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VARIATION IN THE RUSSIAN ARCTIC REINDEER (*Rangifer tarandus*) RUMEN MICROBIOME RELATED TO SEASON CHANGE

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Abstract

Reindeer (*Rangifer tarandus*) is a large Holarctic herbivore animal, the habitat of which, including its existence at low temperatures and poor diets, has led to the evolutionary development of their unique rumen microbiota, which is necessary for the efficient assimilation of the Arctic flora. In winter, lichens rich in secondary metabolites which can influence the representatives of the microbial consortium of the digestive tract, make up a large proportion of reindeer fodder plants. The toxic effects of certain lichen metabolites (e.g., usnic acid) on a number of microorganisms (*Clostridiales*, *Enterococcus*, *Staphylococcus aureus*, *Escherichia coli*, etc.) as well as ruminants (elk) were previously reported. However, little is known about the effect of lichen consumption on the reindeer rumen microbiome. Using molecular analysis, we were the first to study the seasonal patterns of the formation of the microbial communities of the rumen of the reindeer *Rangifer tarandus*, living in the Russian Arctic. The purpose of the study was to compare the composition of the bacterial community of the reindeer rumen in the summer-autumn and winter-spring periods using the method of NGS-sequencing. In the analysis of microbial communities, biodiversity, taxonomic structure, and the relationship of these indicators with the characteristics of reindeer nutrition in connection with seasonal changes were evaluated. Samples of the rumen content were collected in the summer-autumn and winter-spring periods in 2017-2018 from 20 Nenets reindeer (calves 4-8 months old and adult animals 3-6 years old, $n = 3$ per each age group) in the Nenets Autonomous District (AD). Seasonal differences, in contrast to gender and age, turned out to be the main factor influencing the reindeer rumen bacterial community, which, most likely, is due to differences in the composition of the pasture diet. In the summer-autumn period, a significant increase in the α-biodiversity of the rumen microbiome was noted compared to the winter-spring time for the number of OTUs, Chao1 and Shannon indices. A comparison of the β-diversity of the reindeer rumen microbiota composition has demonstrated the presence of pronounced cluster formation for samples collected in different seasons of the year. Despite the fact that in the winter period the diet of reindeer was mainly represented by lichens which are not typical food for other ruminants (such as cattle, sheep, etc.), it was interesting to note that, on the whole, the obtained microbiome profiles correspond to modern ideas about the ruminant rumen microbiota. Nevertheless, during different seasonal periods, significant changes in the representation of a number of

taxa were noted, the clearest of which were detected for microorganisms associated with feed polysaccharide fermentation. So, in the winter-spring season, a significant increase in microorganisms that decompose polysaccharides of lichens, including hemicellulose (*Butyrivibrio*, *Ruminococcus*), and lichenin (*Succiniclasticum*, *Paraprevotellaceae*, and *Prevotella*). In the summer-autumn period, a significant increase in the proportion of cellulolytic bacteria (*Clostridium*, *Blautia*, *Clostridiales*, *Christensenellaceae Mogibacteriaceae*, and *Prevotellaceae*) is noted. In addition, it has been shown that in the summer period a whole spectrum of microorganisms that belong to bacterial pathogens, including *Erysipelotrichaceae*, *Coriobacteriaceae*, *Mycoplasmataceae*, and *Rickettsiales*, proliferate in the reindeer rumen. On the whole, the results obtained allow us to conclude that the reindeer rumen microbiome is quite clearly associated with nutritional characteristics during various seasonal periods, which determine adaptation to environmental conditions.

Keywords: *Rangifer tarandus*, reindeer, rumen, microbiome, seasonal changes, NGS, Russian Arctic

Scarcity of the diet, especially during the long cold season, aggravated by the severity of the weather conditions poses a serious challenge for reindeer physiology. The shortage of available feed determines one of the causes of death of reindeer in winter [1, 2]. The reindeer forage composition varies considerably by seasons. In the summer-autumn period, the diet can be based on up to 300 plant species, including cereals, sedges, leaves of willows, dwarf birches. In this period, lichens account for no more than 15%. In the winter-spring time, the share of lichens in the diet of reindeer increases to 75%, and the remaining 25-30% are the remains of green plants, mosses, twig feed and various impurities [2]. Lichens are extremely poor in nutrients, nitrogenous and mineral compounds, which leads to a slowdown in the growth and development of youngsters, depletion of animals, especially those who have been ill in the summer, pregnant cows and bulls [3, 4]. In addition, lichens produce secondary metabolites, the organic compounds with bactericidal activity, in particular usnic acid [5, 6]. Some researchers have noted its toxic effects on animals. Thus, it was reported about the death of 300 elks who ate lichens in the absence of alternative food [7]. It is also known that usnic acid in high concentration is toxic to sheep [8]. However, it was found that reindeer can consume lichens without negative consequences [9] due to the ability of anaerobic microorganisms of the rumen to detoxify secondary phenolic metabolites of lichens [10]. Moreover, usnic acid and its metabolites are not found in the contents of the rumen, urine, and excrement of reindeer [10]. These facts give rise to interest in in-depth insight into physiological characterization of nutrition of these ruminants, and above all of their unique ruminal microbiota, necessary for the efficient utilization of the Arctic flora.

Previous characterization of various ruminants have revealed that the composition of the rumen microbiome can depend on many factors, including the genotype of animals [11], age [12], habitat [13], season of the year [4, 14], diet and feeding regime [15], health status, use of antimicrobial compounds [16], daylight regime [17], stress [18)] and environmental conditions [19]. Therefore, no doubt, the study of the reindeer adaptability should be based on an assessment of the conditions of their habitat, nutrition, and other factors.

Little is known about changes in the composition of the rumen microbiome of reindeer in winter associated with an increase in the lichen consumption. The report of M.A. Olsen et al. (20), based on classical microbiological methods, demonstrated a decrease in the total number of microorganisms in the rumen of *Rangifer tarandus platyrhynchus* in winter compared to summer by an order of magnitude. Similar results on a decrease in the number of viable zoospores of chytridiomycetes in winter have been described for other ruminant species [21, 22].

Molecular genetic prefiling of the microbiome of the gastrointestinal tract come into conflict with the data obtained with culture methods. Thus, a significant

change was reported in the abundance of some microorganisms in the rumen of a reindeer depending on the season [23]. It was shown that the proportion of cellulolytic bacteria *Butyrivibrio fibrisolvens* increased in winter (22% in summer and 30% in winter), while amylolytic bacteria *Streptococcus bovis* decreased (17% in summer and 4% in winter). Other authors, on the contrary, did not reveal significant changes in the rumen biota in *Rangifer tarandus platyrhynchus* of the Svalbard archipelago with regard to the number of methanogens, bacteria, and protozoa due to the change in the composition of vegetation of natural pastures in autumn and spring [24]. Similar results were obtained by A. Salgado-Flores et al. [25] who assessed the differences in the microbiota of the rumen and cecum of reindeer *Rangifer tarandus tarandus* inhabiting the territory of Norway when feeding lichens and pelleted fodder. The authors established the absence of a significant effect of the diet on the number of the main groups of microorganisms (bacteria, fungi, archaea), but nevertheless showed the presence of significant differences in the composition of the bacterial and archaeal communities both in the rumen and in the cecum. In particular, in the rumen of animals that received a lichen diet, a significant decrease was noted in the proportion of some bacteria of the genus *Ruminococcus* of the order *Bacteroidales*, participating in the decomposition of plant fiber.

Here, we first applied molecular analysis to investigate the seasonal features of bacterial rumen communities in *Rangifer tarandus reindeer* from the Russian Arctic. The results characterize the confinement of microbiota changes to the structure of the forage and their relationship with age and sex differences. For the first time, it has been shown that seasonal changes are among the key factors in the formation of the rumen microbiome in reindeer, which is probably related to the peculiarities of the animal feed base.

Our objective was to compare the bacterial community composition in the rumen of reindeer from the regions of the Russian Arctic zone in the summer-autumn and winter-spring periods using the NGS sequencing method.

Materials and methods. Specimens of the rumen content were sampled from 20 Nenets reindeer (Rangifer tarandus), including 4-8-month old calves and 3-6-year old adults, in summer-autumn season and winter-spring season of 2017 and 2018 (n = 3 for each age) in Nenets Autonomous Okrug (AO), Nelmin-Nos settlement (tundra climate zone). Simultaneously, samples were collected of pasture vegetation which comprised basal reindeer diet corresponding to the seasonal period. A botanical description of the vegetation samples was carried out according to the "Definition of forage plants for reindeer" [26], the ratio of different types of vegetation in the diet and its nutritional value were measured [27].

To profile the reindeer rumen bacterial community composition by NGS (Next generation sequencing) method, total DNA was extracte from the samples using Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) as per the manufacturer's recommendations. The final concentration of total DNA preparation was measured on a Qubit fluorometer (Invitrogen, Inc., USA) with Qubit dsDNA BR Assay Kit (Invitrogen, Inc., USA) according to the manufacturer's description.

NGS sequencing was performed on the next generation sequencing platform MiSeq (Illumina, Inc., USA) with primers to the V3-V4 region of 16S rRNA gene, the forward primer 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′, the reverse primer 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′.

The DNA libraries were constructed using Nextera® XT IndexKit (Illumina, Inc., USA). Agencourt AMPure XP kit (Illumina, Inc., USA) was used for purification of PCR products, and MiSeq® ReagentKit v2 (500 cycle) (Illumina, Inc, USA) was used for sequencing.

Processing of the reads (Q30 quality filtration, primer trimming) were performed using Illumina bioinformatics platform (Illumina, Inc, USA). The quality of reads and the taxonomic composition of bacteria were assessed using QIIME2 v.2019.10 software (https://docs.qiime2.org) with the Green-Genes database version 13.5 (https://greengenes.secondgenome.com).

To compare bacterial communities, indices of α- and β-diversity were calculated. To assess α-diversity, the species richness indices (the number of Operational Taxonomic Units, OTUs, the Chao1 abundance index) and the Shannon diversity index were calculated using QIIME2 software [28] with default parameters. Additionally, the number of common and unique OTUs was determined for the samples, grouped according to the season, with the use of the VennDiagram package [29] in the R software (https://bioinfogp.cnb.csic.es/tools/venny/index.html). β-Diversity was assessed with the R software using the nonmetric multidimensional scaling (NMDS) algorithm [30] with the Bray-Curtis distance metric from the 'vegan' package (RDocumentation. Package 'vegan', 2019, https://cran.r-project.org/web/packages/vegan/vegan.pdf).

Heatmaps characterizing differences in the composition of the rumen microbiome in the winter and summer seasons were constructed using the 'pheatmap' Version 1.0.12 package for the R software (https://cran.r-project.org/web/packages/pheatmap/pheatmap.pdf). When constructing heatmaps, the numerical matrix was centered and scaled by rows and hierarchical clustering was performed using the Ward's method based on the matrix of the squared Euclidean distances.

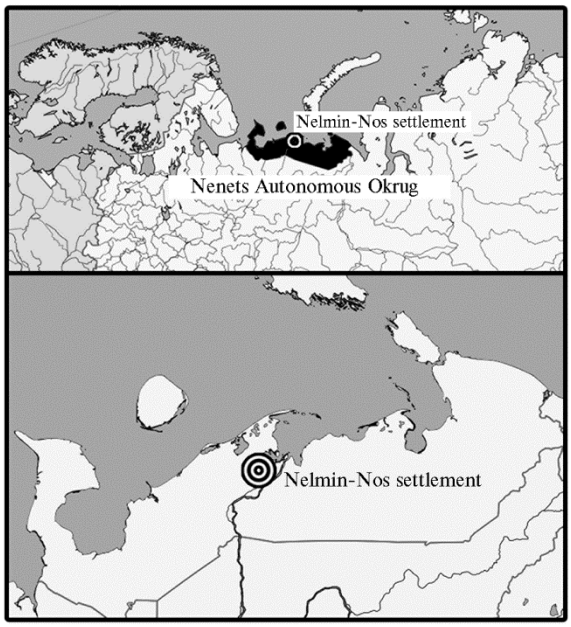

Fig. 1. Region of sampling the rumen contents of the Nenets reindeer (*Rangifer tarandus*) (Nenets Autonomous Okrug, 2017-2018).

The results of NGS sequencing of the bacterial community of the reindeer rumen were deposited in the NCBI (National Center for Biotechnology Information) on the BioProject service at the Sequence Read Archive (SRA) under number PRJNA576999.

Statistical analysis was carried out using Microsoft Excel 2010 software. The average values of indicators (*M*) and their standard errors (±SEM) are given. The significance of differences was assessed using the Student's *t*-test.

Results. Figure 1 shows the location in the Nenets Autonomous District where reindeer rumen content samples were collected. Table 1 presents the averaged composition and nutritional value of the summer-autumn and winter-spring pasture ration of reindeer.

1. Seasonal averaged composition and nutritional value of the pasture ration of the Nenets reindeer (*Rangifer tarandus*) (*M*±SEM, Nenets Autonomous Okrug, 2017-2018)

Parameter	Summer-autumn	Winter-spring
Nutritional value		
Soluble carbohydrates (sugars), g/kg	66.86±3.50	40.22±1.82
Mass fraction of dry matter, %	82.04±1.46	79.15±3.01
Crude fat, g/kg	15.46±0.54	10.62±0.26
Crude protein, g/kg	64.03±3.50	40.69±1.40
Crude ash, g/kg	23.95±1.80	30.95±1.12
Crude fiber, g/kg	160.55±8.60	168.45±7.40
Ingredients		
Lichens (*Cladonia*)	10	75
Silver birch (*Betula pendula* Roth)	20	5
Boreal willow (*Salix borealis* Fries)	15	–
Bog bilberry (*Vaccinium uliginosum* L.)	5	5
Dwarf birch (*Betula nana* L.)	20	–
Perennial herbage	30	15

Note. Dashes indicate the absent of the plant in the sample.

Table 2 describes the rumen content samples we involved in NGS sequencing.

2. Samples of Nenets reindeer (*Rangifer tarandus*) rumen content used in NGS sequencing (Nenets Autonomous Okrug, 2017-2018)

No.	Number of reads	Season	Age	Sex
7SNAM	86227	Summer-autumn	Adult	Bull
12SNAF	84970	Summer-autumn	Adult	Cow
8SNAM	81850	Summer-autumn	Adult	Bull
16SNCM	77574	Summer-autumn	Calf	Bull
15SNCM	76548	Summer-autumn	Calf	Bull
14SNAF	57229	Summer-autumn	Adult	Cow
10SNAF	56651	Summer-autumn	Adult	Cow
13SNCM	54770	Summer-autumn	Calf	Bull
9SNAM	44070	Summer-autumn	Adult	Bull
11SNAF	41268	Summer-autumn	Adult	Cow
16WNAF	31759	Winter-spring	Adult	Cow
15WNAF	29221	Winter-spring	Adult	Cow
20WNAM	28141	Winter-spring	Adult	Bull
18WNCM	26760	Winter-spring	Calf	Bull
19WNCM	26538	Winter-spring	Calf	Bull
22WNAM	25629	Winter-spring	Adult	Bull
14WNAF	24944	Winter-spring	Adult	Cow
17WNCM	20437	Winter-spring	Calf	Bull
13WNAF	17683	Winter-spring	Adult	Cow
21WNAM	14443	Winter-spring	Adult	Bull

Reindeer are the only animals that can effectively use the scarce plant resources of vast areas of the tundra, forest-tundra, and northern taiga [31]. One of the features of the diet of these animals is the high proportion of lichens. The scarcity of the northern diet forces the reindeer, even in summer, to actively eat various types of lichens [1, 2], containing large amounts of toxic metabolites, for example, usnic acid [9]. Moreover, in the winter diet of reindeer lichens can reach 75% [2]. Mushrooms and algae are very different chemically and structurally from vascular plants. In plants, the cell walls consist mainly of cellulose (34-68%), hemicellulose (34-60%), and lignin (5-17%) [32], while in lichens hemicellulose and lichenin are the main component [3].

In ruminants the ruminal bacteria constitute the largest fraction which is diverse in taxonomic composition and spectrum of produced enzymes necessary for decomposition of plant polysaccharides [33-35]. In this work, we for the first time studied the seasonal changes in the structure of the bacterial community of the reindeer rumen. The high-throughput sequencing method was chosen, the use of which previously allowed a number of authors [11, 36] to significantly

expand, compared to classical culture methods, the knowledge about rumen microbiomes of ruminants, including reindeer.

The NGS library we have constructed contained 906712 sequences. The average number of analyzed sequences (reads) in one sample was 45336, the minimum was 14443, and the maximum was 86227. The sequenced fragments were de novo clustered into operational taxonomic OTUs (OTUs) with a 97% identity threshold.

3. Characterization of α-biodiversity of Nenets reindeer (*Rangifer tarandus*) rumen bacterial community based on NGS sequencing (*M*±SEM, Nenets Autonomous Okrug, 2017-2018)

Parameter	Value for groups		p-value
Factor of seasonal differences			
Comparison group	Winter-spring	Summer-autumn	
Shannon index	7.68±0.09	8.26±0.06	0.0000523
OTUs	423.90±25.03	535.00±24.14	0.0075542
Chao1 index	435.71±25.87	548.82±25.09	0.0084612
Factor of age differences			
Comparison group	Calves	Adults	
Shannon index	8.03±0.10	7.95±0.11	0.7685305
OTUs	497.67±7.87	471.64±30.19	0.5885066
Chao1 index	511.16±8.07	484.17±31.08	0.5963144
Factor of sex differences			
Comparison group	Bulls	Cows	
Shannon index	8.01±0.10	7.93±0.15	0.4976555
OTUs	482.33±24.90	475.13±39.74	0.8484414
Chao1 index	495.60±25.85	487.26±40.60	0.8311257

Note. For description of the groups, see *Material and methods.*

Table 3 shows the values of the α-biodiversity parameters (OTUs, Chao1 and Shannon indices). The data indicate that statistically significant differences in the α-biodiversity parameters of the reindeer rumen bacterial community occur between the groups of samples collected in different seasons. Thus, the biodiversity coefficients for the samples collected in the summer-spring period were significantly higher than those for the samples in the winter-spring period according to the Chao1 ($p = 0.0084612$) and Shannon ($p = 0.0000523$) indices and the OTUs number ($p = 0.0075542$). We did not reveal any significant differences in the α-biodiversity depending on sex and age of the reindeer.

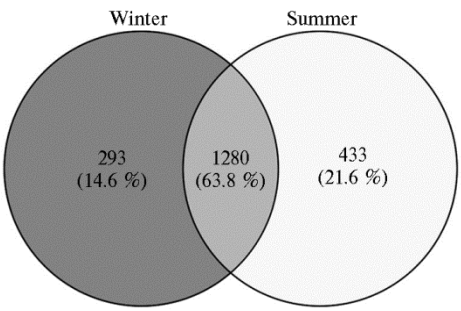

Fig. 2. Comparison of α-biodiversity of Nenets reindeer (*Rangifer tarandus*) rumen microbiomes in summer-autumn and winter-spring seasons by Venn graph analysis method (Nenets Autonomous Okrug, 2017-2018).

These biodiversity parameters indicate not only a significant expansion of the qualitative composition of species (OTUs) in the summer-autumn period, but also an increase in their relative abundance (or evenness) reflected by the Shannon index which accounts for both the species richness and the uniformity of OTUs distribution [28]. The increased value of Chao1 index, which, in addition to the species richness, gives more weight to rare species, also indicates an increase in the biodiversity of the microbial community of the reindeer rumen in the summer-autumn period compared to winter-spring time.

Since the indices of α-biodiversity indicate the greatest influence of seasonal factor on the reindeer rumen microbial community, we calculated the number of common and unique OTUs using the VennDiagram statistical package to

visualize the differences (Fig. 2). The results showed that each season had its own unique set of OTUs. The total number of unique OTUs that were found in the rumen microbiome at least once was 1280. The unique OTUs identified in the summer period accounted for 1713, while in winter their number was 1573.

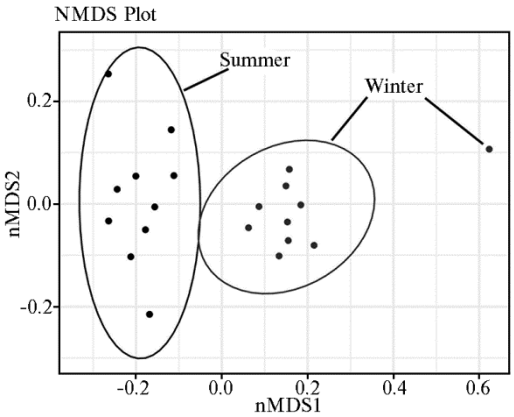

Fig. 3. Comparison of β-biodiversity of Nenets reindeer (*Rangifer tarandus*) rumen microbiomes in summer-autumn and winter-spring seasons by Non-Metric Multidimensional Scaling method (NMDS) (Nenets Autonomous Okrug, 2017-2018).

Seasonal changes in β-biodiversity of the rumen microbiome were visualized by the NMDS method (algorithm of non-metric multidimensional scaling) as a two-dimensional graph in Figure 3. Comparison of β-diversity of the composition of the rumen microbiota of reindeer from different - subgroups demonstrated the presence of a pronounced joint clustering of samples by seasons of the year. A significant shift along the first axis of nMDS1 observed for samples from the summer-autumn and winter-spring subgroups confirms the uniqueness of the composition of the reindeer microbiome in different seasons.

These results are consistent with the studies which reported the presence of specific microbial taxa in the digestive tract, which can vary in animals of the same species or genotype [37]. Such intra- and interspecific variations in the microbiota composition can serve as indicators of ecological processes that form a microbial community associated with the host. Given this, the fact of changes in the indicators of biodiversity in the rumen microbiota of the individuals involved in our study confirms the existing opinion that the microbial community can reflect the physiological state of animals. In our opinion, the detected changes in the rumen microbiota in reindeer are logical, since nutrition is one of the most significant factors affecting the composition of the rumen microbiome [11, 33].

The histogram (Fig. 4) shows the composition of rumen bacterial community of the reindeer at the phylum level. In general, the rumen microbiome comprised 20 bacterial phyla.

At the phylum level, the *Firmicutes* (29.98-52.67%) and *Bacteroidetes* (33.55-51.87%) dominated with no significant differences between seasons. Bacteria of the phyla *Proteobacteria* (0.20-1.64%), *Verrucomicrobia* (1.67-5.21%), TM7 (0.69-4.67%), *Spirochaetes* (1.20-6.88%), *Actinobacteria* (0.40-3.50%), *Planctomycetes* (0.27-3.50%) and SR1 (0.14-6.09%) were less abundant. The bacteria from the remaining phyla (*Cyanobacteria*, *Nitrospirae*, *Chloroflexi*, *Synergistetes*, *Fibrobacteres*, *Fusobacteria*, *Elusimicrobia*, *Tenericutes*, OD1, *Synergistetes*, and two unidentified phyla) accounted for less than 1% of the total bacterial community.

At the family level, *Ruminococcaceae* (5.87-16.17%) and *Prevotellaceae* (12.02-29.16%) prevailed in most samples. Other dominant taxa with a high relative abundance included unclassified bacteria of the order *Bacteroidales* (9.76-16.00%) and families *Clostridiales* (4.31-15.83%), *Lachnospiraceae* (4.17-14.29%), and *Veillonellaceae* (1.67-14.88%).

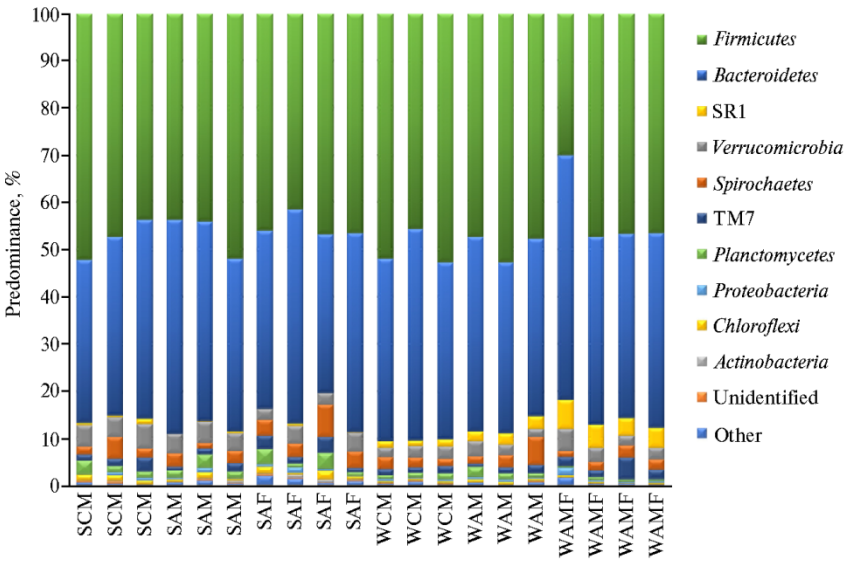

Fig. 4. Seasonal abundance of bacterial phyla in Nenets reindeer (*Rangifer tarandus*) rumen microbiomes: SCM, SAM, SAF — summer-autumn samples from male calves, adult bulls and adult cows; WCM, WAM, WAF — winter-spring samples from male calves, adult bulls and adult cows (Nenets Autonomous Okrug, 2017-2018).

At the genus level, *Prevotella* (8.83-27.04%), *Succiniclasticum* (0.27-12.22%), unidentified bacteria of the genera from the families *Veillonellaceae* (0.21-2.71%), *Lachnospiraceae* (2.12-8.14%), *Ruminococcaceae* (5.02-14.14%) and from the orders *Clostridiales* (2.42-7.71%) and *Bacteroidales* (9.64-17.00%) occupied a dominant position.

Note, minor counts of microorganisms that traditionally belong to causative agents of various diseases of mammals, including members of the families *Fusobacteriaceae* and *Mycoplasmataceae* were present in some individuals.

Despite the fact that the winter ration of reindeer was mainly the lichens, which are not typical feed for other ruminants (cattle, sheep, etc.), it is important to note that, in general, the obtained profiles of the microbiomes correspond to the modern understanding of rumen microbiota in ruminants. Our data serve as a direct confirmation of the results of G. Henderson et al. [11], who studied rumen microbiomes of different ruminants of 32 species and subspecies and showed the presence of a core community which remained stable in all studied species and subspecies. In the core community, these researchers detected bacteria of the genera *Prevotella*, *Butyrivibrio*, and *Ruminococcus* among the representatives of the phyla *Firmicutes* and *Bacteroidetes*. According to their data, the number of other microorganisms (e.g., bacteria of the families *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidales*, and *Clostridiales*) varied depending on the diet and the environment, thereby determining the uniqueness of each species of ruminant, while the differences between groups which determine specificity of adaptation to environmental conditions were manifested at the level of minor taxa.

It is worth noting the important physiological role of microorganisms of the phyla *Firmicutes* and *Bacteroidetes* in the life of ruminants. Many microorganisms that are part of these taxa (for example, of the families *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiaceae*, *Bacteroidaceae*, etc.) are active producers of enzymes (cellulases, hemicellulases, xylanases, glycoside hydrolases, etc.) that the host's body is not able to produce on its own. Microbial enzymes allow the body

of ruminants to assimilate a wide range of plant polysaccharides, providing the body of animals with volatile fatty acids (VFA), such as acetate, propionate, butyrate, and other nutritive compounds [33, 34].

The proportion of phyla *Firmicutes* and *Bacteroidetes* in the rumen was reported to depend on the habitat and type of feed [13]. Thus, many bacteria of the phylum *Firmicutes* (genera *Ruminococcus*, *Clostridium*, *Butirivibrio*, etc.) actively produce cellulolytic enzymes, which allows them to break down plant fiber, while representatives of *Bacteroidetes* synthesize mainly amylolytic enzymes and promote the utilization of easily fermented carbohydrates [34]. Therefore, a change in the profile of microorganisms of these groups can also lead to a change in the spectrum of metabolites produced by them, thereby influencing the host organism.

Our research revealed that the change of seasons caused significant differences in the abundance of microorganisms in the phyla *Firmicutes* and *Bacteroidetes*, as well as in a number of representatives of other taxa. Figures 5 and 6 show heat maps reflecting prevalence of microorganisms the abundance of which in the reindeer rumen significantly differed in the winter-spring and summer-autumn periods.

Figure 5 shows that in the summer-autumn period as compared to winter-spring time, the proportion between SR1 phyla (0.33±0.11% vs. 3.09±0.52%, $r = 0.00004$, $p < 0.05$) and *Planctomycetes* (0.01±0.006% vs. 0.24±0.15%, $r = 0.02$, $p < 0.05$) were significantly less.

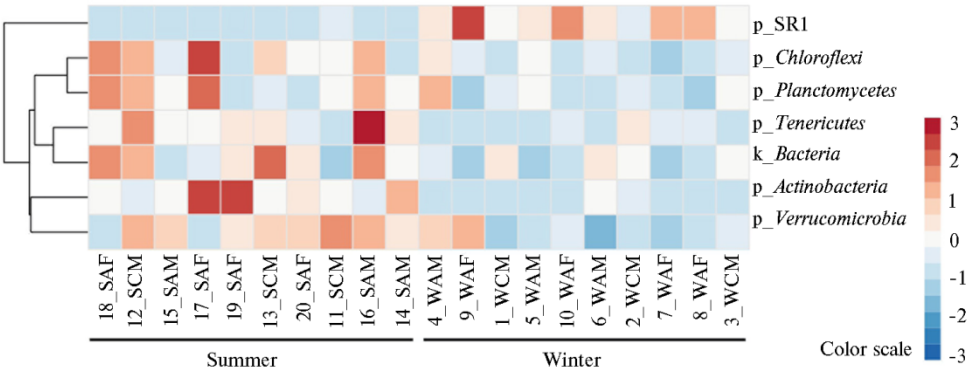

Fig. 5. Heatmap of seasonal difference in the abundance of bacterial phyla in Nenets reindeer (*Rangifer tarandus*) rumen microbiomes: S — summer, W — winter, A — adult, C — calf, M — male, F — female; k — kingdom, p — phylum (Nenets Autonomous Okrug, 2017-2018).

In the winter-spring period, abundance of a number of phyla significantly decreased, including *Actinobacteria* (0.26±0.08% vs. 0.03±0.01%, $r = 0.004$), *Chloroflexi* (0.95±0.15% vs. 0.43±0.06%, $r = 0.004$), *Tenericutes* (3.10±0.08% vs. 0.05±0.02%, $r = 0.006$), *Verrucomicrobia* (0.27±0.06% vs. 0.09±0.03%, $r = 0.01$), unidentified phyla (1.96±0.32% vs.1.01±0.24%, $r = 0.02$); for all r $p < 0.05$. A lower proportion of phyla *Actinobacteria*, *Verrucomicrobia*, and *Chloroflexi* in the rumen in winter appeared to be a regularity; according to some research, these microorganisms are associated with soil and plant ecosystems [38].

Moreover, in general, NGS sequencing did no revealed significant seasonal differences in total proportion of the phyla *Firmicutes* and *Bacteroidetes* dominating in the reindeer rumen. However, as can be seen from Figure 6, at a lower taxonomic level, the abundance of some members of these taxa in the winter significantly increased. Interestingly, significant differences occurred primarily for microorganisms associated with fermentation of plant polysaccharides. Thus, in the winter there was a significant increase in the number of bacteria of the genera

Succiniclasticum (from 1.59±0.26% in the summer-autumn period to 9.55±0.68% in the winter-spring period, $r = 0.000000001$), *Paraprevotellaceae* (from 1.08±0.14% to 2.41±0.20%, $r = 0.00002$), *Coprococcus* (from 0.08±0.03% to 1.18±0, 21%, $r = 0.00004$), *Butyrivibrio* (from 0.77±0.10% to 3.58±0.55%, $r = 0.00007$), *Prevotella* (from 14.13±1.13% to 20.10±0.91%, $r = 0.0005$), and *Ruminococcus* (from 0.28±0.01% to 0.97±0.253%, $r = 0.01$); for all r p <0.05.

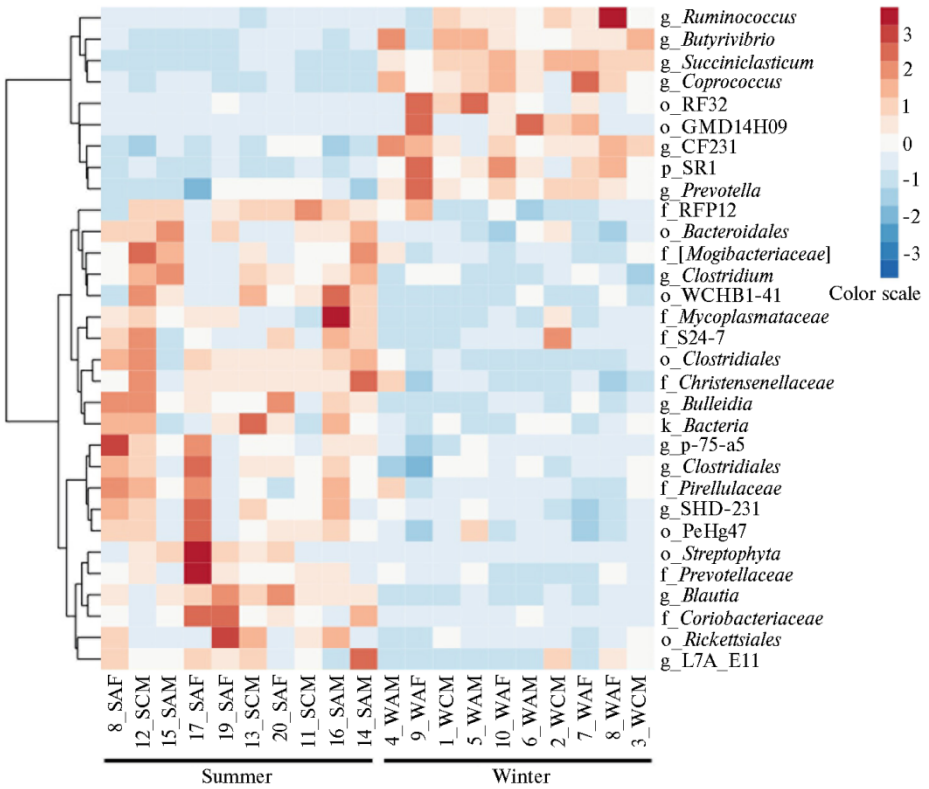

Fig. 6. Heatmap of seasonal difference in the abundance of bacterial genera in Nenets reindeer (*Rangifer tarandus*) rumen microbiomes: S — summer, W — winter, A — adult, C — calf, M — male, F — female; g — genus, f — family, o — order, k — kingdom, p — phylum (Nenets Autonomous Okrug, 2017-2018).

On the contrary, other microorganisms from the phyla *Firmicutes* and *Bacteroides* became significantly more abundant in the winter period. Such regularities were observed for the genera *Clostridium* (from 0.98±0.13% to 0.41±0.07%, $r = 0.001$), *Blautia* (from 1.20±0.15% to 0.18±0.04%, $r = 0.000006$), unidentified genera from the *Clostridiales* order (from 5.71±0.43% to 2.81±0.21%, $r = 0.000006$), from the families *Christensenellaceae* (from 2.39±0.213% to 1.11±0.17%, $r = 0.00001$), *Mogibacteriaceae* (from 2.23±0.41% to 0.89±0.19%, $r = 0.007$) and *Prevotellaceae* (from 3.29±0.50% to 1.99±0.17%, $r = 0.02$); for all r p <0.05.

Our findings confirm the opinion of G. Henderson et al. [11] that the differences in the rumen microbiome of ruminants, which determine characteristics of animal adaptation to environmental conditions, are apparent at the level of minor taxa.

The findings draw us to the conclusion about a fairly clear association of the reindeer rumen microbiome with nutritional habits in different seasons. Considering that lichens consist mainly of structural carbohydrates [3, 32], such as

hemicellulose, it seems natural that bacteria of the genera *Butyrivibrio* and *Ruminococcus* capable of hydrolyzing this polysaccharide predominate in the rumen in the winter-spring period. It is known that the role of other ruminal bacteria, namely the members of genera *Prevotella* and *Paraprevotella* of phylum *Bacteroidetes*, is associated with the degradation of starchy polysaccharides due to production of amylases [39]. An increase in the number of these bacteria in winter may be an expected consequence of the high content of lichen starch, the lichenin in the reindeer diet. In this regard, the discovered increase in the abundance of *Prevotellus* in the rumen of the Chinese sika deer, whose diet included a large amount of oak leaves containing a significant amount of secondary plant metabolites with antimicrobial action is also of interest [40].

Note that the abundance of another ruminal prevotellas from an unidentified genus of the family *Prevotellaceae*, on the contrary, was higher in the summer-autumn period (see Fig. 6). This can be explained by the high genome variability of the microorganisms of this family, as a result of which different genera and species of *Prevotellaceae* show the ability to synthesize a wide range of enzymes that hydrolyze plant polysaccharides. Thus, an increase in the bacterial abundance of *Prevotellus* that we identified in the summer-autumn period is possible due to its ability to form cellulases that break down plant fiber the proportion of which in the reindeer diet increases in summer.

The genera *Clostridium* and *Blautia* also belong to the cellulose-decomposing ruminal bacteria, which explains their significantly higher level in the reindeer rumen in summer and autumn and indicates an increase in the potential of the microbial community for the fermentation of plant polysaccharides. In addition, a significant antimicrobial effect of lichen usnic acid was previously reported in a number of clostridial strains [5]. Note that the findings of T.H. Aagnes et al. [3] come into some contradiction with our data, since these authors reported an increase in the abundance of *Clostridia* in winter, which, obviously, can also be explained by the specific metabolic characteristics of various species of the genus *Clostridium*.

F. Li et al. [41] when analyzing the rumen microbiome in 709 cattle, revealed that the presence of a complex of bacteria, including unidentified bacteria of the order *Clostridiales*, families *Christensenellaceae*, *Mogibacteriaceae*, directly and positively correlates with the amount of acetate, while the genus *Succiniclasticum* abundance correlate with the propionate level. The data obtained by F. Li and colleagues indicate a certain metabolic relationship between these microorganisms. Thus, it is known that the formation of acetate in the rumen is associated with degradation of plant cellulose, while the formation of propionate is associated with the starch metabolism [34]. Thus, our results explain the increase in the abundance of bacteria synthesizing acetate in summer when plants are the main component of the reindeer diet, and those producing propionate in winter when the animals predominantly eat lichens.

In addition, we revealed a slight decrease in the proportion of the phylum *Cyanobacteria* (from 0.07±0.02% to 0%, $r = 0.00004$, $p < 0.05$) in the winter-spring period. In our study, as already noted, the presence of cyanobacteria in the rumen of reindeer inhabiting the territory of the Nenets Autonomous Okrug, both in the summer-autumn and winter-spring periods, was generally minor. Nevertheless, other works [26, 42] mention a sufficiently high number of cyanobacteria in reindeer rumen microbial communities. This seems quite expectable, since cyanobacteria are symbionts of lichens which constitute up 10-15% of reindeer grazing diet in summer and up to 75% in winter [43].

The patterns that we identified for the set of opportunistic microorganisms deserve special attention. NGS analysis showed that many microorganisms that are causative agents of infections preferably reproduce in reindeer rumen in summer and autumn. In winter and spring their abundance significantly decreases, from 1.12±0.17% to 0.50±0.06% (r = 0.003) for *Erysipelotrichaceae*, from 0.26±0.08% to 0.02±0.01% (r = 0.005) for *Coriobacteriaceae*, from 0.22±0.06% to 0.04±0.02% (r = 0.02) for *Mycoplasmataceae*, and from 0.39±0.11% to 0.10±0.02% (r = 0.017) for *Rickettsiales*; for all r p <0.05.

These results are to some extent confirmed by the study [44], in which the *Coriobacteriaceae* family was found to be the most sensitive indicators responding to changes in the rumen microbiome of ruminants. A significantly higher abundance of Mycoplasmataceae family comprising bacterial pathogens, that we revealed during the summer-autumn period, also corresponds to the available data on the higher incidence of reindeer in the summer season [45].

For cattle, a positive correlation was revealed between the abundance of *Erysiopelotrichaceae* family gram-positive bacteria and the feeding habits [46]. Like species of genus *Lactobacillus*, most members of *Erysiopelotrichaceae* ferment a wide variety of sugars to form mainly lactic acid, resulting in increased rumen acidity. It is known that an increase in the amount of lactic acid in the rumen leads to impaired digestion of fiber and the development of lactic acidosis. J.E. Nocek et al. [47] showed that higher lactic acid level in the rumen of cattle can lead to *Fusobacterium necrophorum* infection and necrobacteriosis as a result of the pathogen penetration into the blood through damaged rumen epithelium. Given that necrobacteriosis in reindeer herds is seasonal, i.e. occurs in the summer, the increased abundance of *Erysipelothrihaceae* family that we have identified in the summer seems logical and noteworthy.

To summarize, the survey of Nenets reindeer (*Rangifer tarandus*) in the Russian Arctic showed a significantly higher α-biodiversity level in the summer-autumn period compared to the winter-spring period as per the number of OTUs (p = 0.0075542) and the values of Chao1 (p = 0.0084612) and Shannon (p = 0.0000523) indices. We did not find statistically significant differences in the biodiversity of the rumen bacterial community with regard to sex and age of the reindeer. Regardless of the season, the phyla *Firmicutes* and *Bacteroidetes* dominate in the rumen content, the phyla *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, TM7, *Spirochaete*, *Planctomycetes*, and SR1 are less abundant, and other detected taxa are minor. Nevertheless, we revealed significant changes in the composition of certain taxonomic groups of bacteria depending on the season. In the winter-spring period, the number of ruminal bacteria involved in decomposition of lichen polysaccharides increase, including those decomposing hemicellulose (*Butyrivibrio*, r = 0.00007; *Ruminococcus*, r = 0.01) and lichenin (*Succiniclasticum*, r = 0.000000001; *Paraprevotellaceae*, r = 0.00002; *Prevotella*, r = 0.0005). During summer and autumn, the abundance of microorganisms associated with the decomposition of plant fiber increases, including members of genera *Clostridium* (r = 0.001), *Blautia* (r = 0.000006), unidentified genera from the order *Clostridiales* (r = 0.000006) and from the families *Christensenellaceae* (r = 0.00001), *Mogibacteriaceae* (r = 0.007), and *Prevotellaceae* (r = 0.02). The abundance of some infectious pathogens in the reindeer rumen is higher in summer, including bacteria of the families *Erysipelotrichaceae* (r = 0.003), *Coriobacteriaceae* (r = 0.005), *Mycoplasmataceae* (r = 0.02), and *Rickettsiales* (r = 0.017). For all r values, p < 0.05.

Thus, the obtained microbiome profiles of the reindeer rumen are generally consistent with modern concepts of the rumen microbiota in ruminants. In the summer-autumn period, there is a significant increase in the indices of α-

biodiversity of the rumen microbiome as comparison to the winter-spring period. Comparison of the β-diversity of the reindeer rumen microbiota shows a pronounced clustering of the samples by seasons. In the winter-spring season, a significant increase occurs in the abundance of microorganisms that decompose lichen polysaccharides, including hemicellulose (*Butyrivibrio*, *Ruminococcus*) and lichenin (*Succiniclasticum*, *Paraprevotellaceae*, *Prevotella*). In the summer-autumn period, there is a significant increase in the proportion of cellulolytic bacteria *Clostridium*, *Blautia*, *Clostridiales*, *Christensenellaceae*, *Mogibacteriaceae*, and *Prevotellaceae*. In addition, the summer period is preferable for the development of infectious agents (*Erysipelotrichaceae*, *Coriobacteriaceae*, *Mycoplasmataceae*, and *Rickettsiales*). In general, these findings allow us to conclude that there is a fairly clear association of the reindeer rumen microbiome with nutritional habits in different seasonal periods. The differences in the rumen microbiome of ruminants, which determine adaptation to environmental conditions, are apparent at the level of minor taxa. The data we obtained can be used to develop means facilitating the reindeer adaptation to the ecological conditions of the territory, as well as means for the prevention and treatment of diseases, one way or another associated with seasonal changes in the rumen microbiome.

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