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Bacterial Community Composition and Fermentation Patterns in the Rumen of Sika Deer (*Cervus nippon*) Fed Three Different Diets

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Abstract Sika deer (*Cervus nippon*) rely on microorganisms living in the rumen to convert plant materials into chemical compounds, such as volatile fatty acids (VFAs), but how the rumen bacterial community is affected by different forages and adapt to altered diets remains poorly understood. The present study used 454-pyrosequencing of bacterial 16S ribosomal RNA (rRNA) genes to examine the relationship between rumen bacterial diversity and metabolic phenotypes using three sika deer in a 3×3 latin square design. Three sika deer were fed oak leaves (OL), corn stover (CS), or corn silage (CI), respectively. After a 7-day feeding period, when compared to the CS and CI groups, the OL group had a lower proportion of *Prevotella* spp. and a higher proportion of unclassified bacteria belonging to the families Succinivibrionaceae and Paraprevotellaceae ($P<0.05$). Meanwhile, the concentration of isobutyrate was significantly lower ($P<0.05$) in the OL group than in the CS and CI groups. There was no significant change of dominant bacterial genera in the OL group after 28 days of feeding. Conversely, total volatile fatty acids (TVFAs) showed an increase after 28 days of feeding, mainly due to the increasing of acetate, propionate, and valerate ($P<0.05$). The interplay between bacteria and metabolism in the OL group differed from that in the CS and CI groups, especially for the interaction of TVFAs and acetate/propionate. Overall, the current study suggested that *Prevotella* spp. played critical roles in the fermentation of feed in the rumen of sika deer. However, the differences in interplay patterns between rumen bacterial community composition and metabolic phenotypes were altered in the native and domesticated diets indicating the changed fermentation patterns in the rumen of sika deer.

Abbreviations

OTU	Operational taxonomic units
VFAs	Volatile fatty acids
TVFAs	Total volatile fatty acids
BLAST	Basic local alignment search tool
PCoA	Principal coordinate analysis
PLS-DA	Partial least squares discriminant analysis
AP	Acetate/propionate
FDR	False discovery rate
NH_3-N	Ammonia nitrogen
ANOVA	Analysis of variance
ISAPS	Institute of Special Animal and Plant Sciences
CAAS	Chinese Academy of Agricultural Sciences
ACUC	Animal Care and Use Committee
CFB	Cytophaga–Flexibacter–Bacteroides
DM	Dry matter

Introduction

The rumen harbors a complex consortium of microorganisms including bacteria (10^{11}–10^{12} cells/ml), fungi (10^{3}–10^{6} cells/

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ml), archaea (10^7–10^9 cells/ml), and protozoa (10^4–10^6 cells/ml) [1]. During rumen fermentation, rumen bacteria play a primary role in the conversion of feed components to peptides, ammonia, and volatile fatty acids (VFAs), such as acetate, propionate, and butyrate [2]. These end products provide the host with essential nutrients and metabolic energy (i.e., VFAs) [3], thereby affecting its performance (i.e., animal productivity) and health.

Sika deer (*Cervus nippon*) are ancient and primitive members of the genus *Cervus* because their velvet antlers are distinctive from reindeers and are prized for Chinese medicine. In addition, sika deer yield high quality meat and skin. Therefore, sika deer are farmed in China, mainly for the production of antlers. At present, the number of domesticated sika deer in China is approximately 550,000 head, most of which are distributed in northwestern China. However, very little information is known about the rumen bacterial community of sika deer, based on limited sequences from 16S ribosomal RNA (rRNA) gene clone libraries. Kobayashi et al. [4] reported that most of the bacterial species in the rumen of wild sika deer in Japan were unknown bacteria belonging to the *Cytophaga–Flexibacter–Bacteroides* (CFB) group. In our previous study, *Prevotella* spp. were the dominant bacteria in the rumen of Chinese sika deer [5]. However, the rapid advances in sequencing technologies over the past decade will allow us to comprehensively investigate the composition and densities of the rumen bacterial community of sika deer.

In nature, sika deer have been observed to prefer tannin-rich plants, such as oak leaves [6]. Additionally, in the production practice, oak leaves are beneficial to the growth of antler compared to corn silage, which is the main forage in the growing period of antler under the domesticated condition, while corn stover is the main forage of sika deer in other periods. Moreover, the hydrolysable tannin contents in oak leaves are approximately 1 %, while that in corn stover and corn silage are close to zero [7]. Thus, how forages affect the rumen bacterial community composition of sika deer and how the bacteria adapt to these diets is worthy of investigation.

Recently, several studies provided evidence for the relationship between the particular bacteria groups and fermentation products in cattle [8–12]. For example, Carberry et al. [10] identified negative associations between *Prevotella* spp. and isobutyrate and isovalerate concentrations in the rumen liquor. Hernandez-Sanabria et al. [11] suggested that *Succinivibrio* spp. were negatively correlated with valerate proportion of total VFA. Therefore, this shift in focus from determining "who is there" toward understanding "what are they doing" will help us to understand the process of the adaptation to the varied diets [13]. However, the relationship between rumen bacterial community and metabolic phenotypes of sika deer is poorly known. Moreover, the fermentation patterns in the rumen of sika deer fed native and domesticated diets may be distinct.

Using 454-pyrosequencing of bacterial 16S rRNA gene, the objectives of the present study are to (i) investigate differences of bacterial community composition in the rumen of sika deer fed native and domesticated diets; (ii) compare rumen fermentation under these different diets; (iii) explore the co-occurrence relationship between rumen bacteria and metabolic phenotypes; and (iv) test the hypothesis that the fermentation patterns in the rumen of sika deer are related to the diets.

Materials and Methods

Animals

Three rumen-cannulated adult male sika deer (*C. nippon*), maintained at the research farm (44.04° N, 129.09° E) of the Institute of Special Animal and Plant Sciences (ISAPS), Chinese Academy of Agricultural Sciences (CAAS), in Jilin Province, were used in this study. All animals were individually housed in pens, and all animal procedures were approved and authorized by the CAAS Animal Care and Use Committee (ACUC) and by the ISAPS Wild Animal and Plant Subcommittee.

Study Design and Sampling

Three sika deer were used in a 3×3 latin square design (Table S1). We firstly prepared the concentrate diet (64.5 % corn, 19.7 % soybean meal, 12.8 % distiller dried grains with solubles and a 3 % mixture of vitamins and mineral salts). And then, the same concentrate diet was mixed with either corn stover (CS group), corn silage (CI group), or oak leaves (OL group), to form three different diets. The ratio of forage to concentrate was 50:50 on the base of dry matter (DM). On a DM basis, the concentrate contained 12.68 % crude protein and 3.54 % crude fat, corn stover contained 37.57 % cellulose and 4.03 % crude protein, oak leaves contained 35.58 % cellulose and 6.40 % crude protein, and corn silage contained 69.63 % cellulose and 7.50 % crude protein. All sika deer were fed twice each day at 8:00 am and 4:00 pm and had free access to water.

After one week of adaption to the diets, sika deer received each diet for 28 days. After feeding each diet for 7, 14, and 28 days, the rumen contents including solid and liquid fractions were obtained via rumen cannula before the morning. Rumen samples were stored at −80 °C for later analysis.

DNA Extraction and 454 Pyrosequencing

Total genomic DNA was extracted from the whole rumen content of each animal using the PowerSoil® DNA Isolation Kit (MOBIO, Carlsbard, CA) according to the manufacturer's

Table 1 Number of sequences, OTUs, and alpha-diversity indices from three groups

Groups	Total sequences (mean±S.E)	Sequences per sample (mean±S.E)	OTUs per sample (mean±S.E)	Good's coverage (mean±S.E)	Shannon (mean±S.E)	Simpson (mean±S.E)	Chao 1 (mean±S.E)
OL	107,662	5981±740	783±60	0.92±0.01	6.8±0.3	0.93±0.02	1202±85
CS	119,684	6649±492	815±101	0.94±0.01	6.6±0.4	0.92±0.02	1224±150
CI	103,089	5727±476	764±68	0.93±0.01	6.6±0.3	0.92±0.02	1216±97

instructions. Universal bacterial primers 8F and 533R were used to amplify the V1 to V3 regions of the bacterial 16S rRNA gene [14]. The forward primer was made up of adaptor A, a seven-nucleotide barcode, and the 8F primer, and the reverse primer contained adapter B, a seven-nucleotide barcode, and the 533R primer. The following protocol was used to amplify bacterial 16S rRNA gene sequences: 94 °C for 3 min; followed by 25 cycles for 30 s at 94 °C, 30 s at 57 °C, and 30 s at 72 °C; and a final extension at 72 °C for 10 min. The purified PCR products (approximately 200 ng) were sequenced using a Roche GS-FLX 454 pyrosequencer (Roche, Mannheim, Germany). Each sample had two PCR replicates for pyrosequencing.

Analysis of Pyrosequencing Reads

Pyrosequencing data were processed and analyzed using QIIME 1.7.0 following the pipeline described by Caporaso et al. [15]. Sequences were sorted according to barcode and were examined for quality control using the following criteria: the minimum sequence length was 400 nt; the maximum sequence length was 600 nt; minimum qual score was 25; the maximum number of errors in the barcode was 0; the maximum length of homopolymer run was 6; the number of mismatches in the primer was 0; ambiguous and unassigned characters were excluded. The adaptor B and reverse primer from all assigned sequences were also removed from the raw sequences. Sequencing errors were removed from filtered sequences using denoiser 0.91 [16]. The denoised sequence was clustered into operational taxonomic units (OTUs) using Usearch61 according to the sequence identity of 97 % [17]. Representative sequences were aligned to the Greengenes database [18] using PyNAST [19] with a minimum alignment length of 400 and a minimum identity of 75 %. Potential chimera sequences were removed using Chimera Slayer [20]. OTUs that were found in at least nine samples with at least three sequences were retained for the further analysis. Principal coordinate analysis (PCoA) and partial least squares discriminant analysis (PLS-DA) were performed on a comprehensive web server for comparative metagenomics, METAGENassist [21]. All raw sequences in this study were deposited to the NCBI Sequence Read Archive (SRP033500).

Measurement of Ruminal Fermentation Parameters

Rumen pH was determined immediately using a pH meter (model PB-10/C, Sartorius, Goettingen, Germany). The concentration of ammonia nitrogen (NH_3-N) was measured using a commercial kit (R-Biopharm Roche Inc., South Marshall, MI) according to the manufacturer's instructions at a wavelength of 340 nm (SpectraMax 190; Molecular Devices, Sunnyvale, CA). Rumen fluid was centrifuged at 15,000*g* for 10 min at 4 °C, and 0.2 ml of 2-ethylbutyric acid (internal standard, 2 g/L) in metaphosphoric acid (25 % *w*/*v*) was added to 1 ml of clarified rumen fluid. The concentrations of VFAs in the rumen were determined by gas chromatography with a flame ionization detector and a DB-FFAP column (30 m× 0.25 μm×0.25 μm, Agilent Technologies 6890GC, USA). The carrier gas was N_2 at a flow rate of 2.2 ml/min. The analysis was a gradient oven temperature of 80–170 °C with an incremental rate of 10 °C/min for optimal separation and a detector temperature of 250 °C.

Co-occurrence Network Analyses Between Rumen Bacteria and Metabolic Phenotypes

The co-occurrence pattern was constructed between the OTUs and the rumen fermentation profiles using the method as described by Zhang et al. [22]. Briefly, Each OTU's abundance and the concentrations of metabolic phenotypes were calculated using the mean value from all samples in each group. Statistic *P* values were corrected using the false discovery rate (FDR) method of the *p.adjust* package in R. Correlations have an absolute Pearson's correlation above 0.70 with an FDR-corrected significance level under 0.05, and these correlations were transformed into links between two OTUs in the co-occurrence network. The co-occurrence networks were then visualized using Cytoscape 2.8.2 with a force-directed algorithm [23].

Statistical Analysis

Statistical analysis was performed using the SigmaPlot 12.0 (Systat Software, Inc.) and R software packages. General characteristics were expressed as mean or percentages. Variations in different groups were checked for normal distribution using the Shapiro-Wilk test (significance value of $P<0.05$).

When normally distributed, multiple samples' comparisons were performed using one-way analysis of variance (ANOVA) (parametric) and using Kruskal-Wallis one-way ANOVA on ranks (non-parametric) for un-normal distribution with the significant value of $P<0.05$.

Results

Summary of Pyrosequencing Data

Overall, the current study obtained 661,564 raw 16S rRNA gene sequences from 54 samples. A total of 330,435 high quality sequences were obtained with an average of 6119±344 (S.E) sequences for each sample. Using these sequences, we identified 2931 core OTUs based on 97 % sequence identity (equal to species level), with 783 OTUs per sample in the OL group, 815 OTUs per sample in the CS group, and 764 OTUs per sample in the CI group (Table 1).

Comparison of Bacterial Community Composition in the Three Groups

In all, these core OTUs were assigned to 12 phyla, 34 families, and 61 genera. The top three phyla were Bacteroidetes (64.9 %), Proteobacteria (17.3 %), and Firmicutes (16.1 %) (Fig.1a). *Prevotella* (50.69 %), *Succinivibrio* (11.14 %), and unclassified bacteria belonging to the family Succinivibrionaceae (6.08 %) were the top three genera in the three diet groups (Fig. 1b). Analysis of Good's coverage showed that 93±0.8 (S.E)% of bacterial species were captured in each sample (Table 1). The PCoA and PLS-DA plots showed that the bacterial community in the OL group was clearly separated from those in the CS and CI groups (Fig. 2), indicating that the native diet (OL group) potentially altered rumen bacterial community composition in sika deer.

Bacterial composition was compared between three diets across the three time points. After 7 days of feeding, there were no differences at the phylum level in the three groups (Fig. 3a). At the genus level, the OL group has a lower proportion of *Prevotella* spp. than the CI group ($P=0.006$) and had higher abundance of unidentified bacteria belonging to the families Succinivibrionaceae and Paraprevotellaceae than the CS ($P=0.031$ and $P=0.002$) and CI ($P=0.043$ and $P=0.002$) groups (Fig. 4a). After 14 days of feeding, bacteria belonging to TM7 (OL vs CS, $P=0.018$; OL vs CI, $P=0.017$) and Cyanobacteria (OL vs CS, $P=0.027$) were increased in the OL group (Fig. 3a). At the genus level, the unclassified bacteria belonging to the family Porphyromonadaceae (OL vs CS, $P=0.034$; OL vs

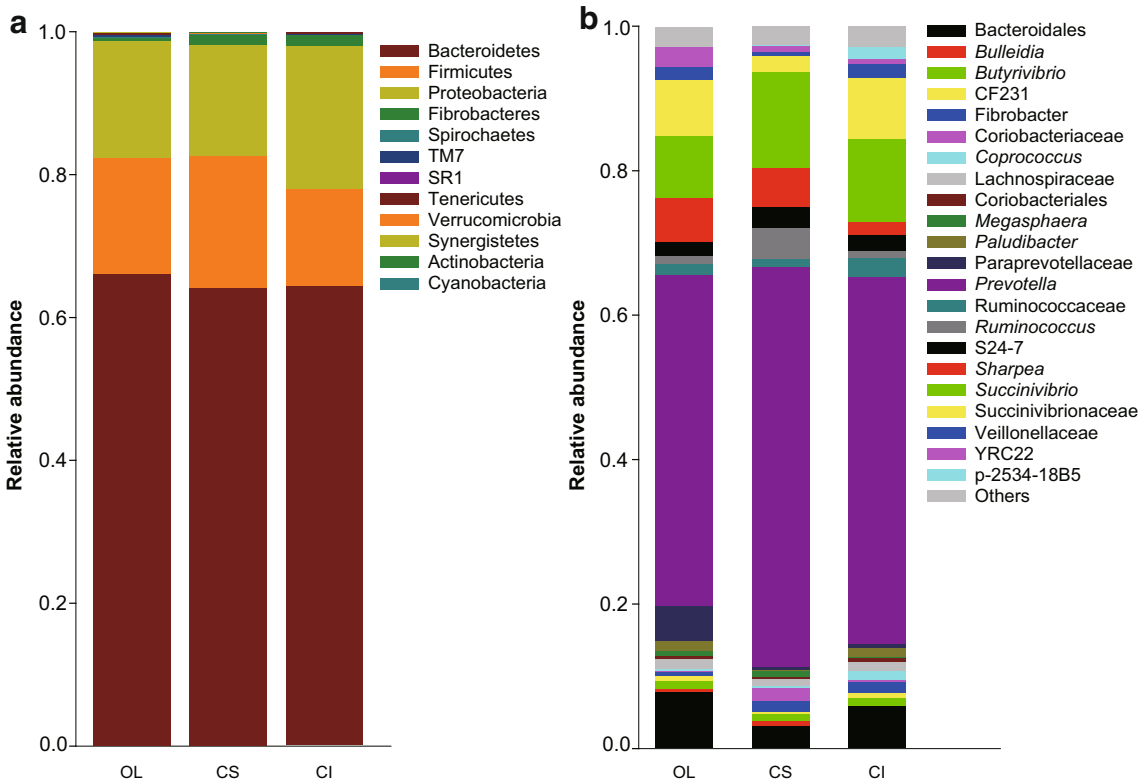

Fig. 1 Rumen bacterial compositions at phylum (**a**) and genus (**b**) levels across three groups. OL oak leaves, CS corn stover, CI corn silage

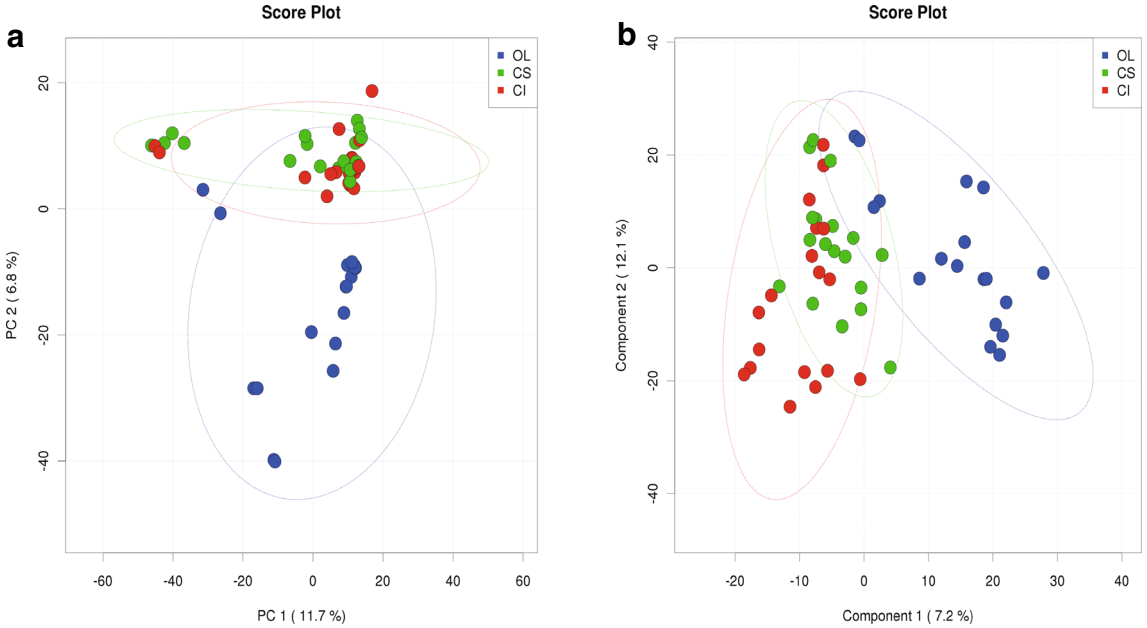

Fig. 2 Comparison of bacterial community in the rumen of sika deer from the OL, CS, and CI groups based on the PCoA (**a**) and PLS-DA (**b**) plotting. (OL oak leaves, CS corn stover, CI corn silage, PCoA principal coordinate analysis, PLS-DA partial least squares discriminant analysis)

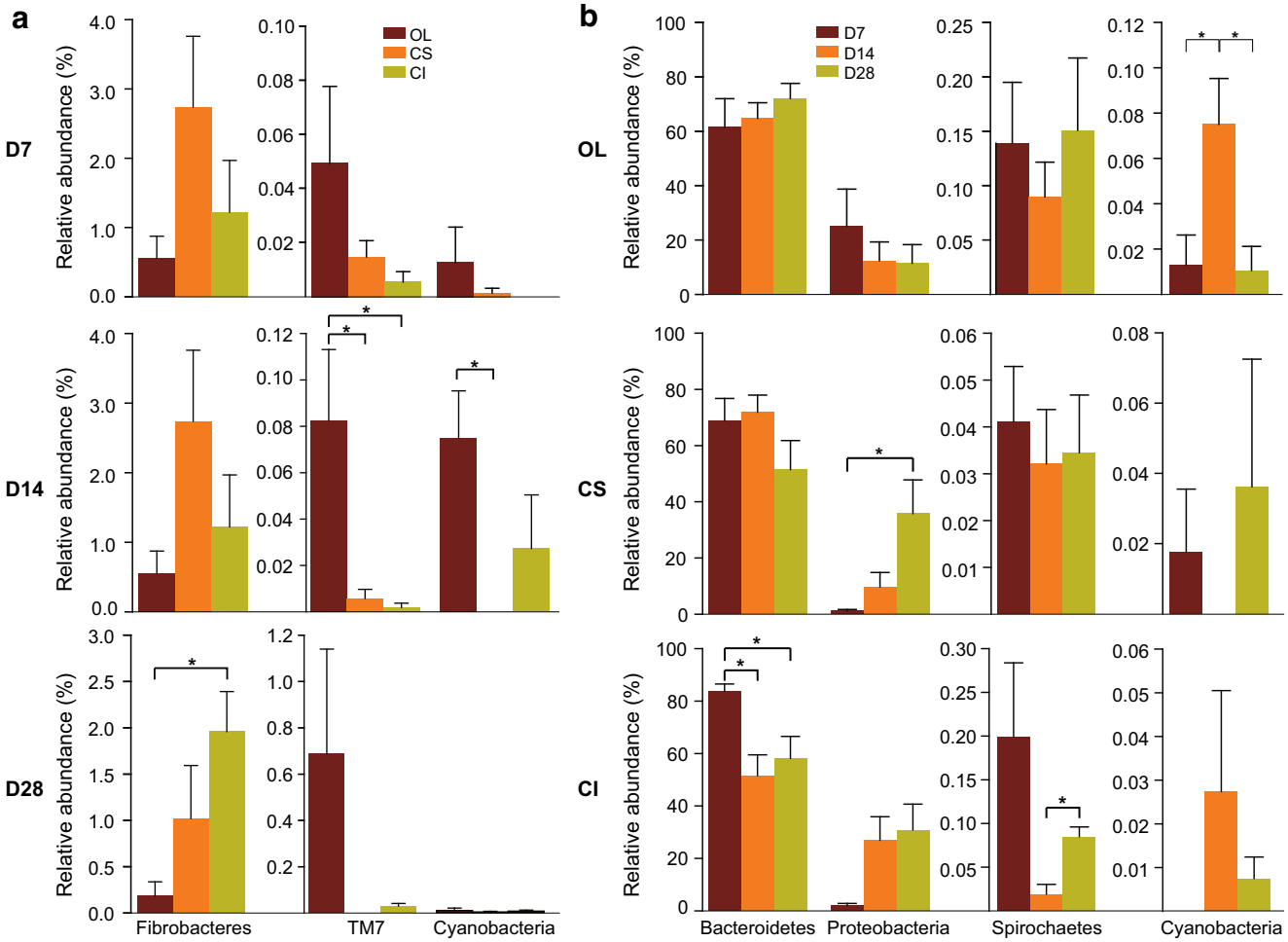

Fig. 3 Phyla with significant differences across three sampling time points (**a**) and three groups (**b**). OL oak leaves, CSCI corn silage, D7 day 7, day 14, D28 day 28. *$P<0.05$

CI, P=0.023) were more abundant in the OL group than the CS and CI groups, and unclassified bacteria in the family Catabacteriaceae were reduced in the OL group compared to the CI group (P=0.043, Fig. 4a). After 28 days of feeding, bacteria belonging to the phylum Fibrobacteres were lower in the OL group than in the CI group (P=0.029, Fig. 3a), but at the genus level, there were no differences. Moreover, for the OL group, the relative abundance of phylum Cyanobacteria was more prevalent at day 14 than at day 7 (P=0.023) and day 28 (P=0.027, Fig. 3b). However, there were no significant differences among the three time points.

Rumen Metabolic Phenotypes in the Three Groups Across Three Time Points

After 7 days of feeding, the concentrations of isobutyrate (OL vs CS, P<0.001; OL vs CI, P<0.001) and NH_3-N (OL vs CS, P<0.001; OL vs CI, P<0.001) in the OL group were lower than in the CS and CI groups, but the ratio of acetate to propionate (AP) in the OL group was higher than in the CI group (P=0.005, Fig. 5a).

After 14 days of feeding, the concentrations of valerate in the OL group were lower than in the CI group (P=0.006, Fig. 5a). The amounts of NH_3-N in the CS and OL groups

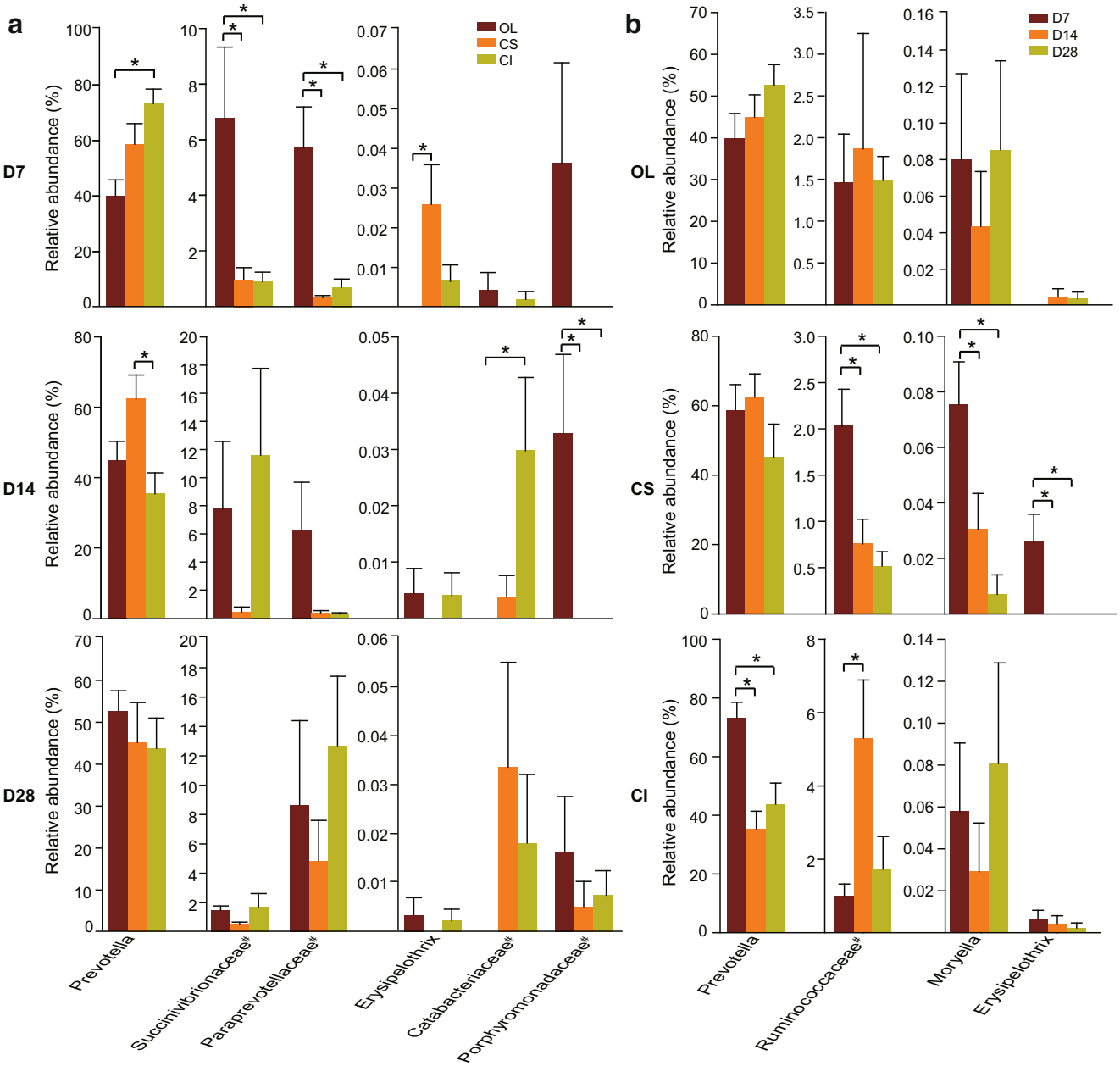

Fig. 4 Significant difference at genera level across three sampling time points (**a**) and three groups (**b**). OL oak leaves, CS corn stover, CI corn silage, D7 day 7, D14 day 14, D28 day 28. *P<0.05. # indicates unclassified bacteria taxa

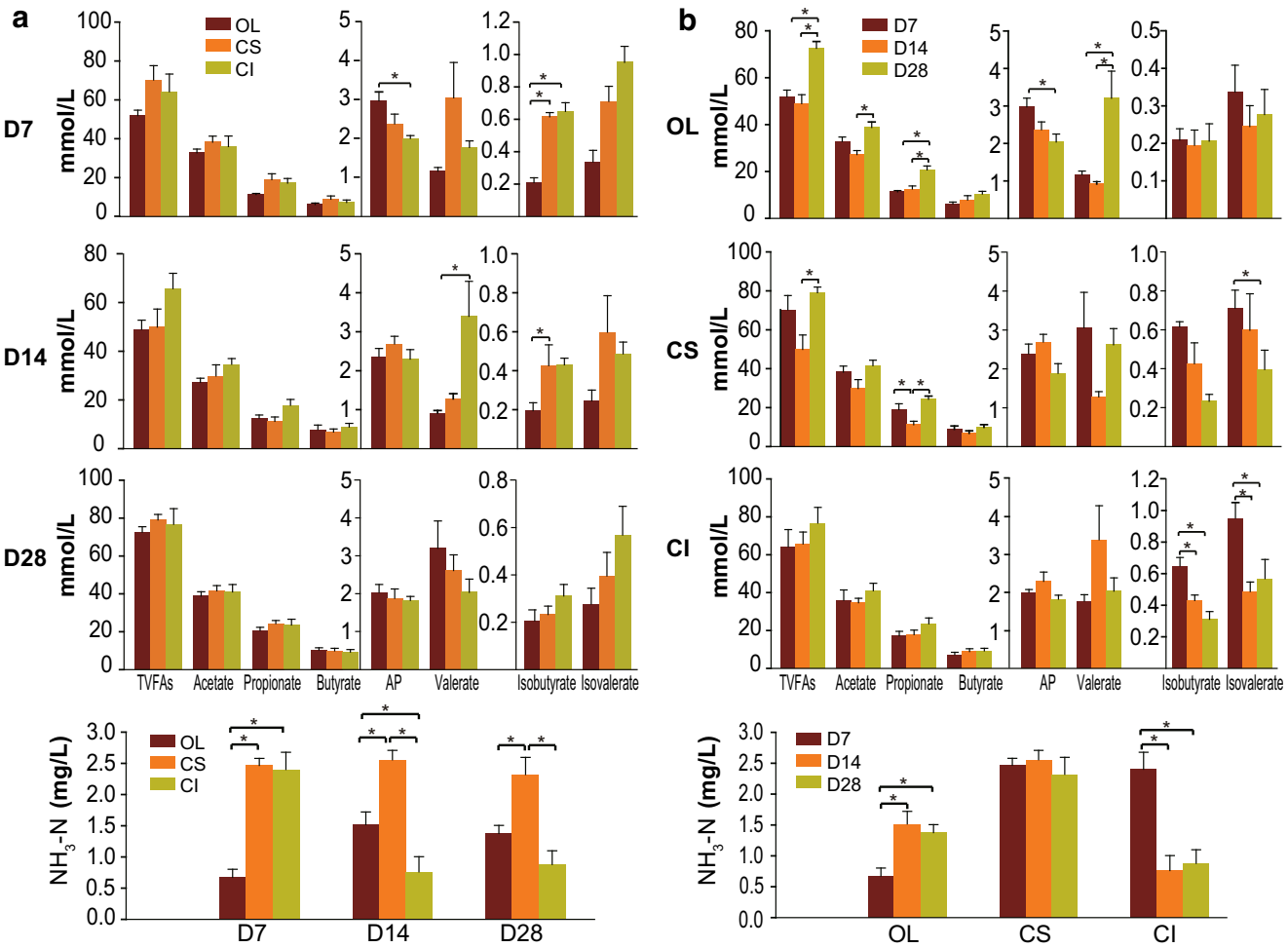

Fig. 5 Comparing the concentrations of volatile fatty acids and NH_3-N in the rumen of sika deer across three sampling time points (**a**) and three groups (**b**). OL oak leaves, CS corn stover, CI corn silage, D7 day 7, D14 day 14, D28 day 28. *P<0.05

were increased than in the CI group (CS vs CI, P<0.001; OL vs CI, P=0.011, Fig. 5a).

After 28 days of feeding, the concentration of NH_3-N in the CS group was higher than in the OL and CI groups (CS vs OL, P=0.007; CS vs CI, P<0.001 Fig. 5a). In addition, for the OL group, the concentrations of total volatile fatty acids (TVFAs) (D7 vs D28, P<0.001; D14 vs D28, P<0.001), propionate (D7 vs D28, P<0.001; D14 vs D28, P<0.001), and valerate (D7 vs D28, P=0.003; D14 vs D28, P=0.002) at day 7 and day 14 were lower than at day 28, but the concentrations of NH_3-N at day 14 (P=0.001) and day 28 (P=0.005) were increased compared to day 7 (Fig. 5b).

The Interplay Patterns Between Rumen Fermentation and Bacterial Community

In the OL group (Fig. 6a), TVFAs were directly positive with butyrate and acetate through 7 OTUs. Most of OTUs associated with butyrate were classified as *Prevotella* spp. AP was negatively correlated to propionate, and isovalerate was interactive with 16 OTUs. However, in the CS (Fig. 6b) and CI (Fig. 6c) groups, TVFAs were positively associated with acetate, propionate, butyrate, and valerate. AP was positively correlated with isovalerate and isobutyrate in the CS and CI groups, respectively (Fig. 6b, c). Across this network, many novel *Prevotella* spp. were positively associated with various metabolic phenotypes.

Discussion

The present study used high-throughput sequencing of the 16S rRNA gene to provide a comprehensive view into understanding the alteration of bacterial compositions and explore the association between bacterial community and metabolic phenotypes in the rumen of sika deer fed three diets. The co-occurrence analyses showed diet-driven pattern of rumen microorganisms and the metabolic phenotype interaction.

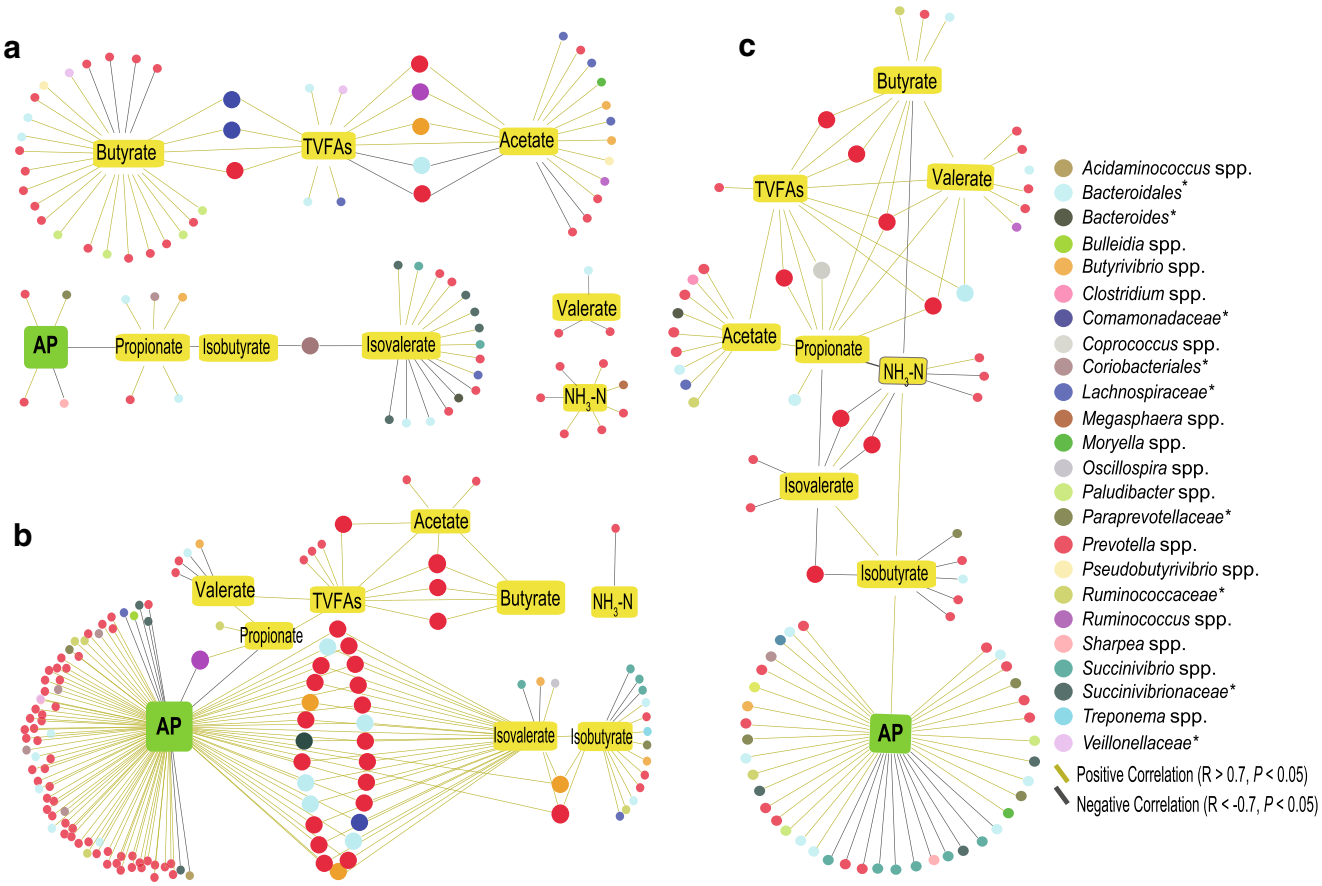

Fig. 6 Co-occurrence network analysis between bacterial OTUs and rumen metabolic phenotypes for the OL (**a**), CS (**b**), and CI (**c**) groups. *Circle nodes* represent bacterial OTUs (or species) and *rounded rectangle nodes* do metabolic phenotypes. Each co-occurring pair between bacterial OTUs (or species) and metabolic phenotypes has an absolute Spearman rank correlation above 0.70 [*gold line* indicates positive correlation ($R>0.70$); *gray line* indicates negative correlation ($R<-0.70$)] with an FDR-corrected significance level under 0.05. *Circles with different color* represent the OTUs from the different genera. OL oak leaves, CS corn stover, CI corn silage. *Asterisk* indicates unclassified bacteria taxa

This study found highly abundant bacteria belonging to the phylum Bacteroidetes, mostly *Prevotella* spp. Other studies on cows, goats, elk, and white-tailed deer also found that *Prevotella* spp. were predominant [24–29]. However, the proportion of *Prevotella* spp. (50.7 %) in the present study was relatively higher than previous findings on cattle (20–30 %) [30–32], moose (10 %) [33], macropods (38 %) [34], horses (0.23 %) [35], elk, and white-tailed deer (about 20 %) [28]. A possible explanation was the co-evolution between rumen bacteria and sika deer [36]. Furthermore, a recent study demonstrated that the enterotype of human gut microbiome dominated by *Prevotella* spp. was strongly associated with long-term diets having abundant carbohydrates [37]. Moreover, metagenomic analyses suggested that *Prevotella* spp. played a potential role in cellulose degradation in the foregut of the Tammar wallaby and in the rumen of Svalbard reindeer [38, 39]. Some members of the genus *Prevotella* contained highly active hemicellulolytic and proteolytic enzymes [40], which could degrade xylan, pectin, and starch [41–44], and comprised a large part of the genetic and metabolic diversity in rumen microbial community [45–47]. Recent studies also showed that the genus *Prevotella* displayed a central niche in maintaining the community structure of human gut microbiome [48] and could provide the host with the ability to adapt various diets [49]. Therefore, our findings suggested that *Prevotella* spp. likely played a key role in the rumen fermentation of sika deer. Interestingly, most of *Prevotella* spp. were identified as novel species, which was similar to the findings of Gruninger et al. [28], who also indentified a number of core OTUs that were classified as uncultured *Prevotella* in the rumen of elk and white-tailed deer in Canada. This suggested that the rumen of cervidae animals may harbor unique microbiota, which needed to further examine in future studies.

The distribution of bacteria, such as *Prevotella* spp., unclassified Succinivibrionaceae and Paraprevotellaceae, and TVFAs was changed within the OL group, as well as in comparison with the CS and CI groups (Figs. 4 and 5), suggesting that the rumen bacterial community composition and fermentation patterns were altered by the diets [50, 51]. The co-occurrence patterns further revealed the interaction between bacteria and fermentation products in the rumen of

sika deer fed three diets. The present study found that different groups of *Prevotella* spp. took important roles in the interaction with various metabolic phenotypes, given that *Prevotella* spp. was the dominant bacteria in the rumen of sika deer. In addition, the results found that bacterial patterns of butyrate were considerably altered after the feed was changed from the CS and CI groups (Fig. 6b, c) to the OL group (Fig. 6a), indicating that butyrate metabolism mediated by specific rumen bacteria may play a major role in the energy acquisition of sika deer fed oak leaves. Butyrate contributed to approximately 70 % of the daily metabolic energy of ruminants [3]. Moreover, butyrate production has recently been shown to play a special role in modulating bacterial energy metabolism in the gut ecosystem [52]. The different interplays in the OL group compared to the CS and CI groups may be related to the tannins in the oak leaves, which could decrease methane emissions causing energy loss to the host [53, 54].

This study showed that many novel *Prevotella* spp. were positively related to butyrate. By surveying non-redundant protein sequence database in NCBI (http://www.ncbi.nlm.nih.gov/), the results showed that 24 species (31 isolates) of the *Prevotella* genus could express potential butyrate kinase responsible for the production of butyrate (Supplementary Fig. S1). Moreover, other butyrate producers, such as unclassified bacteria belonging to the family Lachnospiraceae [55], and *Paludibacter* spp. [56], were positively correlated to butyrate. These results indicated that the phylogenetically diverse butyrate-producing bacteria may contribute to host-specific butyrate biosynthesis in the rumen ecological system of sika deer [56–59]. On the other hand, the current study did not find that valerate was associated with TVFAs in the OL group, which may be caused by tannins contained in oak leaves, as tannins could affect the metabolism of proteins, and inhabit the growth of proteolytic bacteria to generate branch fatty acids, such as valerate [60–62].

The interactive patterns between the AP and other metabolic phenotypes in the OL group were different from that in the CS and CI groups. Many unclassified bacteria in the family Ruminococcaceae were higher and were positively associated with the AP in CS and CI groups (Figs. 4 and 6). The Ruminococcaceae bacteria were enriched in endo-1,4-beta-xylanase and cellulose genes, which played key roles in cleaving the cellulose and hemicellulose components of plant material [63]. Therefore, these bacteria may enhance the degradation of fiber plants in the rumen of sika deer, which could cause the increase of production of hydrogen and possibly methane. This was in agreement with the finding that these bacteria were positively associated with the AP, as the methane production was positively associated with AP [53, 64–66].

On the other hand, many bacterial OTUs belonging to the family Succinivibrionaceae, which were lower in the CS and CI groups than in the OL group, were negatively associated with AP. Similarly, Pope et al. [67] found that Succinivibrionaceae bacteria were also abundant in the gut of the Tammar wallaby, which produced relatively lower methane as compared to ruminants. Bacteria belonging to the family Succinivibrionaceae depended on carbon dioxide to support its metabolism via succinate biosynthesis and, in turn, inhibited the bacterial NADH-H hydrogenases and the subsequent inhibition of methanogens producing lower methane [11, 68]. This suggested that the corn forages may cause more loss of metabolic energy of sika deer than the oak leaves mediating by rumen bacteria. Additionally, the concentrations of isobutyrate and isovalerate were positively associated with the AP in the CS and CI groups. Isobutyrate and isovalerate were derived from branched-chain amino acids such as leucine, valine, and isoleucine [69], which were required for the growth of most ruminal fiber-degrading bacteria generating lots of hydrogen during rumen fermentation [70]. In consideration of the side effects of tannins on the proteins, these results indicated that the composition and concentration of some amino acids in the diets may have a fundamental role in energy metabolism, which need to be evaluated in future study.

In conclusion, the current study suggested that *Prevotella* spp. played critical roles in the fermentation of feed in the rumen of sika deer. However, the interplay patterns between rumen bacterial community composition and metabolic phenotypes were altered in the native and domesticated diets, especially for the butyrate, AP, and TVFAs, indicating that the fermentation patterns were changed in the rumen of sika deer fed different diets. It is important to note that the fungi and protozoa in the rumen of sika deer were not investigated here and require future attention.

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Conflict of Interest The authors declare that they have no competing interests.

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