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Original Article

Gut microbiota dysbiosis in alcoholic fatty liver disease: distinct microbial communities and biochemical alterations

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Abstract: Objectives: To compare the gut microbiota composition among patients with alcoholic fatty liver disease (AFLD), individuals with alcohol use disorder (AUD) without liver damage, and healthy controls, and to investigate correlations between microbial profiles and liver health. Methods: A retrospective analysis was conducted on 34 participants: 14 with AFLD, 10 with AUD without liver damage, and 10 healthy controls. Blood biochemical markers, liver function tests, lipid profiles, and gut microbiota composition were assessed. Gut microbiota was analyzed via high-throughput 16S rRNA gene sequencing. Alpha and beta diversity indices were calculated, and group-specific microbial taxa were identified. Results: AFLD patients showed a decreased Firmicutes/Bacteroidetes ratio and an increased abundance of Bacteroidetes, indicating gut dysbiosis compared to the other groups. Biochemical markers, including triglycerides, alanine aminotransferase, gamma-glutamyl transferase, and cholinesterase, were significantly altered in AFLD patients (all $P > 0.05$). Beta diversity analysis revealed distinct microbial communities in the AFLD group. Notably, taxa such as Megamonas and Selenomonadales were enriched in AFLD, while beneficial genera like Ruminococcus and Faecalibacterium were significantly reduced. Conclusion: AFLD is associated with marked gut microbiota alterations and distinct microbial signatures, which correlate with liver dysfunction and biochemical abnormalities, highlighting the role of dysbiosis in disease pathogenesis.

Keywords: Gut microbiota, alcoholic fatty liver disease, dysbiosis, alcohol use disorder, 16S rRNA sequencing, microbial communities

Introduction

The human gut microbiota is a complex and dynamic community of microorganisms that plays a vital role in maintaining host health and homeostasis [1]. Recent advances in metagenomic sequencing and bioinformatics have enhanced our understanding of the gut microbiome's influence on metabolism, immune regulation, and the pathogenesis of various diseases [2, 3]. Among these, liver diseases - particularly alcoholic fatty liver disease (AFLD) and its progression to more severe hepatic conditions - have attracted increasing attention due to the close interplay between gut microbes, alcohol consumption, and liver function [4].

Alcohol use disorder (AUD) is a major global health concern, affecting millions and contributing to a spectrum of liver diseases, including AFLD, alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma [5, 6]. Alcohol metabolism has profound effects on both liver physiology and the gut microbiota, altering microbial diversity and composition [7]. It also damages the intestinal barrier, leading to increased gut permeability - a condition commonly referred to as a "leaky gut" - which facilitates endotoxemia and promotes chronic inflammation [8]. These disruptions underscore the importance of the gut-liver axis in the pathophysiology of AFLD [9].

Several studies have documented gut microbiota alterations in individuals with AUD and liver disease [10, 11]. Typically, there is a depletion of beneficial taxa such as Lactobacillus and Bifidobacterium, alongside an expansion of potentially pathogenic bacteria, including certain Enterobacteriaceae species [12]. Such dysbiosis may aggravate liver injury via multiple

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mechanisms, including increased endotoxin production, altered bile acid metabolism, and disruption of gut-liver immune crosstalk [13]. However, considerable heterogeneity in microbial profiles has been observed both within and between populations [14], highlighting the need for focused investigations in well-defined subgroups.

Despite growing interest in the gut-liver axis in AUD populations, comparative analyses specifically examining patients with AFLD, individuals with AUD but no liver damage, and healthy controls are still limited [15]. A detailed characterization of microbiota profiles across these distinct cohorts is essential to clarify how microbial dysbiosis contributes to AFLD onset and progression. Such insights may pave the way for microbiota-targeted therapies and personalized interventions aimed at preventing or mitigating liver damage in high-risk individuals. This study aims to comprehensively assess and compare gut microbiota compositions in three groups: patients with AFLD, individuals with AUD without liver disease, and healthy controls.

Materials and methods

Research framework and ethical approval

This retrospective study included 24 patients diagnosed with AFLD or AUD who were admitted to Beijing Tiantan Hospital, Capital Medical University, between December 2020 and October 2022. Additionally, 10 healthy individuals were included as controls. The participants were categorized into three groups: the AFLD group (n = 14), comprising patients with AFLD; the AUD group (n = 10), comprising individuals with AUD but without liver damage; and the healthy control group (n = 10). The study protocol was approved by the Institutional Review Board and Ethics Committee of Beijing Tiantan Hospital, Capital Medical University.

Demographic data, blood test results, and microbiota-related metrics - including operational taxonomic unit (OTU) profiles, alpha diversity, and beta diversity analyses - were extracted from the hospital's electronic medical record system. Bacterial community structure was examined, and characteristic microbial taxa associated with AFLD were identified.

Participant selection and criteria

Eligibility for the AFLD group included adults aged 18-70 years who met the diagnostic criteria for AFLD established by the American College of Gastroenterology [16]; those who had a history of long-term alcohol consumption (≥ 5 years) with daily ethanol intake ≥ 40 g for males or ≥ 20 g for females; or individuals who reported heavy drinking within two weeks prior to enrollment, with ethanol intake exceeding 80 g/day. Participants were also required to have complete clinical data and follow-up records.

Participants in the AUD group were aged 18-70 years, scored at least within the high-risk range on the Alcohol Use Disorders Identification Test, met the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for AUD [17], and had no signs of liver damage as confirmed by imaging studies.

Healthy controls were aged 18-70 years, had no history of alcohol consumption or alcohol-related disorders, and showed no liver abnormalities on imaging.

Exclusion criteria included: Viral hepatitis, cirrhosis, or hepatocellular carcinoma; co-infection with hepatitis viruses, HIV, or syphilis; autoimmune liver diseases or hereditary metabolic disorders; drug-induced liver injury; use of gut microbiota preparations, antibiotics, immunosuppressants, corticosteroids, or hepatotoxic drugs within the previous three months; diagnosed hypertension or diabetes mellitus; malignancies or severe dysfunction of vital organs; and severe neurological or psychiatric disorders.

Outcome measurement and data collection

Hematological and biochemical tests: Fasting venous blood samples (5 mL) were collected from each participant. A portion of the sample was used to assess white blood cell (WBC) count, hemoglobin (Hb) levels, and platelet (PLT) count using an automated hematology analyzer (BC-6900, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

The remaining blood was centrifuged at 3,000 rpm for 10 minutes using a high-speed refrigerated centrifuge (Centrifuge 5810/5810R,

Eppendorf AG, Germany). The resulting plasma was stored at -80°C. Plasma biomarkers, including alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), cholinesterase (CHE), triglycerides (TG), fasting glucose (Glu), aspartate aminotransferase (AST), albumin (ALB), total bilirubin (TBIL), total cholesterol (CHO), low-density lipoprotein cholesterol (LDL-C), urea nitrogen (Ur), creatinine (Cr), and alkaline phosphatase (ALP), were measured using an automatic biochemical analyzer (BS-2000M, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

Bioinformatics analysis: Gut microbiota composition was assessed by high-throughput sequencing of the V3-V4 region of the 16S rRNA gene using the Illumina HiSeq 2500 platform. Bioinformatic processing was performed as follows:

Paired-end reads were merged into contiguous sequences using FLASH. After quality control and filtering, high-quality sequences were compared against reference databases to generate clean reads. OTU clustering was performed using QIIME (version 1.9.3), and taxonomic annotation was conducted by aligning OTU sequences to the Silva138 database.

Alpha diversity indices - including Observed OTUs, Chao1, Shannon, and Simpson indices - were calculated using QIIME. Rarefaction curves, Shannon-Wiener plots, and Simpson index curves were generated in R (version 2.15.5). Intergroup comparisons of alpha diversity were also performed using R.

For beta diversity, UniFrac distances were computed in QIIME, and Principal Coordinate Analysis (PCoA) plots were generated in R. Beta diversity differences between groups were further analyzed using R.

Statistical analysis

Based on previous studies employing 16S rRNA sequencing, a minimum of 10 participants per group was considered sufficient. Species accumulation curves were used to assess sampling adequacy.

Statistical analyses were conducted using SPSS software (version 29.0; SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as n (%), with between-group comparisons performed using the chi-square test. The Shapiro-Wilk test was used to assess the normality of continuous variables. For normally distributed continuous variables, data were reported as mean ± standard deviation ($\bar{x}$ ± sd), and comparisons among groups were conducted using one-way analysis of variance (ANOVA). Post hoc pairwise comparisons were performed using Tukey's Honestly Significant Difference (HSD) test.

For non-normally distributed data, results were presented as median with interquartile range (M [Q1, Q3]), and intergroup comparisons were performed using nonparametric rank-sum tests. A p-value < 0.05 was considered statistically significant.

Results

Comparison of basic demographic characteristics

There were no statistically significant differences in mean age among the AFLD, AUD, and healthy control groups (P = 0.821) (**Table 1**). Similarly, height did not differ significantly across the groups (P = 0.668). Although weight and body mass index (BMI) showed marginal differences, they did not reach statistical significance (weight: P = 0.096; BMI: P = 0.103). Gender distribution was also comparable among the groups (P = 0.995). These results indicate that the groups were demographically matched, supporting the validity of subsequent microbiota comparisons.

Comparison of hematological and biochemical parameters

The WBC count was higher in the AFLD group compared to the AUD and healthy groups, with a difference approaching significance (P = 0.067) (**Table 2**). PLT levels did not differ significantly (P = 0.466), and Hb levels, presented as median with interquartile range, showed near-significant variation (P = 0.075), suggesting subtle intergroup differences in hematological indices.

Glu and LDL-C levels were comparable across the three groups (Glu: P = 0.321; LDL-C: P = 0.240) (**Table 3**). Total CHO was slightly elevated in the AFLD group, but the difference was not statistically significant (P = 0.099). In contrast, TG levels were significantly higher in the

Table 1. Comparison of demographic characteristics

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	F/χ^2	P
Age (years)	40.57 ± 7.71	42.10 ± 8.81	39.40 ± 12.45	0.198	0.821
Height (cm)	173.49 ± 7.69	174.20 ± 6.12	171.75 ± 3.57	0.409	0.668
Weight (kg)	73.11 ± 9.26	73.41 ± 8.17	64.25 ± 4.05	2.528	0.096
BMI (kg/m^2)	24.20 ± 1.72	24.19 ± 2.44	22.62 ± 1.47	2.448	0.103
Male/Female (n)	14/3	10/2	10/2	0.100	0.995

BMI: Body Mass Index.

Table 2. Comparison of routine blood test results

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	P
WBC (109/l)	7.12 ± 1.77	5.90 ± 1.10	5.96 ± 1.02	0.067
PLT (109/l)	248.93 ± 72.95	218.20 ± 42.67	236.50 ± 51.06	0.466
Hb (g/l)	161.50 (157.50, 173.75)	160.00 (158.50, 167.00)	151.00 (146.75, 162.25)	0.075

WBC: white blood cell; PLT: platelet; Hb: hemoglobin.

Table 3. Comparison of blood glucose and lipid profiles

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	P
Glu (mmol/l)	5.06 ± 0.47	4.78 ± 0.39	4.97 ± 0.50	0.321
LDL-C (mmol/l)	3.02 ± 0.77	3.03 ± 0.81	2.56 ± 0.44	0.240
CHO (mmol/l)	5.14 ± 0.79	4.84 ± 0.89	4.46 ± 0.44	0.099
TG (mmol/l)	2.05 (1.67, 2.58)*,#	1.58 (0.68, 2.92)	0.76 (0.60, 1.06)	0.001

Glu: glucose, fasting; LDL-C: low-density lipoprotein cholesterol; CHO: cholesterol; TG: triglycerides. *: Comparison of the AFLD group and the AUD group; #: Comparison of the AFLD group and the Healthy group.

Table 4. Comparison of liver function indices

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	P
ALB (g/l)	46.47 ± 2.66	46.88 ± 1.22	46.26 ± 2.06	0.807
ALT (u/l)	33.77 ± 16.87#	25.63 ± 13.18	18.08 ± 8.46	0.033
AST (u/l)	22.78 ± 6.21	19.79 ± 6.07	17.93 ± 2.64	0.100
ALP (u/l)	71.00 (60.20, 78.40)#	56.65 (51.50, 82.90)	58.70 (47.23, 65.60)	0.050
TBIL (umol/l)	13.15 (9.83, 19.38)	17.01 (13.19, 32.83)	11.45 (9.90, 13.70)	0.070
GGT (u/l)	45.65 (29.10, 61.30)#	41.65 (21.03, 74.05)	22 (16.55, 24.45)	0.003
CHE (u/l)	11919.50 (10474.75, 11923.25)*,#	9631.00 (8578.50, 11113.50)	8734.50 (7685.50, 9351.50)	0.007

ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TBIL: total bilirubin; GGT: gamma-glutamyl transferase; CHE: cholinesterase. *: Comparison of the AFLD group and the AUD group; #: Comparison of the AFLD group and the Healthy group.

AFLD group compared to both the AUD and healthy groups (P = 0.001), highlighting a notable alteration in lipid metabolism.

No significant differences were observed in ALB levels (P = 0.807) (**Table 4**). ALT was significantly elevated in the AFLD group compared to the healthy group (P = 0.033), while AST levels showed no statistical significance (P = 0.100). ALP levels were marginally higher in the AFLD group (P = 0.050), and TBIL also showed a near-significant difference (P = 0.070). Gamma-glutamyl transferase (GGT) was significantly elevated in the AFLD group versus the healthy group (P = 0.003). CHE levels were significantly higher in the AFLD group than in the other two groups (P = 0.007), indicating substantial hepatic functional alterations. Ur and Cr levels showed no significant differences among the groups (Ur: P = 0.344; Cr: P = 0.133) (**Table 5**), suggesting that renal function was comparable across groups.

Table 5. Comparison of renal function indices

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	P
Ur (mmol/l)	5.10 (3.38, 6.23)	5.70 (5.15, 6.23)	4.55 (4.05, 5.58)	0.344
Cr (umol/l)	68.70 (60.50, 75.13)	72.85 (72.45, 79.3)	73.4 (57.78, 78.43)	0.133

Ur: urea nitrogen; Cr: creatinine.

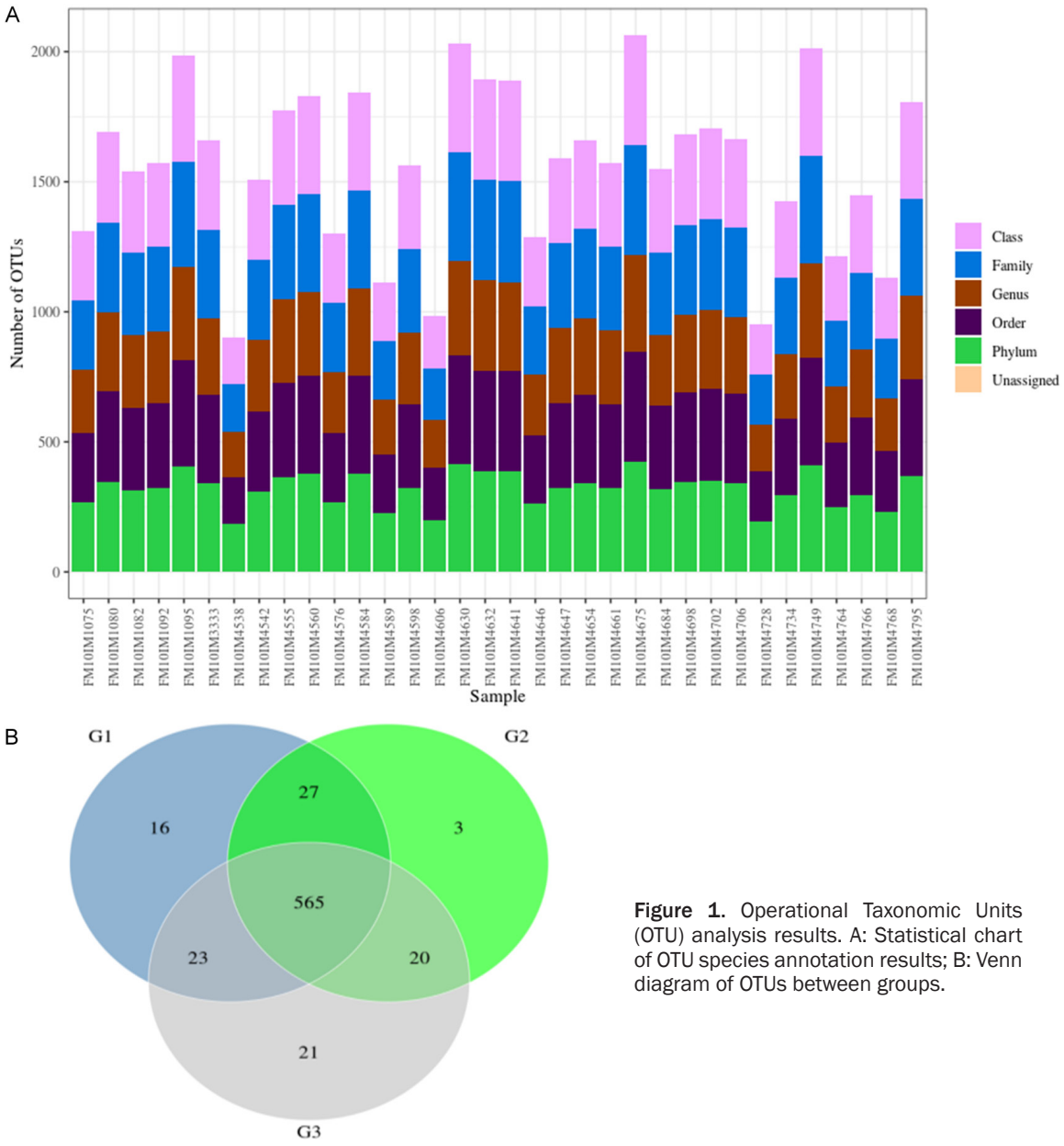

Figure 1. Operational Taxonomic Units (OTU) analysis results. A: Statistical chart of OTU species annotation results; B: Venn diagram of OTUs between groups.

Comparison of OTU composition

Sequencing yielded 4,495,113 high-quality reads. OTUs were classified at various taxonomic levels, and Venn diagram analysis (97% similarity threshold) revealed 631 OTUs in the AFLD group, 615 in the AUD group, and 629 in the healthy control group (**Figure 1**). These findings suggest modest differences in microbial community richness among the groups.

Comparison of alpha diversity

Rarefaction analysis showed that increased sequencing depth did not lead to substantial OTU growth, indicating that the sequencing

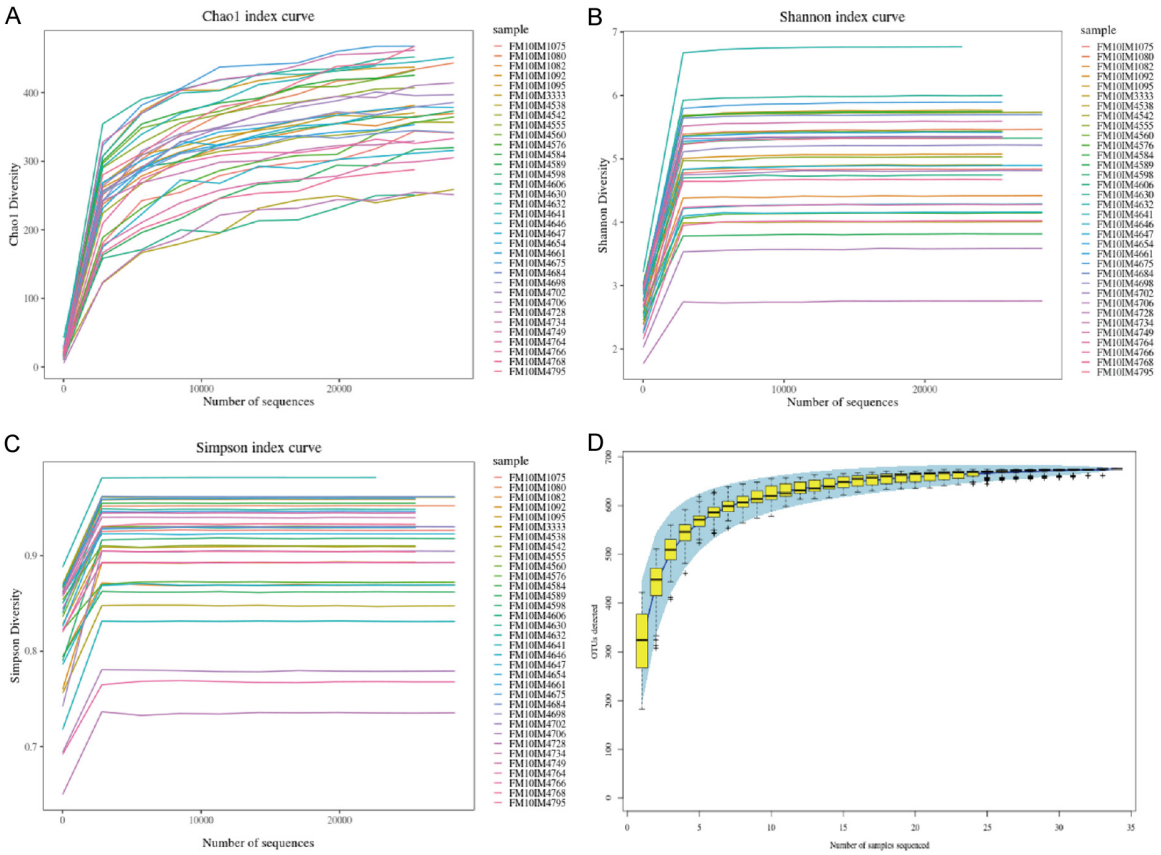

Figure 2. Alpha diversity analysis results. A: Rarefaction curves for each sample; B: Shannon index curves for each sample; C: Simpson index curves for each sample; D: Species accumulation curves for each sample.

Table 6. Comparison of alpha diversity analysis results

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	P
observed species	300.79 ± 59.58	314.9 ± 85.29	352.9 ± 37.05	0.148
chao1	359.27 ± 51.27	376.17 ± 79.00	405.74 ± 53.71	0.202
Shannon	4.66 ± 0.92	4.88 ± 0.63	5.44 ± 0.65	0.061
Simpson	0.89 (0.84, 0, 94)	0.92 (0.89, 0.93)	0.94 (0.93, 0.95)	0.067

depth was sufficient (**Figure 2**). Similarly, Shannon and Simpson indices plateaued, supporting the adequacy of sequencing coverage. The species accumulation curve stabilized at a sample size of 10, suggesting that the sample size met the requirements for diversity analysis.

Although the AFLD group had a slightly lower observed species count, this was not statistically significant (P = 0.148) (**Table 6**). Chao1 index values showed a similar trend (P = 0.202). The Shannon index was lower in the AFLD group compared to healthy controls, approaching significance (P = 0.061). The Simpson index followed a similar pattern (P = 0.067). Although trends in alpha diversity were observed, none reached statistical significance, indicating comparable within-sample diversity across groups.

Comparison of beta diversity

PCoA revealed that the AFLD group was distinctly separated from the healthy group, while the AUD group clustered more closely with healthy controls (**Figure 3**). This suggests notable differences in microbial community composition between AFLD patients and healthy individuals, whereas the AUD group displayed a microbial profile more similar to that of healthy participants.

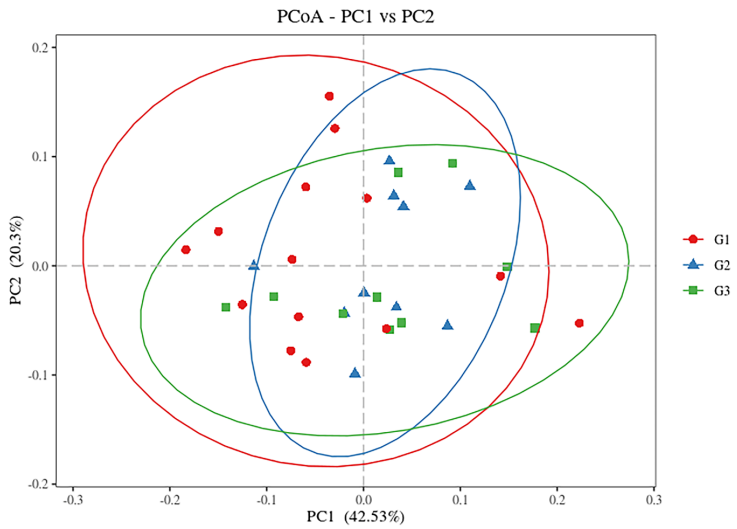

Figure 3. Beta diversity analysis results. PCoA: Principal coordinate analysis; PC: Principal coordinate; G1: Group 1.

Comparison of bacterial community composition

At the phylum level, the top eight most abundant groups were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, Euryarchaeota, and Synergistetes (**Figure 4**). At the class level, the 14 most abundant classes included Bacteroidia, Clostridia, Gammaproteobacteria, Negativicutes, Fusobacteriia, Actinobacteria, Coriobacteriia, Erysipelotrichia, Bacilli, Deltaproteobacteria, Verrucomicrobiae, Alphaproteobacteria, Methanobacteria, and Synergistia.

Firmicutes abundance was significantly lower in the AFLD group compared to the AUD and healthy groups ($P = 0.012$), while Bacteroidetes were more abundant in the AFLD group, though the difference was not statistically significant ($P = 0.121$). The Bacteroidetes/Firmicutes ratio was significantly higher in the AFLD group ($P = 0.013$), indicating a distinct dysbiotic microbial signature. No significant differences were found for Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, Euryarchaeota, or Synergistetes (all $P > 0.05$). See **Table 7**.

Comparison of characteristic microbial taxa in the AFLD group

Distinct microbial taxa were identified in the AFLD group, including Megamonas (genus), Selenomonadales (order), and Negativicutes (class) (**Figure 5**). In contrast, the healthy control group was enriched in beneficial taxa such as Ruminococcus_1, Lachnospiraceae UCG-010, Roseburia, and Faecalibacterium (genus), along with Ruminococcaceae (family), Clostridiales (order), and Clostridia (class). These findings highlight a compositional shift in the microbial communities associated with AFLD.

Discussion

This study aimed to elucidate differences in gut microbiota composition among AFLD patients, individuals with AUD without liver damage, and healthy controls. A key finding was the significantly altered Firmicutes/Bacteroidetes (F/B) ratio in AFLD patients compared to both AUD and healthy individuals. The F/B ratio is widely recognized as an indicator of gut dysbiosis, a microbial imbalance linked to multiple diseases [18, 19]. The reduced F/B ratio observed in the AFLD group may indicate dysbiosis, potentially contributing to fatty liver pathogenesis through increased gut permeability, bacterial translocation, and systemic inflammation [20]. Elevated levels of endotoxins such as lipopolysaccharides (LPS), which translocate from the gut into the bloodstream, can promote systemic inflammation - a hallmark in the progression of fatty liver disease [21].

The increased abundance of Bacteroidetes in the AFLD group further suggests metabolic dysregulation [22]. Bacteroidetes are known to ferment indigestible carbohydrates into short-chain fatty acids (SCFAs), which generally confer health benefits [23]. However, in the context of AFLD, excessive SCFA production may promote hepatic lipogenesis, oxidative stress, and inflammation, thereby exacerbating liver injury [24, 25].

Conversely, the AUD group presented a microbiota composition that was more similar to healthy controls, particularly concerning the abundance of Firmicutes, which suggests that individuals with AUD might not experience gut dysbiosis to the same extent as those with AFLD. A plausible explanation lies in the

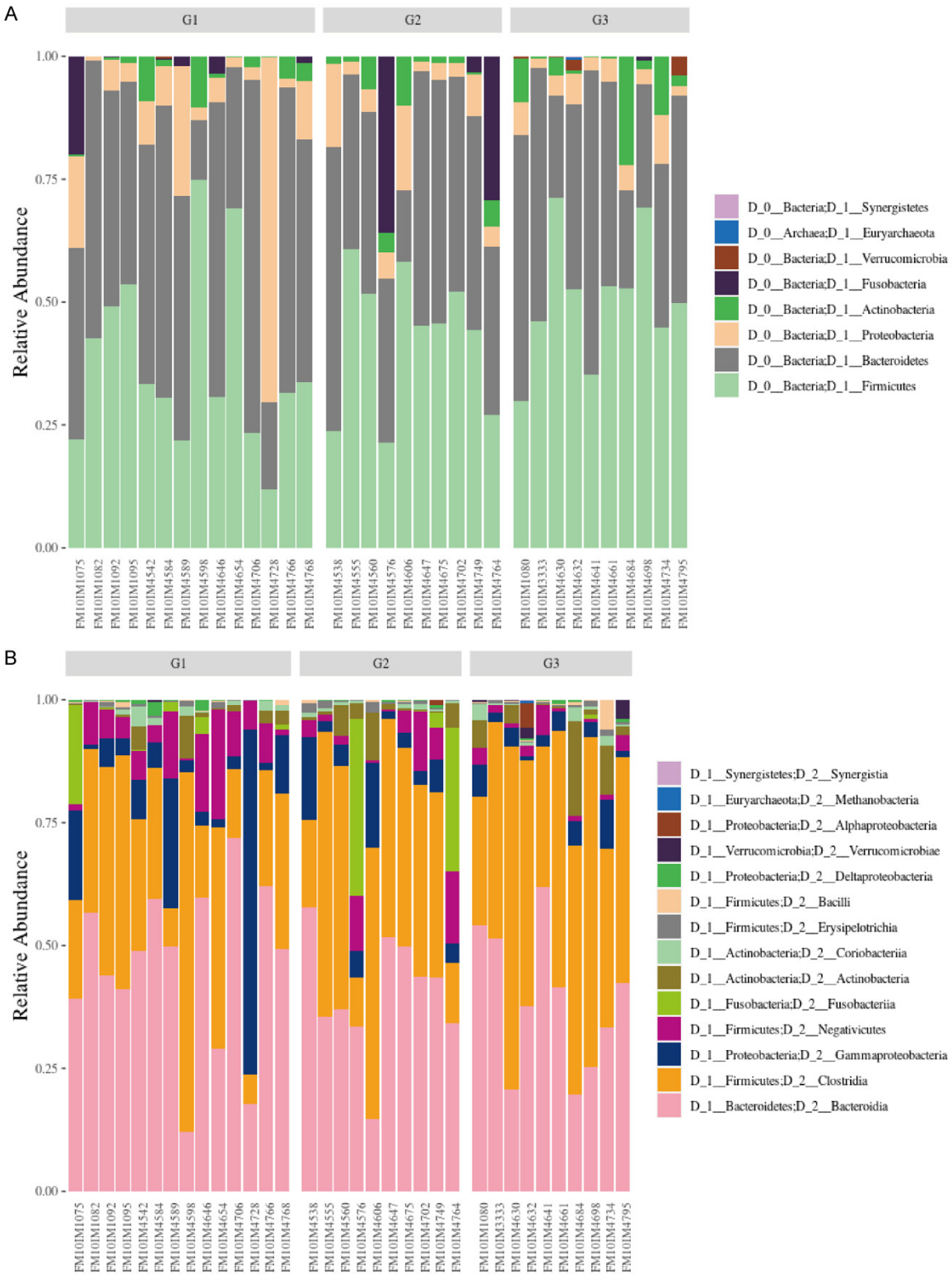

Figure 4. Bar plots of microbial community structure. A: At the Phylum Level; B: At the Class Level.

absence of liver damage in the AUD group, which might prevent inflammatory pathways and metabolic disruptions prevalent in AFLD from becoming established [26, 27]. None-

Table 7. Comparison of bacterial community composition at the phylum level

Parameters	AFLD group (n = 14)	AUD group (n = 10)	Healthy group (n = 10)	P
Firmicutes	32.31% ± 15.04%*,#	42.99% ± 14.21%	50.42% ± 12.89%	0.012
Bacteroidetes	49.86% ± 15.45%	40.15% ± 12.12%	38.72% ± 14.33%	0.121
Proteobacteria	5.62% (2.43%, 13.56%)	4.29% (2.72%, 10.52%)	4.34% (2.41%, 6.26%)	0.759
Actinobacteria	1.20% (0.13%, 3.96%)	1.42% (1.16%, 5.77%)	1.90% (0.36%, 9.57%)	0.387
Fusobacteria	0.40‰ (0.06‰, 13.7‰)	0.10‰ (0.03‰, 9.67%)	0.05‰ (0.04‰, 0.40‰)	0.382
Verrucomicrobia	0.017‰ (0, 0.044‰)	0.000‰	0.14‰ (0, 9.18‰)	0.083
Euryarchaeota	0.000‰	0.000‰	0.000‰	0.258
Synergistetes	0.000‰	0.000‰	0.000‰	0.201
Bacteroidetes/Firmicutes	1.48 (1.33, 2.05)*,#	1.04 (0.68, 1.34)	0.76 (0.37, 1.27)	0.013

*: Comparison of the AFLD group and the AUD group; #: Comparison of the AFLD group and the Healthy group.

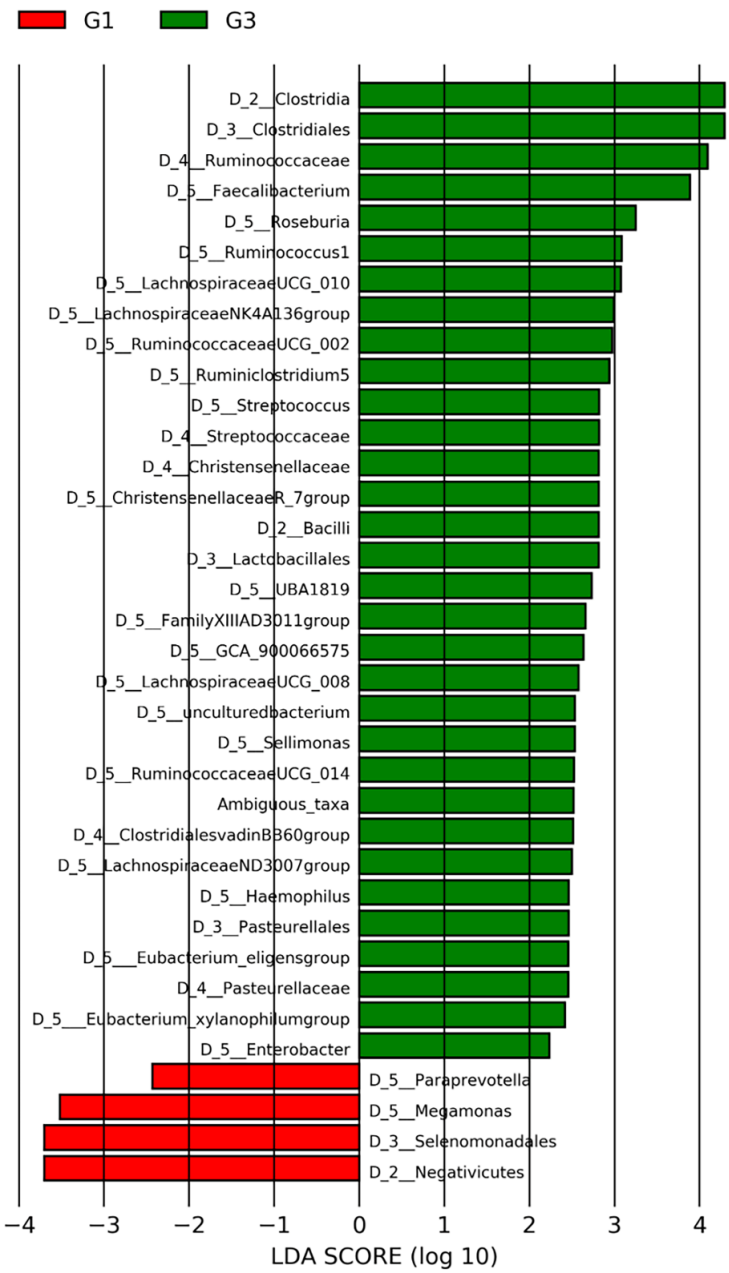

Figure 5. Bar plot of Linear Discriminant Analysis (LDA) score distribution.

theless, it's crucial to note that while the AUD microbiota profile may resemble that of healthy individuals, the potential for metabolic and immune dysregulation cannot be entirely disregarded [28]. Chronic alcohol exposure without liver damage could still harbor risks for subsequent health disorders through subtle gut microbiota modifications not completely captured in this snapshot analysis [29].

In contrast, the microbiota composition of the AUD group more closely resembled that of healthy controls, particularly in the relative abundance of Firmicutes. This may reflect the absence of liver damage in the AUD group, which could prevent the activation of inflammatory and metabolic pathways typically seen in AFLD [26, 27]. Nonetheless, it is important to note that chronic alcohol consumption, even in the absence of overt liver pathology, may still subtly alter microbial communities and predispose individuals to future metabolic or immune dysfunction [28, 29].

Beta diversity analysis further confirmed that the AFLD group harbored a distinct microbial

structure compared to healthy controls, whereas the AUD group clustered more closely with healthy individuals. This finding underscores the role of the liver-gut axis in shaping gut microbial composition [30, 31]. Liver diseases such as AFLD are often part of broader metabolic syndromes, where insulin resistance, lipid abnormalities, and hormonal imbalances interact with gut microbiota in a feedback loop that accelerates disease progression [32, 33].

The enrichment of specific taxa - Megamonas, Selenomonadales, and Negativicutes - in the AFLD group points to potential microbial markers or contributors to disease. Megamonas has been associated with altered bile acid metabolism, which is commonly disrupted in liver disease [34, 35]. Bile acids themselves serve as signaling molecules that modulate both host metabolism and microbial composition, and disruptions in this axis may drive microbial imbalances in AFLD [36]. In contrast, beneficial taxa such as Ruminococcus, Lachnospiraceae, and Faecalibacterium, which possess anti-inflammatory properties, were underrepresented in the AFLD group [37]. Their depletion may impair the gut's ability to modulate inflammation, thereby exacerbating liver damage.

Biochemical findings further supported the microbiota data. Elevated TG and altered liver enzyme levels in the AFLD group were consistent with gut microbial shifts. Increased TG levels may reflect dysregulated lipid metabolism, potentially influenced by microbial alterations in both gut and hepatic environments [38, 39]. Alcohol consumption, a shared factor in both AUD and AFLD, likely contributes to microbial dysbiosis by damaging the intestinal barrier. Increased gut permeability facilitates the translocation of microbial products into circulation, promoting systemic inflammation and linking gut dysbiosis directly to liver injury [40-42].

While our findings provide insight into the gut-liver axis in AFLD and AUD, several limitations must be acknowledged. First, the cross-sectional design precludes causal inference. Longitudinal studies are required to clarify temporal relationships between microbiota changes and liver disease progression. Second, the modest sample size, while adequate for preliminary analysis, may not fully capture population-level variability. Third, potential confounders such as diet, medication use, and lifestyle factors were not controlled. Lastly, the use of 16S rRNA sequencing limits functional insight into microbial metabolism; future studies incorporating metagenomic or metabolomic profiling are warranted to explore microbial functionality in AFLD.

In conclusion, our study highlights distinct alterations in gut microbiota composition among individuals with AFLD, AUD, and healthy controls, emphasizing the potential role of microbial dysbiosis in liver disease pathogenesis. Specifically, shifts in the F/B ratio, enrichment of potentially pathogenic taxa, and depletion of beneficial microbes were closely associated with biochemical indicators of liver dysfunction. These findings suggest that gut microbiota may serve as both a biomarker and therapeutic target for AFLD.

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Disclosure of conflict of interest

None.

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