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Multiomics approach reveals the comprehensive interactions between nutrition and children's gut microbiota, and microbial and host metabolomes

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Abstract

The gut microbiome can modulate nutrient metabolism to produce many metabolites interacting with the host. However, the intricate interactions among dietary intake, the gut microbiome and metabolites, and host metabolites need to be further explored although some studies have been devoted to it. Here, in a cross-sectional studies, 88 children aged 2–12 years were enrolled from northwestern China. The dietary intake data were collected via a designed food frequency questionnaire to calculate plant-based diet indices (PDIs). Stool and plasma samples were collected for metagenomic and broad-targeted metabolomic analysis. Spearman's rank correlation was used to describe the associations between nutrients/PDIs and the gut microbiota and metabolites. PDI was significantly positively associated with *Bilophila wadsworthia*, *Bacteroides thetaiotaomicron*, and *Alistipes indistinctus*, etc., but was obviously negatively correlated with *Roseburia intestinalis*, *Faecalibacterium prausnitzii*, etc. However, these species showed no significant associations with either healthy PDI (hPDI) or unhealthy PDI (uPDI). Interestingly, hPDI was significantly positively related to species, including *Ruminococcus bicirculans*, and was significantly negatively associated with uPDI, and vice versa. The above correlation trends were also observed between PDIs and predicted gut microbial functional pathways, microbial metabolites and the host metabolome. Notably, the significantly related pathways were focused mainly on substances and energy metabolism. PDI was significantly positively associated with the fecal contents of P-aminobenzoate, chenodeoxycholic acid, 4,6-dihydroxyquinoline, quinoline-4,8-diol, etc., but was significantly negatively associated with those of TMAO, FFA, creatine phosphate, etc. In plasma, PDI was significantly positively associated with sarcosine, ornithine, L-histidine, etc., but was distinctly negatively correlated with FFAs, carnitine C2:0, etc. Strikingly, the healthy plant-based diet index (hPDI) is correlated with increased levels of metabolites related to tryptophan metabolism, whereas the unhealthy PDI (uPDI) is linked to increased levels of metabolites associated with tyrosine and sphingolipid metabolism, which are pathways commonly associated with Western diets. Our studies provide reliable data support and a comprehensive understanding of the effects of dietary intake on the gut

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microbiome and microbial and host metabolites and lay a foundation for further studies of the diet-gut microbiota-microbial metabolites and host metabolism.

Keywords Dietary intake, Plant-based indices, Children, Shotgun metagenomic sequencing, Untargeted metabolomics, Correlation analysis

Introduction

Dietary intake and nutrition could significantly shape the developmental trajectory of children by changing the gut microbiome and its metabolites to modulate host metabolism [1]. Conversely, shifts in gut microbial composition and function can impact host appetite and nutrient absorption, suggesting a potentially modifiable approach to optimize children's growth through targeted manipulation of the gut microbiota and metabolites [2, 3]. A balanced diet is essential, as it not only secures the nutrients required for children but also plays a vital role in modulating the gut microbiota and metabolites, including short-chain fatty acids (SCFAs), to modulate host immunity, hormone production, and intestinal barriers to ultimately impact host metabolism and body health, such as fostering the development of robust muscle bone mass in young individuals [4–6]. Therefore, exploring the intricate interactions and associations between dietary intake and the gut microbiota and its metabolites, as well as host metabolism, is essential.

Many dietary indices have been created to assess the nutritional intake of children in an intuitive manner, such as the Youth Healthy Eating Index (YHEI) [7] in the United States, the Mediterranean Diet Quality Index in Children and Adolescents (KIDMED) [8] in Spanish, and the Chinese Children Dietary Index (CCDI) [9]. These indices emphasize increasing dietary diversity and plant-based food intake while advocating for reduced consumption of solid fats, salt, and sugar [10]. A plant-based diet index (PDI) differentiating between a healthy, unhealthy, and overall plant-based diet index (hPDI, uPDI, and PDI) offers a promising approach to standardize and compare studies and integrate results without completely excluding the consumption of animal-derived foods [11]. PDIs have gained widespread use in nutritional and microbiota research because of their ability to quantify the metabolic impact of diet-microbiota interactions [12, 13]. This study revealed that hPDI, rich in fiber and phytochemicals, was positively associated with beneficial bacteria such as *Eubacterium* and *Faecalibacterium*, the regulation of gut barrier function, and microbial and lipid metabolism [14]. Furthermore, integrating multiomics, especially metagomics and metabolomics, is a pivotal research method for comprehensively elucidating diet-microbe-host dynamics [15]. However, current research on PDIs in children has focused predominantly on body composition, particularly in those who are overweight or obese [16, 17]. The precise and comprehensive relationships among PDIs, the gut microbiota, and microbial and host metabolites in children remain to be elucidated. The development of multiomics joint analysis has made diet-microbiome-metabolite studies possible.

Given the influences of regional and dietary factors on the gut microbiota, we recruited children from Lanzhou city in Northwest China to elucidate the comprehensive interactions among dietary intake, the gut microbiome, and microbial and host metabolites. This region is notable for its distinctive dietary patterns, characterized by high intake of staples such as corn, wheat, and naked oats, along with elevated consumption of beef, mutton, and chicken [18]. Our study focused on exploring the associations between PDIs and the gut microbiome and microbial and plasma metabolome via multiomics in this unique population, aiming to contribute to the current understanding of interactions among dietary intake evaluated by PDIs, the gut microbiota, and microbial and host blood metabolomes in children.

Materials and methods

Study cohort

One hundred healthy subjects aged 2–12 years were recruited from the outpatient department of Gansu Province Hospital Rehabilitation Center (Lanzhou City, Gansu Province) in Northwestern China. The participants were subsequently required to complete a designed questionnaire covering essential information, lifestyle factors, familial medical history, bowel habits, and dietary patterns with the assistance of their guardians. Subjects with gastrointestinal disorders, recent antibiotic use (past one month), infections, medications affecting the gut microbiota, a history of drug allergies, and those consuming fermented foods such as yogurt were excluded. Meanwhile, consent forms were signed by the legal guardians of the participants.

We included 100 participants in the statistical analysis and excluded 12 of them due to missing or inaccurate dietary data, which might affect the reliability of the analysis results. Finally, we used the data of 88 participants for analysis.

Sample collection

Phenotypic characteristics, including age, sex, height, weight, and medical and medication history, were recorded for each subject. Overnight fasting venous blood was collected from each participant and centrifuged at 3,000 rpm (1580×g) for 10 min to collect the plasma (Cence TDZ5-WS, China). One gram of each stool sample was collected into a 2 mL tube containing DNA storage buffer for shotgun metagenomic sequencing, while another gram of each stool sample was collected into a separate 2 mL tube with normal saline for untargeted metabolomics analysis. The collected fecal and plasma samples were stored at −80 °C and transported to the laboratory on dry ice for further examination.

Dietary intake collection

Dietary intake information was obtained via a semiquantitative food frequency questionnaire (FFQ), which contains 87 food items across 25 food categories including Chinese and Western staple foods, cereals and potatoes, legumes, red meats (pork, beef, mutton), poultry, fish and shellfish, egg, dairy products, fried foods, vegetables, fruits, desserts, nuts, preserved foods, sweetened beverages, coffee, tea, alcoholic beverages and etc. To get the frequency of consumption, nine levels were set, ranging from 'never or 1–2 times a month' to '≥3 times a day' (1=never; 2=1–2 times/month; 3=3–4 times/month; 4=1–2 times/week; 5=3–4 times/week; 6=5 or more times/week;7=1 time/day; 8=2 times/day; 9=3 or more times/day). To obtain the quantity of each consumed food, five levels varied from 'none or less than 50 g/time' to ' more than 200 g/time' were made (1=None or not more than 50 g/time; 2=More than 50 g but less than 100 g each time; 3=more than 100 g but less than 150 g each time; 4=more than 150 g but less than 200 g each time; 5=more than 200 g each time.). For details, please refers to the FFQ. Then the daily intake of each food were calculated by frequency multiply amount.

Calculation of plant-based diet indices

Plant-based diet indices (PDIs), encompassing an overall plant-based diet index (PDI), a healthful PDI (hPDI), and an unhealthy PDI (uPDI), are increasingly being utilized to evaluate the quality of children's diets in relation to health outcomes [19, 20]. Based on the research of Satija [11] and Miao et al. [12]., Chinese Food Composition Table 2018 and the Chinese Dietary Guideline 2022, and taken into account the local dietary patterns in northwestern China as a basis for classification, the foods were categorized into 14 distinct food groups. Detailed information on foods that make up the 14 food categories was presented in Table S1B2 of the supplementary file. Then, we classified 14 food categories into the healthy plant-based diet group (hPDI, including whole grains, fruits, vegetables, nuts, and potatoes), the unhealthy plant-based diet group (uPDI, comprising refined grains, fried foods, sweetened beverages, desserts, preserved products), and the animal food group (consisting of dairy, eggs, fish, meat, and western foods). Each of the 14 food items was subsequently stratified into consumption quintiles and assigned either positive or inverted scores. For ascending scores, a rating of 5 was allocated to the highest quintile, and a rating of 1 was allocated to the lowest quintile; conversely, for descending scores, the pattern was reversed such that a score of 5 was given to the lowest quintile, and a score of 1 was given to the highest quintile. When calculating the PDI, we included all the above-mentioned plant-based foods. The plant food groups were assigned ascending scores (from 1 to 5) based on the intake quintiles, while the animal food groups were assigned descending scores (from 5 to 1). The PDI ranges from 14 (the lowest) to 70 (the highest), and a higher score indicates a greater intake of plant-based foods. For the calculation of hPDI, ascending scores were given to the healthy plant food groups, and descending scores were assigned to the unhealthy plant food groups and animal food groups. As for the uPDI, ascending scores were applied to the unhealthy plant food groups, and descending scores were given to the healthy plant food groups and animal food groups. Additionally, PDIs were treated as continuous variables to explore their relationships with the aforementioned factors and food groups.

Shotgun metagenomic sequencing

DNA preparation and sequencing

DNA extraction was performed via the phenol/trichloromethane method after the samples were thawed on ice. The extracts were then treated with DNase-free RNase, and the DNA quality was measured via agarose gel electrophoresis and a Qubit 3.0 fluorimeter (Thermo Fisher, Waltham, MA, USA). Shotgun metagenomic sequencing was conducted on the BGISEQ-500 platform with a single-end read length of 150 bp [21]. Raw reads containing 50% low-quality bases (quality ≤ 20) or more than five ambiguous bases were excluded, and the remaining reads were then aligned to the human reference genome (Hg19) database to remove host DNA as previously described [22]. Finally, an average of 70.28 million clean reads were remained after filtering (Refers to Tab S1I. Filter_data for details). The remaining reads were defined as clean reads and utilized for obtaining taxonomic and functional profiles.

Taxonomic and functional annotation

MetaPhlAn 4.0 (Metagenomic Phylogenetic Analysis 4.0) was employed for taxonomic profiling, following the methodology of a previously published study [23]. HUMAnN 4.0 (the HMP Unified Metabolic Analysis Network) was used to profile the abundance of microbial metabolic pathways and other molecular functions from the metagenomic sequencing data as previously described [24].

Diversity calculation

Alpha diversity was assessed by the Shannon index, Simpson index, and inverse Simpson index of the gut microbiota across various levels. Beta diversity was evaluated by Bray–Curtis distance at various taxonomic levels, as depicted by principal coordinate analysis (PCoA). Permutational multivariate analysis of variance (PERMANOVA) was employed to compare the significance between groups.

Correlation analysis

To investigate the correlations between PDIs and various food types in terms of species, genera, phyla, and predicted functional pathways, we employed Spearman's rank correlation and partial Spearman's rank correlation analyses (adjusted for BMI, sex, and age) [25]. For various food types, Spearman's correlation analysis was used. For PDIs, partial Spearman's rank correlation analysis was used. We used partial correlation analysis (short for pcor in the figures) to study the linear relationship between two variables after excluding the influence of one or more independent factors. This method was applied to analyze the correlations between species, genera, phyla, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, fecal and plasma metabolites, and food intake after adjusting for age, sex and BMI. We employed the false discovery rate (FDR) for correction, and all *p*-values in the article have been adjusted.

Metabolomic profiling of fecal and plasma metabolites

Sample preparation and extraction

The samples stored at −80 °C in a refrigerator were thawed on ice and vortexed for 10 s. A 50 μL sample and 300 μL extraction mixture (ACN: methanol = 1:4, v/v) of internal standards, an internal standard solution containing Lidocaine, L-tryptophan-d5, succinic acid-d4, Indole-3-carboxylic Acid-d5, D-Luciferin, [2H5]-Phenoxy acetic Acid, [2H5]-Phenoxy acetic Acid, Sulfaquinoxaline-13C6 at a concentration of 1 μg/mL, were added to a 2 mL microcentrifuge tube. The sample was vortexed for 3 min and then centrifuged at 12,000 rcf (×g) for 10 min at 4 °C. Two hundred microlitres of the upper layer were collected, stored at −20 °C for 30 min, and then centrifuged at 12,000 rcf (×g). A 180 μL aliquot of the upper layer was removed for UPLC–MS analysis.

UPLC Conditions

All samples were analyzed via a UPLC–MS system according to the instrument instructions (UPLC, SHIMADZU Nexera X2, Kyoto, Japan; MS, Applied Biosystems 6500 Q TRAP, Thermo Fisher Scientific, Waltham, America). The UPLC conditions were as follows: column, water ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm × 100 mm); column temperature, 40 °C; flow rate, 0.4 mL/min; injection volume, 2 μL; solvent system, water (0.1% formic acid): acetonitrile (0.1% formic acid); and column, 5% mobile phase B (0.1% formic acid acetonitrile solution) for 0 min, linearly graded to 90% mobile phase B (0.1% formic acid acetonitrile solution) for 11 min, held for 1 min, and then returned to 5% mobile phase B in 0.1 min, held for 1.9 min. The positive and negative ion conditions for MS were as follows: ion spray voltage (ESI + 5500v, ESI- −4500v), ion source gas 1 (ESI + 50psi, ESI −50 psi), declustering potential (Dp) (ESI + 60v, ESI- −60v), curtain gas (ESI + 35psi, ESI −35psi), temperature (ESI + 550 °C, ESI −550 °C), and ion source gas 2 (ESI + 60psi, ESI −60psi), and collision energy (ESI + 30v, ESI- −30v).

Metabolite information extraction and processing

The raw data files acquired by LC–MS were converted to mzML format via Proteowizard software. Peak extraction, peak alignment, and retention time correction were performed via the xcMS program [26]. The peak areas were corrected via the "SVR" method. Peaks with a retention rate lower than 50% were discarded for each set of samples. Afterward, metabolic identification information was obtained by searching the laboratory's self-built database, integrated public database, AI database, and metDNA.

KEGG annotation and enrichment analysis

Functional analyses were performed via the KEGG Compound Database (http://www.kegg.jp/kegg/compound/) to annotate the identified metabolites, and the annotated metabolites were subsequently mapped to the KEGG pathway database (http://www.kegg.jp/kegg/pathway.html). *P* values for the given list of metabolites were determined via hypergeometric testing to identify significantly enriched pathways.

Statistical analysis

Packages in R (version 4.2.0) were utilized in this study. Student's t-test and the Kruskal–Wallis test were used to determine differences in clinical indicators between children with PDIs and those with various food types.

Table 1 PERMANOVA of various phenotypes and PDIs on the gut microbiota and fecal and plasma metabolites

Variables	Mean ± SD	Genera		Fecal metabolism		Plasma metabolism	
		R^2	*P* value	R^2	*P* value	R^2	*P* value
Sex	41:47(F:M)	0.005	0.971	0.012	0.362	0.006	0.956
BMI	17.17±4.85	0.009	0.633	0.010	0.543	0.012	0.389
Age	5.71±2.30	0.018	0.102	0.018	0.064	0.019	**0.039**
Birth weight	3.40±0.65	0.008	0.749	0.010	0.582	0.010	0.586
Birth height	50.97±2.62	0.015	0.199	0.012	0.395	0.016	0.115
PDI	44.48±5.37	0.023	**0.023**	0.022	**0.022**	0.021	**0.028**
hPDI	44.44±7.40	0.016	0.158	0.012	0.322	0.013	0.284
uPDI	45.01±6.81	0.041	0.238	0.014	0.225	0.011	0.524
Nuts	9.38±12.48	0.014	0.228	0.009	0.694	0.011	0.415
Vegetables and fruits	78.62±88.27	0.007	0.818	0.014	0.221	0.012	0.336
Whole grains	23.38±32.76	0.016	0.156	0.008	0.879	0.010	0.562
Potatoes	14.73±22.85	0.023	**0.037**	0.011	0.406	0.007	0.894
Refined grains	149.27±119.55	0.011	0.511	0.009	0.706	0.011	0.516
Fried foods	5.18±6.71	0.019	0.075	0.013	0.300	0.012	0.130
Desserts	7.71±9.85	0.011	0.560	0.009	0.731	0.016	0.130
Preserved foods	1.01±1.81	0.027	**0.009**	0.011	0.493	0.009	0.646
Sweetened beverages	16.72±48.52	0.027	**0.020**	0.008	0.860	0.009	0.660
Dairy products	22.46±73.84	0.039	**0.001**	0.009	0.642	0.012	0.403
Egg	31.43±19.42	0.010	0.530	0.013	0.286	0.011	0.416
Meat	49.06±45.10	0.003	0.991	0.009	0.766	0.013	0.312
Fish	12.21±10.76	0.003	0.992	0.006	0.968	0.013	0.274
Western foods	3.02±3.71	0.024	**0.021**	0.015	0.131	0.009	0.695

Significant associations between gut microbes, microbial and plasma metabolites, and PDIs were evaluated by partial Spearman's rank correlation. The associations between the microbes and the metabolites were assessed via Spearman's rank correlation test. Differentially enriched KEGG pathways of different metabolites were identified on the basis of their reporter score, which was calculated from the z scores of individual KO groups. All the statistical tests were two-tailed, and a *P* value of less than 0.05 was considered statistically significant. Principal coordinate analysis (PCoA) was performed via the statistics function prcomp within R (www.r-project.org).

Results

PERMANOVA of various phenotypes and PDIs on the gut microbiota and metabolites

A total of 41 healthy female and 47 healthy male subjects (mean BMI ± SD, 17.17 ± 4.85) aged 2–12 years (mean age ± SD, 5.71 ± 2.30) were included in this study (Table S1A). The PDI, hPDI, and uPDI were calculated to assess their relationships with 14 food types (Table S1B, Figure S1). PDI was significantly positively associated with preserved foods, desserts, sweetened beverages, whole grains, vegetables and fruits, potatoes, nuts, and refined grains but negatively associated with eggs and fish (Table S1D). hPDI was significantly positively associated with whole grains, vegetables and fruits but negatively associated with preserved foods, desserts, sweetened beverages, fried foods, dairy products, western foods, and fish (Table S1D). uPDI was significantly positively associated with preserved foods, desserts, sweetened beverages, fried foods, and dairy products but negatively associated with whole grains, vegetables and fruits, potatoes, nuts, meat, eggs, and fish (Table S1D). These findings align closely with the calculations of PDIs.

To evaluate the impact of PDIs, 14 food types, and various phenotypes on the gut microbiota and fecal and plasma metabolites, PERMANOVA was used (Table 1, Table S1D). The results showed that PDI significantly affected the composition at the genus level of the gut microbiota ($P=0.023$) and the fecal and plasma metabolites ($P=0.022$ and $P=0.028$, respectively). In addition, food types such as potatoes ($P=0.037$), preserved foods ($P=0.009$), sweetened beverages ($P=0.020$), dairy products ($P=0.001$), and western foods ($P=0.021$) had significant effects on the gut microbiota but not on microbial and plasma metabolites. No significant effect on the gut microbiota or fecal or plasma metabolites was observed for hPDI or uPDI.

Effects of PDI on the composition and function of the gut microbiota

All the ordered phyla were annotated to the gut microbiota of the children (Figure S2A). Among them, Firmicutes showed a significant negative association with PDI, while Fusobacteria presented a clear negative association with hPDI, and Ascomycota presented an evident association with uPDI. Among the top 20 abundant genera (Figure S2B), *Alistipes* was significantly positively associated with PDI, whereas *Faecalibacterium* and *Roseburia* were negatively associated with PDI. *Ruminococcus* was significantly positively associated with hPDI. In terms of the top 20 abundant species (Figure S2C), *Faecalibacterium prausnitzii*, along with *Roseburia intestinalis*, was significantly negatively associated with PDI. *Bifidobacterium longum* also exhibited a significant negative association with hPDI.

Partial correlation analysis was used to illustrate the effects of PDIs on the composition of the gut microbiota (Fig. 1, Figure S3). At the genus level (Figure S3), *Bilophila*, *Alistipes*, *Hydrogeniiclostidium*, *Anaerofustis*, *Gordonibacter*, and *Eisenbergiella* were significantly positively associated with PDI, whereas *Roseburia*, *Haemophilus*, *Faecalibacterium*, and *Veillonella* were significantly negatively associated with PDI. hPDI was significantly positively associated with *Pseudoruminococcus*, *Ruminococcus*, and *Butyricicoccus* but negatively associated with *Agathobaculum*, *Granulicatella*, *Rothia*, and *Parvimonas*. uPDI was positively associated with *Parvimonas* and *Massiliimalia*. At the species level, PDI was significantly positively associated with *Anaerofustis stercorihominis*, *Enterobacter hormaechei*, *Anaerofustis stercorihominis*, *Gordonibacter pamelaeae*, *Bilophila wadsworthia*, *Clostridium symbiosum, Bacteroides nordii*, *Bacteroides thetaiotaomicron*, and *Alistipes indistinctus* but was negatively associated with *Faecalibacterium prausnitzii*, *Agathobaculum butyriciproducens*, *Haemophilus sputorum, Veillonella dispar, Veillonella atypica, Veillonella parvula, Haemophilus parainfluenzae, Roseburia intestinalis, Streptococcus cristatus*, and *Streptococcus cristatus*. hPDI was positively associated with *Bifidobacterium animalis*, *Eubacterium siraeum, Coprococcus eutactus, Eubacterium* sp. AF34_35BH, and *Ruminococcus bicirculans,* but negatively associated with *Agathobaculum butyriciproducens, Granulicatella adiacens, Abiotrophia defective, Bifidobacterium longum, Streptococcus intermedius, Enterococcus avium,* and *Parvimonas micra*. uPDI was clearly positively associated with *Massiliimalia massiliensis, Massiliimalia timonensis, Abiotrophia* sp. HMSC24B09 and *Parvimonas micra*; however, it was significantly negatively associated with *Fructilactobacillus sanfranciscensis, Ruminococcus bicirculans,* and *Blautia* sp MSK 20_85.

Spearman's rank correlation analysis was used to analyze the associations between each dietary item and the gut microbiota. Nuts were significantly positively associated with *Fructilactobacillus sanfranciscensis* and *Ruminococcus bicirculans* but negatively associated with *Abiotrophia* sp. HMSC24B09. Vegetables and fruits were significantly negatively associated with *Agathobaculum butyriciproducens*, *Granulicatella adiacens,* and *Abiotrophia* sp. HMSC24B09. Whole grains were clearly positively associated with *Coprococcus eutactus* and *Bacteroides thetaiotaomicron*. Desserts were significantly positively associated with *Enterococcus avium* but negatively associated with *Abiotrophia* sp. HMSC24B09 and *Bifidobacterium animalis* Interestingly, preserved foods, sweetened beverages, and dairy products were significantly positively associated with *Parvimonas micra*, *Bifidobacterium longum*, *Bilophila wadsworthia*, *Clostridium symbiosum*, and *Anaerofustis stercorihominis* but negatively associated with *Faecalibacterium prausnitzii*, *Veillonella dispar*, *Veillonella infantium*, *Veillonella atypica*, *Haemophilus parainfluenzae*, *Roseburia intestinalis*, and *Ruminococcus bicirculans*. Western foods were significantly positively associated with *Parvimonas micra* but negatively associated with *Massiliimalia massiliensis* and *Gordonibacter pamelaeae*. Fish was significantly negatively associated with *Massiliimalia massiliensis* and *Bilophila wadsworthia*. Meat was significantly negatively associated with *Massiliimalia timonensis* and *Bifidobacterium animalis*. Eggs were positively associated with *Abiotrophia defectiva*.

HUMAnN 4.0 was used to obtain the functional profile. In total, 90 pathways were found to be significantly correlated with PDIs (Fig. 2). Twenty pathways, including pyruvate fermentation to propanoate I, 4-aminobutanoate degradation V, gluconeogenesis, anaerobic energy metabolism, L-ascorbate biosynthesis IV, peptidoglycan biosynthesis II, glucarate and D-galactarate degradation I, L-alanine biosynthesis, menaquinol biosynthesis, etc., were significantly positively associated with PDI. Forty-four pathways, including the TCA cycle, sulfur amino acid biosynthesis, fatty acid biosynthesis, heterolactic fermentation, and polysaccharide degradation, were significantly negatively associated with hPDI (Fig. 2). Interestingly, these hPDI-related metabolic pathways were significantly positively associated with uPDI. For every single food type, desserts, sweetened beverages, and dairy products, which contributed the most to the uPDI, were significantly positively associated with the uPDI-related metabolic pathways.

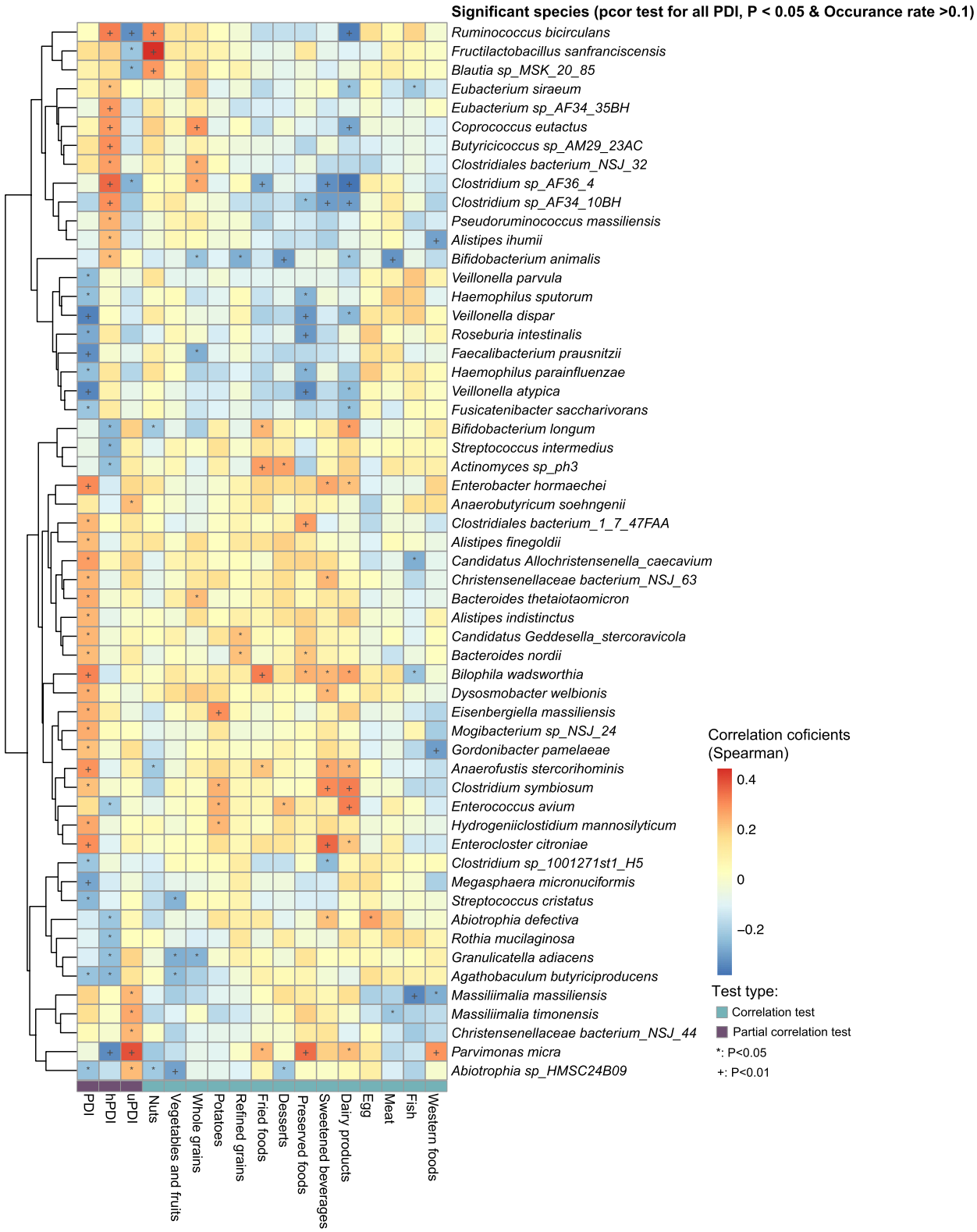

Fig. 1 The gut microbial species significantly associated with PDIs and individual food types. Spearman's rank partial correlation test was employed to assess the relationships between species and PDIs. Spearman's rank correlation test was used to examine the associations between species and each food type. +, $P < 0.01$; *, $P < 0.05$

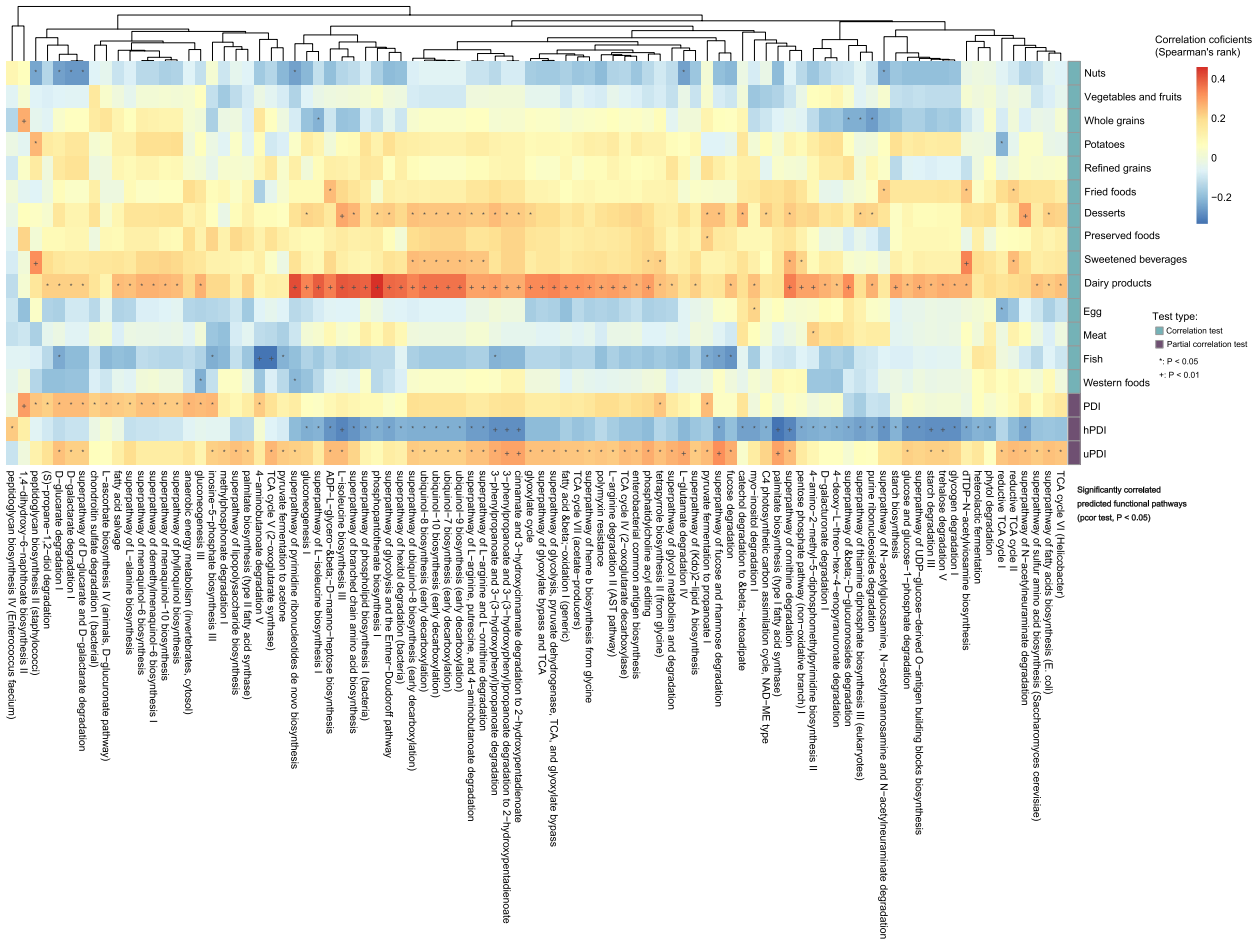

Fig. 2 The predicted functional pathways (PFPs) significantly associated with PDIs and 14 food types. Spearman's rank partial correlation test was used to evaluate the relationships between PFPs and PDIs. Spearman's rank correlation test was used to explore the associations between PFPs and each food type. +, $P < 0.01$; *, $P < 0.05$

Correlation analysis between fecal metabolites and PDIs

Partial correlation analysis revealed 527 fecal metabolites that were significantly correlated with PDIs. A total of 76 metabolites were annotated to the KEGG pathway database. Fifty-six metabolites were found to be significantly associated with PDI (Fig. 3). Fifteen metabolites, including *P*-aminobenzoate, chenodeoxycholic acid, 19-hydroxytestosterone, traumatic acid, 20-hydroxy leukotriene B4, calcitetal acid, 4,6-dihydroxyquinoline, quinoline-4,8-diol, biotin, trans-cinaldehyde, and hydrocinnamic acid, which were involved mainly in tryptophan metabolism, alpha-linolenic acid metabolism, steroid biosynthesis, biotin metabolism, folate biosynthesis, and ubiquinone and other terpenoid-quinone biosynthesis, were positively associated with PDI (Figure S4). The other 41 metabolites, including guanidineacetic acid, greatine phosphate, 12-ethyl-8-propylbacteriochlorophyllide, trimethylamine-N-oxide, 5-aminovaleric acid, D-malic acid, DHA and AA, which were involved mainly in porphyrin and chlorophyll metabolism; D-amino acid metabolism; biosynthesis of unsaturated fatty acids; arginine and proline metabolism; and glyoxylate and dicarboxylate metabolism, were significantly negatively associated with PDI (Figure S4). We then analyzed the correlations between the above metabolites and the significantly PDI-related species (Figure S5). The PDI-positively associated species were significantly positively correlated with the PDI-positively associated fecal metabolites. For the species and fecal metabolites negatively associated with PDI, the same correlation was shown, suggesting a relationship between the gut microbiota and fecal metabolites.

For hPDI, 33 fecal metabolites were significantly associated with hPDI (Fig. 3). Seven metabolites, including heparin, 3-hydroxybutyric acid, 2-hydroxybutyric acid, sterigmatocystin, and (R)-2-hydroxy-3-phenylpropionic acid, which were involved mainly in thiamine metabolism, were significantly positively associated with hPDI (Figure S6). Unsurprisingly, the other 26 metabolites,

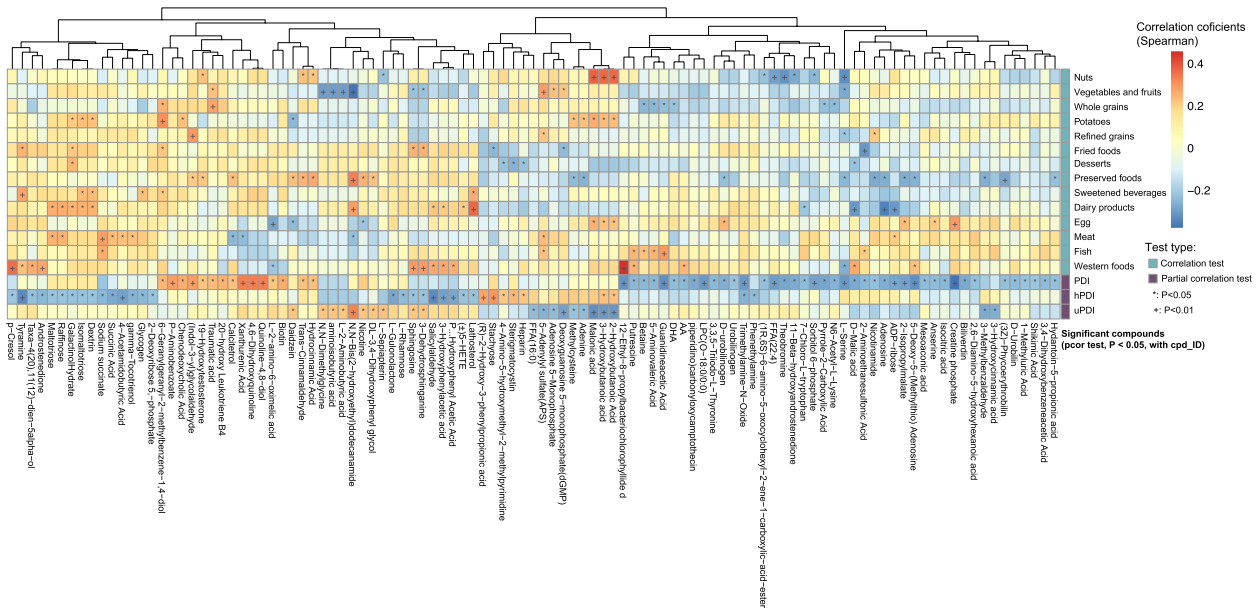

Fig. 3 Fecal metabolites significantly associated with PDIs and 14 food types. Spearman's rank partial correlation test was used to evaluate the relationships between fecal metabolites and PDIs. Spearman's rank correlation test was employed to explore the associations between fecal metabolites and each food type. +, $P < 0.01$; *, $P < 0.05$

including salicylaldehyde, tyramine, 3-hydroxyphenylacetic acid, 4-acetamidobutyric acid, P-hydroxyphenyl acetic acid, L-gluconolactone, succinic acid, trimethylamine-N-oxide, etc., which were involved mainly in tyrosine metabolism, phenylalanine metabolism, galactose metabolism, sphingolipid metabolism, pyruvate metabolism, starch, and sucrose metabolism, oxidative phosphorylation, steroid biosynthesis, and nicotinate and nicotinamide metabolism, were significantly negatively associated with hPDI (Figure S6). An association study revealed that hPDI-positively correlated species, such as *Ruminococcus bicirculans*, exhibit significant positive associations with hPDI-positive fecal metabolites. Conversely, a similar negative correlation was observed between hPDI-negatively associated species and their respective metabolites (Figure S5).

For uPDI, 21 significantly associated fecal metabolites were observed (Fig. 3). Ten metabolites, including aminoisobutyric acid; N,N-bis (2-hydroxyethyl) dodecanamide, which were involved mainly in sphingolipid metabolism; the sphingolipid signaling pathway; apoptosis; necroptosis; cysteine and methionine metabolism; glycine, serine and threonine metabolism; tyrosine metabolism; valine, leucine, and isoleucine degradation; and folate biosynthesis; were positively associated with uPDI. Eleven metabolites, including malonic acid, 3-hydroxybutanoic acid, 2-hydroxybutanoic acid, deoxyguanosine 5-monophosphate (dGMP), 3-methylbenzaldehyde, 3-hydroxycinnamic acid, etc., which were involved in purine metabolism, fatty acid biosynthesis, the cAMP signaling pathway, butanoate metabolism, 4-acetamidobutyric acid, P-hydroxyphenyl acetic acid, L-gluconolactone, succinic acid, trimethylamine-N-oxide, etc., and the Akt signaling pathway, were significantly negatively associated with uPDI (Figure S7). Correlation analysis between the gut microbiota and fecal metabolites revealed that the uPDI-negatively associated species, including *Veillonella dispar* and *Ruminococcus bicirculans,* were significantly positively associated with the uPDI-negatively associated fecal metabolites (Figure S5).

For every single food type, associations were also observed with the above fecal metabolites (Fig. 3). Consistent with PDI, refined grains, potatoes, whole grains, and preserved foods were significantly positively associated with PDI-positively associated metabolites; however, meat was negatively associated with PDI-positively associated metabolites. Notably, uPDI-negatively associated species, namely *Gordonibacter pamelaeae*, *Massiliimalia massiliensis*, and *Massiliimalia timonensis*, were negatively associated with the metabolite trimethylamine-N-oxide. The uPDI-negatively associated species *Veillonella dispar* was negatively associated with the intake of preserved foods and butanoate metabolites. The uPDI-positively associated species *Parvimonas micra* was positively associated with tyrosine metabolites and the intake of Western foods and fried foods.

Relationships between plasma metabolites and PDIs

To explore the associations between plasma metabolites and PDIs, we also conducted a partial correlation analysis. A total of 315 plasma metabolites were significantly associated with PDIs. Sixty-seven metabolites were annotated to KEGG pathways. Among these, 22 plasma metabolites, including D/L-ornithine, phenylacetylene-L-glutamine, L-histidine, sarcosine, thiouric acid, DL-3,4-dihydroxyphenylglycol, carboxylic acid ester, aromatic alcohol, D-phenylalanine, hippuric acid, salicylaldehyde, hercynine, D-mannitol, etc., which mainly participate in pathways such as ABC transporters, D-amino acid metabolism, tyrosine metabolism, phenylalanine metabolism, primary bile acid biosynthesis, nicotinate and nicotinamide metabolism, etc., were significantly positively associated with PDI (Fig. 4, Figure S8). Ten plasma metabolites, including isonicotinic acid, gibberellin A14, amino acids (AAs), 7-methyluric acid, carnitine C2:0, FFA(18:2), and FFA(18:1), which are involved mainly in the biosynthesis of unsaturated fatty acids, insulin resistance, the PPAR signaling pathway, the GnRH signaling pathway, serotonergic synapses, etc., were significantly negatively associated with PDI, suggesting that the intake of food with a lower PDI was beneficial for fatty acid metabolism and the release of hormones. Interestingly, plant groups (whole grains, vegetables, fruits, and potatoes) were positively associated with PDI-positively associated metabolites, including phenylacetylene-l-glutamine, hippuric acid, and salicylaldehyde, but were negatively associated with low-PDI associated metabolites, such as FFAs, 7-methyluric acid, and AA.

Twenty-two plasma metabolites were related to hPDI. Fifteen plasma metabolites, including porphobilinogen, B-nicotinamide mononucleotide, sorbitol 6-phosphate, 4-hydroxybenzaldehyde, pyroglutamic acid, 2-picolinic acid, and 1-pyrroline-4-hydroxy-2-carboxylate, which are involved mainly in tryptophan metabolism, the cAMP signaling pathway, neuroactive ligand-receptor interactions, carbohydrate digestion and absorption, purine and pyrimidine metabolism, D-amino acid metabolism, linoleic acid metabolism, etc., were significantly positively associated with hPDI (Fig. 4, Figure S9). Seven metabolites, including DL-carnitine, pantetheine, creatinine, and cholesterol sulfate, are involved mainly in the metabolism of histidine, phenylalanine, arginine, and proline; steroid hormone biosynthesis; beta-alanine metabolism; bile secretion; butirosin and neomycin biosynthesis; and thermogenesis, were significantly negatively associated with hPDI.

For uPDI, 21 plasma metabolites were found to be related. Six metabolites, including carnitine C2:0, L-cystine, DL-carnitine, and 3-hydroxycinnamic acid, which are involved in cysteine and methionine metabolism, phenylalanine metabolism, bile secretion, and thermogenesis, were positively correlated with uPDI. Fifteen metabolites, including 4-hydroxy-3-methoxybenzaldehyde, 1-pyrroline-4-hydroxy-2- carboxylate, averantin,

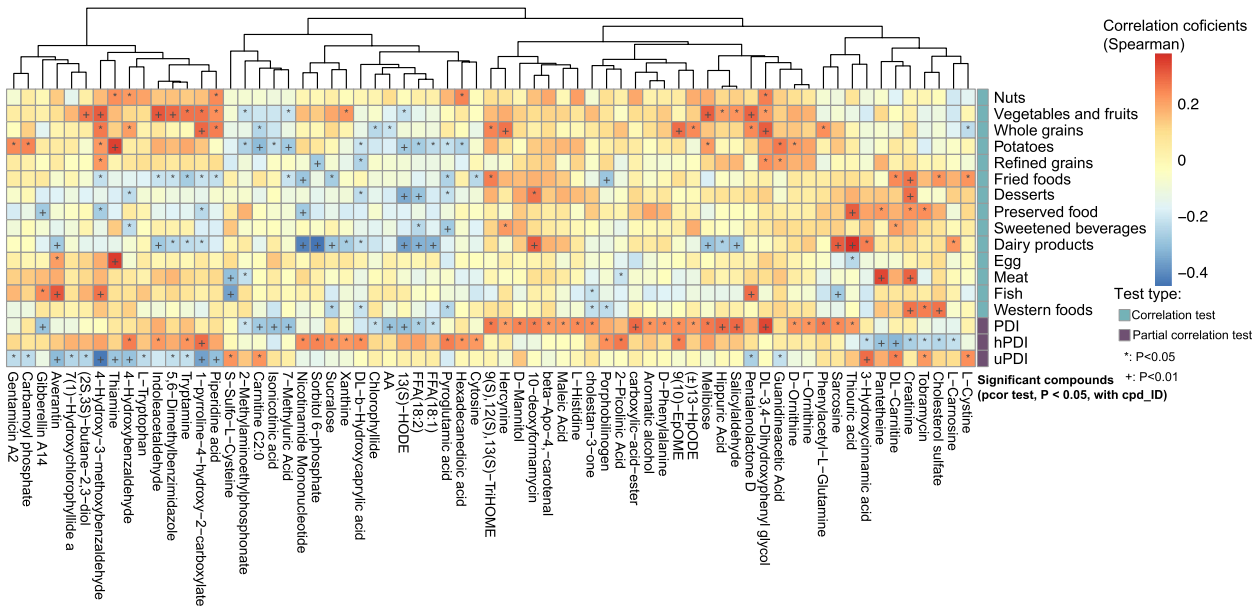

Fig. 4 The plasma metabolites annotated with KEGG maps significantly correlated with PDIs and 14 food types. Spearman's rank partial correlation test was used to evaluate the relationships between plasma metabolites and PDIs. Spearman's rank correlation test was employed to explore the associations between plasma metabolites and each food type. +, $P < 0.01$; *, $P < 0.05$

piperidine acid, 4-hydroxybenzaldehyde, and thiamine, which are involved mainly in glycine, serine and threonine metabolism, tryptophan metabolism, porphyrin, chlorophyll metabolism, D-amino acid metabolism, and purine and pyrimidine metabolism, were significantly negatively associated with uPDI (Fig. 4, Figure S10).

hPDI-positively associated plasma metabolites, like tryptamine and indoleacetaldehyde, had a positive association with *Ruminococcus bicirculans,* but uPDI presented inversely. These metabolites were positively associated with vegetables and fruits. uPDI-positively associated plasma metabolites, like L-Cystine and S-Sulfo-L-Cysteine, had a positive association with *Candidatus Geddesell stercoravicola* and *Candidatus Allochristensenella caecavium.*(Fig. 4, Figure S11.

Intersection analysis of the fecal and plasma metabolomes

We explored the relationship between the fecal and plasma metabolomes. For PDI, nine common metabolites were significantly related to fecal and plasma metabolites. The levels of arachidonic acid (AA), FAHFA (8:0/10:0), FFA (20:4), and N1-acetylspermine increased with the intake of foods with low-PDI score. Cyclamic acid is one of the most widely used artificial sweeteners in food and pharmaceuticals [27], and its level was significantly increased in high-PDI related foods. With respect to the metabolites significantly associated with hPDI, N4-acetylcytidine significantly increased with a reduction in hPDI-related foods. N4-acetylcysteine, the only known type of RNA acetylation in mammalian mRNAs, can promote translation efficiency. For the metabolites significantly related to uPDI, ribosyladenosine and 3-hydroxycinnamic acid were detected in both blood and feces, but with the opposite direction of enrichment, indicating that these two metabolites were positively associated with uPDI in blood but negatively associated with uPDI in feces (Fig. 5).

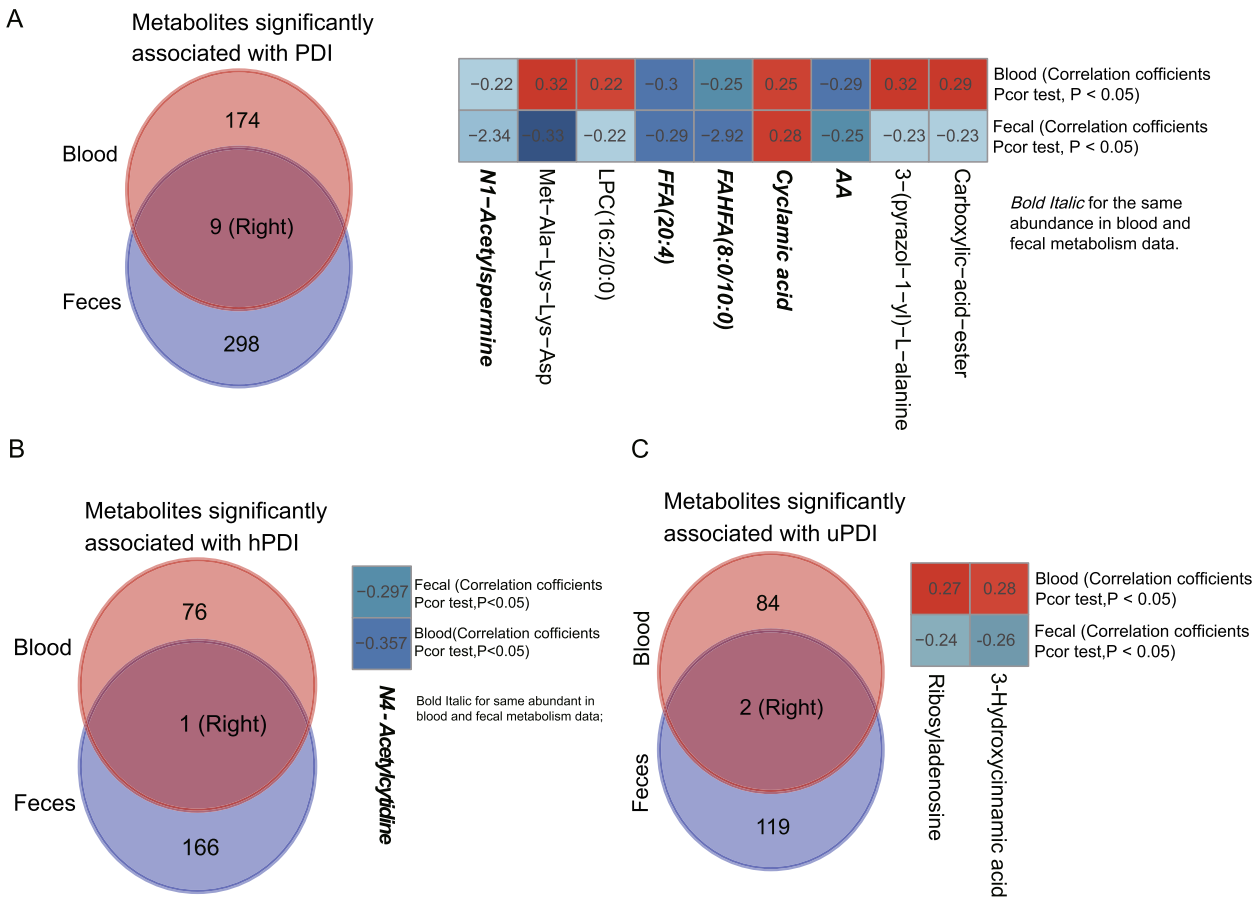

Fig. 5 The Venn diagram shows fecal and blood metabolites that were significantly associated with PDIs. **A** Nine common metabolites associated with PDI were identified in both fecal and plasma samples, among which AA, cyclamic acid, FAHFA (8:0/10:0), FFA (20:4), and N1-acetylspermine exhibited consistent trends. **B** A common metabolite, N4-acetylcytidine, associated with hPDI was detected in both feces and plasma. **C** Two common metabolites associated with uPDI, 3-hydroxycinnamic acid and ribosyladenosine, were observed in both feces and plasma

Discussion

This study provides insights into the complex interrelationships among plant-based diet indices (PDIs), the gut microbiota, and metabolism, revealing that PDIs are significantly correlated with the abundance and metabolic activities of certain bacterial species. A high-PDI score, reflective of a diet rich in plant-based foods, was associated with some beneficial bacteria known to increase fiber, bile acid, and SCFA metabolism. However, the presence of certain species was also linked to the intake of less healthy plant-based foods, such as processed items and sweetened beverages, indicating a potential negative impact on metabolic health. Metabolomic analysis further revealed associations between PDIs and various metabolites, suggesting that dietary plant components can modulate host metabolism. These findings emphasize the importance of diet in shaping the gut microbiome and highlight the need for a more nuanced understanding of dietary quality assessments in children.

Associationsbetween PDIs, food types, and the gut microbiota

Correlation analysis revealed that PDIs significantly influence the gut microbiota, with this effect being contingent upon the quality of the diet consumed. The PDI, an indicator of a predominantly plant-based diet, was positively correlated with several key bacterial species and metabolic processes. Specifically, we identified positive associations with fiber-degrading bacteria such as *Bacteroides thetaiotaomicron* [28], and bile acid-tolerant *Bilophila wadsworthia* positively correlated [29], and with the SCFAs-producing *Roseburia intestinalis* [30] and *Faecalibacterium prausnitzi*i [31], and *Veillonell*a [32] negatively correlated. Additionally, our observation that the PDI positively affects bile acid-tolerant species such as *Bacteroides thetaiotaomicron*, which are associated with increased consumption of whole grains, vegetables, and fruits, aligns with previous studies showing that their abundance correlates with high-fiber diets, potentially promoting anti-inflammatory responses in immune cells and enhancing mucosal integrity via butyrate production [33–35]. We also found that species positively associated with PDI, such as *B. wadsworthia*, were significantly linked to the intake of fried foods, preserved foods, and sweetened beverages. *B. wadsworthia*, in particular, has been shown to have synergistic effects with a fast-food diet, leading to intestinal barrier dysfunction and abnormal bile acid metabolism [36]. Correlation analysis revealed the key roles of *B. wadsworthia* in lipid and phenylalanine metabolism in children's feces and plasma, with phenylalanine metabolism linked to metabolic dysfunctions in obese adults [37]. Therefore, we hypothesize that a healthy diet may contribute to children's health by modulating the relative abundance of beneficial gut microbiota, thereby influencing metabolic pathways and immune responses. Furthermore, the healthy PDI (hPDI), indicative of a diet abundant in nutritious plant foods, exhibited significant positive correlations with SCFA-producing bacteria such as *Bifidobacterium animalis* and *Ruminococcus bicirculans*, which are recognized for their roles in enhancing nutrient absorption and reducing inflammation [38, 39].

Unexpectedly, our findings of a negative correlation between *F. prausnitzii* and PDI diverge from the expected positive correlation reported in previous studies [40, 41]. Our analysis suggests that the inclusion of vegetables and fruits in PDI food types may play a pivotal role in influencing *F. prausnitzii* levels, which aligns with existing evidence from both adult and pediatric populations [14, 41]. We also observed a positive link was found between *F. prausnitzii* and plasma metabolites related to fruit and vegetable intake, including hippuric and caffeic acids. For instance, hippuric acid is recognized as a plasma biomarker indicative of fruit and vegetable intake in both animal and human studies [42]. Caffeic acid, which is found in a variety of dietary plants, is known for its antioxidant and anti-inflammatory properties [43]. Therefore, we speculate that the intake of other foods in the PDI diet, such as fried or pickled foods, may mask the effect of vegetables and fruits on *F. prausnitzii*, which still participates in the metabolism of vegetables and fruits in the body. Additionally, we observed a significant negative association between *R. intestinalis* and the consumption of preserved foods. The intake of preserved foods may mask the positive effects of other healthy foods, such as vegetables, fruits, and whole grains, on *R. intestinalis*. These results highlight the intricate effects of diet on the gut microbiota, indicating the need to refine PDI calculation methods to better assess children's dietary quality, accounting for the complex interactions of dietary components with the microbiome.

The associations between PDIs, each food type, and metabolites from feces and plasma

Metabolomic analyses revealed significant associations between PDIs and various metabolites in fecal and plasma samples. Notably, we found that fecal metabolites related to tryptophan, biotin, and folate metabolism were positively correlated with PDI, whereas those linked to tryptophan metabolism were negatively correlated with meat intake, suggesting a modulatory role for plant-based foods in metabolism [44]. Plasma analyses revealed higher levels of tryptamine and 4-hydroxybenzaldehyde in individuals with high-hPDI score and low-uPDI score, further emphasizing the metabolic impact

of diet quality. Tryptamine, which was associated with increased vegetable and fruit intake, elevates tryptophan metabolites in animal models, modulating the intestinal immune balance [45]. Its suppression under a high-fat diet may contribute to the development of diseases, such as inflammatory bowel and neuropsychiatric disorders, via end metabolites [46, 47]. Conversely, hPDI negatively correlates with tyrosine metabolism (such as tyramine, 3-hydroxyphenylacetic acid, and *P*-hydroxyphenyl acetic acid) and sphingolipid metabolism (such as sphingosine and 3-dehydrosphinganine), and these pathways are positively linked to Western diets and sweetened drinks. Tyrosine is known to be highly enriched in obese individuals compared with the general population [48], and its metabolism was also negatively associated with *Ruminococcus bicirculans*, a species linked to hPDI in our study. *Ruminococcus bicirculans* may play a role in the degradation of complex polysaccharides [49] and cognitive dysfunction [50]. Moreover, a Western diet high in fat notably affects sphingolipid metabolism via the gut microbiota, possibly increasing low-grade inflammation [51, 52]. Notably, metabolite studies indicate that healthy plant food intake benefits tryptophan metabolism, in contrast with the negative impact of unhealthy diets on sphingolipids. These findings underscore the importance of dietary choices in shaping the metabolic landscape and their consequent impact on health.

Intersection analysis revealed that the lipid metabolism-related cometabolites FFA (20:4), FAHFA (8:0/10:0), and AA were negatively correlated with the PDI. This association likely was deprived of that high-PDI scores, indicative of plant-rich food, typically correspond to decreased intake of animal-derived foods, which are primary contributors to dietary lipids. Interestingly, cyclamic acid was uniquely identified in both the fecal and plasma samples and was positively correlated with the PDI. While the microbial mechanisms driving this link remain elusive, they are postulated to be related to the intake of less healthy plant-based items, such as processed foods and sweetened drinks containing artificial sweeteners such as cyclamic acid. This dietary preference may account for the presence of cyclamic acid in children's metabolites, underscoring the substantial impact of plant food choices on metabolic health and warranting further investigation into the intricate interplay among diet, gut microbiota, and host metabolism.

Our study has several limitations for improvement in future research. First, while we established correlations among them, the causal relationships between PDIs and the gut microbiota and metabolites remain unclear. Second, despite our efforts to balance confounding factors in our analysis, inconsistencies in real-world data could not be entirely avoided. Third, although we calculated and used the cumulative average of the PDIs, we could not rule out the potential for misclassification of healthy and unhealthy plant-based diets. We hope to further refine and improve the PDIs to adapt to diets in different cultural backgrounds in the future, which will involve exploring more accurate and nuanced ways to classify diets and calculate the PDI, so as to enhance the reliability and validity of our research findings related to diet assessment.

Conclusions

In summary, our research indicates that plant-based diet indices (PDIs) are significantly associated with the gut microbiota and influence metabolic health. PDIs, which are rich in plant-derived foods, are associated with diverse microbiota that promote nutrient absorption and anti-inflammatory responses. However, the study also suggested that the quality of plant-based foods is essential, as some choices may introduce fewer beneficial species and metabolites. This underscores the necessity for further studies to refine dietary assessments and explore the intricate links between diet, the gut microbiota, and health.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12937-025-01116-6.

Additional file 1: Table S1A. Phenotypic characteristics of 88 participants. Table S1B. Daily intake (g/d) of 14 food groups among 88 participants. Table S1B2. Examples of food items constituting the 14 food groups. Table S1C. Taxonomic and functional annotation of gut microbiota in 88 participants. Table S1D. PERMANOVA of various phenotypes and PDIs on the gut microbiota and metabolites. Table S1E. Associations of plant-based diet indices with bacterial richness and diversity. Table S1F. Associations of plant-based diet indices with bacterial pathways profiled by HUMAnN 4.0. Table S1G. Associations of plant-based diet indices with fecal metabolites. Table S1H. Associations of plant-based diet indices with plasma metabolites. Table S1I. The information screening process for total metagenomic reads. Figure S1. Correlation analysis of PDIs and dietary items. Figure S2. High relative abundance of gut microbiota at phylum, genus and species level. Figure S3. Heatmap of the genera that significantly associated with PDIs, and individual food groups based on the 88 participants. Figure S4A_6A_7A. PDIs-associated gut microbial metabolites in children. Figure S4B. PDI-associated gut microbial pathways in children. Figure S5. Heatmap of the PDI-related species and fecal metabolites based on the 88 participants. Figure S6B. hPDI-associated gut microbial pathway in children. Figure S7B. uPDI-associated gut microbial pathway in children. Figure S8A_9A_10A. PDIs-associated plasma metabolites in children. Figure S8B. PDI-associated plasma pathway in children. Figure S9B. hPDI-associated plasma pathway in children. Figure S10B. uPDI-associated plasma pathway in children. Figure S11. Heatmap of the PDI-related species and plasma metabolites based on the 88 participants

Additional file 2

Additional file 3

Additional file 4

Additional file 5

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Authors' contributions
M.Z.: literature search, formal analysis, writing-original draft & editing; Q.W.: methodology, data curation, formal analysis, writing-review & editing, funding acquisition, and resources; Y.Y.: investigation, writing-review & editing, funding acquisition. X.L.: Conceptualization, Writing-Review & Editing; J.Z.: Investigation, Formal Analysis; G.L.: Investigation, Formal analysis. W.L.: Investigation, Writing-Review & Editing. X.X.: Conceptualization, Funding acquisition, Supervision, Writing-Review & Editing. J.C.: Conceptualization, Methodology, Writing-Review & Editing.

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Data availability
The data underlying this article are available in the article and its online supplementary material. The data that support the findings of this study are openly available at the China National GenBank Database (CNGBdb) with accession number CNP0004326.

Declarations

Ethics approval and consent to participate
The present study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Lanzhou University Second Hospital (protocol code 2022A-221, date of approval 2 March 2022). All participants provided written informed consent.

Consent for publication
Informed consent was obtained from all the subjects involved in the study.

Competing interests
The authors declare no conflicts of interest.

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