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Xinyu Qiao

Xijing Digestive Disease Hospital Fourth Military Medical University

Kunli Du

Xijing Digestive Disease Hospital Fourth Military Medical University

Long Li

Shanghai Tenth People's Hospital

Yumao Yang

Xijing Digestive Disease Hospital Fourth Military Medical University

Ye Tian

Xijing Digestive Disease Hospital Fourth Military Medical University

Gaozan Zheng

Xijing Digestive Disease Hospital Fourth Military Medical University

Ruikai Li

Xijing Digestive Disease Hospital Fourth Military Medical University

Jianting Yang

Xijing Digestive Disease Hospital Fourth Military Medical University

Ziting Zhou

Xijing Digestive Disease Hospital Fourth Military Medical University

Juan Kang

Baoji People's Hospital

Jianyong Zheng

Xijing Digestive Disease Hospital Fourth Military Medical University

Fan Feng

Xijing Digestive Disease Hospital Fourth Military Medical University

Pengfei Wang

wangpengfei118@126.com

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Tables 1 to 4 are available in the Supplementary Files section.

Fecal Microbiota Transplantation Improves Clinical Symptoms and Promotes Intestinal Microecological Remodeling in Patients with Constipation Complicated by Anxiety: A Multi-omics Study

Xinyu Qiao^{1,2#}, Kunli Du^{1#}, Long Li^{3#}, Yumao Yang¹, Ye Tian¹, Gaozan Zheng¹, Ruikai Li¹, Jianting Yang¹, Ziting Zhou¹, Juan Kang⁴, Jianyong Zheng^{1*}, Fan Feng^{1*}, Pengfei Wang^{1,2*}

1. Department of Digestive Surgery, Xijing Hospital of Digestive Diseases, The Fourth Military Medical University, Xi'an, Shaanxi 710032, China.

2. State Key Laboratory of Holistic Integrative Management of Gastrointestinal Cancers, National Clinical Research Center for Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China.

3. Department of Functional Intestinal Diseases, General Surgery of Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, 200072, China.

4. Department of Gastroenterology, Baoji People's Hospital, Jinger Road # 24, Baoji, Shaanxi 721000, China.

[#]These authors contributed equally to this work and should be considered as co-first authorship.

*Fan Feng and Jianyong Zheng as the co-corresponding author and Pengfei Wang as the primary corresponding author.

***Correspondence:**

Pengfei Wang, Department of Digestive Surgery, Xijing Hospital of Digestive Diseases, The Fourth Military Medical University, Chang-Le Xi Street # 169, Xi'an, Shaanxi 710032, China. Email: wangpengfei18@126.com.

Fan Feng, Department of Digestive Surgery, Xijing Hospital of Digestive Diseases, The Fourth Military Medical University, Chang-Le Xi Street # 169, Xi'an, Shaanxi 710032, China. Email: surgeonfengfan@163.com.

Jianyong Zheng, Department of Digestive Surgery, Xijing Hospital of Digestive Diseases, The Fourth Military Medical University, Chang-Le Xi Street # 169, Xi'an, Shaanxi 710032, China. Email: zhjy68@163.com.

Abstract

Background: Constipation is frequently accompanied by anxiety and depressive symptoms, suggesting coordinated disturbances in the gut microbiota, intestinal metabolism, and gut-brain axis. Fecal microbiota transplantation (FMT), as a microbiome-targeted intervention, has shown potential in functional gastrointestinal disorders. However, its clinical response and multi-omics mechanisms in constipation complicated by anxiety remain unclear.

Methods: Eighteen patients with constipation complicated by anxiety received two cycles of oral FMT capsules, and 10 healthy individuals were enrolled as controls. Phenotypes were assessed before and after treatment using Wexner, PAC-QOL, ODS, SDS, and HAMA scores. Fecal samples from patients before and after FMT and from healthy controls were subjected to shotgun metagenomic sequencing and untargeted metabolomics. Microbial composition, MetaCyc pathways, fecal metabolites, efficacy-stratified features, and microbiota-metabolite-clinical phenotype correlation networks were analyzed.

Results: After FMT, Wexner, PAC-QOL, ODS, SDS, and HAMA scores were reduced. Metagenomic analysis showed that baseline gut microbial structure in patients was distinct from healthy controls and shifted toward a healthy-like profile after FMT. Differential species analysis suggested partial microbial recovery, with reversible species mainly involving *Clostridium*, *Blautia*, and *Lachnospira*. Functional pathway analysis showed recovery of selected amino acid metabolism and anaerobic fermentation pathways, whereas pathways related to bacterial surface structures and polysaccharide biosynthesis remained persistently abnormal. Untargeted metabolomics revealed broad fecal metabolic remodeling, characterized by a shift from a relatively suppressed state toward a more active profile. Efficacy-stratified analyses indicated that the Effective and No_Effective groups differed in baseline microbial composition, functional niches, and metabolic background. Integrated correlation network analysis identified associations among differential species, metabolites, and clinical phenotypes. In particular, *Butyricicoccus* sp. AM29-23AC

was negatively correlated with constipation, quality-of-life, anxiety, and depression-related scores and was embedded in a short-chain fatty acid-related microbiota-metabolite network.

Conclusions: Oral FMT capsules were associated with improvement in constipation, quality-of-life, anxiety, and depression-related scores in patients with constipation complicated by anxiety, accompanied by coordinated remodeling of gut microbial composition, microbial functional potential, and fecal metabolic profiles. Baseline microbial and metabolic states may influence clinical response to FMT. *Butyricicoccus* sp. AM29-23AC and short-chain fatty acid-related microbiota-metabolite networks may represent candidate links between FMT-associated ecological remodeling and clinical improvement.

Keywords: Fecal Microbiota Transplantation; Constipation; Anxiety; Metagenomics; Metabolomics; Gut-brain axis

Introduction

Constipation is a common functional bowel disorder characterized by reduced bowel movement frequency, straining, a sensation of incomplete evacuation, and symptoms related to obstructed defecation [1, 2]. Its pathophysiology is heterogeneous and may involve delayed colonic transit, pelvic floor dysfunction, altered intestinal sensation and motility, mucosal immune changes, dysregulation of the enteric nervous system, and disturbances in the luminal metabolic environment [1]. In addition to impairing quality of life, constipation frequently coexists with psychological symptoms such as anxiety and depression, thereby further increasing the overall disease burden [3-5].

In recent years, the gut microbiota has received increasing attention in the pathogenesis and progression of constipation. Previous studies have shown that patients with constipation may exhibit alterations in gut microbial diversity, community structure, and metabolic function [6, 7]. The gut microbiota can regulate epithelial energy metabolism, intestinal barrier function, enteric nervous system activity, and gut motility through short-chain fatty acids (SCFAs), bile acids, amino

acid derivatives, and other neuroactive metabolites [8-10]. In parallel, the gut microbiota and its metabolites participate in gut-brain axis signaling through immune, neuroendocrine, vagal, and blood-brain barrier-related pathways [10-13]. Therefore, gut microbial dysbiosis may not only affect bowel function but also contribute to anxiety and depressive symptoms [14-16].

Fecal microbiota transplantation (FMT) is a therapeutic approach in which intestinal microbiota from rigorously screened healthy donors are transferred to recipients to reconstruct or modulate the recipient gut ecosystem. FMT has been widely used for recurrent *Clostridioides difficile* infection and is increasingly being explored in inflammatory bowel disease, irritable bowel syndrome, chronic constipation, and disorders of gut-brain interaction [17-21]. Current clinical studies and systematic reviews suggest that FMT may improve bowel symptoms in a subset of patients with chronic constipation, although efficacy may be influenced by study design, delivery route, donor screening, and recipient baseline characteristics [19-22]. Large-scale safety and donor-screening studies from China further indicate that FMT is generally acceptable in terms of safety when rigorous donor screening, standardized preparation, and follow-up management are implemented [23-26].

Nevertheless, the clinical efficacy of FMT, interindividual differences in efficacy, and the underlying multi-omics mechanisms in constipation complicated by anxiety have not been systematically investigated. Therefore, this study enrolled patients with constipation complicated by anxiety who received oral FMT capsules, and integrated clinical scoring, shotgun metagenomic sequencing, and untargeted metabolomics to systematically characterize FMT-associated ecological remodeling at the levels of microbial composition, functional pathways, fecal metabolites, and correlation networks. Specifically, this study aimed to address the following questions: (i) whether FMT improves constipation symptoms and anxiety-related clinical scores; (ii) whether the gut microbial composition, functional potential, and fecal metabolome shift toward a healthy state after FMT; (iii) whether the Effective and No_Effective groups differ in baseline microbial and metabolic features; and (iv) whether

differential microbes, metabolites, and clinical phenotypes form biologically meaningful correlation networks.

Methods

Study Design and Participants

This study was approved by the Ethics Committee of the First Affiliated Hospital of the Fourth Military Medical University (approval number: KY20232320-C-1). Eighteen patients with constipation complicated by anxiety were enrolled, and all patients received two cycles of oral FMT capsule treatment. Ten healthy individuals were additionally enrolled as controls. Patients completed clinical scale assessments and provided fecal samples before and after FMT, whereas healthy controls provided fecal samples for baseline comparison. Clinical-omics correlation analyses were subsequently performed (**Fig. 1**). A total of 37 fecal samples were obtained for shotgun metagenomic sequencing, and 36 fecal samples were used for untargeted metabolomics, including samples from 10 patients in the Effective group, 3 patients in the No_Effective group, and 10 healthy controls.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: age 18 years or older, regardless of sex; diagnosis consistent with constipation-related criteria; presence of anxiety symptoms or HAMA score ≥ 14 according to the study criteria; willingness to receive FMT capsule treatment and complete follow-up; and ability to provide fecal samples and complete clinical scale data. The exclusion criteria included severe organic gastrointestinal disease, malignancy, severe infection, immunodeficiency or current immunosuppressive therapy, recent use of antibiotics, probiotics, or other interventions known to affect the gut microbiota, pregnancy or lactation, severe psychiatric disorders, or inability to comply with study procedures.

FMT Donor Screening and Capsule Preparation

The FMT capsules used in this study were purchased from Shanghai Tenth People's

Hospital affiliated with Tongji University School of Medicine. Donors were healthy volunteers who had undergone rigorous screening. Donor screening was performed with reference to domestic and international consensus recommendations regarding FMT donor screening, stool banking, and clinical application [17, 18, 24-26]. Donor fecal material was processed using standardized procedures to generate FMT capsules, with quality control and batch management implemented throughout preparation.

FMT Treatment Protocol

Patients received two cycles of oral FMT capsules. Each cycle consisted of two administrations per day, two capsules per administration, for six consecutive days, with a 7-day interval between the two cycles. Vancomycin was administered for 3 days before FMT, and bowel preparation was performed. Adverse events and compliance were recorded during treatment. Except for medications permitted by the study protocol, antibiotics, probiotics, prebiotics, and other interventions likely to substantially affect the gut microbiota were avoided whenever possible.

Clinical Efficacy Assessment

All patients completed clinical scoring before and after FMT. Constipation severity was assessed using the Wexner constipation score [27]; constipation-related quality of life was assessed using the PAC-QOL questionnaire [28]; obstructed defecation symptoms were evaluated using the Altomare ODS score [29]; depressive symptoms were assessed using the SDS [30]; and anxiety symptoms were assessed using the HAMA [31]. The primary clinical analysis consisted of paired pre- and post-treatment comparisons. According to the prespecified efficacy criterion in this study, patients with a reduction of $\geq 50\%$ in clinical symptom scores from baseline after treatment were classified into the Effective group, whereas those who did not meet this criterion were classified into the No_Effective group. The Wexner constipation score was used as the core indicator for efficacy stratification, and PAC-QOL, ODS, SDS, and HAMA scores were included as treatment-related clinical phenotypes for subsequent analyses.

Fecal Sample Collection and Storage

Fresh fecal samples were collected from patients before and after FMT, and once from healthy controls. Samples were immediately stored at -80°C until DNA or metabolite extraction. All samples were processed using a standardized workflow to minimize batch effects and technical bias.

Fecal DNA Extraction, Library Construction, and Shotgun Metagenomic Sequencing

Microbial genomic DNA was extracted from fecal samples using the MagPure Stool DNA Kit based on magnetic beads. Briefly, 100-200 mg of fecal material was lysed with Buffer ATL and proteinase K, followed by vortexing, bead-beating homogenization, incubation at 65°C , and high-speed centrifugation. The supernatant was then subjected to impurity removal, magnetic bead binding, washing, and elution, and the extracted DNA was stored at -20°C until use.

Metagenomic sequencing libraries were constructed using the VAHTS Universal Plus DNA Library Prep Kit. Qualified DNA was fragmented to approximately 350 bp and subjected to end repair, dA-tailing, adapter ligation/amplification, and magnetic bead purification to obtain final libraries. After quality control, libraries were sequenced on the DNBSEQ-T7 platform using a PE150 (paired-end 150 bp) sequencing strategy.

Metagenomic Bioinformatics Analysis

Raw sequencing data were first quality-filtered to remove low-quality reads and adapter sequences, followed by alignment to the host genome to remove host-contaminating reads, yielding high-quality clean reads. Taxonomic profiling was performed using MetaPhlAn 4.0, which estimates relative abundance at the phylum, genus, and species levels based on clade-specific marker genes. Functional annotation was performed using HUMAnN 3.6 with the UniRef90 database for gene family profiling, followed by mapping to MetaCyc functional pathways.

Alpha diversity was assessed using the Observed, Shannon, and Pielou indices to

evaluate within-sample microbial richness and evenness. Beta diversity was evaluated using principal coordinate analysis (PCoA) based on Bray-Curtis distances, and permutational multivariate analysis of variance (PERMANOVA) was used to test between-group differences in community structure.

Untargeted Metabolomics

For metabolomics, 25 mg of fecal sample was mixed with an extraction solvent containing isotope-labeled internal standards (methanol:acetonitrile:water = 2:2:1). Samples were vortexed, homogenized at 35 Hz for 4 min, sonicated in an ice-water bath with repeated processing, and incubated at -40°C for protein precipitation. After centrifugation, supernatants were filtered and subjected to instrumental analysis.

Samples were analyzed using a Vanquish ultra-high-performance liquid chromatography system coupled to an Orbitrap Exploris 120 high-resolution mass spectrometer. Chromatographic separation was performed on a Waters ACQUITY UPLC BEH Amide column (2.1 mm $\times$ 50 mm, 1.7 μm). Data were acquired in both positive and negative ion modes. The MS1 resolution was 60,000, the MS2 resolution was 15,000, and stepped normalized collision energy was set at 20/30/40. Raw data were converted to mzXML format using ProteoWizard, and peak extraction, alignment, normalization, and statistical analysis were performed using an R-based workflow. Metabolites were annotated against the BiotreeDB (V3.0) database according to the Metabolomics Standards Initiative (MSI) criteria. System reliability was evaluated by QC sample clustering, principal component analysis (PCA), and internal standard stability assessment.

Differential Analysis and Recovery Pattern Classification

Differential analyses of microbial taxa and MetaCyc functional pathways were performed using non-parametric tests with Benjamini-Hochberg correction for multiple comparisons. Because direct Pre vs Post comparisons did not identify significant microbial taxa or pathways after strict multiple-testing correction, an analysis using unadjusted $P < 0.05$ was further performed. Differential metabolites

were identified using the criteria $P < 0.05$, $VIP > 1$, and $|\text{Log2FC}| > 1.5$.

Based on the direction and significance of changes in the Control vs Pre, Control vs Post, and Pre vs Post comparisons, differential microbial taxa, functional pathways, and metabolites were further classified into Reversible, Persistently Dysregulated, Induced Dysregulation, and No change patterns to evaluate whether the post-FMT ecological state shifted toward that of healthy controls.

Correlation and Network Analyses

Spearman correlation analysis was used to assess associations among differential taxa, differential metabolites, functional pathways, and clinical scores. For the global correlation network, strong edges were retained using the criteria $\text{FDR} < 0.05$ and $|\rho| \geq 0.7$, generating an integrated network composed of clinical phenotypes, differential taxa, and differential metabolites.

A focused SCFA-related microbial subnetwork was further constructed. In this subnetwork, microbe-metabolite edges were retained using $\text{FDR} < 0.05$ and $|\rho| \geq 0.60$, whereas phenotype-related edges were retained using $\text{FDR} < 0.05$. Network nodes were classified as species, metabolites, or clinical phenotypes, and edge colors indicated the direction of correlation.

Statistical Analysis

Continuous variables are presented as mean $\pm$ standard deviation. Clinical scores before and after FMT were compared using the Wilcoxon signed-rank test. Between-group comparisons were performed using the Wilcoxon rank-sum test or other appropriate non-parametric tests. Categorical variables are presented as counts and percentages. $P < 0.05$ was considered statistically significant. For high-dimensional omics analyses, FDR correction was applied according to the specific analytical objective.

Results

FMT Improved Clinical Symptoms in Patients with Constipation Complicated

by Anxiety

A total of 18 patients with constipation complicated by anxiety were included, comprising 6 men (33.3%) and 12 women (66.7%), with a mean age of 49.17 ± 17.12 years. Ten healthy controls were also enrolled. According to the prespecified efficacy criteria, 10 patients were classified into the Effective group after FMT, corresponding to an effective rate of 55.6%, whereas the remaining 8 patients were classified into the No_Effective group. Multiple clinical indicators improved significantly after FMT. Specifically, the Wexner constipation score decreased from 20.78 ± 3.00 to 14.17 ± 7.64 ($P = 0.0017$), PAC-QOL decreased from 54.06 ± 16.53 to 37.56 ± 24.01 ($P = 0.0018$), and ODS score decreased from 15.94 ± 2.69 to 9.89 ± 5.29 ($P = 0.0022$). Emotional symptom scores also improved, with SDS decreasing from 61.39 ± 6.84 to 40.78 ± 17.81 ($P = 0.0032$) and HAMA decreasing from 24.56 ± 9.67 to 15.72 ± 13.65 ($P = 0.0218$) (**Table 1**). These findings suggest that FMT intervention was associated with improvements in constipation symptoms and quality of life, together with reductions in anxiety- and depression-related scores.

FMT Promoted Restoration of Gut Microbial Community Structure Toward a Healthy Configuration

Alpha diversity analysis showed no statistically significant overall differences among patients before treatment, patients after treatment, and healthy controls, regardless of whether the Observed or Shannon index was used (**Fig. 2A,B**). However, after stratification by treatment efficacy, the Effective group showed an increasing trend in the Observed index after FMT, and the Shannon index also shifted toward that of healthy controls, whereas the No_Effective group did not exhibit a similar recovery trend (**Fig. 2C,D**). These results suggest that FMT-induced ecological changes may be reflected more strongly in community restructuring than in a simple increase in species richness.

Beta diversity analysis further supported this interpretation. Bray-Curtis PCoA showed that baseline gut microbial communities in patients were clearly separated

from those in healthy controls. After FMT, the overall microbial structure shifted toward healthy controls (**Fig. 2E,F**), indicating directional remodeling of community composition. Nevertheless, post-treatment samples remained significantly different from healthy controls, suggesting that FMT may promote partial recovery toward a healthy state but does not fully normalize the gut microbiota over the short term. Efficacy-stratified analysis further showed that the baseline microbiota of the Effective group was closer to that of healthy controls than that of the No_Effective group, whereas the No_Effective group showed a more pronounced baseline deviation from the healthy state (**Fig. 2G,H**), suggesting that the recipient's initial microbial state may be associated with FMT efficacy.

FMT Partially Reversed Differential Microbial Dysbiosis

At the species level, 137 differential species were identified in the Control vs Pre comparison, including 44 increased and 93 decreased species. In the Control vs Post comparison, 127 differential species were identified, including 64 increased and 63 decreased species (**Table 2**). Overall, baseline differential species in patients were predominantly decreased (93/137, 67.8%), suggesting marked gut microbial dysbiosis in patients with constipation complicated by anxiety. After FMT, the total number of species differing from healthy controls decreased, and the number of species with increased abundance rose, indicating that the overall microbial composition shifted toward a healthy-like microbial profile.

In the direct Pre vs Post comparison, no species remained significant after strict multiple-testing correction. Analysis using $P < 0.05$ identified 29 differential species, of which 22 increased and 7 decreased after treatment. Classification based on abundance-change patterns showed that 52 species (28.7%) were classified as Reversible, 85 species (47.0%) as Persistently Dysregulated, and 42 species (23.2%) as Induced Dysregulation (**Fig. 3A,B**). Among Reversible species, *Clostridium* accounted for the largest proportion (**Fig. 3C-F**), followed by *Blautia* (**Fig. 3G-J**) and *Lachnospira* (**Fig. 3K-M**), suggesting that FMT may partially reverse ecological dysbiosis characterized by loss of core commensal bacteria.

Efficacy-stratified analysis (**Fig. 4A**) showed that Reversible species in the Effective group mainly included *Clostridium*, *Blautia*, and *Eubacterium* (**Fig. 4B-D**), whereas Reversible species in the No_Effective group were more frequently represented by potentially dysbiosis-associated species such as *Klebsiella pneumoniae* (**Fig. 4E**). Baseline comparison between the Effective and No_Effective groups further revealed that *Bifidobacterium*, particularly *Bifidobacterium longum*, was more abundant in the Effective group before treatment (**Fig. 4F**). In contrast, *Synergistes jonesii*, *Prevotella intermedia*, and *Peptostreptococcus anaerobius* were enriched in the No_Effective group (**Fig. 4G-I**). These results suggest that the direction of microbial remodeling after FMT is heterogeneous across patients, and that baseline microbial features may be important determinants of treatment efficacy.

FMT Promoted Partial Recovery of Microbial Functional Profiles

Functional pathway analysis based on the MetaCyc database identified 129 differential pathways in the Control vs Pre comparison, including 43 upregulated and 86 downregulated pathways. In the Control vs Post comparison, 70 differential pathways were identified, including 19 upregulated and 51 downregulated pathways (**Table 3**). Consistent with microbial compositional findings, baseline functional pathways in patients were predominantly downregulated, indicating that constipation complicated by anxiety was associated not only with altered community composition but also with suppressed microbial functional potential. After FMT, the number of pathways differing from healthy controls decreased markedly, indicating partial restoration of microbial functional states.

In the Pre vs Post comparison, no functional pathway remained significant after strict multiple-testing correction. Analysis using $P < 0.05$ identified 45 differential pathways, including 38 upregulated and 7 downregulated pathways after treatment. Heatmap classification showed that 85 pathways (48.9%) were classified as Reversible, 44 (25.3%) as Persistently Dysregulated, and 26 (14.9%) as Induced Dysregulation (**Fig. 5A,B**). Representative pathway boxplots showed that selected pathways related to anaerobic fermentation and amino acid metabolism increased

after FMT and approached healthy control levels, including pyruvate fermentation to butanoate (CENTFERM-PWY), clostridial acidogenic fermentation (PWY-6590), L-serine/glycine biosynthesis (SER-GLYSYN-PWY), and branched-chain amino acid biosynthesis (**Fig. 5C-F**). These findings suggest that FMT may promote partial recovery of microbial energy metabolism and amino acid metabolic functions. Notably, non-recovered pathways were enriched for pathways related to bacterial surface structures and polysaccharide biosynthesis, including colanic acid, dTDP- β -L-rhamnose, and O-antigen construction-related pathways (**Fig. 5G-I**), suggesting that functional abnormalities associated with bacterial ecological adaptation, adhesion/colonization, and surface antigen structures may persist after short-term FMT.

Further efficacy-stratified analysis showed that approximately 34% of functional pathways in the Effective group exhibited a Reversible pattern, 17.9% were Persistently Dysregulated, and 35.3% showed No change. In contrast, 52.0% of pathways in the No_Effective group remained No change, whereas the remaining pathways were mainly persistently abnormal (**Fig. 6A**). Baseline comparison between the Effective and No_Effective groups identified 11 efficacy-associated differential pathways. Five pathways were enriched at baseline in the Effective group, including FASYN-INITIAL-PWY, GLUCUROCAT-PWY, PWY-5497, PWY-5677, and PWY-7242 (**Fig. 6B-F**), whereas six pathways were enriched at baseline in the No_Effective group, including P185-PWY, PWY-5004, PWY-5981, URSIN-PWY, PWY-6396, and PWY-8131 (**Fig. 6G-L**). These findings suggest that the efficacy of FMT may be related not only to microbial compositional differences but also to the recipient's baseline functional ecological niche.

FMT Shifted the Fecal Metabolome from a Suppressed State Toward an Active Remodeling State

Differential metabolite analysis identified 222 differential metabolites in the Control vs Pre comparison, including 60 increased and 162 decreased metabolites (**Fig. 7B**). In the Control vs Post comparison, 225 differential metabolites were detected,

including 125 increased and 100 decreased metabolites (**Fig. 7C**). The Pre vs Post comparison identified 27 differential metabolites, of which 23 increased and 4 decreased after treatment (**Fig. 7D; Table 4**). Overall, baseline differential metabolites in patients were predominantly decreased, whereas the number of increased metabolites rose markedly after FMT. These findings indicate that FMT did not merely induce local metabolic correction but rather triggered broad remodeling of the fecal metabolome, shifting the fecal metabolic state from a relatively suppressed state toward a more active remodeling state.

The overall heatmap of differential metabolites illustrated broad metabolomic remodeling after FMT (**Fig. 7A**). Pattern classification showed that among all differential metabolites, 116 metabolites were classified as Reversible, 106 as Persistently Dysregulated, 119 as Induced Dysregulation, and 17 as No change (**Fig. 7E**), suggesting the coexistence of restoration and newly emerging metabolic remodeling after FMT. Classification by metabolite class showed that differential metabolites were mainly enriched in amino acids and derivatives, lipids, and phenylpropanoids, indicating that these metabolic networks were major contributors to FMT-associated metabolic remodeling. Representative metabolite boxplots showed that 3-amino-5H-pyrimido[5,4-b]indol-4-one, Isorhodoptilometrin, and 3-(3-Hydroxyphenyl)propanoic acid were significantly lower in patients before treatment than in healthy controls and tended to increase after FMT (**Fig. 7F-H**), suggesting that FMT may promote activation of multiple suppressed metabolic categories. In contrast, Pifithrin-Beta remained lower than healthy control levels both before and after treatment and did not change significantly after FMT (**Fig. 7I**), indicating that some metabolic abnormalities were not fully restored after short-term FMT.

After stratification by treatment efficacy (**Fig. 8A**), metabolomic differences became more pronounced. In the Effective group, 31 differential metabolites were identified in the Control vs Pre comparison (**Fig. 8B**), whereas 54 were identified in the No_Effective group (**Fig. 8C**). In the Control vs Post comparison, 13 differential

metabolites were detected in the Effective group (**Fig. 8D**) and 21 in the No_Effective group (**Fig. 8E**). More importantly, the Pre.Effective vs Post.Effective comparison identified 21 differential metabolites (**Fig. 8F**), whereas the Pre.No_Effective vs Post.No_Effective comparison identified only 6 differential metabolites (**Fig. 8G**), suggesting more pronounced metabolic remodeling after FMT in the Effective group and a relatively limited metabolic response in the No_Effective group. Meanwhile, 66 differential metabolites were already present at baseline between Pre.No_Effective and Pre.Effective groups (**Fig. 8H**), indicating distinct pre-treatment metabolic backgrounds between the Effective and No_Effective groups. Metabolite class analysis showed that baseline differences were mainly concentrated in amino acids and derivatives and lipids; treatment-related changes in the Effective group primarily involved amino acids and derivatives, lipids, and phenylpropanoids; in contrast, only six metabolites changed before and after treatment in the No_Effective group, mainly belonging to lipids (**Fig. 8I**). These findings suggest that FMT efficacy is associated not only with microbial compositional differences but also with the recipient's baseline metabolic state and subsequent metabolic plasticity.

Differential Microbes, Differential Metabolites, and Clinical Phenotypes Formed a Significant Correlation Network

To explore potential associations linking gut microbes, fecal metabolites, and clinical phenotype improvement after FMT, Spearman correlation network analysis was performed based on differential microbial taxa, differential metabolites, and clinical scores. Integrated analysis yielded 67,470 correlation pairs, of which 23,810 reached $P < 0.05$. Under more stringent criteria ($FDR < 0.05$ and $|\rho| \geq 0.7$), 361 strong edges were retained, forming a correlation network composed of clinical phenotypes, differential microbial taxa, and differential metabolites (**Fig. 9A**). The network showed that Wexner, PAC-QOL, ODS, SDS, and HAMA scores were significantly negatively correlated with multiple intestinal microbes and metabolites, suggesting that more severe clinical symptoms were associated with lower abundances of selected FMT-associated beneficial microbes and metabolites.

In species-clinical phenotype correlations, *Butyricicoccus* sp. AM29-23AC was significantly negatively correlated with Wexner, PAC-QOL, ODS, SDS, and HAMA scores, with representative scatterplots shown in **Fig. 9B-D**. Among these, the correlation with Wexner score was the strongest ($\rho = -0.799$, FDR = 0.00045). In addition, *Blautia faecis* was significantly negatively correlated with PAC-QOL score ($\rho = -0.754$, FDR = 0.00139) (**Fig. 9E**), and *Blautia* sp. MSK_20_85 was significantly negatively correlated with Wexner score ($\rho = -0.753$, FDR = 0.00139) (**Fig. 9F**), suggesting that recovery of FMT-associated beneficial microbes may be closely related to improvement in constipation symptoms and quality of life. Metabolite-clinical phenotype analysis showed that O-Methylsterigmatocystin, Isorhodoptilometrin, and 3-amino-5H-pyrimido[5,4-b]indol-4-one were significantly negatively correlated with PAC-QOL, Wexner, or SDS scores (**Fig. 9G-I**). A focused SCFA-related microbial subnetwork further revealed tight associations between potential SCFA-producing microbial taxa, including *Butyricicoccus*, *Roseburia*, *Faecalibacterium*, *Coprococcus*, and *Anaerobutyricum*, and multiple differential metabolites. Within this subnetwork, *Butyricicoccus* sp. AM29-23AC was additionally connected to Wexner, PAC-QOL, ODS, SDS, and HAMA clinical phenotype nodes (**Fig. 10**), suggesting that FMT may improve intestinal motility abnormalities and emotional symptoms by restoring SCFA-related microbes and their metabolic interaction networks. Overall, these findings suggest that the therapeutic efficacy to FMT may not be driven by a single microbe or metabolite but rather by an integrated microbe-metabolite-host phenotype interaction network.

Discussion

This study focused on patients with constipation complicated by anxiety, a clinical phenotype closely related to the gut-brain axis. We found that oral FMT capsule treatment was associated with significant improvement in constipation severity, quality of life, obstructed defecation symptoms, and anxiety/depression-related scores. Previous clinical studies and systematic reviews in slow-transit constipation have suggested that FMT can improve bowel symptoms in a subset of patients [19-21].

More recently, FMT has also been explored in disorders of gut-brain interaction [32, 33]. Therefore, the value of the present study lies not in establishing definitive causal efficacy, but in providing integrated clinical, metagenomic, and metabolomic evidence suggesting that FMT-associated clinical benefit may be accompanied by systematic remodeling of the gut ecosystem at the compositional, functional, metabolic, and interaction-network levels.

From a mechanistic perspective, constipation is not a single motility disorder but a heterogeneous condition involving intestinal motility, luminal metabolism, mucosal barrier function, immune regulation, and neuropsychological factors [1, 2]. The gut microbiota may influence gut motility and gut-brain axis signaling through SCFAs, tryptophan and other amino acid derivatives, bile acids, and neuroactive molecules [8-10]. In this study, the baseline microbial structure of patients was separated from that of healthy controls, and differential microbial taxa and functional pathways were predominantly decreased, supporting an ecological signature characterized by loss of commensal microbes and suppressed microbial functional potential in constipation complicated by anxiety. After FMT, microbial structure, MetaCyc functional pathways, and metabolic profiles shifted toward a healthy state, consistent with previous reports linking constipation to gut microbial dysbiosis, microbiota-derived regulation of gut motility, and fecal metabolic abnormalities [4, 6-8, 34].

Efficacy-stratified analysis suggested that the recipient baseline ecological niche may be an important determinant of FMT efficacy. Patients in the Effective group had a higher baseline abundance of *Bifidobacterium longum*, whereas patients in the No_Effective group showed higher abundances of *Synergistes jonesii*, *Prevotella intermedia*, and *Peptostreptococcus anaerobius*. At the metabolomic level, the Effective and No_Effective groups already differed substantially before treatment. These findings suggest that FMT efficacy may depend not only on donor microbiota input but also on whether the recipient gut environment permits colonization, expansion, cross-feeding, and stable metabolic output of beneficial microbes. Previous studies of donor screening, recipient features, FMT procedures, and

treatment outcomes have also suggested that both donor and recipient factors can influence FMT efficacy [18, 22, 24, 25]. These observations provide a rationale for developing efficacy prediction models based on baseline microbial and metabolic features.

At the metabolic level, FMT shifted the fecal metabolome from a globally downregulated baseline profile toward a more active remodeling state, mainly involving amino acids and derivatives, lipids, and phenylpropanoids. Amino acids and their derivatives may serve as neurotransmitter precursors, substrates for epithelial energy metabolism, and mediators of microbial cross-feeding. Lipid- and bile acid-related metabolism is closely related to gut motility, barrier function, and inflammatory regulation, whereas phenylpropanoids and aromatic metabolites may reflect the combined influence of diet and the gut microbiota on the luminal metabolic environment [9, 16, 34]. Because untargeted metabolomics primarily provides candidate signals, the biological significance of these metabolites requires further validation using targeted quantification and functional experiments.

Correlation network analysis further linked microbial and metabolic changes to clinical phenotypes. *Butyricoccus* sp. AM29-23AC was significantly negatively correlated with Wexner, PAC-QOL, ODS, SDS, and HAMA scores, suggesting that it may represent a candidate microbial node connecting FMT-associated microbiota recovery with improvement in constipation and emotional symptoms. Potential SCFA-producing taxa, including *Butyricoccus*, *Roseburia*, *Faecalibacterium*, *Coprococcus*, and *Anaerobutyricum*, formed a metabolite-associated subnetwork, consistent with the known roles of SCFAs in epithelial energy supply, immune homeostasis, and gut-brain axis signaling [10, 35-38]. In addition, microbiota-derived signals can participate in blood-brain barrier permeability, microglial maturation, and emotion-related pathways, providing biological plausibility for a link between SCFA-related microbes, bowel symptoms, and emotional symptoms [12-15].

The strengths of this study include its focus on constipation complicated by anxiety, a clinically common but mechanistically underexplored condition; its integration of

clinical phenotypes, shotgun metagenomics, microbial functional profiling, untargeted metabolomics, and correlation network analysis; and its efficacy-stratified design, which suggests that baseline microbial and metabolic states may influence FMT efficacy. Several limitations should also be acknowledged. First, the sample size was small, particularly the limited number of No_Effective samples available for metabolomic analysis after efficacy stratification. Second, the study lacked a randomized, double-blind, placebo-controlled design, and therefore placebo effects, natural fluctuations, dietary changes, and other confounders cannot be fully excluded. Third, untargeted metabolite annotation and correlation networks remain exploratory and require validation using targeted assays, strain-level tracking, and mechanistic experiments. Future studies should incorporate larger cohorts, randomized controlled designs, longer follow-up, donor-recipient strain tracking, targeted SCFA/bile acid measurement, serum metabolomics, and animal experiments to further clarify the causal mechanisms by which FMT may improve constipation complicated by anxiety.

Conclusion

This study suggests that oral FMT capsule treatment may improve constipation symptoms, quality of life, anxiety and depression-related scores in patients with constipation complicated by anxiety, accompanied by coordinated remodeling of gut microbial composition, microbial functional potential, and fecal metabolic profiles. Baseline microbial composition, functional ecological niches, and metabolic background may influence the treatment efficacy to FMT. *Butyricicoccus* sp. AM29-23AC and SCFA-related microbiota-metabolite networks may represent candidate mechanisms linking FMT-induced ecological remodeling to clinical symptom improvement. These findings provide multi-omics evidence supporting further investigation of FMT for constipation complicated by anxiety and offer a basis for future precision selection of FMT-responsive patients and mechanistic studies of gut-brain axis interventions.

Abbreviations

FMT	Fecal microbiota transplantation
PAC-QOL	Patient Assessment of Constipation Quality of Life
ODS	Obstructed Defecation Syndrome
SDS	Self-Rating Depression Scale
HAMA	Hamilton Anxiety Rating Scale
SCFAs	short-chain fatty acids
PCoA	principal coordinate analysis

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of the Fourth Military Medical University (**approval number: KY20232320-C-1**). All patients provided written informed consent for this study. All methods in this study carried out in accordance with the *Declaration of Helsinki*. Permission to use the patient's samples was obtained from the Ethics Committee of the First Affiliated Hospital of the Fourth Military Medical University.

Clinical trial number: Not applicable.

Consent for publication

Not Applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared no potential conflicts of interest.

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Authors' contributions

Pengfei Wang and Fan Feng designed the study. Jianyong Zheng supervised the study. Xinyu Qiao and Kunli Du wrote the manuscript. Long Li and Yumao Yang revised the manuscript. Ye Tian, Gaozan Zheng, Ruikai Li, Juan Kang assisted with data analysis. Jianting Yang and Ziting Zhou collected the samples.

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Figure Legends

Figure 1. Study design and multi-omics analytical workflow.

Figure 2. FMT Promoted Restoration of Gut Microbial Community Structure Toward a Healthy Configuration. (A,B) Alpha diversity comparisons among healthy controls, pre-treatment patients, and post-treatment patients based on the Observed and Shannon indices. (C,D) Efficacy-stratified alpha diversity analysis showing trends in microbial richness and diversity in the Effective and No_Effective groups before and after FMT. (E,F) Principal coordinate analysis (PCoA) based on Bray-Curtis distances showing separation between baseline patient samples and healthy controls and a post-treatment shift toward the healthy control group. (G,H)

Efficacy-stratified beta diversity analysis showing that baseline microbial communities in the Effective group were closer to healthy controls than those in the No_Effective group.

Figure 3. FMT partially reversed species-level microbial dysbiosis. (A,B) Classification of differential species according to the four abundance-change patterns: Reversible, Persistently Dysregulated, Induced Dysregulation, and No change. (C-F) Representative Reversible *Clostridium* species. (G-J) Representative Reversible *Blautia* species. (K-M) Representative Reversible *Lachnospira*-related species.

Figure 4. Efficacy-stratified microbial features associated with FMT efficacy. (A) Overview of efficacy-stratified patterns of differential species. (B-D) Representative Reversible species in the Effective group, including species from the genera *Clostridium*, *Blautia*, and *Eubacterium*. (E) Representative potentially dysbiosis-associated species classified as Reversible in the No_Effective group, including *Klebsiella pneumoniae*. (F) Baseline abundance of *Bifidobacterium longum*, which was higher in the Effective group than in the No_Effective group. (G-I) Baseline enrichment of *Synergistes jonesii*, *Prevotella intermedia*, and *Peptostreptococcus anaerobius* in the No_Effective group.

Figure 5. FMT promoted partial recovery of microbial functional pathways. (A,B) Pattern classification of MetaCyc functional pathways into Reversible, Persistently Dysregulated, Induced Dysregulation, and No change categories. (C-F) Representative pathways related to anaerobic fermentation and amino acid metabolism that increased after FMT and shifted toward healthy control levels, including pyruvate fermentation to butanoate (CENTFERM-PWY), clostridial acidogenic fermentation (PWY-6590), L-serine/glycine biosynthesis (SER-GLYSYN-PWY), and branched-chain amino acid biosynthesis. (G-I) Representative Persistently Dysregulated pathways related to bacterial surface structures and polysaccharide biosynthesis, including colanic acid, dTDP-beta-L-rhamnose, and O-antigen-related pathways.

Figure 6. Baseline microbial functional niches differed between the Effective and No_Effective groups. (A) Efficacy-stratified distribution of MetaCyc functional pathway patterns after FMT, including Reversible, Persistently Dysregulated, Induced Dysregulation, and No change categories. The Effective group showed a higher proportion of Reversible pathways, whereas the No_Effective group was characterized mainly by No change or persistently dysregulated pathways. (B-F) Baseline pathways enriched in the Effective group, including FASYN-INITIAL-PWY, GLUCUROCAT-PWY, PWY-5497, PWY-5677, and PWY-7242. (G-L) Baseline pathways enriched in the No_Effective group, including P185-PWY, PWY-5004, PWY-5981, URSIN-PWY, PWY-6396, and PWY-8131.

Figure 7. FMT induced broad remodeling of the fecal metabolome. (A) Overall heatmap showing differential metabolites and their abundance-change patterns across groups. (B-D) Differential metabolite profiles in Control vs Pre, Control vs Post, and Pre vs Post comparisons. (E) Pattern classification of all differential metabolites into Reversible, Persistently Dysregulated, Induced Dysregulation, and No change categories. (F-H) Representative metabolites that were decreased at baseline and tended to increase after FMT, including 3-amino-5H-pyrimido[5,4-b]indol-4-one, Isorhodoptilometrin, and 3-(3-Hydroxyphenyl)propanoic acid. (I) Pifithrin-Beta remained persistently lower than healthy control levels before and after FMT.

Figure 8. Efficacy-stratified metabolomic remodeling after FMT. (A) Analytical overview of efficacy-stratified fecal metabolomics. (B,C) Differential metabolites in Control vs Pre comparisons for the Effective and No_Effective groups, respectively. (D,E) Differential metabolites in Control vs Post comparisons for the Effective and No_Effective groups, respectively. (F,G) Treatment-associated differential metabolites in the Effective group (Pre.Effective vs Post.Effective) and the No_Effective group (Pre.No_Effective vs Post.No_Effective), respectively. The Effective group showed more pronounced metabolic remodeling after FMT, whereas the No_Effective group exhibited a limited metabolic response. (H) Baseline differential metabolites between the No_Effective group and the Effective group. (I) Metabolite class composition of

baseline and treatment-associated differential metabolites, indicating that FMT efficacy was related to both baseline metabolic background and post-treatment metabolic plasticity.

Figure 9. Differential microbes, metabolites, and clinical phenotypes formed an integrated correlation network. (A) Global Spearman correlation network constructed from differential species, differential metabolites, and clinical scores. (B-D) Representative negative correlations between *Butyricicoccus* sp. AM29-23AC and clinical scores, indicating that higher abundance of this microbial taxon was associated with lower constipation, quality-of-life impairment, and anxiety/depression-related scores. (E) Negative correlation between *Blautia faecis* and PAC-QOL. (F) Negative correlation between *Blautia* sp. MSK_20_85 and Wexner score. (G-I) Representative negative correlations between differential metabolites, including O-Methylsterigmatocystin, Isorhodoptilometrin, and 3-amino-5H-pyrimido[5,4-b]indol-4-one, and clinical phenotypes.

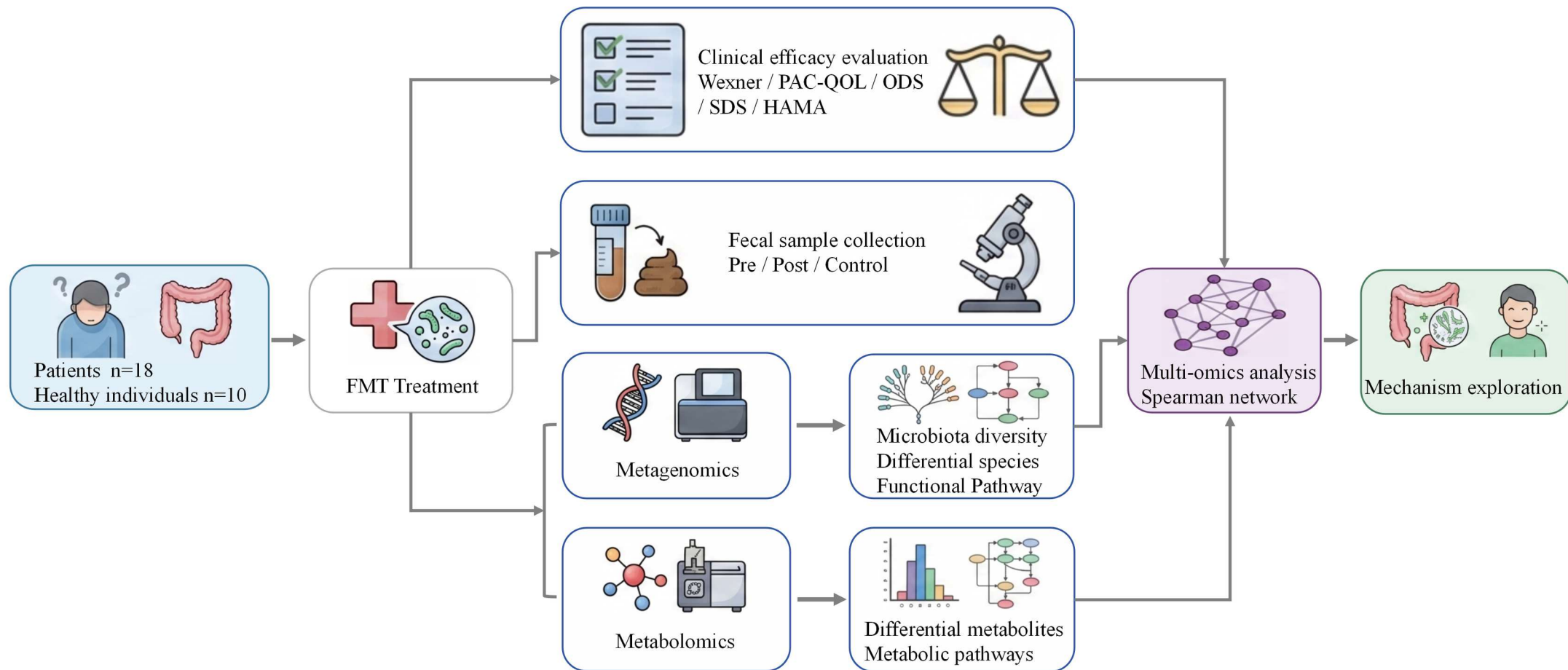
Figure 10. SCFA-related microbiota-metabolite-clinical phenotype subnetwork. A focused correlation subnetwork was constructed for potential short-chain fatty acid (SCFA)-producing microbial taxa, including *Butyricicoccus*, *Roseburia*, *Faecalibacterium*, *Coprococcus*, and *Anaerobutyricum*.

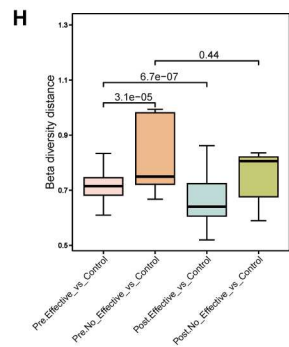
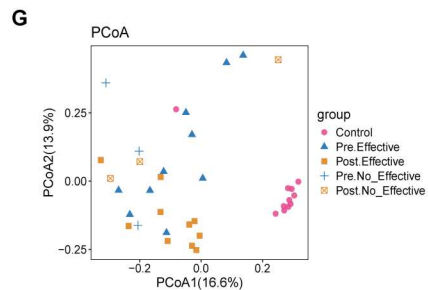
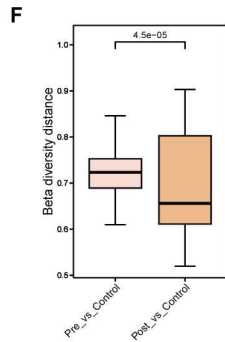
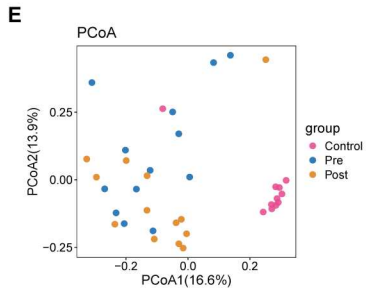
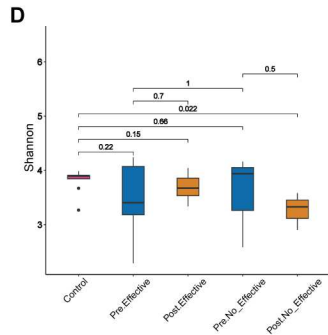
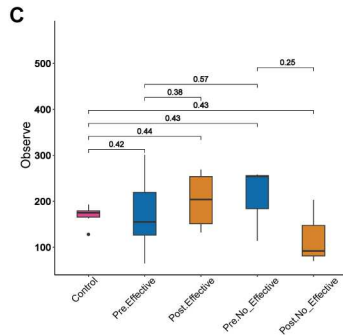
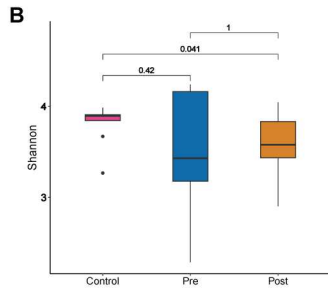
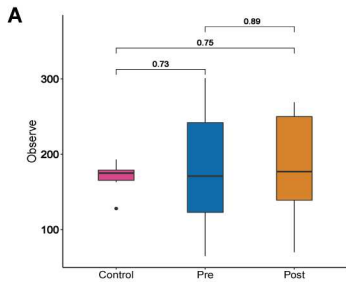
Table 1. Baseline characteristics and comparison of clinical scores before and after treatment.

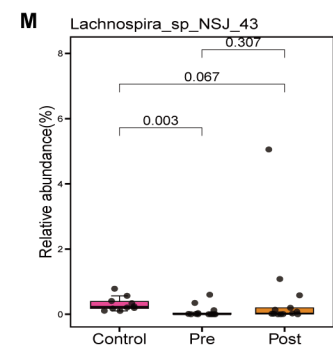
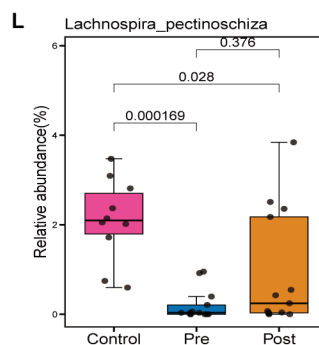
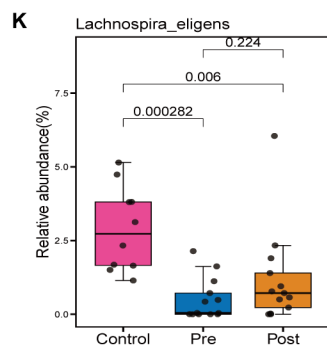
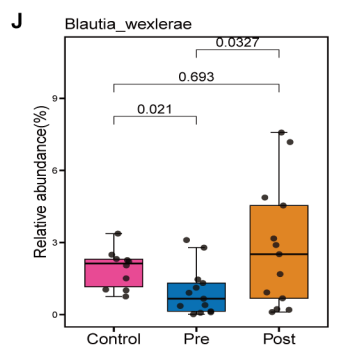
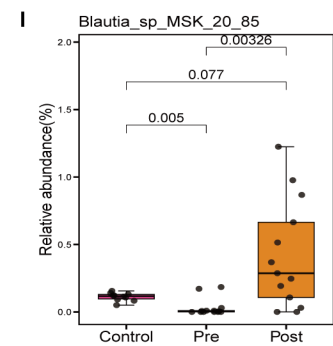
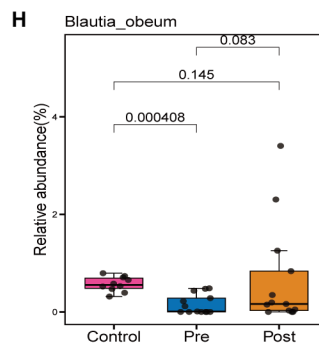
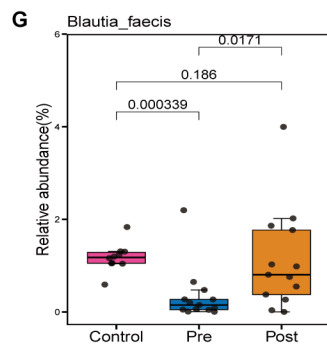
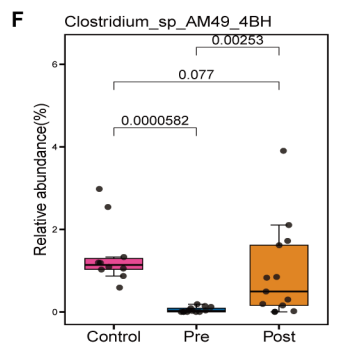
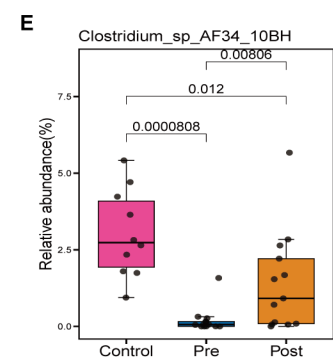
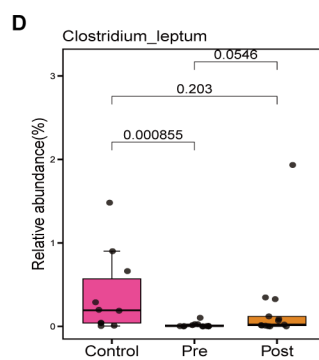
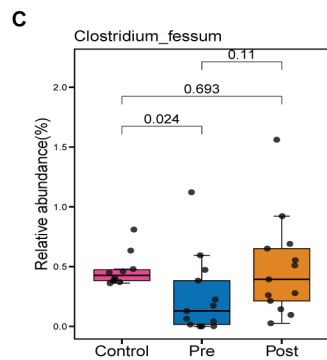
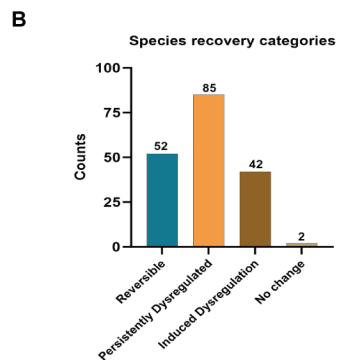
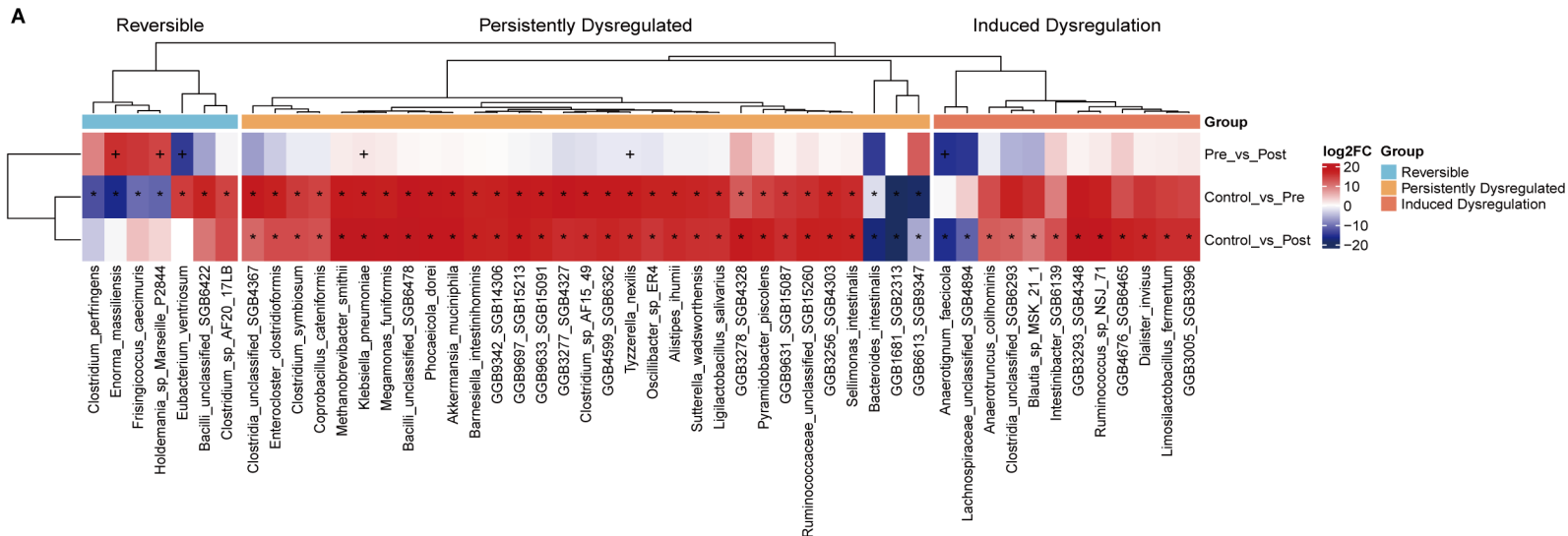
Table 2. Analysis of differential microbial species.

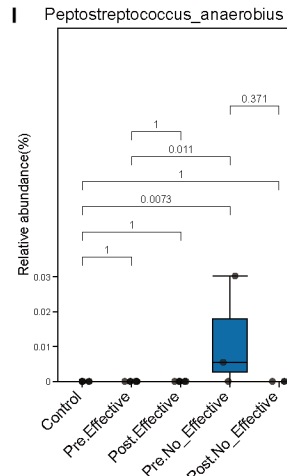
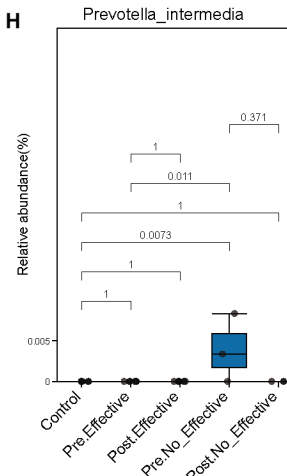
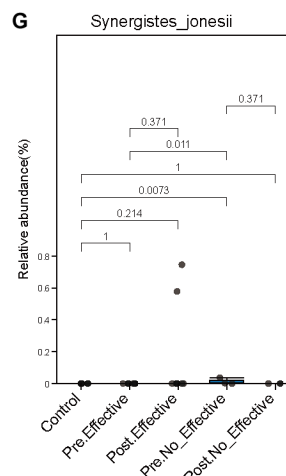
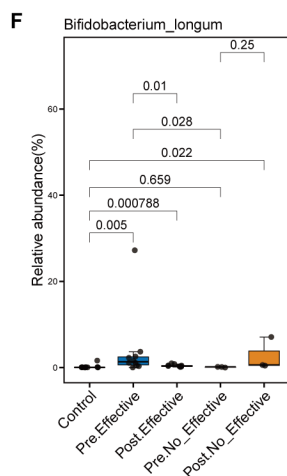
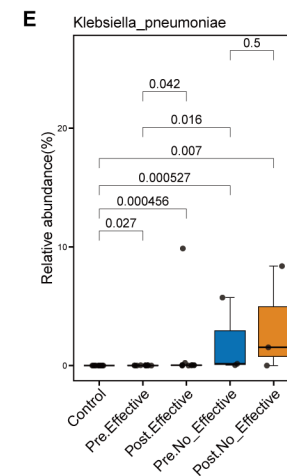
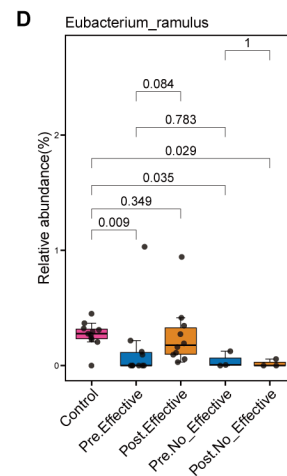
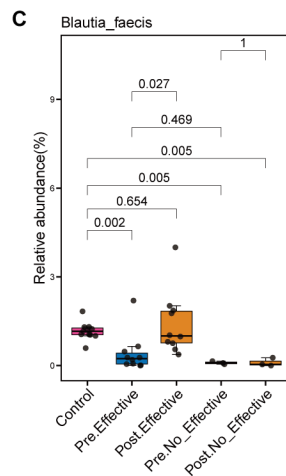
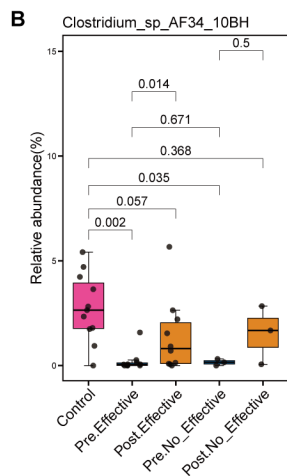
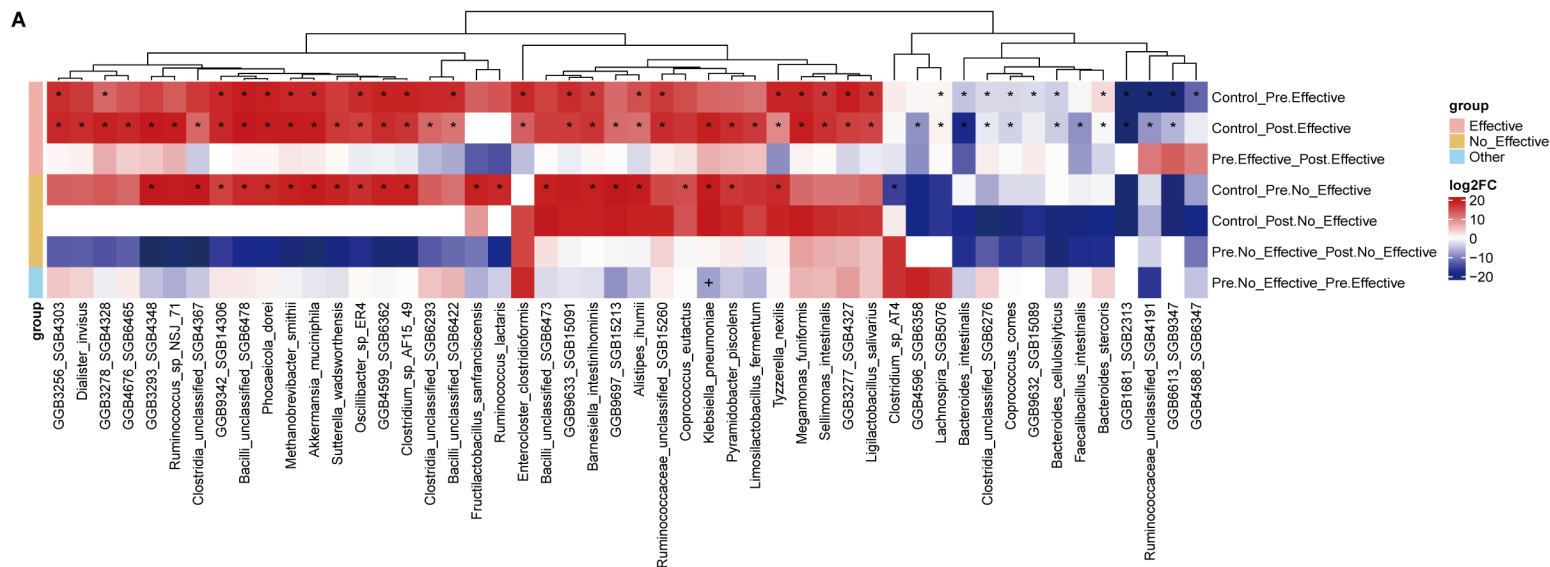
Table 3. Analysis of MetaCyc Differential function.

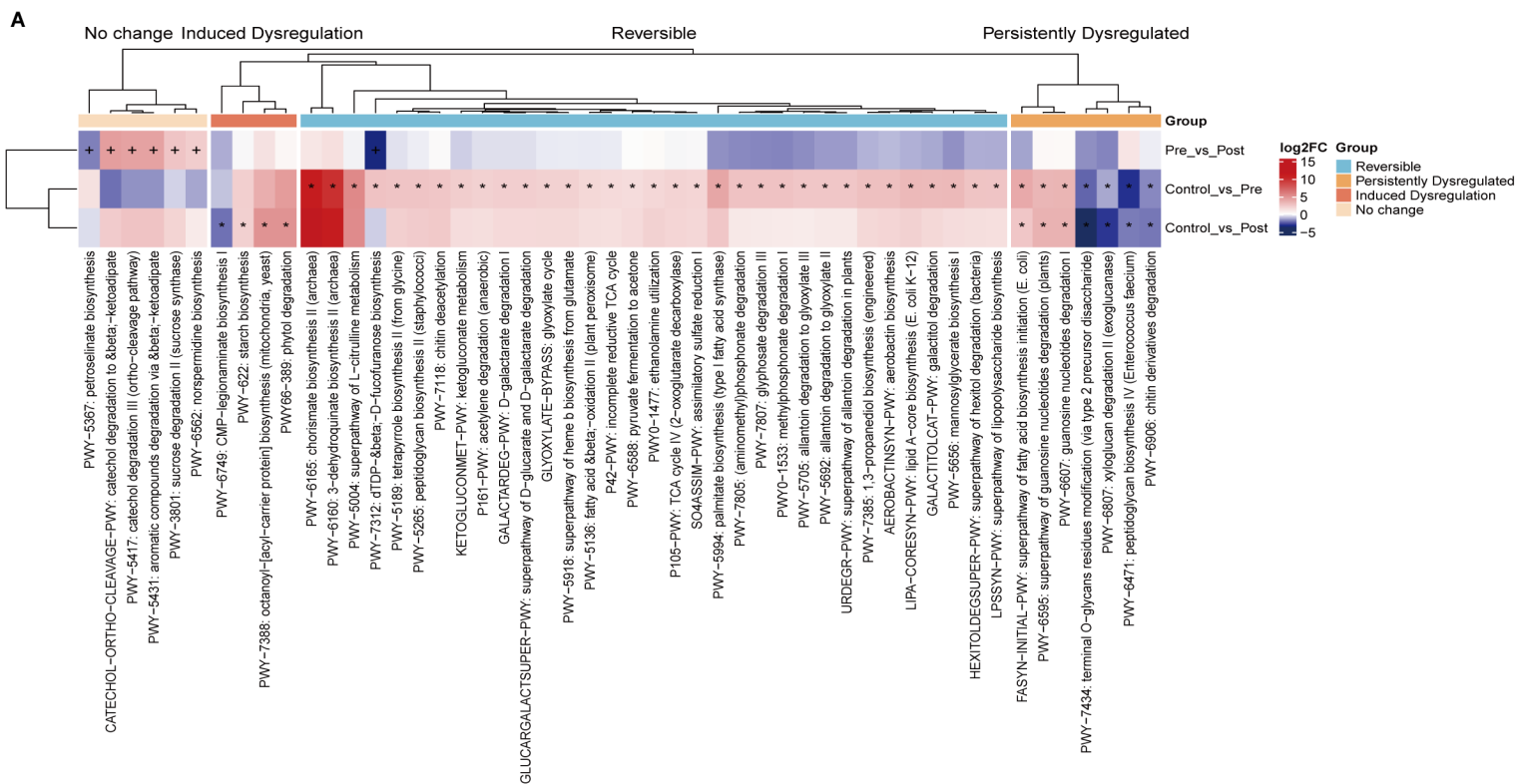
Table 4. Analysis of differential metabolites.



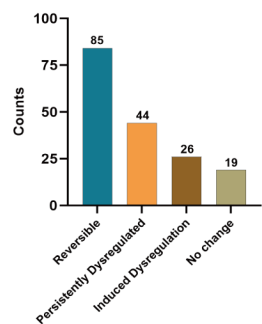




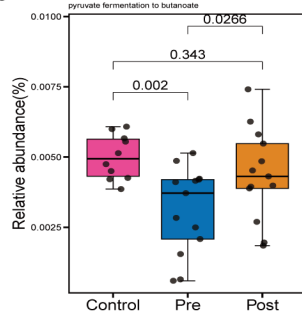




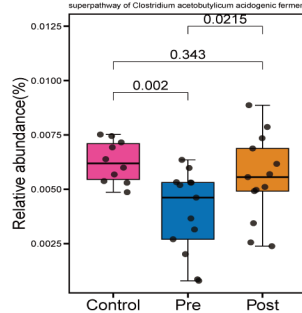
B MetaCyc pathway recovery categories



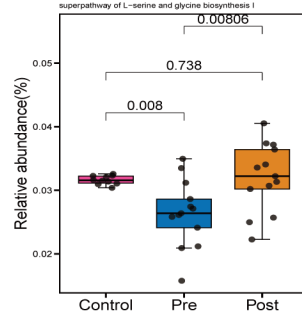
C CENTFERM-PWY



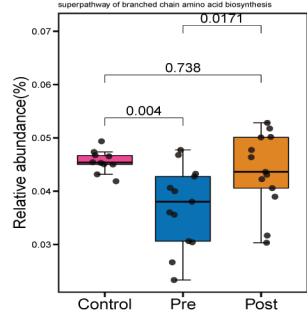
D PWY-6590



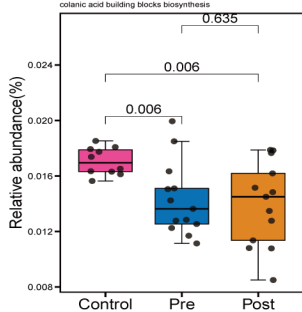
E SER-GLYSYN-PWY



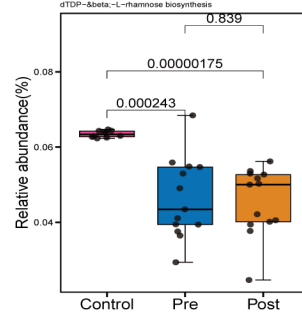
F BRANCHED-CHAIN-AA-SYN-P



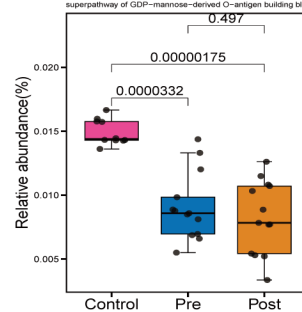
G COLANSYN-PWY

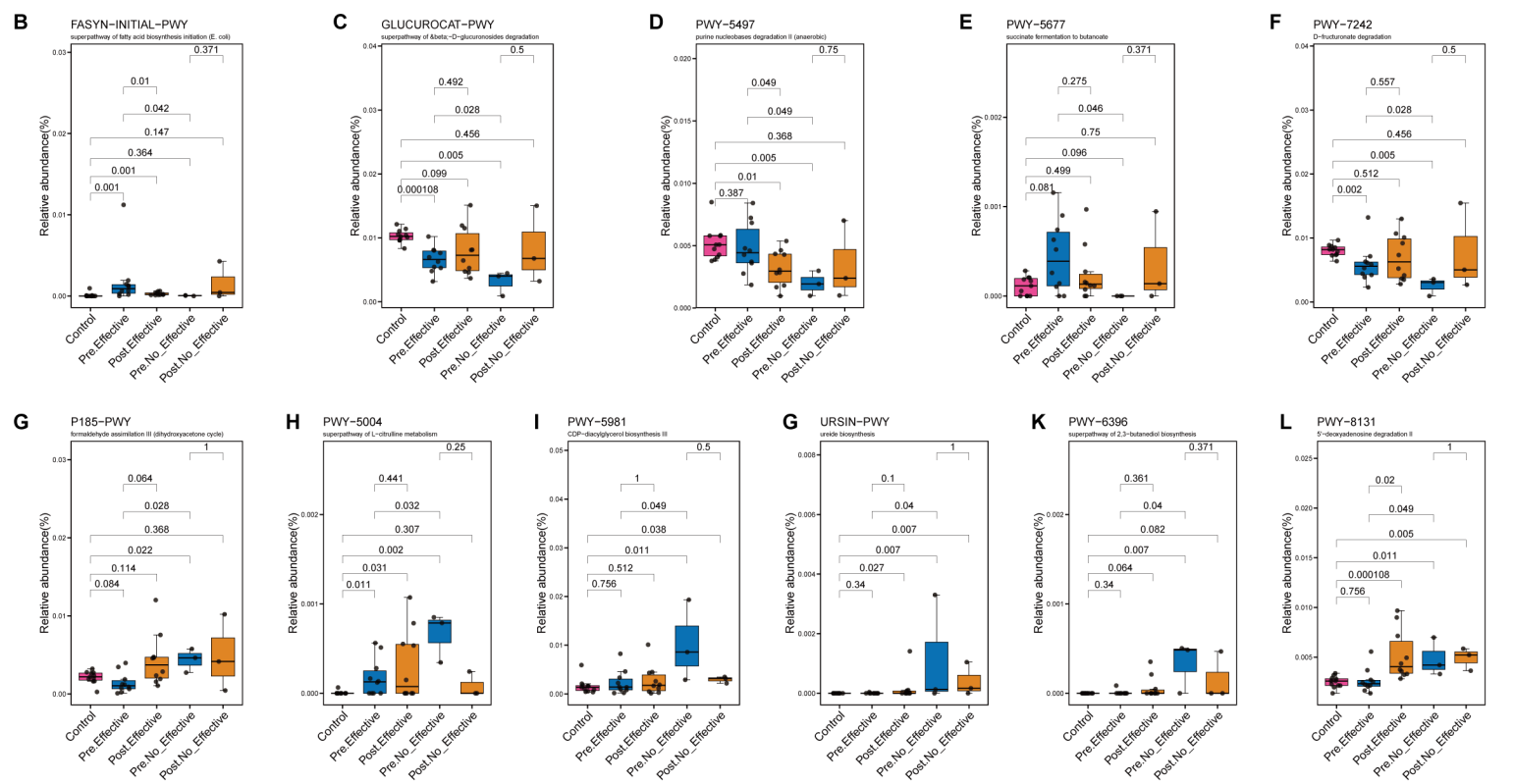
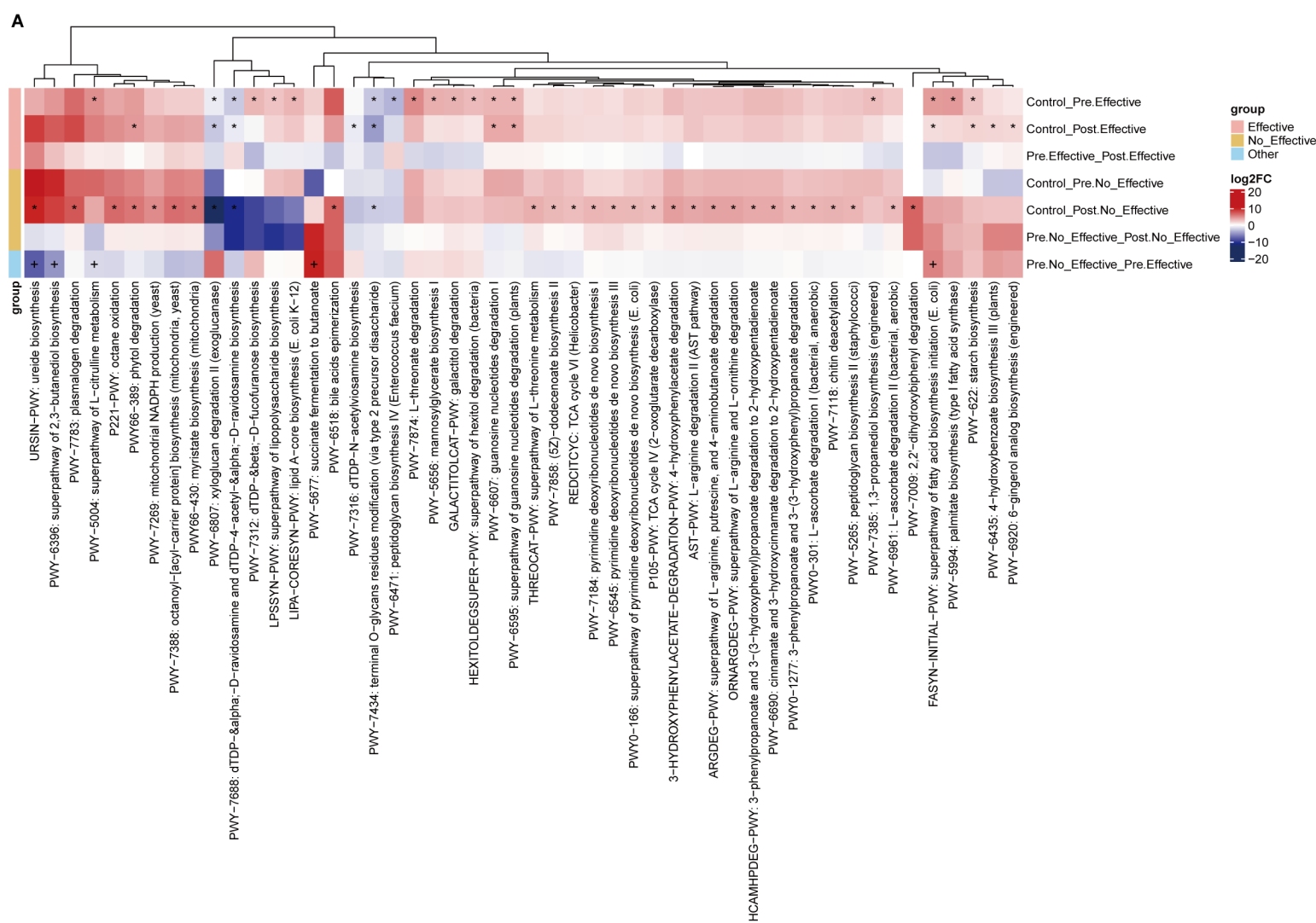


H DTDPRHAMSYN-PWY

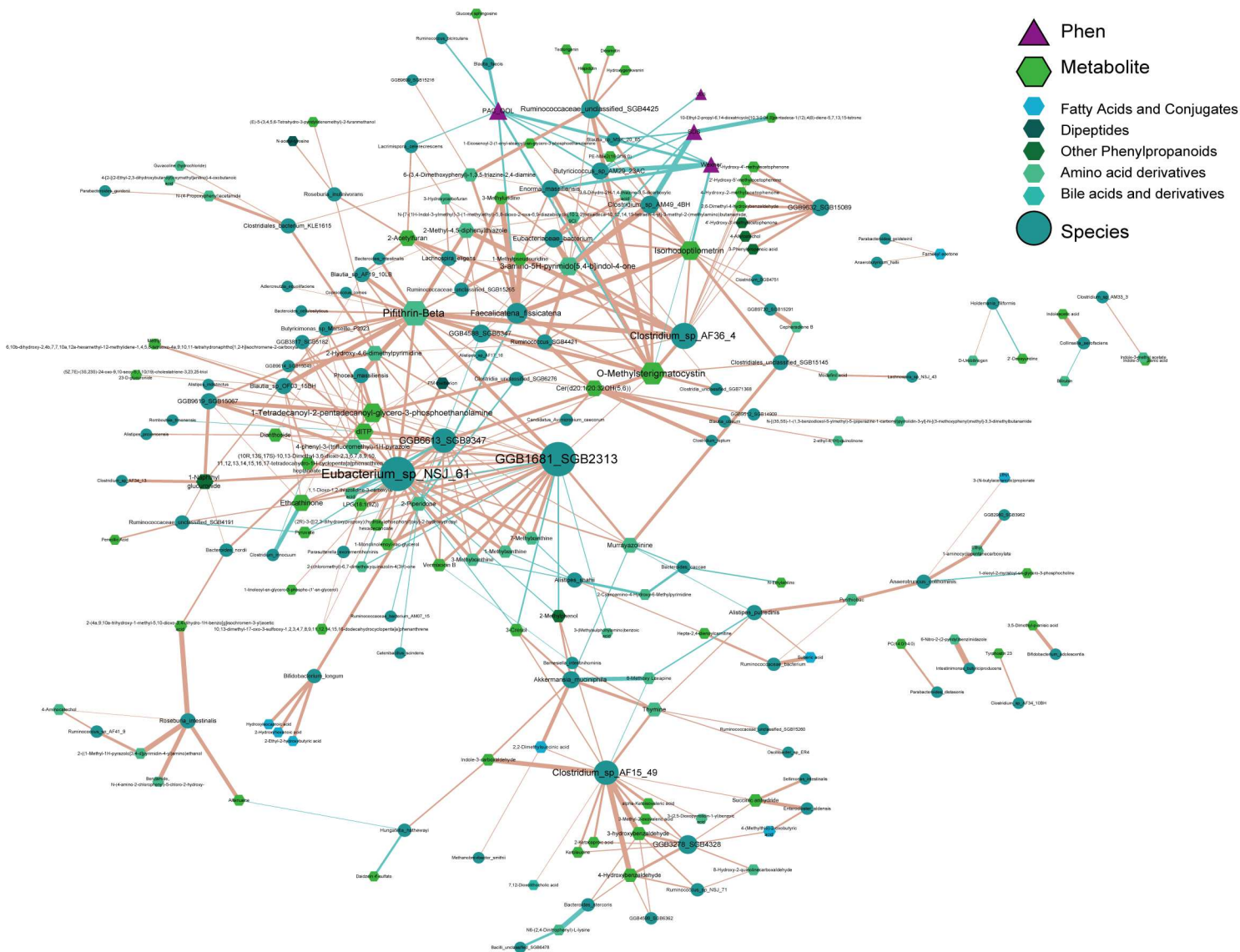


I PWY-7323

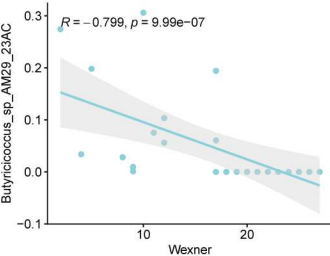




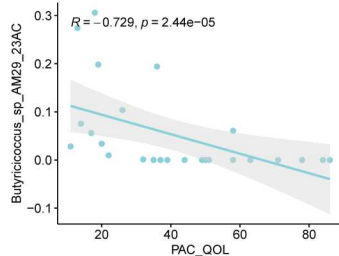
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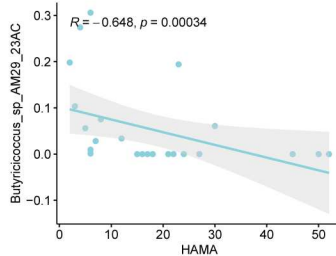
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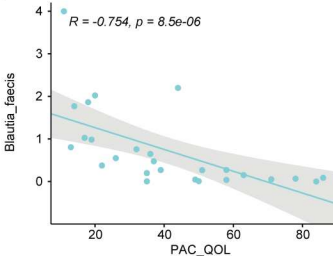
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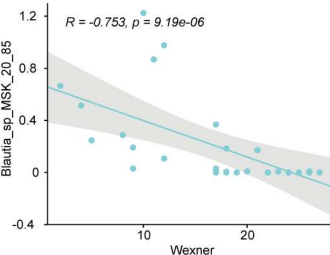
D



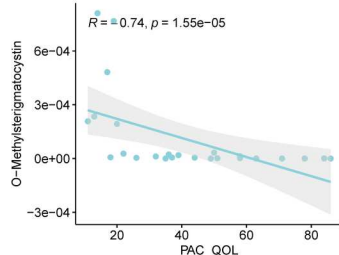
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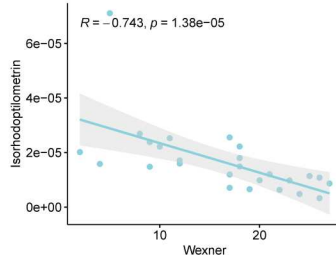
F



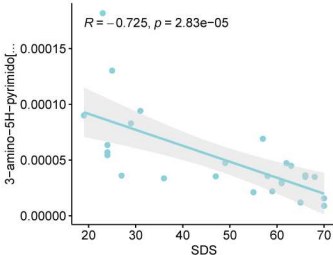
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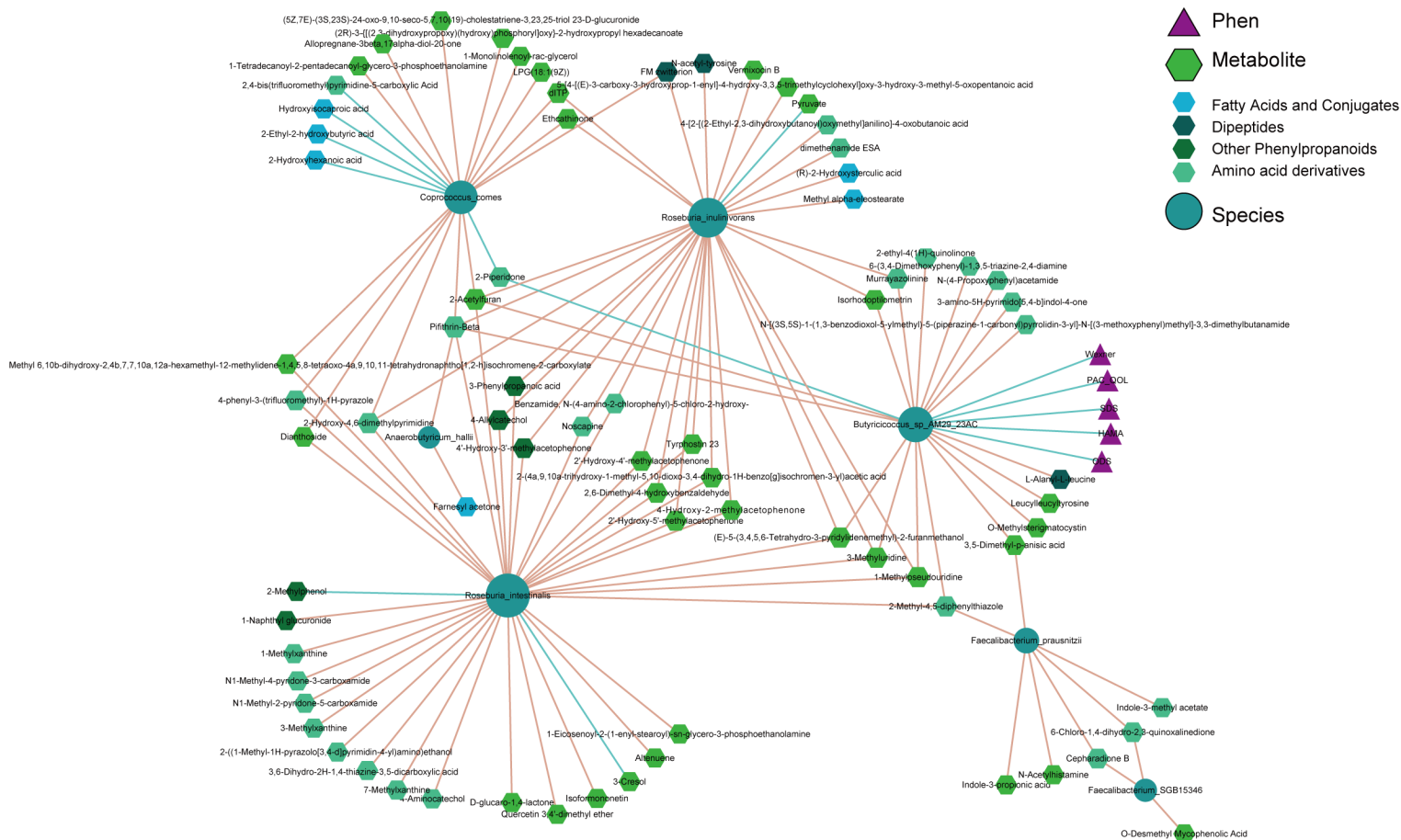


H



I





Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)
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- [Table3.docx](#)
- [Table4.docx](#)