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Fermentable dietary fibers reduce voluntary alcohol intake and modulate gut microbiota composition in rats

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ABSTRACT

Fermentable dietary fibers can reshape the gut microbiota and boost short-chain fatty acid (SCFA) production, processes impaired by chronic alcohol use. We compared five fibers—cellulose, pectin, resistant starch, guar gum, and inulin—in male Wistar rats. After 10 days on the test diets, voluntary alcohol intake was measured with a multiple-scheduled-access paradigm, followed by a 10-day alcohol-intoxication phase. 16S rRNA profiling showed that fermentable fibers (inulin, pectin, guar gum) lowered ethanol consumption by 40–60 % and enriched SCFA-producing Bacteroidia and Muribaculaceae while reducing Proteobacteria. These microbiota shifts persisted after repeated intoxication, indicating ecological resilience. Locomotor testing confirmed that decreased drinking was not attributable to altered activity. Low-/non-fermentable fibers (cellulose, resistant starch) had no effect on intake or community structure. Our results identify fiber fermentability as a critical functional trait and support the incorporation of fermentable fibers into functional foods aimed at microbiota-based modulation of alcohol-related behaviors.

1. Introduction

The gut microbiota has emerged as a critical regulator of host physiology, exerting wide-ranging effects on metabolic, immune, and neural functions. Among the many factors influencing the composition and function of the gut microbiota, diet—and particularly the intake of functional food ingredients such as dietary fibers (DFs)—has been recognized as a key modulator. DFs are edible carbohydrates and closely associated compounds that resist digestion in the human small intestine and undergo partial or complete fermentation in the colon, often promoting health-beneficial microbial shifts and metabolite production. Previous research has shown that various types of dietary fibers, such as cellulose, pectin, resistant starch, and inulin, can differentially affect the gut microbiome and its metabolic outputs (Sonnenburg & Sonnenburg, 2014; Van den Abbeele et al., 2018).

The fermentability of DFs strongly determines their physiological impact. Highly fermentable fibers, such as inulin and pectin, promote the growth of bacterial taxa with beneficial functions, whereas less fermentable fibers like cellulose have more limited effects (Selak et al., 2016; Baxter et al., 2019; Portincasa et al., 2022). Resistant starch and guar gum fall between these extremes, each supporting the growth of specific taxa with differential metabolic contributions. A major consequence of fiber fermentation is the production of short-chain fatty acids (SCFAs), key microbial metabolites that mediate communication between the gut microbiota and host physiology. SCFAs act as signaling molecules in the microbiota–gut–brain axis, and their modulation by

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specific fiber types may therefore influence brain function and behavior, ultimately impacting mental health (Dalile et al., 2019; Silva et al., 2020). In line with this, dysbiotic states characterized by a reduction in SCFA-producing bacteria have been associated with poorer mental health outcomes, while interventions that enhance SCFA-producing taxa have been shown to reduce anxiety- and depressive-like behaviors (Bibbò et al., 2016; Silva et al., 2020).

Alcohol consumption is a well-recognized inducer of gut dysbiosis, disrupting microbial homeostasis and reducing overall diversity. Both preclinical and clinical studies have shown that chronic alcohol intake decreases beneficial SCFA-producing bacteria such as *Lactobacillus* and *Bifidobacterium* (Chayanupatkul et al., 2022; Li et al., 2021), while favoring the overgrowth of potentially pathogenic taxa such as *Proteobacteria* (Litwinowicz & Gamian, 2023). These alterations are thought to contribute to cognitive impairments and behavioral disturbances commonly observed in individuals with heavy alcohol use (Zhan et al., 2016).

This evidence has prompted growing interest in functional food strategies—particularly fermentable dietary fibers with prebiotic properties—as a means of counteracting alcohol-induced dysbiosis. For example, galacto-oligosaccharides have been shown to restore microbial balance and improve intestinal barrier function in alcohol-dependent animal models (Yang et al., 2019). Similarly, fucoidan, a sulfated polysaccharide derived from brown seaweed, has been reported to modulate gut microbiota and attenuate neuroinflammatory responses during alcohol withdrawal (Xue et al., 2021).

Based on this background, the present study aimed to investigate the relationship between dietary fiber consumption, gut microbiota modulation, and voluntary alcohol intake in Wistar rats. Specifically, we explored whether different types of dietary fibers could differentially shape gut microbiota composition and whether these changes were associated with alterations in alcohol consumption.

2. Material and methods

2.1. Animal experiments and procedure

Eighty-four adult male Wistar rats (Envigo, Barcelona, Spain) were used. The animals were individually housed in a temperature and humidity-controlled environment (21 + 1 °C), on a 12-h reverse light/dark cycle (lights off at 8:00 AM). Experimental sessions were conducted during dark phase. Prior to any procedures, the animals were acclimated to our facilities and fed a standard diet for 14 days. The final three days before the procedures started served as the baseline measurement for water and food intake.

After the habituation, the animals were distributed into six groups and fed six different diets: a standard diet and five new dietary preparations, each containing 20 % w/w of different fiber—cellulose, pectin, resistant corn starch, guar gum, or inulin. This level of fiber concentration was selected based on previous studies in rats showing that comparable or higher fiber concentrations are well tolerated and induce clear physiological and MB-related effects (i.e. see Ferreira-Lazarte et al., 2021; Frias & Sgarbieri, 1998; Komatsu et al., 2021; Levrat et al., 1991; Samarghandian et al., 2012; Tazawa et al., 1997; Toden et al., 2007). During this period we analyzed the animals' food intake, weight gain and water consumption. Each group consisted of 12 animals except for the standard diet group which had 24 animals. This group was further divided into two sub-groups: one with alcohol in their diet (alcohol-exposed control group), and the other without any alcohol. The standard diet was used as a control diet (Fig. 1). At the onset of the procedures, the animals weighed between 350 and 400 g. All research was conducted in strict adherence to the European Directive 2010/63/EU and Royal Decree 53/2013 on the protection of animals used for scientific purposes. The Faculty of Psychology of the Complutense University of Madrid and Autonomous Community of Madrid (PROEX262/19) approved the study.

2.2. Preparation of experimental diets

The experimental diets were prepared by incorporating different dietary fibers into a standard rodent diet (LASQCdiet® Rod18-H, Lacs-Vendi, Germany) at a 20 % w/w ratio. All fibers were provided in powdered form. Cellulose (ELAVET S.L., Spain; 100 % cellulose powder, D0592), apple pectin (Riesgo Farma S.L., Spain; ~85 % dietary fiber), resistant corn starch (Mercadiet S.L., Spain; ~75 % dietary fiber), guar gum (HSN S.L., Spain; fiber from *Cyamopsis tetragonoloba* seeds, > 95 % dietary fiber), and inulin (HSN S.L., Spain; 100 % inulin from *Cichorium intybus* roots) were used.

The ingredients were thoroughly mixed to ensure homogeneous distribution of the fibers within the diet. The resulting mixtures were processed into pellet form and dried under controlled conditions to achieve a stable composition suitable for long-term storage and consumption. The diets were stored in sealed containers at low temperatures and renewed periodically to preserve their quality throughout the experimental period.

2.3. Voluntary alcohol consumption

After a 10-day treatment to the new fiber diets, we studied the voluntary alcohol consumption in animals, using the "Drinking in the dark-multiple scheduled access" (DID-MSA) paradigm (Bell et al., 2011; Calleja-Conde et al., 2020). Rats were presented two identical bottles in their home cages, one containing alcohol (10 %) and another containing water. This paradigm involved three 1-h access sessions during the dark cycle, with the first session initiated at lights out and each subsequent period of access separated by 2 h of alcohol deprivation. The bottles containing alcohol and water were weighed before and after each 1-h consumption period. The loss of liquid by dripping was controlled with two control bottles. The position of the bottles was randomly switched every day. Voluntary alcohol consumption was carried out for 15 days. Every three days the alcohol concentration increased (3 %, 6 %, 10 %, 15 % and 20 %). The increasing percentage of alcohol was

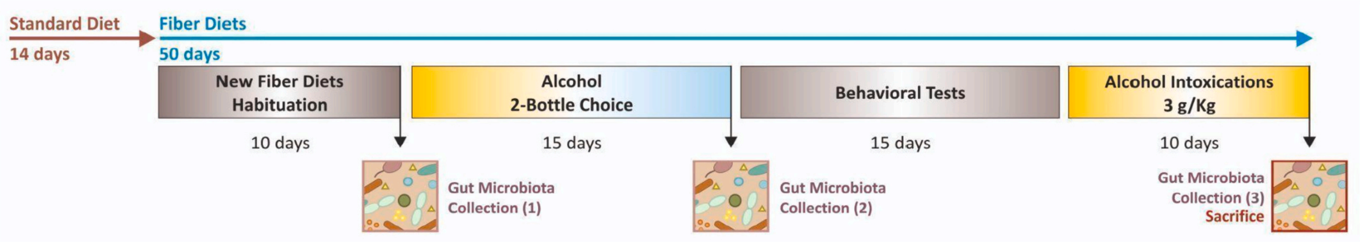

Fig. 1. After a 10-day treatment with the new fiber diets, the voluntary alcohol consumption of the animals was examined for 15 days. At the end of this period, a motor activity test and other behavioral tests were conducted, including the Hot Plate test, Forced Swim test, Elevated Plus Maze, and Novel object recognition. Following the 15-day behavioral studies, the animals were subjected to daily alcohol intoxication at a dose of 3 g/kg at 25 % concentration in a single dose for 10 days. Two hours after the last intoxication episode, the animals were euthanized.

introduced to investigate a potential interaction between diet and different alcohol concentrations. This approach is based on our experience, which shows that, although in some cases no significant interactions were observed (Calleja-Conde et al., 2016), some studies have reported that different alcohol concentrations can induce changes or even reverse the treatment effect on consumption when concentrations reach 15–20 % (Echeverry-Alzate et al., 2019; Roldán et al., 2018).

2.4. Behavioral tests

In order to investigate whether the fiber diets had changed behavior of the animals, Locomotor activity test, Hot Plate test, Forced Swim test, Elevated Plus Maze, and Novel object recognition were conducted at the end of the voluntary alcohol consumption period.

2.4.1. Locomotor activity

The locomotor activity of the rats was assessed in a dimly lit room (20 lx), during 30 min using six custom-made 40 × 35 × 35 cm rectangular boxes, and the boxes were equipped with eight photocells arranged in two lines (4 and 8 cm above the floor) that detected the locomotor activity as beam breaks (Segovia-Rodríguez et al., 2022).

2.4.2. Hot plate test (HPT)

HPT test was used to evaluate pain behavior in rats, following the protocol described in Deuis et al., 2017. Animals were positioned on a heated metallic plate kept at a stable temperature of 50–55 °C, and the latency to the first nocifensive reaction was measured. Such nocifensive behaviors including withdrawal or licking of the forepaws, hind paw withdrawal or licking, stamping, adopting a leaning posture, and jumping. Although forepaw responses were frequently the earliest to emerge, hind paw withdrawal or licking was considered as a more consistent indicator of thermal nociception, since forepaws are often engaged in grooming or exploratory activity and may not remain in continuous contact with the plate surface.

2.4.3. Forced swim test

The forced swim test was conducted over a 24-h schedule according to Slattery & Cryan, 2012. On day 1 (pretest), each rat was placed individually in a transparent acrylic cylinder (approximately 20 cm diameter × 60 cm height), filled with water at a depth of 30 cm, maintained at 23–25 °C. On this day, a 15-min pretest session (habituation) was performed. After 15 min of swimming, rats were removed from the water, dried with towels and returned to their home cages. On day 2 (Twenty-four hours after the pre-test), rats were placed in the cylinder for a 5-min test session, during which passive behavior (immobility) were recorded via a video camera positioned to the side of the swim cylinders. Immobility was defined as floating with minimal movements necessary to keep the head above water. A reduction in immobility time is interpreted as indicative of an antidepressant-like effect. An experimenter blinded to the treatment conditions quantified the active a passive behavior.

2.4.4. Elevated plus maze (EPM)

EPM test was performed to evaluate anxiety-related behavior. The apparatus was constructed from a matte black acrylic surface and consisted of four arms (two closed arms with 30-cm high walls and two open arms without walls, typically considered anxiogenic), 50 cm long and 10 cm wide. The arms were mounted on metal legs, elevating the maze 70 cm above the floor. EPM test was carried out following the protocol described in Walf & Frye, 2007. At the beginning of each trial, rats were placed at the central junction of the open and closed arms, facing the open arm opposite to the experimenter. Animals were allowed to freely explore the apparatus for 5 min. All sessions were recorded using a video camera positioned above the maze. An experimenter blinded to the treatment conditions quantified the time spent in the open and closed arms, as well as the number of entries into each type of arm.

2.4.5. Novel object recognition (NOR)

Novel object recognition (NOR) test was conducted to assess the episodic memory. The test was carried out using six 40 × 40 cm boxes. Two sets of objects were used during this test, one set of objects was made of grey plastic (18-cm high and 6-cm wide) and another set of objects was made of clear blue plastic (17-cm high and 5-cm wide). NOR test was carried out for two days following the protocol described in Bevins & Besheer, 2006. On day 1 (habituation session), each rat was allowed to habituate to the test box during 10 min. On day 2 (familiarization session), rats were placed in the box and allowed to explore two identical sample objects for 10 min. Following, the rats were returned to their cages for 1 h (retention interval). Then, rats were placed in the same box with one familiar and one novel object (counterbalanced across rats) and given 5 min to explore them (test session). All the sessions were monitored by a video camera above the apparatus. An experimenter blind to the treatments measured the time the animals spent exploring each object, the latency of the first approach and the number of approaches to each object. An object approach was any contact with the mouth, nose or paw. Accidental contacts such as backing into the object or bumping the object as it passes were not included as approaches.

2.5. Alcohol intoxication

Once the behavioral testing was completed, we carried out the alcohol intoxications for ten days. Binge alcohol treatment was performed once per day during 10 days. Alcohol was orally administered through gavage (i.g.) at a dose of 3 g/kg using a 25 % alcohol solution in tap water at a volume of 15 ml/kg. Control animals received the same volume of liquid as above but were given tap water alone (López-Moreno et al., 2015). Two hours after the last intoxication episode, the animals were euthanized.

2.6. Collection of rat fecal samples for bacterial analysis and DNA extraction and processing

Rat fecal samples for the bacterial analysis were taken at the following time points: (1) ten days after the incorporation of the diets, (2) on the last day of voluntary alcohol consumption and (3) after the last alcohol intoxication (Fig. 1b). Fecal samples were collected directly from the animals, placing them on a sterilized flat surface and immediately transferring the samples into sterile tubes, which were subsequently frozen at −80 °C. Personnel involved in sample collection used gloves, lab coats, and face masks to prevent the introduction of exogenous microorganisms and replaced these protective materials whenever contact with the samples occurred. The procedure was conducted to minimize the exposure time of the samples, thereby reducing the risk of contamination.

2.6.1. Sequencing

DNA extraction from rat fecal samples (180–200 mg) was performed using the QIAamp® DNA Stool Mini Kit (Qiagen France S.A.S.) following the manufacturer's instructions. DNA concentration and purity were determined by absorbance at 260 nm (A260) and the A260/A280 ratio, respectively, using a NanoDrop spectrophotometer (NanoDrop TM One Spectrophotometer, Thermo Fisher Scientific Inc., Spain). DNA samples were sent to StarSEQ® GmbH (Mainz, Germany) for sequencing the V3–V4 hypervariable regions of the bacterial 16S rRNA gene using the primer pair 341F/806R 5′-CCTACGGGNGGCWGCAG-3′ and 5′-GGATTAGAWACCCBNGTAGTC-3′. DNA amplicons were sequenced on a MiSeq Illumina platform (Illumina, San Diego, CA) following manufacturer's standard procedure for library preparation.

2.7. Statistical analysis

2.7.1. Bioinformatics

The quality of raw sequencing reads was assessed by FastQC (v0.11.9) (Wingett & Andrews, 2018) and summarized by MultiQC (v1.12) (Ewels et al., 2016). The 16S paired raw reads were imported into QIIME2 (v2022.2) (Bolyen et al., 2019) and processed with the DADA2 (Callahan et al., 2016) plugin using default parameters. Trimming and truncation points were selected to maintain a minimum Phred quality score of 25 at the lower quartile, based on random sampling of 10,000 sequences from a total of 9,996,602 without replacement. An overlap of approximately 20 bases between forward and reverse reads was retained to ensure proper pairing. Taxonomic assignment was performed with the q2-feature-classifier (Bokulich et al., 2018) plugin using a pre-trained sklearn-based classifier (Pedregosa et al., 2011). The classifier was generated by extracting sequences corresponding to the 341F/806R primer pair from the SILVA 132.1 database clustered at 99 % identity. Before training, the q2-clawback (Kaehler et al., 2019) plugin was used to incorporate species occurrence probabilities (weights) expected in the animal distal gut, retrieved from qiita.ucsd.edu.

For diversity analyses, rarefaction was applied to normalize sequencing depth across samples. Data were rarefied to 6890 reads per sample based on rarefaction curves of both the Shannon index and Observed_features index (Supplementary Figs. S1 and S2). Alpha diversity was measured using Shannon's diversity index (a quantitative measure of community richness) (Figs. 3A, 5A, 6A). Beta diversity was assessed using principal coordinates analysis (PCoA) based on Bray–Curtis and Jaccard distances (Figs. 3B, 5B, 6B). Linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) was applied with a normalization value (format_input.py -o parameter) of 1,000,000 across all groups. LEfSe was set (run_lefse.py -l parameter) with a threshold of 3.5 on the absolute value of the logarithmic LDA score to identify differentially abundant bacterial taxa (Figs. 3C, 5C, 6C).

Additional quality control metrics, including sequence counts per sample (unique vs. duplicate reads) and mean per-base quality scores, are provided in Supplementary Figs. S3 and S4.

2.7.2. Animal intake and behavioral data

Data from food intake, water consumption, and weight change (Fig. 2A, C, D) were analyzed using two-way mixed ANOVAs (within-subjects: days; between-groups: dietary fiber type). Average food intake from the fourth day (Fig. 2B) was analyzed using one-way ANOVA. Alcohol consumption data (Fig. 4A) were analyzed using three-way mixed ANOVA (within-subjects: days; between-groups: type of diet and alcohol concentration). Locomotor activity (Fig. 4C), forced swim test, hot plate test, elevated plus maze test, and novel object recognition

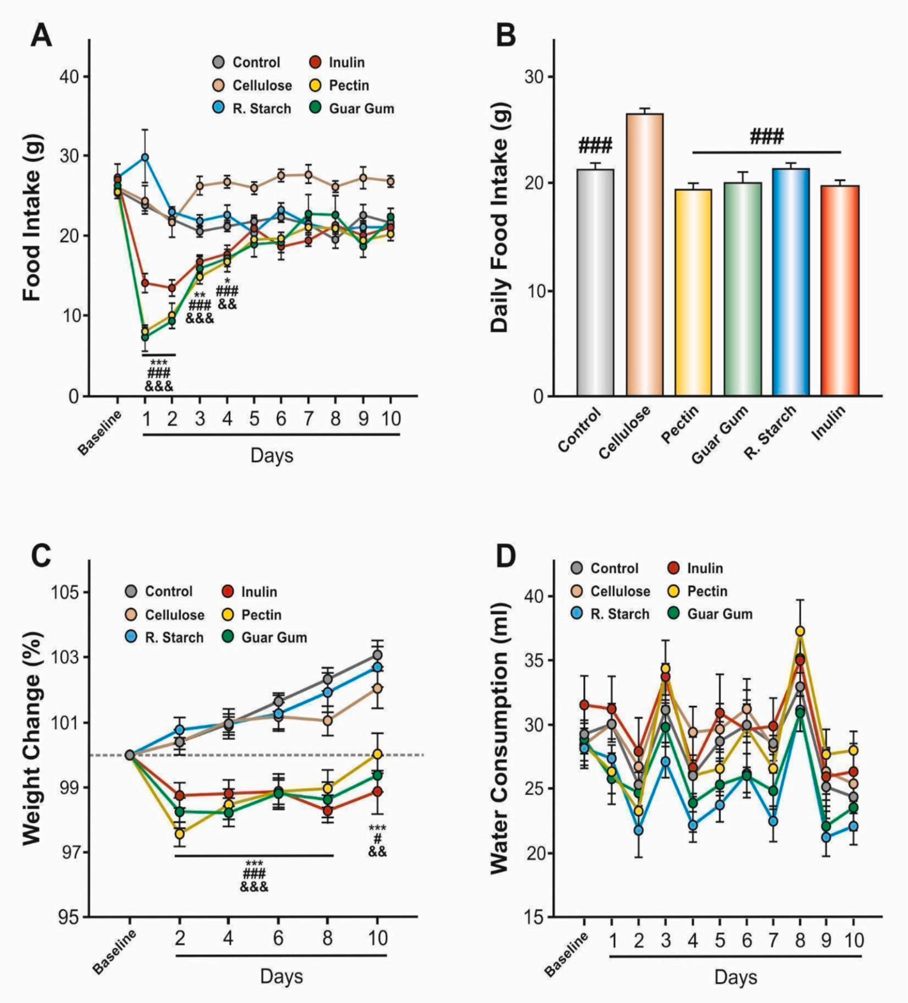

Fig. 2. Effect of dietary fiber types on food intake, weight gain and water intake. (A) Food intake (g) during the first 10 days of exposure to the new dietary fiber types. (B) Average food intake from the fourth day, in which there was a stabilization of this intake. (C) Weight change during the first 10 days of exposure to the new dietary fiber types. (D) Water consumption during the first 10 days of exposure to the new dietary fiber types. Data represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the control group; #$p < 0.05$, ###$p < 0.001$ compared with cellulose group; &&$p < 0.01$, &&&$p < 0.001$ compared with resistant starch group.

were analyzed using one-way ANOVA. Tukey's post hoc analysis was used for multiple group comparisons. All statistical analyses were performed with the SPSS statistical software package (version 29.0; IBM, Chicago, IL, USA).

3. Results

3.1. *Effect of dietary fiber types on food intake, weight gain and water intake*

During the first 10 days of exposure to the new dietary fiber types, we analyzed the animals' food intake, weight gain and water consumption. Fig. 2A shows that the different dietary fiber types had a significant effect on food intake: 2-way mixed ANOVA: dietary fiber type $F_{5,\ 78} = 23.789$, $p < 0.000$; day $F_{10,\ 780} = 41.345$, $p < 0.000$; and interaction $F_{50,\ 780} = 10.767$, $p = 0.000$. During the first 4 days, Inulin, Pectin and Guar Gum groups show a reduction in grams ingested compared to the control, starch and cellulose groups. Fig. 2B shows the average of grams ingested from the 4th day, in which a stabilization of the intake is observed: 1-way ANOVA $F_{5,\ 78} = 15.492$, $p < 0.000$. Once the animals stabilized their food intake, the cellulose group showed statistically significant differences compared to the rest of the groups (25 % increase compared to the control group). As Fig. 2C shows, it is possible that the differences observed in food intake are reflected in weight gain: 2-way mixed ANOVA: dietary fiber type $F_{5,\ 78} = 19.108$, $p < 0.000$; day $F_{5,\ 390} = 20.322$, $p < 0.000$; and interaction $F_{25,\ 390} = 6.023$, $p = 0.000$. On the second day of exposure to the new dietary fiber types, the groups inulin, pectin and guar gum show a slight decrease in weight. This reduction is statistically significant with respect to the rest of the groups. Finally, it should be noted that the differences observed between the groups in food intake and weight change were not accompanied by differences in water consumption, as shown in Fig. 2D: 2-way mixed ANOVA: dietary fiber type $F_{5,\ 78} = 1.635$, NS; day $F_{10,\ 780} = 54.633$, $p < 0.000$; and interaction $F_{50,\ 780} = 1.984$, $p = 0.000$.

3.2. *Effect of dietary fiber types on the rat gut microbiota*

Fig. 3A and B show that the exposure to different dietary fiber types led to statistical differences in Alpha ($p < 0.001$) and Beta diversity ($p < 0.001$). To determine how the gut microbiota differed between the dietary fiber types and control group, we further analyzed the relative abundances of groups for each sample by LEfse (Fig. 3C). At the phylum

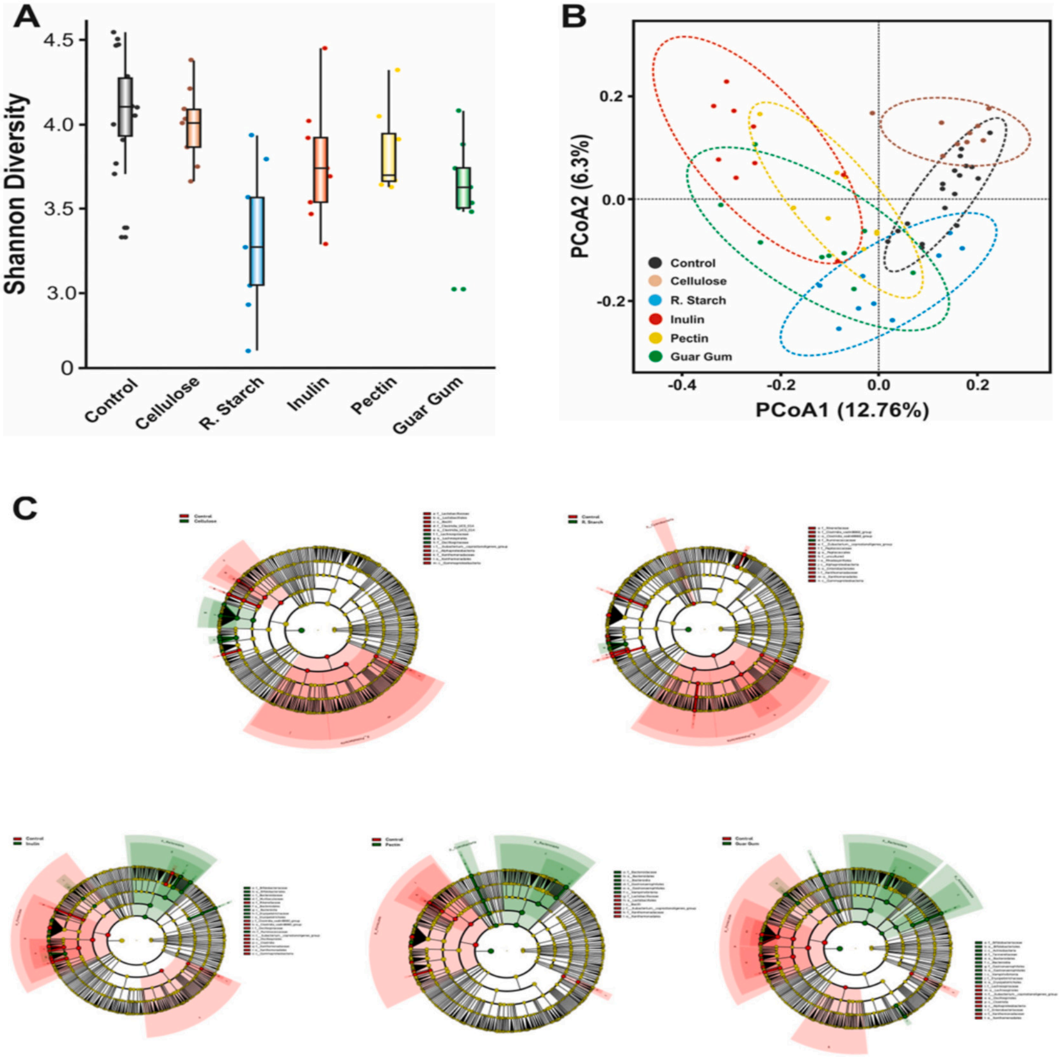

Fig. 3. Effect of dietary fiber types on the rat gut microbiota analyzed by 16S rRNA sequencing. Ten days after the introduction of the different dietary fiber types, the composition of the gut microbiota was analyzed. (A) Shannon diversity index representing stool alpha diversity in groups of fecal specimens. (B) Principal coordinates analysis (PCoA) plot comparing fecal microbial beta-diversity (Bray-Curtis Dissimilarity) among the groups ($n = 9$–18, $N = 63$). (C) Visualization using LefSe cladogram of differential abundant taxa between control and dietary fiber groups. For improved visualization of the cladograms, enlarged versions are provided as Supplementary Fig. S5.

level, the relative abundance of *Proteobacteria* decreased in Cellulose and Resistant Starch groups. In addition, in the Cellulose group, a reduction in the class *Bacilli* and an increase in the order *Lacnospirales* are observed. At the family level, *Lachnospiraceae* and *Oscillospiraceae* are increased. In the Resistant Starch group, there is a reduction in the Class *Gammaproteobacteria* and the family *Rikenellaceae*, while there is an increase in the family *Ruminococcaceae*. On the other hand, at the phylum level, the relative abundance of *Firmicutes* decreased in Inulin, Pectin and Guar Gum groups. On the contrary, the relative abundance of *Bacteroidetes* increased in these groups. Specifically, in the Inulin group, an increase in the relative abundance of class *Bacteroidia*, the order *Bacteroidales* and the families *Bacteroidaceae* and *Muribaculaceae* is observed. On the other hand, a reduction in the relative abundance of the class *Clostridia*, the order *Oscillospirales* and the families *Rikenellaceae* and *Oscillospiraceae* is observed. In the Pectin group, an increase is observed in the phylum *Cyanobacteria*, the classes *Bacteroidia* and *Vampirivibrionia*, the orders *Bacteroidales* and *Gastranaerophilales*, and the families *Bacteroidaceae* and *Gastranaerophilales*. Likewise, the Pectin group shows a decrease in the relative abundance of the class *Bacilli*, the order *Lactobacillales* and the family *Lactobacillaceae*. Finally, the Guar Gum group shows an increase in the phylum *Actinobacteria*, the classes *Actinobacteria*, *Bacteroidia* and *Vampirivibrionia*, the orders *Bifidobacteriales*, *Bacteroidales* and *Gastranaerophilales* and the families *Gastranaerophilales* and *Enterobacteriaceae*. In addition, in the Guar Gum group, a decrease in the class *Clostridia*, the orders *Lachnospirales* and *Oscillospirales* and the family *Lachnospiraceae* is observed.

3.3. *Effect of dietary fiber types on voluntary alcohol consumption and behavioral tests*

Fig. 4A shows that dietary fibers inulin, pectin and guar gum significantly reduced voluntary alcohol consumption in the two-bottle choice paradigm: three-way mixed ANOVA, within subjects days $F_{2,660} = 28{,}95$; $p < 0.001$; between subjects, type of diet $F_{5,330} = 27{,}14$; $p < 0.001$ and alcohol concentration $F_{4,330} = 0{,}48$; $p > 0.05$, NS. There was no interaction between alcohol concentration and fiber type, that is, the reduction in alcohol consumption induced by dietary fibers was independent of alcohol concentration. Fig. 4B shows the marginal means and

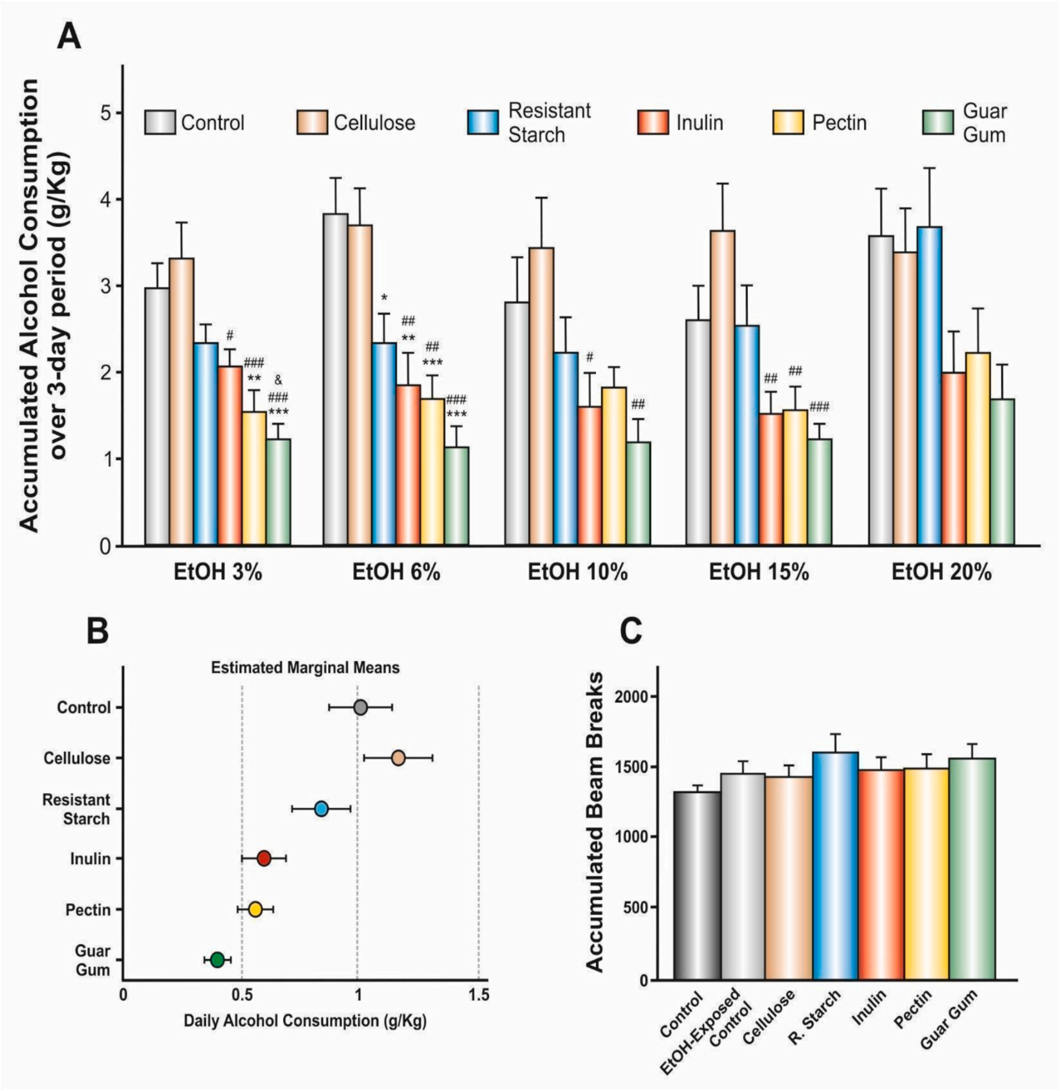

Fig. 4. Effect of dietary fiber types on voluntary alcohol consumption. (A) Inulin, pectin and guar gum significantly reduced voluntary alcohol consumption in the *DID-MSA paradigm*. Every three days the alcohol concentration was changed starting at 3 % until reaching 20 %. Each bar represents the average alcohol consumption in g/kg for those three days ($n = 12$, $N = 72$). Each day consisted of three one-hour alcohol sessions, each separated by two hours. (B) Marginal means and 95 % confidence interval of the 15 days of alcohol consumption regardless of alcohol concentration. (C) Accumulated beam breaks during motor activity test. * $p < 0{,}05$; **$p < 0{,}01$; *** $p < 0{,}001$ compared with Control group; # $p < 0{,}05$; ## $p < 0{,}01$; ### $p < 0{,}001$ compared with Cellulose group; & $p < 0{,}05$ compared with Resistant Starch group.

their corresponding 95 % confidence intervals for alcohol consumption and the effect of dietary fibers. We can see that there was an overlap between the confidence intervals of the control group and the cellulose and resistant starch groups. However, the groups of inulin, pectin and guar gum did not show any overlap. This indicates that, taking into account the total alcohol consumption, any value greater than 0.71 g/kg (inulin upper limit) of alcohol we have 95 % confidence that it does not correspond to any of the groups treated with inulin, pectin or guar gum. Compared to the control group, inulin, pectin, and guar gum caused a reduction in alcohol consumption of 40,4 %, 43,4 %, and 59,6 %, respectively. In order to rule out a depressant effect of some of the fibers or the alcohol consumption on the spontaneous motor activity of the

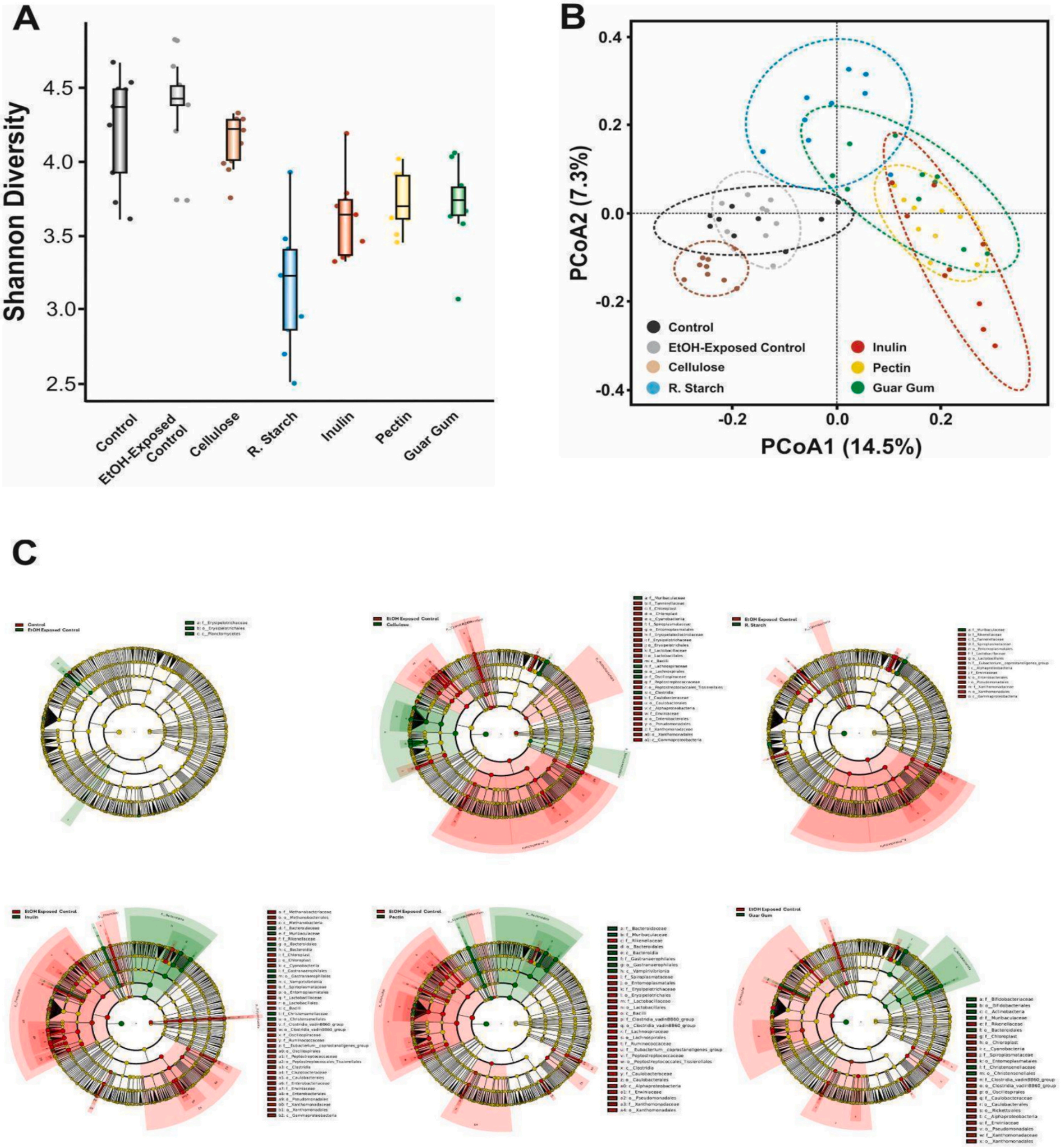

Fig. 5. Effect of dietary fiber types and voluntary alcohol consumption on the rat gut microbiota analyzed by 16S rRNA sequencing. After twenty days of voluntary alcohol consumption, the composition of the gut microbiota was analyzed. (A) Shannon diversity index representing stool alpha diversity in groups of fecal specimens. (B) Principal coordinates analysis (PCoA) plot comparing fecal microbial beta-diversity (Bray-Curtis Dissimilarity) among the groups ($n = 9$, $N = 63$). (C) Visualization using LefSe cladogram of differential abundant taxa between control and dietary fiber groups. For improved visualization of the cladograms, enlarged versions are provided as Supplementary Fig. S6.

animals, we examined their locomotor activity. Fig. 4C shows that there were no significant effects of alcohol or fibers on motor activity, 1-way ANOVA *F* (6, 77) = 0.920, NS. On the other hand, no statistically significant effects of diet or voluntary alcohol consumption were observed on the other behavioral tests: forced swim test, 1-way ANOVA F (6, 76) = 2.312, NS; hot plate test, 1-way ANOVA F (6, 76) = 0.349, NS; elevated plus maze, 1-way ANOVA F (6, 76) = 0.933, NS; novel object recognition, 1-way ANOVA F (6, 72) = 0.198, NS (Unplotted data).

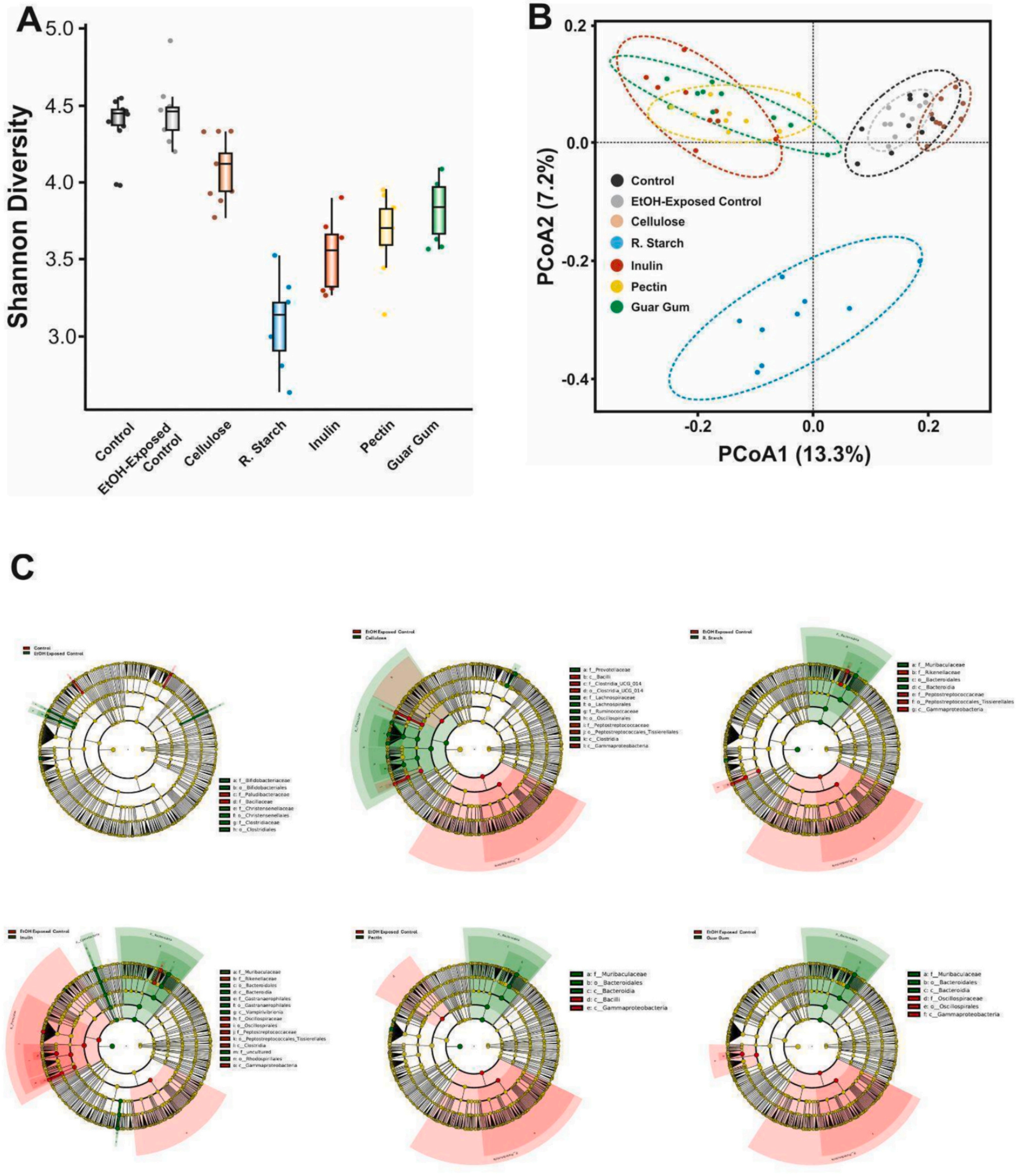

Fig. 6. Effect of dietary fiber types and repeated alcohol intoxications on the rat gut microbiota analyzed by 16S rRNA sequencing. After ten days of alcohol intoxications (3 g/kg/day), the composition of the gut microbiota was analyzed. (A) Shannon diversity index representing stool alpha diversity in groups of fecal specimens. (B) Principal coordinates analysis (PCoA) plot comparing fecal microbial beta-diversity (Bray-Curtis Dissimilarity) among the groups ($n = 9$, $N = 63$). (C) Visualization using LefSe cladogram of differential abundant taxa between control and dietary fiber groups. For improved visualization of the cladograms, enlarged versions are provided as Supplementary Fig. S7.

3.4. *Effect of dietary fiber types and voluntary alcohol consumption on the rat gut microbiota*

Fig. 5A, B shows that exposure to different types of dietary fiber maintains statistical differences in Alpha ($p < 0.001$) and Beta diversity ($p < 0.001$) after twenty days of voluntary alcohol consumption. It is important to note that at this point, the animals exposed to the standard diet were divided into two groups: a control group that consumed alcohol (alcohol-exposed control group) and another control group that was not exposed to alcohol consumption. Subsequently, LEfSe analysis was used to determine how the gut microbiota differed between the alcohol-exposed control group and the rest of the groups (Fig. 5C). Firstly, in relation to the effect of voluntary alcohol consumption, the first cladogram shows that the alcohol-exposed control group presents an increase in relative abundance in the phylum *Planctomycetes*, the order *Erysipelotrichales*, as well as an increase in the family *Erysipelotrichaceae* when we compare it to the control group that was not exposed to alcohol consumption.

The differences observed between the dietary fiber types and alcohol-exposed control group show the differences prior to alcohol consumption. The differences are numerous and occur at all taxonomic levels, so this section will describe the main differences found at the phylum level. Mainly, in Cellulose and Resistant Starch groups we observed a reduction of two phyla: *Proteobacteria* and *Cyanobacteria*. In addition, the Cellulose group presents other differences with the alcohol-exposed control group. For example, at the phylum level, we observed a reduction in *Actinobacteria* and an increase in *Verrucomicrobiota*. Similar to what was observed in the previous analysis, the relative abundance of *Firmicutes* decreased in Inulin, Pectin and Guar Gum groups. On the other hand, Inulin and Pectin groups show an increase in the phylum *Bacteroidetes* and the Guar Gum group shows an increase in the phylum *Actinobacteria*. Although there are numerous significant differences between the groups at lower taxonomic levels (see Fig. 5C), we can conclude that the main effects of dietary fiber types on the gut microbiota are maintained after voluntary alcohol consumption.

3.5. *Effect of dietary fiber types and repeated alcohol intoxications on the rat gut microbiota*

Fig. 6A, B shows that exposure to different types of dietary fiber maintains statistical differences in Alpha ($p < 0.001$) and Beta diversity ($p < 0.001$) after twenty days of voluntary alcohol consumption and ten days of alcohol intoxications (3 g/kg/day). To determine how the gut microbiota differed between the alcohol-exposed control group and the rest of the groups, we further analyzed the relative abundances of groups for each sample by LEfse (Fig. 6C). In relation to the effect of the repeated alcohol intoxications, in the first cladogram we can observe that alcohol leads to an increase in the *Bifidobacteriaceae*, *Christensenellaceae* and *Clostridiaceae* families, as well as *Bifidobacteriales*, *Christensenellales* and *Clostridiales* at the order level. Likewise, alcohol intoxication led to a decrease in the *Paludibacteraceae* and *Bacillaceae* families.

On the other hand, the main differences are found between the dietary fiber types and alcohol-exposed control group at phylum level. In general, all dietary fiber types lead to a reduction in the phylum *Proteobacteria*, except in the case of the Inulin group, although it is important to note that in this group, there is a reduction in the class *Gammaproteobacteria*. Furthermore, all dietary fiber types except the Cellulose group present an increase in the relative abundance of the phylum *Bacteroidetes*. In this group, there is an increase in *Firmicutes* after repeated alcohol intoxications, contrary to what was observed in the Inulin group. Although there are numerous significant differences between the groups at lower taxonomic levels (see Fig. 6C), we observed that after repeated alcohol intoxications, dietary fiber types tend to show a similar gut microbiota pattern, compared to the standard diet.

4. Discussion

The aim of this study was to explore the relationship between dietary fiber consumption, gut microbiota modulation, and voluntary alcohol intake in Wistar rats. Given the established role of the gut microbiota in metabolic and behavioral health, especially through the production of SCFAs, we hypothesized that fermentable fibers would significantly impact both the composition of the microbiota and alcohol consumption behavior. The results largely supported this hypothesis. Fermentable fibers like inulin, pectin, and guar gum increased the abundance of beneficial SCFA-producing bacteria, particularly from the Bacteroidetes phylum, and significantly reduced voluntary alcohol intake compared to less fermentable fibers such as cellulose and resistant starch. These findings support the interpretation that fermentable dietary fibers act as functional food components capable of selectively modulating host physiology through the gut microbiota. Additionally, the study revealed that fermentable fibers reduced food intake and limited weight gain during the first days of administration, further suggesting their role in influencing energy balance and behavior.

The effects of dietary fibers on food intake and weight gain observed in this study suggest that fermentable fibers like inulin, pectin, and guar gum play a significant role in reducing energy intake and limiting weight gain. These fibers likely induce satiety through their fermentation and subsequent production of SCFAs, which have been shown to enhance satiety signaling and reduce food intake (Bastings et al., 2023; Chambers et al., 2014). In contrast, cellulose and resistant starch had little effect on food intake or weight gain, reinforcing the importance of fiber fermentability as a key functional property in regulating energy balance.

The changes in gut microbiota composition provide further insight into the mechanisms driving these effects. The reduced alpha diversity in the fermentable fiber groups, despite lower overall richness, is likely a reflection of selective growth of beneficial taxa such as *Bacteroidetes* and SCFA-producing bacteria. These microbial shifts, as revealed by beta diversity and LEfSe analysis, support the idea that fermentable fibers such as inulin and pectin, act as modulators of intestinal microbial ecology, creating specialized microbial communities with enhanced metabolic functionality. This profile is consistent with the effects of known prebiotics and aligns with previous studies demonstrating similar impacts from galacto-oligosaccharides and fucoidan in alcohol-exposed rodents (Xue et al., 2021; Yang et al., 2019).

Together, these findings reinforce the notion that dietary fibers, particularly those that are fermentable, have a profound impact on both host metabolism and gut microbiota composition, contributing to the observed changes in food intake, weight gain, and microbial profiles. These effects are of particular interest for the development of functional food strategies aimed at modulating the gut-organ axis.

The effects of dietary fibers on voluntary alcohol consumption observed in this study support the hypothesis that fermentable fibers play a pivotal role in alcohol consumption-related symptoms. Several studies have explored the relationship between dietary fibers and various aspects of alcohol use disorder, demonstrating the beneficial effects of dietary fibers in reducing alcohol-related anxiety, restoring sociability, and increasing BDNF (Amadieu et al., 2021, 2022). In addition, Canesso et al. (2014) showed that a high-fiber diet could mitigate alcohol-induced liver injury by preserving gut barrier integrity and preventing dysbiosis. In our study, we have shown for the first time that dietary fibers significantly reduce alcohol consumption across all ethanol concentrations, with guar gum having the most pronounced effect, followed by pectin and inulin. This suggests a potential application of fermentable fibers as nutritional modulators capable of attenuating alcohol-seeking behavior.

The observed increase in *Bacteroidia* and *Muribaculaceae* in the inulin and pectin groups is in line with previous studies reporting that fermentable fibers selectively promote beneficial bacterial taxa (Cronin et al., 2021; Deehan et al., 2024).These microbial shifts may contribute to the reduction in alcohol intake observed in our study by enhancing microbial communities involved in host–microbe interactions relevant to behavior. Previous work has demonstrated that the administration of specific soluble fibers induces antidepressant- and anxiolytic-like effects in rodents, along with a reduction in stress-induced corticosterone levels—factors that may contribute to the reduced alcohol intake observed in our study (Burokas et al., 2017). Similarly, Shen et al. (2025) reported that functional biscuits enriched with medicinal foods, many of them rich in dietary fiber and bioactive compounds, modified gut mucosal microbiota and improved brain and antioxidant functions in mice.

Beyond these microbial shifts, previous studies have suggested that microbial metabolites such as SCFAs may also play a role in gut–brain communication. For example, SCFAs can influence satiety hormones such as GLP-1, with downstream effects on reward-related pathways and alcohol-seeking behavior (Psichas et al., 2015). In addition, other studies have linked microbiota changes driven by fermentable fibers to improvements in gut barrier integrity and reductions in inflammation and oxidative stress (Leclercq et al., 2017). These processes are highly relevant to the pathophysiology of alcohol use disorder and may provide a mechanistic framework for interpreting our results.

Interestingly, the cellulose and resistant starch groups did not show the same reductions in alcohol consumption as the fermentable fiber groups. In particular, resistant starch resulted in alcohol consumption levels comparable to the control group, even at higher ethanol concentrations (15 % and 20 % EtOH). This suggests that less fermentable fibers, like resistant starch, may not exert the same modulatory effect on alcohol intake. This highlights the functional distinction between fermentable and non-fermentable fibers and underscores the relevance of fermentability as a determinant of health-promoting effects. Notably, the microbiota in the resistant starch group showed a shift toward the Firmicutes phylum, which has been associated with increased alcohol consumption in previous studies (Koutromanos et al., 2024; Reyes et al., 2020). Moreover, resistant starch has also been linked to behaviors that may promote alcohol consumption, such as heightened anxiety and increased locomotor activity (Lyte et al., 2016).

Notably, the changes in gut microbiota induced by dietary fibers were maintained even after alcohol exposure. Both alpha and beta diversity analyses showed that while alcohol consumption generally leads to dysbiosis (Leclercq et al., 2014), the fiber-supplemented groups retained distinct microbial profiles, with a stable abundance of beneficial bacteria. This microbial resilience suggests that fermentable fibers may confer protective stability to the intestinal ecosystem, buffering against alcohol-induced disruptions. These findings align with previous studies that have shown the protective role of dietary fibers in mitigating the negative impacts of alcohol on gut health. Specifically, Amadieu et al. (2021) showed that inulin supplementation improves gut microbiota composition in alcohol use disorder patients.

Nevertheless, some limitations of this study should be acknowledged. First, the use of a rodent model restricts the direct application of the findings to humans, as the effects of dietary fibers on alcohol consumption and gut microbiota composition may differ between species. Additionally, the relatively short duration of fiber supplementation and alcohol exposure may not fully reflect the long-term impacts of these interventions. While our analysis focused on major shifts in gut microbiota, more detailed mechanistic studies—such as targeted metabolomics and investigations into neural pathways—are necessary to further elucidate the complex interactions between dietary fibers, gut microbiota, and alcohol-related behaviors.

Furthermore, future research should explore how fermentation-derived metabolites interact with neuroendocrine and immune signaling to influence behavior, and assess the technological feasibility, safety, and bioavailability of incorporating these fibers into functional food formulations aimed at at-risk populations.

Altogether, the present findings reinforce the potential of fermentable dietary fibers as bioactive food components with preventive and modulatory roles in alcohol-related health disturbances.

5. Conclusions

In conclusion, our study demonstrates that dietary fiber intake, particularly fermentable fibers such as inulin, pectin, and guar gum, significantly influences gut microbiota composition and reduces voluntary alcohol consumption in Wistar rats. These fibers were associated with increased abundance of SCFA-producing bacteria and a reduction in Proteobacteria levels, changes that persisted even after alcohol exposure. In contrast, low or negligible fermentable fibers, such as cellulose and resistant starch, did not modify alcohol intake or microbial diversity. These findings highlight the functional importance of fiber fermentability and its capacity to modulate microbiota-mediated host physiology.

Our results support the incorporation of fermentable fibers into dietary strategies aimed at preventing or mitigating alcohol-related health effects through the modulation of the gut-organ axis. Future studies should explore whether similar effects occur in human populations and assess the underlying molecular pathways linking fiber-induced microbiota changes to alcohol consumption patterns. In addition, evaluating the bioavailability, safety, and regulatory implications of functional fiber applications will be critical steps toward potential translation into functional food products.

CRediT authorship contribution statement

Javier Calleja-Conde: Visualization, Methodology, Investigation, Formal analysis. **Kora-Mareen Bühler:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition. **Víctor Echeverry-Alzate:** Writing – review & editing, Methodology, Investigation. **Carlo Bressa:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Lucía Segovia-Rodríguez:** Investigation. **Jose A. Morales-García:** Writing – review & editing, Investigation. **Damián Córdoba-Díaz:** Writing – review & editing, Methodology. **Manuel Córdoba-Díaz:** Writing – review & editing, Methodology. **Carlos Torrado-Salmerón:** Writing – review & editing, Methodology. **Elena Giné:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Jose Antonio López-Moreno:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization.

Ethics statement

Ethical approval for the research procedures in this study was granted by UCM University Research Ethics Committee, Reference number PROEX262/19.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGTP in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2025.107079.

Data availability

Data will be made available on request.

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