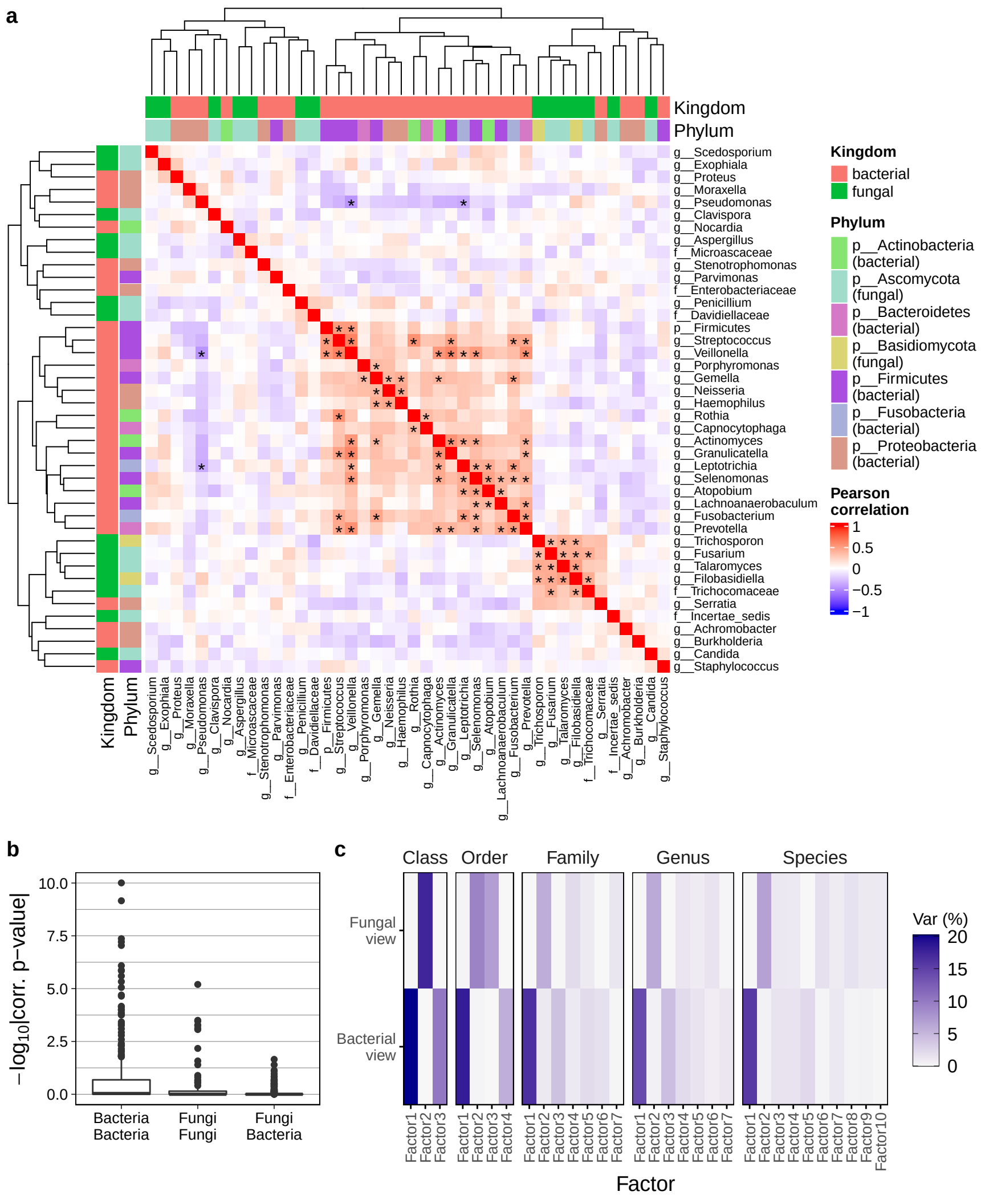
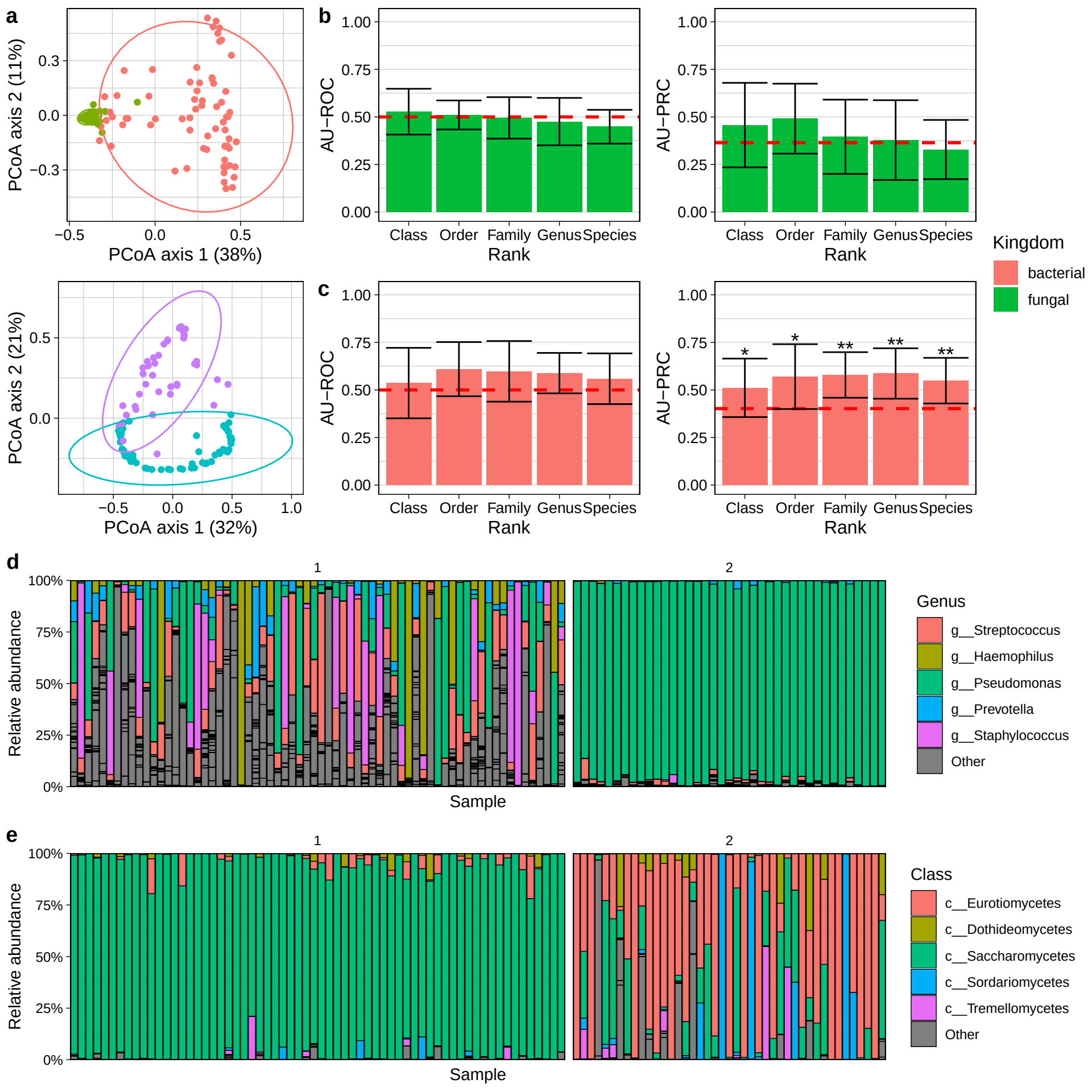

a
BX vs CF
AU−ROC
b
BX vs CF
AU−PRC
c
FB vs NAFD (CF only)
AU−ROC
d
FB vs NAFD (CF only)
AU−PRC
e
CFPE vs No CFPE (CF only)
AU−ROC
f
CFPE vs No CFPE (CF only)
AU−PRC
1.00
0.75
0.50
0.25
0.00
Class
Order
Family
Genus
Species
Rank
Kingdom
bacterial
fungal
both


a
MDA
MDG
Corrected MDG
Importance
Penicillium
Pseudomonas
Neisseria
Campylobacter
Malassezia
Trichocomaceae
Parvimonas
b
Comamonadaceae
Tannerella
Enterobacteriaceae
Treponema
c
Scedosporium
Talaromyces
d
Candida
Exophiala
Aspergillus
e
CF vs BX (both kingdoms)
RF prediction (← CF) (BX →)
Relative abundance
f
CF vs BX (bacteria)
g
CF vs BX (fungi)
h
FB vs NAFD (CF only, fungi)
RF prediction (← NAFD) (FB →)


a
Kingdom
Phylum
g__Scedosporium
g__Exophiala
g__Proteus
g__Moraxella
g__Pseudomonas
g__Clavispora
g__Nocardia
g__Aspergillus
f__Microascaceae
g__Stenotrophomonas
g__Parvimonas
f__Enterobacteriaceae
g__Penicillium
f__Davidiellaceae
p__Firmicutes
g__Streptococcus
g__Veillonella
g__Porphyromonas
g__Gemella
g__Neisseria
g__Haemophilus
g__Rothia
g__Capnocytophaga
g__Actinomyces
g__Granulicatella
g__Leptotrichia
g__Selenomonas
g__Atopobium
g__Lachnoanaerobaculum
g__Fusobacterium
g__Prevotella
g__Trichosporon
g__Fusarium
g__Talaromyces
g__Filobasidiella
f__Trichocomaceae
g__Serratia
f__Incertae_sedis
g__Achromobacter
g__Burkholderia
g__Candida
g__Staphylococcus
Kingdom
bacterial
fungal
Phylum
p__Actinobacteria (bacterial)
p__Ascomycota (fungal)
p__Bacteroidetes (bacterial)
p__Basidiomycota (fungal)
p__Firmicutes (bacterial)
p__Fusobacteria (bacterial)
p__Proteobacteria (bacterial)
Pearson correlation
1
0.5
0
−0.5
−1
b
−log10|corr. p−value|
10.0
7.5
5.0
2.5
0.0
Bacteria Bacteria
Fungi Fungi
Fungi Bacteria
c
Class
Order
Family
Genus
Species
Fungal view
Bacterial view
Var (%)
20
15
10
5
0
Factor1
Factor2
Factor3
Factor4
Factor5
Factor6
Factor7
Factor8
Factor9
Factor10
Factor


a
PCoA axis 2 (11%)
PCoA axis 1 (38%)
b
AU−ROC
AU−PRC
Class
Order
Family
Genus
Species
Rank
Kingdom
bacterial
fungal
PCoA axis 2 (21%)
PCoA axis 1 (32%)
c
d
Relative abundance
Sample
Genus
g__Streptococcus
g__Haemophilus
g__Pseudomonas
g__Prevotella
g__Staphylococcus
Other
e
Class
c__Eurotiomycetes
c__Dothideomycetes
c__Saccharomycetes
c__Sordariomycetes
c__Tremellomycetes
Other