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Longitudinal change of lung microbiome in chronic obstructive pulmonary disease: a prospective cohort study

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Abstract

Background The lung microbiome is increasingly implicated in chronic obstructive pulmonary disease (COPD) pathogenesis. However, its long-term dynamics and interactions with key clinical features—including inhaled corticosteroid (ICS) use, smoking, and lung function—remain poorly defined.

Methods We conducted a prospective two-year study of 43 Korean male patients with COPD who provided sputum samples annually ($n = 129$). Bacterial communities were profiled using 16S rRNA gene sequencing. Associations between microbial composition and clinical characteristics—including inhaled corticosteroid (ICS) use, smoking status, lung function (FEV_1), and recent exacerbations—were evaluated using negative binomial mixed models (NBMMs) with and without time interaction terms, adjusting for potential confounders.

Results At baseline, overall microbial diversity did not significantly differ between patients with COPD and ex-smoker controls without airflow limitation; however, several low-abundance genera showed differential abundance. Cross-sectional NBMMs revealed that ICS use, current smoking, and reduced FEV_1 % predicted were associated with distinct taxonomic profiles. ICS use was associated with reduced relative abundances of *Veillonella*, *Catonella*, and *Saccharimonas*. Persistent smoking was linked to increased abundances of *Actinomyces* and *Bulleidia* and decreased *Lautropia*. Patients with FEV_1 % predicted < 50% exhibited lower *Alloprevotella* levels.
In longitudinal models, ICS use was associated with increasing temporal trends in *Megasphaera* and *Alloprevotella*. Persistent smokers showed attenuated changes in *Butyrivibrio* and *Pseudomonas*, while those with severe airflow limitation exhibited increased *Bacteroides* and decreased *Atopobium*, *Gemella*, *Kingella*, and *Tannerella* over time. Acute exacerbations were not significantly associated with microbial composition at baseline or during follow-up.

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Conclusions Clinical factors in COPD are associated with distinct temporal shifts in the airway microbiome of patients with COPD. Longitudinal profiling combined with NBMMs with time-interaction terms revealed subtle microbial shifts with potential clinical implications that were not evident in cross-sectional analyses. These findings underscore the potential utility of temporal microbiome signatures in stratifying COPD patients and guiding future therapeutic strategies.

Keywords Lung microbiome, Chronic obstructive pulmonary disease, Clinical characteristics, Longitudinal analysis

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic, progressive inflammatory disorder of the lungs characterized by a largely irreversible decline in lung function, most often resulting from prolonged inhalation of tobacco smoke or other noxious environmental irritants such as biomass fuel exposure and air pollution [1, 2]. The pathophysiology of COPD involves persistent airflow limitation due to structural changes in both the small and large airways, mucus hypersecretion, chronic bronchial inflammation, small airway fibrosis, and destruction of the alveolar architecture leading to emphysema [1, 3]. These pathological alterations contribute to impaired gas exchange, chronic respiratory symptoms, and increased susceptibility to exacerbations and respiratory infections.

The healthy human lung harbors diverse commensal microbiota throughout the respiratory tract, with substantial heterogeneity between individuals, temporal variation within the same individual, and spatial differences across regions within the lung [4, 5]. The lung microbiome represents an emerging opportunity for understanding the heterogeneity and exacerbation of COPD. Alterations in the taxonomic composition and relative abundance of these microbial communities—collectively termed dysbiosis—have been implicated in the pathogenesis and progression of multiple chronic lung diseases. In COPD specifically, dysbiosis has been linked to airway inflammation, increased disease severity, and a higher risk of acute exacerbations [6, 7]. Furthermore, recent evidence suggests that the composition of the lung microbiome may serve as a biomarker for predicting clinical outcomes, including hospitalization risk and mortality [8].

The profile of the lung microbiome sampled over longer time frames remains poorly understood [9]. Most studies on the lung microbiome in COPD were cross-sectional or short-term studies [6, 10, 11]. While such studies have provided important snapshots of microbial composition, they cannot capture the dynamic changes that occur over time in a disease that is inherently persistent and often progressive [3]. Given that COPD is characterized by variable disease trajectories, long-term, longitudinal investigations are essential for determining whether specific microbial patterns are stable, transient, or predictive of future clinical events, long term studies could enhance the clinical utility of the lung microbiome as a diagnostic, prognostic, or therapeutic-monitoring tool.

Longitudinal observation of the lung microbiome in COPD has the potential to clarify its association with key clinical features, such as baseline disease severity, rate of lung function decline, exacerbation frequency, response to pharmacologic and non-pharmacologic interventions, and long-term prognosis. By repeatedly sampling the same individuals over time, researchers can disentangle the effects of host factors, environmental exposures, and therapeutic interventions from the background of natural microbial variability [12–14].

In this prospective study, sputum samples, which are reported to be consistent with the respiratory microbiome detected in bronchoalveolar lavage and bronchial samples [15, 16], were collected annually for two years from patients with COPD. Using 16S rRNA gene sequencing, a sensitive and widely adopted molecular technique for profiling bacterial communities, we aimed to characterize longitudinal changes in the lung microbiome of COPD patients and compare these patterns to those observed in healthy individuals. In addition, we investigated whether microbial composition and dynamics varied according to clinically relevant factors, including inhaled corticosteroid use, smoking status, lung function, and history of exacerbations, using negative binomial mixed models (NBMMs), with the goal of identifying microbial signatures that may inform risk stratification and individualized management strategies in COPD.

Materials and methods

Study design and population

At baseline, 10 male ex-smoker participants without airflow limitation and 54 (53 males and 1 female) patients with COPD were recruited between February 2017 and July 2018 at the Severance Hospital. The inclusion criteria for patients with COPD were as follows: post-bronchodilator ratio of forced expiratory volume to forced vital capacity <0.7, and absence of respiratory diseases other than COPD (e.g., previous pulmonary resection, tuberculosis-affected lung, and bronchiectasis). A single female patient was excluded from the analysis due to insufficient numbers to assess sex-related effects on the lung microbiome. Additionally, 10 male patients with COPD were excluded due to no follow-up ($n=9$) or

incomplete clinical information ($n = 1$). Finally, 10 male ex-smoker controls without airflow obstruction (hereafter referred to as 'controls') and 43 patients with COPD were included in this study. Controls were selected on the basis of self-reported health information and clinical assessments performed by a pulmonology specialist. All ten controls demonstrated normal pulmonary function without airflow limitation on spirometry. Two had a remote history of pulmonary tuberculosis, but none had bronchiectasis or asthma. The mean duration of smoking for the controls was 33.5 ± 11.3 pack-years.

After an initial enrolment visit, the patients were followed up annually for 2 years, with clinical assessments and sputum sample collections conducted at baseline, 12 months, and 24 months (three timepoints per participant). Demographic data, exacerbation history, pulmonary function tests, COPD assessment test (CAT) [17], modified medical research council (mMRC) dyspnoea scale [18], St George's Respiratory Questionnaire (SGRQ) scores, sputum samples, and laboratory tests were collected or performed at each visit (Fig. 1). Post-bronchodilator forced expiratory volume in 1 s, % predicted (FEV_1) was dichotomized at 50% to represent the threshold between moderate and severe airflow limitation according to GOLD criteria; this ensured model stability given the small number of very severe cases. Recent exacerbation was defined as the aggravation of one of three symptoms (dyspnoea, cough, or sputum) for two or more days requiring an unscheduled hospital visit for additional treatment with systemic steroids or antibiotics, emergency room visits, or hospitalization within the past three months.

Sputum sample acquisition

Induced sputum samples were obtained using 3% hypertonic saline and collected in DNA-free containers. After collection, samples were immediately transferred to sterile tubes and stored at − 80 °C until further processing. Participants were instructed to rinse their mouths thoroughly before sputum induction to minimize oral contamination, which can confound microbial analyses.

16S rRNA gene amplicon sequencing and analysis

To reduce sputum viscosity, 20 µL of β-mercaptoethanol (Sigma Aldrich, USA) was added to 1 mL of induced sputum. Samples were incubated at 37 °C with shaking (200 rpm) for 30–60 min. DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, USA) following the manufacturer's protocol.

The V3–V4 region of the 16S rRNA gene was amplified using the 2× KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Italy) and primers 318 F and 806R. PCR conditions were as follows: initial denaturation at 95 °C for 3 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and

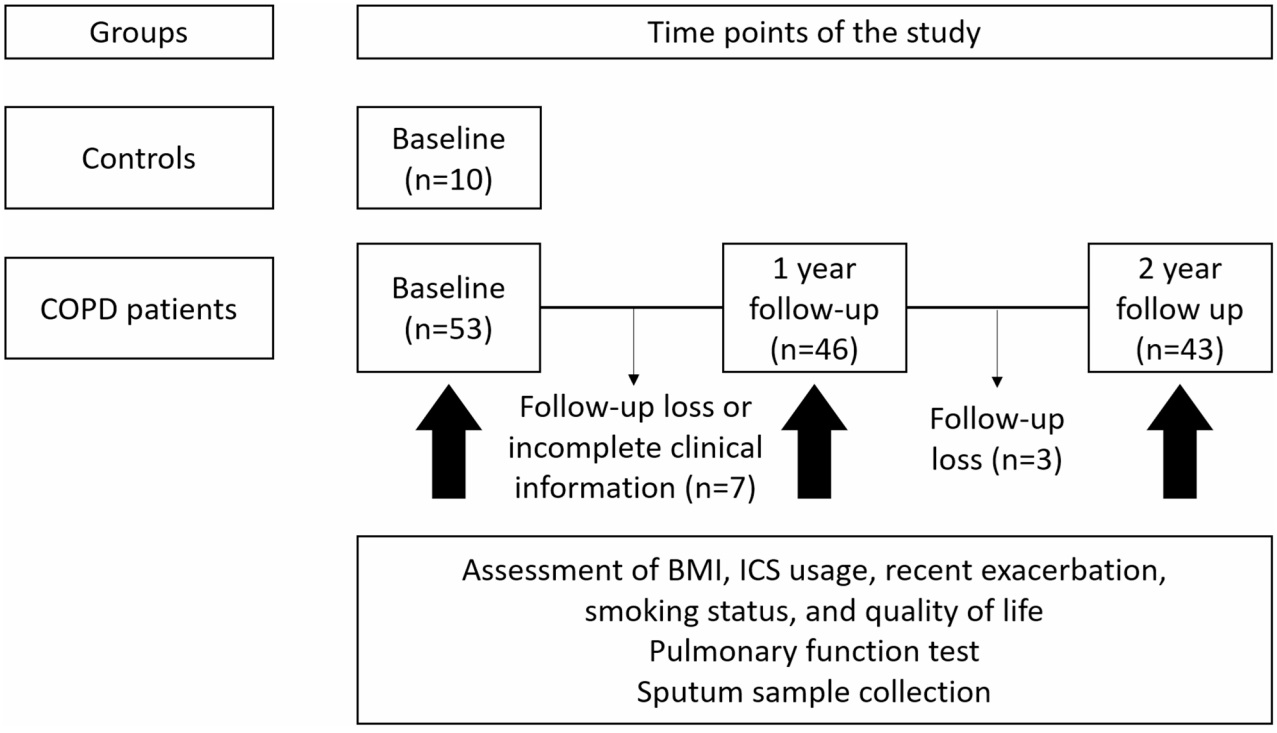

Fig. 1 Overview of the study design and longitudinal sputum sampling schedule. Schematic representation of the two-year prospective study involving 43 male patients with COPD and 10 healthy male controls. Clinical data and sputum samples were collected at baseline (Year 0), 1-year, and 2-year follow-up visits. Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; ICS, inhaled corticosteroids

72 °C for 30 s; and a final extension at 72 °C for 5 min. Amplicons were purified using AMPure XP beads (Beckman Coulter, UK) and libraries were constructed according to Illumina's 16S metagenomic sequencing library preparation protocol. Sequencing was performed using the MiSeq Reagent Kit v3 (Illumina, USA) to generate 2 × 300 bp paired-end reads.

To monitor potential contamination during sample processing, three types of negative controls were included and sequenced in parallel with clinical samples: two DNA extraction blanks (Kitome 1 and Kitome 2, repeated extraction experiments), one PCR blank (PCR NC), and one library preparation blank (Barcoding NC). All negative controls were processed using the same reagents and protocols as those used for the sputum samples, except that no biological material was added.

Raw sequence data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) 2 pipeline (https://qiime2.org; version 2021.4). DADA2 was used for quality filtering, denoising, and chimera removal. Amplicon sequence variants (ASVs) were taxonomically classified using the `q2-feature-classifier` plugin and the EzBioCloud 16S database [19]. A minimum sequencing depth threshold of 20,000 reads per sample was applied to ensure adequate coverage. To reduce sparsity, features with zero counts in > 50% samples were excluded from downstream statistical modeling.

Statistical analysis

All statistical analyses were conducted using R (version 4.1.3), SPSS (version 21, IBM, USA), and OriginPro (version 2023b, OriginLab, USA). Descriptive statistics were presented as means with standard deviations or relative abundances.

Rarefaction was performed to standardize sequencing depth across samples, ensuring that diversity comparisons were not biased by variation in read counts. Alpha diversity indices (Observed OTUs [20], ACE [21], Chao1 [22], Shannon [23], Simpson [24]) were calculated from rarefied data, and group differences were tested using the Kruskal–Wallis test. Bray–Curtis dissimilarity [25] taxon-level differences were assessed using the R package 'MaAsLin2' [26].

To model the associations between microbial genera and clinical variables (recent exacerbation, ICS use, smoking status, FEV_1), we applied NBMMs using the R package `NBZIMM` [27]. Both cross-sectional (without time interaction) and longitudinal (with time interaction) models were fitted, adjusting for age, ICS use, smoking status, FEV_1, and year. Time interaction terms (two-way and three-way) were used to evaluate whether microbial abundance trajectories differed over time by clinical subgroup. For interaction models, participants were classified according to whether the status of at least one clinical characteristic—such as the presence or absence of exacerbation, ICS use, current smoking, or FEV_1 being below or above 50%—remained consistent throughout the follow-up period.

We also evaluated potential seasonal effects by classifying sampling dates according to the four meteorological seasons and employing several alternative grouping strategies such as summer combined with autumn versus winter combined with spring. None of these approaches demonstrated significant differences in microbial diversity (Figure S1). Season was not incorporated into the final models because the seasonal distribution was highly unbalanced, and inclusion of season as an additional covariate substantially destabilized NBMM because of the modest sample size. To evaluate model stability, we calculated intraclass correlation coefficients (ICCs), random-effect (RE) variance estimates, and post-hoc power for each NBMM. All models demonstrated full convergence and moderate ICC values, while post-hoc power varied across taxa, being generally sufficient for major effects. Detailed ICC, RE, and power results are provided in Tables S2–S4. To address multiple testing, we applied Benjamini–Hochberg false discovery rate (FDR) correction to all NBMM results, and both raw and FDR-adjusted *P*-values are presented in the NBMM tables. Statistical significance was defined as $P < 0.05$.

Results

Baseline characteristics

We included 43 male patients with COPD and 10 male controls. Baseline clinical data are summarized in Table 1. Compared to controls, patients with COPD were older and showed significantly reduced lung function. ICS usage, current smoking, and recent exacerbations were variably present among the COPD cohort.

Overall microbial composition in COPD and controls at baseline

To assess differences in microbial composition and diversity according to COPD status, we analyzed sputum samples collected at Year 0 from individuals with COPD ($n = 43$) and controls ($n = 10$). Sequencing depth across samples averaged 79,917 reads per sample (range: 25,674–147,345 reads), with a coefficient of variation of 0.283. A minimum read threshold of 20,000 reads per sample was applied, and no samples were excluded on the basis of this criterion. Features with zero counts in > 50% samples were removed; this resulted in the retention of 60 genera for downstream analyses. Negative control libraries exhibited extremely low sequencing depth (mean 78 reads), which was substantially lower than that exhibited by the clinical samples; this indicated minimal contribution of background contamination. Key taxa prevalence across the cohort and results from negative

Table 1 Summary characteristics of participants included in the study

Baseline characteristics		Control ($n=10$)	COPD ($n=43$)		
		Baseline	Baseline	1-year follow up	2-year follow up
Age, years		62.6 ± 10.3	72.0 ± 6.6	73.0 ± 6.6	74.0 ± 6.6
BMI, kg/m^2		22.8 ± 3.3	23.8 ± 3.5	24.0 ± 3.5	24.1 ± 3.3
Smoking					
	Ex-smoker	8 (80%)	33 (76.7%)	35 (81.4%)	35 (81.4%)
	Current smoker	2 (20%)	10 (23.3%)	8 (18.6%)	8 (18.6%)
Pulmonary function tests					
FVC, L		3.97 ± 0.39	3.37 ± 0.65	3.40 ± 0.71	3.29 ± 0.67
FVC, % predicted		97.8 ± 12.1	94.2 ± 15.0	93.7 ± 16.0	91.5 ± 16.3
FEV_1, L		3.02 ± 0.55	1.56 ± 0.41	1.52 ± 0.43	1.49 ± 0.45
FEV_1, % predicted		102.6 ± 10.6	63.7 ± 16.0	64.1 ± 19.7	61.9 ± 19.1
FEV_1/FVC, %		77.8 ± 2.8	44.2 ± 10.0	42.6 ± 11.0	42.3 ± 11.0
FEV_1, % predicted< 50%		0 (0%)	7 (16.3%)	8 (18.6%)	8 (18.6%)
GOLD group	A	n/a	30 (69.8%)	24 (55.8%)	28 (65.1%)
	B, E	n/a	13 (30.2%)	19 (44.2%)	15 (34.9%)
Average CAT score		n/a	13.3 ± 7.8	13.1 ± 8.1	13.7 ± 9.0
CAT score	< 10	n/a	26 (60.5%)	24 (55.8%)	25 (58.1%)
	≥ 10	n/a	17 (39.5%)	19 (44.2%)	18 (41.9%)
Acute exacerbation within 3 months		n/a	5 (11.6%)	5 (11.6%)	4 (9.3%)
ICS use, yes		n/a	11 (25.6%)	13 (30.2%)	14 (32.6%)

COPD chronic obstructive pulmonary disease, *BMI* body mass index, *FVC* functional vital capacity, *FEV_1* forced expiratory volume in 1 s, *CAT* COPD assessment test, *SGRQ* St George's Respiratory Questionnaire, *GOLD* Global Initiative for Chronic Obstructive Lung Disease, *ICS* Inhaled corticosteroid

control sequencing are summarized in Table S1 and Figure S2. *Streptococcus* was the most abundant genus across all samples (Figure S3A, S3B), which is consistent with prior studies highlighting its dominance in the respiratory tract microbiome of both healthy individuals and those with chronic respiratory diseases. No significant differences were observed in alpha diversity metrics—including observed OTUs, ACE, Chao1, Shannon, and Simpson indices (all $P > 0.05$), suggesting that species richness and evenness are relatively preserved despite disease status. Beta diversity, as measured by Bray-Curtis dissimilarity showed no significant differences ($P = 0.127$) between the two groups (Figure S3C), supporting the observation that microbial composition remains broadly consistent regardless of disease presence. However, *Selenomonas*, *Nanocyncoccus*, *and Bulleidia* showed significantly different relative abundances between patients with COPD and controls, as identified by MaAsLin2 after adjustment for age and smoking status (Figure S3D), which may indicate that subtle shifts in rare taxa contribute to the inflammatory milieu characteristic of COPD.

Microbiome differences according to COPD clinical characteristics

Overall microbiome composition in patients with COPD did not differ significantly among clinical subgroups—including inhaled corticosteroid (ICS) use, smoking status, FEV_1 level, and recent exacerbation history—with respect to alpha or beta diversity metrics (data not shown). These findings indicate that broader community structure remains relatively stable across common clinical features. However, several bacterial taxa exhibited significant differences in relative abundance according to clinical characteristics, as identified by NBMM analysis, a method well-suited for analyzing complex microbiome data as it accommodates overdispersed count data and accounts for subject-level random effects (Table 2). Representative taxa associated with each clinical variable are presented in Fig. 2.

Patients who received ICS treatment continuously for three years had lower relative abundances of *Veillonella*, *Catonella*, and *Saccharimonas* compared to those who never used ICS (Fig. 2A). Higher baseline abundances of *Actinomyces* and *Bulleidia*, and lower abundance of *Lautropia*, were observed among persistent smokers (i.e., those who continued smoking over the three-year period) than among persistent ex-smokers (Fig. 2B). Patients with FEV_1 < 50% over three years exhibited a decreased abundance of *Alloprevotella* compared to those with ≥ 50% (Fig. 2C) over three years. The effect of recent exacerbation could not be calculated due to the small number of patients who experienced an acute exacerbation, highlighting the need for larger cohorts or targeted studies focusing on acute exacerbation events to better understand microbial shifts during these episodes.

Longitudinal microbiome changes by clinical characteristics

The influence of clinical characteristics on longitudinal shifts in bacterial genera was assessed using NBMMs,

Table 2 Genera associated with ICS use, smoking, and lung function in negative binomial mixed models without time interaction. Results from negative binomial mixed models assessing associations between genus-level abundances and ICS use, smoking status, and FEV1. Estimates are adjusted for relevant covariates

Bacteria	Estimate	S.E.	t-value	*P*-value	FDR-adjusted *P*-value
ICS user (*n*=26) vs. non-ICS user (*n*=9) [1]					
Veillonella	-0.505	0.222	-2.273	0.030	0.370
Catonella	-1.128	0.379	-2.975	0.006	0.270
Saccharimonas	-0.915	0.382	-2.396	0.023	0.362
Current smoker (*n*=33) vs ex-smoker (*n*=8) [2]					
Actinomyces	0.479	0.229	2.094	0.043	0.366
Bulleidia	0.726	0.334	2.173	0.036	0.366
Lautropia	-1.204	0.559	-2.152	0.038	0.366
FEV_1, % predicted ≥ 50% (*n*=30) vs. < 50% (*n*=4) [3]					
Alloprevotella	-1.595	0.515	-3.094	0.004	0.131

Abbreviations; *ICS* inhaled corticosteroid, *FEV_1* forced expiratory volume in 1 s, *S.E.* standard error

[1]Adjusted for age, smoking status, FEV_1 % predicted

[2]Adjusted for age, ICS use, FEV_1 % predicted

[3]Adjusted for age, ICS use, smoking status

incorporating age, ICS use, smoking status, and FEV_1 as covariates, along with their two- and three-way interactions with time (year) (Table 3). Representative taxa exhibiting longitudinal variation are shown in Fig. 3.

In the time-interaction model, ICS use was significantly associated with temporal changes in the relative abundances of *Megasphaera* and *Alloprevoteall* (Fig. 3A). *Megasphaera* tended to increase over time in patients with continuous ICS use over three years, whereas *Alloprevotella* remained persistently elevated in the ICS-user group.

Ongoing smoking was linked to attenuated temporal dynamics in *Butyrivibrio* and *Pseudomonas* (Fig. 3B). *Butyrivibrio* showed a decreasing trend over time in patients who continued smoking, while *Pseudomonas* tended to increase over time in non-smokers.

Reduced lung function (FEV_1 < 50%) was associated with longitudinal shifts in several genera (Fig. 3C). Trends in *Bacteroides* increased over time in patients with impaired lung function, whereas *Atopobium*, *Gemella*, *Kingella*, and *Tannerella* declined.

In the models with time interaction, all significant genera met the commonly used FDR threshold of 0.25, whereas in the models without time interaction, raw *P*-values remained significant whereas FDR-adjusted values did not. This result likely indicates that incorporation of time effects captures subject-level temporal structure more effectively and yields more stable associations under multiple-testing correction, while models without the time term explain less of the underlying variation and consequently produce higher FDR-adjusted *P*-values despite significant raw values. Collectively, these findings suggest that ICS use, smoking, and reduced lung function contribute to distinct temporal trajectories of the airway microbiome of patients with COPD.

Discussion

In this longitudinal study of Korean male patients with COPD, we investigated how clinical factors—namely acute exacerbation, ICS use, smoking status, and lung function—affect both the baseline composition and longitudinal dynamics of the sputum microbiome over a two-year period. Utilizing NBMMs with time-interaction terms, we were able to characterize how these variables modulate microbial trajectories beyond simple cross-sectional associations. This analytical approach not only enhances temporal resolution but also allows for identification of context-dependent microbial behaviors, which are often masked in static comparisons.

At baseline, no significant differences were observed in alpha or beta diversity between patients with COPD and controls, consistent with previous studies indicating that global microbial community structure may not fully capture disease-related alterations [6, 28]. This supports the notion that conventional diversity indices may overlook subtle but biologically meaningful changes in the airway microbiota, particularly in diseases characterized by low-grade or localized inflammation. Therefore, greater emphasis should be placed on taxon-level resolution and functional profiling to uncover clinically relevant microbial shifts.

Despite the lack of global diversity differences, several low-abundance genera exhibited significant differential abundance. Notably, *Selenomonas* were enriched in individuals with COPD. *Selenomonas* have been previously associated with anaerobic environments and inflammatory conditions [29], including chronic airway disease and tobacco exposure. Their enrichment may reflect persistent shifts in the microenvironment of the COPD airway, potentially contributing to altered immune responses or microbial-host interactions. Conversely, *Bulleidia* and *Nanosyncoccus* were more abundant in

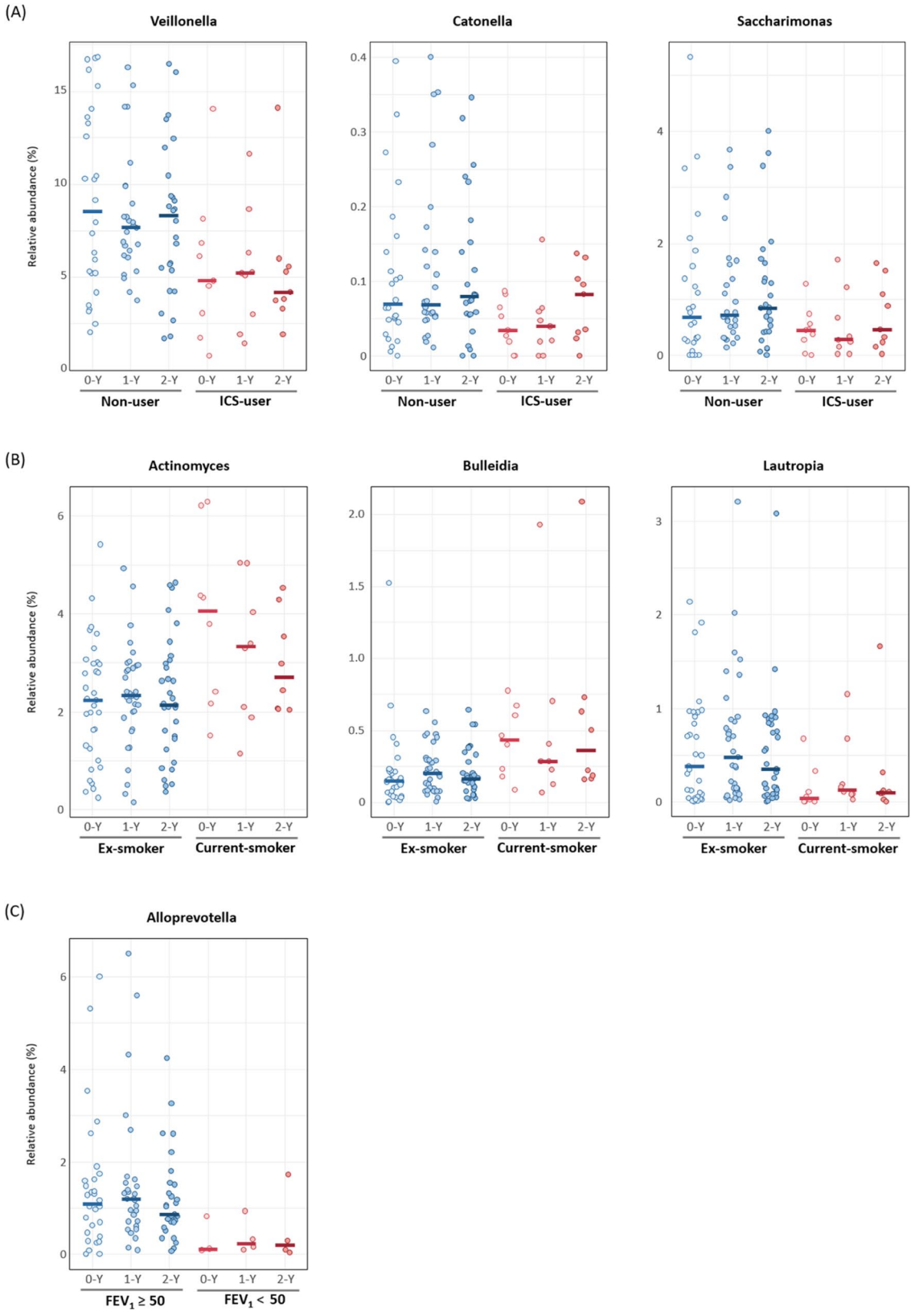

Fig. 2 Changes in key bacterial genera that differed according to ICS use, smoking status, and lung-function level in NBMM models without time interaction. **A** Relative abundances of *Veillonella*, *Catonella*, and *Saccharimonas* in ICS users vs. non-users. **B** Relative abundances of *Actinomyces*, *Bulleidia*, and *Lautropia* in persistent smokers vs. ex-smokers. **C** Relative abundance of *Alloprevotella* in patients with FEV_1 <50% vs. ≥50%. Abbreviations: FEV_1, forced expiratory volume in 1 s % predicted; ICS, inhaled corticosteroids; NBMM, negative binomial mixed model

Table 3 Genera associated with ICS use, smoking, and lung function in negative binomial mixed models with time interaction. Time-interaction negative binomial mixed model results showing genera with significant temporal shifts according to ICS use, smoking status, and FEV1. Estimates are adjusted for confounding variables

Bacteria	Estimate	S.E.	t-value	*P*-value	FDR-adjusted *P*-value
ICS user (*n*=26) vs. non-ICS user (*n*=9) [1]					
Megasphaera	0.638	0.178	3.593	0.001	0.028
Alloprevotella	0.295	0.117	2.532	0.014	0.220
Current smoker(*n*=33) vs ex-smoker (*n*=8) [2]					
Butyrivibrio	-0.437	0.170	-2.565	0.012	0.213
Pseudomonas	-1.090	0.522	-2.089	0.040	0.213
FEV_1 ≥ 50% (*n*=30) vs. < 50% (*n*=4) [3]					
Bacteroides	0.993	0.276	3.600	0.001	0.010
Atopobium	-0.530	0.218	-2.432	0.018	0.080
Gemella	-0.437	0.191	-2.283	0.026	0.089
Kingella	-0.543	0.189	-2.881	0.005	0.042
Tannerella	-0.581	0.214	-2.720	0.008	0.059

Abbreviations; ICS inhaled corticosteroid, *FEV_1* forced expiratory volume in 1 s % predicted, *S.E.* standard error

[1]Adjusted for age, smoking status, FEV_1 % predicted

[2]Adjusted for age, ICS use, FEV_1 % predicted

[3]Adjusted for age, ICS use, smoking status

controls. Bulleidia is considered commensal taxa, with roles in maintaining microbial homeostasis and mucosal integrity [30, 31].

Cross-sectional comparisons within the COPD cohort revealed that long-term ICS use was associated with reduced abundances of *Veillonella*, *Catonella*, and *Saccharimonas*. These genera are commonly regarded as commensal genera that contribute to airway immune tolerance and epithelial homeostasis [32, 33]. Their depletion may reflect ICS-induced suppression of microbial diversity and has been similarly observed in prior studies evaluating the ecological impact of inhaled corticosteroids [34, 35]. Persistent smoking was associated with increased levels of *Actinomyces* and *Bulleidia*, and reduction abundance of *Lautropia*. This pattern is consistent with previous findings linking tobacco exposure to the enrichment of pro-inflammatory or opportunistic taxa [36]. Conversely, *Lautropia* has been described as a marker of microbial stability in healthy individuals [37, 38], and its reduction in smokers may reflect early ecological disruption in the airways. Reduced lung function (FEV_1 < 50%) was associated with an increased abundance of *Alloprevotella*, a genus not commonly highlighted in COPD-related microbiome studies but previously implicated in inflammation and anaerobic overgrowth [39, 40]. Importantly, our longitudinal modeling advances this understanding by identifying taxa whose relative abundances evolve differently over time depending on clinical context. This highlights the utility of integrating dynamic modeling approaches in respiratory microbiome research, where static snapshots may fail to capture complex host–microbe interactions.

In the time-interaction model, ICS use was associated with increasing temporal trends in *Megasphaera* and *Alloprevotella*, thereby complementing cross-sectional findings by highlighting that long-term corticosteroid exposure may promote the expansion of certain taxa—potentially through immunomodulation or changes in the airway microenvironment [32]. Similarly, current smoking was associated with dampened temporal shifts in *Butyrivibrio* and *Pseudomonas*, implying a suppressive effect of smoking on microbial dynamism. In patients with persistently reduced FEV_1, *Bacteroides* exhibited heightened temporal shifts, suggesting an unstable colonization pattern potentially driven by host inflammation or altered mucosal immunity in advanced airway disease. *Bacteroides* species, though typically dominant in the gut, have been detected in the respiratory tract under dysbiotic conditions [41] and are known to respond dynamically to environmental perturbations [42], which supports our observation of increased temporal variability in this genus. While patients with persistently low FEV_1 exhibited dampened temporal shifts in *Atopobium*, *Gemella*, *Kingella*, and *Tannerella*, this likely reflects impaired microbial adaptability and reduced ecological resilience in advanced disease states. These genera, some of which are known to inhabit the oral or mucosal surfaces [43], may become stably maintained within chronically inflamed and structurally altered airways, potentially reflecting reduced ecological resilience in advanced disease states.

Interestingly, acute exacerbation status was not significantly associated with either baseline or longitudinal microbial patterns. This may reflect the limited number of exacerbation events at baseline, or that transient inflammation during exacerbation does not result in sustained microbiome alterations. This is consistent with previous work showing only modest and short-lived effects of exacerbations on airway microbial composition [6, 44].

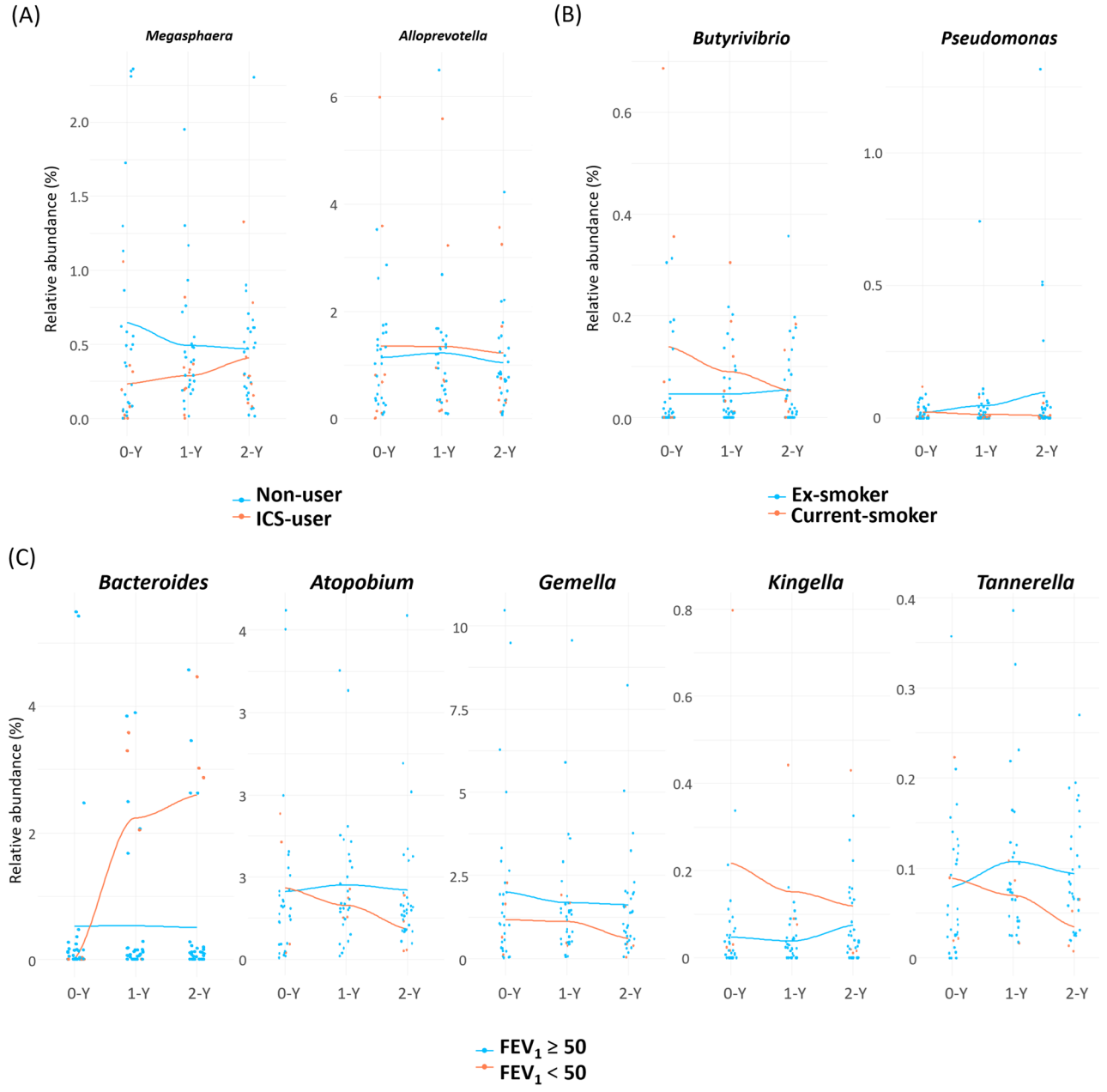

Fig. 3 Changes in key bacterial genera that differed according to ICS use, smoking status, and lung-function level in NBMM models with time interaction. **A***Megasphaera* and *Alloprevotella* show increased trends over time in ICS users. **B** Butyrivibrio and Pseudomonas show altered temporal dynamics by smoking status. **C** Longitudinal shifts are observed in *Bacteroides*, *Atopobium*, *Gemella*, *Kingella*, and *Tannerella* by lung function (FEV_1). Abbreviations; FEV_1, forced expiratory volume in 1 s % predicted; ICS, inhaled corticosteroids; NBMM, negative binomial mixed model

The predominance of oral-associated genera—including *Veillonella*, *Actinomyces*, *Bulleidia*, *Atopobium*, and *Bacteroides*—in relation to COPD traits highlights the potential for oral-lung microbial translocation, a phenomenon increasingly recognized in chronic airway disease. Many of these taxa are known to be enriched in periodontal disease [45–47], which itself is a reported risk factor for COPD [48, 49]. Our findings support the hypothesis that oral microbiota may colonize or persist in the lower airways, potentially contributing to airway inflammation and disease progression. Future studies should investigate whether targeting oral health or reducing microbial translocation could serve as adjunctive strategies for managing COPD-related airway dysbiosis.

This study has several clinical and methodological implications. First, identifying patient subgroups with distinct microbial trajectories—such as those receiving long-term ICS therapy or those with severe airflow limitation—may enable risk stratification for microbiome-related disease progression or therapeutic response.

Second, our use of NBMMs with time-interaction terms provides a robust framework for analyzing complex, overdispersed microbiome data in longitudinal settings. Unlike Poisson models, which assume equality between the mean and variance [50], NBMMs account for the overdispersion commonly observed in real-world microbiome datasets, allowing us to capture subtle temporal shifts in microbial composition that may be missed by traditional approaches [51].

However, it should also be noted that the NBMM approach has inherent limitations. NBMM performance can be affected by small sample sizes and heterogeneous disease characteristics, which may reduce model stability and statistical power [52]. Moreover, convergence issues may arise in datasets with highly sparse taxa or limited repeated measures [53]. Nevertheless, NBMMs provide a suitable framework for longitudinal microbiome analyses, as they accommodate overdispersion and within-subject correlation while enabling temporal modeling in repeated-measures designs.

This study has several limitations. First, our cohort consisted exclusively of male patients from a single center in Korea, limiting generalizability to broader populations, including women. Future larger multicenter studies including both sexes and diverse clinical settings are warranted to validate and extend the results of this study. Second, sputum samples, while clinically practical, may include upper airway contamination and do not provide absolute quantification. Notably, the predominant bacterial constituents of the sputum microbiome were consistent with the lung microbiome detected in bronchoalveolar lavage and bronchial samples reported in previous studies [54]. This suggests that our observations are representative of the bacterial composition of the lung microbiome. Third, the absence of longitudinal sampling in controls precluded assessment of baseline microbial stability. Therefore, it remains unclear whether the results from the annual follow-ups are specific to patients with COPD or whether they differ from those in controls. Fourth, the control group consisted of ex-smokers without airflow limitation, including two individuals with a remote history of pulmonary tuberculosis, and chronic respiratory symptoms were not systematically assessed. These factors may have introduced heterogeneity into the control microbiome profiles despite preserved spirometry. Future studies should incorporate stricter criteria for defining control groups. Finally, we focused on bacterial communities via 16S rRNA sequencing; fungal and viral components of the airway microbiome were not evaluated. Future studies incorporating shotgun metagenomic [55] or integrated multi-omics [56] approaches could provide species-level and functional insights, allowing a more comprehensive understanding of microbial dynamics in COPD.

Despite these limitations, our study provides a novel longitudinal perspective on how host clinical factors modulate the dynamics of the airway microbiome in COPD. Future research integrating multi-kingdom sequencing, host immune profiling, and interventional designs are essential to translate these microbial signatures into clinically actionable biomarkers or therapeutic targets.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

All authors meet the authorship criteria recommended by the International Committee of Medical Journal Editors. H. Lee and J.Y. Jung conceived and designed the study. H. Lee, M. Kim, and C. Kang conducted the data analysis. S.W. Moon and J.Y. Jung drafted the initial manuscript. J.Y. Jung and J.H. Shin performed sputum sample acquisition. H. Lee, M. Kim and C. Kang performed 16S rRNA gene amplicon sequencing and analysis. S.W. Moon, M. Kang and H. Lee performed statistical analyses and prepared figures. E.Y. Kim, S. Won and K. Kim provided statistical support and analytical guidance. J.Y. Jung and J.H. Shin established and curated the COPD cohort. All authors had full access to the data used in this study, take responsibility for the integrity of the work, and provided critical revisions to the final manuscript.

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Data availability

The microbiome sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1379254 and are publicly available. Clinical datasets analyzed during the study are not publicly available due to patient privacy restrictions but can be obtained from the corresponding author (J.Y. Jung and H. Yi) upon reasonable request.

Declarations

Ethics approval and consent to participate

All procedures were conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of the Severance Hospital (Institutional Review Board approval number: 2016-2133-001). Written informed consent was obtained from all patients.

Consent for publication
Written informed consent for publication of clinical details was obtained from the patient.

Competing interests
The authors declare no competing interests.

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