Research article

Alteration of the gut microbiota in patients with lung cancer accompanied by chronic obstructive pulmonary diseases

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A B S T R A C T

Aim: To explore the abundance and diversity of the gut microbiota in patients with lung cancer accompanied by chronic obstructive pulmonary disease (LC-COPD).
Methods: The study cohort comprised 15 patients with LC-COPD, 49 patients with lung cancer, and 18 healthy control individuals. ELISA was used to detect inflammatory factors in venous blood. 16S rDNA sequencing was performed to determine the abundance and diversity of the gut microbiota. Gas chromatography–mass spectrometry was used to determine the concentration of short-chain fatty acids (SCFAs) in feces samples.
Results: The α-diversity index indicated that the richness and diversity of the gut microbiota were lower in patients with LC-COPD compared with patients with lung cancer and controls. Principal component analysis revealed significant differences among the three groups ($P < 0.05$). The linear discriminant analysis effect size algorithm indicated that the o_*Lactobacillales*, g_*Lactobaccillus*, f_*Lactobaccillaceae,* s_*Lactobaccillus_oris*, c_*Bacilli,* g_*Anaerofustis*, s_*uncultured organism*, and s_*bacterium_P1C10* species were prevalent in patients with LC-COPD, while the g_*Clostridium_XIVa* and g_*Butyricicoccus* species were prevalent in patients with lung cancer. Furthermore, the concentrations of the SCFAs butyric acid, isobutyric acid, isovaleric acid, and valeric acid tended to be lower in patients with LC-COPD compared with patients with lung cancer and healthy controls, although these intergroup differences were not significant ($P > 0.05$). Patients with lung cancer had the lowest serum concentration of tumor necrosis factor (TNF)-a. There were no intergroup differences in the concentrations of other inflammatory factors.
Conclusions: The present study indicated that the abundance and structure of the gut microbiota is altered, and the concentrations of SCFAs may be decreased in patients with LC-COPD. In addition, patients with lung cancer had the lowest serum concentration of TNF-a.

1. Introduction

Lung cancer (LC) is one of the most serious malignancies, with the highest number of deaths per population. However, according to

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the global cancer statistics released by GLOBOCAN in 2023, the incidence of LC has declined at a steady pace since 2006–2007 by 2.6 % in men and 1.1 % in women annually. Furthermore, the decrease in LC mortality accelerated from 2005 through 2014 in both men and women [1]. Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disorder that is predicted to be the third most prevalent cause of death by 2030 [2]. Long-chain non-coding RNAs and chronic inflammation are known to be involved in various crucial signaling pathways for cell proliferation, survival, and differentiation in breast cancer [3,4]. Furthermore, COPD is a common comorbid disease in LC and is estimated to affect 40%–70 % of patients with LC, independent of their age, sex, and smoking history [5]. Yi et al. suggested that patients with LC accompanied by COPD (LC-COPD) have more symptoms, such as cough, sputum production, and dyspnea, and have a shorter overall median survival than patients with LC without COPD [6], which suggests that COPD has become an important cause of LC death. It has been reported that plant and animal medicines (e.g., *Quisqualis indica*) have anti-LC activity [7]. However, the mechanism of action of such medicines in LC-COPD requires further investigation.

The human body has a microbiota equivalent to 10 times the number of human cells. The various bacteria that inhabit the human gastrointestinal tract are called intestinal flora, and are mainly composed of the Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria phyla [8]. In recent years, studies have found that the intestinal flora is closely related to chronic pulmonary disease, LC, and other respiratory diseases [9–11]. He et al. reported that the microbial community phenotypes and the components of the induced sputa in patients with LC-COPD differ from those in patients with COPD or LC alone [12]. In addition, Shimizu et al. demonstrated that the richness of *Acidovorax* in tumor tissues is significantly increased in patients with nonsquamous cell carcinoma complicated by COPD [13]. Aside from the microbial phenotypes in COPD and LC, the study of extracellular vesicles has also provided new insights into the understanding of cancer [14]. However, this previous research has several limitations: only the pulmonary microbiome was evaluated, and no healthy population was established as a control. Therefore, there is a need to investigate the diversity and richness of the gut microbiota in patients with LC-COPD.

An increased inflammatory response, enhanced oxidative stress, and alterations in the lung microenvironment are risk factors that promote the aberrant activation of the JAK-STAT pathway in various disease states, including LC-COPD [15]. Short-chain fatty acids (SCFAs), the main metabolites of the intestinal flora, can regulate host inflammatory responses by regulating the acetylation/deacetylation of the histone proteins in the *Foxp3* gene, which affects the release of regulatory cytokines (interleukin (IL)-10, tumor necrosis factor (TNF)-α, interferon-γ, and IL-6) [16]. Ubachs et al. first reported that the SCFA levels are reduced in cachectic patients with cancer, including LC [17]. Additionally, epithelial growth factor receptor (EGFR) is a cell surface transmembrane receptor that mediates the tyrosine signaling pathway to carry extracellular messages inside the cell and is regarded as a therapy target in LC. Emerging evidence has shown that the regulation of SCFA metabolism is associated with the promotion of epithelial proliferation via activation of the EGFR/ERK pathway [18,19]. However, little research has investigated the alterations of inflammatory factors and SCFAs in patients with LC-COPD.

The present study was designed to explore the abundance and diversity of the gut microbiota in patients with LC-COPD for the first time. The concentrations of SCFAs in the gut and the concentrations of inflammatory factors in serum of patients with LC-COPD were also investigated.

2. Materials and methods

2.1. Study design and population

The prospective study was performed in Zhejiang Hospital, China, between January 3, 2022 and May 21, 2022. All participants were consecutively recruited from the Department of Respiratory Medicine and Thoracic Surgery after confirmation that they met the inclusion and exclusion criteria. The inclusion criteria were: 1) Patients with LC had a clear pathological diagnosis of LC based on pulmonary nodule surgery or lung biopsy by puncture. 2) Patients with COPD were diagnosed based on pulmonary function testing according to the GOLD 2022 standard [20]. 3) Patients with LC-COPD were diagnosed in accordance with both of the abovementioned diagnostic standards. 4) Healthy individuals who had no clinical symptoms included cough, expectoration, and dyspnea, go to hospital for regular physical examination with normal pulmonary CT, which indicated no emphysema or other other abnormalities. 5) All participants were aged between 18 and 80 years. The exclusion criteria were: 1) No clear pathological diagnosis. 2) COPD could not be diagnosed based on clinical data. 3) Suspected LC or LC clearly combined with lung infection. 4) Presence of yellow pus or dark sputum. 5) Administration of antibiotics within 2 weeks or unknown antibiotic use status prior to specimen collection. 6) Interventions such as immunotherapy, radiotherapy, and targeted therapy prior to specimen collection. 7) Sequencing results that presented insufficient absolute abundance or a homogeneous composition of flora.

3. Ethics statement

The study was performed in accordance with the principles of the World Medical Association Declaration of Helsinki of 1975, as revised in 1983, and was approved by the Ethics Committee of Zhejiang Hospital (approval no. 2020–90 (K); approval date December 23, 2020). All individual participants provided written informed consent.

3.1. Blood collection and analysis

The serum was extracted from venous blood by centrifuging at 3,000g for 15 min, and was stored at −70 °C for the inflammatory factor analysis. The concentrations of serum IL-1β (Xuran Biological, EH6281 M, Shanghai, China), IL-6 (Xuran Biological, EH6306 M),

TNF-α (Xuran Biological, EH6513 M), and IL-10 (Xuran Biological, EH6257 M) were measured using commercially available ELISA kits according to the manufacturer's instructions. Routine blood test results, such as the white blood cell count, neutrophil count, and C-reactive protein (CRP) concentration were detected in the clinical chemistry laboratory of Zhejiang Hospital.

3.2. Fecal bacterial DNA extraction and 16s RNA sequencing

The E.Z.N.A.® DNA Stool Mini kit (Omega Bio-tek, Norcross, GA, USA) was used to extract fecal bacterial DNA according to the manufacturer's protocols. The DNA concentration and purity were detected using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA). The V3–V4 hypervariable regions of the qualified bacterial 16S rDNA were amplified with the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') via polymerase chain reaction (PCR) using the GeneAmp PCR System 9700 (ABI Co., MA, USA). The PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and then sequenced using the MiSeq PE300 sequencing platform (Illumina, San Diego, CA, USA).

3.3. Bioinformatics analysis of the gut microbiota

After sequence alignment analysis using Usearch software, all sequences were clustered into operational taxonomic units (OTUs) according to 97 % similarity. Venn diagrams were used to demonstrate the common and unique gut microbiota of the three groups. The species accumulation curve was used to reflect the sample size. The Rank abundance distribution curve and the Rarefaction and Shannon indexes were used to reflect the richness and evenness of species. Principal component analysis (PCoA) was applied in the β diversity analysis to evaluate the similarities and differences between groups. The relative species composition abundances at the phylum, class, order, family and genus levels among groups were demonstrated using bar plots. Differential taxonomic features among groups were obtained using linear discriminant analysis (LDA) effect size (LEfSe).

3.4. Metabolite Extraction and gas Chromatography–Mass spectrometry (GC-MS) analysis

The samples were placed into 2-mL Eppendorf (EP) tubes, extracted with 1 mL H_2O, and vortex mixed for 10 s. The samples were then homogenized in a ball mill for 4 min at 40 Hz and ultrasound treated for 5 min in ice water; these procedures were repeated three times. The samples were then centrifuged for 20 min at 5,000g at 4 °C. The supernatant (0.8 mL) was transferred into a fresh 2-mL EP tube and mixed with 0.1 mL of 50 % H_2SO_4 and 0.8 mL of extracting solution (25 mg/L stock in methyl *tert*-butyl ether) as an internal standard. The sample was vortex mixed for 10 s, oscillated for 10 min, and then ultrasound-treated for 10 min in ice water. The sample was then centrifuged for 15 min at 10,000g at 4 °C before being frozen at −20 °C for 30 min. The supernatant was then transferred into a fresh 2 mL glass vial for GC-MS analysis with a SHIMADZU GC2030-QP2020 NX GC-mass spectrometer. The system used a HP-FFAP capillary column. A 1 μL aliquot of the analyte was injected in split mode (5:1). Helium was used as the carrier gas, the front inlet purge flow was 3 mL/min, and the gas flow rate through the column was 1 mL/min. The initial temperature was kept at 80 °C for 1 min, raised to 200 °C at a rate of 10 °C/min for 5 min, and then kept for 1 min at 240 °C at a rate of 40 °C/min. The injection, transfer line, quad, and ion source temperatures were 240 °C, 240 °C, 150 °C, and 200 °C, respectively. The energy was −70 eV in electron impact mode. The MS data were acquired in Scan/SIM mode with the *m/z* range of 33–150 after a solvent delay of 3.5 min.

3.5. Statistical analysis

SPSS26.0 software was used to analyze and classify the data. Statistical differences were analyzed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). Measurement data were expressed as mean ± standard deviation. Normality tests were performed prior to ANOVA. For normally distributed continuous data, ANOVA was applied for intergroup comparisons. If the data did
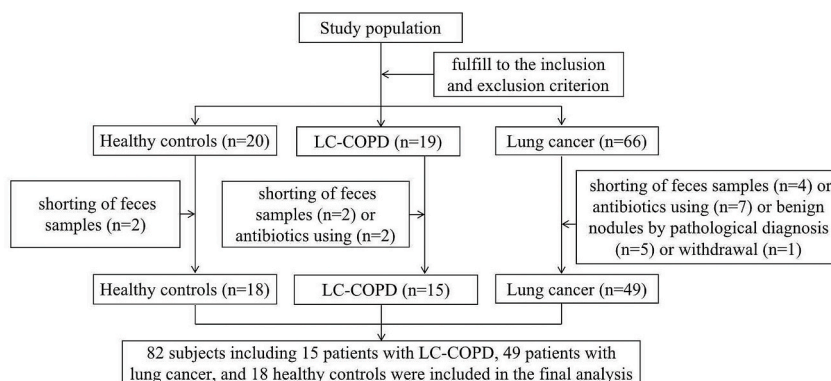

Fig. 1. The flowchart of individuals included into the study.

not conform to normal distribution, the Wilcoxon rank sum test was used to analyze the intergroup differences. All statistical analyses were visualized using R24. P < 0.05 was defined as statistically significant.

4. Results

4.1. Basic clinical characteristics

The study cohort comprised 105 consecutive patients who met the inclusion and exclusion criteria and were recruited into the present study. However, 23 individuals were excluded because of a shortage of fecal samples, use of antibiotics prior to the samples being taken, or other reasons. The final cohort comprised a total of 82 participants, including 15 patients with LC-COPD, 49 patients with LC, and 18 healthy individuals. The study flowchart is shown in Fig. 1. The clinical characteristics of the study participants are shown in Table 1. There were significant differences in age between the LC-COPD versus LC groups and the LC-COPD versus control groups (all P < 0.05). The LC-COPD group had a higher mean age (68.53 ± 5.19 years) than the LC group (57.73 ± 9.83 years) and control group (61.00 ± 7.05 years) (P < 0.05). In addition, the sex distribution significantly differed among the three groups (P < 0.05). There were significant differences between the LC-COPD and LC groups in pulmonary function parameters such as the forced expiratory volume in 1 s (FEV1) and the FEV1/forced vital capacity ratio (all $P < 0.05$). The serum concentrations of albumin, total cholesterol, and low-density lipoprotein cholesterol were significantly lower in the LC group than in the other two groups (all $P < 0.05$), which indicates that patients with LC had a higher nutrient consumption. There were no other differences between the three groups (all $P > 0.05$).

4.2. Analysis of gut microbiota in the three groups

The collected feces samples underwent 16s rDNA analysis to identify the gut microbiota. The similarities and differences in OTUs among the three groups were analyzed using Venn diagrams. The three groups shared 619 OTUs, while the LC-COPD group had its own 410 OTUs, the LC group had its own 1455 OTUs, and the control group had its own 557 OTUs (Fig. 2). According to the number of samples and species OTUs, the species accumulation curve (Fig. 3A), Rank abundance distribution curve (Fig. 3B), Rarefaction curve

Table 1
The basic clinical characteristics of all individuals.

	Control	LC	LC-COPD	Control VS LC		Control VS LC-COPD		LC VS LC-COPD	
	18 (82)	49 (82)	15 (82)	p value	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)
Gender									
Male	6 (33.3)	28 (57.1)	15 (100)	0.000		0.000		0.000	
Female	12 (66.7)	21 (42.9)	0 (0)						
Age	61.00 ± 7.05	57.73 ± 9.83	68.53 ± 5.19	0.518	3.27 (-2.54-9.07)	0.043	7.53 (14.90–0.17)	0.000	−10.80 (-17.01–4.58)
Weight	60.80 ± 11.31	63.49 ± 9.51	68.00 ± 8.01	0.948	−2.69 (-9.22-3.84)	0.111	−7.19 (-15.48–1.09)	0.359	−4.50 (-11.49–2.49)
Height	160.25 ± 8.23	164.67 ± 7.57	168.93 ± 6.62	0.110	−4.43 (-9.52-0.67)	0.005	8.68 (2.22–15.14)	0.180	−4.26 (-9.52-0.67)
FEV1	UN	2.50 ± 0.09	1.95 ± 0.11	UN	UN	UN	UN	0.002	0.54 (0.20–0.89)
FVC	UN	3.20 ± 0.11	3.02 ± 0.11	UN	UN	UN	UN	0.252	0.18 (-0.13-0.49)
FEV1/ FVC	UN	78.63 ± 5.16	64.94 ± 10.02	UN	UN	UN	UN	0.000	13.69 (7.70–19.38)
PEF	UN	8.61 ± 10.90	6.05 ± 2.35	UN	UN	UN	UN	0.372	2.56 (-3.13-0.49)
ALB	42.49 ± 4.47	42.89 ± 3.33	38.68 ± 3.10	1.000	−0.40 (-2.81-2.00)	0.009	3.8 (0.75–6.86)	0.000	4.21 (1.63–6.79)
ALT	23.06 ± 9.38	42.08 ± 13.99	20.55 ± 7.44	1.000	−1.03 (-9.22-7.17)	1.000	2.51 (-7.88-12.9)	0.982	3.53 (-5.24-12.31)
AST	25.38 ± 8.80	23.60 ± 5.85	22.68 ± 5.26	0.972	1.78 (-2.61-6.17)	0.719	2.70 (-2.87-8.26)	1.000	−0.92 (-3.78-5.61)
Cr	63.83 ± 11.53	68.24 ± 15.54	77.33 ± 16.17	0.857	−4.41 (-14.45–5.63)	0.034	13.5 (0.76–26.23)	0.126	−9.08 (-19.83–1.66)
GLU	4.94 ± 0.61	5.14 ± 0.92	6.36 ± 2.60	1.000	−0.2 (-1.10-0.70)	0.010	1.42 (0.28–2.57)	0.008	1.22 (0.26–2.19)
TC	4.89 ± 1.22	4.68 ± 0.74	3.60 ± 0.71	1.000	0.21 (-0.37-0.79)	0.000	1.29 (0.56–2.03)	0.000	1.08 (0.46–1.70)
TG	1.45 ± 0.56	1.25 ± 0.49	1.21 ± 0.44	0.430	0.20 (-0.13-0.54)	0.532	0.24 (-0.19-0.66)	1.000	0.03 (-0.32-0.39)
LDL	2.98 ± 0.85	2.88 ± 0.62	2.07 ± 0.55	1.000	0.10 (-0.34-0.55)	0.001	0.91 (0.34–1.48)	0.000	0.81 (0.33–1.29)
HB	134.5 ± 15.56	134.1 ± 12.35	123.92 ± 26.53	1.000	0.40 (-10.67–11.47)	0.207	10.58 (-3.45-24.63)	0.116	10.19 (-1.66-22.04)

NOTE: ALB: albumin, ALT: alanine aminotransferase.
AST: aspartate aminotransferase, COPD: chronic obstructive pulmonary disease, Cr: creatinine, FEV1: forced expiratory volume in the first second, FVC: forced vital capacity, GLU: blood sugar, HB: hemoglobin, LC: lung caner, LDL: low density lipoprotein, PEF: peak expiratory flow, TC: total cholesterol, TG: triglyceride.

(Fig. 3C) and Shannon curve (Fig. 3D) were calculated to evaluate the richness and diversity of species. The results indicated that the sample size for this study was large enough to reflect species richness. Consequently, the intergroup differences in the α-diversity index were analyzed using the Wilcoxon rank-sum test (Fig. 4). The Simpson index was significantly higher in the LC-COPD group than in the other two groups ($P < 0.05$), while the Shannon index tended to be the lowest in the LC-COPD group ($P = 0.062$), which suggests that the diversity of gut microbiota was decreased in patients with LC-COPD. The PCoA based on the Bray_Curtis distance displayed the similarities and differences between different environments. The contributions of the principal components PC1, PC2, and PC3 were 12.38 %, 6.1 %, and 4.51 %, respectively (Fig. 5A), which showed that there were significant differences among the three groups ($P = 0.038$) (Fig. 5B). The subgroup PCoA results revealed no remarkable differences identified between the control and LC groups ($P = 0.709$) (Fig. 6A), and between control and LC-COPD group ($P = 0.196$) (Fig. 6B), except for a remarkable differences between the LC-COPD and LC groups ($P = 0.005$) (Fig. 6C). The highest species abundances were selected to analyze the distribution differences at the levels of phylum, class, order, family, genus, and species. The dominant phyla were Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Fig. S1). The Metastats analysis was used to analyze the differences at the family and genus levels, which demonstrated that the richness of *f_Coriobacteriaceae*, *f_verrucomicrobiaceae* and *f_Enterococcaceae* was increased at the family level in patients with LC-COPD (Fig. 7A). The richness of *Collinsella*, *Akkermansia,* and *Enterococcus* genera was increased and the abundance of *Faecalibacterium* was decreased in the LC-COPD group (Fig. 7B). In addition, the differences are demonstrated at the phylum, class, order, and species levels in figs. S2–S5. Based on the integration of the Metastats analysis and LDA, LEfSe was also used to analyze the species with significant differences. The Cladogram diagram illustrated that the evolutionary relationship of differential bacteria among three groups (Fig. 8A). The o_*Lactobacillales*, g_*Lactobaccillus*, f_*Lactobaccillaceae,* s_*Lactobaccillus_oris*, c_*Bacilli*, g_*Anaerofustis*, s_*uncultured organism*, and s_*bacterium_P1C10* species were more frequent in patients with LC-COPD, while the species g_*Clostridium_XIVa* and g_Butyricicoccus were more prevalent in patients with LC compared with the control group. In addition, g_*Acetivibrio*, c_*Deltaproteobacteria*, f_*Desulfovibrionaceae*, o_*Desulfovibrionales*, and g_*Sutterella* were abundant in the control group (Fig. 8B).

4.3. Alteration of SCFA concentrations

To explore the alterations in SCFAs, GC-MS was used to detect the concentrations of acetic acid, butyric acid, propionic acid, isobutyric acid, isovaleric acid, and valeric acid in the intestine. The concentrations of butyric acid (Fig. 9B), isobutyric acid (Fig. 9C), isovaleric acid (Fig. 9D), and valeric acid (Fig. 9F) tended to be lower in the LC and LC-COPD groups compared with the control group, although no significant intergroup differences were identified ($P > 0.05$). In addition, no other diferences for acetic acid and propionic acid were determined among three groups ($P > 0.05$) (Fig. 9A and E). There were also no significant differences between the three groups in the concentrations of medium- and long-chain fatty acids (Fig. S6).

4.4. Differences in serum inflammatory factors among the three groups

The CRP concentration was significantly lower in the LC-COPD group compared with the LC and control groups ($P < 0.05$). In addition, the LC group had a significantly lower serum concentration of TNF-a than the LC-COPD and control groups ($P < 0.05$) (Table 2).
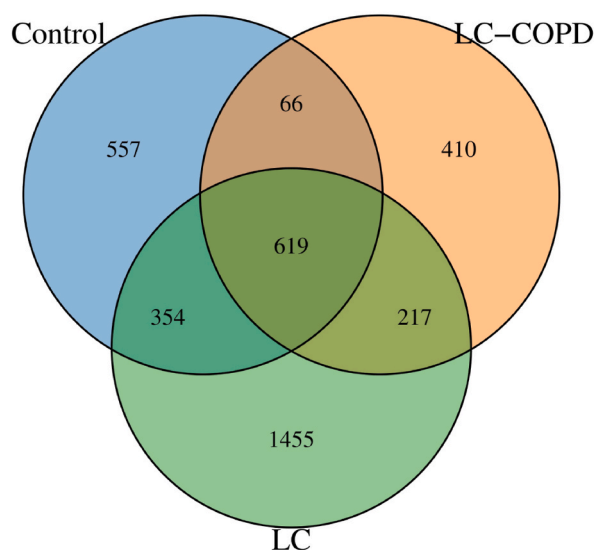

Fig. 2. Venn diagram showing the OTUs based on genera within the gut microbiome among three groups, which indicated that the three groups shared 619 OTUs, while the LC-COPD group had its own 410 OTUs, the LC group had its own 1455 OTUs, and the control group had its own 557 OTUs.
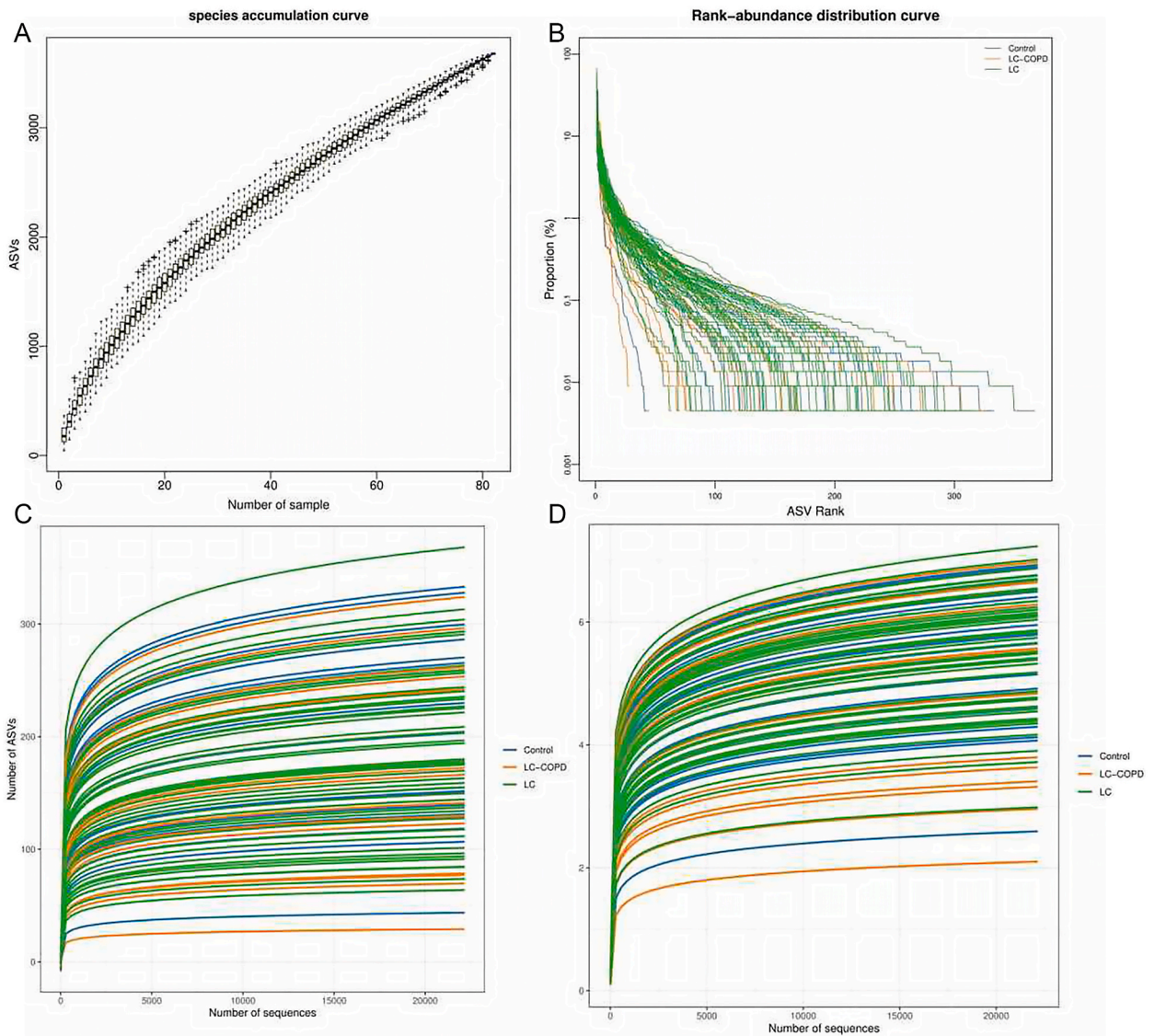
Fig. 3. Alpha diversity analysis (A. Species accumulation curve, B. Rank-abundance distribution curve, C. Rarefaction curve, D. Shannon index) were used to reflect the species richness and diversity, which suggested that the sample size for this study was large enough to reflect species richness.

5. Discussion

The present study was the first to explore the richness and diversity of the gut microbiota in patients with LC-COPD. The results suggest that the richness and diversity of the gut microbiota is decreased in patients with LC-COPD. In addition, the prevalent species in patients with LC-COPD were o_*Lactobacillales*, g_*Lactobaccillus*, f_*Lactobaccillaceae,* s_*Lactobaccillus_oris*, c_*Bacilli,* g_*Anaerofustis*, s_*uncultured organism*, and s_*bacterium_P1C10,* while g_*Clostridium_XIVa* and g_*Butyricicoccus* species were more prevalent in patients with LC. Furthermore, the concentrations of butyric acid, isobutyric acid, isovaleric acid, and valeric acid tended to be lower in patients with LC-COPD and LC compared with the control group, although these intergroup differences were not significant. In addition, the patients with LC had the lowest serum concentration of TNF-α among the three groups.

In the present study, the LC-COPD group a higher mean age than the other two groups and only contained male patients. It is known that the older adult population are more susceptible to COPD, and only patients with very severe COPD seek medical advice in China, which led to the higher mean age in the LC-COPD group. In addition, all patients with LC-COPD were men, and the sex ratio significantly differed among the three groups, which suggests that men had a higher prevalence of smoking than women and subsequently developed COPD or LC. Pulmonary disorders, such as LC and COPD, are usually accompanied by dysbiosis of the intestinal flora and an immune-inflammatory response. Accumulating evidence has indicated that the gut microbiota plays an important role in
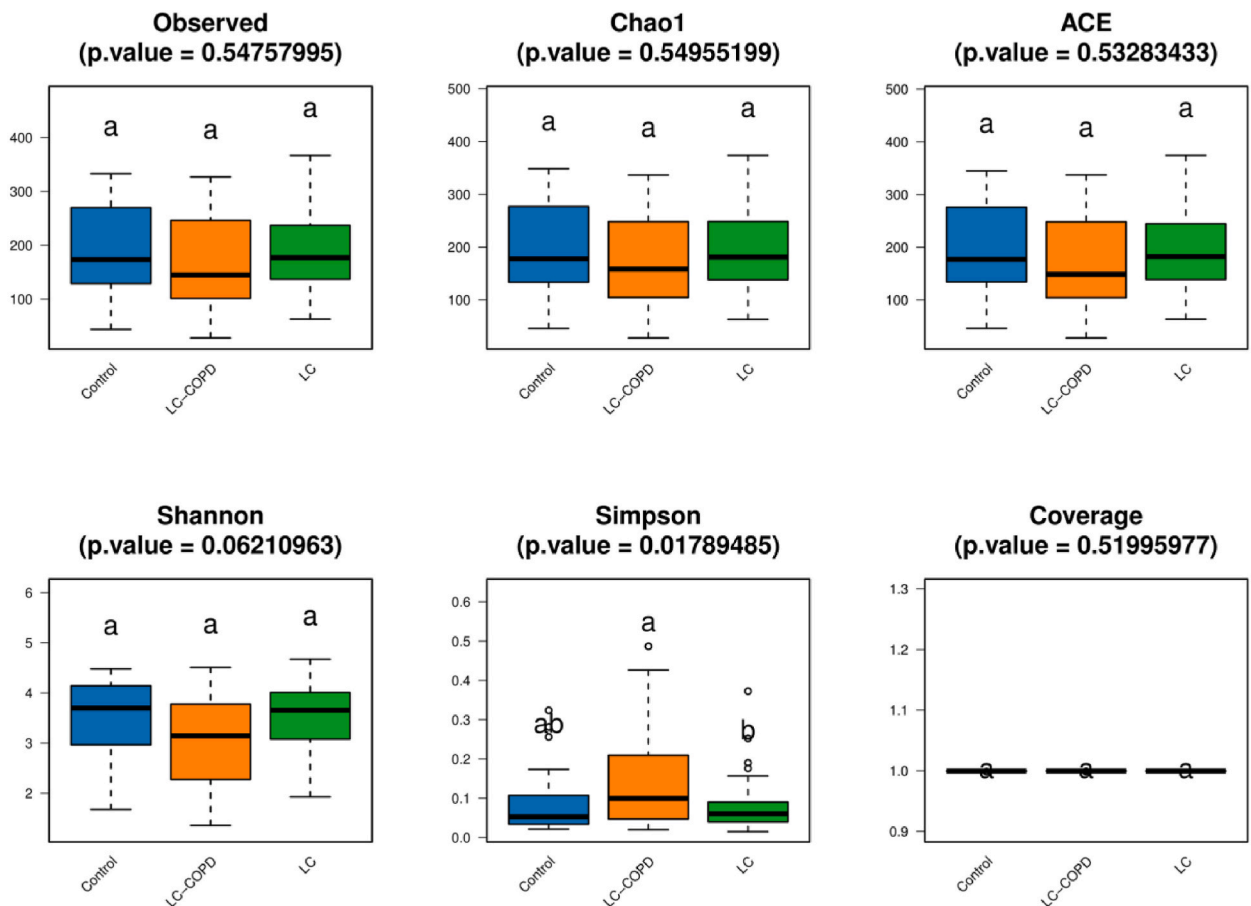
Fig. 4. The Wilcoxon Rank sum test was used to analyze the alpha diversity index differences among three groups. The Simpson index was significantly higher in the LC-COPD group compared with the other two groups (P = 0.017), while the Shannon index tended to be the lowest in the LC-COPD group (P = 0.062), which suggests that the diversity of gut microbiota was decreased in patients with LC-COPD.

carcinogenesis and the progression of cancers by metabolism and inflammation. He et al. reported that the relative abundances of *Streptococcus, Veillonella, Moraxella,* and *Actinomyces* are significantly reduced, while the abundances of *Neisseria* and *Acinetobacter* are significantly elevated, in the lung tissue of patients with LC-COPD compared with patients with COPD or LC alone [12]. Another study also indicated that the abundance of the microbiome in lung tumor tissues is significantly increased in patients with LC-COPD [13]. However, no study has evaluated the diversity and richness of the gut microbiota in patients with LC-COPD. The present study indicated that the diversity and richness of the gut microbiota were lower in patients with LC-COPD compared with patients with LC and healthy controls. In addition, the β analysis also demonstrated significant differences between patients with LC-COPD and those with LC. The LEfSe results suggested that the o_*Lactobacillales*, g_*Lactobaccillus*, f_*Lactobaccillaceae*, s_*Lactobacillus_oris*, c_*Bacilli,* g_*Anaerofustis*, s_*uncultured organism*, and s_*bacterium_P1C10* species were frequently present in patients with LC-COPD, and that the richness of g_*Clostridium_XIVa* and g_*Butyricicoccus* was increased in patients with LC. These findings are in accordance with the findings of previous research. Cong et al. reported that the abundance of *Clostridium XIVa* is increased in pre-treatment LC [21]. It has also been reported that disorder of the gut microbiota is involved in the development of gastrointestinal cancer. Ramli et al. reported that lncRNA UCA1 is a potential molecular target and a potential diagnostic and prognostic marker in patients with gastrointestinal cancer [22]. Another study also revealed that fecal *Anaerotruncus* spp. and *Bacteroides caccae* are abundant and may be associated with the risk of non-small cell lung cancer (NSCLC), regardless of sex and smoking history [23]. Overall, these abovementioned studies suggest that the gut microbiota is altered in patients with LC-COPD. It was not possible to identify a microbiome signature in the present case series, as has been the case in previous studies of many other intestinal diseases, such as inflammatory bowel disease or pediatric celiac disease [24,25]. A previous review reported that there was no evidence to verify the specific gut microbiota signature in pediatric celiac disease [24]. Furthermore, dysbiosis of the gut microbiota is not regarded as a causal factor for inflammatory bowel disease [25]. These heterogeneities may be ascribed to differences between studies in experimental procedures and study design. Therefore, based on the current evidence, the link is not even as clear between gut microbiota dysbiosis and extraintestinal diseases, Further investigation is needed to identify the species that act as biomarkers for predicting the risk of LC-COPD.

It has been reported that SCFAs produced by the gut microbiota play an anti-inflammatory role by regulating Th17/Treg cell
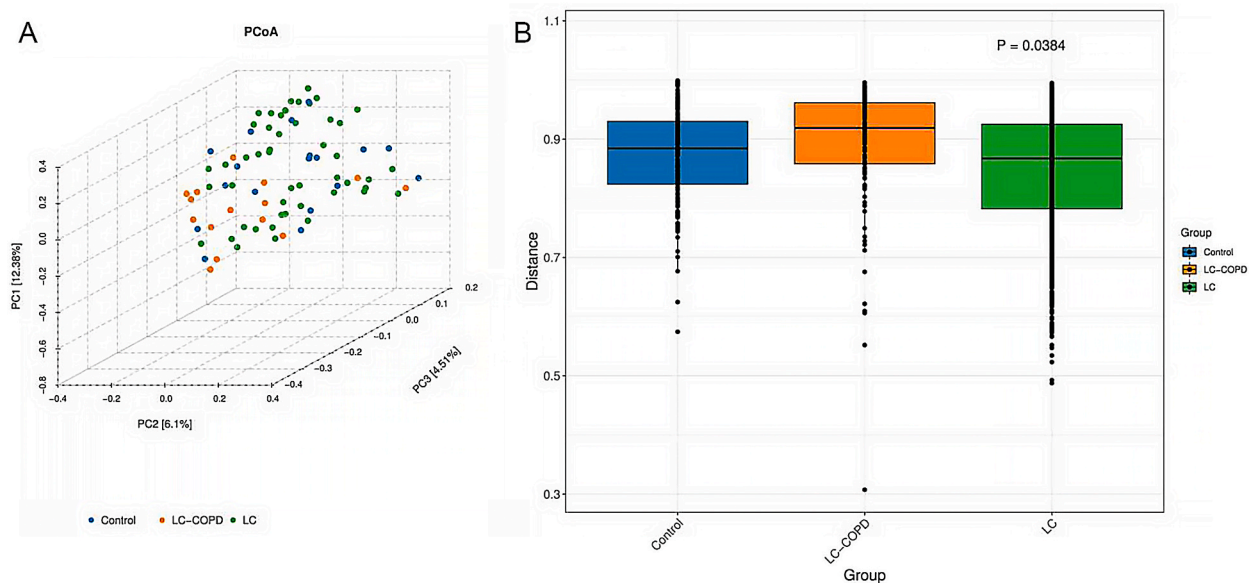
Fig. 5. The beta diversity of the gut microbiome among three groups. The principal coordinate analysis (PCA) graph was obtained by calculating the weighted UniFrac distance indicated that the contributions of the principal components PC1, PC2 and PC3 were 12.38 %, 6.1 % and 4.51 %, respectively (A), which showed that the differences had remarkable among three groups (P = 0.038) (B).
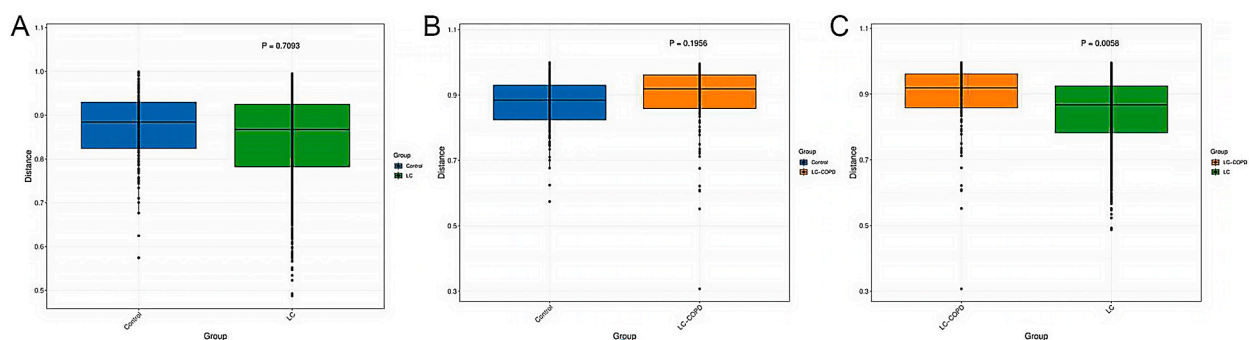

Fig. 6. The subgroups analysis was conducted to certify the intergroup differences among three groups in terms of beta diversity, which revealed no remarkable differences identified between the control and LC groups (P = 0.709) (A), and between control and LC-COPD group (P = 0.196) (B), except for a remarkable differences between the LC-COPD and LC groups (P = 0.005) (C).

differentiation and releasing inflammation factors [26]. Several studies have also reported that SCFAs are crucial in cell homeostasis because they contribute to the modulation of histone deacetylases, resulting in affected cell attachment, immune cell immigration, cytokine production, chemotaxis, and programmed cell death [27]. Other than SCFAs, materials such as chitosan-tamarind gum polysaccharide polyelectrolyte complex also play an important antitumor role [28]. Gui et al. found that most of the gut SCFA-producing bacteria are significantly decreased in patients with NSCLC [29]. Ubachs et al. also found that the fecal levels of all SCFAs tended to be lower in cachectic patients with cancer, including LC [17]. Therefore, the manipulation of SCFA levels in the intestinal tract via alterations in the microbiota structure or the administration of SCFA supplements may be useful in cancer treatment [30]. Chen et al. found that probiotic supplementation attenuated the lung metastasis of melanoma cells in mice associated with the production of SCFAs in the gut [31]. Chen et al. also reported that supplementary valeric acid had a significant therapeutic effect on NSCLC [32]. However, the changes in the concentrations of SCFAs in patients with LC-COPD remain unclear. The present study found that the concentrations of the SCFAs butyric acid, isobutyric acid, isovaleric acid, and valeric acid tended to be decreased in patients with LC-COPD, and similar tendencies were also seen in patients with LC, although no significant intergroup differences were determined. Overall, these studies demonstrate that patients with LC-COPD or LC experience a reduction in SCFAs, which is positively associated with decreased SCFA-producing bacteria in the gut. As mentioned above, g_*Acetivibrio*, c_*Deltaproteobacteria*, f_*Desulfovibrionaceae*, o_*Desulfovibrionales*, and g_*Sutterella* were more prevalent in the control group compared with patients with LC-COPD or LC. This may be because SCFAs are the metabolic product of these species, and thus the decreasing of their prevalence led to the lower concentrations of SCFAs [33–36].
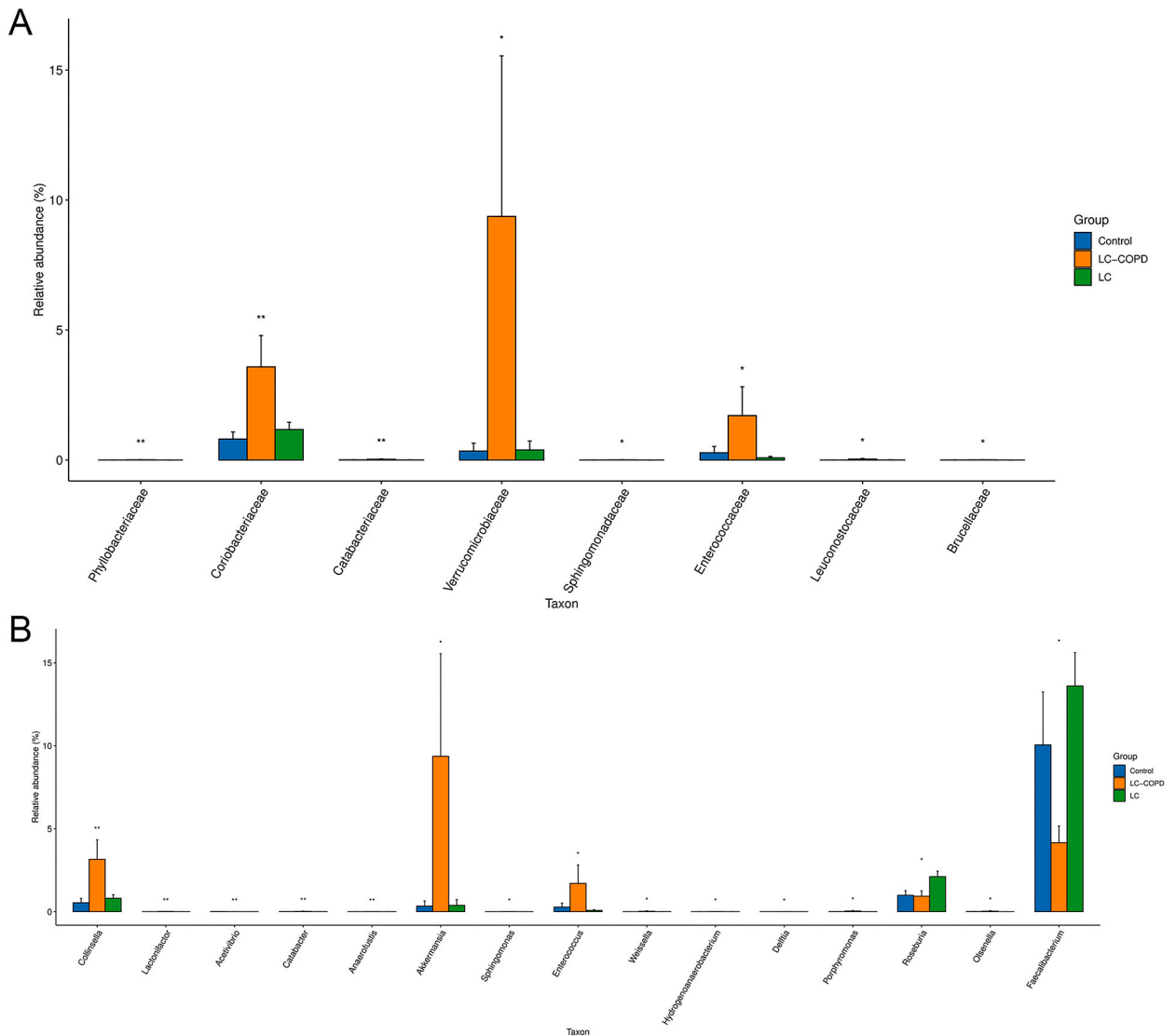
Fig. 7. The Metastats analysis was used to analyze the differences, which demonstrated that the richness of *f_Coriobacteriaceae*, *f_verrucomicrobiaceae* and *f_Enterococcaceae* increased at the family of level in patients with LC-COPD (A), the genus for *Collinsella, Akkermansia* and *Enterococcus* increased and the abundance of *Faecalibacterium* decreased in LC-COPD patients (B).

Abnormal local and systemic inflammation has been implicated in predisposing patients with COPD to LC [37]. The main source of inflammatory factors comes from reactive carbonyl species generated by continuous oxidation of carbohydrates, lipids, and amino acids [38]. Liao et al. found that the circulating levels of Th17-related cytokines, such as IL-23, IL-17, IL-22, and TNF-α, are significantly increased in patients with LC, which shows a clear association with the risk of NSCLC complicated by the presence or absence of COPD [39]. In addition, Kaanane et al. revealed a significant difference in the mRNA expression levels of IL-6, IL-8, IL-10, IL-17, and TNF-α genes in patients with LC compared with healthy individuals in the Moroccan population [40]. In accordance with these previous studies, the present study indicated that the LC-COPD group had an increased CRP concentration, which was related to the lower concentrations of SCFAs. As mentioned above, the richness and diversity of the gut microbiota and the SCFA concentrations were decreased in patients with LC-COPD. However, the present study also found that patients with LC had the lowest serum concentration of TNF-α compared with the other two groups; this was probably because most patients with LC in the current study were still in the early stages, and therefore abnormal local inflammatory factors had not yet been released into the peripheral blood.

The present study has certain limitations. First, because funding was limited, only 15 patients with LC-COPD were recruited. Furthermore, because of the small sample size of patients with small cell lung cancer (SCLC), only three patients with SCLC were recruited into the LC-COPD group; therefore, a subgroup analysis based on the histological type divided into NSCLC or SCLC was not performed. Second, our experience suggests that men in China have a higher prevalence of smoking than women, and subsequently have higher prevalences of COPD and LC. Therefore, only men were recruited into the LC-COPD group, which led to a certain level of
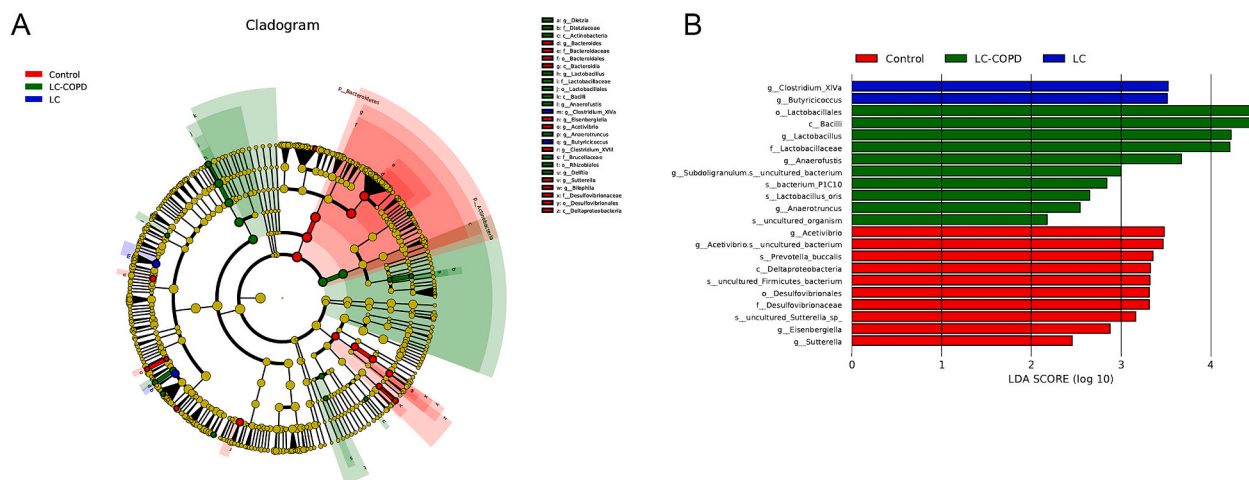
Fig. 8. The Cladogram diagram illustrated that the evolutionary relationship of differential bacteria among three groups (A). The species for o_*Lactobacillales*, g_*Lactobaccillus*, f_*Lactobaccillaceae,* s_*Lactobaccillus_oris*, c_*Bacilli,* g_*Anaerofustis*, s_*uncultured organism*, s_*bacterium_P1C10* were frequent in patients with LC-COPD, and the species for g_*Clostridium_XIVa* and g_Butyricicoccus prevalent in patients with LC compared with healthy population (B).
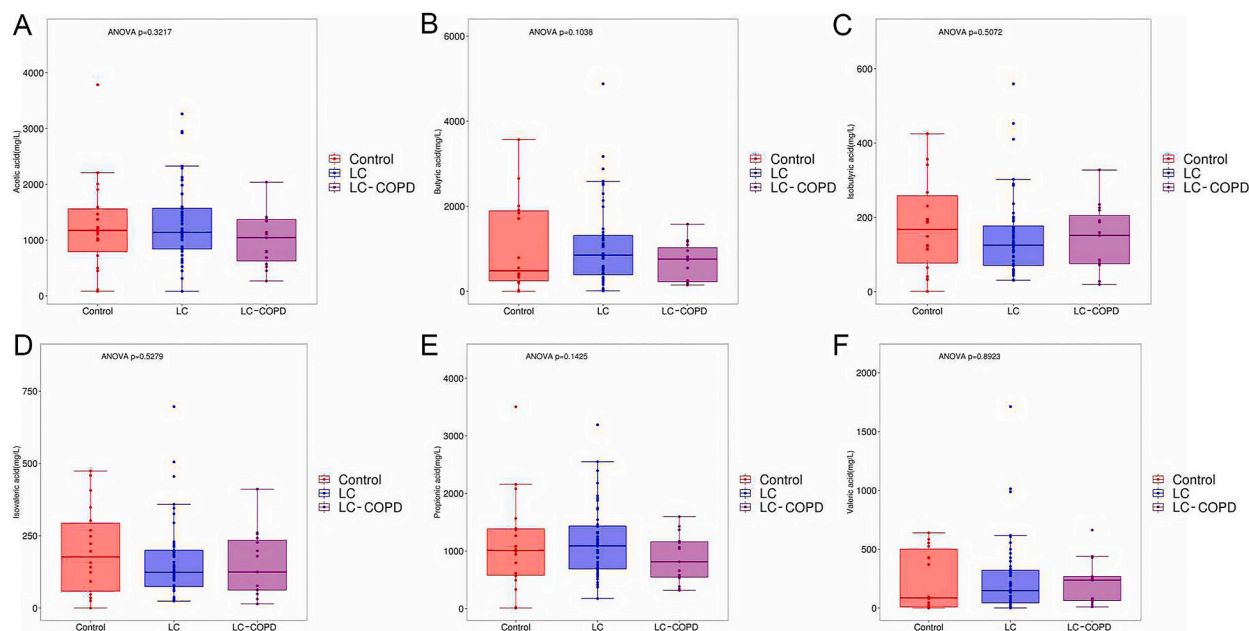

Fig. 9. The compasion of concentration of SCFA among three groups, and no significant intergroup differences were identified (all *P* > 0.05) (A. Acetic acid, B. Butyric acid, C. Isobutyric acid, D. Isovaleric acid, E. Propionic acid, F. Valeric acid).

bias. However, this was a real-world case-control study that reflects the actual clinical situation. To more comprehensively explore the changes in intestinal flora in patients with LC-COPD and the impact of these changes on metabolism and inflammatory factors, we plan to expand the sample size and address the abovementioned limitations in a future study.

In summary, the results of the current study indicated that the richness and diversity of the gut microbiota are decreased in patients with LC-COPD. Furthermore, the concentrations of some SCFAs, namely butyric acid, isobutyric acid, isovaleric acid, and valeric acid, tended to be lower in patients with LC or LC-COPD compared with healthy controls, although these intergroup differences were not significant. In addition, patients with LC had the lowest concentration of TNF-α among the three groups. However, because of the loosely defined relationship between an imbalanced gut microbiota and extraintestinal diseases, the present findings require verification in future studies with larger sample sizes.

Table 2
Inflammatory factor values for all individuals.

	Control	LC	LC-COPD	Control VS LC		Control VS LC-COPD		LC VS LC-COPD	
	18 (82)	49 (82)	15 (82)	p value	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)
WBC	5.46 ± 1.80	5.44 ± 1.39	6.14 ± 1.85	1.000	0.01 (-1.05-1.08)	0.648	−0.69 (-2.03-0.66)	0.406	−0.70 (-1.84-0.44)
NE	61.88 ± 11.51	60.72 ± 0.76	61.50 ± 16.33	1.000	1.15 (-6.64-8.94)	1.000	−0.38 (-9.50-10.27)	1.000	−0.77 (-7.57-9.11)
CRP	3.09 ± 4.41	2.32 ± 3.29	8.31 ± 9.40	0.213	0.77 (-2.69-4.24)	0.014	5.22 (9.62–0.83)	0.001	5.99 (2.28–9.70)
TNF-α	209.52 ± 170.49	153.09 ± 152.90	216.54 ± 127.31	0.046	56.43 (-46.55–159.4)	0.746	−7.02 (-137.64–123.60)	0.025	−63.45 (-173.70–46.80)
IL-1β	33.81 ± 24.81	29.92 ± 22.42	31.84 ± 10.08	1.000	3.88 (-10.51–18.27)	1.000	1.97 (-16.29–20.22)	1.000	−1.92 (-17.33–13.49)
IL-6	27.92 ± 22.42	28.14 ± 11.45	30.34 ± 12.60	1.000	−0.65 (-8.64-7.34)	1.000	−2.85 (-12.99–7.29)	1.000	−2.20 (-10.76–6.36)
IL-10	19.66 ± 14.53	19.87 ± 11.11	20.69 ± 9.52	1.000	−0.21 (-8.09-7.66)	1.000	−1.03 (-11.02–8.96)	1.000	−0.82 (-9.25-7.61)

NOTE: COPD: chronic obstructive pulmonary disease, CRP: C reactive protein, IL: interleukin, LC: lung cancer, NE: neutrophil, TNF: tumor necrosis factor, WBC: white blood cell.

Ethical approval statement

The study was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki of 1975, as revised in 1983. The study was approved by the Ethics Committee of Zhejiang Hospital (approval no. 2020–90 (K)) in 2020-12. All individual participants provided written informed consent.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author or through the link: https://doi.org/10.17632/ycjgjfr56d.1.

CRediT authorship contribution statement

Tingxiang Wang: Writing – original draft. **Wanting Su:** Data curation. **Li Li:** Methodology. **Haiyan Wu:** Formal analysis. **He Huang:** Investigation. **Zhijun Li:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix g. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30380.

Appendix. files

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