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Comparison of gastric microbiota in patients with different gastric lesions in high and low risk areas of gastric cancer

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Abstract

Background Variation in gastric cancer (GC) incidence across different geographic areas persists, even when there are similar prevalence rates of *Helicobacter pylori (H. pylori)* infection. An extensive examination of the gastric microbiota in populations from both high- and low-risk regions of GC could help explain the geographical disparities in GC incidence.

Methods This study enrolled a total of 130 patients with superficial gastritis (SG) and precancerous lesions of gastric cancer (PLGC) from a high-risk area for GC and 205 patients from a low-risk area. Gastric microbial profiling was performed using 16 S rRNA gene sequencing to analyze differences in microbial composition between regions and lesion types.

Results The study revealed significant differences in gastric microbial profiles between patients from high- and low-risk regions, particularly in PLGC patients. PLGC patients from the low-risk region exhibited higher microbial richness than those from the high-risk area, with marked distinctions in microbial community structure between the two regions. Specific differences in microbial composition were observed at the phylum and genus levels between different regions. Six bacterial genera, including *Selenomonas* and *Peptostreptococcus*, were identified as enriched in PLGC patients from the high-risk area. Additionally, there was a noticeable imbalance in the microbiota of the gastric mucosal lining during the progression of gastric lesions.

Conclusion This comparative analysis highlights the potential impact of the gastric microbiome in the development of GC and suggests that regional differences in microbial profiles may provide clues to the varying incidence rates of GC.

Keywords Gastric cancer, Gastric precancerous lesions, Microbiome, 16S rRNA sequencing, Geographical differences

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Background

Gastric cancer (GC) stands as the fifth most prevalent form of cancer on a global scale and is identified as the fourth leading cause of cancer-related mortality worldwide [1]. GC's incidence and mortality rates exhibit significant regional variations, with particularly high rates observed in Eastern Asia, notably in China, Japan, and South Korea [2–4]. According to the latest data from Cancer Statistics in China [5], approximately 358,700 patients were diagnosed with GC, and an estimated 260,400 patients died from GC in 2020, underscoring its persistent impact on public health in China. Similarly, GC displays a markedly diverse geographical distribution within China, predominantly influenced by environmental variabilities, lifestyle habits, and genetic predisposition [6]. Recent studies have proposed the gastric microbiome's potential implication in the GC's initiation and progression.

Helicobacter pylori (*H. pylori*) infection stands out as the most significant risk factor for GC [7, 8], as it can precipitate chronic inflammation and subsequently initiate the Correa's cascade, a stepwise progression from superficial gastritis (SG) to chronic atrophic gastritis (CAG), intestinal metaplasia (IM), dysplasia (DYS) and eventually adenocarcinoma [9, 10]. Nonetheless, it appears that only a fraction of individuals infected with *H. pylori* eventually progress to GC [11]. And the incidence of GC exhibits significant variability within populations characterized by similar rates of *H. pylori* infection, indicating a potential influence of the non-*H. pylori* microbiota and altered gastric microbial profiling in GC tumorigenesis [12]. There has been a growing interest in studying changes in the stomach's microbiota during the development of gastric lesions. A previous study conducted in a region with a high incidence of GC revealed notable differences in microbial diversity and community structure across various gastric lesions [13]. Furthermore, the abundance of certain pathogenic bacteria demonstrates an increase when progressing from the normal state to the early precancerous state in a population with low-*H. pylori* prevalence, as evidenced in a separate study [14].

Despite these advancements, there is a lack of comparative research on the gastric microbiota in patients with different gastric lesions in high-risk and low-risk areas for GC. Such studies could offer critical insights into the potential role of the gastric microbiome in relation to the geographical disparities in GC incidence and might facilitate the development of innovative preventive and therapeutic approaches.

In this study, we undertook a comparative profiling of gastric microbiota in patients presenting with SG and precancerous lesions of gastric cancer (PLGC, encompassing CAG, IM, and DYS) originating from both high-risk and low-risk areas for GC.

Materials and methods

Study population and sample collection

Patients undergoing gastroscopy at the endoscopy centers of Changle District Hospital in Changle (Fuzhou, Fujian province, China), a high-risk area for GC (the incidence of GC was 29.58/100,000 in 2019) and Min Dong Hospital in Fu'an (Ningde, Fujian Province, China), a low-risk area for GC (the incidence of GC was 8.95/100,000 in 2019), were recruited for this study. Individuals residing within the local area and the age range of 20 to 80 were considered eligible to participate. Exclusion criteria included the presence of cancer, severe medical or psychiatric illnesses, current pregnancy, or breastfeeding. All participants were invited to complete a questionnaire on their lifestyle habits, disease, and medication history before undergoing gastroscopy (supplementary questionnaire). We collected 5 mL of fasting venous blood from each patient directly into a blood collection tube containing EDTA. Within 30 min after collection, the sample was centrifuged at 3500 rpm for 10 min. The serum was then immediately frozen at -80 °C and stored for future use. Gastroscopic examinations were performed by skilled endoscopists following a structured endoscopy protocol. Gastric biopsy specimens were taken from the gastric antrum and gastric body, with additional biopsies taken from any suspected precancerous lesions as needed for histopathological evaluation and diagnosis. Subjects were classified according to the most advanced histopathological lesion observed. In addition, cytology brushing samples from the antrum were obtained during the endoscopy for 16 S rRNA sequencing. The samples collected were immediately frozen at − 80 °C and stored for later use.

Between March 2017 and December 2017, 213 eligible subjects were enrolled at Changle District Hospital, with 19 individuals excluded for lack of a precise pathological diagnosis, along with 1 case diagnosed with GC, 4 subjects with missing or incomplete questionnaire information, and 59 who used antibiotics, bismuth or PPI within 4 weeks before endoscopy. From May 2018 to August 2018, 255 eligible subjects were enrolled at Min Dong Hospital, with 12 individuals excluded due to unclear pathological diagnosis, along with 17 subjects with missing or incomplete questionnaire information, 20 who used antibiotics, bismuth or PPI within 4 weeks before endoscopy, and 1 participant with disqualified 16 S rRNA sequencing data. Ultimately, 130 patients from Changle remained, including 27 with SG and 103 with PLGC (85 CAG, 6 IM and 12 DYS). Similarly, 205 patients from Fu'an were left, including 130 with SG and 75 with PLGC (50 CAG, 21 IM, and 4 DYS) for final analysis. The flowchart of participant enrollment is presented in Fig. 1. The research protocol received approval from the Ethics Committee of Fujian Medical University Union Hospital

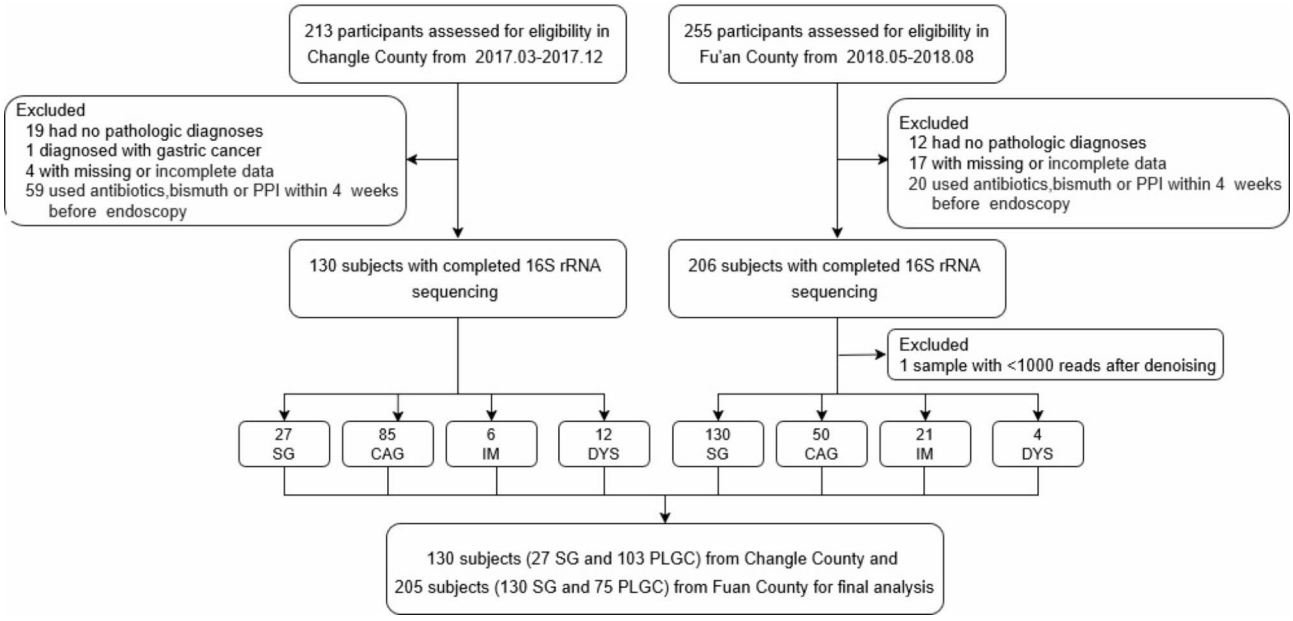

Fig. 1 The flowchart of enrollment of study participants

under reference number 2017KY064. Furthermore, written informed consent was obtained from all participants involved in the study.

DNA extraction and sequencing

The genomic DNA was extracted from antrum brushing samples using a two-step method. First, the samples underwent pretreatment with lysozyme lysis buffer (20 mg/ml lysozyme dissolved in 1 mM EDTA, 20 mM Tris HCl pH 8.0 and 1.2% Triton X-100) and mechanical bead beating, then the genomic DNA was extracted using the Magnetic Universal Genomic DNA Kit (Tiangen Biotech, Beijing, China) in accordance with the manufacturer's instruction.

The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified from the extracted DNA samples with universal primers (341F, 5'-CCTACGGGNGGCWGCAG-3'; 805R, 5'-GACTACHVGGGTATCTAATCC-3'). All PCR reactions were amplified using KAPA HIFI HotStart ReadyMix (KAPA Biosystems, USA), with a reaction system of 12.5 μl of KAPA HIFI HotStart ReadyMix, 1 μl each for upstream and downstream primers (10 μM), along with 50 ng DNA, and finally with ddH_2O used to replenish to 25 μl. The PCR cycling parameters were a 5 min denaturation cycle at 95 °C, followed by 35 cycles of the following: 98 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, followed by a 2 min extension at 72 °C, and finally 4 °C until the end. Next, PCR product was purified using Agencourt AMPure XP (Beckman Coulter, USA). A second-round PCR amplification was performed on the purified PCR product, by adding Illumina sequencing-compatible and barcode-index, and then being purified again. Finally, the library was merged in equimolar ratio and 2 × 300-bp paired-end sequencing was performed on the Illumina MiSeq platform (Illumina, USA) with PhiX control.

Detection of serum gastrin

The serum gastrin concentrations of all samples were measured using the ELISA Kit for Gastrin (GT) (Cloud-Clone Corp., China). The manufacturer reports a detection range of 12.35 pg/mL to 1,000 pg/mL, with a sensitivity of 4.89 pg/mL and intra- and inter-assay coefficients of variation (CV) both less than 15%. The analysis was conducted in a controlled laboratory setting, strictly following the manufacturer's instructions. According to the instructions, gastrin concentrations were calculated using individual standard curves for each plate. Specifically, six standards provided by the manufacturer (12.35–1,000 pg/mL) and blanks were used. The standard curves were generated by subtracting the optical density of the blanks from the standard solutions and plotting the resulting optical densities. The gastrin concentrations of the samples were then calculated using the equations derived from the standard curves of the corresponding plates.

Sequencing data processing

The demultiplexed paired-end 16 S rRNA sequences were processed using QIIME2 (v.2020.8.0) [15]. The primer and adapter sequences were trimmed using Cutadapt. Subsequently, the DADA2 plugin was used for sequence quality filtering and denoising, chimera removal, and finally, the remaining sequences were merged to obtain

amplicon sequence variants (ASVs) table with appropriate parameters (forward and reverse reads were length trimmed at 260 and 201 bases, respectively) [16]. Taxonomic classification of ASVs was carried out using the QIIME2 naive-Bayes classifier, based on the GreenGenes database (version 13.8) [17]. ASVs with a frequency less than 0.001% of the total number of sequences, as well as those assigned to chloroplast, mitochondrial, and eukaryotic sequences, were discarded. One sample with less than 1000 sequence reads was further excluded from subsequent analysis. Finally, the filtered ASV table was created for further downstream analysis. Then, based on representative sequences of ASVs, a rooted phylogenetic tree was constructed using the FastTree plugin [18].

Statistical analysis

A filtered ASV table, taxonomic table, sample information table and phylogenetic tree from raw sequencing data processing were integrated into a microtable class for subsequent data preprocessing using the 'microeco' package [19]. All samples were rarefied to the smallest number of sequences for diversity analysis. Bacterial alpha-diversity was assessed by Observed ASVs, Chao1 and Shannon, Simpson indexes. Beta diversity was estimated using principal coordinate analysis (PCoA) on Bray-Curtis dissimilarity and weighted Unifrac distance and its significance was tested by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations in the 'vegan' package (Adonis test in the "vegan' package). The redundancy analysis (RDA) and Mantel test were applied to evaluate the correlation between environmental variables and microbial beta diversity distance matrices (Bray–Curtis distance matrices). Linear discriminant analysis (LDA) effect size (LEfSe) analysis was conducted to identify differentially abundant genera with relative abundance higher than 0.01% between groups (LDA score > 3.0 and *P* value < 0.05). To further refine our analysis and control for potential confounding factors, we employed mixed-effects models using MaAsLin2 (Multivariate Association with Linear Models). Specifically, we adjusted our models to incorporate smoking, alcohol consumption, and *H. pylori* infection as fixed effects, which are known to influence both gastric microbiota composition and gastric cancer risk.

Participants with a relative abundance of less than 1% of *H. pylori* were grouped as *H. pylori*-negative, while those with greater than 1% were grouped as *H. pylori*-positive as previously described [20, 21]. The baseline characteristics of participants were compared by the Student's t-test for continuous variables and the Chi-squared test for categorical variables. Wilcoxon rank test or Kruskal-Wallis sum-rank test was used to test for differences in alpha-diversity indices and the relative abundance of taxa between groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were utilized to assess the relationship between categorical alpha diversities and the risk of gastric lesion severity using ordinal logistic regression analysis. Analyses were performed without adjustment and with adjustment for age, sex, and variables potentially affecting alpha diversity. To account for the large number of taxa comparisons and reduce the risk of false positives, we applied the false discovery rate (FDR) correction method using the Benjamini-Hochberg procedure. The FDR-adjusted *P*-values were considered significant at a threshold of 0.05. All other analyses were performed with R software version 4.1.0. All tests were 2-tailed, and statistical significance was set to $P < 0.05$.

Results

Basic characteristics of study participants

A total of 27 patients with SG and 103 patients with PLGC from the Changle area were included in the study, along with 145 SG cases and 80 PLGC cases from the Fu'an region. The basic characteristics of these patient groups in both areas are presented in Table 1. In the Changle region, PLGC cases had a lower likelihood of having ever smoked and a higher *H. pylori* infection rate (58.25% vs. 37.04%) compared with SG controls, while age, sex, education level, BMI, alcohol consumption and serum gastrin levels showed no substantive differences between the groups. Similarly, within the Fu'an region, the PLGC patients displayed higher rates of *H. pylori* infection compared to the SG controls (62.67% vs. 48.46%), with the other fundamental characteristics being comparable between the two groups. Among the 180 patients identified as *H. pylori* positive, the average relative abundance of *H. pylori* was 22.78% (range: 1.02-94.25%). Specifically, the average relative abundance of *H. pylori* of the patients with SG was 14.22% in the Changle region and 11.20% in the Fu'an region. For those with PLGC, *H. pylori* showed high dominance, with average relative abundances of 35.64% in the Changle region and 23.70% in the Fu'an region.

Summary of sequencing data

Illumina high-throughput sequencing for the brushing samples generated 19,163,031 raw reads with an average of 46,175 reads per sample. A total of 12,701,167 high-quality reads were obtained after quality filtering, which were clustered into 27,185 ASVs. After filtering out ASVs with a frequency less than 0.001% of the total number of sequences or those assigned to the chloroplast, mitochondrial and eukaryotic sequences, the remaining 2,739 ASVs were retained for further analysis.

The rarefaction curve flattened with an increasing number of measured sequences, indicating that the current sequencing depth was sufficient to ensure adequate

Table 1 Basic characteristics of the study population

Characteristics	Changle			Fu'an		
	SG (*n* = 27)	PLGC (*n* = 103)	*P*-value	SG (*n* = 130)	PLGC (*n* = 75)	*P*-value
Age at endoscopy (years)	49.85 (1.75)	49.87 (1.07)	0.992	49.62 (1.07)	46.65 (1.16)	0.076
Gender			0.149			0.983
Male	16 (59.26)	45 (43.69)		57 (43.85)	33 (44.00)	
Female	11 (40.74)	58 (56.31)		73 (56.15)	42 (56.00)	
Education level			0.950			0.464
Less than high school	18 (66.67)	68 (66.02)		93 (71.54)	50 (66.67)	
High school or above	9 (33.33)	35 (33.98)		37 (28.46)	25 (33.33)	
BMI (kg/m^2)			0.529			0.936
<24	18 (66.67)	75 (72.82)		96 (73.85)	55 (73.33)	
≥ 24	9 (33.33)	28 (27.18)		34 (26.15)	20 (26.67)	
Tabacco smoking			0.048			0.988
No	19 (70.37)	89 (86.41)		90 (69.23)	52 (69.33)	
Yes	8 (29.63)	14 (13.59)		40 (30.77)	23 (30.67)	
Alcohol drinking			0.870			0.682
No	23 (85.19)	89 (86.41)		120 (92.31)	68 (90.67)	
Yes	4 (14.81)	14 (13.59)		10 (7.69)	7 (9.33)	
H. pylori status [b]			0.049			0.049
Negative	17 (62.96)	43 (41.75)		67 (51.54)	28 (37.33)	
Positive	10 (37.04)	60 (58.25)		63 (48.46)	47 (62.67)	
Gastrin (pg/ml)[c]			0.182			0.347
< 32.47	10 (37.04)	50 (51.55)		61 (48.80)	38 (55.88)	
≥ 32.47	17 (62.96)	47 (48.45)		64 (51.20)	30 (44.12)	

[a] *P*-values were determined by a 2-sided Student's t test for age and Chi-squared tests for other variables

[b] *H. pylori* status was determined based on 16 S rRNA sequencing

[c] Gastrin levels were not measured in 18 patients due to insufficient serum samples and divided into two groups based on the median expression level of total population

coverage (Fig. 2A). As illustrated in the Venn diagram of Fig. 2B, our analysis identified 1,383 ASVs in SG cases, 2,248 ASVs in PLGC cases from the Changle region, and 2,479 ASVs in SG cases, 2,201 ASVs in PLGC cases from the Fu'an region. Notably, there was an overlap of 1,115 ASVs common across all groups examined.

The gastric microbiota profile in SG and PLGC patients differs across regions

We initially compared the microbial alpha diversity and community structures between Changle and Fu'an regions in patients with different degrees of gastric lesions. For the alpha diversity, microbial richness was estimated using the observed ASVs and Chao1 indices, and diversity was determined with Shannon and Simpson indices. We observed no significant differences in microbial community richness and diversity between the two regions among the SG patients (Fig. 3A). However, in PLGC patients, those from Fu'an region exhibited significantly higher microbial richness than their counterparts in Changle, as indicated by the observed ASVs (Wilcoxon test, $P = 0.010$) and Chao1 index (Wilcoxon test, $P = 0.009$), although diversity, as measured by the Shannon and Simpson indices, did not differ significantly (Fig. 4A). For the beta diversity, PCoA plots based on the Bray-Curtis dissimilarity matrix revealed significant differences in community composition between two regions both in SG (Adonis $R^2 = 0.02$, $P = 0.004$) and PLGC patients (Adonis $R^2 = 0.02$, $P < 0.001$) (Figs. 3B and 4B). Similar results were obtained based on weighted_UniFrac distance (Figs. 3C and 4C).

The composition of microbial communities in the SG and PLGC patients from the Changle and Fu'an regions are shown in Supplementary Fig. 1 and Supplementary Table 1). The predominant phyla identified in both groups include Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteria. Remarkably, within the SG group, there was a statistically significant difference in the relative abundance of Proteobacteria ($P = 0.003$), with Changle having a lower abundance compared to Fu'an. Conversely, the relative abundance of Firmicutes ($P = 0.030$), Bacteroidetes ($P = 0.005$), and Fusobacteria ($P = 0.003$) was notably higher in Changle than in Fu'an (Supplementary Fig. 1A). Conversely, in the PLGC group, differences in the microbial community composition at the phylum level between Changle and Fu'an regions appear less pronounced (Supplementary Fig. 1C).

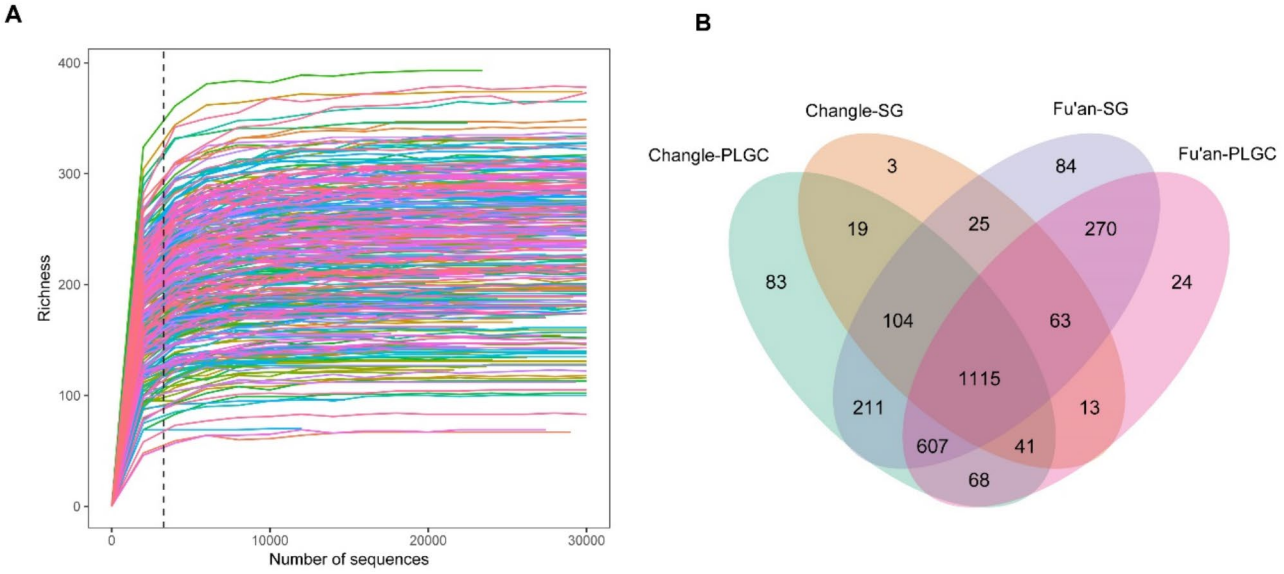

Fig. 2 Rarefaction curves of gastric microbial communities (**A**); Venn diagram of the ASVs (**B**)

Subsequent analysis delved into the relative abundance of microorganisms at the genus level. The top 10 genera with the highest relative abundance are presented in Supplementary Fig. 1B and D. Within the SG group, three genera, namely *Veillonella*, *Fusobacterium*, and *Porphyromonas*, exhibited significantly higher relative abundances in Changle compared to Fu'an. Conversely, *Ralstonia* displayed a marked decrease in relative abundance in Changle (Supplementary Fig. 1B). In the PLGC group, *Helicobacter* emerged as the most abundant genus, with no observable disparities between the regions. However, both *Ralstonia* and *Burkholderia* presented notably diminished relative abundances in Changle as opposed to Fu'an (Supplementary Fig. 1D). Upon further stratification into CAG and IM/DYS patients (Supplementary Fig. 2A and B), *Helicobacter* emerged as the predominant genus in CAG patients, demonstrating a substantial decrease in abundance in IM/DYS patients. Noteworthy, the relative abundance of *Helicobacter* exhibited no significant variation between the two regions across both patient subgroups (Supplementary Table 2).

Next, we identified the most significantly associated taxa with geographical distribution by using LEfSe analysis. At the genus level, a total of 16 taxa exhibited significant differences in abundance between Changle and Fu'an patients in the SG group. Notably, *Veillonella*, *Porphyromonas*, *Fusobacterium*, *Capnocytophaga*, *Leptotrichia*, *Moraxella*, *Peptostreptococcus* and *Selenomonas* were markedly enriched in Changle patients, whereas *Ralstonia*, *Pseudomonas*, *Acinetobacter*, *Achromobacter*, *Brevundimonas*, *Stenotrophomonas*, *Micrococcus* and *Corynebacterium* were predominantly found in Fu'an patients (Fig. 3D). In the PLGC group, Changle patients displayed an increased abundance of *Selenomonas*, *Phascolarctobacterium*, *Massilia*, *Peptostreptococcus*, *Wautersiella* and *Faecalibacterium*, whereas Fu'an patients were characterized by a higher prevalence of *Ralstonia*, *Pseudomonas*, *Burkholderia*, *Treponema*, *Acholeplasma*, *Stenotrophomonas*, *Paenibacillus*, *Corynebacterium* and *Brevibacillus* (Fig. 4D). Additionally, even after controlling for smoking, alcohol intake, and *H. pylori* infection with the MaAsLin2 methodology, the disparities in the abundance of some gastric microbiota between high- and low-risk regions for gastric cancer remained significant (FDR adjusted P-value < 0.05, Supplementary Table 3).

Mucosal Microbiome dysbiosis in gastric lesions

We further examined the changes in mucosal bacteria during different histopathological stages of gastric carcinogenesis by combining populations from two regions. In comparison to SG, the microbial community of CAG demonstrated a significant reduction in richness and evenness, as determined by the Observed ASVs, Chao1, Shannon, and Simpson indexes (all $P < 0.01$). The indexes in IM/DYS were similar to those in SG (Supplementary Fig. 3A). Additionally, a notable discrepancy in the alpha diversity of gastric microbiota was identified among groups classified by alcohol consumption and *H. pylori* status, but not for other examined factors, including serum gastrin level (Supplementary Table 4). However, the association between low alpha diversity and increased risk of gastric lesion progression was insignificant in multivariable ordinal logistic regression analysis (Supplementary Table 5). PCoA plots derived from beta diversity analysis demonstrate a significant divergence in

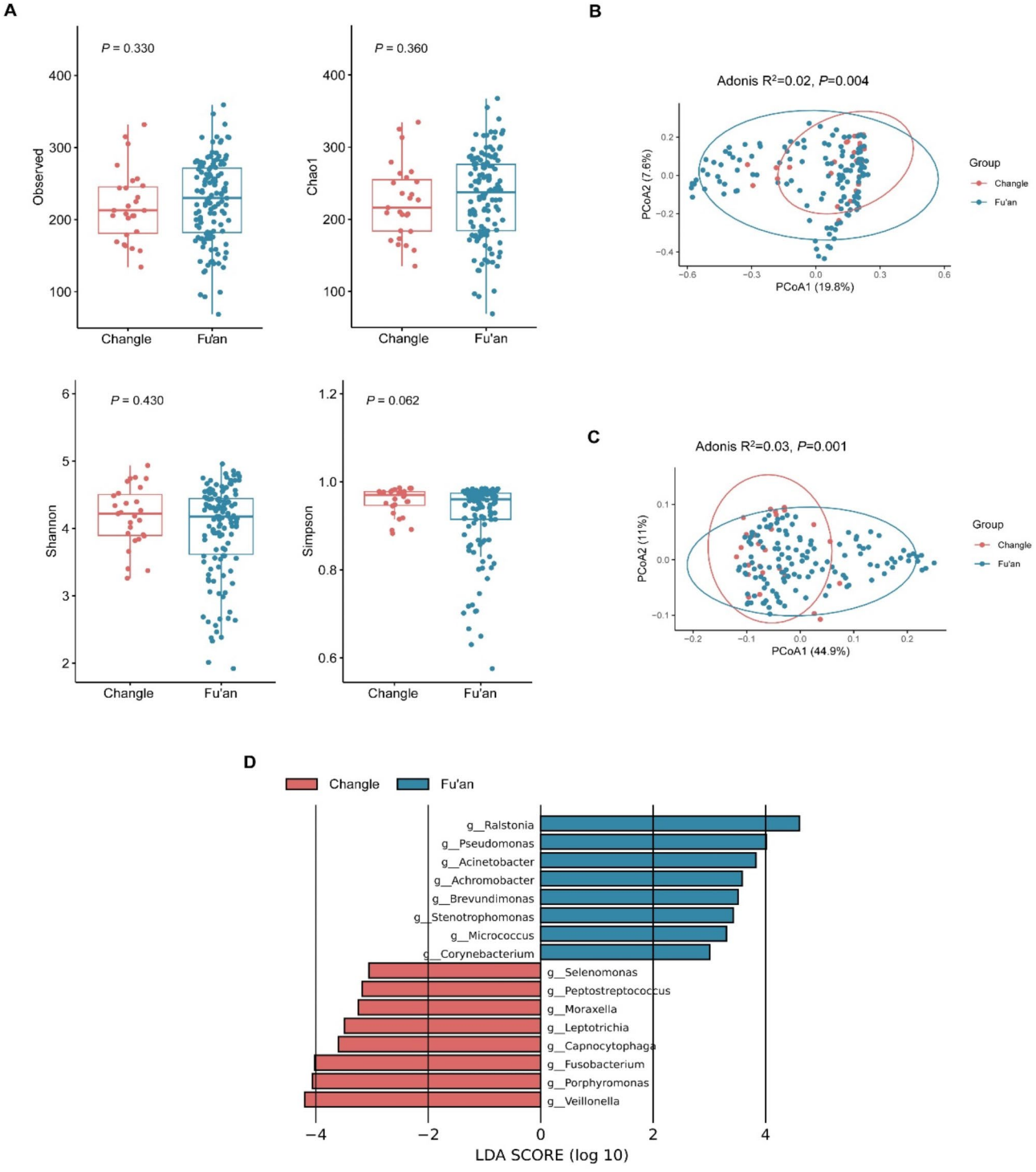

Fig. 3 Diversity analysis for microbial community and genera-specific differences between Changle and Fu'an regions in patients with superficial gastritis (SG). Boxplots of alpha diversity as measured by Observed ASVs, Chao1 richness, Shannon diversity index and Simpson diversity index (calculated by Wilcoxon signed-rank test) (**A**); Beta diversity of the microbial community using PCoA analysis with the Bray-Curtis dissimilarity matrix (**B**) and the weighted_Unifrac metric (**C**); Linear discriminant analysis Effect Size (LEfSe) identified the most differentially abundant genera between groups (LDA score > 3 and $P < 0.05$) (**D**)

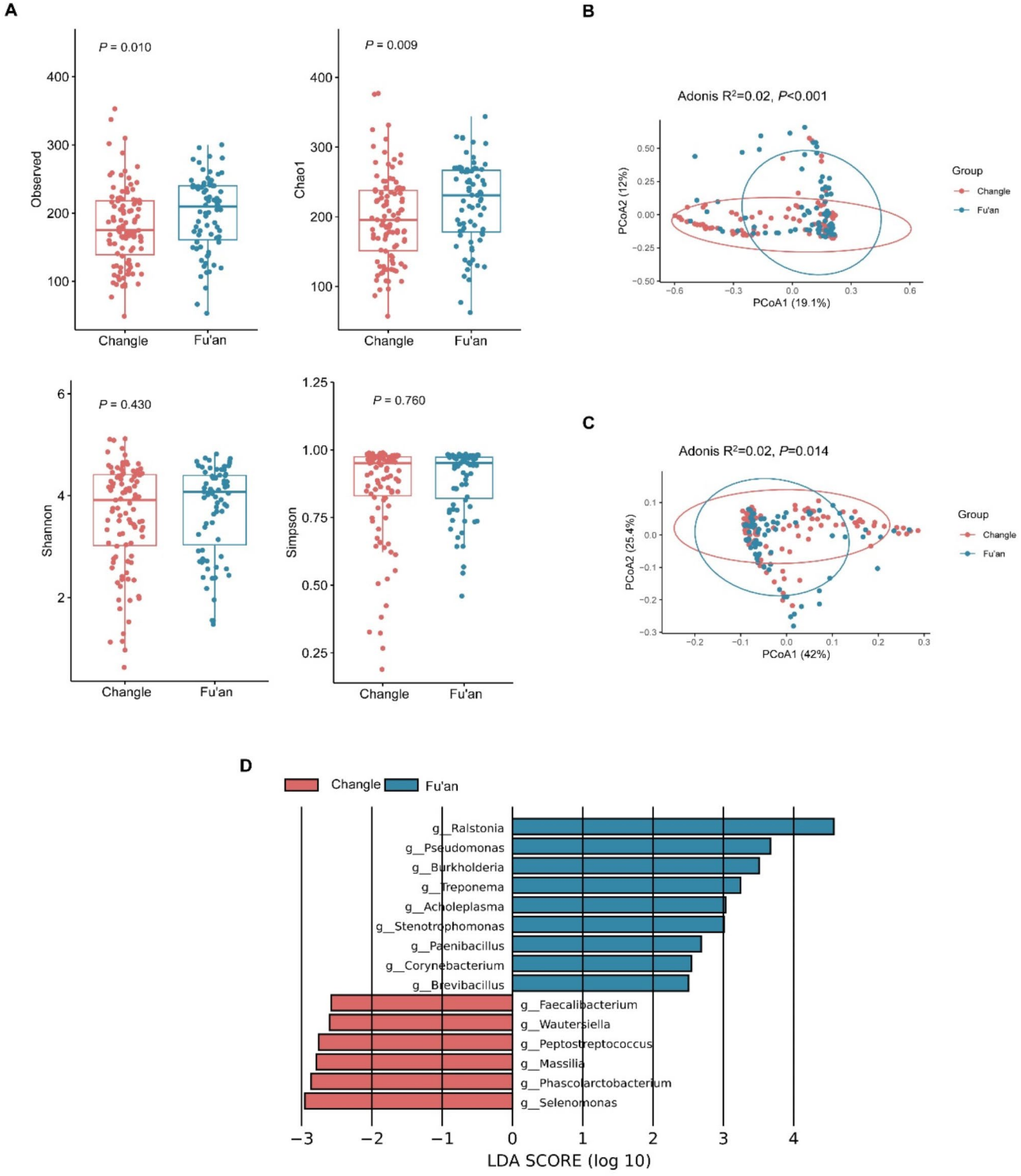

Fig. 4 Diversity analysis for microbial community and genera-specific differences between Changle and Fu'an regions in patients with gastric precancerous lesions (PLGC). Boxplots of alpha diversity as measured by Observed ASVs, Chao1 richness, Shannon diversity index and Simpson diversity index (calculated by Wilcoxon signed-rank test) (**A**); Beta diversity of the microbial community using PCoA analysis with the Bray-Curtis dissimilarity matrix (**B**) and the weighted_Unifrac metric (**C**); Linear discriminant analysis Effect Size (LEfSe) identified the most differentially abundant genera between groups (LDA score > 3 and $P < 0.05$) (**D**)

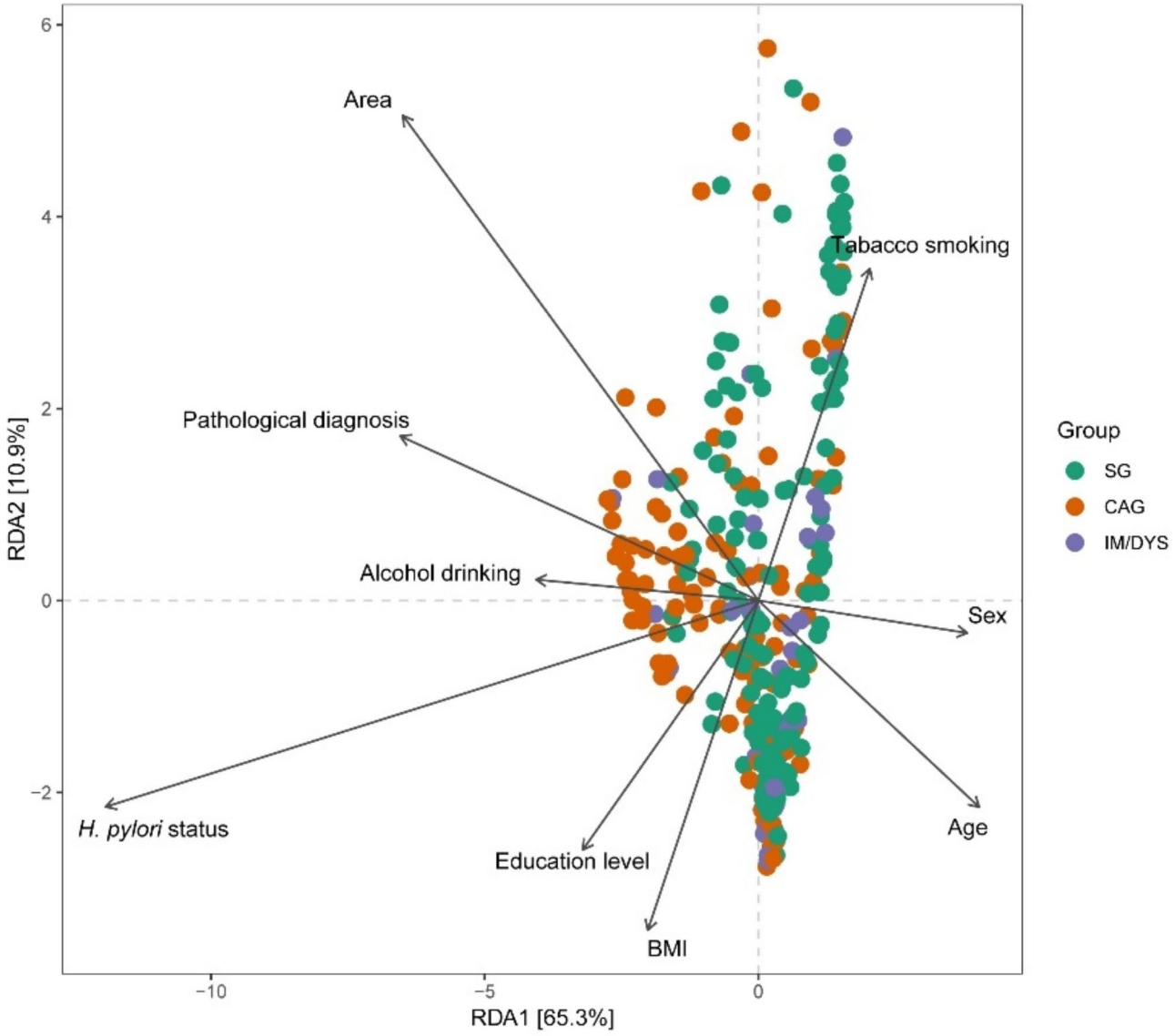

Fig. 5 Redundancy analysis (RDA) showing the relationship between gastric microbial composition and environment factors. The correlation between environment factors and RDA axes is represented by the length and angle of arrows

overall microbial composition among the three patient groups ($P < 0.001$ for Bray-Curtis distances; $P < 0.001$ for weighted_UniFrac distances) (Supplementary Fig. 3B and 3 C).

As illustrated in Supplementary Fig. 4, our examination of the changing trend in the top 10 genera throughout disease stages reveals that none of them displayed a sustained and consistent change in abundance from SG to IM/DYS. Utilizing LEfSe analysis to discern taxa significantly enriched among disease stages, we found *Pseudomonas*, *Acinetobacter*, *Micrococcus*, *Brevibacillus*, *and Cupriavidus* to be the most enriched genera in SG patients. Specifically, *Helicobacter* and *Ammoniphilus* were significantly enriched in CAG patients, while five genera, including *Prevotella*, *Fusobacterium*, *Treponema*, *Campylobacter* and *Acholeplasma*, were significantly enriched in IM/DYS patients (Supplementary Fig. 3D).

We further assessed the effect of environmental factors on the gastric microbiota using RDA analysis. The result showed that *H. pylori* status, area of residence, and gastric lesion severity had the most significant effects on the gastric microbiota (Fig. 5 and Supplementary Table 6).

Discussion

In this study, we meticulously analyzed the composition of gastric microorganisms in patients displaying diverse gastric lesions from regions characterized by high and low risks of gastric cancer. Our primary findings indicate a lack of significant disparity in alpha diversity among SG patients across the two regions. Nevertheless, we

observed a marked elevation in microbial community richness, based on observed ASV and Chao1 indices, in PLGC patients from the low-risk Fu'an region compared to their counterparts in the high-risk Changle region. Beta diversity exhibited notable distinctions between patients in these distinct regions. Additionally, we successfully discerned specific bacterial genera linked to gastric lesions across diverse geographic locations. Furthermore, our observations suggest a correlation between microbial dysbiosis and gastric lesion progression. These discoveries bear profound implications for advancing our comprehension of the contributions of gastric microbiota toward the development of gastric lesions within both high- and low-risk areas for GC.

In spite of comparable rates of *H. pylori* infection in Changle, identified as a high-risk area for gastric cancer (GC), and Fu'an, acknowledged as a low-risk area, the occurrence of GC is notably reduced in Fu'an. This inconsistency strongly indicates that additional factors, aside from *H. pylori* infection, are involved in the development of GC [22]. Our study reveals no substantial disparity in alpha diversity between patients with SG in both regions, which may be due to the fact that the early stage of gastric disease is primarily associated with *H. pylori* infection [23]. However, among patients with PLGC, there are notable differences in the diversity and structure of gastric microbial communities between the two areas. Moreover, we identified distinct bacterial genera associated with PLGC in each region, suggesting that these specific microbial constituents may influence the progression of gastric lesions differently, ultimately leading to the observed disparity in GC incidence.

Previous research has highlighted the intricate interaction between *H. pylori* and the gastric microbiota in modulating inflammation and carcinogenesis [23–25]. Certain microorganisms, such as *Prevotella*, *Bacillus*, and *Lactobacillus*, have been documented to play a role in the pathogenesis of GC [13, 26–28]. In a study comparing *H. pylori*-positive individuals from two regions in Colombia with high and low GC risk, *Neisseria* and *Streptococcus* were found to be more abundant in the low-risk area [29]. However, in our study, we did not observe a significant variance in the abundance of *Neisseria* and *Streptococcus* among patients with SG or PLGC in both regions. Instead, we identified six genera, including *Selenomonas* and *Peptostreptococcus*, that were enriched in PLGC patients from Changle. *Selenomonas*, as an oral bacterium, was continuously enriched from SG to GC [26]. Analysis revealed a profound shift in the gastric microbiota composition within the tumoral group, with certain bacterial species demonstrating significant correlations to immunosuppressive cells in the tumor microenvironment. Notably, *Selenomonas* exhibits a marked increase in the tumoral group relative to the peritumoral group and showed a positive correlation with $Foxp3^+$ regulatory T cells, which lead to tumor escape from immune surveillance [30]. Operational taxonomic units (OTUs) identified as *Peptostreptococcus* were observed to be markedly enriched in the GC relative to precancerous stages, exhibiting a strengthening co-occurrence network with the advancement of the disease [20]. It has been established that the onset of atrophy or IM is correlated with an increased prevalence of *Peptostreptococcus* species, particularly in the absence of *H. pylori* [31]. Furthermore, *Peptostreptococcus anaerobius* has been implicated in the promotion of colorectal carcinogenesis and the modulation of tumor immunity [32]. It is plausible that the differential abundance of such bacteria in Changle and Fu'an could partially explain the divergent cancer rates.

Our findings align with an increasing amount of evidence suggesting that alterations in the gastric microbiota are associated with the progression from initial gastric lesions to GC. The findings of a study conducted in Mexico revealed a decline in microbial diversity progressing from non-atrophic gastritis to IM and intestinal-type GC. Furthermore, principal component analysis illuminated a distinct separation in the microbial profiles between GC patients and individuals with non-atrophic gastritis, while the microbial composition of those with IM exhibited some degree of overlap with both aforementioned groups [27]. A prospective cohort study carried out in Linqu County, China, illustrated substantial variations in microbial diversity and community structure across different gastric lesions, identifying specific bacterial genera linked to these alterations [13]. Similarly, in this study, we observed a significant decrease in microbial richness and evenness in the CAG group compared to SG and a distinct overall microbial composition among SG, CAG, and IM/DYS patients. These findings suggest that the influence of gastric microbiota changes on the progression of gastric lesions may be more profound in populations residing in high-risk areas for GC. Nevertheless, our research failed to establish a link between decreased microbial diversity and an elevated risk of PLGC, possibly due to the relatively modest sample size of this study. While our study primarily focused on describing the microbial differences across geographic regions and gastric lesion stages, the clinical significance of these findings cannot be overstated. For instance, the presence or abundance of these bacteria could potentially serve as biomarkers for early detection of gastric cancer, especially in high-risk populations. Furthermore, understanding the mechanisms by which these bacteria contribute to gastric carcinogenesis may lead to the development of novel therapeutic strategies targeting the gastric microbiome. Future studies should focus on validating these findings in larger cohorts and exploring the

feasibility of using these bacterial markers as diagnostic or prognostic tools in clinical practice.

Results obtained from the RDA analysis enabled a comprehensive assessment of how environmental variables influence the composition of the gastric microbiota. Our results indicate that *H. pylori* status, area of residence, and gastric lesion severity are the most significant determinants of the composition of the gastric microbiota. This is consistent with previous studies which showed *H. pylori* infection can alter the composition of the gastric microbiota, resulting in an elevated presence of potentially deleterious bacteria and a concurrent reduction in beneficial bacterial populations [12]. Furthermore, our results confirm the distinctive microbial profiles observed in patients residing in regions with a high risk for GC when compared with those in lower-risk areas, suggesting a likely contribution of environmental factors, encompassing dietary patterns, other lifestyle factors, and exposure to pollutants, in driving gastric lesion progression through alterations in the gastric microbiota [33, 34]. Noteworthy differences in the microbial composition are also discerned between patients presenting with varying degrees of lesion severity, possibly attributable to changes in the gastric microenvironment elicited by the lesions themselves, including heightened acidity or inflammatory responses that may influence certain bacterial proliferation [35].

In the present study, we investigated the relationship between intragastric pH, as reflected by serum gastrin levels, and gastric microbiota composition in patients with different gastric conditions. Our findings demonstrate that serum gastrin levels, measured using ELISA as a surrogate marker for gastric hypoacidity, did not significantly differ between patients with SG and those with PLGC across both the Changle and Fu'an regions. When participants were stratified into low and high gastrin level groups, no significant differences in alpha diversity metrics of the gastric microbiota were detected between the two groups. These findings suggest that, in our study population, intragastric pH may not be a primary determinant of gastric microbiota composition.

While our study provides valuable insights into the gastric microbiota in relation to gastric lesions, several limitations warrant consideration. The generalizability of our findings may be constrained by the relatively modest sample size and the cross-sectional study design. Longitudinal studies are imperative for elucidating the temporal dynamics of microbial changes throughout the process of gastric carcinogenesis. Moreover, although we have adjusted for smoking, alcohol consumption, and *H. pylori* infection using MaAsLin2, the potential confounding effects of dietary habits, living conditions, and socioeconomic factors cannot be fully excluded. Future studies should aim to collect comprehensive data on these and other relevant lifestyle factors to allow for more robust analyses and to better understand the complex interplay between environmental factors, gastric microbiota, and GC development. Additionally, regarding the relatively high proportion of *H. pylori*-negative patients with gastritis or premalignant changes in our study, we emphasize that the prevalence and incidence of *H. pylori* infection can vary within different regions and subpopulations. Furthermore, other factors such as age, diet, and genetic susceptibility may also influence the development of gastritis or premalignant changes in the absence of *H. pylori* infection. While our results may not be generalizable to all Chinese populations, they highlight the complexity of gastric microbiota and its relationship with gastric lesions, which warrants further investigation.

In conclusion, while *H. pylori* infection remains a critical factor in gastric carcinogenesis, our findings underscore the importance of considering the broader gastric microbiota in understanding regional differences in GC incidence. This provides a clue for explaining variations in GC incidence across different regions. Future research should aim to elucidate the exact mechanisms by which specific microbial taxa contribute to gastric disease progression, and whether modulating the gastric microbiota can serve as a preventive or therapeutic strategy for GC.

Supplementary information

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Supplementary Material 1

Author contributions

WY and FC designed the study. LL, MZ and XG were responsible for field investigation. LL and WL conducted the laboratory work. LL, WL and LY performed data curation and analysis. WY, FC, LL, WL and LY were involved in interpretation of results. WL and LY drafted the manuscript, and all authors revised it for important intellectual content.

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Data availability

The original data was deposited in the BIG Sub database in the National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences, and China National Center for Bioinformation, under Accession Number: PRJCA028994.

Declarations

Ethics approval and consent to participate

The research protocol received approval from the Ethics Committee of Fujian Medical University Union Hospital under reference number 2017KY064. Written informed consent was obtained from all participants involved in the study. The research was carried out in compliance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
No potential conflict of interest was reported by the authors.

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