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Distinct airway mycobiome signature in patients with pulmonary hypertension and subgroups

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Abstract

Background The association between lung microbiome and pulmonary hypertension (PH) remain unknown. This study aims to define the airway mycobiome signature and its potential correlation with clinical parameters of PH.

Methods Overall, 244 patients with PH and 120 healthy controls (CON) were recruited from three independent centers. The PH group was divided into subgroups not using antibiotics or corticosteroids (non-ANT/CORT), and those using ANT, CORT, or ANT + CORT within 1 month, and clinical classification (Groups 1, 3, and 4), World Health Organization functional class (I–IV), and disease severity based on mean pulmonary artery pressure or pulmonary vascular resistance levels for in-depth comparison.

Results Distinct airway mycobiome profiles were observed in PH, CON, and PH subgroups. Linear discriminant analysis effect size analysis showed increased *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* and decreased *Peroneutypa*, *Simplicillium*, and *Metarhizium* in patients with PH (non-ANT/CORT, ANT, CORT, and ANT + CORT) than in CON. Receiver operating characteristic analysis indicated a strong prediction of the two fungal genera sets in distinguishing PH and its subgroups from CON. The two major fungal phyla, *Ascomycota* and *Basidiomycota*, correlated differently with major clinical factors. Increased connections among the top fungal phyla or genera were observed in the PH than in the CON group. Dominant enrichment (*Purpureocillium*, *Issatchenkia*, and *Cyberlindnera*) and diminishment (*Peroneutypa*, *Simplicillium*, and *Metarhizium*) of fungal genera consistently and strongly predicted PH without being influenced by different PH subgroups.

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Conclusions This study provides the first description of the unique airway mycobiome signature in PH and among different PH subgroups.

Keywords Airway, Fungus, Microbiome, Mycobiome, Pulmonary hypertension

Background

Pulmonary hypertension (PH) is a cardiopulmonary vascular disease syndrome diagnosed by a mean pulmonary artery pressure (mPAP) of > 20 mmHg at rest, as measured by right heart catheterization (RHC) [1]. Major pathophysiological changes in PH include sustained pulmonary vasoconstriction, concentric vascular remodeling, occlusive intimal lesions, in situ thrombosis, vascular wall stiffening, right heart dysfunction, and failure in the late stages of the disease [2]. PH is a cardiopulmonary disease syndrome associated with multiple other diseases, and recent study has proposed that it could be considered a systemic disease affecting multiple organs, such as the lungs, heart, central nervous system, bone marrow, and gut [3].

Emerging studies have reported a strong association between altered host microbiota and human diseases [4], including lung diseases [5]. The host-microbiota is a complex ecosystem that influences numerous host functions, such as metabolism, vitamin synthesis, gut permeability and function, and immunity [6]. Although most of the studies have focused on the bacteriome, the mycobiome (fungal community) and virome (viral community) have attracted increasing attention as they also play vital roles in numerous human diseases [7, 8], including lung diseases [9, 10]. Compared to bacteriomes, mycobiomes constitute a relatively small part of the human microbiome. Among the uncovered 100,000 fungi identified to date, approximately 100 have been defined as pathogenic [11]. Pulmonary mycosis is usually caused by inhalation of environmental fungal spores [12]. Given the current lack of knowledge, further exploration of the associations and functions of host mycobiomes in human diseases is required. Previous studies have defined the altered airway mycobiome and its correlation with many chronic respiratory diseases, such as severe asthma with fungal sensitization, chronic obstructive pulmonary disease (COPD), and cystic fibrosis [13–15]. However, the specific mycobiome signature in patients with PH remains unclear.

Based on this background, we aimed to first define the characteristics of the airway mycobiome in PH cases and among different subgroups of patients with PH, and to describe the internal correlation between different fungal taxa and between the airway mycobiome and clinical parameters. This study may enrich our understanding of the PH-specific airway mycobiome signature and provide novel insights into the etiology and mechanisms of PH.

Methods

Study recruitment

The PH population, non-PH control, and control subjects were recruited from communities as described in Additional file 1: Fig. S1. The baseline characteristics of all participants are provided in Table 1 and Additional file 2: Table S1. Extended methods are provided in Additional file 3: Supplementary text. According to previously practiced methods [16, 17], pharyngeal swab samples were collected, amplified, quality-controlled, and ITS sequenced (Additional file 1: Fig. S2 and Additional file 4: Uncropped gel images) to systematically analyze the unique airway mycobiome diversity and composition between PH and CON as well as among different PH subgroups according to clinical classification, World Health Organization (WHO) functional class (WHOFC), disease severity (based on mPAP levels), and usage history of ANT and/or CORT. For the PH cohort, the diagnostic criterion for patients with PH was a resting mPAP > 20 mmHg, as measured by RHC. Patients meeting the following criteria were excluded: (a) receiving immunosuppressive (corticosteroids are not included) therapy treatment within 1 month; (b) receiving treatment for malignancies and/or active tuberculosis or mycobacterial disease; and (c) oral or pulmonary infection within 1 month. The PH group was further divided into the subgroups of non-use of antibiotics and/or corticosteroids (non-ANT/CORT) and use of ANT, CORT, or ANT + CORT within 1 month. For the non-PH cohort, the patients were diagnosed with chronic obstructive pulmonary disease, bronchiectasis, interstitial lung disease, and other relevant conditions. All of the patients experienced dyspnea, but their echocardiographic probability of PH was low. The non-PH group was also further divided into the subgroups of non-ANT/CORT and ANT and/or CORT. The healthy-control (CON) group was free of respiratory diseases and other serious diseases and had no oral and respiratory infections within 1 month. Using the above filter conditions, 244 patients with PH (including 124 non-ANT/CORT, 45 ANT, 24 CORT, and 51 ANT + CORT cases) were recruited from three independent medical centers including the First Affiliated Hospital of Guangzhou Medical University (GZMU), Guangdong Provincial People's Hospital (GDPH), and Guangzhou First People's Hospital (GZFH), while 120 CON were recruited from communities. The PH population enrolled from the three

Table 1 Demographics and clinical characteristics of the participants

Characteristics	PH ($n=244$)	CON ($n=120$)	*P* value
Age, years; Medium (range)	63 (15–86)	56 (23–79)	0.15
Female, *n* (%)	150 (61.48%)	68 (56.67%)	0.38
BMI (kg/m^2)	21 (18.48–23.86)	21.95 (19.88–23.51)	0.05
NT-proBNP (pg/mL)	664 (124.1–2188)		
Smoking status, *n*(%)			> 0.99
Current	30 (12.30%)	15 (12.50%)	
Former	32 (13.11%)	16 (13.33%)	
Never	182 (74.59%)	89 (74.17%)	
Clinical classification, *n*(%)			
Group 1	84 (34.43%)		
IPAH	28 (33.33%)[a]		
CTD	28 (33.33%)[a]		
CHD	20 (23.81%)[a]		
Group 3	87 (35.66%)		
Group 4	73 (29.92%)		
Right heart catheter			
mPAP (mmHg)	40 (31.25–53)		
PVR (Wood Units)	6.05 (4–9.03)		
RAP (mmHg)	6 (4–10)		
PAWP (mmHg)	8 (6–11)		
CO (L/min)	5.5 (4.28–6.8)		
CI (L/min/m^2)	3.5 (2.8–4.5)		
SvO_2 (%)	72 (68–76)		
mPAP degree, *n* (%)			
Low (20 < mPAP < 35 mmHg)	87 (35.67%)		
Medium (35 ≤ mPAP < 45 mmHg)	62 (25.41%)		
High (mPAP ≥ 45 mmHg)	95 (38.93%)		
WHO function class, *n*(%)			
I	10 (4.10%)		
II	78 (31.97%)		
III	139 (56.97%)		
IV	17 (6.97%)		
Use of anti-PH medication, *n*(%)			
None	38 (15.57%)		
Prostacyclin pathway agents	13 (5.33%)		
Endothelin pathway antagonists	14 (5.74%)		
NO-sGC-cGMP pathway agents	74 (30.33%)		
Prostacyclin pathway agents + Endothelin pathway antagonists	3 (1.23%)		
Prostacyclin pathway agents + NO-sGC-cGMP pathway agents	13 (5.33%)		
Endothelin pathway antagonists + NO-sGC-cGMP pathway agents	70 (28.69%)		
Prostacyclin pathway agents + Endothelin pathway antagonists + NO-sGC-cGMP pathway agents	19 (7.79%)		
6-min walk distance (m)	384 (310–467)		

PH Pulmonary hypertension, *CON* Healthy control, *BMI* Body mass index, *NT-proBNP* N-terminal-pro-B-type natriuretic peptide, *IPAH* Idiopathic pulmonary arterial hypertension, *CTD* Connective tissue disease, *CHD* Congenital heart disease, *mPAP* Mean pulmonary artery pressure, *PVR* Pulmonary vascular resistance, *RAP* Right atrial pressure, *PAWP* Pulmonary arterial wedge pressure, *CO* Cardiac output, *CI* Cardiac index, SvO_2 Mixed venous oxygen saturation, *NO* Nitric oxide, *sGC* Soluble guanylate cyclase, (c)GMP, (cyclic) guanosine monophosphate

The chi-square test and Mann–Whitney U test were used to produce the *P* values

[a] indicates % of Group 1 PAH

independent medical centers was mostly hospitalized patients who had a higher chance of using ANT, CORT, or ANT + CORT. Among the PH population, 35.7% (87/244) of Group 3 patients with PH were potentially associated with chronic lung diseases (mostly COPD); therefore, 77.0% (67/87) of these patients required treatment with ANT, CORT, or ANT + CORT. Overall, 49.2% (120/244) of the patients with PH used ANT, CORT, or ANT + CORT. The use of ANT, CORT, or ANT + CORT is considered a key factor in the lung mycobiome and was systematically analyzed in this study. A group of patients with dyspnea ($n = 74$) with chronic lung diseases (mostly COPD) but without PH (based on echocardiographic probability of PH as low) was also recruited from three independent medical centers including the First Affiliated Hospital of Guangzhou Medical University (GZMU, $n = 29$), the Second Affiliated Hospital of Guangzhou Medical University (SGZMU, $n = 9$), and the Second People's Hospital of Foshan (SPHF, $n = 36$), serving as a non-PH dyspnea group for comparison with the Group 3 PH. This study was approved by the ethics committee of all participating hospitals and institutions, including the First Affiliated Hospital of Guangzhou Medical University (Ethical approval numbers: 2020–04), the Second Affiliated Hospital of Guangzhou Medical University (Ethical approval numbers: 2019–05-ks-04), Guangdong Provincial People's Hospital (Ethical approval numbers: GDREC2015254H(R1)), Guangzhou First People's Hospital (Ethical approval numbers: K-2022–083-02), and the Second People's Hospital of Foshan (Ethical approval numbers: 2022–0145), and complied with the Declaration of Helsinki. All subjects obtained and signed broad informed consents before enrollment. The study outline is summarized in Additional file 5: STROBE checklist.

Results

Distinct airway mycobiome profile between CON and PH populations involving Group 1, 3, and 4 patients with PH

The recruitment procedure for the participants is outlined in Additional file 1: Fig. S1, and the demographic and clinical parameters of the participants in the CON and PH groups are described in Table 1. As shown in Fig. 1a, α-diversity indicated no statistical difference in mycobiome richness (Sobs index and Ace index), while significantly lower diversity (indicated by decreased Shannon index ($P < 0.001$) and increased Simpson index ($P < 0.001$)) was observed in patients with PH than in CON. For β-diversity, both non-metric multidimensional scaling (NMDS) using the Bray–Curtis distance algorithm ($P = 0.001$, Fig. 1b) and Partial Least Squares Discriminant Analysis (PLS-DA) (Fig. 1c) showed that the mycobiome of CON and PH groups tended to separate at the two-dimensional level. We analyzed the differences at both phylum and genus levels to visualize the different mycobiome compositions in the CON and PH groups. At the phylum level, *Ascomycota* and *Basidiomycota* were major phyla in both the CON and PH groups (Fig. 1d). At the genus level, *Candida*, *Cutaneotrichosporon*, *Apiotrichum*, and *Sarocladium* were the most abundant genera with the highest proportion in the PH group, while *Candida*, *Fusarium*, *Cutaneotrichosporon*, and *Cladosporium* in the CON group (Fig. 1e). Linear discriminant analysis effect size (LEfSe) multilevel species difference discriminant analysis suggested that the genera *Candida*, *Issatchenkia*, *Cyberlindnera*, and *Purpureocillium* had the highest scores for indexing PH (LDA > 3), whereas *Fusarium*, *Cladosporium*, *Metarhizium*, *Simplicillium*, and *Peroneutypa* were the top genera in the CON group (Fig. 1f). Notably, the abundance of *Ascomycota* was significantly higher in the PH group compared to that in the CON group ($P = 0.034$, Fig. 1g). We further analyzed the most comparable genera between the PH and CON groups. The multispecies difference test bar plots showed that at the genus level, *Candida* ($q = 0.003$), *Sarocladium* ($q = 0.012$), *Purpureocillium* ($q = 0.015$), *Issatchenkia* ($q = 0.003$), and *Cyberlindnera* ($q = 0.002$) were significantly enriched, whereas *Fusarium* ($q = 0.007$), *Cladosporium* ($q = 0.004$), *Metarhizium* ($q = 0.002$), *Simplicillium* ($q = 0.005$), *Peroneutypa* ($q = 0.003$), *Aspergillus* ($q = 0.025$), and *Schizophyllum* ($q = 0.009$) were the major genera with dramatically decreased abundance in patients with PH compared to CON (Fig. 1h).

Airway mycobiome composition among different PH subgroups based on clinical classifications and mPAP level

To further analyze the internal variation among different subtypes of PH, we compared the mycobiome diversity and composition among different PH subgroups based on clinical classification, WHOFC, and mPAP levels. First, as shown in Fig. 2a, the mycobiome diversity within different clinical classification subgroups (Groups 1, 3, and 4) was analyzed. The diagnostic process, hemodynamics, and treatment strategies for Groups 1, 3, and 4 are described in Additional file 1: Fig. S3 and Additional file 2: Table S1. Generally, a divergent mycobiome profile was observed, with Group 3 being more different than Group 1 or 4 by α-diversity (Shannon index, $P < 0.001$ and $P = 0.002$) and β-diversity (NMDS) analyses ($P = 0.001$). Significantly higher abundance of *Candida* ($P < 0.001$), *Issatchenkia* ($P = 0.002$), *Cyberlindnera* ($P = 0.016$), and *Purpureocillium* ($P < 0.001$) were observed in PH Groups 1, 3, and 4 than in the CON group. Notably, Group 3 PH showed a significant reduction in diversity and an increased presence of *Candida* compared to Groups 1 and 4. This difference can be attributed to the fact that Group 3 included a much higher percentage of patients

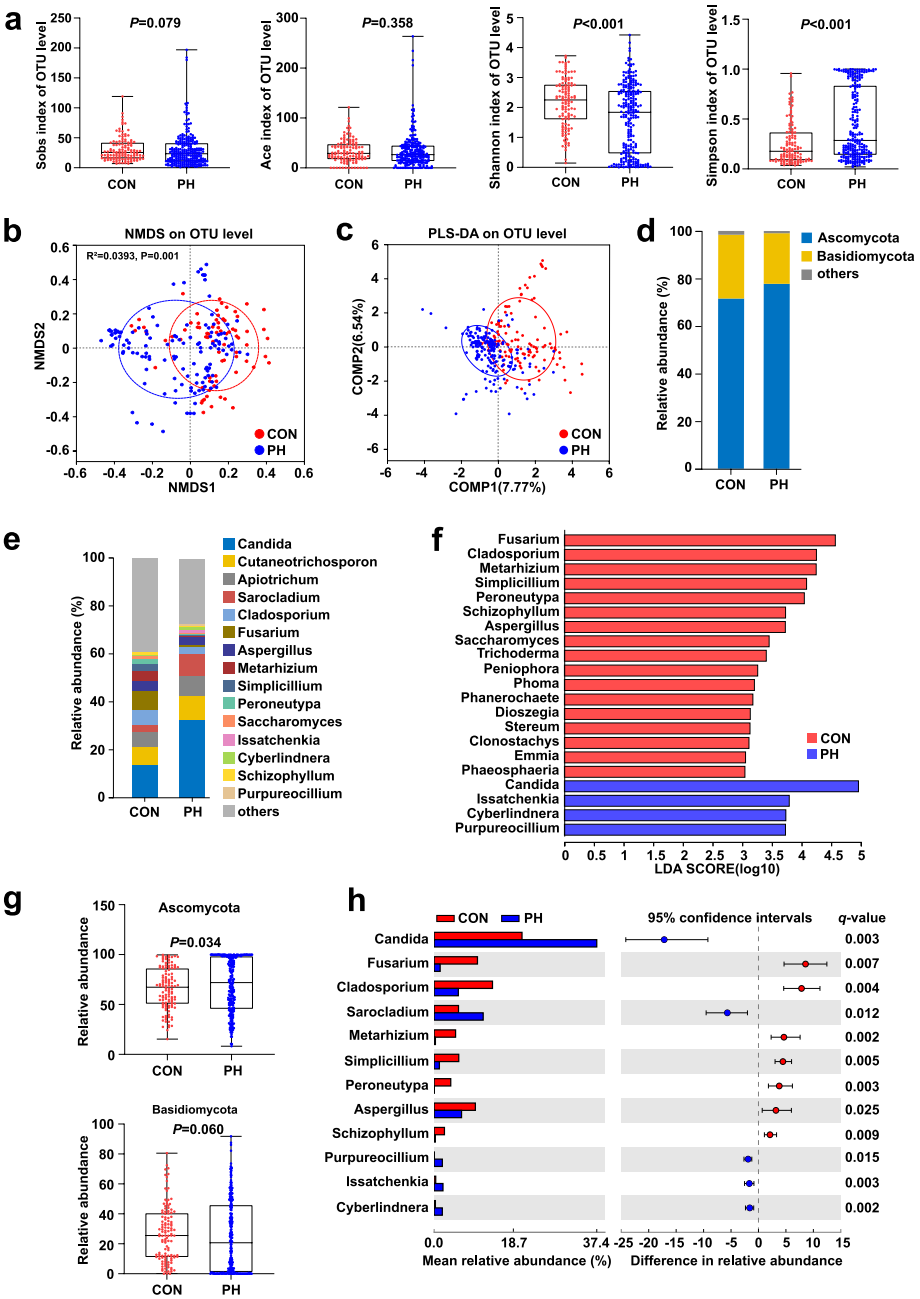


Fig. 1 Airway mycobiome diversity and composition between healthy control (CON) and pulmonary hypertension (PH) groups. **a** Boxplots showing α-diversity (Sobs, Ace, Shannon and Simpson index) between CON (n=120) and PH (n=244) using Mann–Whitney U test. **b** Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity index (Adonis, P=0.001). **c** Partial least squares discriminant analysis (PLS-DA) representing distinct mycobiome. **d**, **e** Percent community abundance (present at > 1% relative abundance) at phylum (**d**) and genus (**e**) levels. **f** Linear discriminant analysis (LDA) effect size (LEfSe) showing the most discriminant fungal genera (only taxa with LDA scores > 3 was presented). **g**Boxplots showing a comparative analysis of *Ascomycota* and *Basidiomycota*in the CON and PH groups. **h** Multispecies difference test bar plots at genus level using White's non-parametric t-test after univariate analysis

who used antibiotics, corticosteroids, or both (67 out of 87), whereas Group 1 had 32 out of 84 patients and Group 4 had 21 out of 73 patients. Previous studies have reported that *Candida* colonization in the respiratory tract is associated with the acute respiratory distress syndrome and prolonged mechanical ventilation in patients with COVID-19 [18–20]. Similar increased *Candida* abundance were also found in patients with interstitial

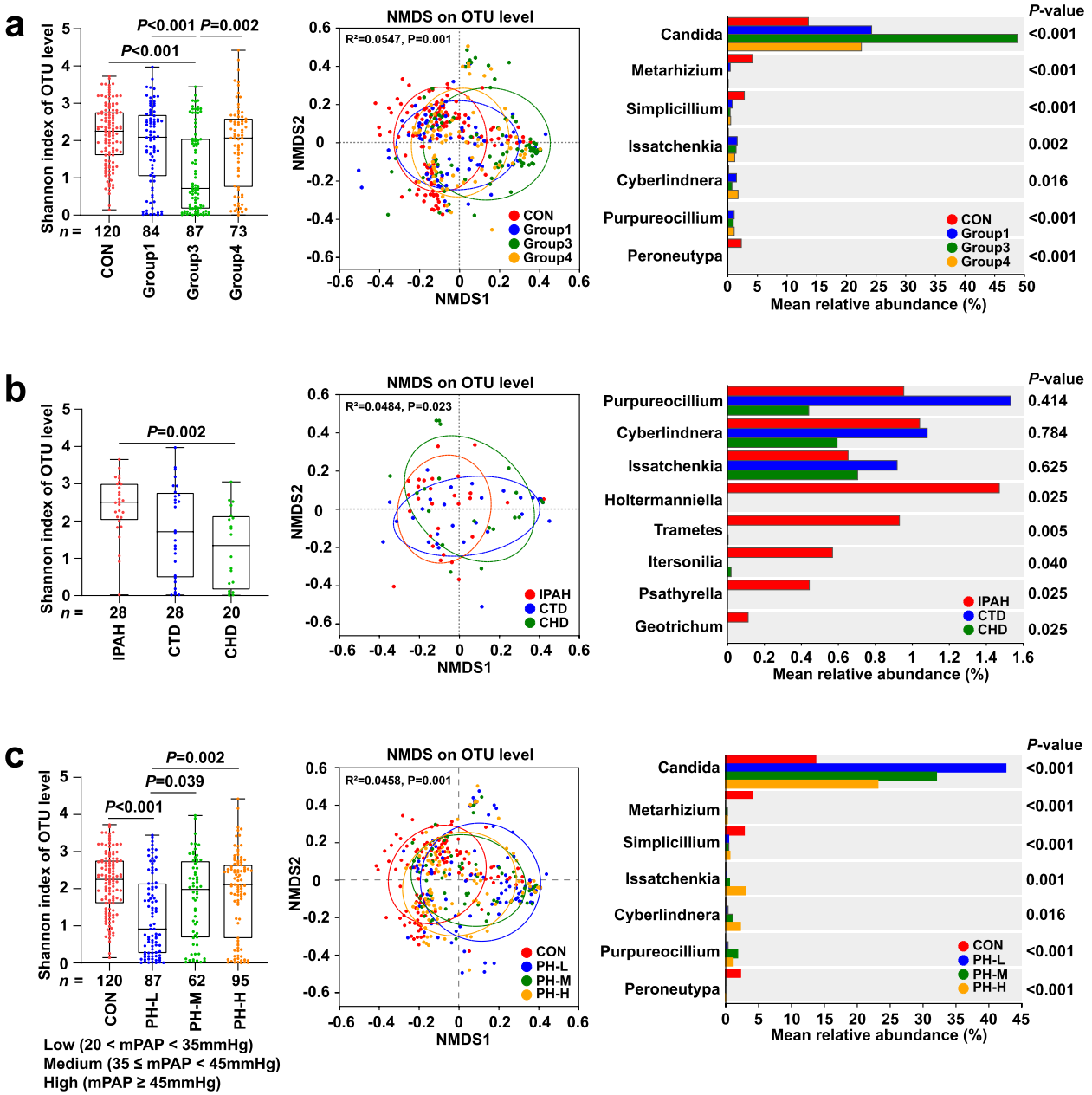


Fig. 2 Airway mycobiome profile comparison between CON and PH subgroups based on clinical classifications and mPAP level. **a–c** Showing α-diversity (Shannon index), NMDS based on Bray-Curtis dissimilarity index and multispecies difference test bar plots at genus level among CON (*n*=120) and different PH groups based on clinical classification (**a** Group 1: *n*=84, Group 3: *n*=87, and Group 4: *n*=73), subgroups of Group 1 PH based on etiology (**b** IPAH: n=28, CTD: 28, and CHD: 20), different mPAP degree (**c** PH-L: *n*=87, PH-M: *n*=62, PH-H: *n*=95)

lung disease [21–23]. Conversely, *Issatchenkia*, *Cyberlindnera*, and *Purpureocillium* exhibited similar elevations, whereas *Metarhizium* ($P<0.001$), *Simplicillium* ($P<0.001$), and *Peroneutypa* ($P<0.001$) showed similar decreases in Groups 1, 3, and 4, indicating that these fungal genera are likely specific features of PH and are not influenced by antibiotic or corticosteroid use. Based on the disease etiology, Group 1 PH was further divided into idiopathic pulmonary arterial hypertension (IPAH), PAH associated with congenital heart disease (CHD-PAH), and connective tissue disease (CTD-PAH). As seen in Fig. 2b, divergent fungal diversity was found by both α-diversity (Shannon index) and β-diversity (NMDS, $P=0.023$) analyses among three subgroups, with genera *Holtermanniella* ($P=0.025$), *Trametes* ($P=0.005$), *Itersonilia* ($P=0.040$), *Psathyrella* ($P=0.025$), and

Geotrichum ($P=0.025$) showing dramatic enrichment in IPAH but not in CHD-PAH or CTD-PAH.

Considering the potential influence of basic chronic lung diseases on airway mycobiomes, we recruited patients with dyspnea ($n=74$) and chronic lung diseases but without PH (based on the low echocardiographic probability of PH) from three medical centers, serving as a non-PH control group (Additional file 1: Fig. S1). The demographic and clinical characteristics of the participants in the non-PH and Group 3 PH groups are presented in Additional file 2: Table S2. Cross-comparison revealed distinct mycobiome profile changes between Group 3 PH ($n=87$) and the non-PH group ($n=74$) (Additional file 1: Fig. S4), as well as between the non-ANT/CORT subgroup (Additional file 1: Fig. S5) and the ANT and/or CORT subgroups (Additional file 1: Fig. S6). Specifically, the increased phylum *Ascomycota* and decreased phylum *Basidiomycota*, together with consistent and dramatically increased proportions of the genera *Purpureocillium* and *Sarocladium* were observed in the overall and subgroups of Group 3 PH versus the non-PH group (Additional file 1: Figs. S4–S6), validating the major findings observed in the PH (subgroups) versus the CON group.

Mycobiome within different subgroups of mPAP levels, including the Low (PH-L, $20<$ mPAP <35 mmHg), Medium (PH-M, $35\leq$ mPAP <45 mmHg), and High (PH-H, mPAP ≥ 45 mmHg) subgroups, were also analyzed. The hemodynamics and treatment strategies for the PH-Low, PH-Medium, and PH-High subgroups are described in Additional file 2: Table S3. As seen in Fig. 2c, divergent α-diversity (Shannon index) and β-diversity (NMDS, $P=0.001$) were observed in different PH subgroups versus CON. At the genus level, a gradually increased abundance of *Issatchenkia* ($P=0.001$) and *Cyberlindnera* ($P=0.016$), and consistently increased abundance of *Purpureocillium* ($P<0.001$) were observed in different PH subgroups based on the mPAP levels. Simultaneously, *Candida* increased dramatically in the PH-low and PH-medium groups (Fig. 2c). Comparing to data shown in PH subgroups divided by mPAP levels, similar trends of mycobiome data were obtained in PH subgroups divided by the levels of PVR, defined as PH-L (PVR <3 Wood Units), PH-M ($3\leq$ PVR ≤ 5 Wood Units), and PH-H (PVR >5 Wood Units). Related data are shown in Additional file 1: Fig. S7.

In addition, mycobiomes within different WHOFC subgroups (WHO-I, WHO-II, WHO-III, and WHO-IV) were also analyzed. Divergent α-diversity (Shannon index) and β-diversity (NMDS, $P=0.001$) were observed in different PH subgroups based on WHOFC. An increase in the WHOFC cardiac function grade was associated with a gradual decrease in the Shannon index and a shift in the NMDS, suggesting that worsened cardiac function may be associated with lower airway mycobiome diversity in patients with PH. At the genus level, gradually increased abundance of *Candida* and consistently increased abundance of *Issatchenkia*, *Cyberlindnera*, and *Purpureocillium* were seen, whereas *Metarhizium*, *Simplicillium*, and *Peroneutypa* showed similar decrease in different WHOFC PH subgroups compared with the CON group (Additional file 1: Fig. S8).

The enriched fungal genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* and the decreased fungal genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* were also consistently captured as the top specific fungal genera in PH (Additional file 1: Fig. S9a), and in most of the PH subgroups, including non-ANT/CORT (Additional file 1: Fig. S9b), and ANT (Additional file 1: Fig. S9c), and CORT (Additional file 1: Fig. S9d), and ANT + CORT groups (Additional file 1: Fig. S9e), using Random Forest analysis.

Effects of ANT, and CORT, or both usage on airway mycobiome profile in PH subgroups

To further assess the roles of ANT, CORT, and their combinations, in the airway mycobiome, we analyzed and compared the airway mycobiome profiles of the CON, non-ANT/CORT, ANT, CORT, and ANT + CORT groups. As seen in Fig. 3, analysis of α-diversity indicated no statistical difference in mycobiome richness (Sobs index and Ace index) and diversity (Shannon index and Simpson index) in non-ANT/CORT PH versus CON (Fig. 3a). The use of CORT, or both significantly decreased diversity (Shannon index, $P=0.002$, and Simpson index, $P=0.001$), whereas the ANT group only showed a statistical difference in the Simpson index ($P=0.041$). It lowered the richness (Sobs index and Ace index), with CORT inducing more dramatic effects than ANT (Fig. 3a). For β-diversity, NMDS (Fig. 3e) showed the CON and different PH subgroups were separate on a two-dimensional level ($P=0.001$). Similarly, *Ascomycota* and *Basidiomycota* were major phyla in the CON and PH subgroups (Fig. 3b). The abundance of *Ascomycota* exhibited a gradual increase in non-ANT/CORT, ANT, CORT, and ANT + CORT subgroups, whereas the abundance of *Basidiomycota* showed a gradual decline, and only ANT + CORT group and CON group had statistical difference ($P<0.001$, Fig. 3d). At the genus level, *Candida*, *Sarocladium*, *Cutaneotrichosporon*, and *Apiotrichum* were the genera with the highest proportions in the PH group, and *Candida*, *Fusarium*, *Cutaneotrichosporon*, and *Cladosporium* were the genera with the highest proportions in the CON group (Fig. 3c), with a significantly increased proportion of *Candida* ($P<0.001$), *Issatchenkia* ($P=0.009$), *Cyberlindnera* ($P=0.016$), and

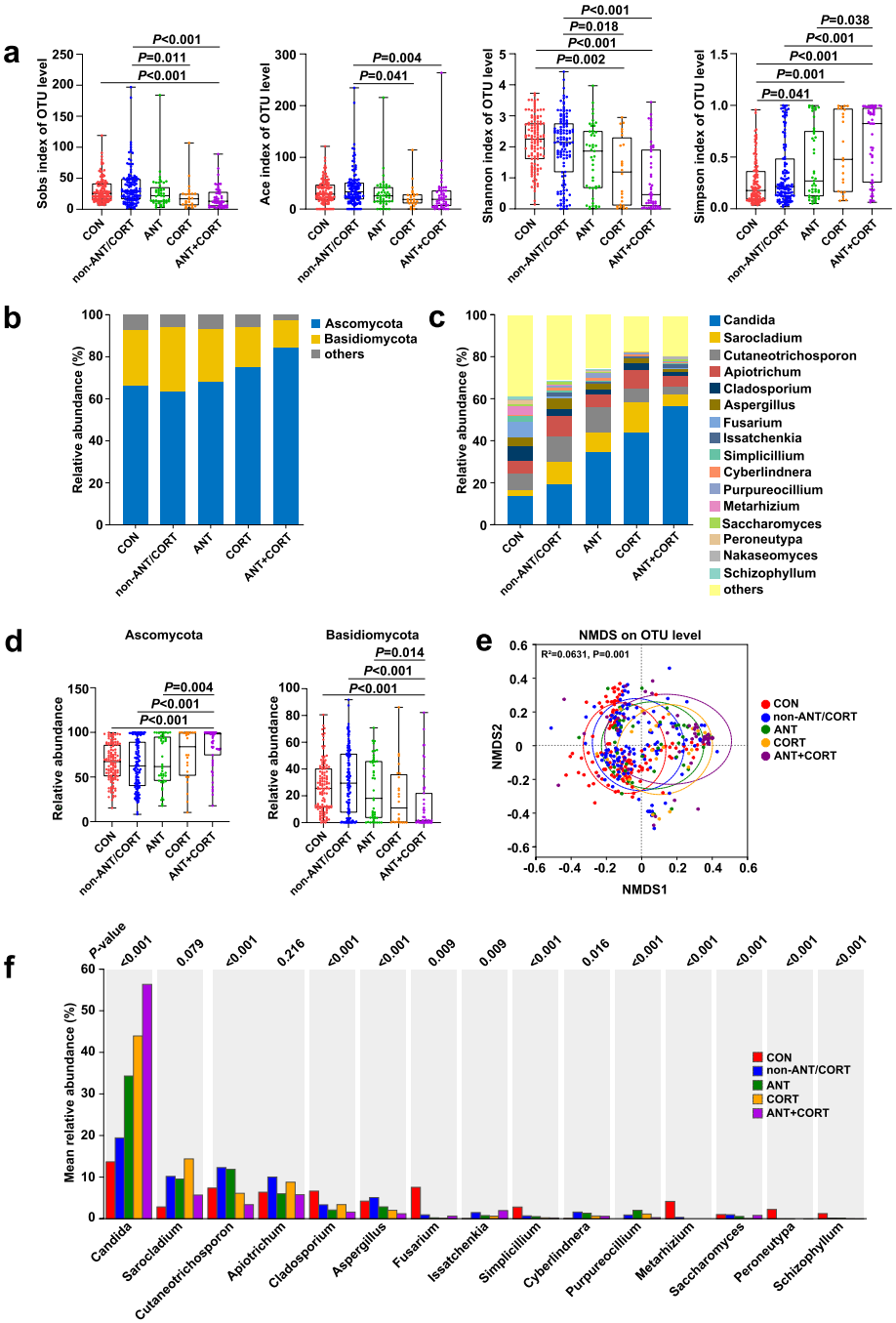


Fig. 3 Airway mycobiome diversity and composition among CON, PH, non-ANT/CORT, usage of ANT, CORT, or both PH subgroups. **a** Boxplots showing α-diversity (Sobs, Ace, Shannon and Simpson indexes) among CON (*n*=120), PH (*n*=244), non-ANT/CORT (*n*=124), ANT (*n*=45), CORT (*n*=24), and ANT+CORT (*n*=51).**b**, **c** Percent community abundance (present at > 1% relative abundance) at phylum (**b**)and genus (**c**) levels. **d** Boxplots showing a comparative analysis of *Ascomycota* and *Basidiomycota*in the CON and PH (subgroups). **e** NMDS based on Bray-Curtis dissimilarity index (Adonis, *P*=0.001). **f** Multispecies difference test bar plots at genus level

Purpureocillium ($P<0.001$) and a decreased proportion of *Cladosporium* ($P<0.001$), *Fusarium* ($P=0.009$), *Simplicillium* ($P<0.001$), *Peroneutypa* ($P<0.001$), *Metarhizium* ($P<0.001$), and *Schizophyllum* ($P<0.001$) observed in different PH subgroups compared to the CON group (Fig. 3f). LEfSe multilevel species difference discriminant analysis also indicated similar fungal genera between the CON and non-ANT/CORT (Fig. 4a), or ANT (Fig. 4b),

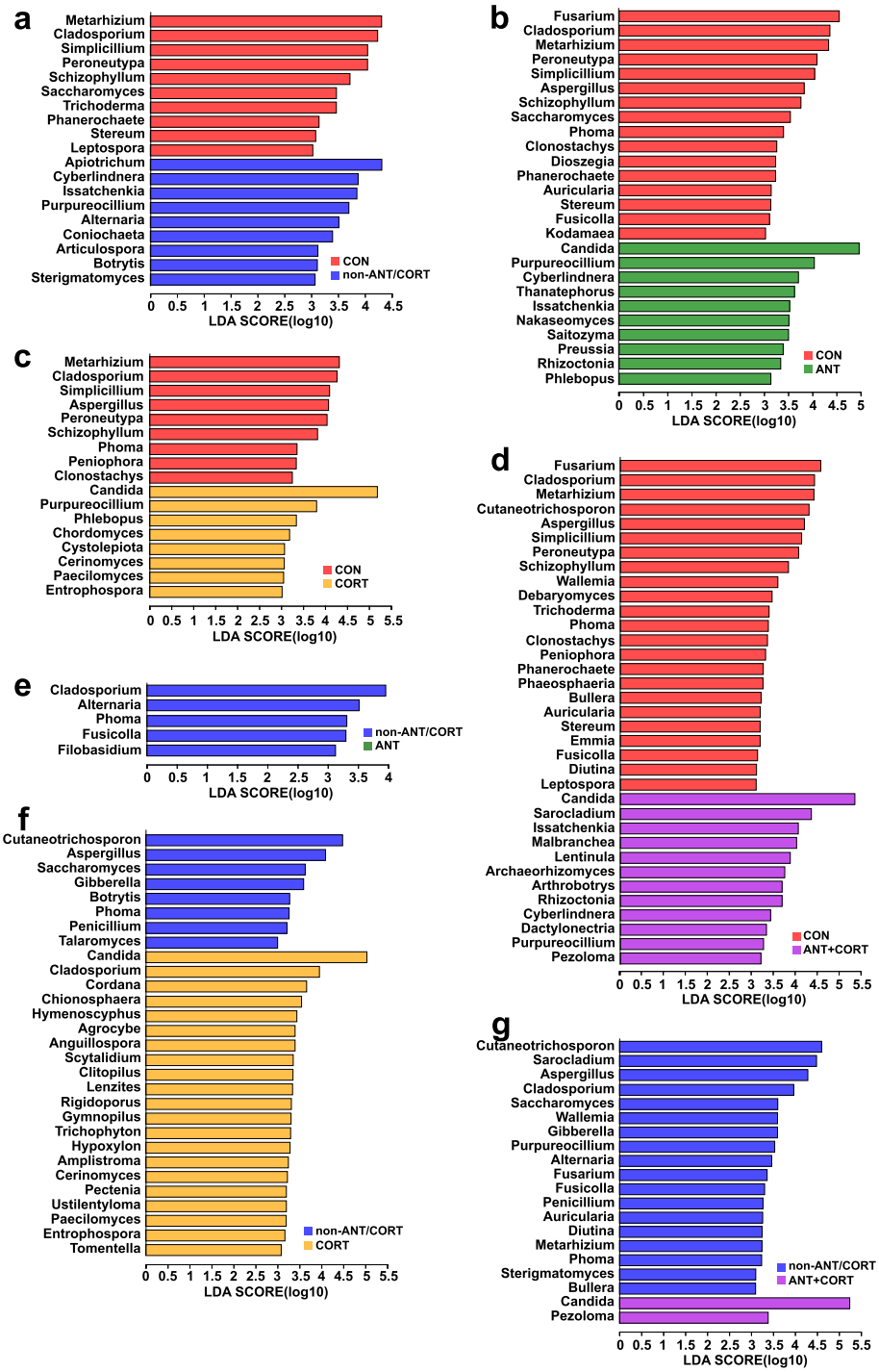


Fig. 4 Distinct airway mycobiome signature between CON and non-ANT/CORT and PH subgroups. **a–d** LEfSe analysis showing the most discriminant fungal genera between CON and non-ANT/CORT (**a**), ANT (**b**), CORT (**c**), or ANT+CORT (**d**), respectively. **e–g** LEfSe analysis showing the most discriminant fungal genera between non-ANT/CORT and ANT (**e**), CORT (**f**), or ANT+CORT (**g**), respectively. Only taxa with LDA scores > 3 was presented

or CORT (Fig. 4c), or ANT + CORT (Fig. 4d) groups. Moreover, the LEfSe multi-level species difference discriminant analysis also suggested similar and distinct genera between the non-ANT/CORT and ANT (Fig. 4e), or CORT (Fig. 4f), or ANT + CORT (Fig. 4g) groups.

Predictive efficiency of specific differential genera for CON and PH

Based on the above analysis, we identified significant and consistent enrichment of the genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* and a decreased proportion of the genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* in the PH and PH subgroups (non-ANT/CORT, ANT, CORT, and ANT + CORT), as defined by the clinical classification, WHOFC, and mPAP levels. Therefore, analyses were performed to evaluate the predictive efficiency of these fungal genera for indexing PH and CON by ROC curve analysis. As seen in Fig. 5, on the one hand, ROC analysis on PH-enriched fungal genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* had an AUC = 0.76 (95% confidence interval [CI], 0.71–0.81) in identifying PH from CON (Fig. 5a), AUC = 0.77 (95% CI, 0.72–0.83) in identifying non-ANT/CORT from CON (Fig. 5b), AUC = 0.77 (95% CI, 0.69–0.85) in identifying ANT from CON (Fig. 5c), AUC = 0.71 (95% CI, 0.59–0.82) in identifying CORT from CON

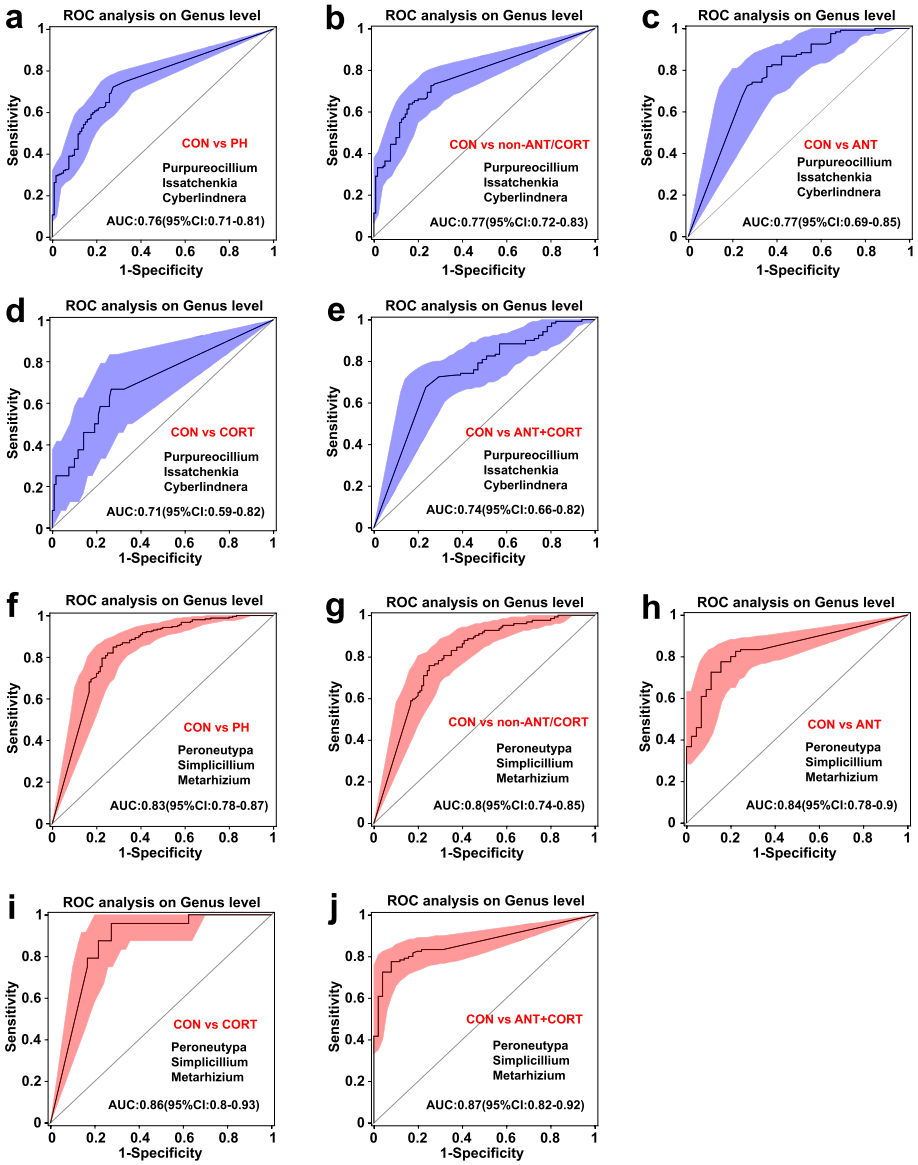


Fig. 5 Prediction analysis for PH, non-ANT/CORT, ANT, CORT, and ANT + CORT versus CON using specific enriched or decreased fungal genera. **a–e** Receiver operating characteristic curves (ROC) of *Purpureocillium*+ *Issatachenkia* + *Cyberlindnera* in identifying PH (**a**), non-ANT/CORT (**b**), ANT (**c**), CORT (**d**), or ANT+CORT (**e**) from CON. **f–j** ROC of *Peroneutypa* + *Simplicillium* + *Metarhizium* in identifying CON from PH (**f**), non-ANT/CORT (**g**), ANT (**h**), CORT (**i**), or ANT+CORT (**j**)

(Fig. 5d), and AUC = 0.74 (95% CI, 0.66–0.82) in identifying ANT + CORT from CON (Fig. 5e). On the other hand, ROC analysis on CON-enriched fungal genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* had an AUC = 0.83 (95% CI, 0.78–0.87) in identifying CON from PH (Fig. 5f), AUC = 0.8 (95% CI, 0.74–0.85) in identifying CON from non-ANT/CORT (Fig. 5g), AUC = 0.84 (95% CI, 0.78–0.9) in identifying CON from ANT (Fig. 5h), AUC = 0.86 (95% CI, 0.8–0.93) in identifying CON from CORT (Fig. 5i), and AUC = 0.87 (95% CI, 0.82–0.92) in identifying CON from ANT + CORT (Fig. 5j).

Moreover, ROC analyses for the PH-enriched fungal genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* and the CON-enriched fungal genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* were also performed in the PH subgroups (Groups 1, 3, and 4). The former showed that AUC = 0.78 (95% CI, 0.72–0.85) in identifying Group 1 from CON (Additional file 1: Fig. S10a), AUC = 0.74 (95% CI, 0.68–0.81) in identifying Group 3 from CON (Additional file 1: Fig. S10b), AUC = 0.75 (95% CI, 0.68–0.82) in identifying Group 4 from CON (Additional file 1: Fig. S10c). The latter analysis on CON-enriched fungal genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* had an AUC = 0.79 (95% CI, 0.72–0.85) in identifying CON from Group 1 (Additional file 1: Fig. S10d), AUC = 0.86 (95% CI, 0.81–0.91) in identifying CON from Group 3 (Additional file 1: Fig. S10e), AUC = 0.84 (95% CI, 0.78–0.89) in identifying CON from Group 4 (Additional file 1: Fig. S10f). The predictive efficiency of these fungal genera sets was generally stronger than the three PH-specific airway bacterial genera, *Streptococcus*, *Lautropia*, and *Ralstonia*, identified in our previous study, which showed an AUC = 0.73 (95% CI, 0.67–0.8) in identifying PH (after exclusion of ANT, CORT, or both usage) from CON. Importantly, the predictive efficiency of these specific fungal genera was not affected by the use of ANT, CORT, or both and was stable across different PH subgroups and independent study populations.

Furthermore, ROC analyses were performed to determine the effects of these selective fungal genera in Group 1 vs. Group 3, Group 1 vs. Group 4, Group 3 vs. Group 4, respectively. ROC analyses were performed to assess the selected increased fungal genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* associated with PH, which show poor predictive values in Group 1 vs. Group 3 (Additional file 1: Fig. S11a, AUC = 0.55 (95% CI, 0.47–0.64)), Group 1 vs. Group 4 (Additional file 1: Fig. S11b, AUC = 0.51 (95% CI, 0.42–0.6)), and Group 3 vs. Group 4 (Additional file 1: Fig. S11c, AUC = 0.54 (95% CI, 0.45–0.63)), respectively. ROC analyses on the decreased fungal genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* associated with PH also show poor predictive values in Group 1 vs. Group 3 (Additional file 1: Fig. S11d, AUC = 0.39 (95% CI, 0.32–0.47)), Group 1 vs. Group 4 (Additional file 1: Fig. S11e, AUC = 0.44 (95% CI, 0.36–0.52)), and Group 3 vs. Group 4 (Additional file 1: Fig. S11f, AUC = 0.55 (95% CI, 0.48–0.63)), respectively. Random Forest analysis identified *Cutaneotrichosporon* as the top different fungal genus in Group 1 vs. Group 3 (Additional file 1: Fig. S12a) and in Group 3 vs. Group 4 (Additional file 1: Fig. S12b), and *Malassezia* as the top different fungal genus in Group 1 vs. Group 4 (Additional file 1: Fig. S12c). ROC analyses show an AUC = 0.72 (95% CI, 0.65–0.8) in Group 1 vs. Group 3 (Additional file 1: Fig. S12d) and an AUC = 0.71 (95% CI, 0.63–0.79) for *Cutaneotrichosporon* in Group 3 vs. Group 4 (Additional file 1: Fig. S12e), while an AUC = 0.55 (95% CI, 0.47–0.64) for *Malassezia* in Group 1 vs. Group 4 (Additional file 1: Fig. S12f).

Correlation analysis between mycobiome and clinical factors and among different mycobiome taxa

We used a variance inflation factor analysis to exclude clinical factors with strong collinearity (threshold > 10). White blood cells and cardiac index were excluded, and the correlation between the remaining 30 clinical factors and different mycobiome taxa (phyla and genera) was analyzed. The results showed that phylum *Ascomycota* was negatively correlated with pulmonary vascular resistance (rho = −0.437, $P < 0.001$), mPAP (rho = −0.293, $P < 0.001$), right atrial pressure (rho = −0.318, $P < 0.001$), mean pulmonary artery diameter (rho = −0.173, $P = 0.007$), right ventricular Tei index (rho = −0.255, $P < 0.001$), body mass index (rho = −0.214, $P < 0.001$), and lymphocyte count (rho = −0.180, $P = 0.005$), while positively correlated with cardiac output (rho = 0.330, $P < 0.001$), tricuspid annular plane systolic excursion (rho = 0.245, $P < 0.001$), right ventricular fractional area change (rho = 0.245, $P < 0.001$), WHOFC (rho = 0.312, $P < 0.001$), age (rho = 0.331, $P < 0.001$), blood urea nitrogen (rho = 0.258, $P < 0.001$), neutrophil count (rho = 0.186, $P = 0.003$), and D-dimer (rho = 0.197, $P = -0.002$). However, the phylum *Basidiomycota* showed the opposite correlation profile to *Ascomycota*, indicating potential mutual competition between these two dominant fungal phyla (Fig. 6a and Additional file 2: Table S4). At the genus level, a specific correlation network was analyzed between the clinical and physical indices and the top 30 major fungal genera (Fig. 6b and Additional file 2: Table S4).

We further analyzed the correlation between mycobiomes in the CON and PH groups. Correlation analyses of the mycobiome at the phylum (top 10 phyla in abundance) and genus (top 50 genera in abundance) levels were performed for the different groups. An increased inner correlation in the airway mycobiome

was observed in the PH group and subgroups (including non-ANT/CORT, and ANT and/or CORT) than in the CON group at both the phylum (Additional file 1: Fig. S13 and Additional file 2: Table S5) and genus (Additional file 1: Fig. S14 and Additional file 2: Table S6) levels. In the CON group, there were 5 positive and 2 negative correlations at phylum level (Additional file 1: Fig. S13a), while there were 181 positive and 45 negative correlations at genus level (Additional file 1: Fig. S14a). In PH group, there were 22 positive and 6 negative correlations at the phylum level (Additional file 1: Fig. S13b), while there were 441 positive and 48 negative correlations at the genus level (Additional file 1: Fig. S14b). In the non-ANT/CORT, 18 positive and 4 negative correlations can be seen at the phylum level (Additional file 1: Fig. S13c), and 264 positive and 28 negative correlations can be seen at the genus level (Additional file 1: Fig. S14c). In the ANT and/or CORT, 11 positive and 5 negative correlations can be seen at phylum level (Additional file 1: Fig. S13d), and 298 positive and 33 negative correlations can be seen at genus level (Additional file 1: Fig. S14d).

Function analysis of mycobiome in PH and CON

There are three types of fungal nutrients: pathotrophs, symbiotrophs, and saprotrophs, which are further subdivided into undefined saprotrophs, animal pathogens, plant pathogens, and wood saprotrophs. The functions in the PH group were dominated by undefined saprotrophs, which were more abundant than those in the CON group (Additional file 1: Fig. S15). Furthermore, a phylogenetic investigation of the communities was performed to predict the functional abundance profile of the two groups using MetaCyc pathway abundance statistics, as outlined in Additional file 1: Fig. S16 and described in Additional file 2: Table S7.

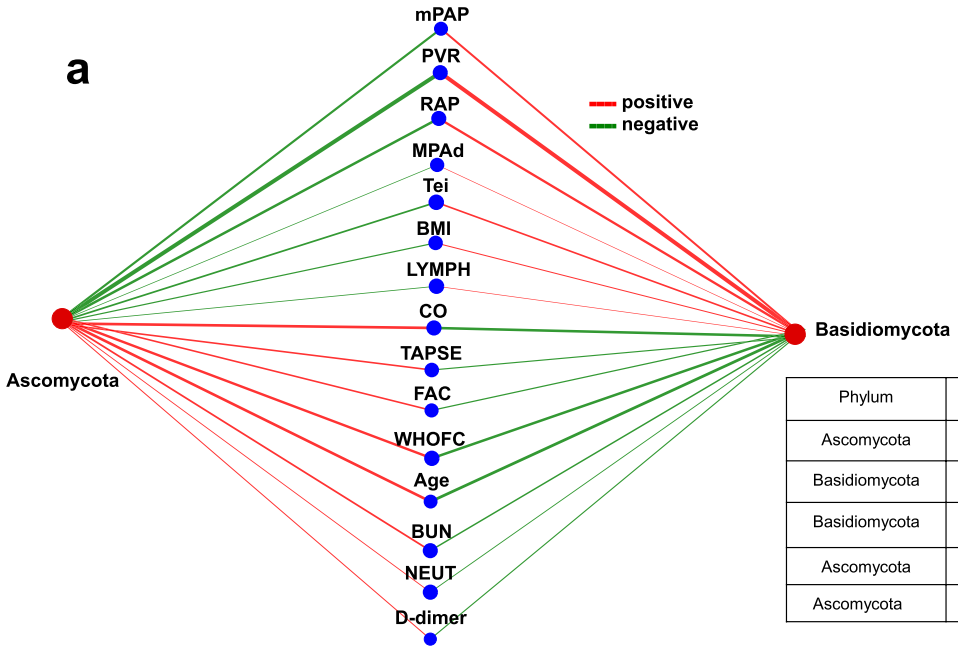
Discussion

The association between host microbiota, especially the gut microbiota, and PH is an emerging research topic. Previous studies have uncovered altered gut microbiome profiles and pathological changes, known as gut dysbiosis, in patients with PH [24, 25] and in experimental animal models [26–29]. Blockage of the metabolite, trimethylamine N-oxide, which is derived from the intestinal microbial flora and has been proven to be enriched in patients with PH [25], can effectively inhibit the disease development of experimental PH in rats [30], suggesting a potential causal role of the altered gut microbiome in driving PH pathogenesis. Compared to the gut microbiota, the specific airway microbiome signature and its role in PH remain poorly studied and are largely unknown. Through an observational study involving 118 patients and 79 reference participants, we collected pharyngeal swab samples and described the PH-specific airway bacteriome signature, including the enrichment of the bacterial genera *Streptococcus*, *Lautropia*, and *Ralstonia* [17]. In a subsequent study, we found that airway delivery of *Streptococcus salivarius*, a major PH-specific bacterium, was sufficient to induce experimental PH in rats, proving the causality between an altered airway bacteriome and PH development [31].

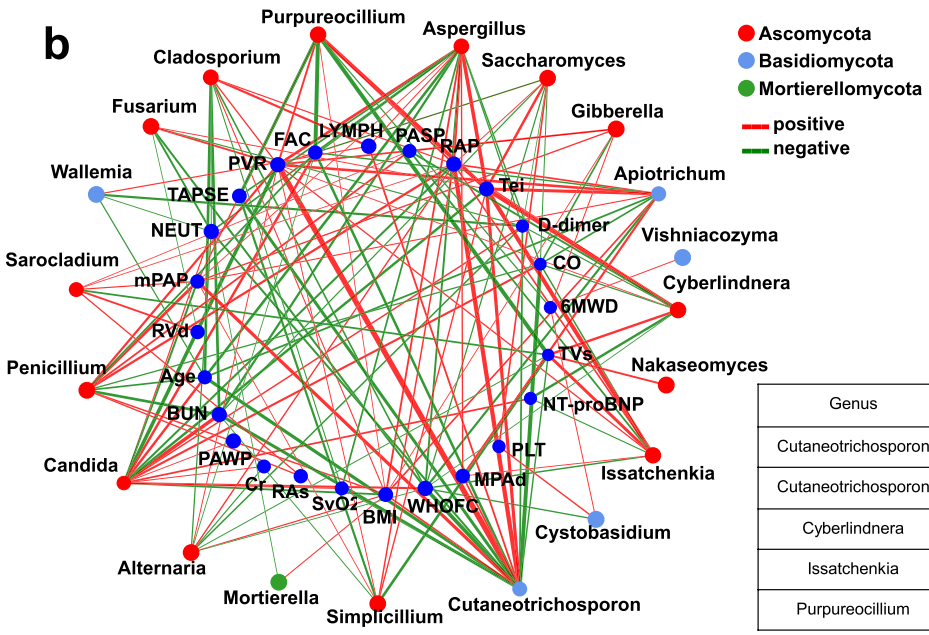
In addition to the bacteriome, evidence indicates that the airway mycobiome plays a key role in maintaining respiratory physiology by interacting with the host system [32–34]. A close association between fungal mycobiome changes and many lung diseases has been reported, such as the distinct airway mycobiome profile between patients with COPD and non-diseased controls, and the specifically altered airway mycobiome associated with frequent exacerbation and high mortality of COPD cases [15]. Although antibiotics normally impact bacterium and do not directly target fungi, the reshaped bacteria microbiota, especially the antibiotics-resistant bacteria, could indirectly affect the fungal community [35]. The effects of antibiotic therapy on fungal loads or fungal

(See figure on next page.)

Fig. 6 Correlation analysis between airway mycobiome taxa and clinical factors. **a** Representing the correlation between phylum level (*Ascomycota*and *Basidiomycota*) and clinical factors of PH by Spearman's rank test. The red circles represented the specific fungal phyla and blue circles represented clinical factors. **b** Representing the correlation between mycobiome taxa at the genus level(top thirty in abundance) and clinical factors of PH by Spearman's rank test. The inner blue circles represented clinical factors, the outer circles represented fungal genera, and different colors represented different phyla as indicated. The red lines represented positive correlation, and green lines represented negative correlation, while thickness of the lines indicated strength of the correlation, and all represented lines have absolute value of correlation coefficient (| rho |) > 0, $P < 0.05$. Tables list top five highest correlation pairs. Detailed correlations can be found in Additional file 2: Table S4. mPAP: pulmonary artery pressure; PVR: pulmonary vascular resistance; RAP: right atrial pressure; MPAd: mean pulmonary artery diameter; Tei: right ventricular Tei index; BMI: body mass index; LYMPH: lymphocyte count; CO: cardiac output; TASPE: tricuspid annular plane systolic excursion; FAC: fractional area change; WHOFC: WHO functional class; BUN: blood urea nitrogen; NEUT: neutrophil count; NT-proBNP: N-terminal-pro-B-type natriuretic peptide; 6MWD: 6-minute walk distance; RVd: right ventricular diastolic diameter; SvO_2: mixed venous oxygen saturation; RAs: right atrial systolic diameter; PASP: pulmonary artery systolic pressure; TVs: tricuspid annular systolic velocity; PAWP: pulmonary arterial wedge pressure; PLT: platelet



Phylum	Clinical indexes	Coefficient
Ascomycota	PVR	-0.44
Basidiomycota	PVR	0.44
Basidiomycota	Age	-0.34
Ascomycota	Age	0.33
Ascomycota	CO	0.33



Genus	Clinical indexes	Coefficient
Cutaneotrichosporon	PVR	0.41
Cutaneotrichosporon	RAP	0.34
Cyberlindnera	Tei	0.34
Issatchenkia	Tei	0.33
Purpureocillium	TVs	-0.32

Fig. 6 (See legend on previous page.)

species composition in the lung varied in different studies. Several studies have indicated that antibiotic usage precedes the increased detection of fungi in sputum [36–40]. Antibiotic therapy in other systems correlates with an increased incidence of fungal infections, such as bloodstream infections [41–44], oral candidosis [45], vaginal candidiasis [46], and increased fungal loads in the gastrointestinal tract [22, 47, 48]. So far, it remains less understood about the potential communication among lung bacterium, mycobiome, and virome under healthy and disease conditions. As summarized by Zhao L and colleagues in an emerging review article [49], secondary

or coinfection with bacteria, viruses, and fungi is common and usually progressive. Complex interactions between the bacteriome, mycobiome, and virome pose a great challenge to infection control in patients with chronic respiratory diseases. This study systematically assessed and profiled the airway mycobiome composition of the PH and CON populations using pharyngeal swab samples and ITS amplicon sequencing. Considering the PH subgroups based on clinical classification, WHOFC, mPAP levels, and usage history of ANT, CORT, or both, the airway mycobiome signatures among the different PH subgroups were further analyzed and described. The results showed distinct airway mycobiome profiles between the PH and CON groups and among the PH subgroups, based on the aforementioned classifications. Notably, through multiple pairwise comparisons, we identified stable and consistent mycobiome signatures in PH (and subgroups) compared to CON without being influenced by ANT, CORT, or both, characterized by specific enrichment of the fungal genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera* and a decrease in the fungal genera *Peroneutypa*, *Simplicillium*, and *Metarhizium* in PH (and subgroups) compared to CON. Further analyses validated that these two fungal genera have stable and strong predictive values for indexing PH and different subgroups from CON. Moreover, a specific correlation pattern was observed between the airway mycobiome signature and clinical parameters of PH, strongly indicating a potential association between the altered airway mycobiome and disease progression of PH. This study provides the first comprehensive analysis of the airway mycobiome signature in patients with PH, enables internal comparison among different PH subgroups, and uncovers stable and consistent PH-specific changes in the airway mycobiome throughout all the assessed PH subgroups.

Antibiotics change the composition of host microbiome rapidly and remarkably [50, 51]. Ng et al. reported that antibiotics significantly reshape the gut microbiota composition, and postantibiotic recovery depends on multiple factors, including the host diet, community context, and environmental reservoirs [52]. Ward et al. showed that antibiotics can alter the α-diversity and β-diversity of lung mycobiome [53]. Corticosteroids are immunosuppressive agents that affect host microbiomes. In a meta-analysis of patients with COPD, asthma, and chronic rhinosinusitis, Hartmann et al. demonstrated that corticosteroids significantly affected the airway microbiome composition [54]. Our study showed similar results: the α-diversity, β-diversity, and mycobiome composition were remarkably reduced in the ANT, CORT, or ANT + CORT groups versus the non-ANT/CORT group. However, *Candida* was mildly enriched in the ANT group and more significantly enriched in the CORT and ANT + CORT groups, suggesting a positive role of ANT, CORT, or ANT + CORT usage on *Candida* in competing with other fungal genera. In contrast, neither the PH-enriched genera *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera*, nor the CON-enriched genera *Peroneutypa*, *Simplicillium*, and *Metarhizium*, were altered by ANT, CORT, or ANT + CORT usage, highlighting their values as PH-specific airway mycobiome signatures. Further analyses showed consistent differential expression patterns of the two mycobiome genera sets in different PH subgroups and different centers, which were not influenced by clinical classification (Groups 1, 3, and 4), WHOFC, mPAP levels, or ANT, CORT, or both usage. ROC analyses validated the strong and stable prediction of these two mycobiome genera sets in the total PH population and different subgroups, which were higher than the PH-specific airway bacterial genera *Streptococcus*, *Lautropia*, and *Ralstonia*, which were identified after the exclusion of ANT usage in our previous study [17]. Nevertheless, the lack of influence of these genera strengthens their value as a stable airway mycobiome signature for indexing patients with PH, without considering the interference from clinical factors and individual medical history of ANT, CORT, or both.

Among these differentially detected fungal genera, *Purpureocillium* was one of the most significant genera predicted by Random Forest analysis in the overall PH, non-ANT/CORT, ANT, and CORT subgroups. *Purpureocillium* is a fungal genus that belongs to the *Ophiocordycipitaceae* family in the *Ascomycota* phylum. *Purpureocillium* contains at least five species, including *Purpureocillium lilacinum* (*P. lilacinum*), a saprobic and filamentous fungus [55] that is linked to lung infection [56], fungi-related humidifier lungs [57], and opportunistic fungal cellulitis [58] in humans. As reviewed by Chen and Hu, *P. lilacinum* secretes a group of secondary metabolites with various biological activities, including anticancer, antimicrobial, and insecticidal [59]. Reduced abundance of gut *P. lilacinum* has been observed in patients with pulmonary TB receiving anti-TB treatments [60]. *P. lilacinum* is considered a rare but emerging non-*Aspergillus* mold that may cause invasive fungal diseases and induce significant mortality in patients with malignancies or after hematopoietic stem cell transplantation for whom blood culture and ITS sequencing are strongly recommended [61]. Voriconazole and posaconazole are recommended for managing *P. lilacinum* infections. However, amphotericin B is frequently resistant [61, 62]. As summarized in 101 cases from the FungiScope registry and the literature, invasive *P. lilacinum* infection is observed in patients with hematological and oncological diseases, steroid treatment, solid organ transplantation,

and diabetes mellitus, with the most prevalent infection sites being skin and lungs [62]. Shen et al. showed that *Purpureocillium* produces a defensin called purlisin, which exhibits antibacterial activity and inhibits potassium channel activity [11]. Suppressed potassium channel activity has been well documented to induce PH and PH-associated pulmonary vascular remodeling [63]. However, the detailed etiological and functional associations between *Purpureocillium* and human diseases, particularly lung diseases and PH, remain unclear and warrant further investigation.

The major strength of this study is that it provided the first comprehensive description of the airway mycobiome signature in a population with PH from three independent medical centers and among different PH subgroups divided by clinical classification (Groups 1, 3, and 4), WHO functional class, disease severity, and usage history of ANT, CORT, or ANT + CORT. Given the lack of fundamental knowledge, these data provide essential resources for future studies on the etiology and pathobiology of lung/airway mycobiomes and PH. However, a major limitation of this study is the lack of data from metagenomic or metatranscriptomic sequencing due to the nature of low microbial load and contamination of host tissue/cell in samples collected from pharyngeal swabs. Also, during the progression of this study, only four Group 2 PH patients were recruited since the study was basically conducted by doctors and researchers from the lung departments. Due to the small sample size, Group 2 PH patients were excluded and only Groups 1, 3, and 4 were analyzed in this study. Another limitation is the lack of cross-comparisons with other internal or external studies, given the lack of similar reports. Future studies should include correlation analyses of fungal taxa and explore the correlations among airway bacteria, mycobiomes, and viromes. This approach will help build a comprehensive understanding of the interactions within the airway microbiome under both CON and PH conditions. Such efforts will enhance our knowledge of the microbiome signature in individuals with PH. In addition, timeline observations will provide evidence of the dynamic features of the host mycobiome. Moreover, there is no direct evidence supporting whether the altered mycobiome abundance of specific fungal genera can causally drive the pathogenesis of PH or act as a consequence of the disease. We have previously shown that airway delivery of specific PH-enriched bacteria, *Streptococcus salivarius*, in the airway microbiome of the PH population [17] was sufficient to induce experimental PH in rat [31], indicating a strong association between the exposure of lung/airway microbiota and the disease progression of PH. Therefore, similar work is also worth conducting to determine the potential causality between an altered airway mycobiome and PH development. Future studies should focus on the following aspects: (a) validation of the PH-specific lung/airway mycobiome signature using internal larger-scale studies with increased enrollments/centers and external independent studies by other groups; and (b) investigation of the pathogenic role of PH-enriched mycobiome genera, such as *Purpureocillium*, *Issatchenkia*, and *Cyberlindnera*, to induce typical PH and PH airway microenvironments, including microbiota composition and host immunological responses, and identify novel therapeutic strategies by targeting these specific fungal genera. Moreover, ITS sequencing is often associated with a lack of accuracy in mycobiome identification at the species level. Shotgun metagenomics analysis is an advanced approach for identifying specifically diminished and enriched fungal species under PH conditions. However, as a non-amplicon sequencing method, shotgun metagenomics fails to capture useful signals from pharyngeal swab samples because of low-microbial loads. In addition, bronchoalveolar lavage fluid samples with a relatively high microbial load were not obtained from the clinical PH population for ethical approval. Therefore, we employed ITS amplicon sequencing was used in this study. Additionally, it is advisable for future research to conduct correlation analyses among different fungal taxa and explore relationships between airway bacteria, mycobiomes, and viromes. These efforts will contribute to a comprehensive understanding of airway microbiome interactions under both CON and PH conditions, ultimately enhancing our understanding of the microbiome signatures in individuals with PH.

Conclusions

To our knowledge, this is the first comprehensive analysis using pharyngeal swab samples and ITS sequencing to analyze the airway mycobiome in 244 patients with PH from three independent medical centers, compared to healthy donors or non-PH controls. It describes the profound changes in the diversity and composition of airway fungal signatures between PH and CON and among different PH subgroups divided by clinical classification, mPAP levels, WHO functional class, and usage history of ANT, CORT, or ANT + CORT. We found consistent enrichment and diminishment of specific fungal genera in the total PH population and in different PH subgroups without the influence of ANT, CORT, or ANT + CORT usage histories. Moreover, specific correlations between the mycobiome and clinical parameters and between different mycobiome taxa were also captured, distinguishing PH (and subgroups) from CON. These data provide compelling evidence and a fundamental resource for the future elucidation of the potential causal relationship

between altered lung/airway mycobiomes and disease development in PH.

Abbreviations

ANT	Antibiotics
AUC	Area under the curve
CHD	Congenital heart disease
CI	Confidence interval
CON	Healthy controls
COPD	Chronic obstructive pulmonary disease
CORT	Corticosteroids
CTD	Connective tissue disease
IPAH	Idiopathic pulmonary arterial hypertension
LEfSe	Linear discriminant analysis Effect Size
mPAP	Mean pulmonary artery pressure
PAH	Pulmonary arterial hypertension
NMDS	Non-metric multidimensional scaling
PH	Pulmonary hypertension
RHC	Right heart catheterization
ROC	Receiver operating characteristic curve
WHO	World Health Organization
WHOFC	World Health Organization functional class

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-03982-7.

Additional file 1: Fig. S1: Study design and flowchart illustration. Fig. S2: Quality control for pharyngeal swab samples. Fig. S3: PH diagnostic flowchart. Fig. S4: Distinct Airway mycobiome signature between non–PH and Group 3 PH groups. Fig. S5: Distinct Airway mycobiome signature between non–PH and Group 3 PH in non–ANT/CORT subgroups. Fig. S6: Distinct Airway mycobiome signature between non–PH and Group 3 PH in ANT and/or CORT subgroups. Fig. S7: Airway mycobiome profile comparison between CON and PH subgroups based on PVR levels. Fig. S8: Airway mycobiome profile comparison between CON and PH subgroups based on WHOFC. Fig. S9: Top specific fungal genera in CON versus PH or subgroups. Fig. S10: Prediction analysis for Group 1, Group 3, and Group 4 versus CON using specific enriched or decreased fungal genera. Fig. S11: Prediction analysis for between different PH subgroups using specific enriched or decreased fungal genera. Fig. S12: Random Forest analysis and ROC analysis on different PH subgroups. Fig. S13: Correlation analysis of mycobiome at phylum level in CON, PH and different PH subgroups. Fig. S14: Correlation analysis of mycobiome at genus level in CON and PH (subgroups). Fig. S15: Variations in composition of fungal functional groups inferred by FUNGuild between CON and PH. Fig. S16: Significant MetaCyc pathway abundance for the pharynx mycobiome between CON and PH.

Additional file 2: Table S1: Hemodynamics and treatment strategy for PH subgroups based on clinical classification. Table S2: Demographic and clinical characteristics of the participants in non-PH and Group 3 PH group. Table S3: Hemodynamics and treatment strategy for PH subgroups based on mPAP level. Table S4: Correlation analysis between fungi and clinical factors at phylum and genus level. Table S5: Correlation analysis between different fungi in CON, PH, non-ANT/CORT and ANT and/or CORT groups at phylum level. Table S6: Correlation analysis between different fungi in CON, PH, non-ANT/CORT and ANT and/or CORT groups at genus level. Table S7: *P* values of MetaCyc pathways at CON vs PH.

Additional file 3: Supplementary text. Expanded Methods. Supplementary Figure Legends.

Additional file 4: Uncropped gel images.

Additional file 5: STROBE checklist.

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Authors' contributions
C.Z. (Chenting Zhang), B.Z., Q.J., and H.W. collected samples, performed bioinformatics analysis, analyzed data and prepared figures; Y.X. and Z.Z. (Zizhou Zhang) performed bioinformatics analysis; X.W., Y.Z. (Yulin Zheng), P.L., Z.L. (Zhenxiang Li), Z.L. (Ziying Lin), Y.C., C.H., Z.Z. (Zhuxiang Zhao), T.Z., W.L. (Weiquan Liang), Y.Z. (Yi Zhang), and C.Z. (Caojin Zhang) collected samples; W.L. (Wenju Lu) and J.X.-J.Y provided critical consultation and advice to the project and critical revision of the manuscript; J.W. and C.L. initiated the project, designed the study, provided critical consultation and advice to the project and revised the manuscript; K.Y. initiated the project, designed the study, wrote and revised the manuscript; All authors approved for the final submission of the manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding authors upon reasonable request. The ITS DNA Sequence data associated with this project are available from the NCBI Sequence Read Archive (SRA) database with accession number PRJNA1068074 (BioProject: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1068074) [64].

Declarations

Ethics approval and consent to participate
This study conforms to the principles outlined in the Declaration of Helsinki and was approved by the ethics committee of all participating hospitals and institutions, including the First Affiliated Hospital of Guangzhou Medical University (Ethical approval numbers: 2020–04), the Second Affiliated Hospital of Guangzhou Medical University (Ethical approval numbers: 2019–05-ks-04), Guangdong Provincial People's Hospital (Ethical approval numbers: GDREC2015254H(R1)), Guangzhou First People's Hospital (Ethical approval numbers: K-2022–083-02), and the Second People's Hospital of Foshan (Ethical approval numbers: 2022–0145). All subjects obtained and signed broad informed consents before enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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