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A 6-year longitudinal study investigating factors associated with the onset and progression of metabolic dysfunction-associated steatotic liver disease and gut microbiota in the general population

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A 6-year longitudinal study investigating factors associated with the onset and progression of metabolic dysfunction-associated steatotic liver disease and gut microbiota in the general population

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Abstract

Background: Longitudinal data on changes in hepatic steatosis and gut microbiota during the development and persistence of metabolic dysfunction-associated steatotic liver disease (MASLD) are limited. In this study, we compared 6-year trajectories of liver fat, cardiometabolic measures, and gut bacterial composition between individuals who developed MASLD and those with persistent MASLD.

Methods: This community-based, observational study used data from the Iwaki Health Promotion Project in Japan and included 91 adults with MASLD in 2024: 50 without steatotic liver disease in 2018 (Normal–MASLD group) and 41 with MASLD in 2018 (MASLD–MASLD group). Controlled attenuation parameter (CAP), liver stiffness, cardiometabolic measures, and stool microbiota were assessed in 2018, 2019, 2022, 2023, and 2024. Gut microbiota were characterized by 16S rRNA gene sequencing, and differentially abundant genera were identified using linear discriminant analysis effect size. Longitudinal trajectories were assessed using linear mixed-effects models with participant-specific random intercepts.

Results: In the Normal–MASLD group, median CAP increased from 208 dB/m in 2018 to 257 dB/m in 2023 and 283 dB/m in 2024, corresponding to an average increase of 12.5 dB/m per year over 6 years. The median CAP first exceeded the steatosis threshold of 248 dB/m during

the 2023 assessment. On the other hand, in the MASLD–MASLD group, CAP remained elevated throughout the follow-up period without an upward trend. The group-by-year interaction for CAP was significant, indicating different trajectories between groups. Liver stiffness and cardiometabolic measures did not show any significant group-by-year interactions. At baseline, *Faecalibacterium* spp. were associated with the normal–MASLD group, and *Ruminococcus* and *Bifidobacterium* spp. were associated with the MASLD–MASLD group in the year-specific microbiota analyses. *Faecalibacterium* spp. abundance decreased after 2019 in both groups, and the numerical difference between the groups narrowed in later assessments.

Conclusions: Our findings support the monitoring of cardiometabolic risk and hepatic steatosis in individuals at risk of developing MASLD. The changing association between *Faecalibacterium* spp. and MASLD status highlights the need for a longitudinal assessment of the gut microbiota. Whether interventions targeting the gut microbiota can alter the progression of steatosis requires further investigation.

Keywords MASLD, Controlled attenuation parameter, Gut microbiota, Longitudinal Study

1 | Background

In 2023, the condition formerly known as nonalcoholic fatty liver disease (NAFLD) was renamed as metabolic dysfunction-associated steatotic liver disease (MASLD) [1]. The diagnosis of MASLD requires hepatic steatosis to be present along with at least one of the following cardiometabolic risk criteria: overweight or central obesity, hyperglycemia or diabetes, elevated blood pressure, hypertriglyceridemia, and low high-density lipoprotein (HDL) cholesterol level. The revised nomenclature reflects the metabolic basis of the disease. Although MASLD is often asymptomatic initially, it may progress to cirrhosis and other serious complications. Therefore, it is important to characterize its natural history.

Liver biopsy was traditionally used to diagnose hepatic steatosis; however, now, several noninvasive methods are available. FibroScan provides a rapid, noninvasive assessment of liver fat by using the controlled attenuation parameter (CAP). A CAP threshold of 248 dB/m is commonly used to define steatosis [2]. However, the rate of liver fat accumulation in healthy individuals leading to steatotic liver disease (SLD) remains unclear. This is because most previous studies have been cross-sectional, and very few cohort studies have followed the same population, including healthy individuals, over the long term [3-5].

Gut microbiota have been implicated in the development of MASLD. Specifically, patients with MASLD have been reported to show reduced microbial diversity, increased abundances of *Bacteroides* and *Ruminococcus* spp., and decreased abundances of butyrate-producing genera such as *Faecalibacterium* and *Eubacterium* [6, 7]. However, the longitudinal trajectories of MASLD-associated bacteria remain poorly understood, partly because few population-based cohorts have been followed up repeatedly for extended periods.

Because MASLD diagnosis also depends on cardiometabolic risk criteria, understanding how these factors relate to longitudinal changes in liver fat and gut microbiota may inform prevention and treatment. In this community-based study, we repeatedly assessed liver fat by using FibroScan, cardiometabolic factors, and gut microbiota over a period of 6 years in participants who developed MASLD and those with persistent MASLD. We aimed to characterize the trajectories of hepatic steatosis and its related factors during MASLD development and persistence, with particular focus on gut microbiota.

2 | Methods

2.1 | Study Design and Participants

105 This observational study was conducted in accordance with the Strengthening the Reporting
106 of Observational Studies in Epidemiology (STROBE) guidelines. It was part of the Iwaki
107 Health Promotion Project, a community health initiative for residents of the Iwaki district of
108 Hirosaki City, Aomori Prefecture, Japan, whereby annual health examinations are conducted in
109 June [8]. The participant selection process is illustrated in Fig. 1. All participants were adult
110 volunteers recruited through a public invitation. Of the 260 individuals who participated in all
111 five surveys with CAP measurements in 2018, 2019, 2022, 2023, and 2024, one who tested
112 positive for the hepatitis B surface antigen and 17 with missing data were excluded. Surveys
113 were not conducted in 2020 and 2021 owing to the COVID-19 pandemic. Among the remaining
114 participants, 111 had SLD (CAP, ≥248 dB/m) in 2024, and 103 met the MASLD criteria. The
115 analysis included 50 participants without SLD in 2018 who developed MASLD by 2024 (the
116 normal–MASLD group) and 41 with MASLD in both 2018 and 2024 (the MASLD–MASLD
117 group), for a total of 91 participants.
118
119 **2.2 | Transient Elastography**
120 CAP and liver stiffness measurement (LSM) were assessed using a FibroScan 530 device
121 (Echosens, Paris, France) with an M or XL probe. All examinations were performed by a trained
122 hepatologist. Measurements with fewer than 10 valid acquisitions or an interquartile range-to-

123 median ratio greater than 0.30 were excluded as unreliable. SLD was defined as CAP of ≥248
124 dB/m, in accordance with previous research [2].
125
126 **2.3 | Clinical Parameters**
127 The following parameters were assessed: age, sex, height, weight, body mass index (BMI),
128 waist circumference, blood pressure, aspartate aminotransferase, alanine aminotransferase, γ-
129 glutamyl transferase, fasting plasma glucose, glycated hemoglobin (HbA1c), triglycerides, HDL
130 cholesterol, low-density lipoprotein cholesterol, and alcohol consumption. Excessive alcohol
131 consumption was defined as ≥30 g/day in men and ≥20 g/day in women.
132
133 **2.4 | MASLD Diagnosis**
134 MASLD was diagnosed in individuals with SLD who met at least one of the following five
135 cardiometabolic risk criteria: (1) overweight or central obesity, defined as BMI of ≥23 kg/m² or
136 waist circumference of ≥94 cm in men or ≥80 cm in women; (2) dysglycemia, defined as a
137 fasting plasma glucose level of ≥100 mg/dL, 2-h post-load glucose levels of ≥140 mg/dL,
138 HbA1c of ≥5.7%, presence of type 2 diabetes, or treatment for type 2 diabetes; (3) elevated
139 blood pressure, defined as ≥130/85 mmHg or use of antihypertensive treatment; (4) elevated
140 triglycerides, defined as ≥150 mg/dL or use of dyslipidemia treatment; and (5) low HDL

cholesterol, defined as ≤40 mg/dL in men or ≤50 mg/dL in women, or use of dyslipidemia treatment. These criteria were based on the multisociety Delphi consensus statement on SLD nomenclature [1]. Although the waist circumference thresholds for MASLD differ from the Japanese metabolic syndrome thresholds (≥85 cm in men and ≥90 cm in women), agreement between the definitions has been reported to exceed 95% [9].

2.5 | Next-Generation Sequencing Analysis of Gut Microbiota

At each survey, participants collected stool samples at home using a sampling kit (TechnoSuruga Laboratory, Shizuoka, Japan). DNA was extracted from bead-homogenized stool suspensions using the NR-201 reagent kit and a GENE PREP STAR PI-480 automated nucleic acid extraction system (Kurabo, Osaka, Japan). The V3–V4 region of the 16S rRNA gene was amplified using universal primers as previously described [10]. Polymerase chain reaction PCR products were purified with a PCR purification filter plate (Merck Millipore, Burlington, MA, USA) and quantified by real-time quantitative PCR. Purified amplicons underwent paired-end sequencing on a MiSeq system (Illumina, San Diego, CA, USA; 2 × 284 cycles in 2018,2019 and 2 × 301 cycles in 2022,2023) and a MiSeq i100 Series (Illumina; 2 × 301 cycles in 2024). Paired-end reads were processed as follows: Adapter sequences and low-quality bases (Q, <20) were trimmed from the 3′ ends by using Cutadapt.

159 Reads containing ambiguous bases (N) or those measuring <150 base pairs (bp) were discarded.
160 Quality-filtered read pairs were merged, and merged reads shorter than 370 bp or longer than
161 470 bp were removed using the fastq_mergepairs command in VSEARCH [11]. Merged reads
162 with at least one sequencing error were excluded. After chimeric reads were removed using
163 uchime_denovo in VSEARCH, the remaining reads were clustered into operational taxonomic
164 units at ≥97% sequence similarity. The representative operational taxonomic unit sequences
165 were taxonomically assigned using the Ribosomal Database Project classifier (commit hash
166 701e229dde7cbe53d4261301e23459d91615999d) [12]. Assignments with confidence scores of
167 <0.8 were classified as unassigned. The relative abundance was calculated from the number of
168 reads assigned to each genus. Taxa with a relative abundance of ≥0.1% were analyzed.
169
170 **2.6 | Statistical Analysis**
171 Nonparametric tests were used because the data were not normally distributed. Categorical
172 variables are presented as counts and percentages and continuous variables as medians and
173 interquartile ranges. Groups were compared using the chi-square test or Mann–Whitney U test,
174 as appropriate. Within each group, the differences in measurements across the study years were
175 assessed using the Friedman test. MicrobiomeAnalyst was used for gut microbiota analyses [13,
176 14].

Alpha diversity was evaluated using Shannon and Simpson indices, and beta diversity was assessed using principal coordinate analysis and permutational multivariate analysis of variance. Gut microbiota were characterized by 16S rRNA gene amplicon sequencing, and differentially abundant genera were identified using linear discriminant analysis effect size (LEfSe), with a linear discriminant analysis score threshold of 2.

Repeated measurements from 2018, 2019, 2022, 2023, and 2024 were analyzed using linear mixed-effects models. Each model included a participant-specific random intercept and fixed effects for group, survey year, and group-by-year interactions. The year was treated as a categorical variable, with 2018 as the reference. Between-group differences in trajectories were assessed by comparing models with and without interaction by using a likelihood ratio test after maximum likelihood estimation. Bacterial abundances were transformed using log(1 + measured values) before analysis.

Statistical analyses were performed using R version 4.1.1 (R Foundation for Statistical Computing) and SPSS version 28.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

3 | Results

3.1 | Participant Characteristics

The participant characteristics in 2018 and 2024 are presented in Tables 1 and 2, respectively. Age and sex did not differ significantly between the normal–MASLD and MASLD–MASLD groups. At baseline, CAP was 94.0 dB/m higher in the MASLD–MASLD group, and several other measures also differed significantly. Except for hyperglycemia or diabetes, each cardiometabolic criterion was more prevalent in the MASLD–MASLD group; the proportion of patients meeting at least three criteria was approximately twice as high. In 2024, when all analyzed participants had MASLD, CAP, BMI, and waist circumference remained significantly higher in the MASLD–MASLD group, whereas the other assessed variables did not differ between the groups. In the comparison of gut microbiota diversity, no significant differences were observed in either α-diversity or β-diversity at baseline or in 2024 between the two groups (Fig. 2).

3.2 | Longitudinal Changes in MASLD-Related Parameters

Longitudinal changes in MASLD-related measures are presented in Tables 3 and 4. In the normal–MASLD group, CAP, LSM, BMI, waist circumference, fasting plasma glucose level,

systolic and diastolic blood pressure values, and γ-glutamyl transferase level increased between 2018 and 2024. HbA1c, triglyceride, and HDL cholesterol levels showed no significant changes. The median CAP exceeded the steatosis threshold of 248 dB/m in 2023, 5 years after baseline. From 2018 to 2024, the median CAP increased from 208 to 283 dB/m, equivalent to an average change of 12.5 dB/m per year over 6 years.

In the MASLD–MASLD group, most measures fluctuated across surveys without a consistent upward or downward pattern. The CAP remained high at every assessment and was 18.0 dB/m higher than that in the normal–MASLD group in 2024. The median LSM was in the 4-kPa range in 2018 and 2019 and in the 5-kPa range from 2022 onward.

The percentage changes from baseline to year 6 are presented in Table 5. In the normal–MASLD group, CAP, LSM, triglycerides, alanine aminotransferase, and systolic blood pressure showed comparatively large percentage increases, whereas increases in BMI and waist circumference were smaller. No measure changed substantially in the MASLD–MASLD group.

In the linear mixed-effects models, the group-by-year interaction for CAP was significant, indicating different trajectories between the groups (Table 6). In contrast, no significant group-by-year interactions were observed for LSM or any cardiometabolic criteria-related parameter. Across the survey years, the normal–MASLD group generally had lower median BMI, waist circumference, fasting plasma glucose, systolic and diastolic blood pressure, and triglycerides,

and higher median HDL cholesterol than the corresponding values in the MASLD–MASLD group.

3.3 | Between-Group and Longitudinal Comparisons of Gut Bacteria

The year-specific LEfSe results are shown in Figure 3, and the genera identified in each survey are summarized in Table 7. *Faecalibacterium* was associated with the normal–MASLD group in 2018, but not thereafter. *Ruminococcus* and *Bifidobacterium* were associated with the MASLD–MASLD group in 2018. *Ruminococcus* was also associated with this group in 2022, whereas the direction of the *Bifidobacterium* association varied; it was associated with the MASLD–MASLD group in 2019 and with the normal–MASLD group in 2022. In 2024, only *Bacteroides* was identified as associated with the MASLD–MASLD group.

We compared the relative abundances of the nine genera identified using year-specific LEfSe across the five survey years (Tables 8 and 9). *Faecalibacterium* abundance declined after 2019 in both groups. It was numerically higher in the normal–MASLD group in 2018 and 2019; however, both groups showed similar values from 2022 onward. The other genera varied by year, with no consistent trajectories.

In the linear mixed-effects models, the group-by-year interaction was significant for

246 *Bacteroides*, indicating different trajectories. No other genus showed a significant between-
247 group difference in trajectory (Table 10).
248
249
250 **4 | Discussion**
251 In this study, we repeatedly assessed liver fat, cardiometabolic factors, and gut microbiota
252 over a period of 6 years in participants who developed MASLD and those with persistent
253 MASLD so as to characterize the trajectories of hepatic steatosis and its related factors during
254 MASLD development and persistence, with particular focus on gut microbiota. We compared
255 CAP and the relative abundances of gut bacteria at five time points between 2018 and 2024 in
256 individuals who developed MASLD (the normal–MASLD group) and those with persistent
257 MASLD (the MASLD–MASLD group). CAP increased in the normal–MASLD group, and its
258 trajectories differed significantly between the groups. The median CAP first exceeded the
259 steatosis threshold at the 2023 assessment, approximately 5 years after baseline.
260 *Faecalibacterium* abundance declined after 2019 in both groups. Previous longitudinal studies
261 on incident steatosis often compared only two time points over 2–5 years [3-5]. Our study
262 provides five repeated assessments over 6 years, although measurements were unavailable for
263 2020 and 2021. LEfSe analysis for each year identified the genera associated with each group.

However, a longitudinal analysis of the selected genera did not yield results suggesting an association. Thus, the associations observed in the individual assessments did not necessarily represent consistent differences in trajectories.

In the normal–MASLD group in this study, the median CAP increased from 208 dB/m in 2018 to 257 dB/m in 2023, exceeding the 248 dB/m steatosis threshold, and reached 283 dB/m in 2024. Nevertheless, it remained below the MASLD–MASLD group median of 301 dB/m in 2024. Cervera et al. reported an increase from 236 to 304 dB/m over 5 years among participants who developed steatosis, at an average of 13.6 dB/m per year, which is broadly consistent with our estimate [3]. Their study included only baseline and 5-year measurements. In contrast, CAP changed slightly over 6 years in the MASLD–MASLD group in our study, whose baseline median was already approximately 300 dB/m. Prior studies of participants with steatosis at baseline have reported an increase of 19 dB/m over 5 years or a ≥20% increase in only 16.5% of participants [3, 4]. These findings suggest that CAP may increase during steatosis development and change more slowly after reaching approximately 300 dB/m. This interpretation requires caution because the group assignment was partly based on the CAP. LSM, however, increased modestly over time, even in the MASLD–MASLD group. Persistent steatosis may promote lipotoxic hepatocellular injury and chronic inflammation, activating hepatic stellate cells and extracellular matrix deposition despite a slight increase in liver fat [15, 16].

Among the five cardiometabolic criteria, overweight or central obesity is considered particularly relevant for steatosis [3, 17, 18]. However, in the normal–MASLD group, the percentage increases from baseline were larger for systolic blood pressure, triglycerides, fasting plasma glucose, and diastolic blood pressure than for BMI and waist circumference. Overweight or central obesity was already present in 64.0% of the participants in this group at baseline, which may partly explain the smaller relative changes in BMI and waist circumference. Hyperglycemia or diabetes was also present in 66.0% of the patients at baseline; however, fasting plasma glucose levels increased substantially over 6 years. Previous longitudinal studies have associated fasting glucose with incident NAFLD independently of age, BMI, triglycerides, and blood pressure [19, 20]. Elevated fasting glucose levels can reflect inadequate insulin-mediated suppression of hepatic glucose production. Intracellular glucose metabolites may activate carbohydrate response element-binding protein (ChREBP), promoting de novo lipogenesis. Concurrent adipose tissue insulin resistance may increase lipolysis and the delivery of free fatty acids to the liver. Taken together, these pathways may contribute to hepatic fat accumulation [21].

At baseline, LEfSe associated *Faecalibacterium* with the normal–MASLD group, and *Ruminococcus* and *Bifidobacterium* with the MASLD–MASLD group. The findings for *Faecalibacterium* and *Ruminococcus* were in broad agreement with reports of lower

Faecalibacterium and higher *Ruminococcus* abundances in MASLD [6, 7]. *Faecalibacterium* was not associated with either group by LEfSe after baseline. Its relative abundance at baseline was 1.39 percentage points higher in the normal–MASLD group; however, the difference narrowed thereafter. In both groups, the abundance increased temporarily in 2019 and subsequently declined. *Faecalibacterium* is a major butyrate-producing genus that is often reported to be less abundant in patients with NAFLD or MASLD [22-24]. Animal studies suggest that *Faecalibacterium* may reduce hepatic fat accumulation through effects on adipose inflammation, hepatic lipid metabolism, and intestinal barrier function [25, 26]. The mixed-effects analysis did not establish a difference in the trajectory between the groups, which is consistent with their shared downward patterns after 2019. However, these observational findings do not establish whether declining *Faecalibacterium* contributes to the development of MASLD.

LEfSe associated *Ruminococcus* with the MASLD–MASLD group at baseline and in 2022. Although the abundance of this genus fluctuated, it was generally higher in the MASLD–MASLD group. An animal study reported that *Ruminococcus* may promote hepatic fat accumulation through metabolites in the tryptophan and phenylalanine pathways [27]. Higher *Ruminococcus* abundance has also been reported in patients with NAFLD and hepatic fibrosis [6].

318 *Bifidobacterium* may protect against hepatic fat accumulation through production of short-
319 chain fatty acids, improvement of intestinal barrier function, and promotion of fatty acid β-
320 oxidation [28, 29]. In our study, however, its abundance was higher in the MASLD–MASLD
321 group at baseline and in 2019 and slightly higher in the normal–MASLD group thereafter.
322 However, the reasons for this difference remain unclear. Neither *Bifidobacterium* nor
323 *Ruminococcus* showed a consistent longitudinal pattern, which limited the interpretation of their
324 associations in this cohort.
325 *Ruminococcus 2* was associated with the MASLD–MASLD group by LEfSe in 2019, 2022,
326 and 2024, and was more abundant in this group at every survey. The *Ruminococcus 2* detected
327 here belongs to the family *Lachnospiraceae* and is taxonomically distinct from *Ruminococcus*
328 assigned to the family *Ruminococcaceae*. Previous reports have linked *Ruminococcus 2* to body
329 weight measures, including waist circumference and BMI, and lipid indices, including low-
330 density lipoprotein cholesterol, triglycerides, and total cholesterol [30]. Other studies have
331 suggested potential protective effects on the intestinal barrier and inflammation in fatty liver
332 disease [31] and a possible role in hepatocellular carcinoma through immune and bile acid
333 pathways has also been proposed [32]. However, the role of the genus remains uncertain, and
334 our data cannot establish a causal role in MASLD. Similarly, *Bacteroides* was associated with
335 the MASLD–MASLD group in the 2024 assessment, and the linear mixed-effects model

indicated a difference in its longitudinal trajectory between the groups. However, its relative abundance varied substantially across the survey years, precluding a clear conclusion regarding its association with MASLD in this study.

This study had some limitations. First, groups were defined by the MASLD status in 2018 and 2024, and CAP contributes to this classification; thus, differences in CAP trajectories partly reflect the group definitions. Without measurements in 2020 and 2021, we could not precisely determine when MASLD developed in the normal–MASLD group or how bacterial abundances changed around onset. Second, the sample comprised only 91 participants, which limited the precision of the estimates for genus-specific longitudinal differences. Third, genus-level relative abundances derived from 16S rRNA sequencing cannot resolve species or strains, absolute bacterial counts, or metabolic functions. The nine genera in this study were selected from year-specific LEfSe analyses of the same participants; therefore, these exploratory findings require validation in an independent cohort. Fourth, changes in diet, physical activity, antibiotic exposure, and other medication use were not adequately accounted for, leaving potential residual confounding. Therefore, a causal relationship between microbial changes and hepatic fat accumulation cannot be inferred.

5 | Conclusion

In this study, the median CAP increased over 6 years among participants who transitioned from no SLD to MASLD and first exceeded the steatosis threshold at the 5-year assessment. *Faecalibacterium* abundance decreased after 2019 in both groups, but longitudinal analysis did not establish a distinct microbial trajectory associated with incident MASLD. Even in 2024, when all participants had MASLD, CAP and measures of adiposity remained higher in those with MASLD at baseline. These findings support the need to monitor cardiometabolic risk and hepatic steatosis before the development of MASLD. Whether interventions targeting the gut microbiota can alter the progression of steatosis requires further investigation.

List of abbreviations

MASLD: metabolic dysfunction-associated steatotic liver disease

CAP: controlled attenuation parameter

NAFLD: nonalcoholic fatty liver disease

HDL: high-density lipoprotein

SLD: steatotic liver disease

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

LSM: liver stiffness measurement

BMI: body mass index

HbA1c: glycated hemoglobin

bp: base pair

OTU: operational taxonomic unit

LEfSe: linear discriminant analysis effect size

CI: confidence interval

ChREBP: carbohydrate response element-binding protein

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Ethics Committee of Hirosaki University School of Medicine (approval number: 2025-068). Informed consent was obtained from all participants. All participants were fully informed of the purpose and procedures of the study and provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The main contributions of this study are included in this article. Further inquiries can be

406 directed to the corresponding authors.

407

408 **Competing interests**

409 The authors declare that they have no competing interests.

410

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414

415 **Authors' contributions**

416 Author Contributions: Conceptualization, S.S.; methodology, S.S. and C.I.; validation, S.S.;

417 investigation, S.S., C.I., K.Y., K.F., R.K., N.I., K.F., D.C., T.M., S.N., K.M., and H.S.; data

418 curation, S.S., C.I., and T.M.; writing original draft preparation, S.S.; review and editing, S.S.,

419 C.I., K.Y., K.F., R.K., N.I., K.F., D.C., T.M., S.N., K.M., and H.S.; supervision, T.M., S.N., and

420 H.S.; funding acquisition, T.M., K.M., and S.N. All authors have read and agreed to the

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Data Availability Statement

The FASTQ data for gut bacteria generated in this study are available in the DDJB database under accession numbers PRJDB42539, PRJDB42542, PRJDB42711, PRJDB42714, and PRJDB46070. Raw data were generated at Hirosaki University Graduate School of Medicine. The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon request.

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Figure captions

Figure 1. Study enrollment flowchart.

Figure 2. The diversity of gut microbiota.

(a) 2018 survey and (b) 2024 survey

Figure 3. The LEfSe results for the relationship between 2018, 2019, 2022, 2023, and 2024. The threshold of the logarithmic LDA score for discriminative features was set to 2.0 at an FDR-adjusted p-value of <0.05.

LEfSe: linear discriminant analysis effect size; LDA: linear discriminant analysis; FDR: false discovery rate

Figure 1

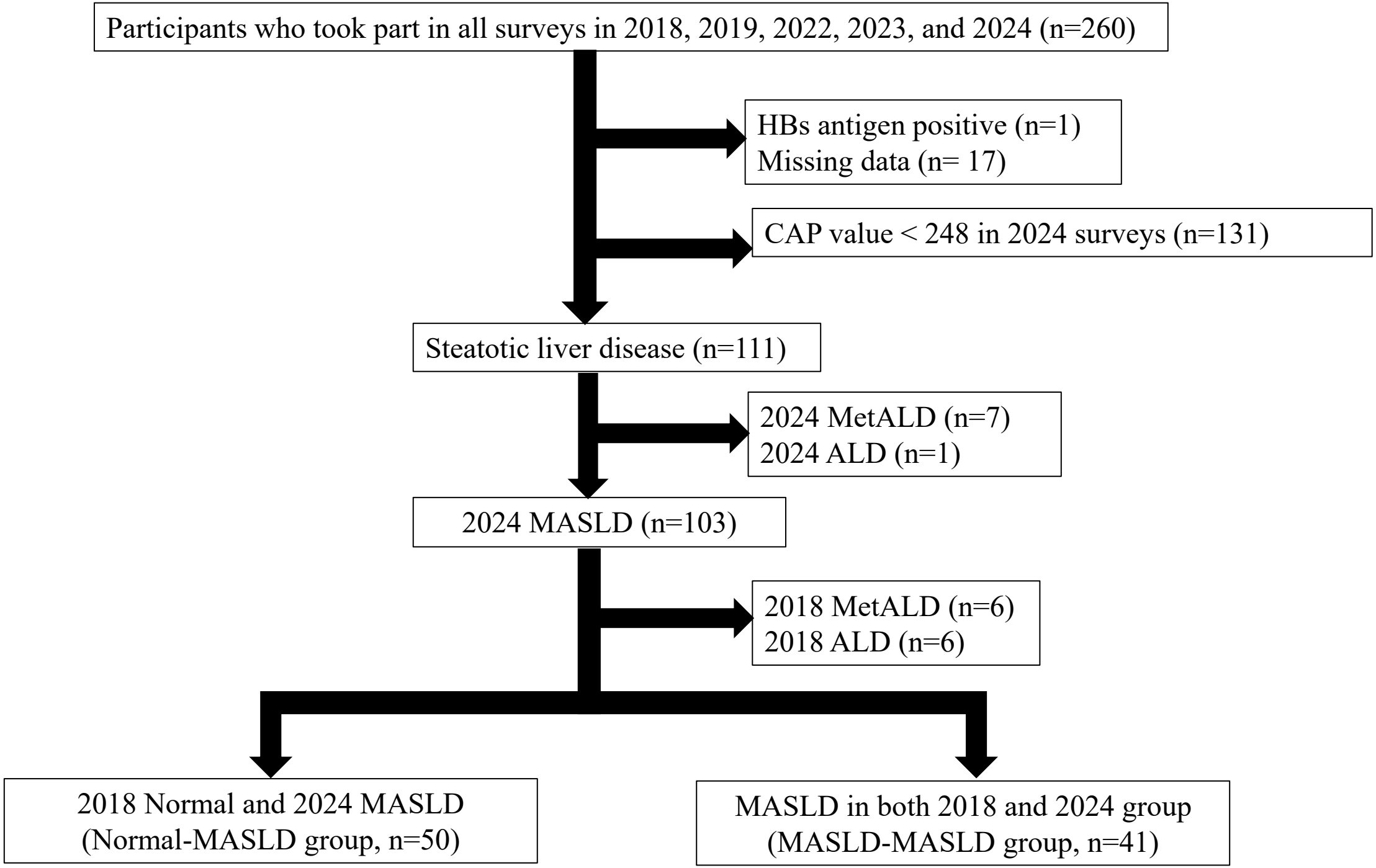
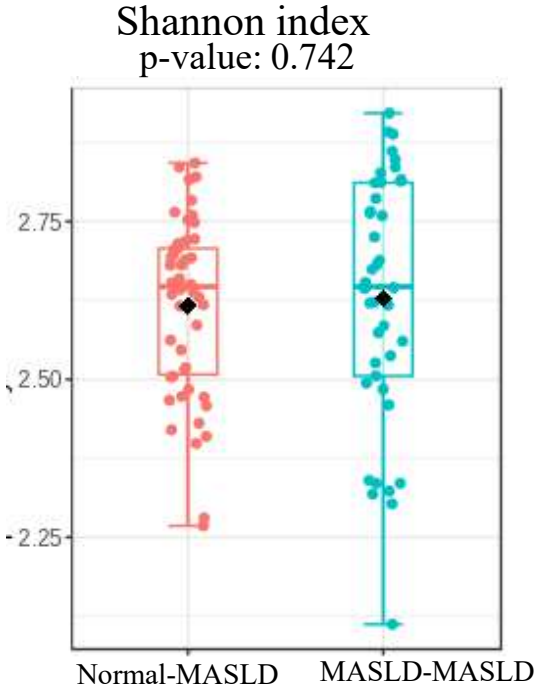
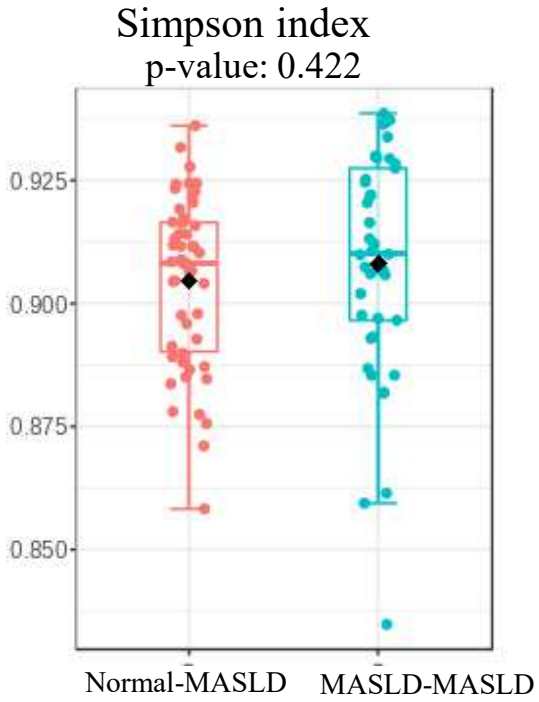

Participants who took part in all surveys in 2018, 2019, 2022, 2023, and 2024 (n=260)
HBs antigen positive (n=1)
Missing data (n= 17)
CAP value < 248 in 2024 surveys (n=131)
Steatotic liver disease (n=111)
2024 MetALD (n=7)
2024 ALD (n=1)
2024 MASLD (n=103)
2018 MetALD (n=6)
2018 ALD (n=6)
2018 Normal and 2024 MASLD
(Normal-MASLD group, n=50)
MASLD in both 2018 and 2024 group
(MASLD-MASLD group, n=41)

Fig 2a

2018





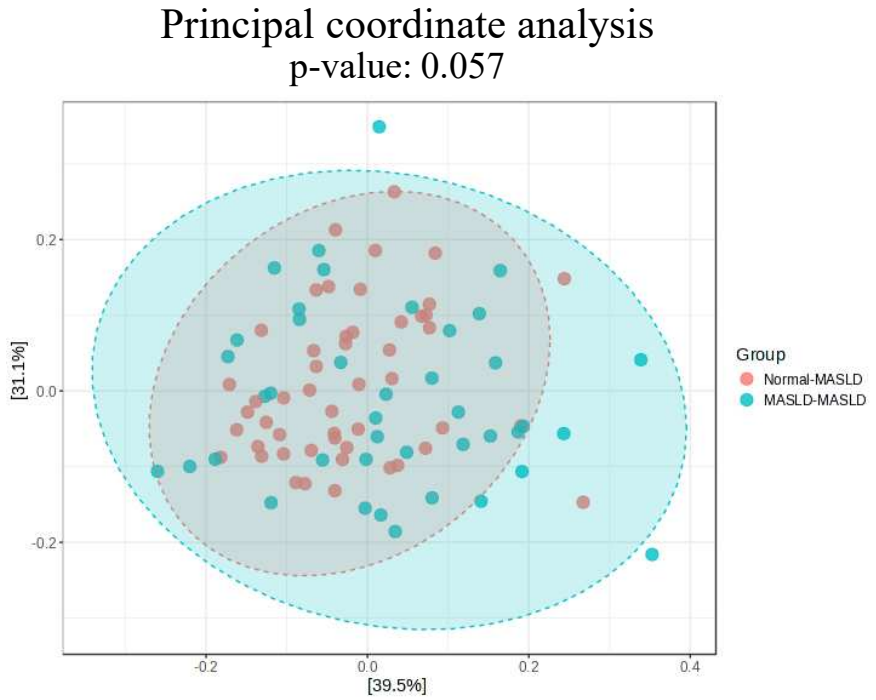
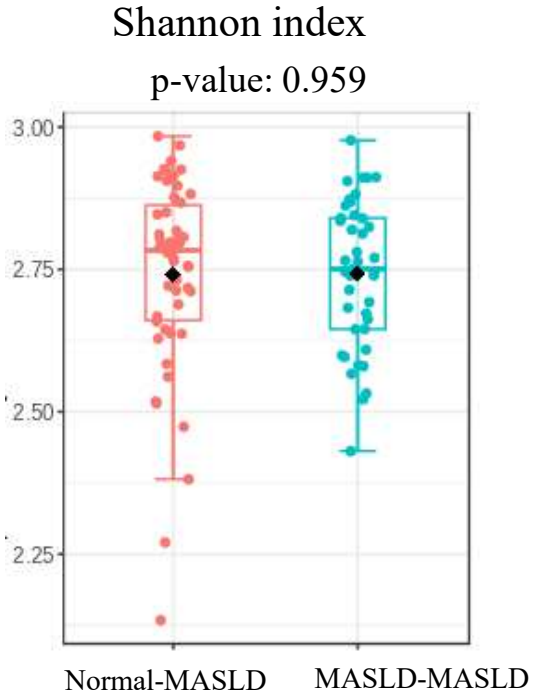
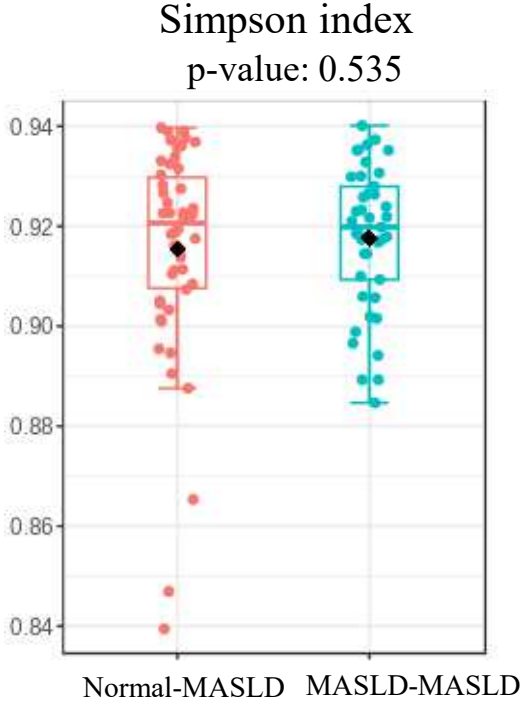


Fig 2b

2024





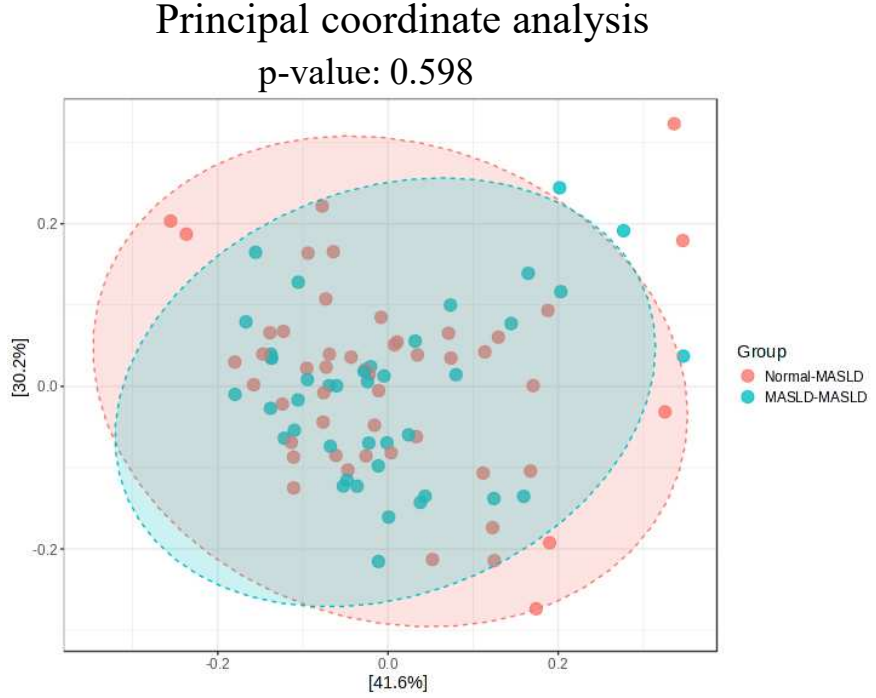
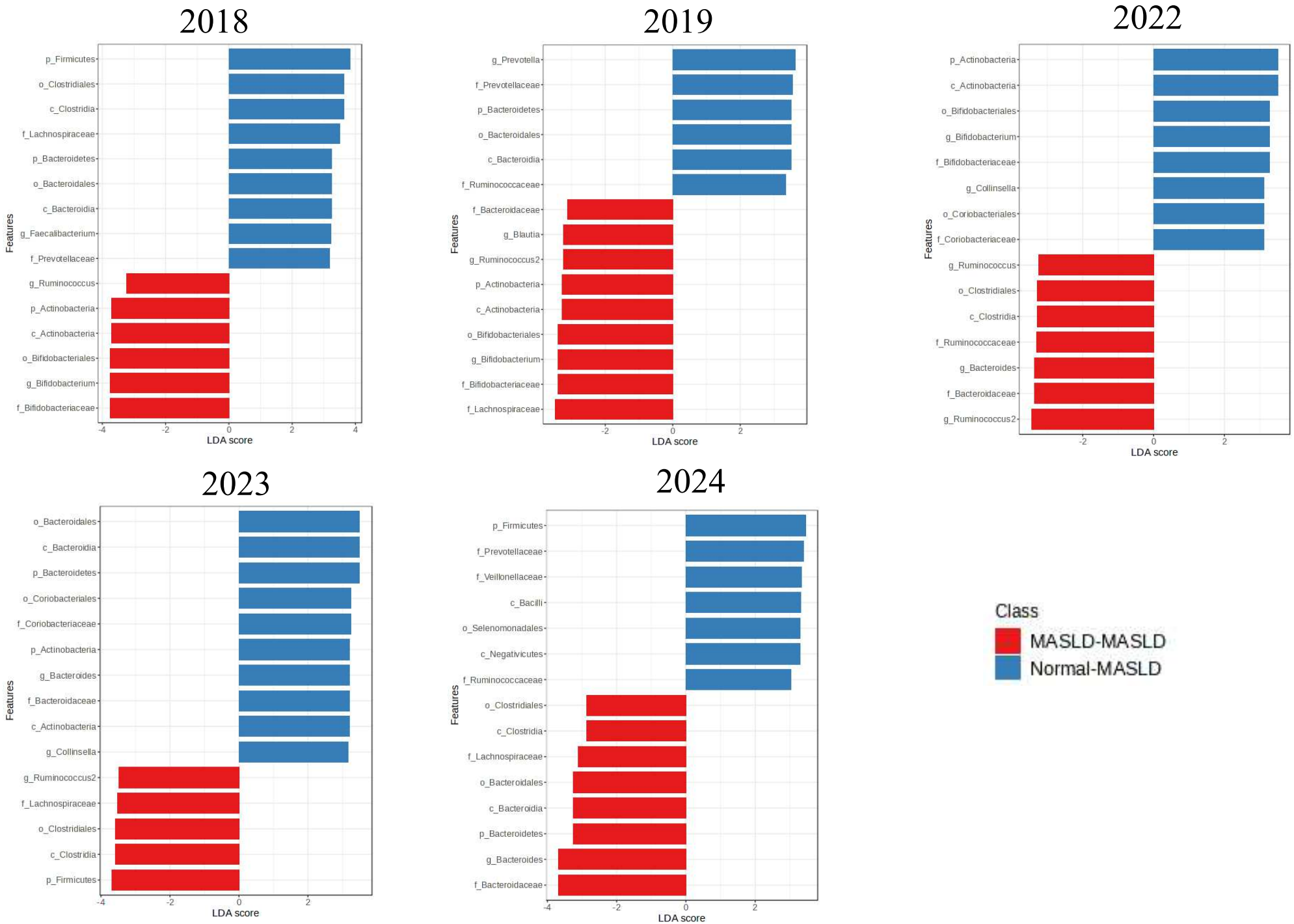


Fig 3


2018
p_Firmicutes
o_Clostridiales
c_Clostridia
f_Lachnospiraceae
p_Bacteroidetes
o_Bacteroidales
c_Bacteroidia
g_Faecalibacterium
f_Prevotellaceae
g_Ruminococcus
p_Actinobacteria
c_Actinobacteria
o_Bifidobacteriales
g_Bifidobacterium
f_Bifidobacteriaceae
Features
-4
-2
0
2
4
LDA score
2019
g_Prevotella
f_Prevotellaceae
p_Bacteroidetes
o_Bacteroidales
c_Bacteroidia
f_Ruminococcaceae
f_Bacteroidaceae
g_Blautia
g_Ruminococcus2
p_Actinobacteria
c_Actinobacteria
o_Bifidobacteriales
g_Bifidobacterium
f_Bifidobacteriaceae
f_Lachnospiraceae
Features
-2
0
2
LDA score
2022
p_Actinobacteria
c_Actinobacteria
o_Bifidobacteriales
g_Bifidobacterium
f_Bifidobacteriaceae
g_Collinsella
o_Coriobacteriales
f_Coriobacteriaceae
g_Ruminococcus
o_Clostridiales
c_Clostridia
f_Ruminococcaceae
g_Bacteroides
f_Bacteroidaceae
g_Ruminococcus2
Features
-2
0
2
LDA score
2023
o_Bacteroidales
c_Bacteroidia
p_Bacteroidetes
o_Coriobacteriales
f_Coriobacteriaceae
p_Actinobacteria
g_Bacteroides
f_Bacteroidaceae
c_Actinobacteria
g_Collinsella
g_Ruminococcus2
f_Lachnospiraceae
o_Clostridiales
c_Clostridia
p_Firmicutes
Features
-4
-2
0
2
LDA score
2024
p_Firmicutes
f_Prevotellaceae
f_Veillonellaceae
c_Bacilli
o_Selenomonadales
c_Negativicutes
f_Ruminococcaceae
o_Clostridiales
c_Clostridia
f_Lachnospiraceae
o_Bacteroidales
c_Bacteroidia
p_Bacteroidetes
g_Bacteroides
f_Bacteroidaceae
Features
-4
-2
0
2
LDA score
Class
MASLD-MASLD
Normal-MASLD

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