



Contribution of Ruminal Bacteriome to the Individual Variation of Nitrogen Utilization Efficiency of Dairy Cows

Min Li, Huiyue Zhong, Ming Li, Nan Zheng, Jiaqi Wang* and Shengguo Zhao*

State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

OPEN ACCESS

Edited by:

Anil Kumar Puniya,
National Dairy Research Institute
(ICAR), India

Reviewed by:

Sabine Marie Podmirseg,
University of Innsbruck, Austria
Fabio S. Lima,
University of California, Davis,
United States

*Correspondence:

Jiaqi Wang
jiaqi.wang@vip.163.com
Shengguo Zhao
zhaoshengguo1984@163.com

Specialty section:

This article was submitted to
Systems Microbiology,
a section of the journal
Frontiers in Microbiology

Received: 15 November 2021

Accepted: 07 February 2022

Published: 18 March 2022

Citation:

Li M, Zhong H, Li M, Zheng N,
Wang J and Zhao S (2022)
Contribution of Ruminal Bacteriome
to the Individual Variation of Nitrogen
Utilization Efficiency of Dairy Cows.
Front. Microbiol. 13:815225.
doi: 10.3389/fmicb.2022.815225

High nitrogen utilization efficiency (NUE) is important for increasing milk protein production and decreasing the feed nitrogen cost and nitrogen emission to the environment. Currently, there is a limited whole picture of the relationship between ruminal bacteriome and the NUE of dairy cows, even though some information has been revealed about the bacteriome and milk or milk protein production of dairy cows. The purpose of this study was to compare the rumen bacterial community in dairy cows with different nitrogen utilization efficiency under the same diet. The natural abundance of ^{15}N between the animal proteins and diet ($\Delta^{15}\text{N}$) was used as a simple, non-invasive, and accurate biomarker for NUE in ruminants to mark the individual variation. Dairy cows with high NUE (HE_HP, $n = 7$), medium NUE (ME_MP, $n = 7$), and low NUE (LE_LP, $n = 7$) were selected from 284 Holstein dairy cows with the same diet. Measurement of the rumen fermentation indices showed that the proportion of propionate was higher in HE_HP cows and ME_MP cows than in LE_LP cows ($P < 0.05$). The diversity of rumen bacterial community was higher in LE_LP cows than in ME_MP cows and HE_HP cows by 16S rRNA sequencing analysis ($P < 0.05$). Moreover, at the genus level, the relative abundances of *Succinivibrionaceae*_UCG_001, uncultured_*Selenomonadaceae*, and *Acidaminococcus* were higher in HE_HP cows than in LE_LP cows ($P < 0.05$). Interestingly, we found that these bacteria were positively correlated with milk protein yield and negatively correlated with $\Delta^{15}\text{N}$ ($P < 0.05$). However, *Clostridia*_UCG_014, *Saccharofermentans*, *Bacilli*_RF39, and *Desulfovibrio* were lower in HE_HP cows and ME_MP cows than in LE_LP cows ($P < 0.05$), which were negatively correlated with milk protein yield and positively correlated with $\Delta^{15}\text{N}$ ($P < 0.05$). In conclusion, the study showed that the diversity and relative abundances of rumen bacteria differed among different NUE cows, indicating that rumen bacteriome contributes to nitrogen metabolism in dairy cows.

Keywords: rumen, nitrogen utilization efficiency, bacteriome, dairy cows, milk

INTRODUCTION

With the development of animal husbandry, most of the nitrogen intake by dairy cows is excreted by feces and urine. Nitrogen excretion leads to emission of greenhouse gases by livestock, followed by nitrogen loss in soil (N_2O), volatilization of NH_3 , and leaching of NO_3^- , to environment pollution, aquatic and terrestrial eutrophication, and acidification (Angelidis et al., 2019). Oenema and Tamminga (2005) showed that the global amount of nitrogen

annually excreted by livestock ranged from 8×10^7 T to 1.3×10^8 T, and the production of cattle contributed 60% of the total emissions. Therefore, it is necessary to improve the nitrogen utilization efficiency (NUE) of dairy cows by dietary regulation. NUE can be affected by heredity, nutrition, and management (Wang et al., 2007; Broderick et al., 2008; Cantalapiedra-Hijar et al., 2014)(Broderick et al., 2008; Wang et al., 2007; Cantalapiedra-Hijar et al., 2014). It was reported that the NUE of dairy cows was still at a low level (only ~25%) (Huhtanen and Hristov, 2009), and more nitrogen was emitted to the environment (Calsamiglia et al., 2010).

Stable isotope fractionation, a result of the change in the ratio of heavy to light isotope ($^{15}\text{N}/^{14}\text{N}$), can be used to characterize the nutrition status of animals (Lavery and Ferris, 2021). $\delta^{15}\text{N}$ is the ratio of $^{15}\text{N}/^{14}\text{N}$ in the animal minus $^{15}\text{N}/^{14}\text{N}$ ratio in the standard. N partitioning is different in plasma, feces, and milk compared to diet. It was reported that N isotope fractionation can predict NUE (Cheng et al., 2013). Plasma nitrogen isotopic fractionation ($\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{plasma}} - \delta^{15}\text{N}_{\text{diet}}$) can be especially used as a predictor to effectively assess NUE (Cheng et al., 2016). Cantalapiedra-Hijar et al. (2018) suggested that $\Delta^{15}\text{N}$ could be used to predict NUE variation across diets and individuals. $\Delta^{15}\text{N}$ was demonstrated to be negatively correlated with NUE and the synthesis of microbe protein under different conditions (Cheng et al., 2016).

Rumen microbiota can convert fiber into digestible substances such as volatile fatty acid (VFAs) and microbial proteins. Ruminal VFAs produced by rumen microbial fermentation can provide 70% of the energy requirements in dairy cows (Matthews et al., 2019). Moreover, the bacteria in the rumen accounts for more than 95% of the microbial community, which is the main contributor to the performance of dairy cows (Castillo-Gonzalez et al., 2014). Li et al. (2019) found that the microbial composition and function between high residual feed intake steers and low residual feed intake steers were different, suggesting that microbiome interactions are the major contributor to the variations of feed efficiency. Xue et al. (2018) revealed that pan and core bacteriome were associated with milking traits in lactating dairy cows which were fed the same diet under the same environment, and they also found that the abundance of *Sharpea* was higher in high-milk-protein-yield (MPY) cows than in low-MPY cows. *Succinivibrio* and *Clostridium* were enriched in low-MPY cows compared to high-MPY cows. Although specific bacteria were related to milk composition (Jami et al., 2014), there are few studies on uncovering the function of rumen bacteria to NUE. The interaction of rumen bacteria and NUE is not clear.

In this study, $\Delta^{15}\text{N}$ and MPY, as NUE indicators, were used to discuss NUE's relationship with the rumen bacteriome. The objective of the study was to reveal the potential effects of rumen bacteriome on NUE in dairy cows.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment procedures were approved by the animal care committee (approval number: ISA2020-82). A total of 284

healthy lactating Holstein dairy cows (parity = 2; days in milk = 48 ± 1 , mean \pm SEM) were selected from 4,086 dairy cows in a commercial dairy farm. The dairy cows were fed an identical diet (**Supplementary Table 1**) and had free access to water. Milk yield was recorded for a month, and milk samples were collected on the last day at a volume ratio of 4:3:3 homologized to the morning (0500h), afternoon (1400h), and evening (1700h). Milk protein was tested by infrared analysis using near-infrared spectroscopy. Milk protein yield was calculated using milk protein content multiplied by the milk yield. Milk urea nitrogen was measured with the Urea Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing). Blood was collected using a 10-ml heparin anticoagulant tube from the caudal vein within 2 h after morning feeding. The blood samples were centrifuged at $4,000 \times g$ for 5 min to obtain plasma and stored at -20°C . The plasma ^{15}N and diet ^{15}N were determined by Elemental Analysis-Isotope Ratio Mass (Isoprime100, England). $\Delta^{15}\text{N}$ was calculated using $\delta^{15}\text{N}_{\text{plasma}} - \delta^{15}\text{N}_{\text{diet}}$ (Oenema and Tamminga, 2005). Based on $\Delta^{15}\text{N}$ and MPY, 7 high NUE (HE_HP: $1.9\text{‰} < \Delta^{15}\text{N} < 2.2\text{‰}$; $1.7 \text{ kg/day} < \text{MPY} < 2.0 \text{ kg/day}$, days in milk (DIM) = 59 ± 9) dairy cows, 7 medium NUE (ME_MP: $2.2\text{‰} < \Delta^{15}\text{N} < 2.4\text{‰}$; $1.4 \text{ kg/day} < \text{MPY} < 1.7 \text{ kg/day}$, DIM = 54 ± 15) dairy cows, and 7 low NUE (LE_LP: $2.4 \text{‰} < \Delta^{15}\text{N} < 2.7 \text{‰}$; $1.0 \text{ kg/day} < \text{MPY} < 1.4 \text{ kg/day}$, DIM = 63 ± 9) dairy cows were selected (**Figure 1**). The rumen liquid was collected within 2 h after the morning feeding using oral stomach and tube and stored in liquid nitrogen immediately.

Rumen Fermentation Parameter Detection

The rumen liquid was centrifuged at $13,000 \times g$ at 4°C for 5 min to obtain the supernatant. Then, 600 μl of the supernatant was mixed with 50 μl of 25% metaphosphoric acid and was centrifuged at $12,000 \times g$ for 20 min to obtain the

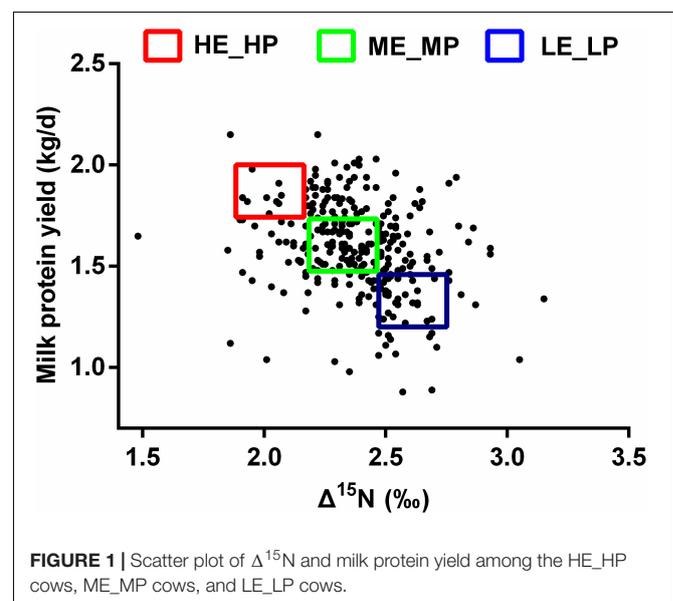


FIGURE 1 | Scatter plot of $\Delta^{15}\text{N}$ and milk protein yield among the HE_HP cows, ME_MP cows, and LE_LP cows.

supernatant for the analysis of ammonia nitrogen and VFAs. The ammonia nitrogen concentrations were determined using phenol-sodium hypochlorite colorimetric analysis (Broderick and Clayton, 1997). The proportion of VFAs was determined using GC (Agilent, 7890A) (Liang et al., 2017).

DNA Extraction and 16S rRNA Gene Sequencing

Total DNA of rumen liquid was extracted from the rumen liquid of 21 dairy cows using cetyl trimethyl ammonium bromide (Coolaber, Beijing) plus bead-beating method (Minas et al., 2011). The qualities and quantities of the DNA samples were measured using NanoDrop (Thermo, Beijing). The DNA was amplified using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTATCTAAT-3'), which target the V3–V4 region of the bacterial 16S rRNA gene. The reactions were performed in a 50- μ l reaction mixture containing 2 μ l DNA, 3.0 μ l of each primer, 1.0 μ l of dNTP, 0.25 of DNA polymerase (Takara, Dalian), 5 μ l of 10 \times PCR buffer, and 38.75 of ddH₂O. The PCR cycling procedures were as follows: 95°C for 5 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, and followed by 72°C for 15 min. The amplicon sequencing library was constructed using TruSeq Nano DNA LT Library Prep Kit (Illumina, America). After the library quality control was finished, the library was sequenced on an Illumina HiSeq platform (Illumina, America).

Bioinformatic Analysis

Reads were removed primers, demultiplexed, and quality-filtered using Vsearch (Rognes et al., 2016). The reads were truncated at any site of >3 sequential bases receiving a quality score of <Q20, and the reads with <75% (of total read length) consecutive high-quality base calls were removed. Operational taxonomic units (OTU) were analyzed using QIIME 2.0 and clustered at 97% similarity using Vsearch (Haas et al., 2011). OTUs were

filtered based on the number of samples in an out <10 and aligned using MAFFT. The phylogenetic tree was constructed using FASTTREE. OTUs were further conducted for species annotation using SKLEARN based on SILVA-138 database. Data rarefying was performed using the minimum size of reads in a sample. The statistical bioinformatics analysis of this study was performed using MicrobiomeAnalyst (Chong et al., 2020). The OTU-level alpha diversity of rumen bacteria was obtained using Chao1 index, ACE index, Shannon index, and Simpson index. The OTU-level beta diversity was performed based on weighted UniFrac and visualized by principal coordinate analysis (PCoA) using the phyloseq package in R (McMurdie and Holmes, 2013). Linear discriminant analysis effect size (LEfSe) was performed on non-parametric factorial Kruskal–Wallis sum-rank test to identify bacteria with significantly different abundances in the three groups (Segata et al., 2011), followed by linear discriminant analysis (LDA) to calculate the effect size of each differently abundant bacteria. The heat map of bacteria and MPY, $\Delta^{15}\text{N}$ was generated using RevolverMaps.¹

Statistical Analysis

Lactation performance (DIM, milk yield, milk protein, milk urea nitrogen, and milk protein yield), $\Delta^{15}\text{N}$, rumen fermentation parameter concentrations (VFA, NH₃-N), and alpha diversity indices of HE_HP cows, ME_MP cows, and LE_LP cows were analyzed using one-way ANOVA. The relative abundances of bacteria and alpha diversity indices among the three groups were compared using Kruskal–Wallis test. Dunn's multiple-comparisons test was used for multiple comparisons of means among the three groups. Statistical analysis was performed using GraphPad Prism 7.0, with $P < 0.05$ considered as statistical significance. LEfSe was used to compare the relative abundances of rumen bacteria in HE_HP cows, ME_MP cows, and LE_LP

¹<https://www.revolvermaps.com>

TABLE 1 | The production, nitrogen utilization efficiency indexes, and rumen fermentation parameters of dairy cows.

	Group			SEM	P-value
	HE_HP	ME_MP	LE_LP		
Milk yield (kg/day)	51.89a	46.06b	35.16c	1.75	<0.01
Milk protein (%)	3.49	3.31	3.39	0.06	0.49
Milk urea nitrogen (mg/dl)	14.80	14.90	15.14	0.38	0.93
Milk protein yield (kg/day)	1.79a	1.52b	1.19c	0.06	<0.01
$\Delta^{15}\text{N}$ (‰)	2.05c	2.30b	2.54a	0.05	<0.01
Ruminal VFA composition (%)					
Acetate	58.89b	59.11b	62.67a	0.60	<0.01
Propionate	28.87a	28.25a	24.34b	0.57	<0.01
Isobutyrate	0.44	0.51	0.47	0.02	0.27
Butyrate	9.34	9.87	10.15	0.22	0.21
Isovalerate	0.79	0.86	0.86	0.04	0.64
Valerate	1.66	1.40	1.49	0.08	0.57
A:P ratio	2.05b	2.10b	2.60a	0.22	<0.01
Ruminal NH ₃ -N (mg/dl)	7.11	10.25	9.17	0.67	0.15

Lowercase letters indicate different shoulder markers of peer data. A:P ratio, ratio of acetate to propionate; VFA, volatile fatty acid.

cows, and significant differences were considered by a LDA score >2 and $P < 0.05$. Correlation analysis between the specific bacteria and NUE was performed using Spearman's correlation.

RESULTS

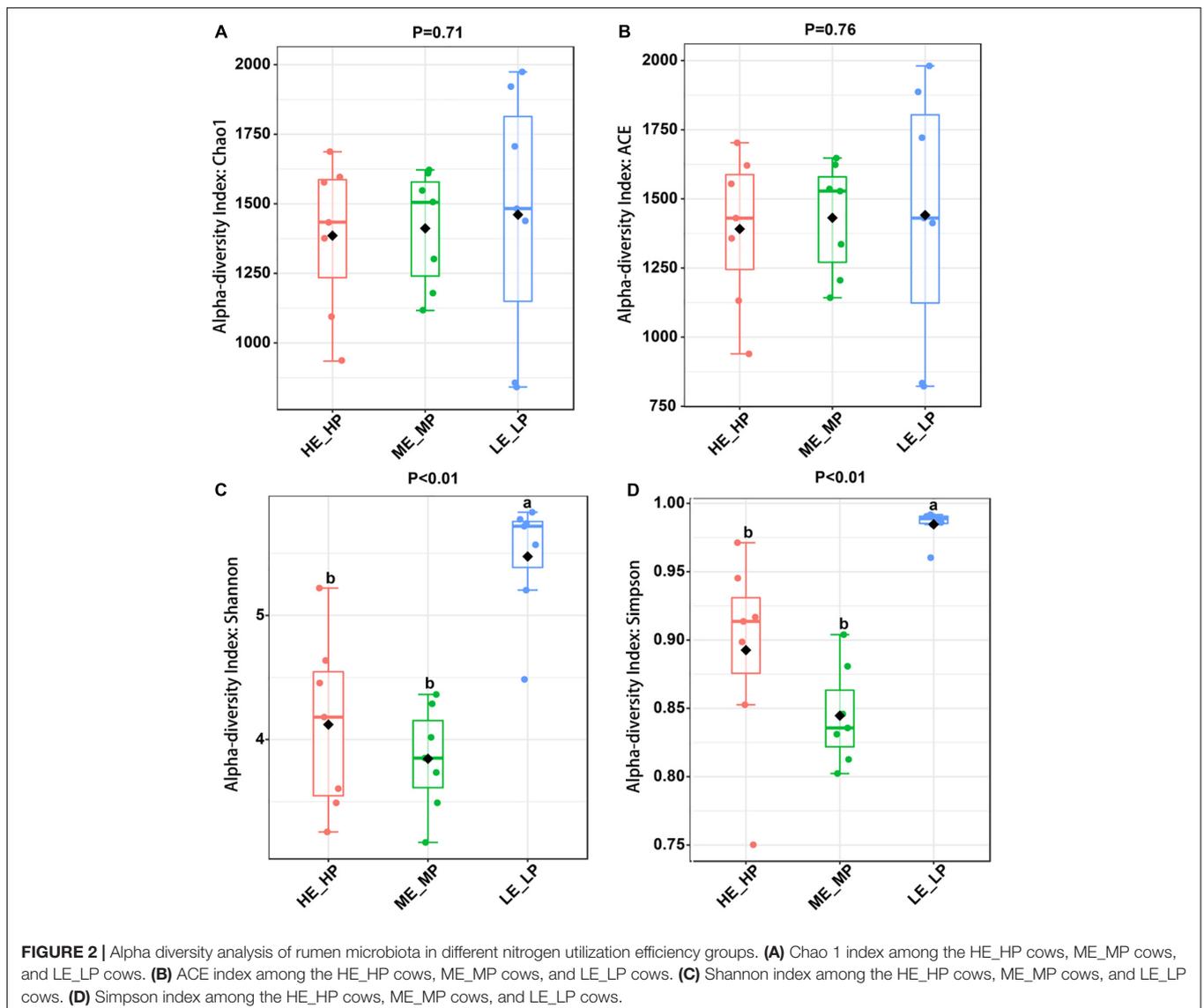
Production and Rumen Volatile Fatty Acid Related to Nitrogen Utilization Efficiency

As to the selected 21 dairy cows, the milk yield was 44.37 ± 1.75 kg/day (mean \pm SEM), the MPY of 21 dairy cows was 1.50 ± 0.06 kg/day, $\Delta^{15}\text{N}$ was 2.30 ± 0.05 ‰, the composition of acetate was $60.22 \pm 0.60\%$, propionate was $27.15 \pm 0.57\%$, isobutyrate was $0.47 \pm 0.02\%$, butyrate was $9.79 \pm 0.22\%$, isovalerate was $0.84 \pm 0.04\%$, valerate was $1.52 \pm 0.08\%$, and $\text{NH}_3\text{-N}$ was 8.85 ± 0.67 mg/dl. Milk yield

and MPY in HE_HP were the highest among the three groups ($P < 0.01$) and in LE_LP cows were the lowest. In contrast, $\Delta^{15}\text{N}$ in HE_HP cows was the lowest and in LE_LP cows was the highest ($P < 0.01$; **Table 1**). The DIM, milk protein, and milk urea nitrogen showed no significance among the HE_HP cows, ME_MP cows, and LE_LP cows ($P > 0.05$). Furthermore, the ruminal levels of $\text{NH}_3\text{-N}$ and the proportion of butyrate and valerate were not different among the three groups ($P > 0.05$). However, the HE_HP cows exhibited a significantly lower concentration of acetate (58.89%) and a higher concentration of propionate (28.87%) compared to LE_LP cows ($P < 0.05$).

Rumen Bacterial Diversity Related to Nitrogen Utilization Efficiency

Amplicon sequencing of the 16S rRNA generated a total of 1,647,027 high-quality sequences and 8,987 OTUs across 21



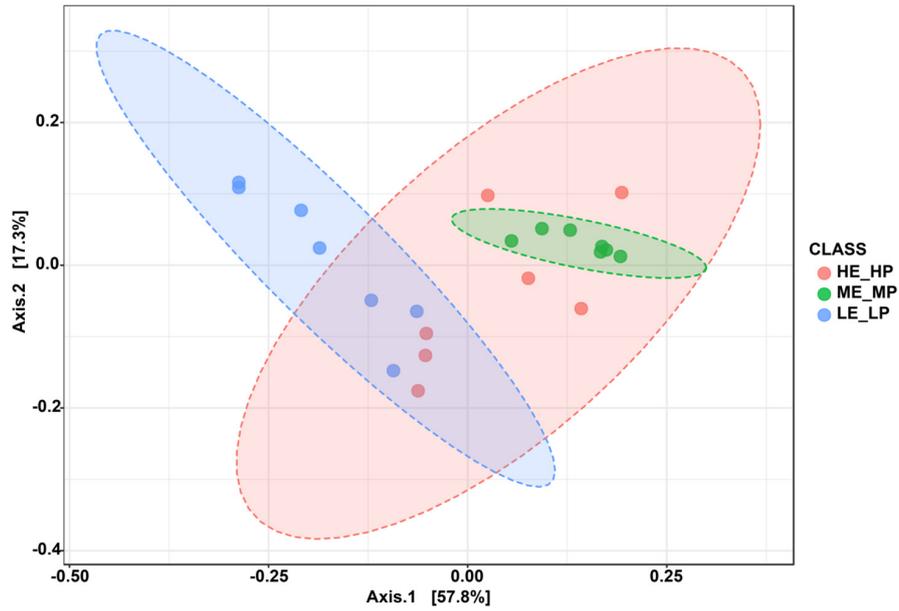


FIGURE 3 | Principal coordinate analysis of bacteria beta diversity at species level among the HE_HP cows, ME_MP cows, and LE_LP cows.

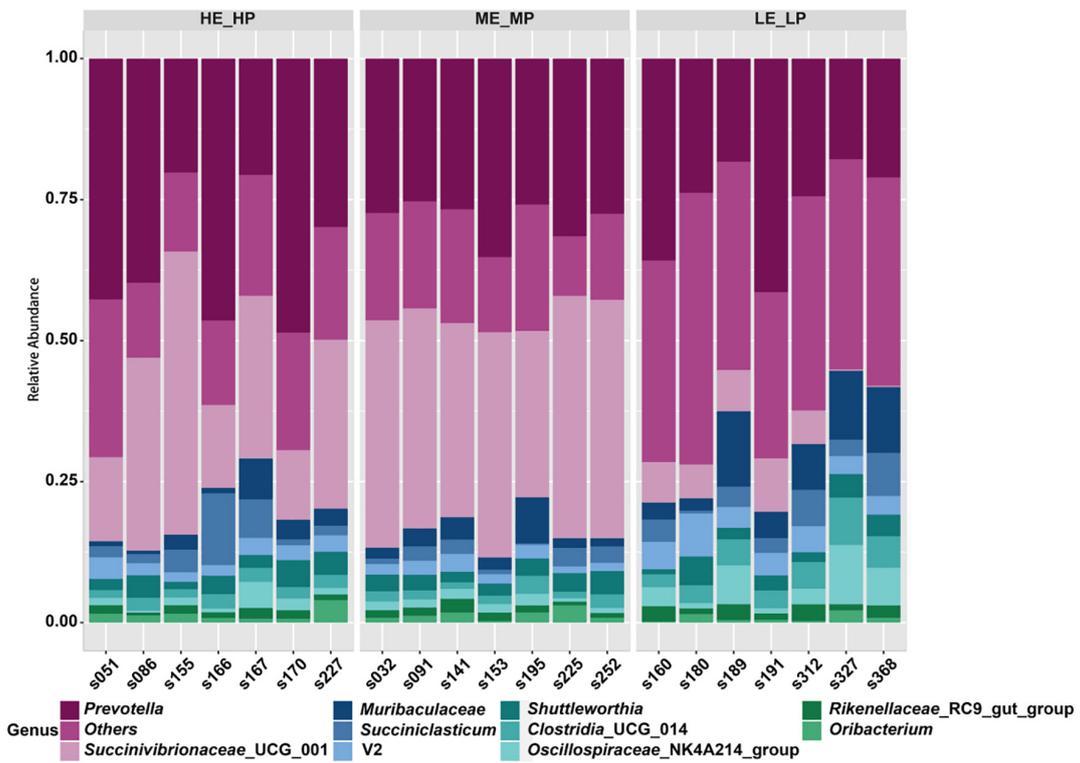


FIGURE 4 | Relative abundances of rumen microbiota at the genus level among the HE_HP cows, ME_MP cows, and LE_LP cows.

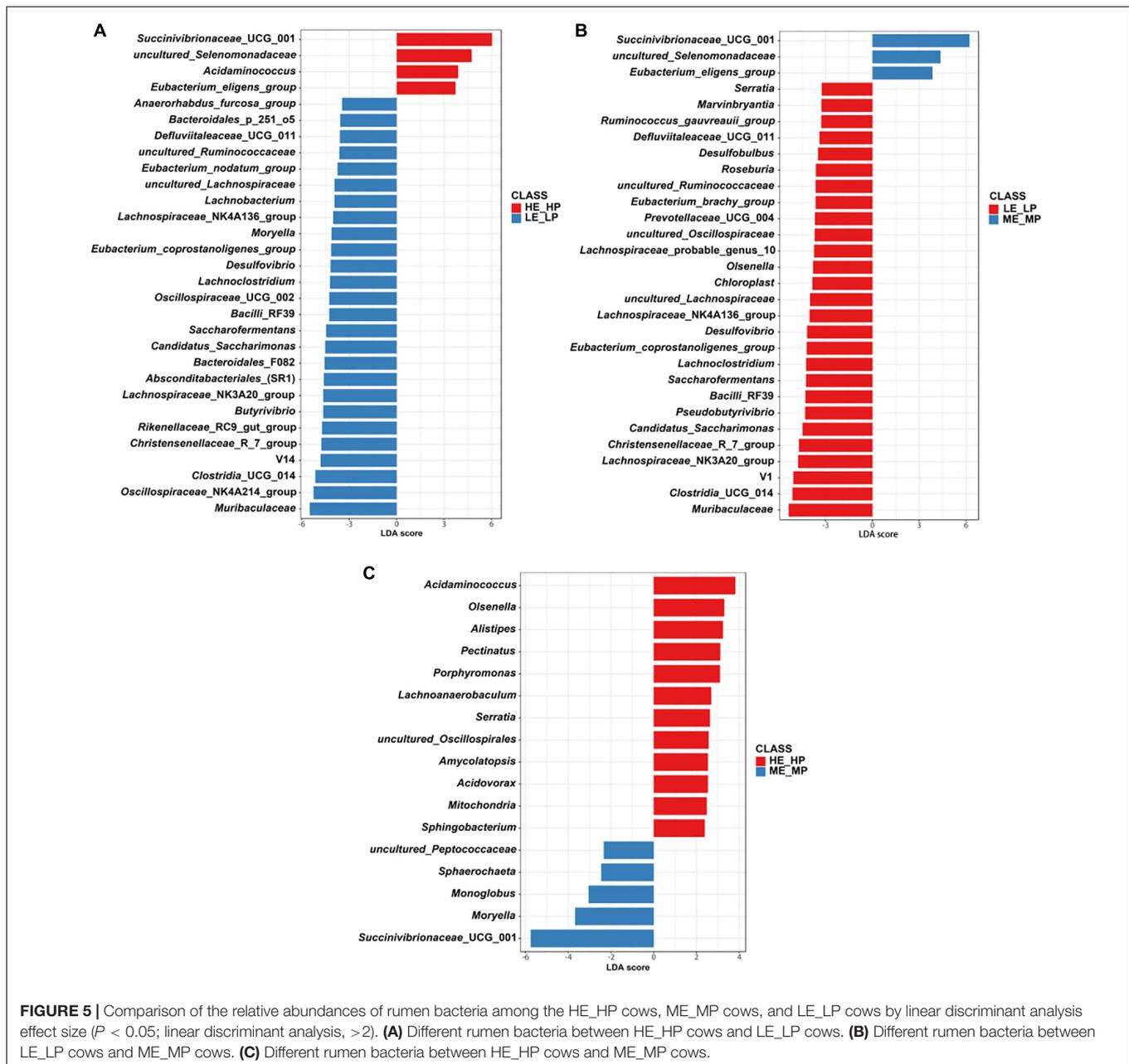


FIGURE 5 | Comparison of the relative abundances of rumen bacteria among the HE_HP cows, ME_MP cows, and LE_LP cows by linear discriminant analysis effect size ($P < 0.05$; linear discriminant analysis, > 2). **(A)** Different rumen bacteria between HE_HP cows and LE_LP cows. **(B)** Different rumen bacteria between LE_LP cows and ME_MP cows. **(C)** Different rumen bacteria between HE_HP cows and ME_MP cows.

samples (Supplementary Figure 1). The rarefaction curves revealed that the sequencing depth was sufficient to cover most species of rumen bacteria (Supplementary Figure 2). The Chao1 index and ACE index ($P > 0.05$) were not significantly different in HE_HP cows, ME_MP cows, and LE_LP cows (Figures 2A,B). The Shannon index and Simpson index ($P < 0.01$) were significantly higher in LE_LP cows compared to HE_HP cows and ME_MP cows (Figures 2C,D). The PCoA based on weighted UniFrac distances showed that the bacterial composition between the HE_HP and LE_LP groups was different (PERMANOVA, $P < 0.01$), while the ME_MP group had no significant difference compared to the HE_HP group (Figure 3), and the ME_MP group had the

most homogeneous community distribution compared to the other two groups.

Different Rumen Bacterial Taxa in Dairy Cows With Different Nitrogen Utilization Efficiency

A total of 24 bacterial phyla were identified; the predominant bacterial phyla were Bacteroidota ($41.24 \pm 8.02\%$), Firmicutes ($30.25 \pm 11.96\%$), and then Proteobacteria ($25.78 \pm 14.97\%$) (Supplementary Figure 3). At the family level, the predominant bacterial families were Prevotellaceae ($32.76 \pm 9.4\%$), Succinivibrionaceae ($23.48 \pm 16.25\%$), and

TABLE 2 | Relative abundances of specific bacteria in dairy cows with different nitrogen utilization efficiency.

Genus	Relative abundance (%)			SEM	P-value
	HE_HP	ME_MP	LE_LP		
Succinivibrionaceae_UCG_001	26.43a	38.33a	5.19b	3.54	<0.01
uncultured_Selenomonadaceae	1.42a	1.10a	0.60b	0.17	0.04
Acidaminococcus	0.16a	0.03b	0.03b	0.03	0.03
Eubacterium_eligens_group	0.14ab	0.21a	0.08b	0.02	0.02
Clostridia_UCG_014	2.09b	1.85b	4.57a	0.38	<0.01
Lachnospiraceae_NK3A20_group	0.86ab	0.40b	1.60a	0.22	<0.01
Candidatus_Saccharimonas	0.34ab	0.30b	0.93a	0.09	<0.01
Saccharofermentans	0.32b	0.39b	0.81a	0.07	0.03
Bacilli_RF39	0.29b	0.26b	0.66a	0.05	<0.01
Desulfovibrio	0.19b	0.17b	0.48a	0.05	<0.01
Lachnoclostridium	0.20ab	0.16b	0.51a	0.06	<0.01
uncultured_Lachnospiraceae	0.13ab	0.08b	0.27a	0.03	0.03
Lachnospiraceae_NK4A136_group	0.18b	0.22ab	0.42a	0.04	0.03
Eubacterium_coprostanoligenes_group	0.20b	0.17b	0.47a	0.04	<0.01
Defluviitaleaceae_UCG_011	0.06b	0.05b	0.14a	0.01	<0.01
Olsenella	0.04ab	0.02b	0.14a	0.02	<0.01
Uncultured_Ruminococcaceae	0.03b	0.03b	0.14a	0.01	<0.01
Lachnospiraceae_probable_genus_10	0.05b	0.06ab	0.18a	0.02	0.02

The bacteria with relative abundances were greater than 0.01% among HE_HP cows, ME_MP cows, and LE_LP cows. The different lowercase letters mean the significance ($P < 0.05$).

Lachnospiraceae ($12.84 \pm 4.79\%$) (**Supplementary Figure 4**). At the genus level, a total of 326 genera were identified; *Prevotella* ($29.85 \pm 9.53\%$), *Succinivibrionaceae_UCG_001* ($23.42 \pm 16.38\%$), *Muribaculaceae* ($4.61 \pm 3.95\%$), and *Succiniclasticum* ($3.35 \pm 2.91\%$) were the most abundant genera (**Figure 4**).

The different abundant bacteria at the genus level among the HE_HP cows, ME_MP cows, and LE_LP cows were revealed by LEfSe (**Figure 5**). The relative abundance of *Succinivibrionaceae_UCG_001*, uncultured_Selenomonadaceae, and *Acidaminococcus* was higher in HE_HP cows ($P < 0.05$). Moreover, *Clostridia_UCG_014*, *Saccharofermentans*, *Bacilli_RF39*, and *Desulfovibrio* were higher in LE_LP cows ($P < 0.01$). There were differences in the abundances of bacterial taxa among the three groups (**Table 2**). Meanwhile, LEfSe was used to screen the different OTUs (**Supplementary Table 2**). The genera corresponding to OTUs with significant differences included *Succinivibrionaceae_UCG_001*, *Shuttleworthia*, *Clostridia_UCG_014*, uncultured_Selenomonadaceae, *Lachnospiraceae_NK3A20_group*, *Christensenellaceae_R-7_group*, *Desulfovibrio*, *Saccharofermentans*, *Allisonella*, and *Eubacterium_eligens_group* ($P < 0.05$), and there was 20% less genus *Succinivibrionaceae_UCG_001* in LE_LP cows than in the other two groups. Moreover, the most differently abundant OTUs belonged to the phylum of Firmicutes.

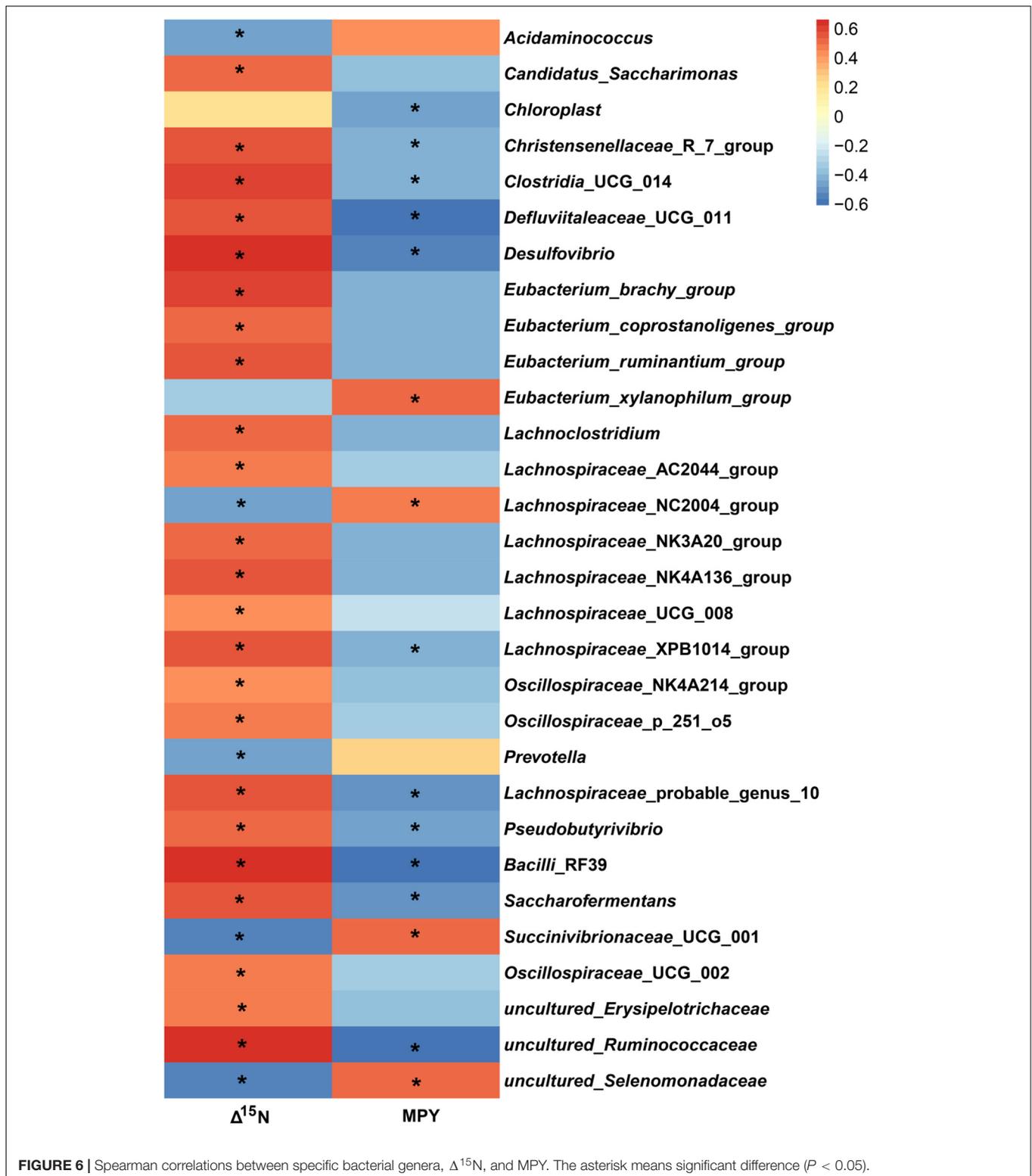
Relationships of Different Rumen Bacteria With $\Delta^{15}\text{N}$ and Milk-Protein-Yield

In the study, in all of the 326 encountered genera, the relative abundance of 127 bacterial taxa was $>0.01\%$.

We analyzed the 127 bacterial taxa and $\Delta^{15}\text{N}$ and MPY using Spearman's correlation. The correlation analysis revealed that 32 bacterial taxa were significantly correlated with $\Delta^{15}\text{N}$ and MPY (**Figure 6**). The results showed that $\Delta^{15}\text{N}$ and MPY possessed remarkable relationships with bacterial taxa, including five positive relationships and 30 negative relationships ($P < 0.05$). Among them, *Succinivibrionaceae_UCG_001*, uncultured_Selenomonadaceae, *Acidaminococcus*, *Eubacterium_xylanophilum_group*, and *Lachnospiraceae_NC2004_group* were positively correlated with MPY and negatively correlated with $\Delta^{15}\text{N}$. The scatter plots of the specific bacteria and $\Delta^{15}\text{N}$ and MPY are shown in **Figure 7**, which shows that the same genus of dairy cows with different NUE had different abundance levels, but *Clostridia_UCG_014*, *Saccharofermentans*, *Bacilli_RF39*, and *Desulfovibrio* were negatively correlated with MPY and positively correlated with $\Delta^{15}\text{N}$ ($P < 0.05$).

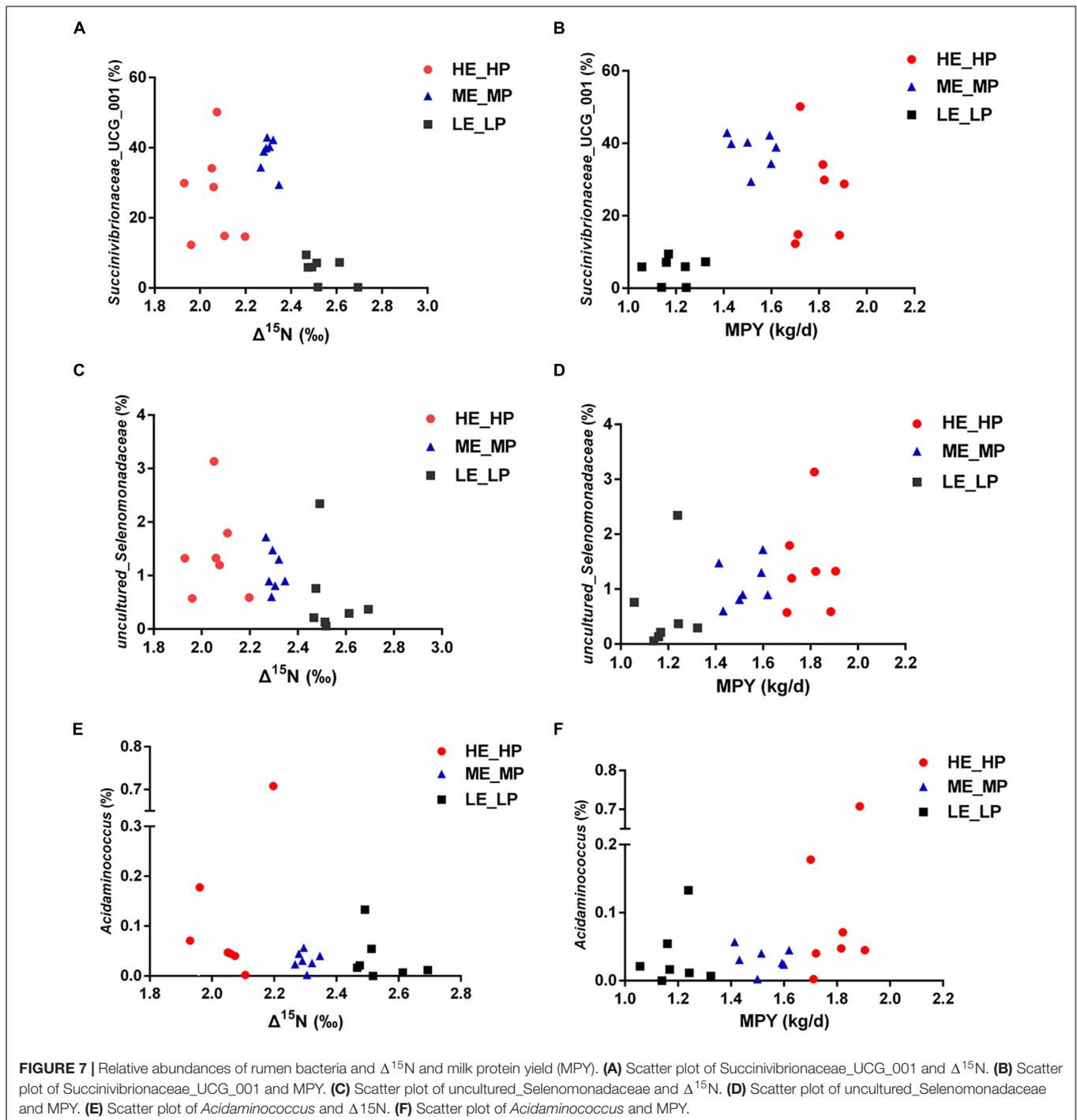
DISCUSSION

At present, the most effective way to improve the NUE of dairy cows is to match the dietary nitrogen content and energy supply during rumen fermentation by changing the diet composition (Reid et al., 2015). In addition, maximum NUE is usually achieved at the cost of some loss-of-production performance, but it can be achieved by understanding the key mechanisms that regulate nitrogen metabolism. The indexes commonly used to evaluate the NUE of dairy cows include blood urea nitrogen, milk urea nitrogen, milk protein yield, and $\Delta^{15}\text{N}$ (Kohn et al., 2005; Burgos et al., 2007; Warinner and Tuross, 2010). The $\Delta^{15}\text{N}$,



compared to other indicators, can overcome the differences at an individual level and is better for large groups of cows (Cantalapiedra-Hijar et al., 2016). In recent years, several studies have proved $\Delta^{15}\text{N}$ as a biomarker to assess NUE, and many

scholars have pointed out that $\Delta^{15}\text{N}$ was negatively correlated with NUE and rumen microbial protein synthesis efficiency (Cheng et al., 2011, 2014; Cantalapiedra-Hijar et al., 2015). MPY is an important index to measure the lactating performance of



dairy cows, which is a new biomarker for high milk protein and high yield (Wu et al., 2018; Xue et al., 2019). However, the MPY is not necessarily high when NUE is high, which is not in line with our aims of efficiency and production. Thus, the cows were divided into HE_HP cows, ME_MP cows, and LE_LP cows according to $\Delta^{15}\text{N}$ and MPY, and the rumen fermentation indexes were compared. The proportion of propionate and the ratio of acetate to propionate, which is mainly involved in

carbohydrate metabolism, were higher in HE_HP cows and ME_MP cows than in LE_LP cows, suggesting that propionate had a closed correlation with the rumen nitrogen metabolism. The propionate produced in the rumen was absorbed and transferred to glucose which provided energy for milk protein synthesis. Similarly, Xue et al. (2019) also found that high-milk-production and high-protein-content cows had significantly more ruminal propionate compared to those with low milk

production and protein content. However, ruminal $\text{NH}_3\text{-N}$ showed no significant changes among the three groups, which may be due to its low variation and levels (<11 mg/dl).

The rumen bacteria play an important role in dietary nutrient digestion and absorption. Furthermore, we detected that the diversity of rumen bacteria and specific rumen bacterial taxa contributed to NUE among the HE_HP cows, ME_MP cows, and LE_LP cows. The alpha diversity indices of Shannon and Simpson showed a lower bacterial diversity in HE_HP cows. Xue et al. (2020) compared the rumen microbial structure of high-MPY cows and low-MPY cows and found that the ruminal microbial richness in high-MPY cows was lower. It has been reported that the higher feed efficiency of dairy cows possessed a lower diversity, indicating that HE_HP cows can have higher feed efficiency than ME_MP cows and LE_LP cows and also suggesting that HE_HP cows have higher ruminal fermentation efficiency (Li and Guan, 2017). A lower diversity of the rumen microbiota has been identified in cows with a higher milk yield (Shabat et al., 2016), which is consistent with our results.

We revealed the difference in the relative abundances of rumen bacteria that may be associated with NUE. The family Succinivibrionaceae was higher in HE_HP cows and ME_MP cows than in LE_LP cows. Succinivibrionaceae, including 6 genera (*Anaerobiospirillum*, Succinivibrionaceae_UCG_001, *Ruminobacter*, Succinivibrionaceae_UCG_002, *Succinimonas*, and *Succinivibrio*), was the most active rumen bacterial family in beef (Li and Guan, 2017). Succinivibrionaceae is one of the contributors to producing succinate, and succinate can be used by other microorganisms to synthesize propionate and valerate, and the abundance of Succinivibrionaceae has been reported to be related to energy metabolism and methane emission (Wallace et al., 2015). Xue et al. (2020) pointed out that the methanogens in rumen had a lower relative abundance in high-MPY dairy cows, suggesting that these cows can produce less methane and sufficiently satisfy the higher performance of milk protein and milk yield. Succinivibrionaceae_UCG_001 composed the core rumen microbiota in young cattle and is the most abundant genus of the phylum Proteobacteria (Wang et al., 2019). In the study, we found that *Succinivibrionaceae_UCG_001* was higher in HE_HP cows and ME_MP cows than in LE_LP cows, indicating that HE_HP cows and ME_MP cows have higher levels of propionate, a major substrate for gluconeogenesis, and it is enough to supply glucose for milk production to influence NUE. Furthermore, there are 6 OTUs belonging to *Succinivibrionaceae_UCG_001* in the top 50 specific OTUs. It has been reported that *Succinivibrionaceae_UCG_001* was negatively correlated with the ratio of acetate to propionate (Cao et al., 2020). The Spearman correlation analysis showed that *Succinivibrionaceae_UCG_001* was positively related with MPY and negatively with $\Delta^{15}\text{N}$, which supported the important contribution of *Succinivibrionaceae* to nitrogen metabolism in dairy cows. Our results revealed that the production of propionate and acetate might affect the glucose supply, which could influence the NUE of dairy cows. The uncultured *Selenomonadaceae* also had more abundance in HE_HP cows and ME_MP cows, and it was positively correlated with MPY and negatively correlated with $\Delta^{15}\text{N}$. It indicated that the following research on the uncultured *Selenomonadaceae*

was needed to reveal its contribution to NUE. Moreover, *Acidaminococcus* was higher in HE_HP cows than in ME_MP cows and LE_LP cows, and it was negatively related with $\Delta^{15}\text{N}$. *Acidaminococcus* has been demonstrated to utilize amino acids as the sole energy source to produce acetic and butyric acids, which were negatively associated with metabolic diseases such as obesity (Rogosa, 1969; Li et al., 2021). The effect of *Acidaminococcus* on ruminant NUE was still unclear, and more culturing studies are needed to find out its role in nitrogen metabolism.

However, the results also revealed that the relative abundances of *Clostridia_UCG_014*, *Saccharofermentans*, *Bacilli_RF39*, and *Desulfovibrio* were higher in LE_LP cows than in HE_HP cows and ME_MP cows (relative abundance, $>0.1\%$). Thus, it might demonstrate that these four bacteria were negatively associated with NUE. *Clostridia_UCG_014* is a kind of pro-inflammatory bacteria (Wang et al., 2021), and its high abundance potentially led to the production of more proinflammatory metabolites to interfere with nitrogen metabolism. *Saccharofermentans* isolated from sludge treating brewery wastewater can ferment cellulose and starch to produce acetate (Chen et al., 2010; Dai et al., 2016). A recent study has indicated the higher abundance of *Saccharofermentans* in the rumen of cows with laminitis (Opdahl et al., 2018). *Bacilli_RF39* was a novel Tenericutes lineage and reported in goats and the human gut (Zhang et al., 2015; Nayfach et al., 2019). Due to the loss of ability to biosynthesize fatty acids, it is difficult to degrade the cellular membranes of plants for dairy cows. *Desulfovibrio* can reduce sulfate to sulfide compounds, which can block the oxidation of short-chain fatty acids in the rumen and compete with Firmicutes for energy substance and carbon source (Jia et al., 2012). In our study, *Saccharofermentans*, *Bacilli_RF39*, and *Desulfovibrio* were negatively correlated with MPY and were positively correlated with $\Delta^{15}\text{N}$, suggesting that those bacteria had a negative function in improving the rumen nitrogen metabolism. Although $\Delta^{15}\text{N}$ is a good biomarker for NUE, it is not the actual NUE. Here we just found out the presence of related bacterial species to $\Delta^{15}\text{N}$. The activity and function of these bacteria contributing to the NUE which are more direct and important need to be revealed in the future. More productive data, e.g., individual feed intake, are beneficial to reveal the closely related rumen microbes to NUE, which needs to be addressed in future studies. In addition, microbes such as archaea, protozoa, and anaerobic fungi also had roles in ruminal nitrogen metabolism, but we were solely focused on the bacteria community in this study. Therefore, the contribution of archaea, protozoa, and anaerobic fungi on nitrogen utilization efficiency has to be revealed in the following studies.

CONCLUSION

The relative abundances and diversity of rumen bacteria in dairy cows were clearly different among high- and low-NUE cows. The relative abundances of *Succinivibrionaceae*, *Selenomonadaceae*, and *Acidaminococcus* were higher in the rumen of high-NUE cows, which positively related with MPY and negatively with $\Delta^{15}\text{N}$. The study indicated that ruminal bacteria was a potential regulation target to enhance the nitrogen utilization of dairy

cows. However, the activity and function of these bacteria contributing to the NUE need to be studied in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care and Use Committee for Livestock of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (approval number: ISA2020-82).

AUTHOR CONTRIBUTIONS

MnL contributed to conceptualization, software, data curation, and writing—original draft. HZ performed data curation. MgL

took charge of the software. NZ was in charge of supervision. SZ contributed to writing—review and editing. JW was in charge of administration. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the State Key Laboratory of Animal Nutrition (2004DA125184G2108), Scientific Research Project for Major Achievements of Agricultural Science and Technology Innovation Program (CAAS-ZDXT2019004), Agricultural Science and Technology Innovation Program (ASTIP-IAS12), and China Agriculture Research System of MOF and MARA.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.815225/full#supplementary-material>

REFERENCES

- Angelidis, A., Crompton, L., Misselbrook, T., Yan, T., Reynolds, C. K., and Stergiadis, S. (2019). Evaluation and prediction of nitrogen use efficiency and outputs in faeces and urine in beef cattle. *Agr. Ecosyst. Environ.* 280, 1–15.
- Broderick, G. A., and Clayton, M. K. (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80, 2964–2971. doi: 10.3168/jds.S0022-0302(97)76262-3
- Broderick, G. A., Stevenson, M. J., Patton, R. A., Lobos, N. E., and Olmos Colmenero, J. J. (2008). Effect of supplementing rumen-protected methionine on production and nitrogen excretion in lactating dairy cows. *J. Dairy Sci.* 91, 1092–1102. doi: 10.3168/jds.2007-0769
- Burgos, S. A., Fadel, J. G., and Depeters, E. J. (2007). Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: relation of milk urea nitrogen to urine urea nitrogen excretion. *J. Dairy Sci.* 90, 5499–5508. doi: 10.3168/jds.2007-0299
- Calsamiglia, S., Ferret, A., Reynolds, C. K., Kristensen, N. B., and van Vuuren, A. M. (2010). Strategies for optimizing nitrogen use by ruminants. *Animal* 4, 1184–1196. doi: 10.1017/S1751731110000911
- Cantalapiedra-Hijar, G., Dewhurst, R. J., Cheng, L., Cabrita, A. R. J., Fonseca, A. J. M., Nozière, P., et al. (2018). Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: a meta-analysis. *Animal* 12, 1827–1837. doi: 10.1017/S1751731117003391
- Cantalapiedra-Hijar, G., Fouillet, H., Huneau, J. F., Fanchone, A., Doreau, M., Nozière, P., et al. (2016). Relationship between efficiency of nitrogen utilization and isotopic nitrogen fractionation in dairy cows: contribution of digestion v. metabolism? *Animal* 10, 221–229. doi: 10.1017/S1751731115002025
- Cantalapiedra-Hijar, G., Ortigues-Marty, I., Sepchat, B., Agabriel, J., Huneau, J. F., and Fouillet, H. (2015). Diet-animal fractionation of nitrogen stable isotopes reflects the efficiency of nitrogen assimilation in ruminants. *Br. J. Nutr.* 113, 1158–1169. doi: 10.1017/S0007114514004449
- Cantalapiedra-Hijar, G., Peyraud, J. L., Lemosquet, S., Molina-Alcaide, E., Boudra, H., Nozière, P., et al. (2014). Dietary carbohydrate composition modifies the milk N efficiency in late lactation cows fed low crude protein diets. *Animal* 8, 275–285. doi: 10.1017/S1751731113002012
- Cao, N., Wu, H., Zhang, X. Z., Meng, Q. X., and Zhou, Z. M. (2020). Calcium propionate supplementation alters the ruminal bacterial and archaeal communities in pre- and postweaning calves. *J. Dairy Sci.* 103, 3204–3218. doi: 10.3168/jds.2019-16964
- Castillo-Gonzalez, A. R., Burrola-Barraza, M. E., Dominguez-Viveros, J., and Chavez-Martinez, A. (2014). Rumen microorganisms and fermentation microorganismos fermentación ruminal. *Aerch. Med. Vet.* 46, 349–361. doi: 10.4067/S0301-732X2014000300003
- Chen, S., Niu, L., and Zhang, Y. (2010). Saccharofermentans acetigenes gen. nov., sp. nov., an anaerobic bacterium isolated from sludge treating brewery wastewater. *Int. J. Syst. Evol. Microbiol.* 60, 2735–2738. doi: 10.1099/ijs.0.017590-0
- Cheng, L., Edwards, G. R., Dewhurst, R. J., Nicol, A. M., and Pacheco, D. (2016). The effect of dietary water soluble carbohydrate to nitrogen ratio on nitrogen partitioning and isotopic fractionation of lactating goats offered a high-nitrogen diet. *Animal* 10, 779–785. doi: 10.1017/S1751731115002335
- Cheng, L., Kim, E. J., Merry, R. J., and Dewhurst, R. J. (2011). Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. *J. Dairy Sci.* 94, 2031–2041. doi: 10.3168/jds.2010-3763
- Cheng, L., Sheahan, A. J., Gibbs, S. J., Rius, A. G., Kay, J. K., Meier, S., et al. (2013). Technical note: nitrogen isotopic fractionation can be used to predict nitrogen-use efficiency in dairy cows fed temperate pasture. *J. Anim. Sci.* 91, 5785–5788. doi: 10.2527/jas.2012-5378
- Cheng, L., Woodward, S. L., Dewhurst, R. J., Zhou, H., and Edwards, G. R. (2014). Nitrogen partitioning, energy use efficiency and isotopic fractionation measurements from cows differing in genetic merit fed low-quality pasture in late lactation. *Anim. Prod. Sci.* 54, 1651–1656. doi: 10.1071/AN14171
- Chong, J., Liu, P., Zhou, G., and Xia, J. (2020). Using microbiome analyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nat. Protoc.* 15, 799–821. doi: 10.1038/s41596-019-0264-1
- Dai, Y., Yan, Z., Jia, L., Zhang, S., Gao, L., Wei, X., et al. (2016). The composition, localization and function of low-temperature-adapted microbial communities involved in methanogenic degradations of cellulose and chitin from Qinghai-Tibetan Plateau wetland soils. *J. Appl. Microbiol.* 121, 163–176. doi: 10.1111/jam.13164
- Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V., Giannoukos, G., et al. (2011). Human microbiome consortium, Petrosino, J.F., Knight, R., Birren, B.W. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* 21, 494–504. doi: 10.1101/gr.112730.110

- Huhtanen, P., and Hristov, A. N. (2009). A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92, 3222–3232. doi: 10.3168/jds.2008-1352
- Jami, E., White, B. A., and Mizrahi, I. (2014). Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PLoS One* 9:e85423. doi: 10.1371/journal.pone.0085423
- Jia, W., Whitehead, R. N., Griffiths, L., Dawson, C., Bai, H., Waring, R. H., et al. (2012). Diversity and distribution of sulphate-reducing bacteria in human faeces from healthy subjects and patients with inflammatory bowel disease. *FEMS Immunol. Med. Microbiol.* 65, 55–68. doi: 10.1111/j.1574-695X
- Kohn, R. A., Dinneen, M. M., and Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *Anim. Sci.* 83, 879–889. doi: 10.2527/2005.834879x
- Lavery, A., and Ferris, C. P. (2021). Proxy measures and novel strategies for estimating nitrogen utilisation efficiency in dairy cattle. *Animals* 11:343. doi: 10.3390/ani11020343
- Li, F., and Guan, L. L. (2017). Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle. *Appl. Environ. Microbiol.* 83, e00061–17. doi: 10.1128/AEM.00061-17
- Li, F., Hitch, T. C. A., Chen, Y., Creevey, C. J., and Guan, L. L. (2019). Comparative metagenomic and metatranscriptomic analyses reveal the breed effect on the rumen microbiome and its associations with feed efficiency in beef cattle. *Microbiome* 7:6. doi: 10.1186/s40168-019-0618-5
- Li, J., Dong, L., Liu, Y., and Gao, J. (2021). Stimulation of Codonopsis pilosula polysaccharide on bifidobacterium of human gut bacteria *in vitro*. *Evid. Based Complement Alternat. Med.* 2021:9524913. doi: 10.1155/2021/9524913
- Liang, Y. S., Li, G. Z., Li, X. Y., Lü, J. Y., Li, F. D., Tang, D. F., et al. (2017). Growth performance, rumen fermentation, bacteria composition, and gene expressions involved in intracellular pH regulation of rumen epithelium in finishing Hu lambs differing in residual feed intake phenotype. *J. Anim. Sci.* 95, 1727–1738. doi: 10.2527/jas.2016.1134
- Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W., and Cotter, P. D. (2019). The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes* 10, 115–132. doi: 10.1080/19490976.2018.1505176
- McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217
- Minas, K., McEwan, N. R., Newbold, C. J., and Scott, K. P. (2011). Optimization of a high-throughput CTAB-based protocol for the extraction of qPCR-grade DNA from rumen fluid, plant and bacterial pure cultures. *FEMS Microbiol. Lett.* 325, 162–169. doi: 10.1111/j.1574-6968.2011.02424.x
- Nayfach, S., Shi, Z. J., Seshadri, R., Pollard, K. S., and Kyrpides, N. C. (2019). New insights from uncultivated genomes of the global human gut microbiome. *Nature* 568, 505–510. doi: 10.1038/s41586-019-1058-x
- Oenema, O., and Tamminga, S. (2005). Nitrogen in global animal production and management options for improving nitrogen use efficiency. *Sci. China C Life Sci.* 2, 871–887. doi: 10.1007/BF03187126
- Opdahl, L. J., Gonda, M. G., and St-Pierre, B. (2018). Identification of uncultured bacterial species from firmicutes, bacteroidetes and CANDIDATUS saccharibacteria as candidate cellulose utilizers from the rumen of beef cows. *Microorganisms* 6:17. doi: 10.3390/microorganisms6010017
- Reid, M., O'Donovan, M., Elliott, C. T., Bailey, J. S., Watson, C. J., Lalor, S. T., et al. (2015). The effect of dietary crude protein and phosphorus on grass-fed dairy cow production, nutrient status, and milk heat stability. *J. Dairy Sci.* 8, 517–531. doi: 10.3168/jds.2014-8437
- Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *Peer J.* 4:e2584. doi: 10.7717/peerj.2584
- Rogosa, M. (1969). Acidaminococcus gen. n., Acidaminococcus fermentans sp. n., anaerobic gram-negative diplococci using amino acids as the sole energy source for growth. *J. Bacteriol.* 98, 56–66. doi: 10.1128/jb.98.2.756-766.1969
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12:R60. doi: 10.1186/gb-2011-12-6-r60
- Shabat, S. K., Sasson, G., Doron-Faigenboim, A., Durman, T., Yaacoby, S., and Berg Miller, M. E. (2016). Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. *ISME J.* 10, 2958–2972. doi: 10.1038/ismej.2016.62
- Wallace, R. J., Rooke, J. A., McKain, N., Duthie, C. A., Hyslop, J. J., Ross, D. W., et al. (2015). The rumen microbial metagenome associated with high methane production in cattle. *BMC Genomics* 16:839. doi: 10.1186/s12864-015-2032-0
- Wang, B., Ma, M. P., Diao, Q. Y., and Tu, Y. (2019). Saponin-induced shifts in the rumen microbiome and metabolome of young cattle. *Front. Microbiol.* 10:356. doi: 10.3389/fmicb.2019.00356
- Wang, C., Liu, J. X., Yuan, Z. P., Wu, Y. M., Zhai, S. W., and Ye, H. W. (2007). Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 90, 2960–2965. doi: 10.3168/jds.2006-129
- Wang, Y., Nan, X., Zhao, Y., Jiang, L., Wang, H., Zhang, F., et al. (2021). Dietary supplementation of inulin ameliorates subclinical mastitis via regulation of rumen microbial community and metabolites in dairy cows. *Microbiol. Spectr.* 9:e0010521. doi: 10.1128/Spectrum.00105-21
- Warinner, C., and Tuross, N. (2010). Brief communication: tissue isotopic enrichment associated with growth depression in a pig: implications for archaeology and ecology. *Am. J. Phys. Anthropol.* 141, 486–493. doi: 10.1002/ajpa.21222
- Wu, X. H., Sun, H. Z., Xue, M. Y., Wang, D. M., Guan, L. L., and Liu, J. X. (2018). Serum metabolome profiling revealed potential biomarkers for milk protein yield in dairy cows. *J. Proteomics* 184, 54–61. doi: 10.1016/j.jprot.2018.06.005
- Xue, M. Y., Sun, H. Z., Guan, L. L., and Liu, J. X. (2018). Assessment of rumen microbiota from a large dairy cattle cohort reveals the pan and core bacteriomes contributing to varied phenotypes. *Appl. Environ. Microbiol.* 84, e00970–18. doi: 10.1128/AEM.00970-18
- Xue, M. Y., Sun, H. Z., Wu, X. H., Guan, L. L., and Liu, J. X. (2019). Assessment of rumen bacteria in dairy cows with varied milk protein yield. *J. Dairy Sci.* 102, 5031–5041. doi: 10.3168/jds.2018-15974
- Xue, M. Y., Sun, H. Z., Wu, X. H., Liu, J. X., and Guan, L. L. (2020). Multi-omics reveals that the rumen microbiome and its metabolome together with the host metabolome contribute to individualized dairy cow performance. *Microbiome* 8:64. doi: 10.1186/s40168-020-00819-8
- Zhang, L., Huang, X., Xue, B., Peng, Q., Wang, Z., and Yan, T. (2015). Immunization against rumen methanogenesis by vaccination with a new recombinant protein. *PLoS One* 10:e0140086. doi: 10.1371/journal.pone.0140086

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Li, Zhong, Li, Zheng, Wang and Zhao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.