










Influence of symbiotic administration on gut microbiota: an exploratory study at different levels and intensity of spinal cord injury

Elisa García-Vences^{1,2†} , Estefania De la Cruz Castillo^{1†}, Roxana Rodriguez-Barrera¹ , Adrian Flores Romero¹, Teresa Ponce-López¹ , David R. Mayen-Quinto², Hector Faustino Noyola-Villalobos² , Ivan Ignacio Mejía², Marco Antonio Vargas-Hernández² , Exsal Manuel Albores-Méndez², Andrea P. Ibarra-García¹ , Antonio Ibarra^{1,2*} 

¹Centro de Investigación en Ciencias de la Salud (CICSA), FCS, Universidad Anáhuac México Campus Norte, Huixquilucan, Mex. 52786, México

²Escuela Militar de Graduados de Sanidad, Centro de Investigación y Desarrollo del Ejército y Fuerza Aérea Mexicanos, Universidad del Ejército y Fuerza Aérea, México City 11200, México

[†]These authors shared the first authorship and contributed equally to this work.

***Correspondence:** Antonio Ibarra, Centro de Investigación en Ciencias de la Salud (CICSA), FCS, Universidad Anáhuac México Campus Norte, Av. Universidad Anáhuac No. 46, Col. Lomas Anáhuac, Huixquilucan, Mex. 52786, México. jose.ibarra@anahuac.mx

Academic Editor: Rodrigo Pacheco, Universidad San Sebastián, Chile

Received: August 11, 2025 **Accepted:** April 24, 2026 **Published:** June 25, 2026

Cite this article: García-Vences E, De la Cruz Castillo E, Rodriguez-Barrera R, Flores Romero A, Ponce-López T, Mayen-Quinto DR, et al. Influence of symbiotic administration on gut microbiota: an exploratory study at different levels and intensity of spinal cord injury. *Explor Neuroprot Ther.* 2026;6:1004160. <https://doi.org/10.37349/ent.2026.1004160>

Abstract

Aim: Spinal cord injury (SCI) disrupts autonomic regulation of the gastrointestinal tract, resulting in altered motility, microbial dysbiosis, and decreased production of short-chain fatty acids such as butyrate, a metabolite with neuroprotective properties. This study aimed to evaluate the effects of SCI level and severity on gut microbiota composition and butyrate concentration, and to determine the impact of symbiotic supplementation with *Enterococcus faecium* and agave inulin on microbial and metabolic recovery.

Methods: Twenty female Sprague Dawley rats were randomized into five groups: Sham, thoracic five moderate (T5M), thoracic five severe (T5S), thoracic nine moderate (T9M), and thoracic nine severe (T9S). Fecal samples were collected four weeks post-injury and again after four weeks of daily symbiotic administration. Butyrate levels were quantified by gas chromatography, and microbial composition was analyzed by 16S ribosomal RNA (16S rRNA) sequencing (V3–V4 region). Locomotor recovery was assessed weekly using the Basso, Beattie, and Bresnahan (BBB) scale.

Results: All SCI groups displayed significant dysbiosis compared with Sham, characterized by decreased relative abundance of butyrate-producing genera such as *Clostridium* at week four, as confirmed in the main dataset. Following symbiotic supplementation, *Bifidobacterium*, *Lactobacillus*, and *Clostridium* levels showed partial restoration, although responses varied by injury site and severity. The T9M group demonstrated the most consistent recovery of butyrate-producing taxa; however, butyrate concentration



decreased after treatment, while T9S showed a significant increase in butyrate concentration, and T5S exhibited minimal change. Motor recovery was greatest in T9M, followed by T9S and T5M, with no significant improvement in T5S.

Conclusions: SCI level and severity strongly influenced microbial dysbiosis, butyrate metabolism, and motor recovery. Symbiotic supplementation partially restored butyrate-producing bacterial taxa, with the most pronounced metabolic and functional benefits observed in the T9M group. These findings support a level-dependent role of gut-brain axis modulation as a potential therapeutic strategy after SCI.

Keywords

spinal cord injury, gut microbiota, symbiotic administration

Introduction

Gut microbiota, particularly that located in the cecum and colon, express carbohydrate-activated enzymes, allowing them to produce complex carbohydrates through anaerobic fermentation, generating metabolites such as short-chain fatty acids (SCFAs) [1] which work as pleiotropic signaling compounds [2] in the microbiota-gut-brain/spinal cord axis. Briefly, in this axis, signals from the brain influence motor, sensory, and secretory function of the gut, while visceral messages from the gut impact brain function [3], constituting a continuous feedback loop.

The gastrointestinal tract (GIT) serves as the main interface for interaction and nutritional exchange between the animal and the external environment [4]. Bacterial composition can be influenced by several factors, including immune mechanisms, diet, and gut motility, and stressors such as sepsis, burns, trauma, and infection [5, 6].

Spinal cord injury (SCI) affects sensory, motor, and autonomic functions, exerting significant impacts on the physical and psychosocial well-being of patients [7, 8]. Complications following SCI depend directly on the level, the intensity, and the type of injury. Symptoms can be classified by severity based on the neurological level: cervical, thoracic, lumbar, or sacral. For instance, patients with damage above the T5 level require additional care to prevent comorbidities related to bowel dysfunction (BD) or neurogenic bladder. At this level, supraspinal sympathetic pathways to the splanchnic and pelvic nerves are disrupted, impairing autonomic control of intestinal motility and urinary sphincter tone. This autonomic imbalance contributes to chronic constipation, fecal impaction, and urinary retention, which are common and clinically significant complications in high-thoracic injuries [7, 9].

BD is one of the most common complications after traumatic SCI, affecting 25–41% of all SCI cases [10–12]. BD involves changes in the extrinsic autonomic innervation to the bowel, with clinical repercussions in motility (i.e., constipation in 46%, fecal incontinence in 41%), sphincter control (31%), and abdominal cramping (18%) [12, 13]. While intrinsic enteric innervation remains intact, it may lose its integrity over time due to alterations in the extrinsic system [14].

This series of events leads to gut dysbiosis, which has been documented by various authors in both patients and animals [15–17]. Some studies have explored therapeutics aimed at re-establishing these alterations, such as nutraceuticals or sodium butyrate administration [16, 18]. The role of *Enterococcus faecium* as a nutraceutical has been investigated, demonstrating immunomodulatory effects in various pathologies [19]. It has been shown to increase transforming growth factor-beta (TGF- β) expression in the GIT during infectious disorders [20].

The *E. faecium* + agave inulin combination was selected due to its complementary mechanisms targeting both microbial restoration and host immune modulation. *E. faecium* exhibits strong mucosal adhesion, acid and bile tolerance, and proven efficacy in restoring gut eubiosis after inflammatory insults. It enhances intestinal TGF- β expression, reduces IL-6 and TNF- α release, and modulates toll-like receptor signaling, collectively supporting anti-inflammatory and barrier-protective effects [19, 20]. Agave inulin, a highly fermentable fructan, selectively stimulates the growth of beneficial genera such as *Bifidobacterium*

and *Lactobacillus*, promoting the synthesis of SCFAs including acetate and butyrate [21, 22]. The synergistic action of this symbiotic pair has been reported to improve gut permeability, immune tolerance, and microbial diversity in models of gastrointestinal inflammation [23, 24]. Therefore, this formulation was considered mechanistically suitable to counteract SCI-induced dysbiosis and to enhance butyrate recovery through both microbial and host-mediated pathways.

One reported combination therapy involved agave inulin and *E. faecium*; both in vitro and clinical studies have indicated that agave inulin promotes the growth of *Bifidobacterium*, enhancing lactic and acetic acid production while reducing pathogenic bacterial strains [23, 24]. On the other hand, *E. faecium* has effects on both innate and adaptive responses of the immune system and can modulate pro-inflammatory cytokines, evidenced by its ability to reduce the expression of IL-8 and IL-10 while increasing TGF- β expression in the GIT mucosa during infectious processes [23, 24].

The main goal was to characterize gut microbiota and fecal butyrate concentrations in rats with different intensities and levels of SCI before and after a symbiotic administration using *E. faecium* and agave inulin.

In addition, identifying correlations between changes in gut microbiota composition, fecal butyrate levels, and motor recovery may help to elucidate the functional interplay between microbial metabolism and neurophysiological outcomes following SCI, providing a foundation for future mechanistic studies.

Materials and methods

Animals and experimental design

SCI and all experimental procedures were conducted in accordance with protocols approved by the National Institutes of Health (US) guidelines and the Guide for the Care and Use of Animals. The Animal Bioethics and Welfare Committee of the Universidad Anáhuac México approved all animal procedures (Registry number: 201702-CI) in accordance with the National Institutes of Health Guide for the care and use of laboratory animals, and the Mexican Official Norm on Principles of Laboratory Animal Care (NOM 062-ZOO-1999).

Twenty female Sprague-Dawley rats, aged eight to eleven weeks old, weighing between 200 g and 300 g, were housed into five groups of four rats each ($n = 4/\text{group}$) under a 12-hour light/dark cycle, in thermally controlled rooms. They were fed standard pellets and given water ad libitum. To minimize stress, all animals were handled at least once daily for 30 days prior to surgery, and environmental enrichment was provided. Sterile bedding and filtered water were replaced daily. Females were selected to minimize variability in body weight, stress-related autonomic responses, and post-injury urinary complications, which are more frequent in males and could confound gastrointestinal outcomes and fecal sampling. This approach also ensured consistency with prior SCI studies from our group that employed the same sex and strain, allowing for valid comparisons across experiments. The symbiont was administered daily by orogastric tube for four consecutive weeks (weeks 4–8 post-injury). To minimize potential stress or motility alterations due to the orogastric procedure, animals were gently handled and acclimated for three days before administration, and no signs of distress or reduced food intake were observed.

The rats were allocated into five groups using the STATS TM v.2.0 software: 1) Sham ($n = 4$), 2) severe injury at T5 level (thoracic five severe, T5S) ($n = 4$), 3) moderate injury at T5 level (thoracic five moderate, T5M) ($n = 4$), 4) severe injury at T9 level (thoracic nine severe, T9S) ($n = 4$), and 5) moderate injury at T9 level (thoracic nine moderate, T9M) ($n = 4$). The five groups were studied over a period of eight weeks. Four weeks post-injury (wpi), all groups received a symbiotic consisting of *E. faecium* and inulin. Stool samples were collected at 4 wpi (prior to symbiotic administration) and at 8 wpi (after four weeks of symbiotic treatment). Gut microbiota composition and butyrate concentrations were analyzed from fecal samples at both time points. Motor recovery was assessed weekly in all groups until the end of the experiment (8 wpi). The sample size calculation for studies evaluating the taxonomic composition of the gut microbiota was performed using the formula proposed by Scheff et al. [25], 2003 which estimates power based on pairwise distance matrix comparisons between groups. This calculation indicated that four rats

per group would provide 90% power to detect significant differences in microbial composition between pre- and post-treatment conditions, taking into account both injury level and severity.

The study followed a pre- and post-test longitudinal design in which each rat served as its own control. Fecal samples collected at 4 wpi (pre-treatment) and 8 wpi (post-treatment) were compared within each group to assess the effect of the symbiotic. This approach minimized inter-animal variability and reduced total animal use in accordance with ethical principles.

Traumatic SCI

Rats were weighed and anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. After fifteen minutes, a laminectomy was performed at the thoracic vertebra to expose the dura mater. Stabilization clamps were used to access the T5 or T9 segment of the spinal cord. Injuries were inflicted using the Infinite Horizons SCI device 5.0, programmed for moderate injury at 200 kilovolts and severe at 300 kilovolts.

Post-surgery, all rats received 10 mg/kg of paracetamol orally twice a day and 5 mg/kg of ketoprofen, once a day via subcutaneous injection for one week, along with fluid replenishment with saline solution. Manual emptying of the bladder and rectum was performed three times daily until sphincter control was recovered. Sham-operated animals underwent all surgical procedures to expose the spinal cord but were not injured and served as controls. Animals were carefully monitored for signs of infection, dehydration, or self-mutilation, with appropriate veterinary care provided as needed. All experiments were designed and reported in accordance with established guidelines. At the end of the experiment (8 wpi), for euthanasia, the animals were first anesthetized intraperitoneally, with an injection of a ketamine (90 mg/kg) and xylazine (10 mg/kg) mixture. Once the animals no longer exhibited reflexes, an intraperitoneal injection of sodium pentobarbital (200 mg/kg, lethal dose) was administered.

Symbiotic administration

Treatment began four wpi, rats were weighed every third day, and a homogeneous mixture of *E. faecium* (0.8 g equivalent to 1.6×10^{10} CFU/kg body weight) plus agave inulin (0.86 g/kg body weight) was administered via an orogastric tube, diluted in drinking water until the end of the experiment (8 wpi). The symbiotic consisted of *E. faecium* [1×10^7 CFU; Alimentos Esenciales para la Humanidad (AEH) S.A. de C.V., Ciudad de México, México] and agave inulin (8 g; AEH S.A. de C.V., Ciudad de México, México).

Characterization of the gut microbiota

Fecal samples were collected individually from each rat at 4 and 8 wpi using sterile forceps in a laminar flow hood. Each sample was stored at -80°C until processing. For microbial characterization, genomic DNA was first extracted individually from 0.25 g of feces using the QIAamp PowerFecal DNA Kit (QIAGEN, 11993). To obtain representative community profiles for each experimental group and time point, equal amounts of purified DNA from each individual within the same group were pooled prior to 16S ribosomal RNA (16S rRNA) gene amplification and sequencing on the Illumina MiSeq platform. This approach was chosen to ensure sufficient DNA yield and sequencing depth while reducing technical variability between samples. Consequently, microbial composition data reflect group-averaged taxonomic profiles, whereas metabolic (butyrate) and behavioral analyses were performed on individual samples. For sequencing, equal volumes of DNA extracted from each animal of the same experimental group and time point were pooled to obtain one composite sample per group. The pooled DNA was subsequently divided into four technical aliquots, each processed independently through the 16S V3–V4 library preparation (Nextera XT kit, Illumina) and sequenced on the MiSeq platform.

Stool samples were collected at 4 wpi (before symbiotic administration) and at 8 wpi (after four weeks of symbiotic administration) through manual extraction in a disinfected laminar flow hood. After handwashing and donning gloves, gentle pressure was applied to the rectal ampulla, and samples were collected with sterile Adson tissue forceps, previously sterilized with 12% Krit germicidal solution. Samples were stored in 2 mL centrifuge tubes at -80°C for further processing.

To isolate and purify bacterial DNA, 0.25 g of feces was processed using the QIAamp PowerFecal DNA Kit (QIAGEN Model: 11993), following the manufacturer's protocol. Sample quality was assessed using the Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer, measuring at a wavelength range 260–320 nm to obtain quantity (ng/mL) and assess purity using the ratios 260/280 nm and 260/320 nm (values above 1.8 were included). Electrophoresis (1% agarose gel) was performed to evaluate DNA integrity.

Sequencing

Sequencing was performed on MiSeq following the workflow for preparing 16S rRNA Gene Amplicons for the Illumina MiSeq System (Part # 15044223 Rev. B): amplicon primers, library preparation, and sequencing on MiSeq.

For the 16S library preparation, modifications were made. The conditions for the first stage PCR were 98°C for 30 seconds, followed by 5 cycles of 98°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, after which a final elongation step at 72°C for 5 minutes, and then held at 4°C. The second stage PCR involved 98°C for 30 seconds, followed by 8 cycles of 98°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, concluding with a final elongation step at 72°C for 5 minutes, and then held at 4°C.

Metagenomic analysis

Once genetic material was obtained, PCR was performed, and sequences were purified. Operational Taxonomic Units (OTUs) were identified. For classification analysis, we used the 16S Metagenomics software, available on the BaseSpace platform (Illumina, Inc.), utilizing a modified version of the Greengenes taxonomic database. The following metrics were reported for each sample: 1) alpha diversity: using the Shannon index and the number of identified species (R), and 2) beta diversity.

Chimeric sequences were discarded, and all non-chimeric input sequences were required to match at least one OTU with over 97% identity. Dissimilarity scores were calculated using the Bray-Curtis distance metric, considering relative abundance at the genus level for each rat and lesion group. Hierarchical clustering and principal coordinate analysis (PCoA) were employed to graphically summarize inter-sample relationships, with a heat map representing the relative abundance of the eight principal genera before and after symbiotic administration.

Butyrate determination

Fecal samples were collected by groups 4 wpi and 8 wpi. Prior to collection, sterile bedding was changed, and samples were harvested after an 8-hour waiting period using sterile tissue forceps. Pooled samples were stored in 50 mL centrifuge tubes at –80°C until processing.

Stool was lyophilized at –65°C under 1.33×10^{-6} torrs. One gram was weighed into a 50 mL centrifuge tube and 5 mL of distilled, deionized water was added. The mixture was homogenized for 10 minutes at maximum speed in a vortex mixer. Subsequently, the pH was evaluated with a pH meter, and it was acidified with 5 M HCl to the range of 2 to 3. The sample was centrifuged at 4,000 rpm for 25 minutes at 16°C, and 2 mL of chloroform was added. After a second centrifugation at 4,000 rpm for 2 minutes at 16°C, the aqueous phase was transferred to sterile 50 mL centrifuge tubes.

Butyric acid was determined by gas chromatography (GC) with a Shimadzu GC2010 (Japan), equipped with a silica capillary column for free fatty acids (model DB-1701, 30 m length, 0.25 mm internal diameter and 0.25 μ m thickness, Agilent, USA). GC conditions included helium as carrier gas with a flow of 4 mL/min. The oven temperature was set to 250°C, with the column maintained at 95°C. The flow rates for hydrogen and air were set at 40 and 400 mL/min, respectively. The flame ionization detector (FID) was operated at 300°C. The retention time for butyric acid was between 3.5 and 3.6 minutes, and the injected sample volume was 2 μ L, with butyric acid concentration at 0.1% w/v and the molecular weight of 88.11 g/mol. Each analysis lasted 20 minutes, with butyrate levels determined using Sigma-Aldrich standard 107-92-6. For each pooled sample, butyrate concentration was reported in μ g butyric acid/2 μ L. All the pools were processed in triplicate.

Clinical assessment

To evaluate motor recovery, animals were examined weekly by blinded raters using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale [25, 26] from the time of surgery until the end of the study.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism v9.0 and PAST v.4.11. Normality was evaluated using the Shapiro-Wilk test. Since most data (e.g., BBB scores, microbial diversity indices) did not follow a normal distribution, non-parametric analyses were performed. For alpha-diversity indices [Richness (R) and Shannon; Figure 1], comparisons between groups were analyzed by the Kruskal-Wallis test, and pre- vs. post-treatment comparisons within each group by the Friedman test. Beta-diversity analysis (Figure 2) was performed using Bray-Curtis dissimilarity matrices and analyzed with PERMANOVA (Adonis) to estimate the explained variation (EV, EV%) between groups. Butyrate concentrations were compared between groups using the Kruskal-Wallis test, and pre- vs. post-treatment differences were evaluated with the Wilcoxon signed-rank test. Locomotor performance (BBB scores) was reported as median \pm interquartile range (IQR). Longitudinal analysis of BBB recovery was performed using the Friedman test, and between-group comparisons at final time points by the Kruskal-Wallis test. All analyses were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Characterization of the gut microbiota

A total of 40 samples were sequenced. The characterization of the microbiota was performed on individual samples from each lesion group, both before and after symbiotic administration (4 wpi and 8 wpi).

Alpha diversity

In Figure 1, we report the number of identified species (R) and the Shannon index. Notably, after the administration of symbiotics, the variability of (R) decreased at 8 wpi across all experimental groups, except the T9M group, where it only decreased in some cases. In contrast, the Shannon index showed greater dispersion following administration; however, no statistical differences were observed between the measurements taken before (4 wpi) and after (8 wpi) the administration of symbiotic.

Quantitatively, comparisons between 4 wpi (before symbiotic administration) and 8 wpi (after symbiotic administration) showed that the R index decreased by approximately 10–15% in T5S and T9S groups, remained stable in T5M, and increased by about 8% in T9M. Similarly, the Shannon index showed a slight increase in the T9M group (0.21 ± 0.04 units), suggesting a mild improvement in microbial evenness following symbiotic supplementation.

Thus, alpha-diversity analysis confirmed similar overall R between groups, with only mild trends toward increased evenness in the moderate injury models.

Although these results were not statistically significant, moderate injuries (particularly T9M) tended to show slightly higher R and Shannon values following symbiotic supplementation, suggesting a mild increase in evenness and species distribution compared with severe injuries.

Beta diversity

Relative abundance was calculated, and the most abundant genera per sample are presented below in Figure 2. The most prevalent genera were *Bifidobacterium*, *Lactobacillus*, *Clostridium*, and *Alkaliphilus*. After treatment (8 wpi), the microbiome composition across groups became more homogeneous. The predominant genera—*Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Alkaliphilus*, *Allobaculum*, and *Sarcina*—together represented approximately 90% of the total abundance.

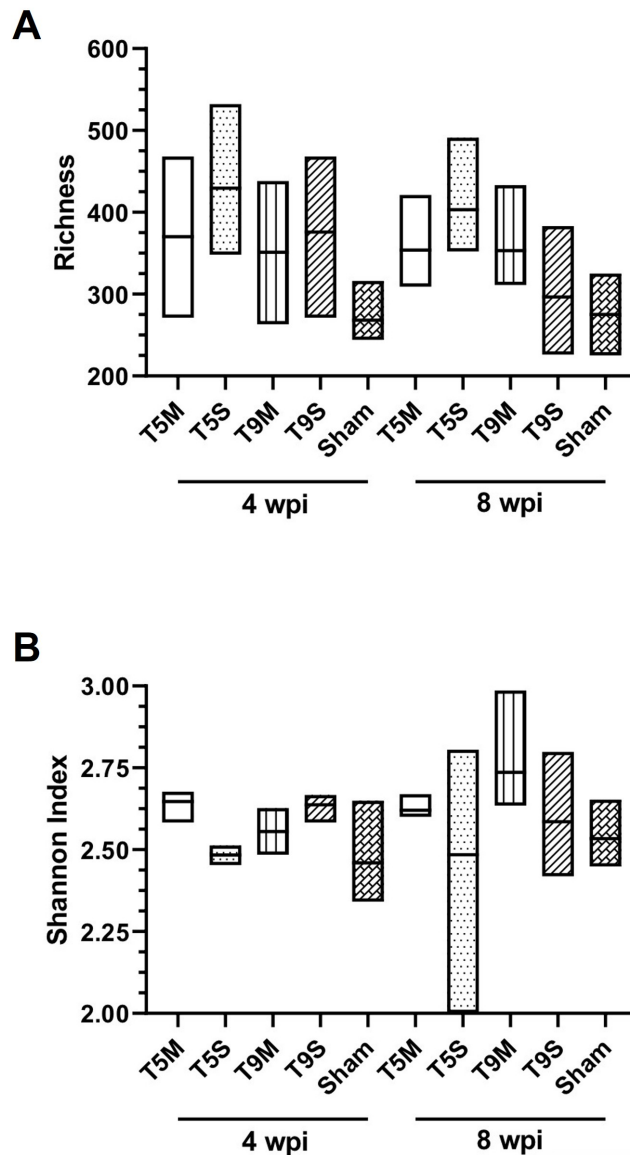


Figure 1. Comparison of means of Richness (R) and Shannon index before (4 wpi) and after (8 wpi) symbiotic administration. Box plots showing R (A) and Shannon index (B) across experimental groups (T5M, T5S, T9M, T9S, Sham) at 4 wpi (before symbiotic administration) and 8 wpi (after symbiotic administration). wpi: weeks post-injury; T5M: thoracic five moderate; T5S: thoracic five severe; T9M: thoracic nine moderate; T9S: thoracic nine severe.

Quantitative analysis of microbial composition revealed time-dependent changes following symbiotic administration. In the T9M group, *Clostridium* decreased from 18.6% at 4 wpi to 7.0% at 8 wpi, while *Bifidobacterium* increased from 32.1% to 34.9%. In parallel, *Lactobacillus* increased from 17.1% to 32.8%, indicating a shift toward beneficial taxa after treatment.

In the T5M group, *Bifidobacterium* increased from 30.8% at 4 wpi to 37.1% at 8 wpi, whereas *Lactobacillus* decreased from 28.7% to 18.5%. Changes in *Clostridium* were modest, increasing from 9.3% to 12.1%. At the family level, *Enterobacteriaceae* showed a reduction after symbiotic administration across groups; this taxon is represented accordingly in Figure 2 (see legend).

In severe models (T5S and T9S), compositional shifts were less pronounced, indicating a severity-dependent microbial response. All comparisons correspond to changes between before (4 wpi) and after (8 wpi) symbiotic administration, consistent with the graphical representation.

The Shannon diversity index mirrored these findings, indicating greater evenness in T9M and T5M after symbiotic supplementation.

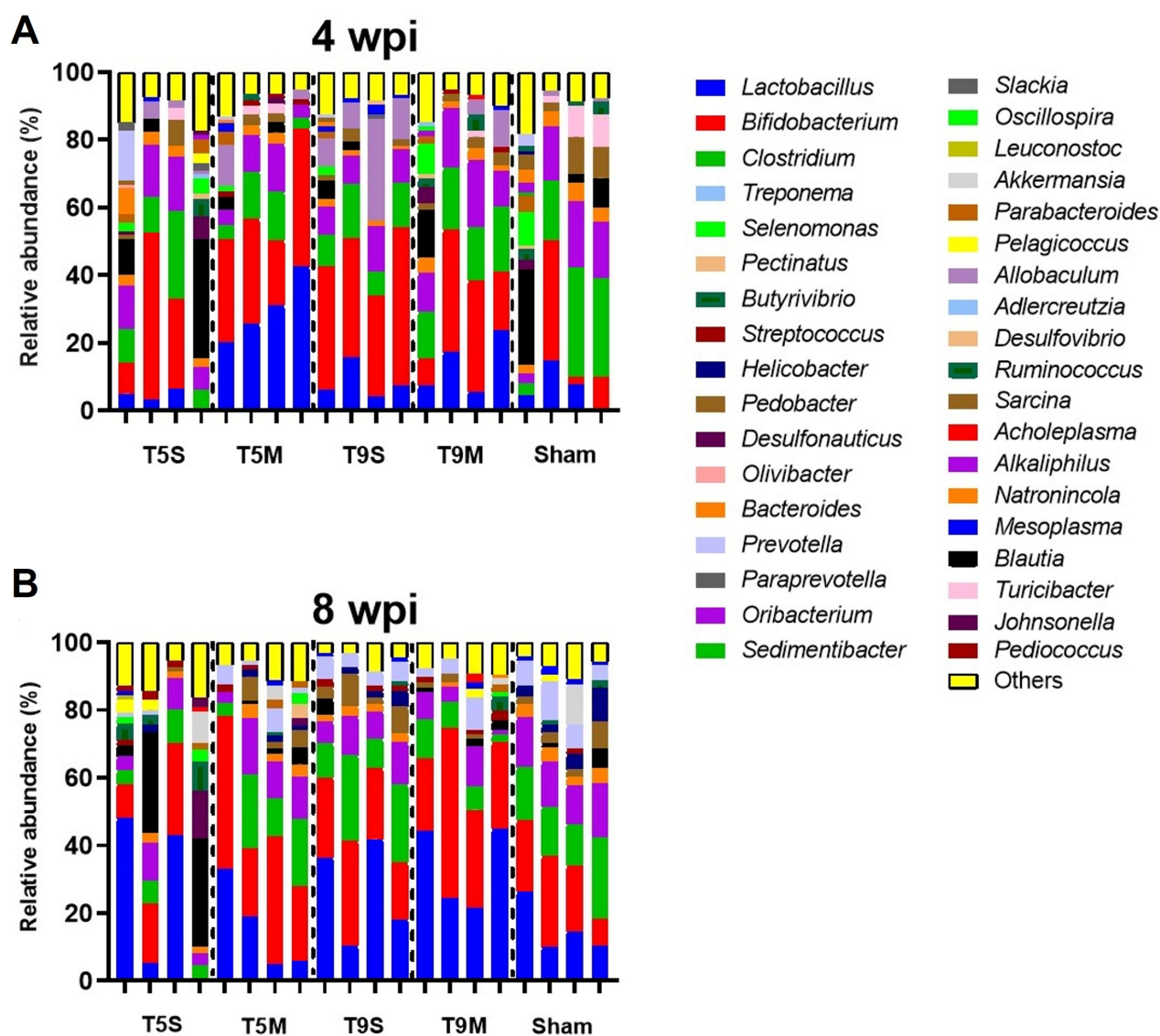


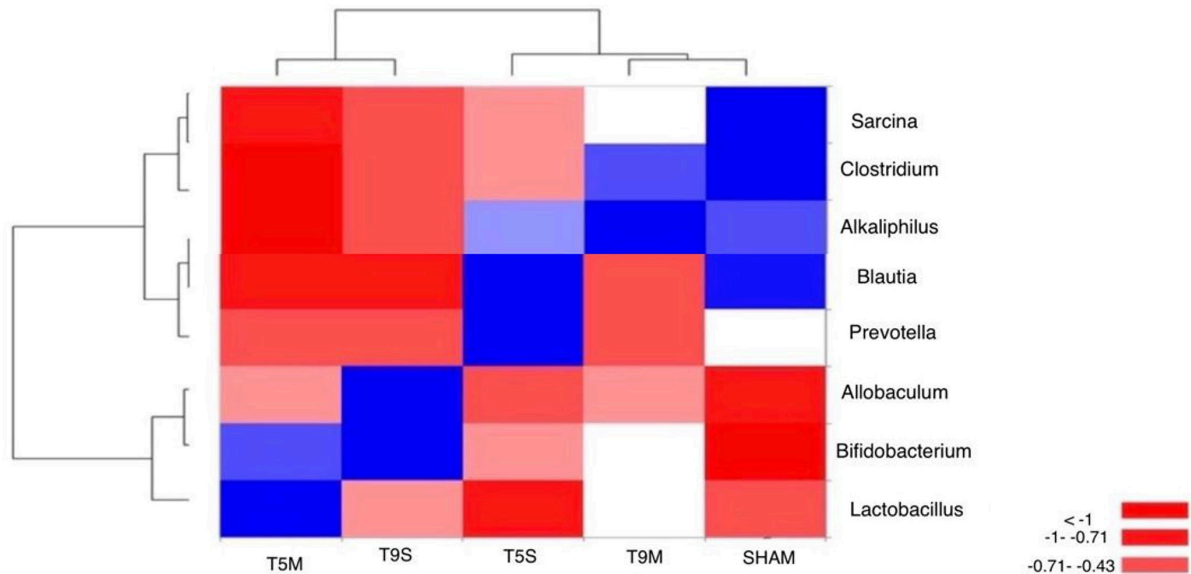
Figure 2. Relative abundance analysis of the gut microbiome (Beta diversity) before and after symbiotic administration. Agglomerative hierarchical clustering highlights the OTUs with the most significant differences in abundance between groups. Stacked bar plots showing the relative abundance of bacterial taxa across experimental groups (T5M, T5S, T9M, T9S, Sham) at 4 wpi (A—before symbiotic administration) and 8 wpi (B—after symbiotic administration). Each bar represents a technical replicate derived from pooled DNA samples of all animals within each group and time point. Colors indicate different bacterial taxa, and bar length corresponds to relative abundance. Following symbiotic supplementation, microbiome composition showed a trend toward homogenization across groups, with increased representation of beneficial genera such as *Bifidobacterium* and *Lactobacillus*. *Enterobacteriaceae* is presented as a family-level taxon (not genus-level) and is labeled accordingly in the figure. OTUs: Operational Taxonomic Units; wpi: weeks post-injury; T5M: thoracic five moderate; T5S: thoracic five severe; T9M: thoracic nine moderate; T9S: thoracic nine severe.

Before symbiotic administration at 4 wpi, the most abundant genera across groups were *Bifidobacterium*, *Lactobacillus*, *Clostridium*, and *Alkaliphilus*. After symbiotic administration at 8 wpi, all the groups exhibited a similar microbiome composition where *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Alkaliphilus*, *Allobaculum*, *Turicibacter*, *Natronincola*, *Sarcina*, *Akkermansia*, *Johnsonella*, and *Blautia* together constituted 89.96% of the relative abundance. The eight principal genera of each group were represented in a heat map, as seen in Figure 3.

The main genera before and after symbiotic administration were grouped by category, as represented in Table 1, arranged from highest to lowest abundance.

To determine significant dissimilarities in microbiome composition between the experimental groups, PERMANOVA analysis was performed to assess the effects of injury intensity and level. First, we evaluated dissimilarities between the groups before symbiotic administration, which resulted in an explained variation (EV) of 23.5% with a significant difference ($p = 0.004$). After symbiotic administration, the

A 4wpi



B 8wpi

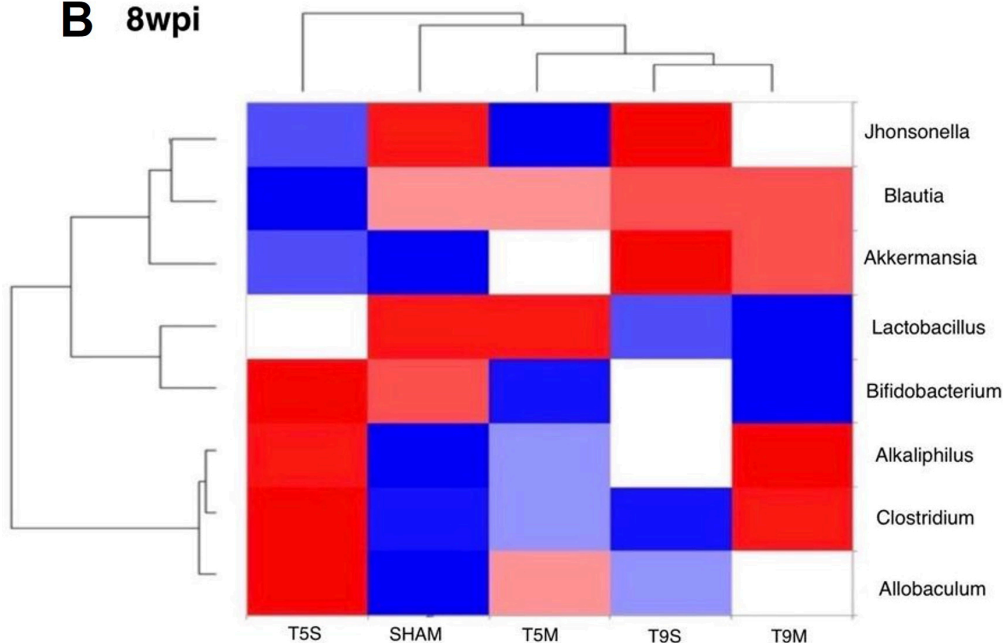


Figure 3. Analysis of species abundance clustering in the heat map. The longitudinal axis shows the group information, while the transverse axis represents the species annotation information. The clustering tree on the left of the figure displays species clustering, and the tree above illustrates the clustering among sample groups. The values in the heat map represent the z-scores obtained after standardization of the relative abundance of species in each row, reflecting gut microbial distribution at the genus level for each experimental group at 4 wpi and 8 wpi. The blue color indicates a higher percentage of relative abundance. wpi: weeks post-injury.

Table 1. Comparative relative abundance percentages of the gut microbiome before and after symbiotic administration.

Group % of the total relative abundance		
	Before symbiotic administration (4wpi)	After symbiotic administration (8 wpi)
Sham	<i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Lactobacillus</i> , <i>Sarcina</i> , <i>Turicibacter</i> , and <i>Natronincola</i> (78.46%).	<i>Lactobacillus</i> , <i>Blautia</i> , <i>Bifidobacterium</i> , <i>Alkaliphilus</i> , <i>Clostridium</i> , <i>Ruminococcus</i> , <i>Johnsonella</i> , <i>Akkermansia</i> , <i>Natronincola</i> , and <i>Oscillospira</i> (81.13%).
T5S	<i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Prevotella</i> , <i>Blautia</i> , <i>Lactobacillus</i> , <i>Sarcina</i> , and <i>Natronincola</i> (72.87%).	<i>Lactobacillus</i> , <i>Blautia</i> , <i>Bifidobacterium</i> , <i>Alkaliphilus</i> , <i>Clostridium</i> , <i>Ruminococcus</i> , <i>Johnsonella</i> , <i>Akkermansia</i> , <i>Natronincola</i> , and <i>Oscillospira</i> (81.13%).
T5M	<i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Allobaculum</i> , and <i>Natronincola</i> (83.22%).	<i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Allobaculum</i> , <i>Sarcina</i> , and <i>Natronincola</i> (82.18%).

Table 1. Comparative relative abundance percentages of the gut microbiome before and after symbiotic administration. (continued)

Group % of the total relative abundance		
	Before symbiotic administration (4wpi)	After symbiotic administration (8 wpi)
T9S	<i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Allobaculum</i> , <i>Lactobacillus</i> , and <i>Blautia</i> (83.56%).	<i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Allobaculum</i> , <i>Sarcina</i> , <i>Natronincola</i> , and <i>Turicibacter</i> (91.9%).
T9M	<i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , <i>Lactobacillus</i> , <i>Allobaculum</i> , <i>Blautia</i> , <i>Sarcina</i> , and <i>Natronincola</i> (84.94%).	<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Alkaliphilus</i> , and <i>Allobaculum</i> (82.99%).

wpi: weeks post-injury; T5M: thoracic five moderate; T5S: thoracic five severe; T9M: thoracic nine moderate; T9S: thoracic nine severe.

dissimilarities between the groups showed an EV of 12.7% with a significant difference ($p < 0.05$), suggesting that symbiotic administration promotes homogenization of microbiota genera irrespective of injury level or severity.

Next, we compared each group to itself before and after symbiotic administration using PERMANOVA, yielding the following results: T5S with an EV of 25.6% ($p = 0.2$); T5M with an EV of 12.3% ($p = 0.439$); T9S with an EV of 41.4% ($p = 0.058$); T9M with an EV of 83.3% ($p = 0.035$); and Sham with an EV of 45.4% ($p = 0.1$). The principal observation of EV was noted in the T9 level and Sham. See [Table 2](#).

Table 2. PERMANOVA analysis with the ten principal genera identifies the different groups.

Before symbiotic administration		After symbiotic administration		EV and PERMANOVA
Sham				
Genus	%	Genus	%	EV and PERMANOVA
<i>Clostridium</i>	27.3	<i>Bifidobacterium</i>	24.2	45.4% ($p = 0.1$)
<i>Alkaliphilus</i>	17.5	<i>Lactobacillus</i>	16.3	
<i>Bifidobacterium</i>	16.1	<i>Clostridium</i>	14.7	
<i>Sarcina</i>	7.9	<i>Alkaliphilus</i>	13.7	
<i>Turicibacter</i>	7.6	<i>Allobaculum</i>	9.4	
<i>Lactobacillus</i>	6.2	<i>Natronincola</i>	3.7	
<i>Blautia</i>	5.6	<i>Akkermansia</i>	3.5	
<i>Natronincola</i>	4.6	<i>Turicibacter</i>	3.4	
<i>Ruminococcus</i>	2.3	<i>Sarcina</i>	2.7	
<i>Allobaculum</i>	1.2	<i>Mesoplasma</i>	2.0	
T5S				
Genus	%	Genus	%	EV and PERMANOVA
<i>Bifidobacterium</i>	15.5	<i>Lactobacillus</i>	23.1	25.6% ($p = 0.2$)
<i>Clostridium</i>	6.8	<i>Blautia</i>	19.5	
<i>Alkaliphilus</i>	5.9	<i>Bifidobacterium</i>	15.1	
<i>Prevotella</i>	5.4	<i>Alkaliphilus</i>	7.9	
<i>Blautia</i>	3.8	<i>Clostridium</i>	6.9	
<i>Lactobacillus</i>	3.7	<i>Ruminococcus</i>	4.4	
<i>Sarcina</i>	3.5	<i>Johnsonella</i>	3.8	
<i>Natronincola</i>	2.1	<i>Akkermansia</i>	2.5	
<i>Bacteroides</i>	1.5	<i>Natronincola</i>	2.2	
<i>Allobaculum</i>	1.3	<i>Pelagicoccus</i>	1.9	
T5M				
Genus	%	Genus	%	EV and PERMANOVA
<i>Bifidobacterium</i>	30.8	<i>Bifidobacterium</i>	37.1	12.3% ($p = 0.439$)
<i>Lactobacillus</i>	28.7	<i>Lactobacillus</i>	18.5	

Table 2. PERMANOVA analysis with the ten principal genera identifies the different groups. (continued)

Before symbiotic administration		After symbiotic administration		EV and PERMANOVA
<i>Clostridium</i>	9.3	<i>Clostridium</i>	12.1	
<i>Alkaliphilus</i>	8.7	<i>Alkaliphilus</i>	9.7	
<i>Allobaculum</i>	5.1	<i>Allobaculum</i>	5.2	
<i>Blautia</i>	2.4	<i>Sarcina</i>	2.8	
<i>Natronincola</i>	2.1	<i>Natronincola</i>	2.4	
<i>Sarcina</i>	1.8	<i>Akkermansia</i>	1.7	
<i>Pediococcus</i>	1.8	<i>Pediococcus</i>	1.4	
<i>Parabacteroides</i>	1.6	<i>Turicibacter</i>	1.4	
T9S				
Genus	%	Genus	%	EV and PERMANOVA
<i>Bifidobacterium</i>	41.4	<i>Bifidobacterium</i>	26.5	41.4% ($p = 0.058$)
<i>Clostridium</i>	13.3	<i>Lactobacillus</i>	21.9	
<i>Lactobacillus</i>	10.2	<i>Clostridium</i>	19.7	
<i>Allobaculum</i>	9.9	<i>Alkaliphilus</i>	10.4	
<i>Alkaliphilus</i>	9.4	<i>Sarcina</i>	7.0	
<i>Blautia</i>	2.9	<i>Allobaculum</i>	4.7	
<i>Sarcina</i>	2.5	<i>Natronincola</i>	2.8	
<i>Natronincola</i>	1.8	<i>Turicibacter</i>	2.2	
<i>Mesoplasma</i>	1.3	<i>Pediococcus</i>	1.2	
<i>Oscillospira</i>	1.1	<i>Blautia</i>	0.9	
T9M				
Genus	%	Genus	%	EV and PERMANOVA
<i>Bifidobacterium</i>	32.1	<i>Bifidobacterium</i>	34.9	83.3% ($p = 0.035$)
<i>Clostridium</i>	18.6	<i>Lactobacillus</i>	32.8	
<i>Lactobacillus</i>	17.1	<i>Clostridium</i>	7.0	
<i>Alkaliphilus</i>	16.8	<i>Alkaliphilus</i>	6.7	
<i>Allobaculum</i>	3.9	<i>Allobaculum</i>	4.7	
<i>Sarcina</i>	2.9	<i>Pediococcus</i>	1.7	
<i>Natronincola</i>	2.3	<i>Blautia</i>	1.5	
<i>Ruminococcus</i>	1.1	<i>Sarcina</i>	1.5	
<i>Pediococcus</i>	1.0	<i>Ruminococcus</i>	1.3	
<i>Turicibacter</i>	0.7	<i>Mesoplasma</i>	1.0	

EV: explained variation; T5M: thoracic five moderate; T5S: thoracic five severe; T9M: thoracic nine moderate; T9S: thoracic nine severe.

Butyrate determination

Before symbiotic administration (4 wpi), butyrate levels varied across experimental groups and did not consistently decrease in all SCI conditions compared with the Sham group. While some injury groups showed lower concentrations, others (e.g., T5S) exhibited comparable or even higher baseline levels. In contrast, animals with moderate T9 injury showed relatively higher baseline concentrations overall (Figure 4).

After four weeks of symbiotic supplementation (8 wpi), butyrate concentrations showed a heterogeneous response across experimental groups. While some groups (e.g., T9S) exhibited an increase, others, such as T9M, showed a decrease. Changes in T5 groups were modest, whereas the Sham group remained relatively stable across both time points. A significant increase in butyrate concentration was observed in the T9S group ($p < 0.05$), whereas other groups showed no consistent significant changes.

Overall, these results suggest that symbiotic supplementation may enhance butyrate production, particularly in animals with moderate T9 injury.

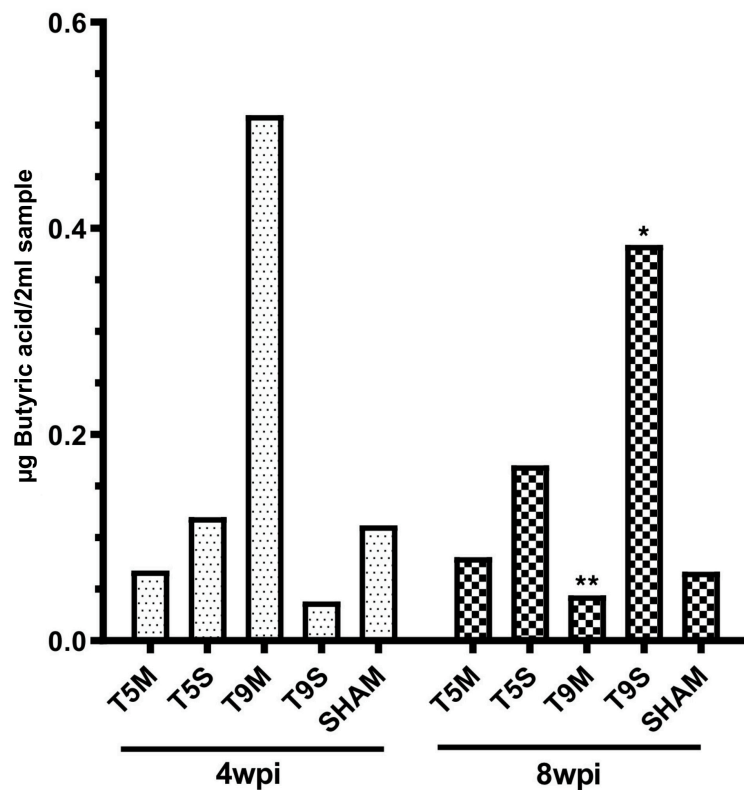


Figure 4. Butyric acid concentration before and after symbiotic administration. Bar graph showing butyric acid levels in pooled fecal samples from each experimental group at 4 wpi (before symbiotic administration) and 8 wpi (after symbiotic administration). Values are expressed as mean \pm SD of technical replicates. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$. Comparisons correspond to within-group differences between 4 wpi and 8 wpi. wpi: weeks post-injury; SD: standard deviation.

Clinical assessment: BBB

To verify the consistency of the contusion model, we evaluated motor recovery in all groups following injury. A mixed-effects model (REML) with Tukey's multiple comparisons test was used to assess motor recovery over time, comparing the period before (4 wpi) and after (8 wpi) symbiotic administration.

During the first four weeks, we observed the expected spontaneous recovery of locomotor function across all groups. However, following treatment, this improvement continued to progress, despite the lesion having reached its chronic phase.

The recovery was statistically significant in the T9M ($p < 0.0001$), T9S ($p = 0.0166$), and T5M ($p = 0.0013$) groups (Figure 5).

Overall, the symbiotic formulation with *E. faecium* and agave inulin promoted compositional and metabolic restoration of the gut microbiota primarily in moderate injuries. However, since no untreated SCI controls were maintained at 8 weeks, the observed effects should be interpreted cautiously as potential improvements rather than definitive treatment effects.

Discussion

Although the study of the microbiota-intestine-brain axis continues to shed light on the symbiotic dynamics that allow us to maintain homeostasis with our external environment, our current knowledge remains limited. This project aimed to identify how the level and intensity of SCI influence the composition of the intestinal microbiota and butyrate levels in feces. Injuries at the T5 level, although less commonly studied in SCI models, are known to cause more severe physiological consequences than injuries at T9. This study evaluated two scenarios: a) a T5 lesion, which interrupts extrinsic innervation of the small and large intestine, rectum, and spleen without affecting ganglia from other plexuses such as the cardiac plexus, and b) a T9 lesion, affecting only the extrinsic innervation to the colon and rectum.

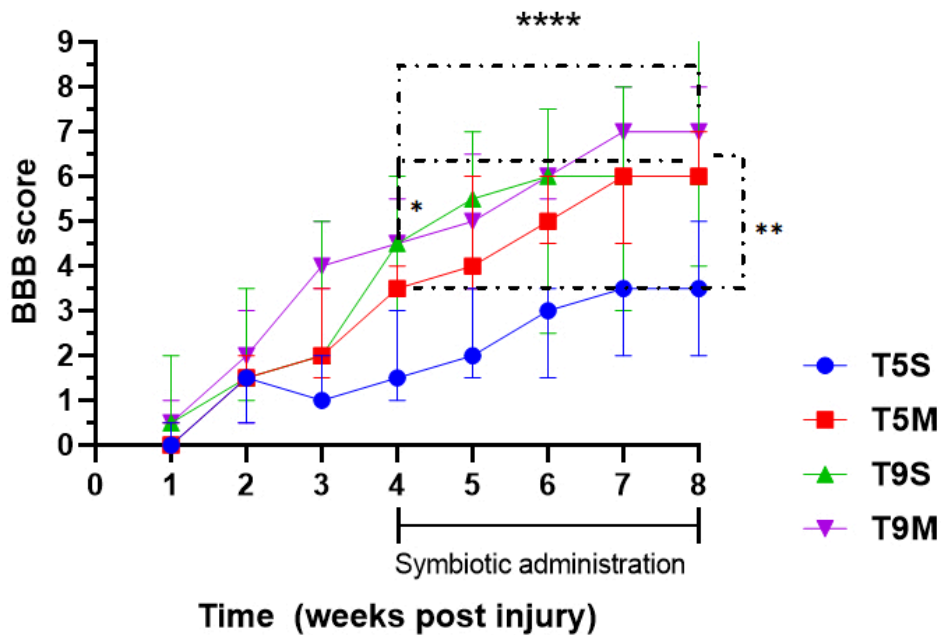


Figure 5. Motor recovery throughout the experiment. Statistical analysis was evaluated with REML with Tukey's multiple comparisons to evaluate all animals per group over time. Values are expressed as mean \pm SD. Significant improvements were observed during the symbiotic administration period, particularly in T9 groups. Asterisks indicate statistically significant differences as follows: * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Specifically, * indicates within-group differences during the early phase of treatment (around week 4–5), ** indicates significant differences between groups at week 8 (notably between T5S and treated groups), and **** indicates highly significant improvements across time points during the symbiotic administration period (4–8 wpi), particularly in T9M and T9S groups. wpi: weeks post-injury; REML: mixed-effects model; SD: standard deviation; T5S: thoracic five severe; T9M: thoracic nine moderate; T9S: thoracic nine severe.

SCI has been shown to negatively affect the gut microbiota, as described by other authors [17, 27]. In our study, microbial species diversity was most affected in the T5S group at four wpi. By eight weeks, intergroup variation decreased, while intragroup variation increased, although the total number of identified species and variability showed an overall decreasing trend.

Notably, a general increase in *Bifidobacterium* was observed across all injured groups four weeks post-SCI. In contrast, *Clostridium* accounted for 27.28% of the microbiota in the Sham group, but remained below 20% in all other experimental groups. The T5S group displayed the most pronounced alterations, with many genera appearing in less than 50% of the samples, indicating significant disruption in microbial stability. The impact of SCI level and severity on microbial recovery was further evidenced after symbiotic administration. Two main trends were observed. 1. In all groups except Sham, there was a homogenization in the relative abundance of bacterial species, leading to greater similarity between groups. 2. This effect was absent in the T5S group, which maintained a distinct profile. In most treated groups, *Bifidobacterium*, *Clostridium*, and *Lactobacillus* predominated, suggesting a partial return to eubiosis [15]. However, in T5S, the dominant genera were *Blautia* and *Lactobacillus*, indicating a persistent imbalance likely due to greater neural disruption. These findings align only partially with previous studies. For instance, Lin et al. [27] (2020) reported significant alterations in the fecal microbiota composition of patients with SCI, including changes in the relative abundance of several bacterial taxa associated with gut dysbiosis. It is important to note that interpretation of microbial changes at the genus level should be made with caution, as some genera, such as *Clostridium* and *Bifidobacterium*, contain both beneficial and pathogenic species. The increase in *Clostridium* observed after symbiotic treatment in our study likely reflects the enrichment of beneficial butyrate-producing species (e.g., *C. butyricum*), consistent with the concomitant rise in fecal butyrate levels. Similarly, the elevation in *Bifidobacterium* abundance may correspond to probiotic strains that contribute to carbohydrate fermentation and epithelial health. The differences with previous reports may relate to variations in injury level, severity, post-injury phase, or sequencing resolution. Our analysis was based on 16S rRNA V3–V4 region sequencing, which provides genus-level but not species-level identification; therefore, the results should be interpreted as indicative of functional recovery rather than exact taxonomic reconstitution.

Hierarchical clustering analyses supported some of these differences in microbial composition, although not all findings were statistically significant. It is worth noting that such predictive models rely on dissimilarity measures and do not identify individual taxa responsible for group differences [22, 23]. In clinical studies, dysbiosis has been reported to be more severe in upper motor neuron injuries. Predominant genera in lesion groups include *Blautia*, *Bifidobacterium*, *Faecalibacterium*, and *Ruminococcus*, while *Roseburia*, *Pseudobutyrvibrio*, *Dialister*, and *Megamonas* are often depleted [15, 27]. These patterns are consistent with findings in the T5-injured animals of our study.

Regarding butyrate levels, the data suggest that T5-level injuries may impair the assimilation of symbiotics, likely due to disruptions in gut innervation. Although fecal pH was not directly measured in this study, it is known that butyrate-producing bacteria contribute to the acidification of the colon through SCFA production. Based on this, one could hypothesize that animals in the Sham, T9S, and T9M groups might present a more acidic fecal environment, whereas those in the T5S and T5M groups might exhibit a more alkaline profile, consistent with a lower abundance of butyrogenic bacteria. These suppositions, however, remain speculative and highlight the need for future studies to include direct fecal pH measurements, which could serve as a functional readout of SCFA activity and microbiota composition in SCI models. Assessing pH alongside microbiota profiling and SCFA quantification (e.g., butyrate, acetate, propionate) would provide a more comprehensive understanding of gut environment alterations following SCI.

Notwithstanding, one limitation of our study was the pooling of fecal samples, which may have masked individual variability. Ideally, fecal microbiota analysis should be performed per subject to improve resolution and accuracy [28]. Additionally, the relatively small sample size ($n = 4$ per group) may limit the generalizability of the findings and reduce the ability to detect subtle inter-individual differences. Despite these constraints, the observed microbial and metabolic trends were consistent across experimental groups, supporting the robustness of our findings. Another limitation of this study is the absence of additional untreated SCI cohorts maintained until 8 wpi. However, the longitudinal pre- or post-test design allowed each animal to serve as its own control, reducing biological variability. The Sham group provided the physiological reference for baseline microbial and metabolic conditions. Moreover, the study design did not include SCI groups that remained untreated at eight wpi. As a result, it is difficult to fully distinguish the effects of the symbiotic treatment from the natural progression of recovery or time-dependent microbial fluctuations. This limitation affects the interpretation of metagenomic, butyrate, and locomotor outcomes, which should therefore be regarded as indicative of potential functional improvement rather than definitive proof of treatment efficacy. Future studies should incorporate untreated SCI controls at equivalent time points to better isolate the specific contribution of the symbiotic intervention.

Looking more closely at *Clostridium*, a well-established butyrate producer, its abundance was reduced in all experimental groups at four weeks. After symbiotic therapy, *Bifidobacterium*, *Lactobacillus*, and *Clostridium* populations increased, yet the T5S group again showed limited improvement, potentially due to greater impairment of the gut-brain axis [29–31].

To better understand the distinct roles of SCFAs, future studies should include quantification of other SCFAs, such as acetate and propionate, to differentiate their contributions to gut and systemic homeostasis. From a functional perspective, the motor recovery patterns observed via BBB scoring reflected what is well-established in SCI models. Rats with lower-level injuries (T9) exhibited better locomotor recovery than those with higher-level injuries (T5). Similarly, animals with moderate injuries recovered more than those with severe injuries, confirming the well-documented correlation between injury severity, lesion level, and functional outcome. These differences became especially clear by week 8, with the T9M group showing the greatest improvement, followed by T5M, T9S, and finally T5S. These findings are consistent with the natural progression of SCI and emphasize that lesion level and intensity are key determinants of motor function recovery. Furthermore, discrepancies among studies reporting divergent trends in *Bifidobacterium* and *Clostridium* abundance following SCI (e.g., Jeffrey et al. [17], 2018 vs. Kigerl et al. [28], 2016) may be explained by methodological heterogeneity, including differences in post-injury sampling windows, dietary composition, and the use of antibiotics or fiber sources, as highlighted by Gungor et al.

[15] (2016). Integrating these contextual factors provides a more coherent framework for understanding microbial variability across experimental models.

In summary, this study provides important insights into how the level and severity of SCI influence gut microbiota composition and butyrate metabolism. The microbial alterations were most profound in animals with high-level and severe injuries (T5S), which also exhibited less favorable bacterial recovery. Meanwhile, motor function improved in line with expectations, with better outcomes in lower and moderate injuries, independent of symbiotic intervention.

These findings underscore the need to consider injury-specific factors—especially neural disruption and gastrointestinal innervation—when interpreting microbial and physiological outcomes in SCI. Future research should focus on individual-level microbiota analyses, early interventions, and mechanistic studies exploring SCFA-specific effects on host health.

Conclusion

The present study demonstrates that symbiotic administration with *E. faecium* and agave inulin significantly influenced gut microbiota composition and metabolic function following SCI. Treatment led to the recovery of beneficial bacterial genera such as *Bifidobacterium*, *Clostridium*, and *Lactobacillus*. Locomotor recovery followed the expected pattern based on lesion level and injury severity, with better outcomes observed in moderate and lower thoracic injuries. Separately, the microbial and metabolic changes observed after symbiotic administration suggest a potential modulatory effect on the microbiota-gut-spinal cord axis. However, the observed changes in butyrate concentration were inconsistent with the expected relationship between butyrate levels and injury severity.

Although limited by the small sample size and pooled sequencing design, the consistent post-treatment trends indicate that symbiotic administration exerted a restorative and modulatory effect on intestinal microbial ecology and SCFA metabolism. These findings underscore the therapeutic potential of symbiotic supplementation as a complementary strategy to improve gastrointestinal and neurological function after SCI. Future studies with larger cohorts and individual-level analyses will help confirm these effects and explore their underlying mechanisms.

Abbreviations

16S rRNA: 16S ribosomal RNA

AEH: Alimentos Esenciales para la Humanidad

BBB: Basso, Beattie, and Bresnahan

BD: bowel dysfunction

EV: explained variation

GC: gas chromatography

GIT: gastrointestinal tract

OTUs: Operational Taxonomic Units

PCoA: principal coordinate analysis

R: Richness

REML: mixed-effects model

SCFAs: short-chain fatty acids

SCI: spinal cord injury

SD: standard deviation

T5M: thoracic five moderate

T5S: thoracic five severe

T9M: thoracic nine moderate

T9S: thoracic nine severe

TGF- β : transforming growth factor-beta

wpi: weeks post-injury

Declarations

Author contributions

EGV: Conceptualization, Investigation, Writing—review & editing, Visualization, Formal analysis. EDICC: Investigation, Writing—original draft. RRB: Investigation. AFR: Investigation. TPL: Validation. DRMQ: Investigation, Validation. HFNV: Supervision. IIM: Investigation, Writing—review & editing. MAVH: Supervision. EMAM: Supervision. APIG: Writing—review & editing. AI: Conceptualization, Writing—review & editing, Validation, Supervision. All authors read and approved the submitted version.

Conflicts of interest

Antonio Ibarra, who is the Editorial Board Member and Guest Editor of Exploration of Neuroprotective Therapy, had no involvement in the decision-making or the review process of this manuscript. The other authors declare no conflicts of interest.

Ethical approval

All experimental procedures were conducted in accordance with protocols approved by the National Institutes of Health (US) guidelines and the Guide for the Care and Use of Animals. The Animal Bioethics and Welfare Committee of the Universidad Anáhuac México approved all animal procedures (Registry number: 201702-CI) in accordance with the National Institutes of Health Guide for the care and use of laboratory animals, and the Mexican Official Norm on Principles of Laboratory Animal Care (NOM 062-ZOO-1999).

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The data supporting the findings will be made available upon request.

Funding

This study was supported by Universidad Anáhuac México, Grant/Award Number: 201702; Universidad Anáhuac Mexico, Grant/Award Number: 201707. The funder(s) had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright

© The Author(s) 2026.

Publisher's note

Open Exploration maintains a neutral stance on jurisdictional claims in published institutional affiliations and maps. All opinions expressed in this article are the personal views of the author(s) and do not represent the stance of the editorial team or the publisher.

References

1. Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunology*. 2016;5:e73. [DOI] [PubMed] [PMC]
2. Robles Alonso V, Guarner F. Linking the gut microbiota to human health. *Br J Nutr*. 2013;109:S21–6. [DOI] [PubMed]
3. Dinan TG, Cryan JF. Melancholic microbes: a link between gut microbiota and depression? *Neurogastroenterol Motil*. 2013;25:713–9. [DOI] [PubMed]
4. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour-epigenetic regulation of the gut-brain axis. *Genes Brain Behav*. 2014;13:69–86. [DOI] [PubMed]
5. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? *Neurochem Int*. 2016;99:110–32. [DOI] [PubMed]
6. Forsythe P, Kunze WA. Voices from within: gut microbes and the CNS. *Cell Mol Life Sci*. 2013;70:55–69. [DOI] [PubMed] [PMC]
7. Kirshblum SC, Waring W, Biering-Sorensen F, Burns SP, Johansen M, Schmidt-Read M, et al. Reference for the 2011 revision of the International Standards for Neurological Classification of Spinal Cord Injury. *J Spinal Cord Med*. 2011;34:547–54. [DOI] [PubMed] [PMC]
8. Furlan JC, Sakakibara BM, Miller WC, Krassioukov AV. Global incidence and prevalence of traumatic spinal cord injury. *Can J Neurol Sci*. 2013;40:456–64. [DOI] [PubMed]
9. Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. *Spine (Phila Pa 1976)*. 2001;26:S2–12. [DOI] [PubMed]
10. Braddom R. *Physical Medicine and Rehabilitation*. Philadelphia: WB Saunders Company; 2000.
11. Stone JM, Nino-Murcia M, Wolfe VA, Perkash I. Chronic gastrointestinal problems in spinal cord injury patients: a prospective analysis. *Am J Gastroenterol*. 1990;85:1114–9.
12. Glickman S, Kamm MA. Bowel dysfunction in spinal-cord-injury patients. *Lancet*. 1996;347:1651–3.
13. Lynch AC, Antony A, Dobbs BR, Frizelle FA. Bowel dysfunction following spinal cord injury. *Spinal Cord*. 2001;39:193–203. [DOI]
14. Levi R, Hultling C, Nash MS, Seiger Å. The Stockholm spinal cord injury study: 1. Medical problems in a regional SCI population. *Spinal Cord*. 1995;33:308–15. [DOI]
15. Gungor B, Adiguzel E, Gursel I, Yilmaz B, Gursel M. Intestinal Microbiota in Patients with Spinal Cord Injury. *PLoS One*. 2016;11:e0145878. [DOI] [PubMed] [PMC]
16. Kigerl KA, Mostacada K, Popovich PG. Gut Microbiota Are Disease-Modifying Factors After Traumatic Spinal Cord Injury. *Neurotherapeutics*. 2018;15:60–7. [DOI] [PubMed] [PMC]
17. O'Connor G, Jeffrey E, Madorma D, Marcillo A, Abreu MT, Deo SK, et al. Investigation of Microbiota Alterations and Intestinal Inflammation Post-Spinal Cord Injury in Rat Model. *J Neurotrauma*. 2018;35:2159–66. [DOI] [PubMed] [PMC]
18. Lanza M, Campolo M, Casili G, Filippone A, Paterniti I, Cuzzocrea S, et al. Sodium Butyrate Exerts Neuroprotective Effects in Spinal Cord Injury. *Mol Neurobiol*. 2019;56:3937–47. [DOI] [PubMed]
19. Khalkhali S, Mojgani N. *Enterococcus faecium*; a Suitable Probiotic Candidate for Modulation of Immune Responses Against Pathogens. *Int J Basic Sci Med*. 2017;2:77–82. [DOI]
20. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant Properties of Probiotic Bacteria. *Nutrients*. 2017;9:521. [DOI]
21. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GC, Swanson KS. Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *J Nutr*. 2015;145:2025–32. [DOI]

22. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14:491–502. [DOI]
23. Bao T, Wang Z, Zhu L, Lu H, Wang T, Zhang Y, et al. Inulin increases the proportion of monocytic myeloid-derived suppressor cells in peripheral blood, liver, spleen and regulates the secretion of plasma inflammatory cytokines in mice with non-alcoholic fatty liver disease. *Chin J Cell Mol Immunol*. 2020;36:228–35.
24. Divyashri G, Krishna G, Muralidhara, Prapulla SG. Probiotic attributes, antioxidant, anti-inflammatory and neuromodulatory effects of *Enterococcus faecium* CFR 3003: *In vitro* and *in vivo* evidence. *J Med Microbiol*. 2015;64. [DOI]
25. Scheff SW, Rabchevsky AG, Fugaccia I, Main JA, Lumppp JE Jr. Experimental modeling of spinal cord injury: characterization of a force-defined injury device. *J Neurotrauma*. 2003;20:179–93. [DOI] [PubMed]
26. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma*. 1995;12:1–21. [DOI]
27. Lin R, Xu J, Ma Q, Chen M, Wang L, Wen S, et al. Alterations in the fecal microbiota of patients with spinal cord injury. *PLoS One*. 2020;15:e0236470. [DOI] [PubMed] [PMC]
28. Kigerl KA, Hall JCE, Wang L, Mo X, Yu Z, Popovich PG. Gut dysbiosis impairs recovery after spinal cord injury. *J Exp Med*. 2016;213:2603–20. [DOI]
29. Wang Z, Burwinkel M, Chai W, Lange E, Blohm U, Breithaupt A, et al. Dietary *Enterococcus faecium* NCIMB 10415 and zinc oxide stimulate immune reactions to trivalent influenza vaccination in pigs but do not affect virological response upon challenge infection. *PLoS One*. 2014;9:e87007. [DOI] [PubMed] [PMC]
30. Reineccius G, Health H. *Flavor Chemistry and Technology*. 2nd ed. Taylor & Francis; 2006.
31. D'hoë K, Conterno L, Fava F, Falony G, Vieira-Silva S, Vermeiren J, et al. Prebiotic Wheat Bran Fractions Induce Specific Microbiota Changes. *Front Microbiol*. 2018;9:31. [DOI] [PubMed] [PMC]