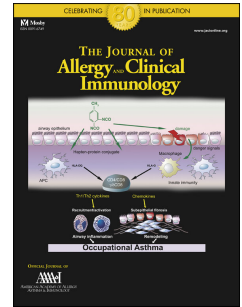


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Early-life gut microbiome composition and milk allergy resolution

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Abstract

Background: Gut microbiota may play a role in the natural history of cow's milk allergy

Objective: To examine the association between early life gut microbiota and the resolution of cow's milk allergy

Methods: We studied 226 children with milk allergy who were enrolled at infancy in the Consortium of Food Allergy (CoFAR) observational study of food allergy. Fecal samples were collected at age 3-16 months, and the children were followed longitudinally with clinical evaluation, milk-specific IgE levels, and milk skin prick test performed at enrollment, 6 months, 12 months, and yearly thereafter up until age 8 years. Gut microbiome was profiled by 16s rRNA sequencing and microbiome analyses performed using QIIME (Quantitative Insights into Microbial Ecology), PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), and STAMP (Statistical Analysis of Metagenomic Profiles).

Results: Milk allergy resolved by age 8 years in 128 (56.6%) of the 226 children. Gut microbiome composition at age 3-6 months was associated with milk allergy resolution by age 8 years (PERMANOVA $P = 0.047$), with enrichment of *Clostridia* and *Firmicutes* in the infant gut microbiome of subjects whose milk allergy resolved. Metagenome functional prediction supported decreased fatty acid metabolism in the gut microbiome of subjects whose milk allergy resolved ($\eta^2 = 0.43$, ANOVA $P = 0.034$).

Conclusions: Early infancy is a window during which gut microbiota may shape food allergy outcomes in childhood. Bacterial taxa within *Clostridia* and *Firmicutes* could be studied as probiotic candidates for milk allergy therapy.

Key messages

- *Clostridia* and *Firmicutes* were enriched in the gut microbiome of milk allergic children at age 3-6 months whose milk allergy resolved by age 8 years.

- Metagenome functional prediction showed decreased fatty acid metabolism in the early infant gut microbiome of children whose milk allergy resolved.

Capsule summary

Examination of early-life gut microbiota in milk allergic children from a longitudinal, multicenter observational study showed that *Clostridia* and *Firmicutes* are associated with resolution of milk allergy.

Key Words: cow's milk allergy, microbiome, microbiota, Clostridia, Firmicutes, Bacteroidetes, metagenome, fatty acid, food allergy, 16s rRNA sequencing

Abbreviations:

CoFAR: Consortium of Food Allergy Research

LDA: linear discriminant analysis

LEfSe : Linear discriminant analysis effect size

OTU: operational taxonomic units

PCoA: principal coordinate analysis

PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

QIIME: Quantitative Insights into Microbial Ecology

slgE: specific IgE

SPT: skin prick test

STAMP: Statistical Analysis of Metagenomic Profiles

Introduction

Cow's milk allergy is the most common food allergy in young children, affecting 2-3%.^{1,2} Individuals with milk allergy are at risk for allergic reactions and also face challenges in finding suitable replacements for the nutritional content that milk-based products provide.³ A child may have to live with the limitations imposed by milk allergy for several years, as recent studies have shown that milk allergy often continues into later childhood and adulthood.^{1,4} Parents of milk allergic children often ask allergists to gauge whether their child's milk allergy will resolve.

We hypothesized that gut microbiota play a role in the natural history of milk allergy, as the etiology of food allergy is thought to involve deviation from the default state of mucosal immune tolerance that may be driven by diet, commensal microbiota, and interactions between them.^{5,6} Variations in infant gut flora have been associated with allergy skin prick test response,⁷ specific IgE levels,⁸ atopic dermatitis,^{8,9} and food allergy status.¹⁰ Much of this previous work has relied upon culture methods, which allow for examination of species specifically targeted and cultured, but exclude the large majority of bacterial organisms that cannot be cultured. These excluded organisms may play key roles in the natural history of milk allergy.

In this study, we used high-throughput sequencing to comprehensively characterize the gut microbiota of 226 children age 3-16 months with cow's milk allergy. We followed these children up to age 8 years and examined for associations between early-life gut microbiota diversity, composition, and milk allergy resolution.

Methods

Study protocols were approved by the institutional review boards of the participating institutions.

Study design and subjects

The subjects of this study are a subset of a larger observational cohort study by the Consortium of Food Allergy Research (CoFAR) of 512 participants with milk allergy, egg allergy, and/or moderate-to-severe atopic dermatitis but without known peanut allergy.^{1,11} Participants were recruited at age 3-15 months from five study centers across the United States and were followed over time. The study sites included the Icahn School of Medicine at Mount Sinai, New York, New York; Duke University School of Medical Center, Durham, NC; Johns Hopkins University School of Medicine, Baltimore, MD; National Jewish Health, Denver, CO; and Arkansas Children's Hospital, Little Rock, AR. The goal of the CoFAR observational study was to identify factors associated with the development of peanut allergy in a high risk cohort. Examination of the natural histories of milk and egg allergy were secondary objectives.

We collected stool from 234 of the 244 CoFAR observational study participants who had milk allergy at study entry. Stool samples were collected at or near the time of enrollment. Samples were collected during a study visit or by the parent at home using a stool collection kit provided by CoFAR. For this study, we excluded from the analysis 3 subjects who submitted stool samples after age 17 months, yielding 231 stool samples from 231 participants for DNA isolation.

DNA isolation and sequencing

DNA was isolated using the MoBio Power Soil DNA Isolation kit (Carlsbad, CA). The V4 region of the 16S *rRNA* gene was amplified with barcoded primers and 16S *rRNA* sequencing was

performed on the Illumina MiSeq platform using 2x250bp paired-end read. Stool samples from 5 participants yielded less than 2,000 sequences/sample (2.2% of 231 samples sequenced) and these subjects were removed from the analysis, yielding a final sample size for analysis of 226 subjects. In total, 5,478,379 sequences were assigned to 16S rRNA gene sequences for the 226 samples, with a median of 23,682 sequences per sample.

Outcomes

Data were prospectively collected from study enrollment up through a visit at approximately age 8 years. Participants were considered to have milk allergy if they had either (1) positive physician-supervised oral food challenge or a convincing reaction and sensitization to milk by milk-specific IgE level ≥ 0.35 kU_A/L and/or skin prick test (SPT) > 3 mm, or (2) a flare of atopic dermatitis associated with milk ingestion along with a milk-specific IgE level > 5 kU_A/L. At entry, dietary, medical, and social histories were obtained by questionnaires administered to the parents of participants. Milk-specific IgE levels and milk skin prick test were performed at entry and periodically thereafter as clinically indicated. Atopic dermatitis severity was graded based on criteria previously described.¹ Participants were evaluated in person at enrollment, 6 months, 12 months, and yearly thereafter with additional telephone follow-up between each visit. Milk allergy was considered persistent if the subject had milk allergy according to the above definitions at the last documented encounter.

We used logistic regression implemented in R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) to test for the associations between early life exposures that shaped the infant gut microbiome (mode of delivery, breastfeeding, solid food intake, antibiotic use) and milk allergy resolution by age 8 years or milk allergen sensitization.

Microbiome analyses

Based on observations from previous studies^{12,13} of marked shifts in the gut microbiome composition in early life associated with developmental stages (e.g. predominant nutrition from breastmilk and formula until age 6 months, predominant nutrition from solid food and transition to whole milk or milk alternatives around 12 months), we *a priori* stratified the participants into the following 3 groups for microbiome analysis: participants whose stool samples were obtained at (1) age 3 to 6 months, (2) age 7 to 12 months, and (3) age 13 to 16 months. The members of the investigative team who conducted the microbiome and bioinformatic analyses had no role in acquiring or assessing the clinical results of the cohort study.

Unless noted otherwise, we performed all analyses using QIIME 1.8.0 (Quantitative Insights into Microbial Ecology), an open-source bioinformatics pipeline for performing analysis of microbiome sequence data. Operational taxonomic units (OTUs), defined as taxonomic units based on DNA sequences that share high identity¹⁴ were constructed using a >97% similarity threshold. Within the three age groups for analysis, alpha diversity (the richness of a sample in terms of the diversity of OTUs observed in it) was estimated using Faith's phylogenetic diversity¹⁵. Beta diversity (distance between samples based on differences in OTUs present in each sample) was measured using unweighted UniFrac¹⁶. Principal coordinate analysis (PCoA) was used to visualize clustering patterns between samples based on beta diversity distances. Linear discriminant analysis effect size (LEfSe)¹⁷, a method for biomarker discovery, was used to determine taxa that best characterize each population, i.e. bacterial groups associated with milk allergy resolution or persistence. LEfSe scores measure the consistence of differences in relative abundance between taxa in the groups analyzed (i.e. resolution vs. persistence), with a higher score indicating higher consistency. Association between microbiome composition and covariates was tested using PERMANOVA, a non-parametric test similar to ANOVA but that does not require the data to be normally distributed. Significance of PERMANOVA tests was determined using 999 permutations with adjustment for multiple testing.

Prediction of metagenome functional content

We used PICRUST 1.0.0 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)¹⁸ to predict metagenome function from the 16S *rRNA* data. Bray-Curtis distances were used to determine similarity of samples based on metagenomic composition. We used STAMP (Statistical Analysis of Metagenomic Profiles)¹⁹ to analyze the predicted metagenome and identify pathways associated with resolution or persistence of milk allergy. The Benjamini-Hochberg procedure was used to control the false discovery rate due to multiple testing.

Baseline characteristic comparisons and logistic regression results were considered statistically significant with unadjusted $P < 0.05$, significant results from linear discriminant analysis (LDA) required $P < 0.05$ and LDA score > 2.0 , and PERMANOVA and metagenome analyses were considered statistically significant with adjusted $P < 0.05$.

Results

Study population

The baseline characteristics of the participants are shown in **Table 1**. The study population was mostly white (77%) with more male (65.9%) than female children. The majority of children had been born vaginally (61.9%) with about half breastfeeding and almost all (96.9%) taking solid foods at entry. The median age of participants at the time of stool collection was 10.6 months (IQR = 4.2) with a range of 3.6 to 16.9 months. The median age at last follow-up was 80.0 months (IQR = 18.0), with no significant difference in age at last follow-up between those with resolution or persistence of milk allergy. Participants whose milk allergy resolved by age 8 years had significantly smaller milk SPT, lower milk sIgE levels, and milder atopic dermatitis at entry.

The baseline characteristics of the participants stratified by age at stool collection (age 3-6 months (n=29), 7-12 months (n=144), 13-16 months (n=53)) are shown in **Supplementary Table 1**.

There were no associations between early life exposures that shaped the infant gut microbiome (mode of delivery, breastfeeding, solid food intake, antibiotic use) and milk allergy resolution by age 8 years or milk allergen sensitization (**Supplementary Table 2**).

Distinct gut microbiome in 3-6 month old subjects with milk allergy resolution by age 8 years

Subjects sampled at age 3-6 months who achieved milk allergy resolution by age 8 years had a distinct gut microbiome composition compared to subjects sampled at the same age whose milk allergy persisted (PERMANOVA $P = 0.047$). This distinction was not observed in subjects sampled later in life at age 7-12 months (PERMANOVA $P = 0.39$) or at age 13-16 months (PERMANOVA $P = 0.96$). There were no significant effects of antibiotic use or atopic dermatitis severity on bacterial composition in the subjects sampled at age 3- 6 months (PERMANOVA $P = 0.53$ and 0.80 , respectively), indicating that neither antibiotic use nor atopic dermatitis were confounders of the association between gut microbiome composition and milk allergy resolution. Results from linear discriminant analysis effect size (LEfSe) and principal coordinate analysis (PCoA) confirmed the distinct gut microbiome composition associated with milk allergy resolution.

Linear discriminant analysis revealed distinct taxa in the microbiome of 3-6 month subjects with milk allergy resolution vs. milk allergy persistence (**Figure 1**). Among subjects sampled at age 3-6 months, the *Firmicutes* phylum and *Clostridia* class were enriched in the infant gut microbiome of subjects whose milk allergy resolved by age 8 years, while *Bacteroidetes* and *Enterobacter*, were characteristic of subjects whose milk allergy did not resolve by age 8 years

(**Figure 1b**). These findings were consistent when covariates (antibiotics, solid food intake, mode of delivery, race, and breastfeeding) were taken into account in the LEfSe analysis (**Supplementary Figure 1**).

PCoA demonstrated separation in gut microbiome composition between subjects sampled at age 3-6 months who achieved milk allergy resolution vs. subjects with persistent milk allergy (**Figure 2a**). PCoA did not show separation reflecting distinct gut microbiome composition related to milk allergy resolution in subjects sampled at age 7-12 or 13-16 months (**Figures 2b and 2c**).

Trend toward increased bacterial diversity in subjects with milk allergy resolution by age 8 years
Compared to subjects whose milk allergy did not resolve by mid-childhood, the gut microbiome of participants with milk allergy resolution showed a trend of increased bacterial diversity. This trend was most pronounced in subjects sampled at age 3-6 months, with decreasing difference in those sampled at age 7-12 months and little difference at age 13-16 months (**Figure 3**).

Distinct metagenome in subjects with milk allergy resolution

PCoA of predicted metagenome function showed a significant separation in bacterial functional pathways related to milk allergy resolution in subjects sampled at age 3-6 months (**Figure 4a**, PERMANOVA $P = 0.004$), but not in those sampled later in life (**Figures 4b and 4c**, PERMANOVA $P = 0.79$ at age 7-12 months and PERMANOVA $P = 0.72$ at age 13-16 months). The only metabolic pathway that was differentially enriched in the metagenome of subjects sampled at age 3-6 months was fatty acid metabolism, which was decreased ($\eta^2 = 0.43$, $P = 0.034$) in subjects whose milk allergy resolved (**Figure 5 and Supplementary Table 3**). Subjects sampled at older ages did not show differential abundance of any metabolic pathways (**Supplementary Table 3**).

Correlation between gut microbiome diversity, composition, and milk sIgE level or milk SPT

Milk sIgE levels or milk SPT size at baseline, change in levels, or slope of these measures over time were not significantly correlated with abundance of any microbial taxa. Alpha diversity was not significantly correlated with milk sIgE in any age group (PERMANOVA $P = 0.40$ in age 3-6 months; PERMANOVA $P = 0.08$ in age 7-12 months; PERMANOVA $P = 0.18$ in age 13-16 months), nor with milk SPT in any age group (PERMANOVA $P = 0.32$ in age 3-6 months; PERMANOVA $P = 0.10$ in age 7-12 months; PERMANOVA $P = 0.27$ in age 13-16 months).

Discussion

We found that gut microbiome composition of milk allergic children was associated with resolution of milk allergy by age 8 years, with enrichment of *Clostridia* and *Firmicutes* in the 3-6 month old gut microbiome of subjects whose milk allergy later resolved. Our examination of predicted metagenome function showed differential bacterial function associated with milk allergy resolution, with decreased fatty acid metabolism in resolvers.

Comparison with other studies

Our study is the first to examine the relationship between the microbiome and food allergy resolution. Previous studies have examined associations between the gut microbiome and food allergy status. Using bacterial cultures, Thompson-Chagoyan et al. found that compared to healthy infants, cow's milk allergic infants had higher total bacteria and anaerobic counts; after 6 months during which milk allergic subjects took extensively hydrolyzed formula and healthy control subjects took cow's milk formula, the milk allergic group had higher proportions of lactobacilli and lower proportions of enterobacteria and bifidobacteria.¹⁰ Major limitations of this study included reliance on bacterial culture (which cannot characterize the majority of bacterial taxa, which are unculturable) and differential diet as a confounder of the association between

gut microbiome and food allergy status. Ling et al. reported enrichment of *Clostridium sensu stricto*, *Butyricicoccus*, *Clostridaceae 1*, and *Ruminococcaceae* sequences in 34 Chinese infants with food allergy to a variety of foods compared to 45 healthy controls, but half of the “food allergic” had non-IgE-mediated food allergy.²⁰ Employing a murine model of food allergy, Noval Rivas et al. reported that OVA-sensitized IL4raF709 mice had differential abundance of *Lachnospiraceae*, *Lactobacillaceae*, *Rikenellaceae*, and *Porphyromonadaceae* compared to wild type mice, and reconstitution of wildtype mice with the microbiota from these allergen-sensitized mice promoted OVA-specific IgE responses.²¹ Although the above studies reported differences in bacterial composition related to food allergy status, none examined resolution of food allergy as we did in this study.

We found taxa from the *Clostridia* class and *Firmicutes* phylum to be enriched in human subjects with milk allergy resolution. Such taxa have been previously linked to immune tolerance in mouse models of allergy and protection from allergic inflammation in subjects with eosinophilic esophagitis. Atarashi et al. identified 17 strains of bacteria from human stool that enhanced Treg cell abundance and induced key anti-inflammatory markers such as IL-10 and inducible T-cell co-stimulator (ICOS) in Treg cells upon inoculation into germ-free mice, with genome sequencing revealing that all 17 strains were taxa within *Clostridia*.²² Oral administration of these 17 *Clostridia* strains into mice attenuated disease in models of colitis and allergic diarrhea.²² Stefka et al. found that gnotobiotic mice colonized with *Clostridium* clusters protected against sensitization to peanut/cholera toxin, with reduced levels of peanut-specific and total IgE compared to germ-free controls and no temperature drop upon allergen challenge.²³ Additionally, *Clostridia*-colonized mice had significantly increased proportions of Foxp3+ Tregs in their colonic lamina propria and increased concentrations of fecal IgA compared to germ-free mice.²³ *Clostridia* colonization induced IL-22 production by RORgt+ innate lymphoid cells and T cells, which reduced the concentration of serum allergen,

supporting the concept that *Clostridia* play a critical role in regulating sensitization to food allergens.²³ A strength of our study is that in contrast to these previous murine studies, it provides human data to support that *Clostridia* play a role in allergy and inflammation. With regards to *Firmicutes*, a study of 33 pediatric subjects with eosinophilic esophagitis and 35 normal controls showed that taxa from the *Firmicutes* phylum predominated the esophageal microbiota of control subjects.²⁴

The gut microbiome of our 3-6 month subjects with persistent milk allergy was enriched with taxa from phylum *Bacteroidetes*, which is consistent with a prior study demonstrating a predominance of this phylum in infants with milk allergy and high risk of chronic allergic disease. Specifically, Kirjavainen et al. found high abundance of *Bacteroides* in the gut microbiome of infants with a high degree of milk allergy (as demonstrated by reaction to extensively hydrolyzed formula), early onset atopic eczema, and a strong family history of atopic disorders.²⁵ Our results and those of Kirjavainen et al. are in contrast to findings from murine models, where colonization with *Bacteroides* were observed to correct Th1/Th2 imbalances²⁶ and induce regulatory T cell development.^{27,28}

The results of our predicted metagenome analyses showed that bacterial functional pathways related to fatty acid metabolism were significantly reduced in subjects whose milk allergy resolved with time. This finding suggests that decreased fatty acid metabolism by gut microbiota may be helpful toward reducing long-term allergy risk. Gas liquid chromatography studies of infant stool composition show lower levels of branched-chain short fatty acids in healthy infants relative to cow's milk allergic infants.²⁹ These levels were observed cross-sectionally, so it is not known if lower levels of fatty acids would be found in infants whose milk allergy resolved with time versus those with persistent milk allergy. Overall, lipids are known to facilitate the passage

of food allergens through intestinal epithelial barriers and affect the allergenicity of food proteins.³⁰ Lipids in cow's milk itself can induce the expansion of iNKT cells and release of IL-4, IL-5, and IL-13, leading to a Th2-dominant milieu.³¹ Attenuation of such lipid-driven pro-inflammatory effects would be ostensibly beneficial in allergy resolution, and our results suggest that gut microbiota may contribute to this via reduced fatty acid metabolism.

Early infancy as a time window shaping the course of food allergy

The associations between microbiome composition, metagenome functional content, and milk allergy resolution were specific to subjects sampled at 3-6 months in our study, with no significant associations in subjects sampled at 7-12 months or 13-16 months. Two potential interpretations of this include: (1) early infancy (i.e. 3-6 months) is a particular developmental time window during which gut microbiota shapes the course of food allergy, and gut microbiota at later ages (e.g. 7-16 months) have little effect on food allergy course, and (2) gut microbiota is associated with food allergy course at different age stages, but confounders obscure this signal when subjects are sampled at > 6 months age. Early infancy could be a key developmental time window, as we and others have shown age-dependent associations between infant gut microbiome and allergy. Azad and colleagues found that gut microbial richness at age 3 months was associated with increased likelihood of food sensitization by 1 year, while there was no association between microbial richness at 12 months and food sensitization.³² Similarly, Abrahamsson et al. found that gut microbiota diversity at age 1 month but not at age 12 months was associated with atopic dermatitis during the first 2 years of life.³³ Porcine models have shown that the postnatal environment from as early as the first day of life influences gut microbiota and subsequent metabolic phenotypes.³⁴ Murine models also support that age-sensitive contact with microbiota is critical for establishing tolerance to later exposures.³⁵ Our collective findings support the theory that factors influencing early immune system development may be particularly important for subsequent allergy development.³⁶

However, the intestinal microbiome in early life is markedly dynamic with dramatic shifts after the introduction of solid foods leading to an “adult-like” profile.^{12,37} Thus, it is possible that associations between gut microbiota and allergy outcomes are obscured after the introduction of solid food in the diet, e.g. at approximately 6 months of age.

No association between gut microbiota and milk-specific IgE or milk SPT

We did not find an association between gut microbiota and sensitization (by sIgE or SPT) to milk at baseline, confirming previously reported results. Adlerberth et al. found no association between culturable bacteria and food sensitization by 18 months in 3 cohorts of European infants.⁸ Similarly, Kendler et al. reported no association between culturable gut microbiota and sensitization to common food allergens including milk, casein, egg, peanut and hazelnut.³⁸ Experimental models suggest that food antigen sensitization results from inhibition of the immune system default of tolerance.³⁹ The cross-sectional design of our study precludes us from determining whether changes over time in gut microbiota composition correlate with the decline in IgE levels associated with food allergy resolution. It is also possible that the mechanisms by which early life microbiome composition are associated with resolution are indirect through the production of bacterial metabolites. Indeed, short-chain fatty acids such as butyrate have been shown to have anti-inflammatory properties²³ and to regulate macrophage function.⁴⁰ Overall, our results suggest that gut microbiota may not play a direct role in tolerance inhibition leading to food allergen sensitization, but may instead help reestablish the default state of tolerance.

Limitations and future directions

Although we examined the gut microbiome of 226 subjects with milk allergy, significant associations between gut microbiome composition, predicted metagenome function, and milk

allergy resolution were observed only in those sampled at age 3-6 months. This subset proved to be informative and supports the notion that the gut microbiome of young infants may be of particular interest and should be targeted with larger sample sizes in future studies that include longitudinal sampling. We note that previous studies of the early-life microbiome have had similar or smaller sample size for young infants^{41,42} A secondary analysis of microbiota associated with tolerance of baked milk would have been of interest, but we did not characterize baked milk consumption in a rigorous manner. Ability to consume products with baked milk is associated with accelerated resolution of milk allergy.^{43,44} Additionally, although our findings support the concept that specific gut microbial taxa are associated with milk allergy resolution, we caution that our results cannot determine causality and do not imply that probiotic supplementation with these taxa should be implemented as treatment for milk allergy without prospective study. Probiotic supplementation, particularly with *Lactobacillus* and *Bifidobacterium* species, has been explored as potential treatment for cow's milk allergy in randomized controlled trials with mixed results.^{45,46} The taxa we identified differ from those already targeted and would require prospective study for efficacy as well as safety before their potential use as treatment for cow's milk allergy.⁴¹

Conclusions

We found enrichment of *Clostridia* and *Firmicutes* in the infant gut microbiome of 3-6 month old subjects whose milk allergy resolved by age 8 years. The microbiota of these young infants whose milk allergy resolved were associated with decreased fatty acid metabolism. Our results are the first to address the relationship between gut microbiota and food allergy resolution, and our findings suggest directions for prospective examination of the highlighted taxa for potential therapeutic consideration. Early infancy is a window during which gut microbiota may shape food allergy outcomes in childhood.

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Figure legends

Figure 1: Linear discriminant analysis shows distinct gut microbiome composition

associated with milk allergy resolution. Taxa within *Clostridia* and *Firmicutes* were enriched in children sampled at age 3-6 months with milk allergy resolution vs. milk allergy persistence by age 8 years, while taxa within *Bacteroidetes* were underrepresented. (a) Linear Discriminant Analysis scores as calculated by LEfSe of taxa differentially abundant in milk allergy resolution and milk allergy persistence (b) LEfSe cladogram representing differentially abundant taxa. All taxa were significantly associated with either resolution or persistence of milk allergy ($P < 0.05$). Only taxa with LDA scores >2 are presented.

Figure 2. Principal coordinate analysis shows separation of bacterial composition at age

3-6 months associated with milk allergy resolution. PCoA plots of bacterial composition and diversity of the gut microbiome of children sampled at age (a) 3-6 months, (b) 7-12 months, and (c) 13-16 months. Plots are based on unweighted UniFrac distances, with each point corresponding to a subject and colored by milk allergy resolution vs. milk allergy persistence by age 8 years. Only children sampled at age 3-6 months had a significantly distinct microbiome composition associated with milk allergy resolution by age 8 years (PERMANOVA $P = 0.047$).

Figure 3. Trend toward increased bacterial diversity in subjects with milk allergy

resolution by age 8 years. Rarefaction curves for bacterial diversity of the gut microbiome of children sampled at age (a) 3-6 months, (b) 7-12 months, and (c) 13-16 months. The vertical axis indicates alpha diversity measured using Faith's phylogenetic diversity, while the horizontal axis indicates sequencing depth. Mean and standard deviation alpha diversity at each sequencing depth were estimated by subsampling all samples 10 times at the specified rarefaction.

Figure 4. Principal coordinate analysis shows separation of metagenome functional content at age 3-6 months associated with milk allergy resolution. PCoA plots of metagenome functional content of the gut microbiome of children sampled at age (a) 3-6 months, (b) 7-12 months, and (c) 13-16 months. Plots are based on Bray-Curtis distances, with each point corresponding to a subject and colored by milk allergy resolution vs. milk allergy persistence by age 8 years. Only children sampled at age 3-6 months had a significantly distinct metagenome composition associated with milk allergy resolution by age 8 years (PERMANOVA $P = 0.004$).

Figure 5. Metagenome functional enrichment of fatty acid metabolism in milk allergy persistence. Prediction of metagenome functional content using PICRUST showed differential enrichment of fatty acid metabolism pathways in children sampled at age 3-6 months ($\eta^2 = 0.43$, ANOVA $P_{\text{corrected}} = 0.034$). The vertical axis indicates the relative abundance of predicted KEGG orthologs annotated as being part of fatty acid metabolism. Error bars indicate standard deviation from the mean. **Supplementary Table 3** includes information on all pathways.

464 **Table 1: Baseline characteristics of the participating subjects with milk allergy**

Characteristics	All subjects with milk allergy included in the analysis	Milk allergy persistent at age 8 years	Milk allergy resolved by age 8 years	P value*
Subjects	226	98 (43.4%)	128 (56.6%)	
Age at stool collection				0.83
3-6 months	29	11 (37.9%)	18 (62.1%)	
7-12 months	144	64 (44.4%)	80 (55.6%)	
13-16 months	53	23 (43.4%)	30 (56.6%)	
Age at last followup (months) – median (IQR)	80.0 (18.0)	79.9 (18.4)	80 (15.8)	0.68 [^]
Sex				1.0
Female	77	33 (42.9%)	44 (57.1%)	
Male	149	65 (43.6%)	84 (56.4%)	
Race				0.13
White	174	72 (41.4%)	102 (58.6%)	
Black/African-American	30	12 (40.0%)	18 (60.0%)	
Asian	19	13 (68.4%)	6 (31.6%)	
Other	3	1 (33.3%)	2 (66.7%)	
Baseline milk IgE level				2.1 x 10 ⁻⁹
<2	91	20 (22.0%)	71 (78.0%)	
≥ 2-10	68	30 (44.1%)	38 (55.9%)	
≥10	67	48 (71.6%)	19 (28.4%)	
Baseline milk SPT response (wheal in mm)				1.1 x 10 ⁻⁵
<5	63	12 (19.0%)	51 (81.0%)	
5-10	82	41 (50.0%)	41 (50.0%)	
>10	81	45 (55.6%)	36 (44.4%)	

Baseline AD severity				0.02
None	29	6 (20.7%)	23 (79.3%)	
Mild	32	11 (34.4%)	21 (65.6%)	
Moderate	126	62 (49.2%)	64 (50.8%)	
Severe	39	19 (48.7%)	20 (51.3%)	
Breast-feeding at entry				0.47
Never	36	14 (38.9%)	22 (61.1%)	
Yes, currently	115	47 (40.9%)	68 (59.1%)	
Yes, but no longer	75	37 (49.3%)	38 (50.7%)	
Mode of delivery**				0.78
Vaginal	140	62 (44.3%)	78 (55.7%)	
C-section	85	36 (42.4%)	49 (57.6%)	
Solid food intake				0.70
Yes	219	96 (43.8%)	123 (56.2%)	
No	7	2 (28.6%)	5 (71.4%)	
Antibiotics – lifetime prior to sampling				0.89
Yes	142	61 (43.0%)	81 (57.0%)	
No	84	37 (44.0%)	47 (56.0%)	

*Fisher Exact test

^Wilcoxon rank sum test

**Data N/A for 1 subject

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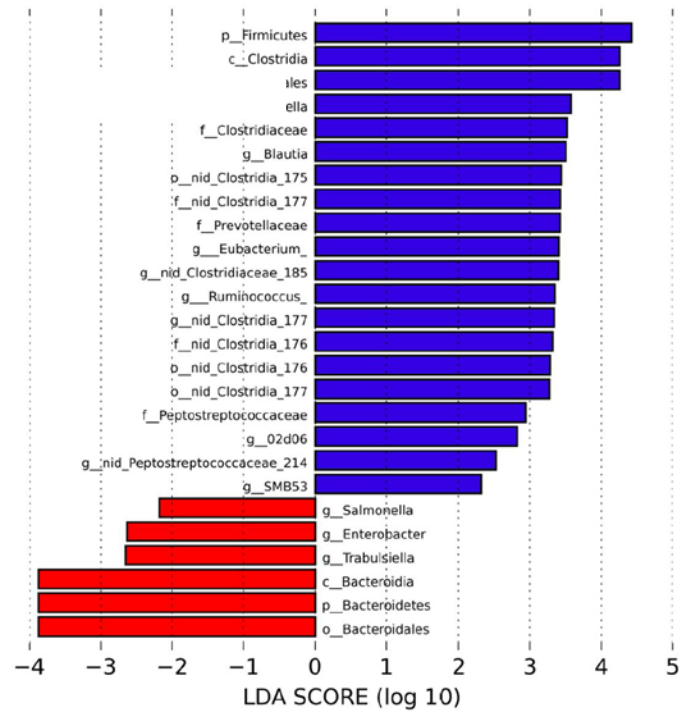
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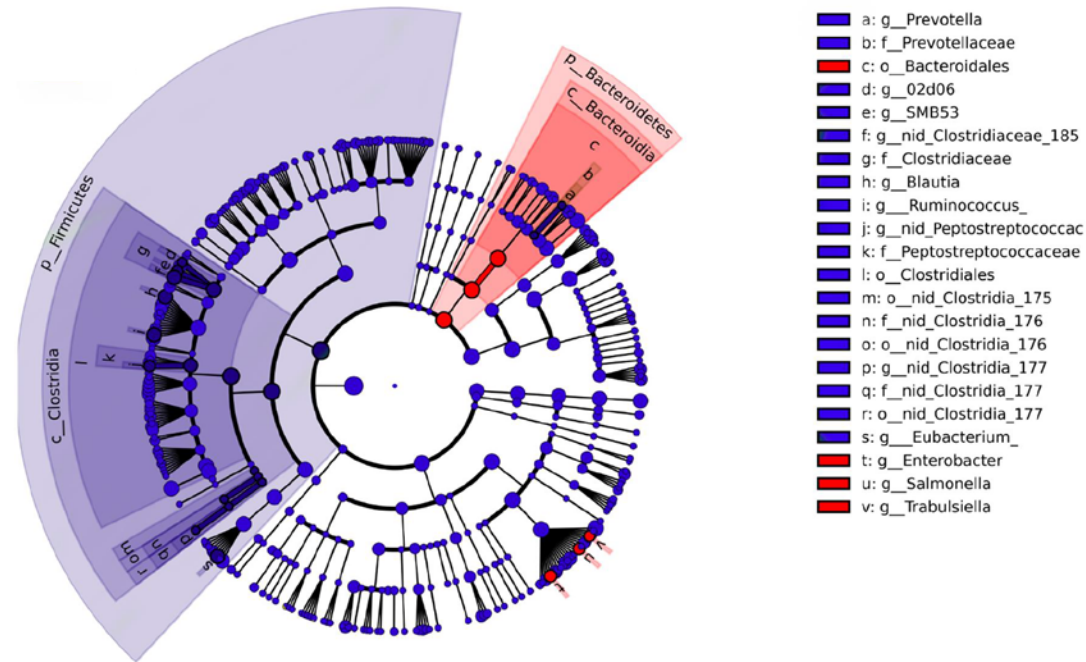
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Figure 1

a



b



Resolved Persistent

Figure 2

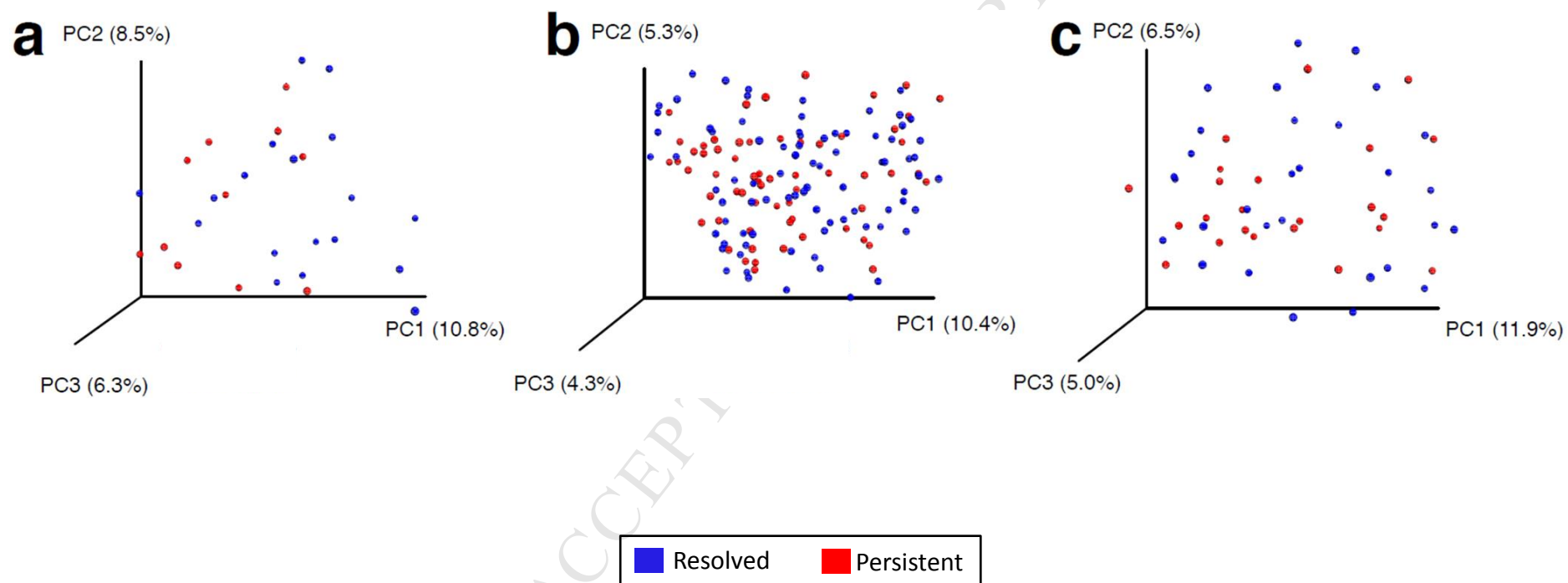


Figure 3

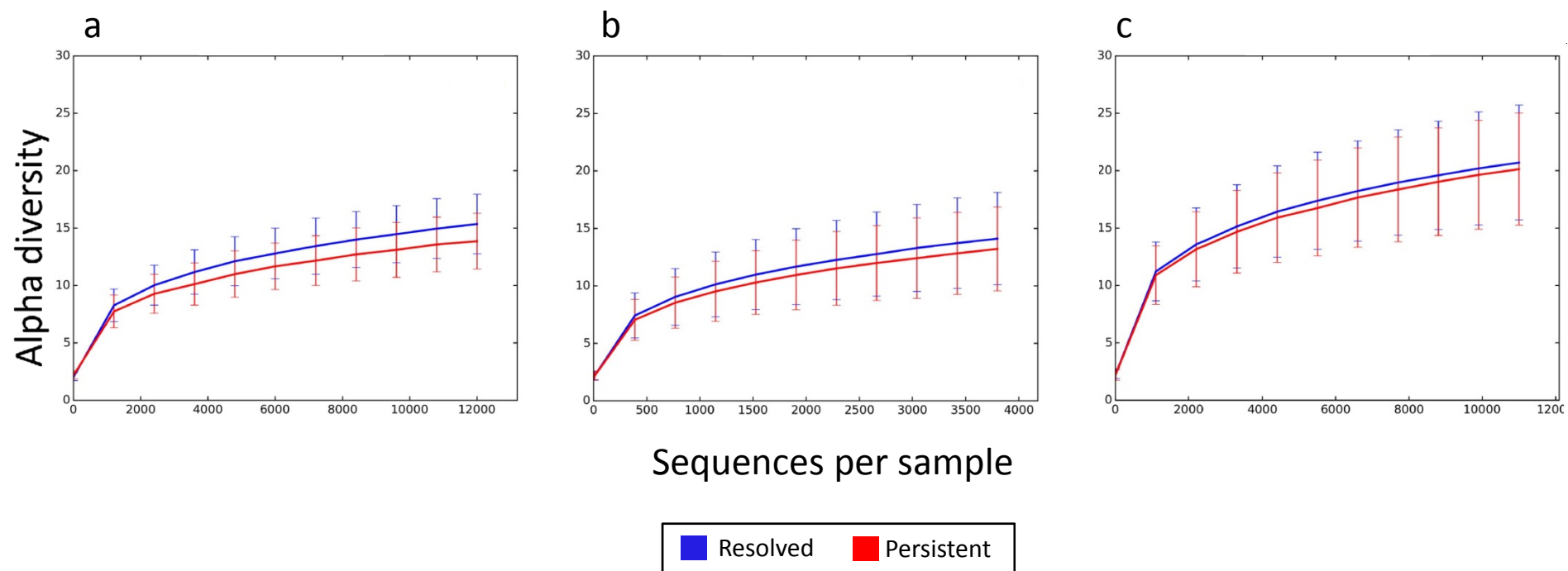


Figure 4

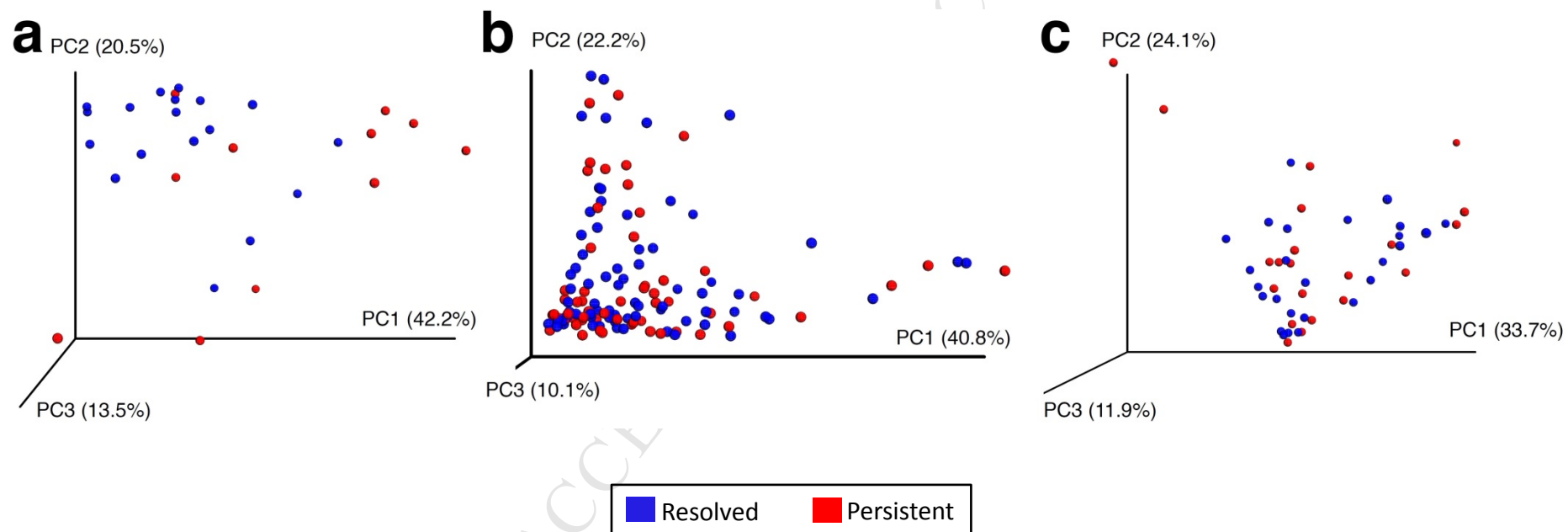
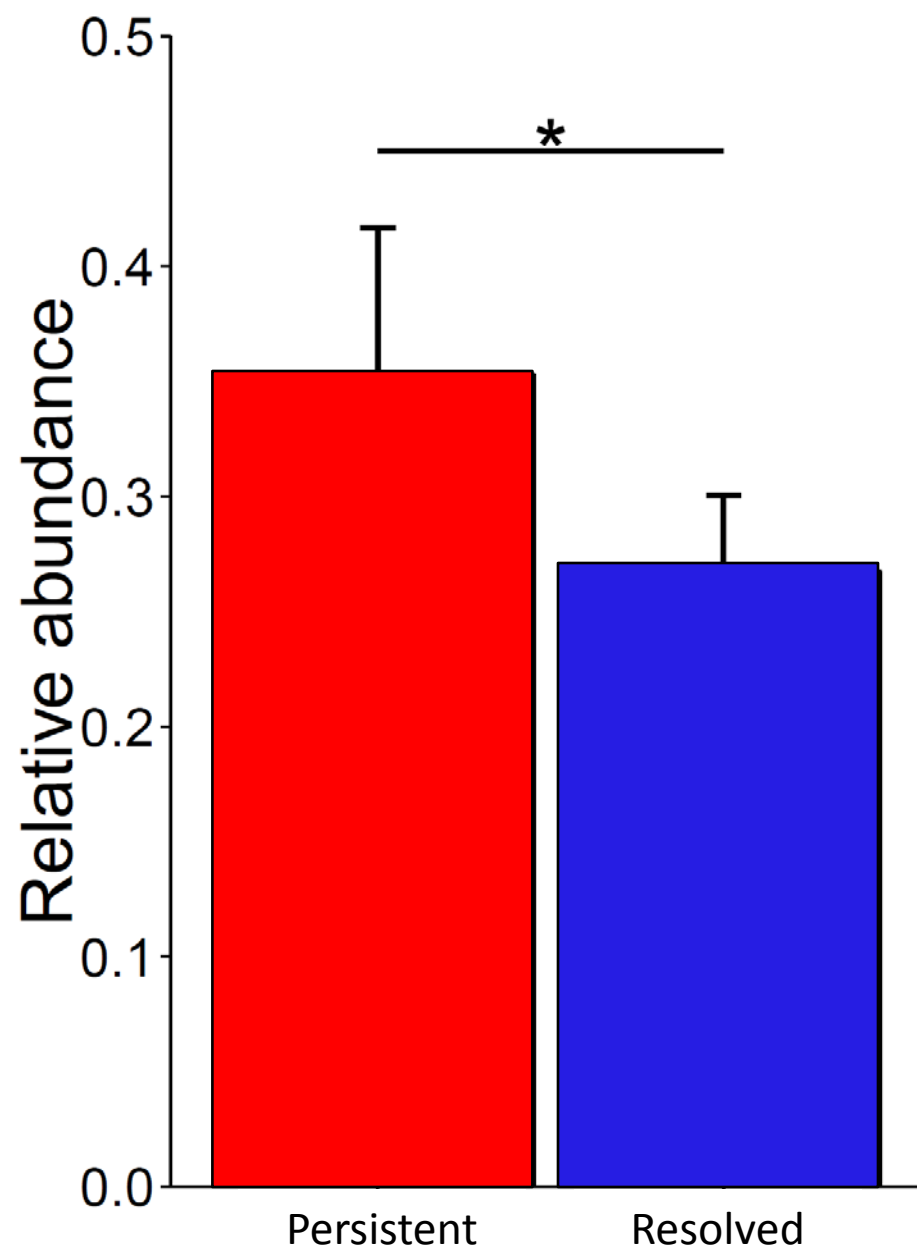


Figure 5



SUPPLEMENTARY MATERIAL**Early-life gut microbiome composition is associated with milk allergy resolution**

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Supplementary Table 1: Baseline characteristics of the participants stratified by age at time of stool collection

Characteristics	All subjects with milk allergy included in the analysis	Age 3-6 months	Age 7-12 months	Age 13-16 months	P-value*
Subjects	226	29 (12.8%)	144 (63.7%)	53 (23.4%)	
Age at last followup (months) – median(IQR)	80.0 (18.0)	72.4 (18.8)	79.4 (22.5)	83.6 (10.8)	
Sex					0.23
Female	77	14 (18.2%)	46 (59.7%)	17 (22.1%)	
Male	149	15 (10.1%)	98 (65.8%)	36 (24.2%)	
Race					0.02
White	174	16 (9.2%)	112 (64.4%)	46 (26.4%)	
Black/African-American	30	8 (26.7%)	16 (53.3%)	6 (20%)	
Asian	19	5 (26.3%)	13 (68.4%)	1 (5.3%)	
Other	3	0 (0.0%)	3 (100.0%)	0 (0.0%)	
Baseline milk IgE level					0.17
<2	91	18 (19.8%)	52 (57.1%)	21 (23.1%)	
≥ 2-10	68	6 (8.8%)	46 (67.6%)	16 (23.5%)	
≥10	67	5 (7.5%)	46 (68.7%)	16 (23.9%)	
Baseline milk SPT response (wheal in mm)					0.0005
<5	63	18 (28.6%)	29 (46.0%)	16 (25.4%)	
5-10	82	7 (8.5%)	56 (68.3%)	19 (23.2%)	
>10	81	4 (4.9%)	59 (72.8%)	18 (22.2%)	
Baseline AD severity					0.01
None	29	4 (13.8%)	15 (51.7%)	10 (34.5%)	
Mild	32	0 (0.0%)	24 (75.0%)	8 (25.0%)	

Moderate	126	14 (11.1%)	83 (65.9%)	29 (23.0%)	
Severe	39	11 (28.2%)	22 (56.4%)	6 (15.4%)	
Breast-feeding at entry					0.15
Never	36	6 (16.7%)	22 (61.1%)	8 (22.2%)	
Yes, currently	115	14 (12.2%)	67 (58.3%)	34 (29.6%)	
Yes, but no longer	75	9 (12.0%)	55 (73.3%)	11 (14.7%)	
Mode of delivery**					0.04
Vaginal	140	23 (16.4%)	90 (64.3%)	27 (19.3%)	
C-section	85	6 (7.1%)	53 (62.4%)	26 (30.6%)	
Solid food intake					2.8x10 ⁻⁷
Yes	219	22 (10.0%)	144 (65.8%)	53 (24.2%)	
No	7	7 (100.0%)	0 (0.0%)	0 (0.0%)	
Antibiotics – lifetime prior to sampling					0.01
Yes	142	11 (7.7%)	95 (66.9%)	36 (25.4%)	
No	84	18 (21.4%)	49 (58.3%)	17 (20.2%)	

**Data N/A for 1 subject

*Fisher's Exact test

Supplementary Table 2: Association between early life exposures and main outcomes of interest

	Milk allergy resolution by age 8 years OR (95% CI)	Sensitization to milk at baseline OR (95% CI)
Mode of delivery	1.08 (0.61-1.94)	1.50 (0.72-3.27)
Breastfeeding status		
Never	reference	Reference
Yes, currently	0.92 (0.42-1.97)	0.72 (0.25-1.83)
Yes, no longer	0.65 (0.29-1.46)	0.74 (0.24-2.00)
Solid food intake	0.51 (0.05-3.20)	10.38 (1.63-112.62)
Antibiotic use	1.05 (0.58-1.87)	1.48 (0.73-2.99)

Supplementary Table 3. Results for tests of association between bacterial functional pathways and milk allergy resolution using STAMP. Subjects were stratified by age (3-6 months, 7-12 months, 13-16 months). PICRUST-predicted KEGG metabolic pathways were utilized as input for STAMP analysis. ANOVA with Tukey-Kramer post-hoc test and Benjamini-Hochberg FDR correction for multiple testing were utilized to test for associations between each pathway and resolution or persistence. Effect size was measured as η^2 . Pathways are represented using standard KEGG conventions, e.g. “Metabolism” at the top level, “Lipid Metabolism” as the second level, and “Fatty acid metabolism” at the lowest level. Degenerate cases where the test statistic could not be calculated are indicated. See file Bacterial_functional_pathways_milk_resolution_STAMP.xlsx.

Supplementary Figure 1. Linear discriminant analysis shows distinct gut microbiome composition associated with milk allergy resolution regardless of covariates. After accounting for specific covariates known to play a role in shaping infant microbiome composition, taxa within *Clostridia* and *Firmicutes* were still enriched in children sampled at age 3-6 months with milk allergy resolution vs. milk allergy persistence by age 8 years, while taxa within *Bacteroidetes* were underrepresented. Linear Discriminant Analysis scores as calculated by LEfSe are presented for antibiotics, breastfeeding, mode of delivery, race, and solid food intake. All taxa were significantly associated with either resolution or persistence of milk allergy ($P < 0.05$). Only taxa with LDA scores >2 are presented.

Level_1	Level_2	Level_3	p-values	p-values (corrected)	Effect size	Note	No: mean rel. freq. (%)	No: std. dev. (%)	Yes: mean rel. freq. (%)	Yes: std. dev. (%)
Metabolism	Lipid Metabolism	Fatty acid metabolism	0.00010408	0.0341384	0.433196106		0.352531247	0.064075578	0.267186047	0.033255287
Metabolism	Metabolism of Terpenoids and Polyketides	Tetracycline biosynthesis	0.000781902	0.256463818	0.346518429		0.108713919	0.037671817	0.155088573	0.025905791
Metabolism	Xenobiotics Biodegradation and Metabolism	Drug metabolism - cytochrome P450	0.000928566	0.304569507	0.338619769		0.077008333	0.024343373	0.043192381	0.022023576
Metabolism	Xenobiotics Biodegradation and Metabolism	Metabolism of xenobiotics by cytochrome P450	0.001011995	0.331934317	0.334636432		0.072917622	0.025072607	0.041036874	0.019554501
Metabolism	Energy Metabolism	Methane metabolism	0.001997867	0.655300523	0.234239633		1.057670271	0.115056711	1.234263678	0.138537884
Metabolism	Metabolism of Other Amino Acids	Glutathione metabolism	0.002558446	0.839170222	0.290423605		0.298051334	0.064280601	0.22262497	0.052414604
Metabolism	Amino Acid Metabolism	Tyrosine metabolism	0.003110073	1.020104108	0.280823111		0.400851081	0.032489936	0.355101247	0.037257176
Metabolism	Carbohydrate metabolism	Carbohydrate metabolism_Unclassified	0.003373399	1.106474794	0.276797299		0.227426041	0.034073375	0.193400116	0.020926002
Metabolism	Metabolism of Terpenoids and Polyketides	Geraniol degradation	0.003685055	1.208698073	0.272400806		0.105449431	0.054397867	0.05250474	0.032119727
Metabolism	Lipid Metabolism	Fatty acid biosynthesis	0.005081966	1.666884847	0.256240573		0.399805721	0.066544031	0.459086832	0.034103543
Metabolism	Xenobiotics Biodegradation and Metabolism	Caprolactam degradation	0.005180599	1.699236541	0.255265821		0.084606112	0.044400567	0.043487685	0.025812013
Metabolism	Metabolism of Cofactors and Vitamins	Retinol metabolism	0.005792037	1.899788028	0.249590477		0.05194853	0.016616976	0.031489558	0.017567098
Metabolism	Amino Acid Metabolism	Tryptophan metabolism	0.007573126	2.483985381	0.235826818		0.234064028	0.084182668	0.166085442	0.036729884
Metabolism	Lipid Metabolism	Glycerolipid metabolism	0.008458715	2.774458586	0.230100058		0.36163871	0.043144258	0.412917569	0.046901097
Metabolism	Metabolism of Cofactors and Vitamins	Ubiquinone and other terpenoid-quinone biosynthesis	0.008882677	2.913518013	0.227558552		0.294170623	0.087287398	0.193064464	0.09222745
Metabolism	Amino Acid Metabolism	Valine, leucine and isoleucine degradation	0.00891621	9.24516719	0.227362514		0.268559502	0.060050905	0.212585246	0.042835049
Metabolism	Carbohydrate Metabolism	Fructose and mannose metabolism	0.009152974	3.002175574	0.225998079		0.944858712	0.143520617	1.173267517	0.234915685
Metabolism	Amino Acid Metabolism	Lysine degradation	0.010273914	3.369843901	0.219964997		0.204600837	0.073778912	0.146185803	0.035544831
Metabolism	Xenobiotics Biodegradation and Metabolism	Toluene degradation	0.01248897	4.096382153	0.209703049		0.142947242	0.038359465	0.098794035	0.043446228
Metabolism	Metabolism of Cofactors and Vitamins	Vitamin B6 metabolism	0.013035819	4.275748498	0.207439378		0.204623142	0.025936796	0.177180306	0.068082901
Metabolism	Amino Acid Metabolism	Phenylalanine metabolism	0.013976945	4.58443802	0.203750137		0.2209566	0.056662407	0.178907346	0.025669505
Metabolism	Nucleotide metabolism	Nucleotide metabolism_Unclassified	0.016974501	5.567636381	0.193415127		0.106427797	0.045476763	0.073313081	0.021696252
Metabolism	Carbohydrate Metabolism	Butanoate metabolism	0.017148577	5.624733335	0.19287044		0.700222464	0.088338463	0.638100676	0.036835371
Metabolism	Glycan Biosynthesis and Metabolism	Peptidoglycan biosynthesis	0.017207509	5.644062913	0.192687251		0.697815343	0.103263214	0.775579163	0.055622682
Metabolism	Lipid Metabolism	alpha-Linolenic acid metabolism	0.020498795	6.723604629	0.183313469		0.02340777	0.01140407	0.011412062	0.007618964
Metabolism	Lipid Metabolism	Biosynthesis of unsaturated fatty acids	0.020952092	6.872286311	0.182138282		0.200665275	0.058705073	0.147209551	0.052544466
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of siderophore group nonribosomal peptides	0.021164898	6.942086465	0.181595051		0.074187167	0.051419704	0.040339372	0.018512514
Metabolism	Enzyme Families	Protein kinases	0.021614478	7.089548772	0.180464599		0.398115647	0.078318249	0.342712546	0.039229507
Metabolism	Biosynthesis and biodegradation of secondary metabolites	Biosynthesis and biodegradation of secondary metabolites_Unclassified	0.02302028	7.550651969	0.177071416		0.115652114	0.059848092	0.074994424	0.026928687
Metabolism	Amino Acid Metabolism	Glycine, serine and threonine metabolism	0.023529358	7.717629371	0.175892077		0.859762967	0.0533977	0.811003909	0.049826595
Metabolism	Glycan Biosynthesis and Metabolism	Lipopolysaccharide biosynthesis proteins	0.024203305	7.938684138	0.174368361		0.47291436	0.184417874	0.296533881	0.187325205
Metabolism	Xenobiotics Biodegradation and Metabolism	Polycyclic aromatic hydrocarbon degradation	0.025487901	8.360031431	0.171574969		0.077402656	0.031275146	0.098819289	0.015655473
Metabolism	Biosynthesis of Other Secondary Metabolites	Flavone and flavonol biosynthesis	0.026240854	8.6070002	0.170000662		0.005904394	0.005249198	0.002595821	0.001853026
Metabolism	Biosynthesis of Other Secondary Metabolites	Isoquinoline alkaloid biosynthesis	0.026254952	8.611624147	0.169971607		0.057053783	0.007253432	0.048903022	0.010240526
Metabolism	Carbohydrate Metabolism	Inositol phosphate metabolism	0.026606604	8.726966109	0.169251742		0.124673929	0.048020587	0.092938169	0.021331084
Metabolism	Metabolism of Cofactors and Vitamins	Porphyrin and chlorophyll metabolism	0.028003488	9.185144017	0.1664809		0.612303391	0.18800453	0.813107455	0.234468594
Metabolism	Metabolism of Terpenoids and Polyketides	Limonene and pinene degradation	0.029603719	9.710019714	0.163467769		0.124683859	0.033948944	0.097676553	0.01891872
Metabolism	Carbohydrate Metabolism	Amino sugar and nucleotide sugar metabolism	0.03267837	10.71850531	0.158100481		1.419899174	0.161495162	1.559001762	0.152135427
Metabolism	Glycan Biosynthesis and Metabolism	Lipopolysaccharide biosynthesis	0.033132593	10.86749044	0.157349772		0.300722074	0.15209546	0.168820764	0.145615129
Metabolism	Xenobiotics Biodegradation and Metabolism	Fluorobenzoate degradation	0.034662766	11.36938726	0.154893001		0.020624766	0.017597738	0.008543837	0.010620659
Metabolism	Metabolism of Terpenoids and Polyketides	Terpenoid backbone biosynthesis	0.034911489	11.45096829	0.154503742		0.463153848	0.09248323	0.520975294	0.041386585
Metabolism	Lipid Metabolism	Linoleic acid metabolism	0.037198106	12.20097887	0.151047553		0.043072532	0.022256342	0.06067593	0.01891872
Metabolism	Glycan biosynthesis and metabolism	Glycan biosynthesis and metabolism_Unclassified	0.03728368	12.22904692	0.150922299		0.06523242	0.044132882	0.037102966	0.022322006
Metabolism	Metabolism of Other Amino Acids	D-Arginine and D-ornithine metabolism	0.037622731	12.34025589	0.150428791		0.002016502	0.001150775	0.004686436	0.003802842
Metabolism	Energy Metabolism	Oxidative phosphorylation	0.041627269	13.65374422	0.144910021		0.980616437	0.127607236	1.067274888	0.082809688
Metabolism	Energy Metabolism	Photosynthesis	0.047929229	15.72078705	0.137206381		0.301253879	0.073964516	0.355336459	0.060277165
Metabolism	Xenobiotics Biodegradation and Metabolism	Bisphenol degradation	0.050078547	16.42576336	0.134807028		0.05513984	0.029131404	0.076080819	0.02342946
Metabolism	Amino Acid Metabolism	Lysine biosynthesis	0.05142667	16.86794766	0.13335372		0.732086794	0.137900777	0.824696541	0.097573917
Metabolism	Energy Metabolism	Photosynthesis proteins	0.053069043	17.40664601	0.131633551		0.309442114	0.069081245	0.359960988	0.058904877
Metabolism	Nucleotide Metabolism	Pyrimidine metabolism	0.053136329	17.42871582	0.131564213		1.542766285	0.234145663	1.680372988	0.117926876
Metabolism	Metabolism of Cofactors and Vitamins	Lipoic acid metabolism	0.053154815	17.43477938	0.131545178		0.056284927	0.011644072	0.041237458	0.022003569
Metabolism	Xenobiotics Biodegradation and Metabolism	Drug metabolism - other enzymes	0.054850403	17.99093213	0.129826731		0.257088429	0.026820016	0.278642355	0.027230979
Metabolism	Carbohydrate Metabolism	Pentose phosphate pathway	0.057756726	18.94420625	0.127008883		0.890240523	0.044315884	0.935933088	0.065142332
Metabolism	Biosynthesis of Other Secondary Metabolites	Trp, piperidine and pyridine alkaloid biosynthesis	0.062972842	20.65509213	0.122268432		0.111846017	0.005429096	0.104096719	0.012062583
Metabolism	Carbohydrate Metabolism	Ascorbate and aldarate metabolism	0.066288089	21.74249335	0.119460762		0.226019573	0.0818637	0.180558336	0.041022491
Metabolism	Enzyme Families	Peptidases	0.069441896	22.77694196	0.116917922		1.734080353	0.127779291	1.813153541	0.089080045
Metabolism	Lipid Metabolism	Arachidonic acid metabolism	0.075742923	24.8436786	0.112169098		0.032664887	0.015941007	0.023098848	0.010929014
Metabolism	Amino Acid Metabolism	Histidine metabolism	0.083988899	27.54835896	0.106526129		0.520407571	0.098210978	0.572117485	0.05111904
Metabolism	Glycan Biosynthesis and Metabolism	Glycosyltransferases	0.086586887	28.4004988	0.104864607		0.370360018	0.091117519	0.314966447	0.069723968
Metabolism	Metabolism of cofactors and vitamins	Metabolism of cofactors and vitamins_Unclassified	0.090601101	29.71716128	0.102394953		0.155918826	0.053407335	0.126543827	0.03355707
Metabolism	Metabolism of Other Amino Acids	beta-Alanine metabolism	0.101024074	33.13589631	0.096472629		0.219494384	0.070847315	0.184741992	0.03497226
Metabolism	Carbohydrate Metabolism	Citrate cycle (TCA cycle)	0.10645824	34.91830258	0.093630244		0.617629878	0.100867634	0.542244197	0.121034968
Metabolism	Metabolism of Cofactors and Vitamins	One carbon pool by folate	0.110401392	36.21165657	0.091660358		0.500867555	0.071128381	0.536689786	0.041614746
Metabolism	Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - lacto and neolacto series	0.11364229	37.27467116	0.090095315		0.000159648	0.000241162	4.21E-05	0.000131988
Metabolism	Energy Metabolism	Nitrogen metabolism	0.115037815	37.73240325	0.089435684		0.729500126	0.062747423	0.689752536	0.060787508
Metabolism	Metabolism of Terpenoids and Polyketides	Polyketide sugar unit biosynthesis	0.118506122	38.87000812	0.087831833		0.152002769	0.048133263	0.17595763	0.029063265
Metabolism	Carbohydrate Metabolism	Pyruvate metabolism	0.140426563	46.05991261	0.078714566		1.014115274	0.09697406	1.055141598	0.041539275

Metabolism Metabolism of Other Amino Acids	D-Alanine metabolism	0.148246616	48.62489007	0.075823028	0.095298926	0.014562896	0.103776102	0.014235034
Metabolism Carbohydrate Metabolism	Propanoate metabolism	0.161821447	53.07743469	0.071172245	0.540659546	0.053393577	0.51547635	0.037378574
Metabolism Biosynthesis of Other Secondary Metabolites	beta-Lactam resistance	0.162652305	53.34995599	0.070901403	0.020672685	0.013067934	0.026761639	0.008940008
Metabolism Carbohydrate Metabolism	Galactose metabolism	0.168107814	55.13936301	0.069159516	0.778378449	0.136398602	0.838706778	0.084918618
Metabolism Xenobiotics Biodegradation and Metabolism	Atrazine degradation	0.171132036	56.13130784	0.068220223	0.039363317	0.031622319	0.023771716	0.025462581
Metabolism Metabolism of Cofactors and Vitamins	Pantothenate and CoA biosynthesis	0.173032282	56.75458837	0.067639249	0.567985734	0.054849179	0.597958946	0.053467688
Metabolism Biosynthesis of Other Secondary Metabolites	Betalain biosynthesis	0.180093266	59.07059136	0.065540083	2.96E-05	8.76E-05	1.89E-07	7.79E-07
Metabolism Metabolism of Other Amino Acids	Phosphonate and phosphinate metabolism	0.18345631	60.17366972	0.064571861	0.068681569	0.01546238	0.060547624	0.014745931
Metabolism Biosynthesis of Other Secondary Metabolites	Phenylpropanoid biosynthesis	0.184713199	60.58592939	0.064215016	0.189783793	0.059616505	0.16561547	0.032507374
Metabolism Lipid Metabolism	Synthesis and degradation of ketone bodies	0.185178942	60.73869308	0.064083467	0.027549185	0.010356431	0.032580124	0.008640974
Metabolism Energy Metabolism	Carbon fixation in photosynthetic organisms	0.19922892	65.34708565	0.06072897	0.594634723	0.042954754	0.615308672	0.037414388
Metabolism Glycan Biosynthesis and Metabolism	N-Glycan biosynthesis	0.200991357	65.92516502	0.059822937	0.021193537	0.016019923	0.014084654	0.012017661
Metabolism Metabolism of Cofactors and Vitamins	Folate biosynthesis	0.214482385	70.35022233	0.056474515	0.393613453	0.053279044	0.362959839	0.064961188
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide products	0.22017008	72.21578621	0.055133604	2.82E-05	8.91E-05	8.47E-07	1.86E-06
Metabolism Amino Acid Metabolism	Arginine and proline metabolism	0.225291651	73.89566149	0.053959525	1.130345621	0.109597128	1.174892906	0.076529266
Metabolism Metabolism of Terpenoids and Polyketides	Carotenoid biosynthesis	0.229469258	75.26591669	0.053024259	0.007389111	0.007009363	0.015315038	0.019888028
Metabolism Xenobiotics Biodegradation and Metabolism	Nitrotoluene degradation	0.229578658	75.30179986	0.053000031	0.07104891	0.027565768	0.085233666	0.029988555
Metabolism Lipid Metabolism	Glycerophospholipid metabolism	0.235943304	77.38940356	0.051612931	0.535908424	0.062258449	0.56341578	0.053898171
Metabolism Xenobiotics Biodegradation and Metabolism	Benzoate degradation	0.24599525	80.686442	0.049508248	0.238397617	0.076058102	0.214171037	0.027184053
Metabolism Xenobiotics Biodegradation and Metabolism	Chlorocyclohexane and chlorobenzene degradation	0.254412733	83.44737626	0.04782165	0.025296193	0.01323348	0.019518007	0.012047254
Metabolism Energy Metabolism	Sulfur metabolism	0.262957725	86.25013392	0.06175479	0.312780668	0.057377337	0.287866078	0.053401692
Metabolism Biosynthesis of Other Secondary Metabolites	Indole alkaloid biosynthesis	0.263300198	86.36246487	0.046110829	1.81E-06	5.73E-06	1.89E-07	7.79E-07
Metabolism Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	0.265813166	87.18671856	0.045639487	0.438623279	0.10345025	0.405157154	0.0484074
Metabolism Lipid Metabolism	Ether lipid metabolism	0.279448943	91.65925333	0.043171658	0.001294184	0.001214587	0.000833363	0.000940059
Metabolism Amino Acid Metabolism	Amino acid related enzymes	0.29146805	95.60152038	0.041114177	1.323149506	0.180361226	1.379833152	0.092436322
Metabolism Xenobiotics Biodegradation and Metabolism	Dioxin degradation	0.298769885	97.99652229	0.039914241	0.08535501	0.029107899	0.076198914	0.015719775
Metabolism Metabolism of Cofactors and Vitamins	Thiamine metabolism	0.299222491	98.14497708	0.039841055	0.454040106	0.072563414	0.480266104	0.055404195
Metabolism Lipid Metabolism	Steroid hormone biosynthesis	0.318123965	104.3446606	0.036902179	0.034867928	0.037701351	0.021809642	0.028625548
Metabolism Xenobiotics Biodegradation and Metabolism	Styrene degradation	0.32256921	105.8027009	0.03624277	0.031297212	0.025076235	0.024004581	0.012334833
Metabolism Metabolism of Terpenoids and Polyketides	Zeatin biosynthesis	0.331616675	108.7702695	0.034935683	0.039835786	0.009757989	0.042809885	0.005873023
Metabolism Metabolism of Other Amino Acids	Taurine and hypotaurine metabolism	0.351561704	115.3122389	0.032209966	0.113956577	0.016432898	0.108572662	0.012858121
Metabolism Amino Acid Metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	0.353043731	115.7983438	0.032015514	0.759401462	0.13123642	0.799316153	0.088011819
Metabolism Metabolism of Other Amino Acids	Selenocompound metabolism	0.367717997	120.6115031	0.030146745	0.404389179	0.04038185	0.394094904	0.017222761
Metabolism Metabolism of Cofactors and Vitamins	Riboflavin metabolism	0.369394938	121.1615397	0.029939534	0.221832474	0.0445812	0.209362075	0.026393945
Metabolism Xenobiotics Biodegradation and Metabolism	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	0.377414346	123.7919056	0.028965892	1.60E-05	3.79E-05	0.000136287	0.000427893
Metabolism Xenobiotics Biodegradation and Metabolism	Aminobenzoate degradation	0.397244289	130.2961269	0.026675276	0.131835597	0.049529199	0.117213237	0.038211074
Metabolism Metabolism of Other Amino Acids	D-Glutamine and D-glutamate metabolism	0.406142645	133.2147877	0.025698478	0.127905716	0.017560439	0.132417578	0.010212699
Metabolism Nucleotide Metabolism	Purine metabolism	0.412896767	135.4301396	0.024977059	2.093166187	0.241366094	2.147211085	0.087448807
Metabolism Carbohydrate Metabolism	Glyoxylate and dicarboxylate metabolism	0.433989143	142.3484389	0.022829452	0.557087828	0.087271356	0.53490101	0.057772629
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of ansamycins	0.45479125	149.17153	0.020857354	0.10256389	0.027386408	0.109644836	0.020844128
Metabolism Carbohydrate Metabolism	Starch and sucrose metabolism	0.46407429	152.216367	0.020020934	1.097139007	0.153188765	1.062479757	0.089231679
Metabolism Amino Acid Metabolism	Cysteine and methionine metabolism	0.493652526	161.9180287	0.017522365	0.967231586	0.096835243	0.94752076	0.050324009
Metabolism Glycan Biosynthesis and Metabolism	Glycosaminoglycan degradation	0.499954788	163.9851703	0.017020995	0.08748571	0.08948113	0.06632098	0.070138476
Metabolism Amino Acid Metabolism	Alanine, aspartate and glutamate metabolism	0.50138699	164.4549328	0.016908511	0.958158145	0.166778248	0.991725291	0.088606664
Metabolism Lipid Metabolism	Steroid biosynthesis	0.519912135	170.5311802	0.01550064	0.010267216	0.020870437	0.005655936	0.01568727
Metabolism Amino Acid Metabolism	Valine, leucine and isoleucine biosynthesis	0.520432396	170.7018257	0.015462335	0.731552579	0.128804007	0.756909844	0.073422427
Metabolism Biosynthesis of Other Secondary Metabolites	Caffeine metabolism	0.520909076	170.8581769	0.015427296	0.003416279	0.006959776	0.001882447	0.005229776
Metabolism Lipid Metabolism	Fatty acid elongation in mitochondria	0.521085386	170.9160068	0.01541435	0.00341531	0.006960248	0.001882059	0.00522991
Metabolism Carbohydrate Metabolism	C5-Branched dibasic acid metabolism	0.538582292	176.6549919	0.014166543	0.340465658	0.0515082	0.330539229	0.031297686
Metabolism Lipid Metabolism	Sphingolipid metabolism	0.551308016	180.8290294	0.01330349	0.213265334	0.099372751	0.19289357	0.075104351
Metabolism Xenobiotics Biodegradation and Metabolism	Chloroalkane and chloroalkene degradation	0.556646	182.5798881	0.012952223	0.1891645	0.03689792	0.197796086	0.036354108
Metabolism Energy metabolism	Energy metabolism_Unclassified	0.568142637	186.3507848	0.012216685	0.785980314	0.144795024	0.810897191	0.078922182
Metabolism Xenobiotics Biodegradation and Metabolism	Naphthalene degradation	0.572345857	187.729441	0.011954777	0.156143856	0.027733836	0.149290042	0.031663665
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of vancomycin group antibiotics	0.576697368	189.1567366	0.01168751	0.051687909	0.017149153	0.054504745	0.00864741
Metabolism Metabolism of Cofactors and Vitamins	Biotin metabolism	0.60569811	198.6689799	0.010003792	0.127811982	0.031199278	0.134368773	0.031920752
Metabolism Glycan Biosynthesis and Metabolism	Various types of N-glycan biosynthesis	0.608288516	199.5186332	0.009861391	1.81E-06	5.73E-06	5.67E-06	2.34E-05
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - ganglio series	0.609344203	199.8648987	0.009803722	0.047145459	0.04536191	0.037711942	0.046388253
Metabolism Biosynthesis of Other Secondary Metabolites	Flavonoid biosynthesis	0.648586074	212.7362323	0.004322586	0.006079166	0.008050815	0.004786389	0.006401251
Metabolism Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	0.650741037	213.4430602	0.007703316	1.195055981	0.110321017	1.216480061	0.12242918
Metabolism Glycan Biosynthesis and Metabolism	Other glycan degradation	0.654816285	214.7797414	0.007512914	0.273544333	0.135049441	0.250254646	0.126624059
Metabolism Energy Metabolism	Photosynthesis - antenna proteins	0.670414282	219.8958845	0.006810055	3.58E-06	8.39E-06	2.31E-06	6.79E-06
Metabolism Carbohydrate Metabolism	Pentose and glucuronate interconversions	0.672022355	220.4233326	0.006739895	0.569167443	0.095761753	0.556580566	0.057026117
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - globo series	0.734724173	240.9895287	0.004322586	0.104844402	0.053428387	0.098035386	0.048024004
Metabolism Biosynthesis of Other Secondary Metabolites	Novobiocin biosynthesis	0.741608385	243.2475502	0.00409341	0.126059361	0.014814924	0.127840845	0.012599739
Metabolism Energy Metabolism	Carbon fixation pathways in prokaryotes	0.747138404	245.0613965	0.00391429	0.901635872	0.02905006	0.890784772	0.090096002
Metabolism Others	Others_Unclassified	0.762155111	249.9868763	0.003449938	1.082356164	0.086127924	1.097438413	0.142795633
Metabolism Metabolism of Other Amino Acids	Cyanoamino acid metabolism	0.824306976	270.3726881	0.001857858	0.277873365	0.052995488	0.27401903	0.036209221
Metabolism Biosynthesis of Other Secondary Metabolites	Penicillin and cephalosporin biosynthesis	0.834197199	273.6166812	0.001651752	0.024683766	0.022064713	0.02319745	0.014455109

Metabolism Lipid metabolism	Lipid metabolism_Unclassified	0.843860869	276.7863649	0.001462523	0.146009196	0.054308209	0.149148573	0.027385534
Metabolism Biosynthesis of Other Secondary Metabolites	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0.876071468	287.3514416	0.00091718	0.001757368	0.004243124	0.002009992	0.003919988
Metabolism Metabolism of Terpenoids and Polyketides	Prenyltransferases	0.897233102	294.2924575	0.000629185	0.287896653	0.068070199	0.290573069	0.038523696
Metabolism Lipid Metabolism	Lipid biosynthesis proteins	0.923254833	302.8275852	0.000350078	0.527921102	0.049050206	0.526067061	0.047464845
Metabolism Biosynthesis of Other Secondary Metabolites	Streptomycin biosynthesis	0.935087267	306.7086237	0.000250243	0.276899212	0.079469545	0.27882474	0.041941986
Metabolism Biosynthesis of Other Secondary Metabolites	Butirosin and neomycin biosynthesis	0.937216743	307.4070917	0.000234062	0.056446665	0.023119498	0.056992273	0.01247638
Metabolism Amino acid metabolism	Amino acid metabolism_Unclassified	0.93995026	308.3036853	0.000214089	0.219963849	0.067246139	0.221536632	0.040227637
Metabolism Lipid Metabolism	Secondary bile acid biosynthesis	0.954781921	313.16847	0.0001213	0.033027318	0.022249225	0.033374427	0.008613916
Metabolism Biosynthesis of Other Secondary Metabolites	Isoflavonoid biosynthesis	0.979875552	321.3991811	2.40E-05	3.31E-07	1.05E-06	3.44E-07	1.42E-06
Metabolism Xenobiotics Biodegradation and Metabolism	Xylene degradation	0.980292846	321.5360534	2.30E-05	0.067656022	0.022473245	0.067478882	0.014434701
Metabolism Xenobiotics Biodegradation and Metabolism	Ethylbenzene degradation	0.981598092	321.9641742	2.01E-05	0.039260169	0.012778844	0.039123785	0.015865002
Metabolism Lipid Metabolism	Primary bile acid biosynthesis	0.989429314	324.5328149	6.62E-06	0.033665751	0.021984565	0.033745806	0.008467424
Metabolism Biosynthesis of Other Secondary Metabolites	Clavulanic acid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Enzyme Families	Cytochrome P450	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Glycosaminoglycan biosynthesis - chondroitin sulfate	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Other types of O-glycan biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of 12-, 14- and 16-membered macrolides	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide backbone	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Sesquiterpenoid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0

Level_1	Level_2	Level_3	p-values	p-values (corrected)	Effect size	Note	No: mean rel. freq. (%)	No: std. dev. (%)	Yes: mean rel. freq. (%)	Yes: std. dev. (%)
Metabolism	Energy Metabolism	Carbon fixation in photosynthetic organisms	0.020097803	6.592079447	0.037465873		0.640588925	0.042979518	0.624248645	0.039455664
Metabolism	Biosynthesis of Other Secondary Metabolites	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0.021828447	7.15973069	0.036486057		0.002131345	0.003152493	0.001206961	0.001483657
Metabolism	Xenobiotics Biodegradation and Metabolism	Bisphenol degradation	0.063317439	20.76811991	0.024074647		0.083789733	0.027110903	0.0762791	0.020707974
Metabolism	Metabolism of Other Amino Acids	Selenocompound metabolism	0.073208915	24.01252426	0.022426844		0.383922263	0.042270892	0.395826632	0.036174917
Metabolism	Carbohydrate Metabolism	Pentose phosphate pathway	0.073268072	40.31233399	0.022417714		0.959183453	0.071815414	0.939354842	0.05901264
Metabolism	Amino acid metabolism	Amino acid metabolism_Unclassified	0.083760815	27.47354743	0.020911383		0.196151656	0.03489651	0.20698284	0.038002508
Metabolism	Energy Metabolism	Methane metabolism	0.09192441	30.15120659	0.019872755		1.29575796	0.161593982	1.252007665	0.144575603
Metabolism	Lipid Metabolism	Linoleic acid metabolism	0.09413307	30.8756469	0.019608732		0.069218509	0.021698151	0.063831162	0.016395258
Metabolism	Carbohydrate Metabolism	Pentose and glucuronate interconversions	0.10289275	33.74882203	0.018623634		0.568980445	0.073487815	0.549257205	0.068866413
Metabolism	Carbohydrate Metabolism	Fructose and mannose metabolism	0.122903457	40.31233399	0.016678418		1.108177686	0.248976358	1.052295611	0.180003366
Metabolism	Carbohydrate metabolism	Carbohydrate metabolism_Unclassified	0.13654035	44.78523476	0.015542249		0.207361107	0.025643881	0.201177759	0.023355618
Metabolism	Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	0.164195664	53.85617763	0.01358277		1.211599716	0.088333589	1.191100157	0.085089119
Metabolism	Amino Acid Metabolism	Arginine and proline metabolism	0.176491285	57.88914157	0.012828006		1.211180477	0.077697703	1.191884828	0.088157234
Metabolism	Biosynthesis of Other Secondary Metabolites	Betalain biosynthesis	0.214421686	70.33031307	0.010833496		1.15E-07	5.62E-07	9.02E-07	4.91E-06
Metabolism	Metabolism of Other Amino Acids	Taurine and hypotaurine metabolism	0.226960385	74.44300631	0.010263548		0.105253182	0.01289516	0.107723912	0.01130167
Metabolism	Lipid Metabolism	Synthesis and degradation of ketone bodies	0.233353196	76.53984839	0.009987193		0.026170108	0.009466412	0.027988657	0.008566941
Metabolism	Lipid Metabolism	Arachidonic acid metabolism	0.240326078	78.82695347	0.009695923		0.016879671	0.010496893	0.018965363	0.010391678
Metabolism	Amino Acid Metabolism	Phenylalanine metabolism	0.24724646	81.09683874	0.00941675		0.176877043	0.028621116	0.171215073	0.028854588
Metabolism	Lipid metabolism	Lipid metabolism_Unclassified	0.250839875	82.27547893	0.0092755		0.13773078	0.040060749	0.130353237	0.035911129
Metabolism	Energy Metabolism	Photosynthesis - antenna proteins	0.255668814	83.85937086	0.009089517		0.002107675	0.016348368	3.26E-05	0.000179465
Metabolism	Carbohydrate Metabolism	Starch and sucrose metabolism	0.264739236	86.83446932	0.008751535		1.136773179	0.130401579	1.11307529	0.120540587
Metabolism	Amino Acid Metabolism	Cysteine and methionine metabolism	0.27529515	90.29680915	0.008375752		0.972270655	0.076101149	0.987027679	0.08198683
Metabolism	Xenobiotics Biodegradation and Metabolism	Chloroalkane and chloroalkene degradation	0.276497154	90.69106662	0.008334099		0.208192909	0.03082414	0.20260846	0.029653262
Metabolism	Carbohydrate Metabolism	Inositol phosphate metabolism	0.27815758	91.23568637	0.00827693		0.098174102	0.019934958	0.094544612	0.019470654
Metabolism	Metabolism of Cofactors and Vitamins	One carbon pool by folate	0.279958385	91.82635032	0.008215409		0.558929743	0.04819773	0.567512798	0.045527009
Metabolism	Nucleotide metabolism	Nucleotide metabolism_Unclassified	0.286841229	94.08392314	0.007984761		0.062490885	0.031040474	0.068154306	0.031419738
Metabolism	Metabolism of Terpenoids and Polyketides	Carotenoid biosynthesis	0.294727588	96.67064897	0.007728916		0.006668243	0.011081759	0.005038854	0.007341755
Metabolism	Metabolism of Terpenoids and Polyketides	Terpenoid backbone biosynthesis	0.305272357	100.1293332	0.007400122		0.539860805	0.063870802	0.550701226	0.060845599
Metabolism	Biosynthesis of Other Secondary Metabolites	Isoflavonoid biosynthesis	0.320925468	105.2635534	0.006938102		7.62E-08	5.95E-07	1.75E-06	1.31E-05
Metabolism	Metabolism of Cofactors and Vitamins	Retinol metabolism	0.3325036	109.0611809	0.006614879		0.031878539	0.016344596	0.029177087	0.016427985
Metabolism	Nucleotide Metabolism	Purine metabolism	0.332548314	109.0758471	0.006613659		2.18443303	0.170618713	2.212924644	0.174624652
Metabolism	Xenobiotics Biodegradation and Metabolism	Chlorocyclohexane and chlorobenzene degradation	0.33522281	109.9530816	0.006541117		0.015177762	0.009883672	0.016756719	0.009442946
Metabolism	Biosynthesis of Other Secondary Metabolites	Butirosin and neomycin biosynthesis	0.340788086	111.7784923	0.006392604		0.067128068	0.014012443	0.064812175	0.014507442
Metabolism	Lipid Metabolism	Fatty acid biosynthesis	0.359834465	118.0257044	0.005908021		0.457055476	0.05595081	0.447955882	0.060264013
Metabolism	Metabolism of Other Amino Acids	Phosphonate and phosphinate metabolism	0.374330147	122.7802883	0.005562095		0.060402302	0.01377266	0.0586575	0.009535538
Metabolism	Enzyme Families	Cytochrome P450	0.38642645	126.7478755	0.005287396		0	0	4.72E-08	4.25E-07
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of vancomycin group antibiotics	0.393091415	128.9339841	0.005141171		0.05683527	0.013257229	0.058785021	0.013558796
Metabolism	Carbohydrate Metabolism	Pyruvate metabolism	0.400614887	131.4016828	0.004980309		1.039905965	0.062539485	1.03128101	0.058667287
Metabolism	Energy Metabolism	Sulfur metabolism	0.408788639	134.0826737	0.004810412		0.272868474	0.042210206	0.278739734	0.04151391
Metabolism	Metabolism of Cofactors and Vitamins	Folate biosynthesis	0.408919349	134.1255466	0.004807736		0.379336462	0.051410923	0.3725746	0.045569483
Metabolism	Biosynthesis of Other Secondary Metabolites	Indole alkaloid biosynthesis	0.419734395	137.6728815	0.004590518		3.72E-08	2.90E-07	1.38E-07	9.39E-07
Metabolism	Metabolism of Cofactors and Vitamins	Biotin metabolism	0.430919068	141.3414544	0.004374436		0.135746115	0.02217808	0.132477735	0.025979056
Metabolism	Xenobiotics Biodegradation and Metabolism	Fluorobenzoate degradation	0.447486774	146.7756619	0.004069475		0.005466862	0.008614562	0.006625467	0.00923772
Metabolism	Biosynthesis of Other Secondary Metabolites	Tropane, piperidine and pyridine alkaloid biosynthesis	0.453071635	148.6074962	0.003970555		0.109568638	0.008129223	0.108556825	0.007785061
Metabolism	Amino Acid Metabolism	Glycine, serine and threonine metabolism	0.457389419	150.0237294	0.003895371		0.83844611	0.046028906	0.832442665	0.048643812
Metabolism	Amino Acid Metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	0.462560948	151.7199911	0.003806776		0.863045494	0.091377582	0.851958316	0.086815516
Metabolism	Lipid Metabolism	Ether lipid metabolism	0.470410847	154.2947578	0.003675257		0.001252734	0.001527373	0.001480958	0.002077393
Metabolism	Carbohydrate Metabolism	Propanoate metabolism	0.479916361	157.4125665	0.003520641		0.502415074	0.036382718	0.506680497	0.034875872
Metabolism	Glycan Biosynthesis and Metabolism	Various types of N-glycan biosynthesis	0.483688569	158.6498506	0.003460654		1.07E-06	5.90E-06	2.23E-06	1.18E-05
Metabolism	Lipid Metabolism	Fatty acid elongation in mitochondria	0.485455794	159.2295005	0.003432814		0.001112243	0.003467326	0.001565439	0.004072074
Metabolism	Metabolism of Terpenoids and Polyketides	Prenyltransferases	0.493380854	161.82892	0.003309997		0.292313329	0.044054457	0.297288057	0.041725973
Metabolism	Xenobiotics Biodegradation and Metabolism	Nitrotoluene degradation	0.494220019	162.1041663	0.003297184		0.089377609	0.034091902	0.085804581	0.027978783
Metabolism	Biosynthesis of Other Secondary Metabolites	Phenylpropanoid biosynthesis	0.50167523	164.5494756	0.003184935		0.185912684	0.061027454	0.179515784	0.051944443
Metabolism	Energy Metabolism	Nitrogen metabolism	0.505252723	165.7228931	0.003132067		0.684932215	0.039622523	0.689921195	0.047157106
Metabolism	Xenobiotics Biodegradation and Metabolism	Toluene degradation	0.51616678	169.3027039	0.002974667		0.084647977	0.036844603	0.088634683	0.035598031
Metabolism	Metabolism of Other Amino Acids	Glutathione metabolism	0.520894476	170.853388	0.002908264		0.20465643	0.069046671	0.211691454	0.060836214
Metabolism	Metabolism of Terpenoids and Polyketides	Tetracycline biosynthesis	0.52241447	171.3519461	0.002887139		0.149802274	0.04039583	0.145488758	0.039152571
Metabolism	Xenobiotics Biodegradation and Metabolism	Metabolism of xenobiotics by cytochrome P450	0.530550362	174.0205189	0.002775889		0.037428655	0.025242072	0.034820278	0.023887112
Metabolism	Biosynthesis of Other Secondary Metabolites	beta-Lactam resistance	0.53098094	174.1617484	0.002770086		0.028059757	0.013342969	0.026789275	0.010750687
Metabolism	Metabolism of Other Amino Acids	Cyanoamino acid metabolism	0.538312577	176.5665252	0.00267256		0.292549613	0.066969301	0.286096773	0.057438842
Metabolism	Metabolism of Other Amino Acids	D-Alanine metabolism	0.547191636	179.4788566	0.002557641		0.100908925	0.00910396	0.102081239	0.012965171
Metabolism	Xenobiotics Biodegradation and Metabolism	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	0.559195885	183.4162503	0.00240767		5.11E-05	0.000207426	7.26E-05	0.00022225
Metabolism	Biosynthesis of Other Secondary Metabolites	Caffeine metabolism	0.563979111	184.9851484	0.002349595		0.001179383	0.003770417	0.001566025	0.004071953
Metabolism	Lipid Metabolism	Steroid biosynthesis	0.566854118	185.9281506	0.002315142		0.003593124	0.010497594	0.00471245	0.012212442
Metabolism	Metabolism of Other Amino Acids	D-Glutamine and D-glutamate metabolism	0.567783343	186.2329364	0.002304078		0.138408141	0.015695607	0.139846203	0.014116933
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide products	0.584036144	191.5638551	0.002116152		6.69E-05	0.000521413	3.08E-05	0.00024223
Metabolism	Metabolism of Cofactors and Vitamins	Porphyrin and chlorophyll metabolism	0.59359312	194.6985433	0.002010456		0.879003658	0.233585502	0.857227655	0.245140948

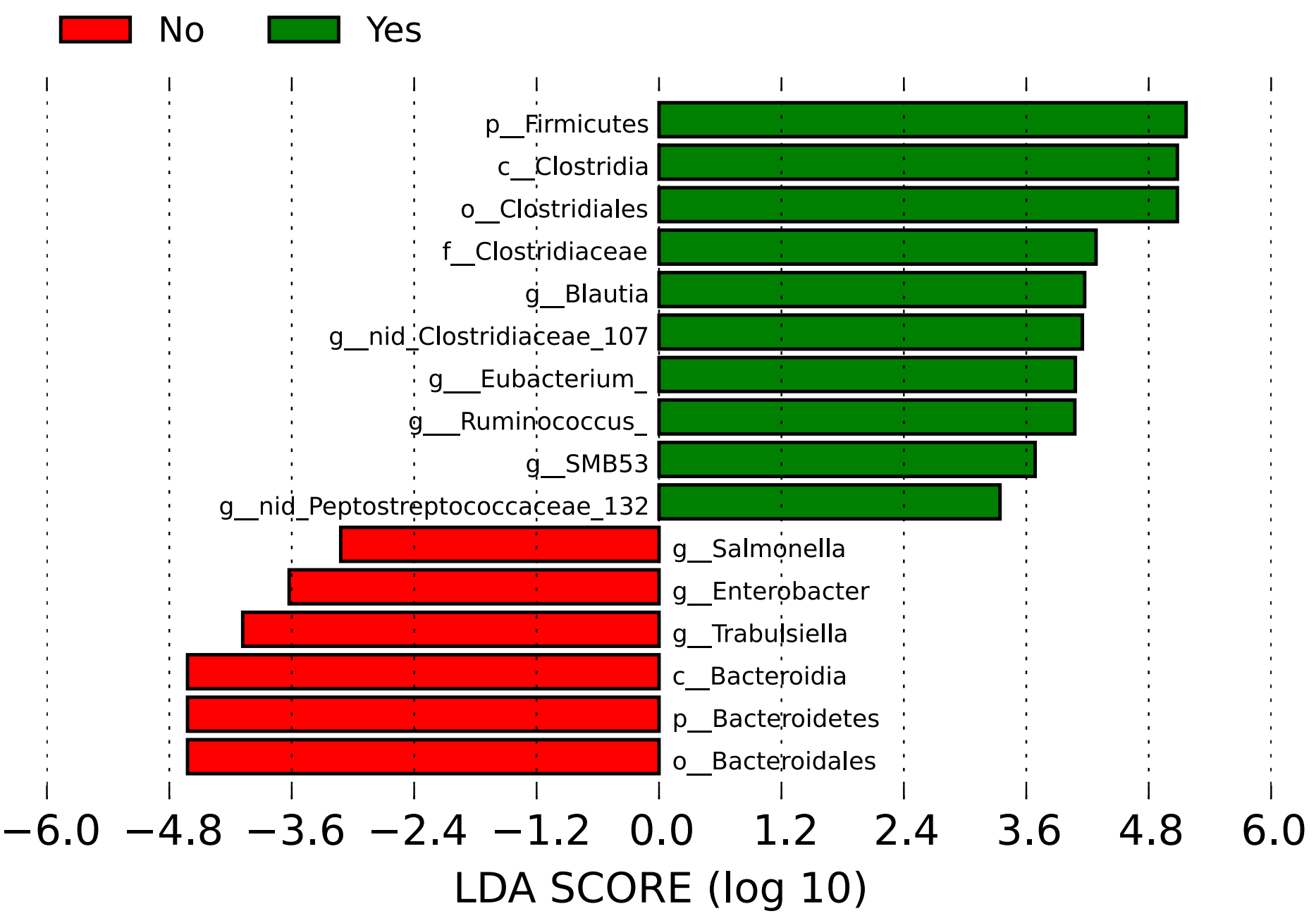
Metabolism Metabolism of cofactors and vitamins	Metabolism of cofactors and vitamins_Unclassified	0.594557575	195.0148845	0.001999982	0.107148125	0.03793703	0.110441136	0.035236595
Metabolism Metabolism of Terpenoids and Polyketides	Limonene and pinene degradation	0.602403306	197.5882843	0.001916074	0.085068293	0.035010108	0.082137838	0.031610705
Metabolism Biosynthesis of Other Secondary Metabolites	Flavonoid biosynthesis	0.604970393	198.4302891	0.001889114	0.004734623	0.006044971	0.004252588	0.00502279
Metabolism Metabolism of Other Amino Acids	D-Arginine and D-orithine metabolism	0.606380382	198.8927652	0.001874409	0.003086529	0.002859962	0.00284241	0.002734737
Metabolism Metabolism of Other Amino Acids	beta-Alanine metabolism	0.609024954	199.760185	0.001847023	0.178862473	0.050278214	0.174614254	0.04783171
Metabolism Lipid Metabolism	Fatty acid metabolism	0.615086849	201.7484865	0.00185203	0.261347213	0.068841002	0.26723625	0.069037457
Metabolism Carbohydrate Metabolism	Glyoxylate and dicarboxylate metabolism	0.625936494	205.3071699	0.001677813	0.522840852	0.052564735	0.517960521	0.063345239
Metabolism Carbohydrate Metabolism	Citrate cycle (TCA cycle)	0.630911149	206.9388567	0.001629947	0.524552436	0.078110148	0.517880777	0.084412481
Metabolism Lipid Metabolism	Biosynthesis of unsaturated fatty acids	0.634836426	208.2263478	0.001592777	0.137665427	0.046160262	0.141377215	0.045904949
Metabolism Xenobiotics Biodegradation and Metabolism	Drug metabolism - cytochrome P450	0.636676675	208.8299495	0.001575531	0.038732482	0.02577047	0.036710891	0.024757568
Metabolism Metabolism of Cofactors and Vitamins	Riboflavin metabolism	0.637241615	209.0152498	0.00157026	0.20486677	0.031855374	0.202332476	0.031480542
Metabolism Xenobiotics Biodegradation and Metabolism	Xylene degradation	0.644484627	211.3909578	0.001503627	0.069049303	0.01594492	0.070392642	0.017992187
Metabolism Amino Acid Metabolism	Alanine, aspartate and glutamate metabolism	0.646951154	212.1999786	0.001481335	1.063574223	0.115320763	1.054982947	0.106612085
Metabolism Biosynthesis of Other Secondary Metabolites	Isoquinoline alkaloid biosynthesis	0.649650232	213.0852762	0.001457171	0.0522333	0.009416138	0.051625903	0.006466229
Metabolism Xenobiotics Biodegradation and Metabolism	Drug metabolism - other enzymes	0.651399932	213.6591778	0.001441634	0.293277468	0.028276709	0.295487834	0.029197622
Metabolism Metabolism of Terpenoids and Polyketides	Polyketide sugar unit biosynthesis	0.655457709	214.9901284	0.001405986	0.186464467	0.037653317	0.189230635	0.035607714
Metabolism Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	0.659359383	216.2698778	0.001372213	0.435808215	0.075926339	0.441744079	0.08174048
Metabolism Xenobiotics Biodegradation and Metabolism	Naphthalene degradation	0.669921837	219.7343627	0.001283221	0.142774557	0.027025823	0.140896376	0.025096313
Metabolism Energy metabolism	Energy metabolism_Unclassified	0.675231619	221.4759709	0.001239811	0.786637383	0.105136385	0.778723506	0.115607279
Metabolism Glycan Biosynthesis and Metabolism	N-Glycan biosynthesis	0.678561617	222.5682105	0.001213033	0.015329472	0.0069825	0.015974312	0.009501671
Metabolism Xenobiotics Biodegradation and Metabolism	Atrazine degradation	0.682145573	223.7437481	0.00148595	0.020037237	0.020701876	0.021503682	0.021369294
Metabolism Xenobiotics Biodegradation and Metabolism	Ethylbenzene degradation	0.69502912	227.9695512	0.001085586	0.032646082	0.013667884	0.0318368	0.010873173
Metabolism Lipid Metabolism	Lipid biosynthesis proteins	0.697982551	228.9382767	0.00106359	0.536013563	0.039001711	0.533506106	0.037314459
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of siderophore group nonribosomal peptides	0.701239405	230.0065247	0.001039634	0.027668943	0.027709253	0.025929956	0.025895139
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - lacto and neolacto series	0.715796702	234.7813182	0.000936345	0.000159756	0.000441508	0.000204485	0.000878475
Metabolism Metabolism of Cofactors and Vitamins	Vitamin B6 metabolism	0.721983137	236.104691	0.000894297	0.187503996	0.030135535	0.189289721	0.029107014
Metabolism Carbohydrate Metabolism	C5-Branched dibasic acid metabolism	0.72768027	238.6791286	0.000856534	0.353203864	0.037656532	0.355501183	0.039723954
Metabolism Nucleotide Metabolism	Pyrimidine metabolism	0.728712299	239.0176341	0.000849791	1.751074225	0.156977171	1.760475394	0.161585891
Metabolism Lipid Metabolism	Glycerophospholipid metabolism	0.733196311	240.4883899	0.000820838	0.551417037	0.05994412	0.548148023	0.053701943
Metabolism Lipid Metabolism	Glycerolipid metabolism	0.735050789	241.0966587	0.000809027	0.400499449	0.058751701	0.403584154	0.049497765
Metabolism Xenobiotics Biodegradation and Metabolism	Dioxin degradation	0.750580164	246.1902939	0.000713834	0.075323975	0.01606868	0.076271247	0.018590797
Metabolism Glycan Biosynthesis and Metabolism	Lipopolysaccharide biosynthesis proteins	0.752911047	246.9548235	0.000700111	0.251810902	0.1496602	0.25996516	0.154685063
Metabolism Lipid Metabolism	Sphingolipid metabolism	0.753504373	247.1494343	0.000696642	0.224905949	0.090650978	0.229412191	0.079549312
Metabolism Glycan Biosynthesis and Metabolism	Peptidoglycan biosynthesis	0.755503463	247.8051358	0.000685021	0.802169859	0.072741894	0.798296038	0.073652923
Metabolism Carbohydrate Metabolism	Ascorbate and aldarate metabolism	0.761176869	249.6660131	0.000652623	0.156401179	0.05067773	0.158965567	0.048926284
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - ganglio series	0.77240893	253.350129	0.000590997	0.037684072	0.035356556	0.035952654	0.035178101
Metabolism Glycan Biosynthesis and Metabolism	Lipopolysaccharide biosynthesis	0.772892704	253.5088069	0.000588417	0.14048981	0.119516495	0.146541269	0.126410567
Metabolism Amino Acid Metabolism	Valine, leucine and isoleucine degradation	0.774710548	254.1050597	0.000578777	0.198647357	0.044129654	0.200820073	0.045136163
Metabolism Glycan Biosynthesis and Metabolism	Glycosaminoglycan degradation	0.775340219	254.3115918	0.000575458	0.066476797	0.054772019	0.063778001	0.056374422
Metabolism Lipid Metabolism	Primary bile acid biosynthesis	0.776098301	254.5602426	0.000571476	0.04197581	0.015057518	0.041261968	0.014569379
Metabolism Xenobiotics Biodegradation and Metabolism	Aminobenzoate degradation	0.777750963	255.1023159	0.000562846	0.098554706	0.029783481	0.100009007	0.030761598
Metabolism Lipid Metabolism	Secondary bile acid biosynthesis	0.786319364	257.9127515	0.000519236	0.041264132	0.015106634	0.040579354	0.014699742
Metabolism Metabolism of Cofactors and Vitamins	Lipoic acid metabolism	0.788650478	258.6773567	0.000507698	0.035011264	0.018783048	0.035822517	0.017061513
Metabolism Glycan Biosynthesis and Metabolism	Other glycan degradation	0.790064463	259.1411438	0.000500767	0.293543809	0.132595708	0.288055376	0.112218676
Metabolism Amino Acid Metabolism	Lysine biosynthesis	0.793630701	260.31087	0.000483512	0.874609802	0.098013207	0.870322975	0.095355798
Metabolism Energy Metabolism	Oxidative phosphorylation	0.796765626	261.3391253	0.000468611	1.050074997	0.095489861	1.045533949	0.109741925
Metabolism Xenobiotics Biodegradation and Metabolism	Benzoate degradation	0.798257021	261.8283208	0.000461609	0.209919535	0.025758077	0.211100596	0.02826326
Metabolism Amino Acid Metabolism	Tyrosine metabolism	0.798976413	262.0642635	0.000458251	0.349196091	0.037071577	0.34754206	0.039117593
Metabolism Metabolism of Cofactors and Vitamins	Thiamine metabolism	0.810714872	265.914478	0.000405301	0.518229197	0.056584832	0.515992865	0.053756329
Metabolism Energy Metabolism	Carbon fixation pathways in prokaryotes	0.817633076	268.1836491	0.0003757	0.908680314	0.063105645	0.906183448	0.064272065
Metabolism Amino Acid Metabolism	Tryptophan metabolism	0.818051476	268.320884	0.000373948	0.142222889	0.059548895	0.144441343	0.054619231
Metabolism Enzyme Families	Protein kinases	0.830939074	272.5480162	0.000322074	0.328993202	0.056114583	0.331004841	0.055019335
Metabolism Xenobiotics Biodegradation and Metabolism	Caprolactam degradation	0.832727648	273.1346687	0.000315195	0.031573666	0.037910177	0.032866829	0.034596954
Metabolism Enzyme Families	Peptidases	0.834852346	273.8315695	0.000307123	1.840908992	0.089282243	1.844257296	0.098412141
Metabolism Lipid Metabolism	Steroid hormone biosynthesis	0.835296489	273.9772485	0.00030545	0.018921811	0.023457984	0.019802583	0.02602105
Metabolism Energy Metabolism	Photosynthesis	0.84595691	277.4738665	0.000266709	0.386930447	0.089339859	0.389314321	0.05600597
Metabolism Amino Acid Metabolism	Lysine degradation	0.847334885	277.9258422	0.0002619	0.129617921	0.06243457	0.127679864	0.056797083
Metabolism Metabolism of Cofactors and Vitamins	Pantothenate and CoA biosynthesis	0.863917043	283.36479	0.000207571	0.619691371	0.046685103	0.618412992	0.041728126
Metabolism Glycan Biosynthesis and Metabolism	Glycosyltransferases	0.875418042	287.1371178	0.000173694	0.294557985	0.062904815	0.296269506	0.065330187
Metabolism Metabolism of Cofactors and Vitamins	Glycosphingolipid biosynthesis - globo series	0.881116657	289.0062637	0.000158053	0.101599567	0.043785367	0.102697253	0.042804978
Metabolism Xenobiotics Biodegradation and Metabolism	Styrene degradation	0.882212775	289.3657902	0.00015513	0.019241527	0.009782259	0.019499722	0.010613296
Metabolism Carbohydrate Metabolism	Butanoate metabolism	0.883113414	289.6611999	0.00015275	0.622856461	0.06566973	0.621286233	0.060729529
Metabolism Others	Others_Unclassified	0.885013076	290.2842888	0.000147791	1.011917647	0.076520821	1.013940238	0.086537698
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of ansamycins	0.885557785	292.4629535	0.000146385	0.11574434	0.02376197	0.11518655	0.022092182
Metabolism Biosynthesis of Other Secondary Metabolites	Streptomycin biosynthesis	0.890824258	292.1903567	0.00013314	0.304385559	0.056674886	0.305687315	0.055232685
Metabolism Biosynthesis of Other Secondary Metabolites	Penicillin and cephalosporin biosynthesis	0.913363959	299.5833787	8.36E-05	0.020701811	0.014569327	0.020436006	0.014252855
Metabolism Carbohydrate Metabolism	Galactose metabolism	0.920736615	302.0016097	7.00E-05	0.860820973	0.124327593	0.858901461	0.104800074
Metabolism Metabolism of Terpenoids and Polyketides	Zeatin biosynthesis	0.922986063	302.7394287	6.00E-05	0.045931654	0.007411572	0.046046268	0.006640717

Metabolism Metabolism of Cofactors and Vitamins	Ubiquinone and other terpenoid-quinone biosynthesis	0.927171539	304.1122649	5.90E-05	0.155495786	0.088443053	0.156838781	0.085074815
Metabolism Biosynthesis of Other Secondary Metabolites	Clavulanic acid biosynthesis	0.928191245	304.4467284	5.74E-05	3.81E-08	2.98E-07	4.35E-08	3.92E-07
Metabolism Lipid Metabolism	alpha-Linolenic acid metabolism	0.931610292	305.5681757	5.20E-05	0.007491134	0.012577925	0.007316072	0.01157112
Metabolism Carbohydrate Metabolism	Amino sugar and nucleotide sugar metabolism	0.939045387	308.0068868	4.13E-05	1.525691638	0.152603198	1.523874396	0.1296052
Metabolism Biosynthesis and biodegradation of secondary metabolites	Biosynthesis and biodegradation of secondary metabolites_Unclassified	0.943409872	309.438438	3.56E-05	0.068022687	0.036682305	0.067604827	0.033071328
Metabolism Xenobiotics Biodegradation and Metabolism	Polycyclic aromatic hydrocarbon degradation	0.956840584	313.8437115	2.07E-05	0.099518043	0.022695343	0.099320652	0.020519053
Metabolism Biosynthesis of Other Secondary Metabolites	Novobiocin biosynthesis	0.959421363	314.6902072	1.83E-05	0.135456232	0.012427849	0.135351882	0.011810023
Metabolism Amino Acid Metabolism	Amino acid related enzymes	0.969044566	317.8466177	1.06E-05	1.445095238	0.115708433	1.445854257	0.114820108
Metabolism Amino Acid Metabolism	Valine, leucine and isoleucine biosynthesis	0.971387912	318.615235	9.09E-06	0.798994124	0.081837924	0.799505127	0.085451796
Metabolism Biosynthesis of Other Secondary Metabolites	Flavone and flavonol biosynthesis	0.976208377	320.1963476	6.29E-06	0.005054524	0.005178453	0.005080003	0.004918558
Metabolism Metabolism of Terpenoids and Polyketides	Geraniol degradation	0.976411912	320.2631072	6.18E-06	0.040314828	0.04608646	0.040090605	0.043562593
Metabolism Glycan biosynthesis and metabolism	Glycan biosynthesis and metabolism_Unclassified	0.980023833	321.4478174	4.43E-06	0.027993974	0.031267095	0.028119965	0.028343259
Metabolism Energy Metabolism	Photosynthesis proteins	0.99315022	325.7532722	5.21E-07	0.39252015	0.099267099	0.392408706	0.053065188
Metabolism Amino Acid Metabolism	Histidine metabolism	0.999621254	327.8757714	1.59E-09	0.60178324	0.059634292	0.60178832	0.065495458
Metabolism Glycan Biosynthesis and Metabolism	Glycosaminoglycan biosynthesis - chondroitin sulfate	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Other types of O-glycan biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of 12-, 14- and 16-membered macrolides	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide backbone	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Sesquiterpenoid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0

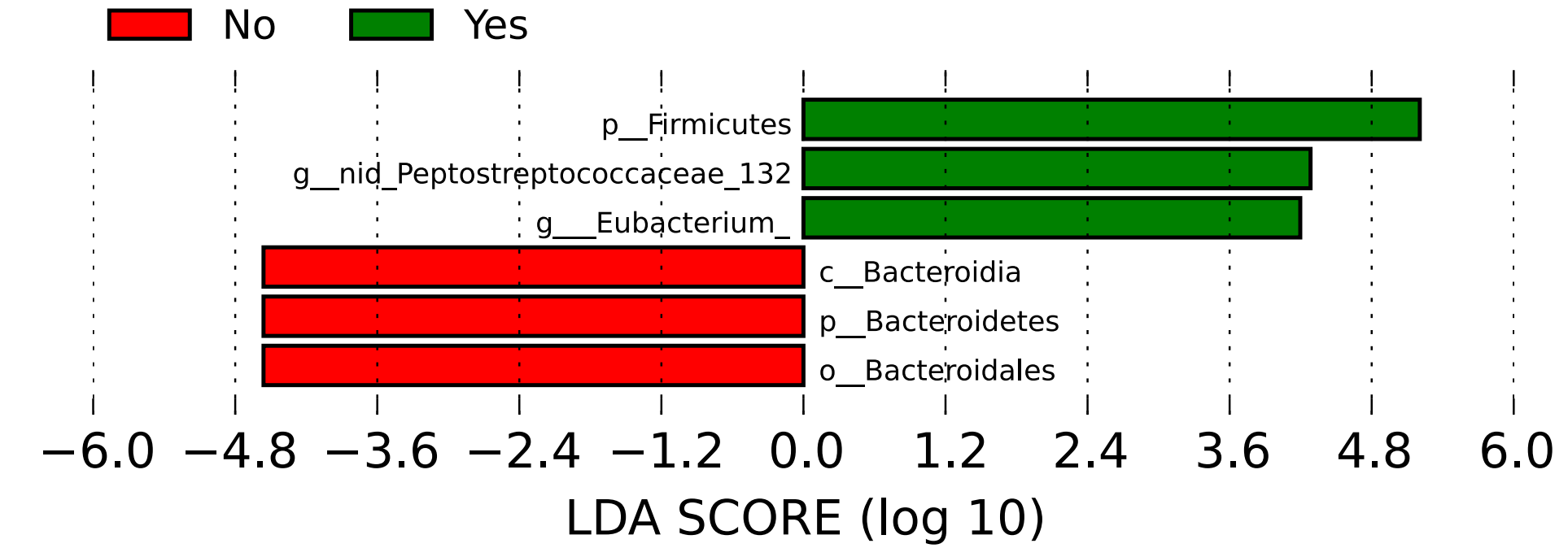
Level_1	Level_2	Level_3	p-values	p-values (corrected)	Effect size	Note	No: mean rel. freq. (%)	No: std. dev. (%)	Yes: mean rel. freq. (%)	Yes: std. dev. (%)
Metabolism	Biosynthesis of Other Secondary Metabolites	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0.09016166	29.57302439	0.05526955		0.001132619	0.000959163	0.000712307	0.000777891
Metabolism	Carbohydrate Metabolism	Amino sugar and nucleotide sugar metabolism	0.134371693	44.07391542	0.043407254		1.501198148	0.077992741	1.543501841	0.11158551
Metabolism	Metabolism of Cofactors and Vitamins	Pantothenate and CoA biosynthesis	0.156176495	51.22589024	0.03903357		0.630913216	0.034968531	0.64480113	0.033513159
Metabolism	Glycan Biosynthesis and Metabolism	Various types of N-glycan biosynthesis	0.189631089	62.19899724	0.033492589		9.98E-07	4.03E-06	0	0
Metabolism	Energy Metabolism	Carbon fixation in photosynthetic organisms	0.2141884	70.25379535	0.030088042		0.633138278	0.039168836	0.64620301	0.034807261
Metabolism	Amino Acid Metabolism	Histidine metabolism	0.228657149	74.99954491	0.02828706		0.60841376	0.055535809	0.624722165	0.040003328
Metabolism	Xenobiotics Biodegradation and Metabolism	Polycyclic aromatic hydrocarbon degradation	0.229821233	75.38136445	0.02814799		0.103746105	0.018296286	0.108701542	0.010549321
Metabolism	Metabolism of Terpenoids and Polyketides	Polyketide sugar unit biosynthesis	0.23075599	75.68796487	0.028036915		0.201182675	0.028565079	0.210724899	0.027281326
Metabolism	Xenobiotics Biodegradation and Metabolism	Caprolactam degradation	0.248040494	81.35728188	0.026074088		0.024646156	0.025205933	0.017853974	0.016146522
Metabolism	Nucleotide metabolism	Nucleotide metabolism_Unclassified	0.252101352	82.68924358	0.025636524		0.053733995	0.02630664	0.046948002	0.015131387
Metabolism	Carbohydrate Metabolism	Pentose phosphate pathway	0.255473252	83.79522678	0.025279579		0.944267688	0.052250729	0.959625101	0.043045959
Metabolism	Xenobiotics Biodegradation and Metabolism	Drug metabolism - other enzymes	0.256452824	84.11652637	0.025176945		0.301778128	0.021910175	0.308761505	0.021245604
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide products	0.25734756	84.40999954	0.025083612		1.57E-07	7.37E-07	0	0
Metabolism	Metabolism of Terpenoids and Polyketides	Limonene and pinene degradation	0.288075391	94.48872818	0.022099051		0.075489339	0.025913034	0.068761307	0.018826944
Metabolism	Energy Metabolism	Photosynthesis	0.290179346	95.17882545	0.021909179		0.395089191	0.054580378	0.411515647	0.054254692
Metabolism	Xenobiotics Biodegradation and Metabolism	Toluene degradation	0.294886532	96.72278253	0.021490534		0.0860043	0.040146739	0.076552648	0.023010468
Metabolism	Glycan biosynthesis and metabolism	Glycan biosynthesis and metabolism_Unclassified	0.304224886	99.78576275	0.020684322		0.020724085	0.020815996	0.016023631	0.011034227
Metabolism	Biosynthesis of Other Secondary Metabolites	Streptomycin biosynthesis	0.30809756	101.0559998	0.020359106		0.322399635	0.045391896	0.333409096	0.030843841
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of vancomycin group antibiotics	0.309932003	101.6576971	0.020206863		0.061555583	0.011583799	0.064370149	0.007988467
Metabolism	Energy Metabolism	Photosynthesis proteins	0.31051557	101.849107	0.020158673		0.397340493	0.052981632	0.412818877	0.053865398
Metabolism	Amino Acid Metabolism	Arginine and proline metabolism	0.351507456	115.2944456	0.017040328		1.221088319	0.07565742	1.240253014	0.069323566
Metabolism	Enzyme Families	Protein kinases	0.379661331	124.5289167	0.015166012		0.317868188	0.048619742	0.308937038	0.020868546
Metabolism	Lipid Metabolism	alpha-Linolenic acid metabolism	0.385430706	126.4212714	0.014805395		0.003580456	0.008099355	0.002158457	0.002845853
Metabolism	Amino Acid Metabolism	Tryptophan metabolism	0.385527214	126.4529263	0.014799427		0.125907887	0.039891316	0.117628286	0.027574976
Metabolism	Enzyme Families	Cytochrome P450	0.386431763	126.7496184	0.01474359		0	0	3.87E-07	2.08E-06
Metabolism	Xenobiotics Biodegradation and Metabolism	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	0.387310211	127.0377492	0.014689539		0.0002548	0.000691508	0.000135676	0.000215538
Metabolism	Metabolism of Cofactors and Vitamins	Ubiquinone and other terpenoid-quinone biosynthesis	0.40137493	131.650977	0.013847011		0.140878065	0.072490993	0.125150649	0.060131628
Metabolism	Carbohydrate Metabolism	Ascorbate and aldarate metabolism	0.401944985	131.837955	0.013813749		0.140404103	0.040492493	0.131781136	0.032350757
Metabolism	Carbohydrate Metabolism	Fructose and mannose metabolism	0.409822284	134.4217092	0.013360937		0.140804666	0.172881334	1.087583874	0.164863193
Metabolism	Metabolism of Cofactors and Vitamins	Thiamine metabolism	0.423084139	138.7715976	0.012626427		0.524790277	0.047062182	0.534510191	0.038833675
Metabolism	Carbohydrate Metabolism	Galactose metabolism	0.4308604	141.3222111	0.012211293		0.877967797	0.092576696	0.897923742	0.08607591
Metabolism	Carbohydrate Metabolism	Butanoate metabolism	0.438479771	143.8213649	0.011815235		0.6191586	0.046749733	0.60973105	0.039373605
Metabolism	Amino Acid Metabolism	Cysteine and methionine metabolism	0.442171784	145.032345	0.011627031		0.985928502	0.068540881	0.97255127	0.054769702
Metabolism	Xenobiotics Biodegradation and Metabolism	Naphthalene degradation	0.44345499	145.4532368	0.011562175		0.145180347	0.025656439	0.139798114	0.023875747
Metabolism	Lipid Metabolism	Biosynthesis of unsaturated fatty acids	0.447904026	146.9125205	0.01133951		0.134437181	0.040288933	0.12676886	0.031311687
Metabolism	Biosynthesis of Other Secondary Metabolites	beta-Lactam resistance	0.465424766	152.6593234	0.010494772		0.028158666	0.011711432	0.030446125	0.010437403
Metabolism	Energy Metabolism	Methane metabolism	0.466931877	153.1536558	0.010424431		1.284267226	0.122016547	1.309963889	0.125649964
Metabolism	Lipid Metabolism	Arachidonic acid metabolism	0.476621856	156.3319688	0.009980629		0.018883392	0.009390899	0.016767838	0.011182396
Metabolism	Metabolism of Terpenoids and Polyketides	Geraniol degradation	0.477589554	156.6493737	0.009937101		0.030061476	0.0315264	0.024930664	0.019396441
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of siderophore group nonribosomal peptides	0.486044674	159.4226531	0.009562752		0.018128113	0.018168013	0.015390645	0.009151947
Metabolism	Xenobiotics Biodegradation and Metabolism	Drug metabolism - cytochrome P450	0.490418826	160.8573748	0.009373225		0.032903339	0.020180365	0.029390121	0.015933113
Metabolism	Amino Acid Metabolism	Alanine, aspartate and glutamate metabolism	0.490875086	161.0070283	0.009353616		1.08781206	0.102438355	1.105258808	0.077103634
Metabolism	Xenobiotics Biodegradation and Metabolism	Fluorobenzoate degradation	0.498093022	163.3745111	0.009047363		0.005426059	0.008050918	0.003960896	0.007234848
Metabolism	Lipid Metabolism	Linoleic acid metabolism	0.500231252	164.0758507	0.008958054		0.06758758	0.017221526	0.070780134	0.01618508
Metabolism	Carbohydrate Metabolism	Inositol phosphate metabolism	0.50291293	164.9554411	0.008846947		0.0944389	0.016461282	0.097523201	0.01596103
Metabolism	Metabolism of Terpenoids and Polyketides	Zeatin biosynthesis	0.512217302	168.007275	0.008469097		0.047841699	0.006529399	0.048966897	0.005624905
Metabolism	Amino Acid Metabolism	Lysine degradation	0.513527185	168.4369168	0.008416841		0.112005162	0.046459475	0.105259671	0.025911919
Metabolism	Carbohydrate Metabolism	Pentose and glucuronate interconversions	0.5138831	168.5536568	0.008402682		0.572737305	0.071028505	0.585696392	0.068793306
Metabolism	Biosynthesis of Other Secondary Metabolites	Flavonoid biosynthesis	0.514574736	168.7805135	0.008375216		0.005257022	0.004671221	0.004480866	0.003771471
Metabolism	Lipid Metabolism	Fatty acid metabolism	0.519277925	170.3231592	0.008190119		0.246817757	0.060283066	0.23765344	0.040341909
Metabolism	Metabolism of Other Amino Acids	Cyanoamino acid metabolism	0.521470211	171.042229	0.008104832		0.303215857	0.079860281	0.315023315	0.05014779
Metabolism	Others	Others_Unclassified	0.52395571	171.857473	0.008008891		0.997622782	0.063320083	0.984800308	0.075918598
Metabolism	Xenobiotics Biodegradation and Metabolism	Xylene degradation	0.525674141	172.4211182	0.007943025		0.071651717	0.017660666	0.068638698	0.015903585
Metabolism	Xenobiotics Biodegradation and Metabolism	Metabolism of xenobiotics by cytochrome P450	0.555283836	182.1330981	0.006865767		0.030612081	0.019342103	0.027779475	0.014725619
Metabolism	Carbohydrate Metabolism	Pyruvate metabolism	0.566337307	185.7586368	0.006490364		1.045563463	0.074975612	1.055787029	0.051317693
Metabolism	Metabolism of Terpenoids and Polyketides	Terpenoid backbone biosynthesis	0.567545832	186.155033	0.006450165		0.560684755	0.048743763	0.566730978	0.02494164
Metabolism	Metabolism of Terpenoids and Polyketides	Tetracycline biosynthesis	0.57095761	187.274096	0.006337564		0.151684712	0.041482485	0.157522111	0.0654110489
Metabolism	Xenobiotics Biodegradation and Metabolism	Dioxin degradation	0.573468119	188.097543	0.006255538		0.075532365	0.018571174	0.072595743	0.018168493
Metabolism	Amino Acid Metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	0.584446016	191.6982933	0.005904987		0.869249233	0.069201447	0.880491283	0.074576697
Metabolism	Metabolism of Cofactors and Vitamins	Folate biosynthesis	0.586997271	192.5351049	0.005825386		0.376184943	0.03933824	0.381903345	0.035148135
Metabolism	Metabolism of cofactors and vitamins	Metabolism of cofactors and vitamins_Unclassified	0.58871111	193.0972442	0.005772303		0.104708311	0.027369566	0.101100543	0.019964723
Metabolism	Amino Acid Metabolism	Valine, leucine and isoleucine biosynthesis	0.595650019	195.3732061	0.005605555		0.812815237	0.074201505	0.823280432	0.065411023
Metabolism	Metabolism of Other Amino Acids	Glutathione metabolism	0.607494427	199.258172	0.005210647		0.194830727	0.048223023	0.188508393	0.039100021
Metabolism	Lipid Metabolism	Primary bile acid biosynthesis	0.610558193	200.2630873	0.005122461		0.04245125	0.011994803	0.043946591	0.008840681
Metabolism	Lipid Metabolism	Fatty acid biosynthesis	0.631929681	207.2729355	0.004533079		0.466453599	0.068844537	0.474137917	0.044649509
Metabolism	Metabolism of Cofactors and Vitamins	Porphyrin and chlorophyll metabolism	0.633173543	207.6809221	0.004500135		0.880348156	0.212784792	0.908205207	0.199459757
Metabolism	Nucleotide Metabolism	Pyrimidine metabolism	0.633632824	207.8315661	0.004488008		1.788860086	0.128178269	1.804263507	0.101207862

Metabolism Glycan Biosynthesis and Metabolism	Peptidoglycan biosynthesis	0.637803247	209.1994652	0.004378801	0.816843478	0.075224778	0.825722152	0.058651119
Metabolism Lipid Metabolism	Secondary bile acid biosynthesis	0.651500173	213.6920566	0.004031522	0.042001542	0.012099506	0.043336228	0.008873611
Metabolism Lipid Metabolism	Ether lipid metabolism	0.652534722	214.0313889	0.00400599	0.001680794	0.002809553	0.002037933	0.00277669
Metabolism Biosynthesis of Other Secondary Metabolites	Penicillin and cephalosporin biosynthesis	0.65410444	214.5462562	0.003967436	0.022324049	0.015125501	0.024084525	0.012738074
Metabolism Metabolism of Other Amino Acids	Taurine and hypotaurine metabolism	0.657313258	215.5987486	0.003889317	0.10691528	0.013329587	0.108546	0.01262299
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - globo series	0.66276703	217.3875859	0.003758659	0.114038428	0.043671644	0.119257192	0.040871817
Metabolism Lipid Metabolism	Synthesis and degradation of ketone bodies	0.667770597	219.0287558	0.003641106	0.02616169	0.008081861	0.027153341	0.008166975
Metabolism Lipid Metabolism	Fatty acid elongation in mitochondria	0.669630001	219.6386404	0.003597982	0.001928215	0.003664351	0.001504035	0.003365891
Metabolism Lipid Metabolism	Steroid biosynthesis	0.669893777	219.7251589	0.003591889	0.005789808	0.010991497	0.004517687	0.010097971
Metabolism Biosynthesis of Other Secondary Metabolites	Caffeine metabolism	0.670033812	219.7710905	0.003588657	0.001928432	0.003664238	0.001504802	0.003366012
Metabolism Carbohydrate Metabolism	C5-Branched dibasic acid metabolism	0.674167333	221.1268852	0.003494017	0.353263914	0.039225552	0.357879577	0.038172751
Metabolism Metabolism of Other Amino Acids	Selenocompound metabolism	0.678428012	222.5243881	0.003398013	0.389532413	0.028404478	0.386251908	0.027407249
Metabolism Glycan Biosynthesis and Metabolism	N-Glycan biosynthesis	0.679630021	222.9186468	0.003371211	0.017752006	0.009384525	0.018727152	0.007380673
Metabolism Metabolism of Cofactors and Vitamins	One carbon pool by folate	0.687785106	225.5935148	0.003192621	0.580420587	0.039274741	0.584584064	0.034148444
Metabolism Amino Acid Metabolism	Amino acid related enzymes	0.688414778	225.8000471	0.003179065	1.47003639	0.101292117	1.479766858	0.070834508
Metabolism Metabolism of Terpenoids and Polyketides	Carotenoid biosynthesis	0.689209341	226.060664	0.003162008	0.005126104	0.005402304	0.00454895	0.004816308
Metabolism Metabolism of Cofactors and Vitamins	Biotin metabolism	0.689410959	226.1267946	0.003157688	0.135676067	0.02596027	0.138549717	0.024791421
Metabolism Metabolism of Cofactors and Vitamins	Lipoic acid metabolism	0.696842745	228.5644204	0.003008823	0.033254937	0.015925373	0.031559959	0.014825081
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - lacto and neolacto series	0.69848189	229.1020599	0.002966842	0.000150571	0.000343584	0.000204656	0.000579741
Metabolism Metabolism of Cofactors and Vitamins	Vitamin B6 metabolism	0.714513624	234.3604686	0.002646067	0.190657328	0.027477197	0.187934543	0.025180324
Metabolism Lipid Metabolism	Sphingolipid metabolism	0.714604749	234.3903576	0.002644303	0.249084953	0.073387507	0.256626249	0.071968828
Metabolism Carbohydrate Metabolism	Glyoxylate and dicarboxylate metabolism	0.716700269	235.0776883	0.002603922	0.517547777	0.056099565	0.52259611	0.041872507
Metabolism Xenobiotics Biodegradation and Metabolism	Benzoate degradation	0.721402208	236.6199243	0.002514589	0.207103493	0.024000391	0.209599747	0.025120959
Metabolism Amino Acid Metabolism	Glycine, serine and threonine metabolism	0.722818428	237.0844444	0.002488025	0.831378739	0.039550803	0.835264837	0.037791943
Metabolism Biosynthesis of Other Secondary Metabolites	Phenylpropanoid biosynthesis	0.725794604	238.0606301	0.002432716	0.19055524	0.08747728	0.197589923	0.054217364
Metabolism Metabolism of Cofactors and Vitamins	Retinol metabolism	0.727313793	238.5589241	0.002404751	0.030134332	0.017490626	0.028604432	0.013668518
Metabolism Metabolism of Other Amino Acids	D-Arginine and D-ornithine metabolism	0.737116603	241.7742457	0.00222863	0.002172472	0.00147666	0.002373144	0.002480329
Metabolism Xenobiotics Biodegradation and Metabolism	Atrazine degradation	0.740584564	242.911737	0.002168101	0.022538737	0.019012871	0.02088267	0.016450308
Metabolism Amino Acid Metabolism	Phenylalanine metabolism	0.742012741	243.3801789	0.002143442	0.171838756	0.032875745	0.168851969	0.031203092
Metabolism Glycan Biosynthesis and Metabolism	Lipopolysaccharide biosynthesis	0.748610457	245.5442299	0.002031541	0.133568588	0.124815313	0.123017025	0.108584175
Metabolism Carbohydrate Metabolism	Starch and sucrose metabolism	0.75779138	248.5555725	0.001881295	1.153586044	0.18302626	1.16664324	0.116495099
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of ansamycins	0.758327876	248.7315433	0.001872711	0.11717345	0.019835204	0.118711965	0.015679564
Metabolism Glycan Biosynthesis and Metabolism	Other glycan degradation	0.765119631	250.9592389	0.001765881	0.312026765	0.016395367	0.322452717	0.127589502
Metabolism Amino Acid Metabolism	Lysine biosynthesis	0.776753015	254.7749889	0.001590777	0.891526126	0.075457278	0.897124194	0.064577268
Metabolism Glycan Biosynthesis and Metabolism	Lipopolysaccharide biosynthesis proteins	0.795589698	260.9534209	0.001327993	0.234669664	0.145101978	0.224729428	0.126901138
Metabolism Amino Acid Metabolism	Tyrosine metabolism	0.797157529	261.4676695	0.00130726	0.343959248	0.040005234	0.341145127	0.037396297
Metabolism Carbohydrate Metabolism	Citrate cycle (TCA cycle)	0.800658904	262.6161205	0.001261585	0.530668485	0.101007757	0.524092422	0.083872675
Metabolism Lipid Metabolism	Glycerophospholipid metabolism	0.804179593	263.7709065	0.001216526	0.652027578	0.054085552	0.54861795	0.043581726
Metabolism Biosynthesis and biodegradation of secondary metabolites	Biosynthesis and biodegradation of secondary metabolites_Unclassified	0.805893321	264.3330092	0.001194907	0.059369363	0.02776042	0.057912985	0.013362858
Metabolism Glycan Biosynthesis and Metabolism	Glycosphingolipid biosynthesis - ganglio series	0.810991758	266.0052968	0.001131801	0.039162691	0.040766807	0.041945537	0.041131876
Metabolism Biosynthesis of Other Secondary Metabolites	Tropane, piperidine and pyridine alkaloid biosynthesis	0.812142174	266.3826329	0.001117812	0.107358854	0.010265448	0.108063189	0.010563494
Metabolism Metabolism of Terpenoids and Polyketides	Prenyltransferases	0.812304384	266.435838	0.001115847	0.29793249	0.035316106	0.300005319	0.026700549
Metabolism Xenobiotics Biodegradation and Metabolism	Bisphenol degradation	0.81521093	267.3891849	0.001080943	0.080840569	0.021877107	0.082204242	0.019463515
Metabolism Carbohydrate metabolism	Carbohydrate metabolism_Unclassified	0.818049613	268.3202731	0.001047418	0.206766647	0.027213882	0.205214968	0.020705513
Metabolism Biosynthesis of Other Secondary Metabolites	Isoquinoline alkaloid biosynthesis	0.827475843	271.4120765	0.000940063	0.051043208	0.008478246	0.050446028	0.010457814
Metabolism Metabolism of Other Amino Acids	D-Glutamine and D-glutamate metabolism	0.829618307	272.1148048	0.000916509	0.142406589	0.012256203	0.143109403	0.01088589
Metabolism Biosynthesis of Other Secondary Metabolites	Butirosin and neomycin biosynthesis	0.840663267	275.7375514	0.000800019	0.069976512	0.013859488	0.07066291	0.010396957
Metabolism Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	0.84834622	278.2575601	0.000723831	1.186458321	0.051390638	1.182871108	0.075395825
Metabolism Energy Metabolism	Nitrogen metabolism	0.853576395	279.9730575	0.000674221	0.681306913	0.049899543	0.678573431	0.05382457
Metabolism Metabolism of Other Amino Acids	D-Alanine metabolism	0.867793303	284.6362034	0.000548528	0.103069601	0.009902178	0.103625977	0.013022758
Metabolism Metabolism of Cofactors and Vitamins	Riboflavin metabolism	0.868900649	284.9994129	0.000539297	0.201200056	0.03184369	0.202680721	0.031397344
Metabolism Amino Acid Metabolism	Valine, leucine and isoleucine degradation	0.87110749	285.7232568	0.000521139	0.185543194	0.038933531	0.187302222	0.037591377
Metabolism Lipid metabolism	Lipid metabolism_Unclassified	0.871361907	285.8067054	0.000519066	0.132982843	0.032613708	0.131711568	0.02312996
Metabolism Metabolism of Other Amino Acids	beta-Alanine metabolism	0.872950464	286.3277521	0.000506219	0.167801522	0.031686272	0.166508185	0.025758167
Metabolism Nucleotide Metabolism	Purine metabolism	0.873447448	286.4907629	0.000502234	2.223376518	0.157131072	2.229809952	0.129675361
Metabolism Xenobiotics Biodegradation and Metabolism	Ethylbenzene degradation	0.879302407	288.4111895	0.000456497	0.031908098	0.012767489	0.031414612	0.010316377
Metabolism Xenobiotics Biodegradation and Metabolism	Aminobenzoate degradation	0.880248923	288.7216467	0.000449313	0.100570316	0.028258127	0.101619354	0.021218011
Metabolism Glycan Biosynthesis and Metabolism	Glycosaminoglycan degradation	0.88731254	291.038513	0.000397535	0.069786819	0.05861964	0.072136112	0.058207062
Metabolism Xenobiotics Biodegradation and Metabolism	Styrene degradation	0.8914758	292.4040623	0.000368529	0.019462518	0.008324008	0.019766784	0.007473524
Metabolism Xenobiotics Biodegradation and Metabolism	Nitrotoluene degradation	0.898051863	294.561011	0.000324989	0.086896849	0.027440727	0.085896451	0.027543499
Metabolism Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	0.898840384	294.8196461	0.000319955	0.431157184	0.068430613	0.428963572	0.054169554
Metabolism Xenobiotics Biodegradation and Metabolism	Chloroalkane and chloroalkene degradation	0.900473662	295.3553612	0.000309655	0.203261314	0.028197812	0.204247181	0.027424352
Metabolism Carbohydrate Metabolism	Propanoate metabolism	0.909597891	298.3481081	0.000255255	0.494864388	0.031979078	0.495803307	0.026727301
Metabolism Lipid Metabolism	Glycerolipid metabolism	0.921665256	302.3062041	0.000191458	0.417832663	0.05660839	0.416457155	0.042788861
Metabolism Xenobiotics Biodegradation and Metabolism	Chlorocyclohexane and chlorobenzene degradation	0.923721119	302.9805269	0.000181511	0.015713221	0.008097931	0.015929649	0.007854523
Metabolism Enzyme Families	Peptidases	0.92572829	303.6388792	0.000172058	1.86548181	0.084981991	1.867345776	0.056786407
Metabolism Energy Metabolism	Oxidative phosphorylation	0.92720826	304.1243093	0.000165251	1.068971532	0.12610309	1.065910195	0.11142493
Metabolism Glycan Biosynthesis and Metabolism	Glycosyltransferases	0.933312859	306.1266178	0.000138635	0.300375202	0.057696191	0.29913162	0.047834174

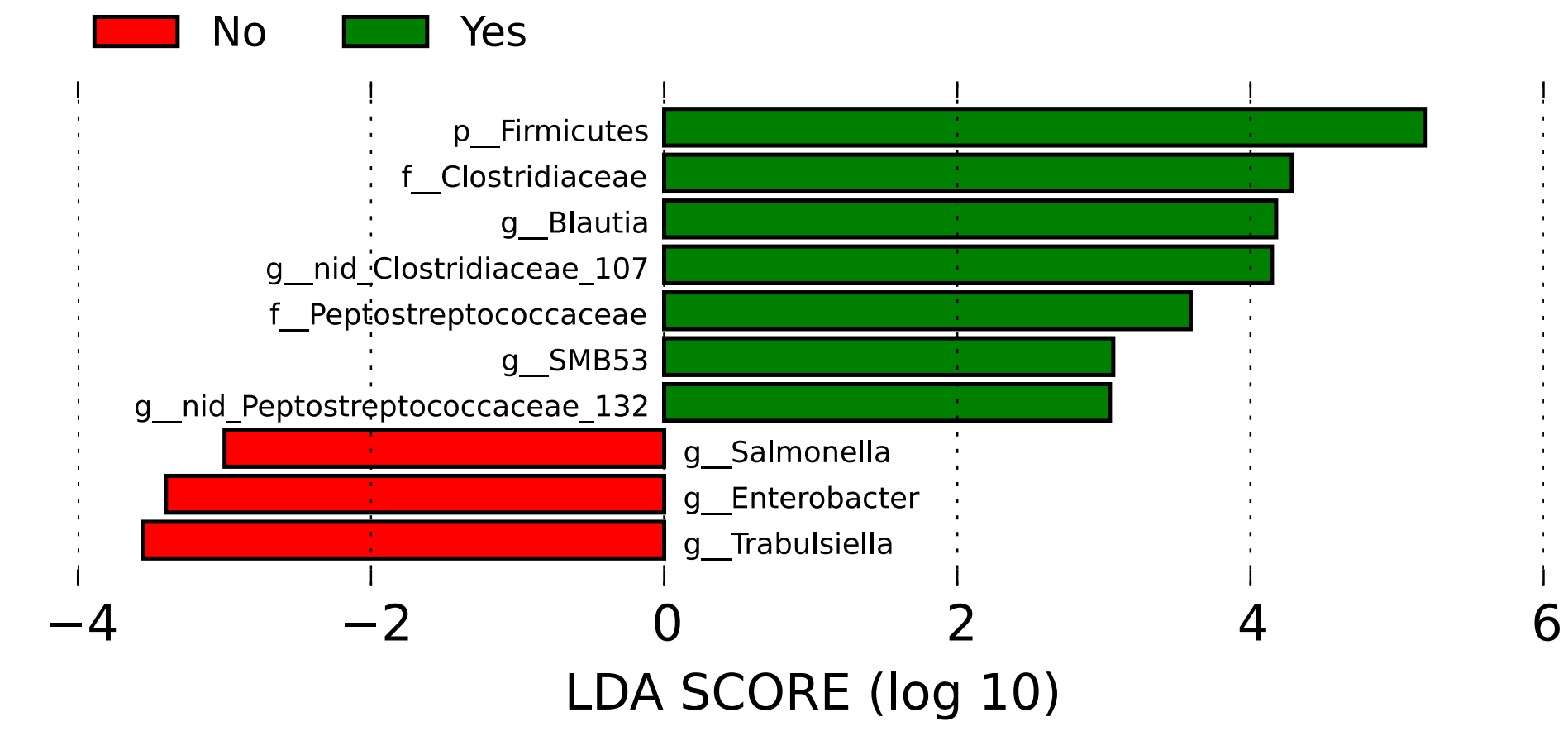
Metabolism Lipid Metabolism	Lipid biosynthesis proteins	0.934867523	306.6365477	0.000132233	0.54893116	0.051213294	0.549974231	0.039488007
Metabolism Biosynthesis of Other Secondary Metabolites	Novobiocin biosynthesis	0.947498573	310.7795321	8.59E-05	0.13426206	0.00986359	0.134438097	0.009057363
Metabolism Energy Metabolism	Sulfur metabolism	0.947562563	310.8005208	8.56E-05	0.278881941	0.035179283	0.278278586	0.029926875
Metabolism Amino acid metabolism	Amino acid metabolism_Unclassified	0.949302819	311.3713247	8.00E-05	0.201792901	0.045628984	0.201025879	0.03991316
Metabolism Biosynthesis of Other Secondary Metabolites	Flavone and flavonol biosynthesis	0.955522814	313.4114829	6.16E-05	0.006963852	0.008258799	0.007084349	0.007071328
Metabolism Metabolism of Other Amino Acids	Phosphonate and phosphinate metabolism	0.959918581	314.8532947	5.00E-05	0.06005206	0.010363232	0.059891407	0.011900241
Metabolism Energy Metabolism	Photosynthesis - antenna proteins	0.976254636	320.2115208	1.75E-05	3.09E-05	6.62E-05	3.03E-05	8.63E-05
Metabolism Energy Metabolism	Carbon fixation pathways in prokaryotes	0.976744082	320.372059	1.68E-05	0.924870865	0.091899017	0.925571139	0.078563156
Metabolism Energy metabolism	Energy metabolism_Unclassified	0.982314928	322.1992965	9.73E-06	0.778388217	0.116936608	0.779094115	0.108358105
Metabolism Lipid Metabolism	Steroid hormone biosynthesis	0.983417245	322.5608563	8.55E-06	0.020566688	0.022671196	0.020433963	0.02235168
Metabolism Biosynthesis of Other Secondary Metabolites	Betalain biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Biosynthesis of Other Secondary Metabolites	Clavulanic acid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Biosynthesis of Other Secondary Metabolites	Indole alkaloid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Biosynthesis of Other Secondary Metabolites	Isoflavonoid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Glycosaminoglycan biosynthesis - chondroitin sulfate	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Glycan Biosynthesis and Metabolism	Other types of O-glycan biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of 12-, 14- and 16-membered macrolides	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Biosynthesis of type II polyketide backbone	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0
Metabolism Metabolism of Terpenoids and Polyketides	Sesquiterpenoid biosynthesis	1	328	-1 degenerate case: failed to calculate p-value	0	0	0	0



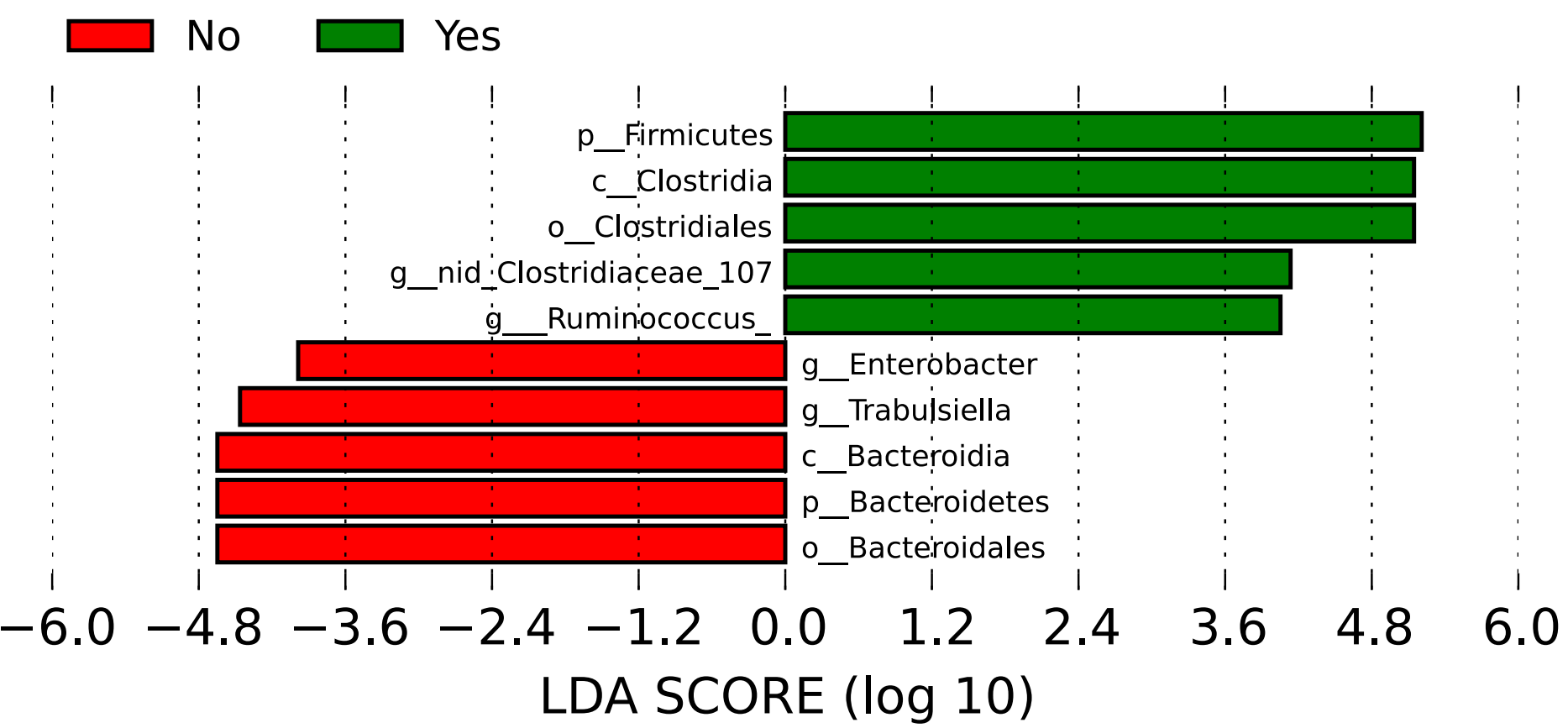
Antibiotics



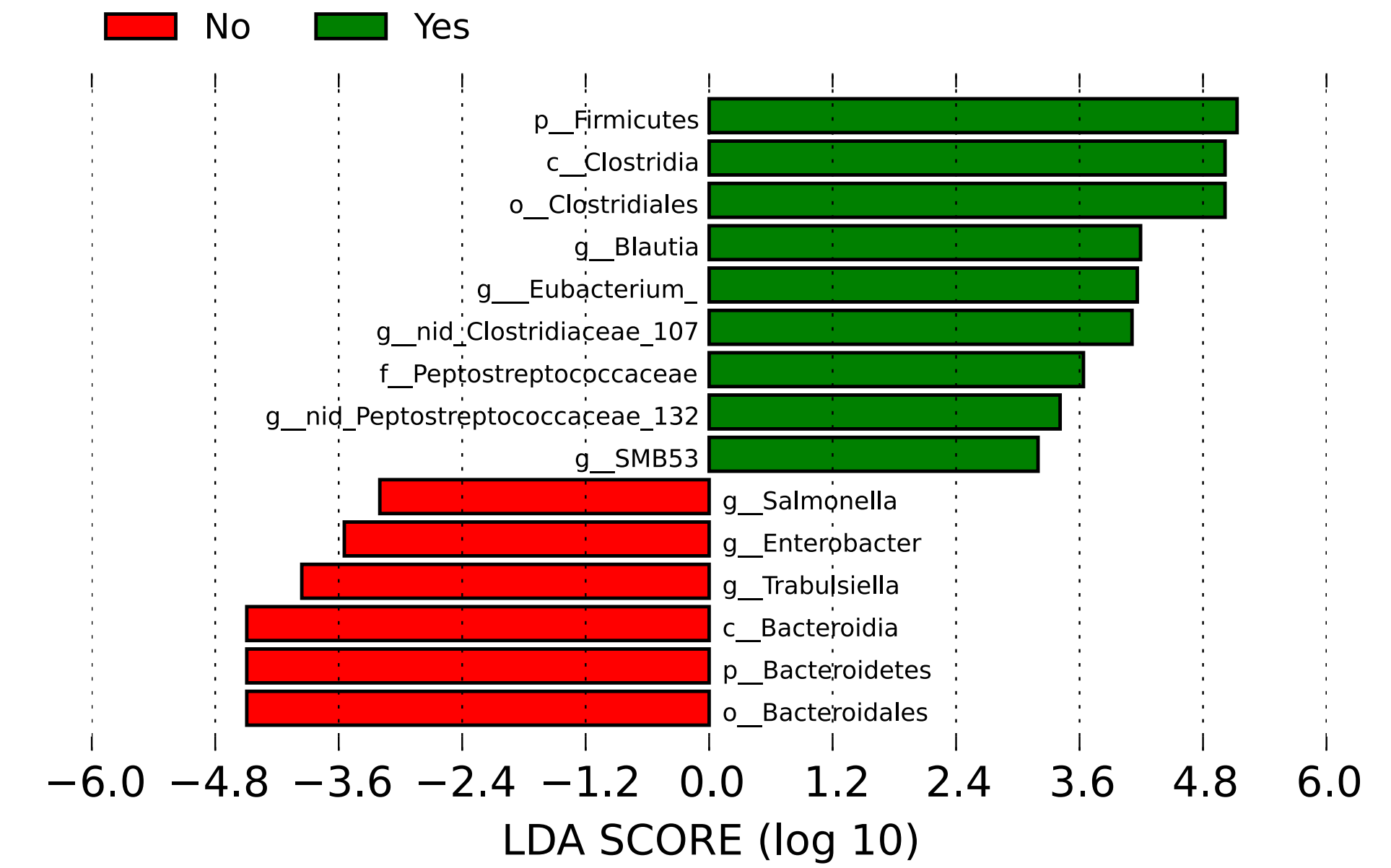
Breastfeeding



C-section



Race



Solid food