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RESEARCH ARTICLE

Effects of Hexavalent Chromium Exposure on Gut Microbiota and Kidney Metabolism

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ABSTRACT

Hexavalent chromium [Cr(VI)] is a highly oxidized form of chromium that finds extensive application in numerous industrial activities, such as pigment manufacturing and stainless-steel production. Notably, these industrial activities generate waste containing Cr(VI), which could be released into the environment in substantial quantities, subsequently entering the body through drinking water contamination, bioaccumulation in crops, and inhalation. However, research examining the effects of Cr(VI) exposure on gut microbiota and kidney metabolism remains limited. Here, this study employed amplicon sequencing and metabolomics techniques to investigate the impacts of Cr(VI) on gut microbiota and kidney metabolism in rats. Results showed that Cr(VI) exposure could result in gut microbial dysbiosis, characterized by reduced α-diversity and significant alterations in microbial composition. Bacterial taxonomic analysis showed that the relative abundances of 2 phyla and 21 genera increased dramatically, while the relative abundance of 4 phyla and 48 genera decreased significantly during Cr(VI) exposure. Furthermore, metabolomic analysis demonstrated that Cr(VI) exposure induces kidney metabolic disorders, involving significant changes in 553 metabolites and 12 metabolic pathways. In summary, this research suggests that Cr(VI) exposure results in gut microbial dysbiosis and kidney metabolic disorders in rats. Given the increasing severity of the risks associated with the use and leakage of Cr(VI), this study provides crucial evidence regarding its health challenges. Moreover, this study also contributes to raising public awareness regarding the health threats associated with Cr(VI) exposure and establishes a foundation for regulating chromium waste discharge and protecting public health.

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INTRODUCTION

Chromium, a widely occurring mineral in the earth's crust, may enter the environment through various human activities, including ore mining, metal smelting, and wastewater treatment (Asri *et al*., 2018). It is considered a highly dangerous and bioaccumulative metal pollutant, exhibiting notable carcinogenic, teratogenic, and biological toxic effects (Baszuk *et al*., 2021). Statistically, the global demand for chromium exceeds 30 million tons annually and continues to rise. Chromium is primarily used in the production of automobile parts, electronic devices, stainless steel, and other products essential to human life. However, the constant advancement of automobiles and electronics, along with increasing demand, leads to higher chromium consumption and waste generation. Unfortunately, most chromium-containing metal products lack effective recycling methods and are often disposed of through deep burial or incineration, resulting in significant metal waste entering the ecosystem and posing risks to the ecological environment and life safety. Land and lakes serve as primary habitats for both aquatic and terrestrial

animals, as well as being the ultimate repository for metal waste. Early investigations have revealed the presence of heavy metals in water and soil, as well as in certain edible plants, aquatic animals, and terrestrial animals, suggesting a potential risk of heavy metal ingestion by humans or animals through the food chain (Soliman *et al*., 2024). Recently, some studies have highlighted reproductive toxicity, liver toxicity, and immune system toxicity in humans due to Cr(VI) exposure (Ni *et al*., 2020). Although the effects of chromium on the ecological environment and host health have garnered significant attention, most studies have concentrated on its distribution and migration in the environment, as well as its impact on wild animals. However, research on the effects of chromium on gut microbiota and renal metabolism remains limited. Given the crucial roles of gut microbiota and kidneys in host health and their interconnectedness, further investigation into the specific alterations in intestinal microbiota and renal metabolism during chromium exposure, as well as the potential interplay between the two during this period, is warranted.

Gut microbiota, recognized as the most complex and extensive microecosystem, plays a vital role in maintaining intestinal homeostasis and supporting host health (Bian *et al*., 2025). Moreover, gut microbiota has also been demonstrated to function in nutrient absorption, metabolism, immune system maturation, and intestinal mucosal barrier (Xu *et al*., 2025; Zhu *et al*., 2026). Notably, the execution and maintenance of these complex physiological functions depend on the gut microbial homeostasis. However, the gut microbiota is susceptible to many internal and external factors. Among the external factors, heavy metals are considered significant drivers of gut microbial imbalance (Porru *et al*., 2024). Previous studies have indicated that gut microbial dysbiosis can lead to the progression of various diseases, including diarrhea, irritable bowel syndrome, and colitis (Ali *et al*., 2026). Recent studies involving the gut microbiota have also revealed its important role in diabetes, nephritis, and atherosclerosis (Iatcu *et al*., 2021). Notably, although the gut microbiota inhabits the intestines, their influence can reach beyond the gastrointestinal tract, impacting various organs at both close and distant sites, including the liver and kidneys.

The kidney is the vital metabolic and excretory organ in animals and humans, which is associated with host health (Kobayashi *et al*., 2021). As a central participant in the interaction of host-microbiota, the health of the kidney has attracted growing attention. Previous studies have indicated that abnormal renal function can lead to the accumulation of toxins in the host, which may affect the intestinal environment through blood circulation, thereby disrupting the gut microbial balance (Kim *et al*., 2020). Several studies involving patients with chronic kidney disease have demonstrated a significant reduction in the diversity of gut microbiota, alongside an increase in the abundance of pathogenic bacteria. Gut microbial dysbiosis could result in the production of harmful metabolites, such as indole sulfate and tosilate, which can exacerbate kidney damage via the gut-kidney axis. Moreover, gut microbial dysbiosis may also cause increased intestinal permeability, making it easier for bacteria, endotoxins, and inflammatory factors to enter the blood, triggering a systemic inflammatory response and further exacerbating the progression of chronic kidney disease (Chen *et al*., 2019). Conversely, a stable gut microbiota can produce beneficial metabolites, including short-chain fatty acids, which inhibit inflammatory responses and protect renal vascular endothelial cells, thereby reducing the risk of kidney disease (Zhao *et al*., 2023). The interaction between the intestine and the kidney is bidirectional, which is a prerequisite for the normal functioning and health maintenance of the gut and kidney. Consequently, maintenance of the gut microbial homeostasis is a promising strategy for alleviating kidney injury.

MATERIALS AND METHODS

Animal experiments and sample collection: A group of 30 healthy rats with similar weight and age purchased from the laboratory animal center (Wuhan, China) were used for animal experiments. Before the formal experiment, the rats underwent a physical examination and acclimatized to the housing environment for a duration of seven days. This acclimatization period was implemented to mitigate the potential impact of stress and health status on the experimental outcomes. The rats were housed in a standardized environment and provided with an adequate diet and drinking water. After the adaptive feeding, the rats were randomly divided into two groups, with 15 rats in each group. The rats in the model group (M) received the same diet as the control group (C) but were gavaged with 0.07424 mg/kg potassium dichromate (K2Cr2O7) to induce Cr(VI) poisoning over the 35-day experiment. Concurrently, the control group (C) received the same volume of saline as the experimental group. Upon completion of the experiment, all rats were euthanized, and ileal contents and kidney tissues were collected. The ileal contents and kidney were placed in cryovials, rapidly frozen with liquid nitrogen, and stored at -80^{o}C for amplicon sequencing and metabolomics analysis.

16S rDNA Amplicon Sequencing: The extraction of bacterial DNA, sequencing of gut microbiota, bioinformatics, and statistical analysis are all based on previous research (Li *et al*., 2021a).

Metabolomics Analysis: Untargeted metabolomics was used for examining the changes in kidney metabolism. The metabolomics methodology, including sample preparation, identification of metabolites, data processing, and analysis of metabolic pathways, was informed by prior research (Li *et al*., 2021b).

RESULTS

Data acquisition and analysis: To investigate the changes in gut microbiota during Cr(VI) exposure, amplicon sequencing was performed on intestinal contents from both treatments, yielding a total of 962,824 raw sequences (C = 482,516, M = 480,308) (Table 1). These data were then subjected to the quality assessment, and 772,539 (C = 388,927, M = 383,612, ranging from 61,396 to 75,665 per sample) valid sequences were screened in both groups, whose qualification rate is over

80%. Following taxonomic assignment, the obtained sequences were clustered into 1,924 OTUs (C = 1274, M = 858, varying from 148 to 374 OTUs) as per the 97% sequence similarity (Fig. 1A-C). Among these identified OTUs, the number of individual OTUs was 1,066 and 650 in the C and M groups, respectively. Notably, there were 208 core OTUs in C and M groups, accounting for approximately 5.04% of the total OTU quantity. Multi-sample rarefaction abundance curves illustrating sequencing depth and uniformity tend to saturate, suggesting that nearly all bacterial species present in the sample can be detected without necessitating an increase in sequencing depth (Fig. 1D-F).

Table 1: Analysis of effective sequences generated by amplicon sequencing.

Sample	Raw Reads	Clean Reads	Denoised Reads	Merged Reads	Effective Reads
C1	79987	73758	70755	68002	64953
C2	79964	72699	69545	65833	62127
C3	82752	77413	77031	76628	75665
C4	79879	72031	68531	64902	61396
C5	79923	72178	69215	65858	62624
C6	80011	71405	68355	64627	62162
M1	80063	73392	70031	67123	62695
M2	79986	74219	71669	69547	64772
M3	80087	74948	72610	70975	65215
M4	79974	73633	70955	68089	63801
M5	80102	74248	71836	69830	64113
M6	80096	73602	70551	67847	63016

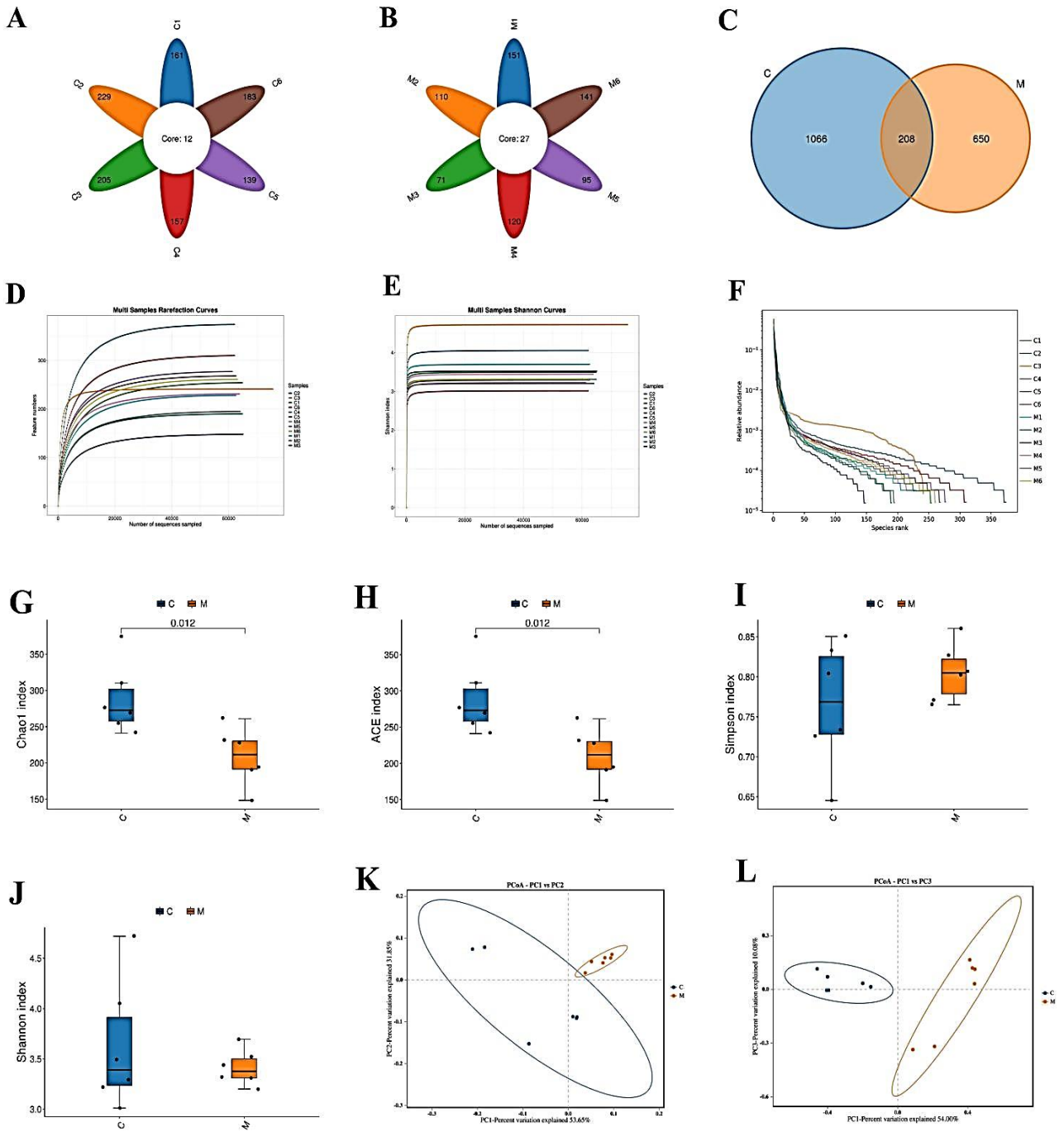

Fig. 1: Analysis of gut microbiota associated with Cr(VI) exposure. (A, B, C) The Venn diagram and the bar diagram show the number of OTUs per group and each sample, respectively. (D, E, F) The rarefaction curve reflects the sequencing depth and uniformity of the gut microbiota. (G, H I, J) The Chao1, ACE, Simpson, and Shannon indices are employed to assess the gut microbial alpha diversity. (K, L) Differences and similarities in the gut microbial structures across various samples, both within and between groups, are visualized using PCoA plots.

Changes of gut microbial diversity related to Cr (VI) exposure: We also calculated the alpha and beta diversity indices based on the abundance of OTUs in each sample to analyze the impact of Cr(VI) exposure on gut microbial diversity. Intergroup analysis demonstrated significant differences in the ACE (287.95±19.92 versus 209.24±16.07, P=0.012) and Chao1 (287345±19.85 versus 208.90±16.11, P=0.012) indices, while no significant difference was observed in the Simpson (0.76±0.03 versus 0.80±0.01, P>0.05) and Shannon (3.63±0.26 versus 3.41±0.07, P>0.05) indices between C and M groups (Fig. 1G-J). These results showed that Cr(VI) exposure dramatically reduced the gut microbial abundance but had no effect on their diversity. Furthermore, we also generated PCoA plots to further investigate the impact of the Cr(VI) exposure on the beta diversity of gut microbiota. Results showed that samples within the same group were clustered together, whereas samples from different groups exhibited the opposite trend, suggesting that Cr(VI) can significantly alter the structure of the gut microbiota (Fig. 1K, L).

Changes of gut microbial composition related to Cr(VI) exposure: The gut microbiota of C and M groups detected 27 phyla and 528 genera. At the phylum level, *Firmicutes* (C = 90.61%, M = 96.16%) was the most prevalent phylum in both groups, followed by *Proteobacteria* (C = 2.60%, M = 0.99%) and *Bacteroidota* (C = 2.31%, M = 0.72%) (Fig. 2A). Other phyla such as *Cyanobacteria* (C = 0.67%, M = 0.31%), *unclassified_Bacteria* (C = 0.60%, M = 0.16%), *Campylobacterota* (C = 0.35%, M = 0.20%), *Acidobacteriota* (C = 0.20%, M = 0.07%) and *Gemmatimonadota* (C = 0.19%, M = 0.006%) in both groups were recognized in low abundance, which accounted for less than 0.5% of the total taxonomic groups identified. Among identified genus, the preponderant genus found in M group were *Lactobacillus* (67.48%), *unclassified_Bacilli* (9.25%), and *Ligilactobacillus* (6.91%) in descending order (Fig. 2B). Additionally, *Ligilactobacillus* (52.59%), *Romboutsia* (22.04%) and *Lactobacillus* (4.57%) were found to be abundant in the C group, accounting for approximately 80% of the total abundance. Notably, the visualised clustering heatmap was also generated to investigate the distribution and changes of various bacterial phyla and genera during Cr(VI) exposure (Fig. 2C).

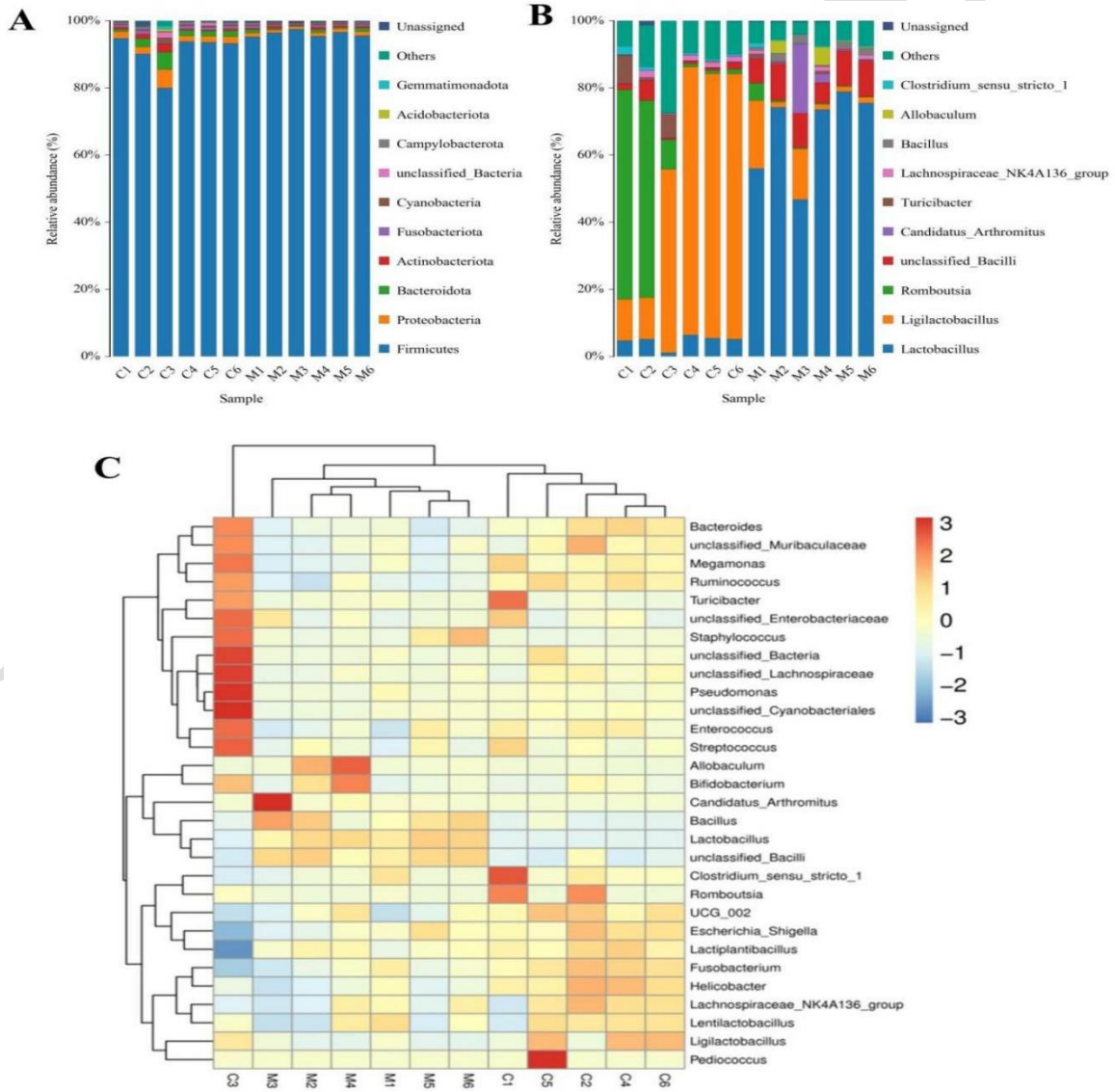

Fig. 2: Changes in composition and relative abundance of microbial taxa (A: phylum; B: genus) associated with Cr(VI) exposure. C: Bacterial species and variation of the top 30 genera in abundance are shown by the heatmap.

To further explore the gut microbial changes during Cr(VI) exposure, we used Metastats and LEfSe analysis to identify differential taxa at different levels. At the phylum level, the M group showed a significant increase in the relative abundances of *Firmicutes* and *Spirochaetota*, whereas the levels of *Campylobacterota*, *Proteobacteria*, *Bacteroidota,* and *Entotheonellaeota* were significantly decreased (Fig. 3A). Additionally, we also found that 69 genera exhibited significant differences between the C and M groups (Fig. 3B, C). Among them, the relative abundances of 48 bacterial genera (*Ruminococcus*, *Bacteroides*, *Acinetobacter*, *Megamonas*, *Helicobacter*, *Ligilactobacillus*, *Prevotellaceae_UCG_001*, *Akkermansia*, *Alloprevotella*, *Moheibacter*, *Erysipelatoclostridium*, *Prevotellaceae_NK3B31_group*, *Parasutterella*, *Blautia*, *Enterococcus*, *Subdoligranulum*, *[Eubacterium]_siraeum_group*, *MND1*, *hgcI_clade*, *Tyzzerella*, *Butyricicoccus*, *Globicatella*, *Delftia*, *unclassified_Lactobacillaceae*, *Anaerobiospirillum*, *unclassified_Clostridia*, *Sphingomonas*, *Gilliamella*, *Pseudolabrys*, *Enterorhabdus*, *Lachnoclostridium*, *Romboutsia*, *Collinsella*, *Alistipes*, *Haemophilus*, *Prevotellaceae_Ga6A1_group*, *Streptococcus*, *Peptococcus*, *Proteus*, *Bradyrhizobium,* etc.) significantly decreased, whereas the relative richness of 21 bacterial genera (*Lactobacillus*, *Bacillus*, *Olsenella*, *Erysipelotrichaceae_UCG_003*, *Family_XIII_UCG_001*, *Limibaculum*, *Sulfurospirillum*, *Treponema*, *Vicinamibacter*, *Allobaculum*, *Coriobacteriaceae_UCG_002*, *Clostridiales_bacterium_42_27*, *Lawsonia,* etc.) significantly increased during Cr(VI) exposure. Moreover, Cr(VI) exposure even resulted in 11 bacterial genera (*[Eubacterium]_siraeum_group*, *MND1*, *hgcI_clade*, *Tyzzerella*, *Globicatella*, *Delftia*, *Anaerobiospirillum*, *Pseudolabrys*, *Enterorhabdus*, *Lachnoclostridium*, *Proteus*) that cannot be observed in the gut microbiota. Besides the above-mentioned differential taxa, the M group was dramatically enriched for *unclassified_Acinetobacter*, *Acinetobacter*, *unclassified_Muribaculaceae*, *Ligilactobacillus,* and *Romboutsia*, while *Candidatus_Arthromitus* was dramatically overrepresented in the C group (Fig. 4A, B). Additionally, we also performed correlation network analysis to reveal the potential relationships among bacterial genera (Fig. 5).

Changes of kidney metabolism related to Cr(VI) exposure: To further investigate the metabolic changes in rats exposed to Cr (VI), multivariate statistical analysis, including PCA and OPLS-DA, was employed to elucidate the potential relationship between metabolomics and biological characteristics. The samples of the Cr(VI) exposure group in PCA and OPLS-DA were distinctly separated from those of the control individuals, indicating the significant differences in intestinal metabolism between C and M groups (Fig. 6A, B). Additionally, the differential metabolites were selected based on the criteria of VIP > 1 and P<0.05, and their abundances were visualized through a clustering heatmap (Fig. 6C). Results indicated significant differences in 553 metabolites between the C and M groups (Fig. 7A). Among them, the proportions of 273 metabolites increased dramatically, whereas 280 metabolites showed a significant decrease during Cr(VI) exposure.

Subsequently, we generated metabolic pathways associated with Cr(VI) exposure based on differential metabolites (Fig. 7B). Among enriched pathways, 12 kidney metabolic pathways with a significant difference were the steroid hormone biosynthesis, ovarian steroidogenesis, parkinson disease, fatty acid biosynthesis, cysteine and methionine metabolism, thiamine metabolism, sulfur metabolism, glutathione metabolism, tryptophan metabolism, fructose and mannose metabolism and tyrosine metabolism, which involved in 49 potential biomarkers including androstenediol, cholesterol, tetrahydrocorticosterone, cortol, urocortisol, L-Tyrosine, dopamine quinone, pyrophosphate, L-Cysteine, L-Cystathionine, sulfite, trypanothione, trypanothione disulfide and glutathionylspermidine, etc (Table 2). The representative metabolic diagram in the kidney (Fig. 7C).

Table 2: Differentially expressed metabolic pathways and corresponding kidney metabolic biomarkers between the C and M groups. Fold change (FC): ratio of relative abundance of metabolites in the C and M groups.

Pathway	Biomarkers	P	VIP	FC	trend
	Androstenediol	0.01	1.89	2.61	up
	Cholesterol	0.02	1.78	0.26	down
Steroid	Tetrahydrocorticosterone	0.01	1.87	0.31	down
hormone	Cortol	0.01	1.70	0.40	down
biosynthesis	Urocortisol	0.04	1.76	1.5×10^{-8}	down
Ovarian	Androstenediol	0.01	1.89	2.61	up
steroidogenesis	Cholesterol	0.02	1.78	0.26	down
	L-Tyrosine	0.008	1.72	0.81	down
Parkinson	Dopamine quinone	0.001	1.86	0.56	down
disease	Pyrophosphate	0.020	1.58	0.59	down
	UDP-glucose	0.01	1.54	1.09	up
Starch and	Isomaltose	0.003	1.73	3.01	up
sucrose	D-Fructose	0.007	1.74	1.55	up
metabolism					
	Caprylic acid	0.02	1.56	5.38	up
Fatty acid	Hexadecanoic acid	0.01	1.67	4.54	up
biosynthesis	(9Z)-Octadecenoic acid	0.03	1.42	1.13	up
	L-Cysteine	0.002	1.78	2.29	up
	L-Cystathionine	0.02	1.43	0.43	down
Cysteine and	Sulfite	0.01	1.52	1.84	up
methionine	5-Methylthio-D-ribose	0.008	1.61	0.35	down
metabolism	Sulfate	0.00001	2.07	1.21	up
	S-Adenosyl-L-homocysteine	0.04	1.35	0.83	down
Thiamine	L-Tyrosine	0.008	1.72	0.81	down
metabolism	L-Cysteine	0.002	1.78	2.29	up
	4-Amino-5-aminomethyl-2-methylpyrimidine	0.0008	1.87	0.55	down
	D-Glyceraldehyde 3-phosphate	0.04	1.27	1.10	up
Sulfur	L-Cysteine	0.002	1.78	2.29	up
metabolism	Sulfite	0.01	1.52	1.84	up
	Sulfate	0.00001	2.07	1.21	up
	Dimethyl sulfoxide	0.04	1.32	1.08	up
Glutathione	L-Cysteine	0.002	1.78	2.29	up
metabolism	Trypanothione	0.03	1.46	0.07	down
	5-Oxoproline	0.01	1.48	1.37	up
	Trypanothione disulfide	0.01	1.52	0.69	down
	Glutathionylspermidine	0.01	1.71	6.88	up
Tryptophan	4,8-Dihydroxyquinoline	0.03	1.43	0.43	down
metabolism	5-(2'-Formylethyl)-4,6-dihydroxypicolinate	0.01	1.49	1.32	up
	Tryptamine	0.007	1.66	0.75	down
	3-Methyldioxyindole	0.02	1.53	1.10	up
	L-Kynurenine	0.01	1.51	0.64	down
	Indole-3-ethanol	0.02	1.50	2.16	up
Fructose and	L-Rhamnose	0.02	1.46	0.67	down
mannose	D-Fructose	0.007	1.74	1.55	up
metabolism	D-Glyceraldehyde 3-phosphate	0.04	1.27	1.10	up
Tyrosine	L-Tyrosine	0.008	1.72	0.81	down
metabolism	3-(3,4-Dihydroxyphenyl)pyruvate	0.007	1.75	0.40	down
	Rosmarinate	0.002	1.78	2.28	up
	5,6-Dihydroxyindole	0.012	1.55	0.58	down
	Maleic acid	0.0006	1.90	1.75	up

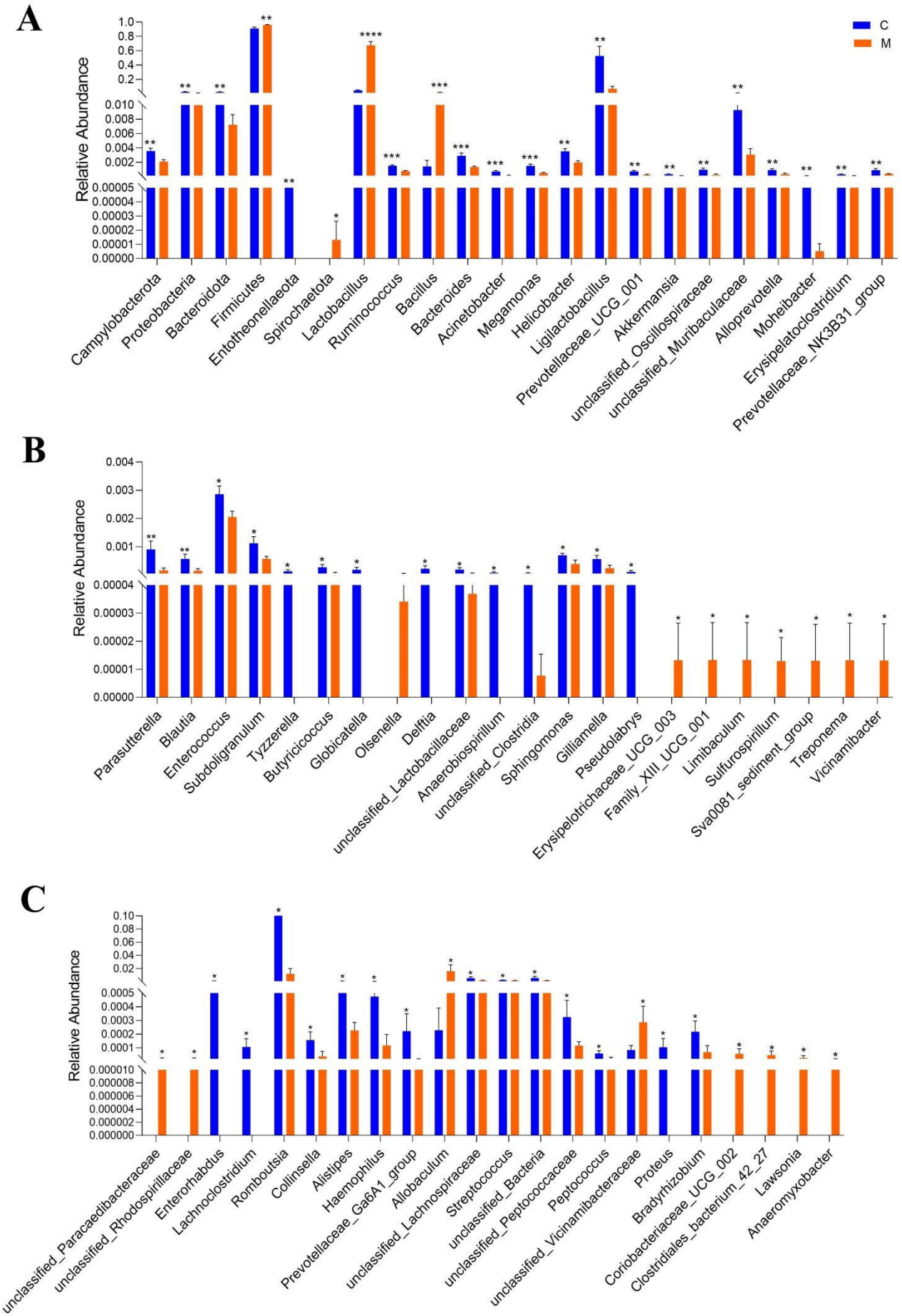

Fig. 3: Statistical analysis of differential phylum (A) and genus (B, C) associated with Cr(VI) exposure.

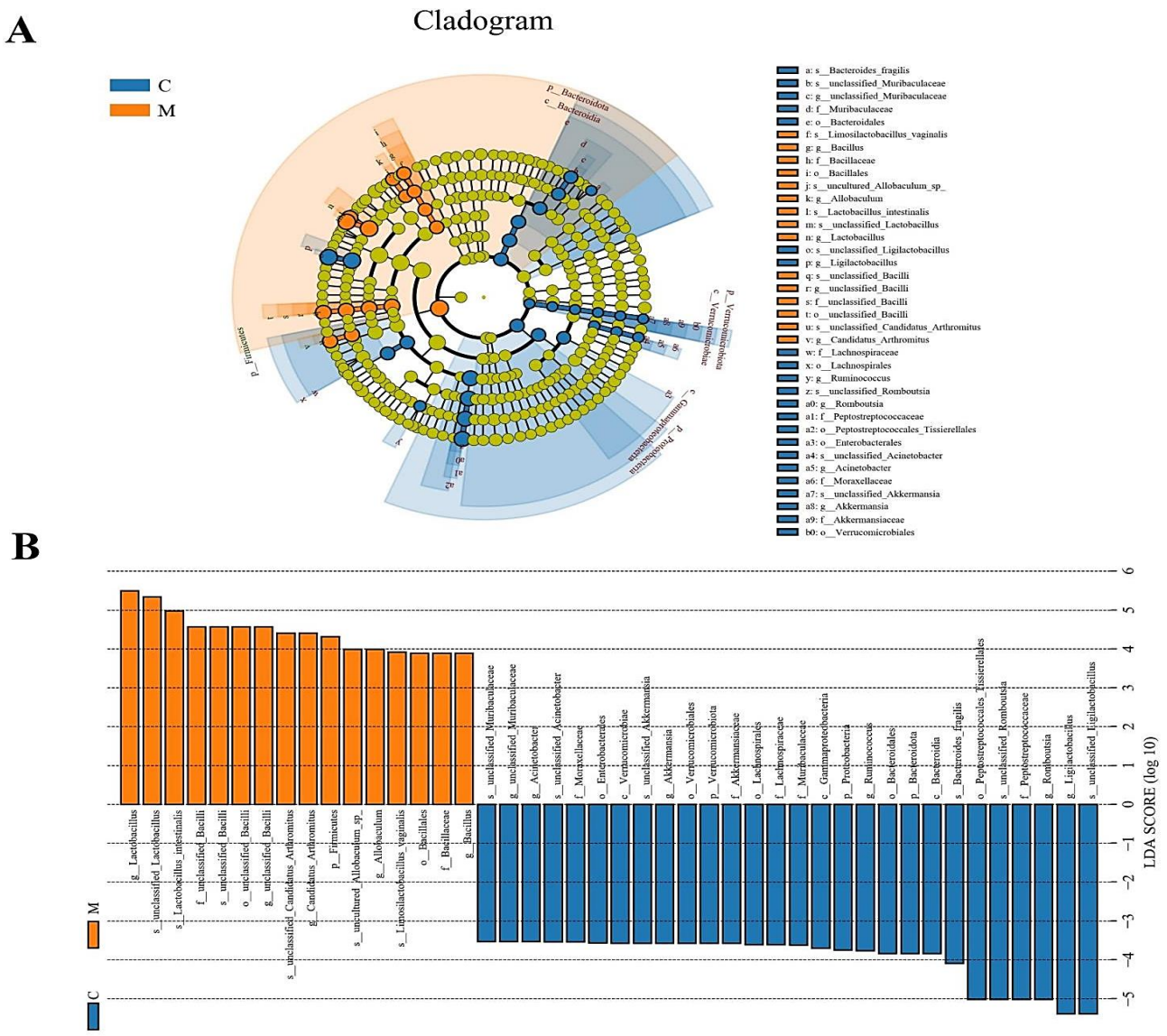

Fig. 4: Phylogenetic distribution of differential biomarkers and gut microbial correlation analysis. A: The cladogram showing the relationships between differential biomarkers. The taxa with no distinct differences were indicated by yellow circles. B: The criterion for distinct difference is LDA scores > 4.

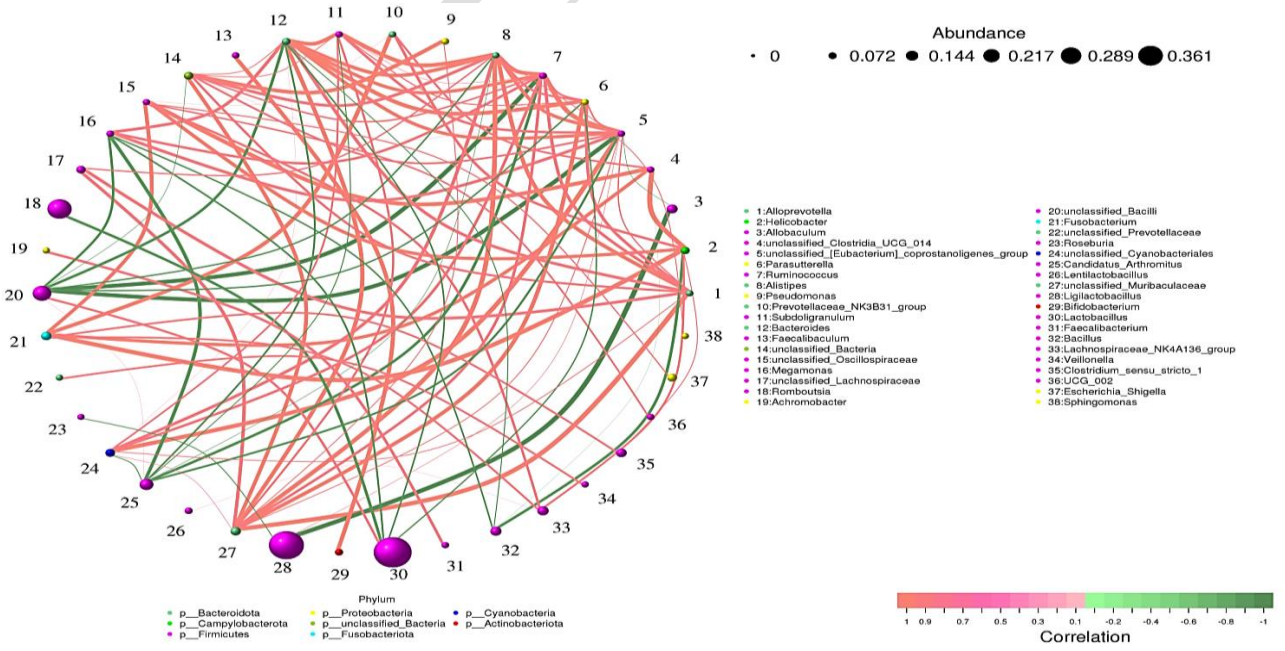

Fig. 5: Species correlation network diagram of gut microbial community.

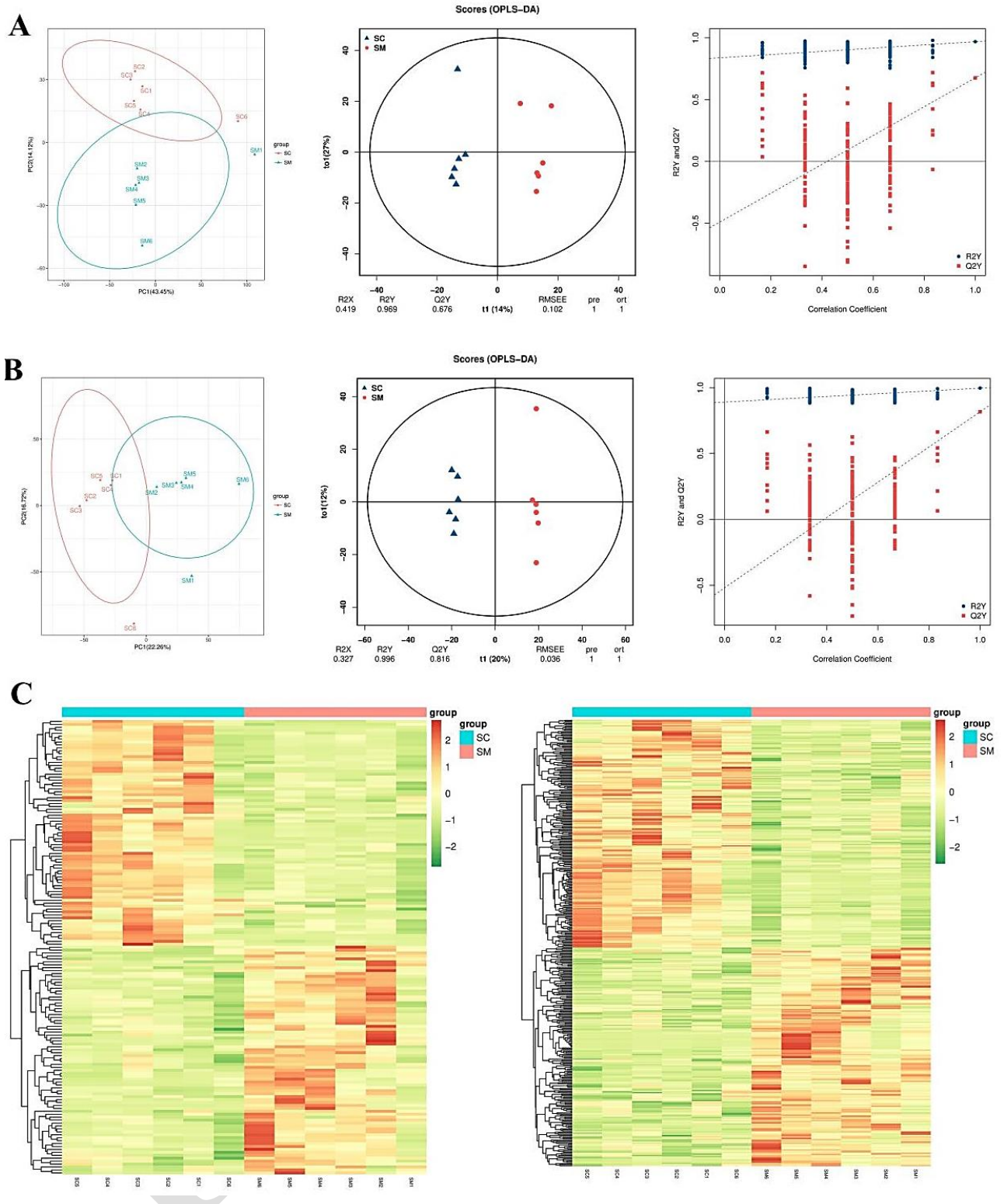

Fig. 6: Cr(VI) exposure disrupts kidney metabolism. PCA score plots, OPLS-DA score plots, and permutation tests of the OPLS-DA model in positive (A) and negative ion modes (B). C: Hierarchical clustering heat map summarizes changes in differential metabolites between groups.

Correlation analysis for differential bacteria and metabolites: To further investigate the relation between gut microbiota and kidney metabolites, we selected representative bacteria and metabolites to create a metabolite-microbe interaction heat map (Fig. 8). Results revealed that L-Tyrosine was positively related to *Acinetobacter*, *Akkermansia*, *Alistipes*, *Alloprevotella*, *Bacteroides*, *Haemophilus*, *Ligilactobacillus*, *Megamonas*, *Prevotellaceae_UCG_001,* and *Ruminococcus*. *L-Cysteine* was positively correlated with *Bacillus* and *Olsenella* but negatively associated with *Delftia*, *Ligilactobacillus*, *Prevotellaceae_NK3B31_group*, and *Ruminococcus*. Trypanothione was positively correlated with *Acinetobacter*, *Akkermansia*, *Alistipes*, *Alloprevotella*, *Bacteroides*, *Blautia*, *Butyricicoccus*, *Gilliamella*, *Haemophilus*, *Ligilactobacillus*, *Megamonas*, *Prevotellaceae_Ga6A1_group*, *Prevotellaceae_UCG_001,* and *Ruminococcus*.

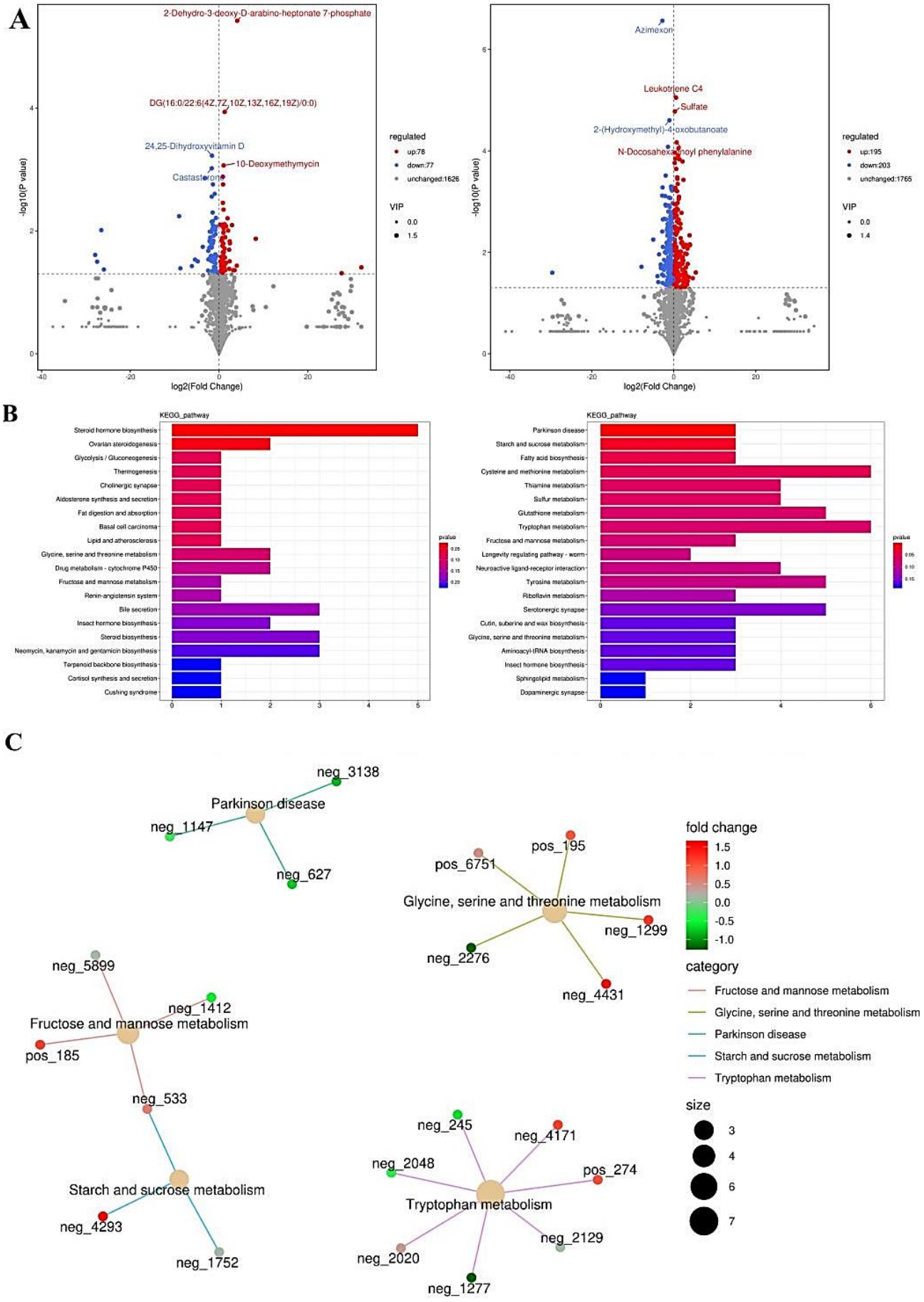

Fig. 7: Differentially expressed kidney metabolites and metabolic pathways linked to Cr(VI) exposure. (A) Volcano plots displaying the differential metabolites captured in both positive-ion and negative-ion modes. (B) Analysis of enrichment concerning metabolic pathways in both modes. (C) The representative network diagram of metabolites and associated metabolic pathways. The numbers represent the corresponding metabolites.

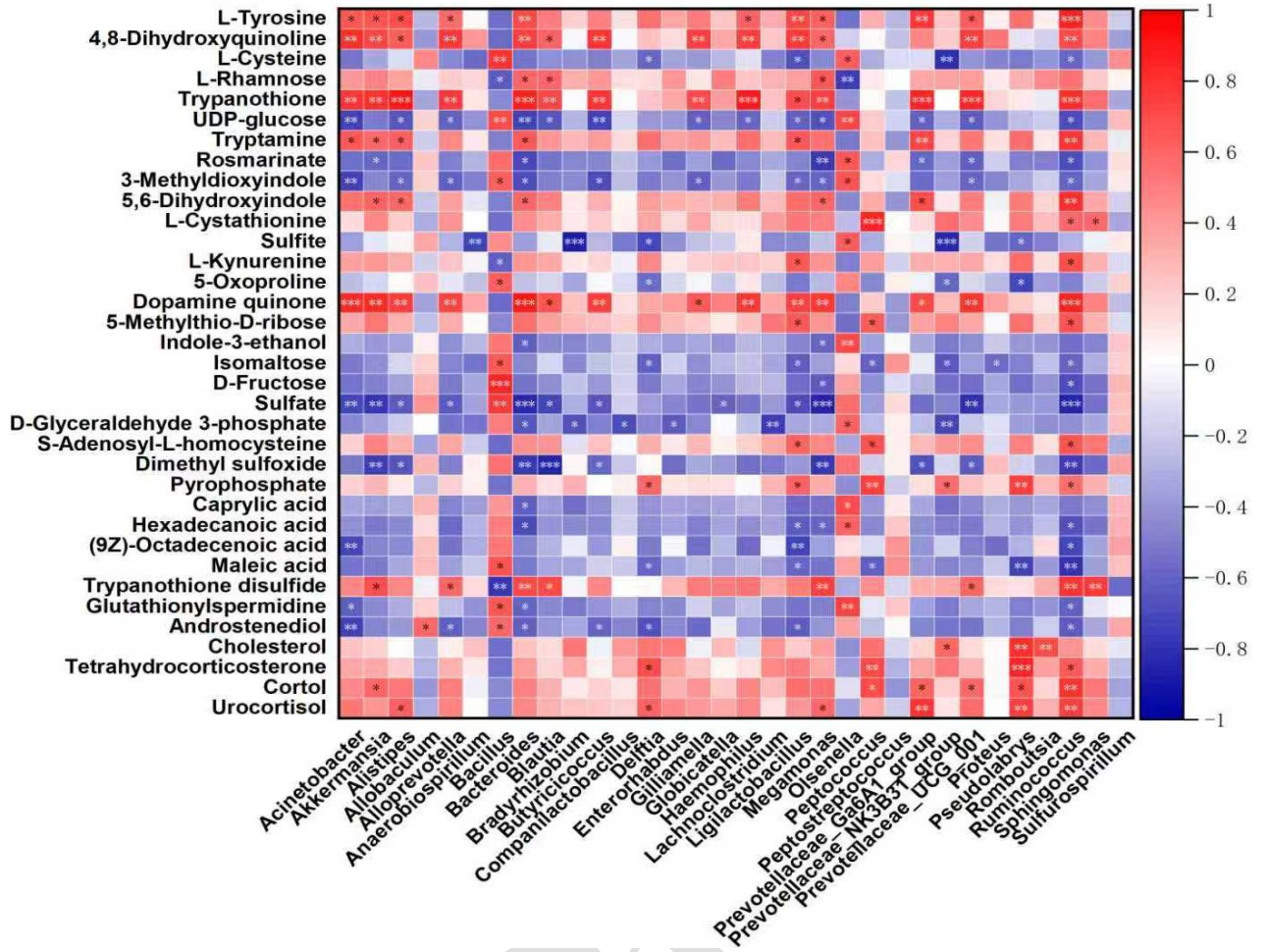

Fig. 8: Analysis of the correlation between gut microbiota and kidney metabolites. The horizontal axis denotes bacterial species, while the vertical axis represents metabolites.

DISCUSSION

Studies have indicated that heavy metals in the environment can accumulate in soil, groundwater, and plants, resulting in global environmental safety and public health issues (Song *et al*., 2024). Currently, numerous studies have reported gastrointestinal, cardiovascular, and respiratory diseases associated with both direct and indirect exposure to metal pollutants (Pan *et al*., 2024). Consequently, the detrimental effects of heavy metal pollution on environmental safety and animal health have garnered increasing attention. Hexavalent chromium, recognized as one of the most hazardous metal pollutants, has been linked to lung cancer, liver and kidney damage, and skin ulcers. However, there remains a paucity of research on the impact of Cr(VI) exposure on the gut microbiota and kidney health of rats. Here, we systematically explored the effects of Cr(VI) exposure on the gut microbiota and kidney health of rats. Results showed that exposure to Cr(VI) may lead to gut microbial dysbiosis and disrupt kidney metabolism in rats.

As a crucial and sensitive indicator, gut microbiota is inevitably influenced and dynamically varies within limits based on animal species, age, and genotype (Hou *et al*., 2025). Typically, these physiological changes could not destroy normal intestinal function. However, long-term strong external stimulus, particularly heavy metals, can influence microbial growth and survival in the intestine, which in turn changes the gut microbial composition and diversity and forces existing microorganisms to adapt to a new environment, causing the disturbance of gut microbial homeostasis. Early survey indicates that long-term exposure to Cr(VI) significantly decreased gut microbial diversity of chicken (Li *et al*., 2021a). Similarly, Yao *et al*. (2019) also found a significant decrease in gut microbiota diversity in bufonid during Cr(VI) exposure. In agreement with earlier research, this study demonstrated that perennial Cr(VI) exposure could decrease gut microbial diversity of rats. These results all emphasized the detrimental effects of Cr(VI) exposure on the host gut microbiota. We speculated that Cr(VI) may further compromise host health by disrupting the homeostasis of gut microbiota.

Notably, this study also showed that Cr(VI) exposure causes significant changes in several functional bacteria, which may play key roles in maintaining intestinal health and function. Moreover, most of these quantitatively decreased bacteria (*Ruminococcus*, *Bacteroides*, *Ligilactobacillus*, *Prevotellaceae_UCG_001*, *Akkermansia*, *Prevotellaceae_NK3B31_group*, *Blautia*, *Alloprevotella*, *Lachnoclostridium*, *Romboutsia*, *Prevotellaceae_Ga6A1_group*, *Butyricicoccus,* and *Alistipes*) in the Cr(VI) group were considered as intestinal beneficial bacteria. *Akkermansia* is an important beneficial gut bacterium that plays a variety of important physiological roles. For instance, *Akkermansia* maintains intestinal homeostasis by degrading mucin and promoting

the secretion of mucus from goblet cells, thereby enhancing the integrity of the intestinal barrier (Regnier *et al*., 2020). *Akkermansia* also exhibits significant potential in the contexts of obesity, diabetes, inflammatory bowel disease, autoimmune disorders, and cancer immunotherapy by increasing the thickness of the mucus layer, regulating immune cell function, and improving metabolic homeostasis (Regnier *et al*., 2020). Moreover, several studies have highlighted the crucial role of *Akkermansia* in cancer prevention, anti-aging, and alleviating depression (Gubernatorova *et al*., 2023). *Romboutsia* plays a crucial role in intestinal function by breaking down carbohydrates such as D-glucose, D-sucrose, and D-maltose (Li *et al*., 2021b). *Butyricicoccus* is a significant component of a healthy microbiome, and its deficiency has been closely associated with the development of colorectal cancer (Eeckhaut *et al*., 2013). Early investigations have indicated that *Alloprevotella* not only produces acetate and succinate but also contributes to reducing the risk of lifelong cardiovascular disease (Han *et al*., 2024). *Ligilactobacillus*, characterized by its unique surface structure and proteins, competes with pathogenic bacteria for adhesion sites on intestinal epithelial cells, effectively preventing the adhesion of harmful bacteria. Furthermore, this bacterium generates beneficial metabolites during its metabolism, thereby improving intestinal barrier function and enhancing host immune responses. In livestock production, *Ligilactobacillus* can serve as a feed additive to enhance the intestinal microecological balance and increase feed conversion rates. *Ruminococcus* is widely distributed in the digestive tract of mammals and can degrade complex carbohydrates, such as cellulose and resistant starch, thereby playing a significant role in host energy metabolism and intestinal homeostasis. Furthermore, *Ruminococcus* has demonstrated cardiovascular protective effects, with its abundance being associated with a reduced cardiovascular risk in obese individuals. *Prevotellaceae* has also been shown to degrade complex plant polysaccharides, including xylan and pectin, and possesses anti-inflammatory potential. Consequently, Cr(VI) may impact host energy intake and hinder growth and development by reducing the abundance of *Ruminococcus* and *Prevotellaceae*. Previous studies indicate that *Bacteroides* not only participate in the degradation of dietary fiber to provide energy for the host, but also regulate the immune system and affect metabolic balance (Qu *et al*., 2025). Notably, some bacteria (*Akkermansia, Ruminococcus*, *Alistipes*, *Blautia, Prevotellaceae, Bacteroides*) mentioned above have been demonstrated to produce short-chain fatty acids (SCFAs), which possess various biological properties (Holmberg *et al*., 2024). SCFAs are core metabolites produced by gut microbiota through the fermentation of dietary fiber, significantly influencing host health via multiple pathways. As a primary energy source for colonic epithelial cells, SCFAs enhance the intestinal barrier function and mitigate the risk of leaky gut by promoting the proliferation of intestinal mucosal cells and thickening the mucus layer (Ikeda *et al*., 2022). Additionally, SCFAs inhibit the colonization of pathogens such as *Escherichia coli* and *Salmonella* by lowering intestinal pH. Consistent with our study, previous studies on chickens and mice also provided evidence that Cr(VI) exposure could lead to a reduction in the abundance of SCFAs-producing bacteria.

In this study, we also conducted untargeted metabolomics to analyze changes in kidney metabolites exposed to Cr(VI). Metabolomic results indicated that 12 metabolic pathways (cysteine and methionine metabolism, thiamine metabolism, glutathione metabolism, tryptophan metabolism, and tyrosine metabolism, etc.) and 49 metabolites were involved in Cr(VI) exposure-induced kidney dysfunction. Amino acids serve as the fundamental building blocks of life and are crucial for protein synthesis, nutrient metabolism, and energy conversion. Consequently, abnormal amino acid metabolism will inevitably affect multiple physiological functions and endanger host health. In this study, we observed that Cr(VI) exposure induces alterations in both tryptophan and tyrosine metabolism. Increasing evidence supports the notion that gut microbiota can affect the production of metabolites through direct or indirect action, thereby affecting other organ systems such as the kidneys, brain, and liver. Notably, tryptophan metabolism is also regulated either directly or indirectly by gut microbiota, and its metabolites possess immune and metabolic functions, which are regarded as bridging substances between the host and gut microbiota. Moreover, tryptophan metabolism has been demonstrated to affect various pathophysiological processes. For instance, Ren *et al*. (2021) demonstrated that fisetin can relieve chronic kidney disease caused by hyperuricemia through regulating aryl hydrocarbon receptor activation and tryptophan metabolism mediated by gut microbiota. Moreover, Zhang *et al*. (2023) reported that Rosa laevigata Michx polysaccharide can alleviate diabetic nephropathy by mediating tryptophan metabolism, indicating the important role of this metabolic pathway in kidney disease. Consequently, Cr(VI) may drive kidney damage by mediating gut microbiota to disrupt tryptophan metabolism. Previous research has indicated that tyrosine metabolism changes significantly during acute kidney injury (Yang *et al*., 2024). For instance, Li *et al*. (2022) indicated that the alleviating effects of anthocyanins on diabetic kidney disease may be closely associated with the regulation of tryptophan metabolism and tyrosine metabolism, suggesting the significant roles these metabolic pathways play in kidney disease. Furthermore, Miao *et al*. (2017) found that pyrethroid insecticides can induce liver and kidney damage in mice, accompanied by significant changes in tyrosine metabolism and energy metabolism. These results all show the importance of tryptophan metabolism and tyrosine metabolism in kidney disease, which is consistent with our findings. Glutathione is a low-molecular-weight antioxidant that is widely distributed in cells and plays a crucial role in enhancing immunity, delaying aging, and maintaining the integrity of red blood cell membranes (Desideri *et al*., 2019). Additionally, it is involved in the tricarboxylic acid cycle and sugar metabolism, contributing to the reduction of cellular and DNA damage caused by harmful substances such as peroxides and heavy metals (Yang *et al*., 2024). Recent studies on glutathione have shown that it is closely related to the development of liver cancer, esophageal cancer, and chronic kidney disease (Peng *et al*., 2017; Qiu *et al*., 2021). Thiamine (vitamin B1) is an

important water-soluble vitamin necessary for ATP production and carbohydrate metabolism (Marrs and Lonsdale, 2021). Meanwhile, it is also a key and rate-limiting cofactor of multiple enzymes involved in the amino acid, glucose, and fatty acid pathways, which is closely related to host health and energy metabolism (Zhu *et al*., 2022). Wen *et al*. (2021) indicated that thiamine could alleviate subacute ruminal acidosis of goats caused by high-concentrate diets as well as improve intestinal antioxidant capacity and intestinal barrier function. Conversely, thiamine deficiency can perturb mitochondrial respiration and induce pseudo-hypoxia, which increases vascular reactivity, cell apoptosis, inflammation, and even organ dysfunction (Marrs and Lonsdale, 2021).

Conclusions: Taken together, this study explored the effects of Cr (VI) exposure on gut microbiota and renal metabolism in rats. Results demonstrated that Cr(VI) exposure leads to dysbiosis of the gut microbiota, primarily manifested as reduced microbial diversity and altered microbiota composition. Furthermore, Cr(VI) also impacts renal metabolic function. This study elucidates a multi-level mechanism of Cr(VI) pathogenesis, highlighting the complex interactions among environmental toxins, gut microbiota, and host metabolism. Additionally, this study provides a reference for the development of early microbial and metabolic biomarkers for monitoring Cr(VI) exposure and health warning systems. Future research should further integrate metagenomics, metabolomics, and host transcriptomics technologies to deeply elucidate the molecular mechanisms of Cr (VI) action, thereby offering new insights for the prevention and treatment of Cr(VI) exposure-related diseases.

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REFERENCES

Ali M, Xu C, Hina Q, *et al*., 2026. Interrelations between probiotics, gut microbiota, intestinal barrier, and immune response focusing on diarrhea in dairy calves. Journal of Integrative Agriculture 25:16–29.

Asri M, El G, Elabed S, *et al*., 2018. Wicherhamomyces anomalus biofilm supported on wood husk for chromium wastewater treatment. Journal of Hazardous Materials 359:554-562.

Bian S, Zhu S, Lu J, *et al*., 2025. Targeting gut microbiota in non-alcoholic fatty liver disease (NAFLD): Pathogenesis and therapeutic insights: A review. International Journal of Biological Macromolecules 330:147995.

Baszuk P, Janasik B, Pietrzak S, *et al*., 2021. Lung cancer occurrence-correlation with serum Chromium levels and genotypes. Biological Trace Element Research 199:1228-1236.

Chen Y, Chen D, Chen L, *et al*., 2019. Microbiome-metabolome reveals the contribution of gut-kidney axis on kidney disease. Journal of Transition Medicine 17:5.

Desideri E, Ciccarone F and Ciriolo M, 2019. Targeting Glutathione Metabolism: Partner in Crime in Anticancer Therapy. Nutrients 11:1926.

Eeckhaut V, Machiels K, Perrier C, *et al*., 2013. Butyricicoccus pullicaecorum in inflammatory bowel disease. Gut 62:1745-1752.

Gubernatorova E, Gorshkova E, Bondareva M, *et al*., 2023. Akkermansia muciniphila - friend or foe in colorectal cancer? Frontiers in Immunology 14:1303795.

Han B, Shi L, Bao M, *et al*., 2024. Dietary ellagic acid therapy for CNS autoimmunity: Targeting on Alloprevotella rava and propionate metabolism. Microbiome 12:114.

Holmberg S, Feeney R, Prasoodanan P, *et al*., 2024. The gut commensal Blautia maintains colonic mucus function under low-fiber consumption through secretion of short-chain fatty acids. Nature Communications 15:3502.

Hou J, Wu P, Cai J, *et al*., 2025. Gut microbiota dysbiosis amplifies thiram hepatotoxicity via a mitochondrial-autophagy-apoptosis nexus orchestrated by the gut-liver axis. Cellular Signalling 136:112104.

Iatcu C, Steen A and Covasa M, 2021. Gut Microbiota and Complications of Type-2 Diabetes. Nutrients 14:166.

Ikeda T, Nishida A, Yamano M, *et al*., 2022. Short-chain fatty acid receptors and gut microbiota as therapeutic targets in metabolic, immune, and neurological diseases. Pharmacology Therapeutics 239:108273.

Kim J, Kim H, Park J, *et al*., 2020. The Association between Gut Microbiota and Uremia of Chronic Kidney Disease. Microorganisms 8:907.

Kobayashi T, Iwata Y, Nakade Y, *et al*., 2021. Significance of the Gut Microbiota in Acute Kidney Injury. Toxins 13:369.

Li A, Ding J, Shen T, *et al*., 2021a. Environmental hexavalent chromium exposure induces gut microbial dysbiosis in chickens. Ecotoxicology and Environmental Safety 227:112871.

Li T, Huang Z, Jia R, *et al*., 2021b. Spirulina platensis polysaccharides attenuate lipid and carbohydrate metabolism disorder in high-sucrose and high-fat diet-fed rats in association with intestinal microbiota. Food Research International 147:110530.

Li Y, Lu Y, Tang D, *et al*., 2022. Anthocyanin improves kidney function in diabetic kidney disease by regulating amino acid metabolism. Journal of Translational Medicine 20:510.

Marrs C and Lonsdale D, 2021. Hiding in Plain Sight: Modern Thiamine Deficiency. Cells-Basel 10.

Miao J, Wang D, Yan J, *et al*., 2017. Comparison of subacute effects of two types of pyrethroid insecticides using metabolomics methods. Pesticide Biochemistry and Physiology 143:161-167.

Ni X, Wan L, Liang P, *et al*., 2020. The acute toxic effects of hexavalent chromium on the liver of marine medaka (Oryzias melastigma). Comparative Biochemistry and Physiology D-Genomics Proteomics 231:108734.

Pan Z, Gong T and Liang P, 2024. Heavy Metal Exposure and Cardiovascular Disease. Circulation Research 134:1160-1178.

Peng L, Linghu R, Chen D, *et al*., 2017. Inhibition of glutathione metabolism attenuates esophageal cancer progression. Experimental and Molecular Medicine 49:e318.

Porru S, Esplugues A, Llop S, *et al*., 2024. The effects of heavy metal exposure on brain and gut microbiota: A systematic review of animal studies. Environmental Pollution 348:123732.

Qiu P, Hou W, Wang H, *et al*., 2021. Sirt1 deficiency upregulates glutathione metabolism to prevent hepatocellular carcinoma initiation in mice. Oncogene 40:6023-6033.

Qu Z, Liu H, Yang J, *et al*., 2025. Selective utilization of medicinal polysaccharides by human gut Bacteroides and Parabacteroides species. Nature Communications 16:638.

Regnier M, Rastelli M, Morissette A, *et al*., 2020. Rhubarb supplementation prevents diet-induced obesity and diabetes in association with increased *Akkermansia muciniphila* in mice. Nutrients 12:2932.

Ren Q, Cheng L, Guo F, *et al*., 2021. Fisetin improves hyperuricemia-induced chronic kidney disease via regulating gut microbiota-mediated tryptophan metabolism and aryl hydrocarbon receptor activation. Journal of Agricultural and Food Chemistry 69: 10932-10942.

Soliman M, Al-Akeel R, Al-Ghamdi M, *et al*., 2024. Trophic transfer of heavy metals across a food chain in a wastewater-irrigated agroecosystem. Environmental Monitoring and Assessment 196:1082.

Song X, Li C, Qiu Z, *et al*., 2024. Ecotoxicological effects of polyethylene microplastics and lead (Pb) on the biomass, activity, and community diversity of soil microbes. Environmental Research 252:119012.

Wen K, Zhao M, Liu L, *et al*., 2021. Thiamine modulates intestinal morphological structure and microbiota under subacute ruminal acidosis induced by a high-concentrate diet in Saanen goats. Animal 15:100370.

Xu C, He Q, Zhu Z, *et al*., 2025. Propolis improves intestinal barrier function against Cryptosporidium parvum via NLRP6 inflammasome. mBio 16:e0231725.

Yang Z, Ni J, Sun X, *et al*., 2024. The prevention effect of Limosilactobacillus reuteri on acute kidney injury by regulating gut microbiota. Microbiology and Immunology 68: 213-223.

Yao Q, Yang H, Wang X, *et al*., 2019. Effects of hexavalent chromium on intestinal histology and microbiota in Bufo gargarizans tadpoles. Chemosphere 216:313-323.

Zhang T, Sun W, Wang L, *et al*., 2023. *Rosa laevigata* Michx. polysaccharide ameliorates diabetic nephropathy in mice through inhibiting ferroptosis and PI3K/AKT pathway-mediated apoptosis and modulating tryptophan metabolism. Journal of Diabetes Research 2023:9164883.

Zhao H, Yang C, Liu T, *et al*., 2023. The roles of gut microbiota and its metabolites in diabetic nephropathy. Frontiers in Microbiology 14:1207132.

Zhu J, Hua X, Yang T, *et al*., 2022. Alterations in gut vitamin and amino acid metabolism are associated with symptoms and neurodevelopment in children with autism spectrum disorder. Journal of Autism and Developmental Disorders 52:3116-3128.

Zhu S, Bian S, Lu J, *et al*., 2026. Pathogenesis of bovine mastitis and influence of the gut microbiota: A Review. Journal of Agricultural and Food Chemistry 74(5):4222-4234.