Research Article

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Rumen bacterial community of young and adult of reindeer (*rangifer tarandus*) from Yamalo-Nenets Autonomous District of Russia

Abstract: The aim of the work was to compare the taxonomic composition of the rumen procariotic community in young and adult individuals of Nenets breed reindeer (*Rangifer tarandus*) from the central part of the Yamal region by using the NGS method (next generation sequencing) and compare the microbiome composition of reindeer with the microbiome of their initial vegetation food material. The obtained data showed that the dominant position in microbial communities, like that of other ruminants, was occupied by representatives of phylum *Firmicutes* and *Bacteroidetes*, whose total share between observed groups did not differ significantly. The composition of the microbiome of the rumen of the investigated group of animals was completely different from the microbiome structure of the initial vegetation cover. Digestion of vegetation by reindeers resulted in complex transformation in the initial plant microbiome and an increase of biological diversity which was expressed in operational taxonomic unit (OTU) numbers increasing and changes in indexes of alpha-diversity parameters. According to the results of alpha- and beta- diversity of the rumen microbial communities, the greatest uniqueness was revealed for the microbiomes of the adults in comparison with calves and young. The presence of changes in the biodiversity indexes of the rumen microbiota in the reindeer, examined by us, confirm the opinion of the researchers that the microbial community may also reflect the physiological state of the animals. It has also been demonstrated

that the presence of the phylum *Verrucomicrobia*, and the genera *Stenotrophomonas*, *Pseudomonas*, etc., may be specific to Nenets breed reindeer and have a pattern with their presence on various plants and lichens that are part of the reindeer diet. This is partially confirmed by data on plants microbiome taxonomy.

1 Introduction

Currently, agriculture in the Russian Arctic is an intensively developing part of the local economic. Strong localization of industrial and agricultural activity in polar environments results in an increased consumption of local production by inhabitants of highly urbanized areas (Russian Arctic is the most urbanized part of the country). The microbiome of agricultural ecosystems in polar environments is under investigation and only a few data are known about the taxonomy and functional composition of the microbiome in compartments of arctic ecosystems environments. The stable state of the population of reindeer in current conditions is faced with a devastation process. The reindeer's rumen microbiota plays an important role in food digestion (Church 1993; Morgavi et al. 2013) due to enzymes produced by symbiotic microorganisms. In this context, the study of living reindeers in natural conditions and the formation of their adaptations is required for corrections of current agricultural practices. This special interest is connected with the possibility for the effective use of poor Arctic region plant resources for feeding, which undoubtedly can exert significant selective pressure on the structural and functional organization of the ruminal microbiome (Tarakanov 2006; Mukhachev and Layshev 2007). This problem became more urgent in

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the context of increasing over pasturing of tundra's in the Yamalo-Nents autonomous region.

Reindeer (*Rangifer tarandus)* take a special place among other herbivorous ruminants. This is a unique animal species, which during the expansion of its habitat, has acquired specific adaptations for life in the severe northern conditions. Agriculture in the Arctic zone is mostly reindeer farming. Nowadays on the territory of the Russian Federation there are more than 1.5 million domestic reindeers, and in the Yamalo-Nenets Autonomous District – there are about 680 thousand individuals. This results in over grazing and degradation of vegetation resources, as well as land use redistribution. The quality of pastures is susceptible to intensive changes. That is why investigation of reindeer food quality, with special reference to microbiological characteristics of their stomach, is urgent for development of current agricultural practices in the polar environments.

The geographical isolation of reindeer from other subspecies of the ruminant family *Cervidae* (Sundset et al. 2007) not only resulted in anatomical and morphological differences in the structure of their digestive system, compared with other ruminants (Dubos 1966; Hofmann 1973; Hackmann et al. 2010), but also in the formation of specific microbial communities of the rumen. These differences could be the result of: microbiome composition of local vegetation resources and adaptation of reindeer to severe environmental conditions in arctic ecosystems. There are some studies that suggest that there is a correlation between the evolution of a host organism and its intestinal microbiota (Brooks et al. 2016; Groussin et al. 2017). It is reasonable to suggest that the difference in the intestinal microbiota may be due to the divergence of their hosts in the process of evolution (Delsuc et al. 2014).

The relation of host phylogeny and their dietary strategies, the connection between host genetic diversification and the gastrointestinal microbiota is still under investigation (Ley et al. 2008; Muegge et al. 2011). It has been shown that the microbial community of the rumen may also reflect regional characteristics of the diet and the general physiological state of the animals. Also, the peculiarities of vegetation used as a feed by reindeers, in terms of initial composition of microbiome, could be a factor which affects gastrointestinal microbiota quality.

The composition of reindeers' food base varies significantly with the season. During the summer-autumn period, the basis of their diet is plants, including cereals, sedges, willow leaves, and dwarf birch trees. The portion of lichens is only up to 15% during this season. In the winter-spring period, the portion of lichens in the diet of reindeer increases to 70%, while the remaining 30% is represented by green plants, mosses, twigs, and various impurities (Borozdin et al. 1990, Mukhachev, Layshev 2007). Among the important functions of reindeer rumen anaerobic microflora is its ability to detoxify the secondary phenolic metabolites of lichens, e.g. usnic acid (Orpin et al. 1985; Sundset et al. 2008). However, by examining 32 livestock species, Henderson et al. (2015) demonstrated that changes in the rumen microbiota were associated with the identity of the host species, and not with the diet. The reindeer rumen microbiome is less studied (Aagnes et al. 1995; Sundset et al. 2007) compared with other ruminants, such as sheep and cattle (Jami and Mizrahi 2012).

Until the 1990s, studies of reindeer ruminal microorganisms were based on observations of cultured strains on artificial nutrient media (Hungate 1966). Certain strains of reindeer ruminal fungi were studied (Orpin et al. 1985; Mathiesen 2005; Sundset et al. 2007). The development of molecular-genetic methods for observing the microorganisms has significantly expanded the understanding of microbiome composition of the rumen. An important feature of metagenomic studies is the absence of the need to cultivate microorganisms, which is a fundamental point, since up to 99% of microorganisms in the biosphere cannot be cultivated on artificial nutrient media. Moreover, in polar environments the presence of cultivated forms of microorganisms are very low. That is why previous studies essentially under evaluation the taxonomic and functional diversity of gastrointestinal microbiota.

According to estimates, the diversity of ruminal microorganisms reaches several thousand species, of which less than 100 have been studied in detail. Most among them are strictly anaerobic uncultivated species, which cannot be investigated without application of metagenomic techniques (Henderson et al. 2015). Therefore, the most informative way of studying the rumen microbial community is molecular genetic methods such as NGS (next generation sequencing), which are not primarily aimed at studying its individual members, but the structure of the community as a whole. NGS is the most modern technique that allows analysis of several hundred thousand genetic sequences at once, determining the structure of the microbial community and assessing the influence of various factors on it.

Few publications can be found about the ruminal microbiocenosis of reindeers in the territory of Norway. Zielińska et al. (2016) studied the microbial communities of feces of *Rangifer tarandus platyrhynchus* reindeer of the Svalbard archipelago. Gruninger et al. (2014) reported on the composition of the rumen microbiota of another deer species – the Sika deer family. Therefore, the study of bio-

diversity, identification and taxonomic description of the reindeer rumen microbiocenosis is relevant for expanding information about the reindeer physiology.

This paper presents the first molecular genetic studies of the rumen microbiocenosis of the Nenets breed of reindeer living in the Yamalo-Nenets Autonomous District of Russian Federation. The aim of the work was to compare the taxonomic composition of the rumen procariotic community in young and adult individuals of reindeer (*Rangifer tarandus*) using the NGS method and to compare the reindeer microbiome composition with the microbiome of the initial feed material.

2 Materials and Methods

2.1 The study sites and sampling strategy

The vegetation material was sampled from the soil surface near the Erkuta research plot, located on first terrace of the Erkuta river. Samples were taken from the soil surface, covered by living plant material and frozen in a mobile expedition refrigerator. This place was characterized by complexity of micro- and nano-relief, the presence of lides, over-moistened micro-depressions, relatively dry micro-elevations and frost mounds (Figure 1). The vegetation cover was presented by hummock tundra with prevalence of mosses and brushes. The sampling plot was located on the first river terrace on the left bank of Erkuta river and river floodplain. The first river terrace is characterized by presence of laydas, over-saturated micro-depressions, micro-elevations and sorted circles. Soils were classified as predominantly Histic Stagnic Cryosols/Peaty Gleyzems underlain by permafrost and Turbic Cryosols/ Typic Cryozems in sorted circles. Vegetation cover is more depended on spatial variation of surface manifested in presence of hummocks, over-moistened micro-depressions and small frost mounds. Vegetation cover ranged from relatively dry tundra (high elevated and drained) associations to wet tundra associations, located in depressions (with *Eriophorum vaginatum* as a determinant). At some places sharp variation of vegetation types is represented - black turfy surfaces with almost no low percentage of higher vegetation (black color) are combined with wet tundra. Five samples of dry tundra vegetation cover were collected in October 2017 for further metagenomic investigations. Samples were frozen until the starting of laboratory research. The study site's location is presented on the Figure 1.

Figure 1: The study sites location (designated by red circle)

Samples of the rumenal content were taken in the autumn-winter period in November 2017 from calves (6-8 months, n = 3), young (1-2 years, n = 3) and adult individuals (3-6 years, n = 6) of the Nenets breed from the reindeer herding brigade No. 2 of the Yamal department of the FGBNU VNIIVEA on the territory of the Priuralsky region of the Yamal-Nenets Autonomous District, 20-100 km from the town of Salekhard and 20-40km from the settlement of Kharp. A totally of twelve animals were investigated. By the nature of the vegetation, the territory where deer were grazed is typical of the forest-tundra of the Ural sector of Western Siberia and the Southern Yamal. The composition of the averaged reindeer pasture ration included 55% lichen, 20% grass, 18% shrubs, up to 5% snowy greens and up to 2% moss (Mukhachev, Layshev 2007).

2.2 Metagenomic study of the vegetation materials

Samples of scar contents were taken using an esophageal probe, which was injected through the mouth opening into the esophagus and pushed to the scar through a pyloric sphincter. The probe, which hit the scar, was characterized by a specific sound and smell. Then samples were

stored in froze state and transported to Saint-Petersburg. Plant material for DNA extraction were not ground before analyses. Samples were frozen in the field and transported to the laboratory.

DNA was extracted from 0.5 g of vegetation material using the PowerSoil DNA Isolation Kit (Mobio Laboratories, Solana Beach, CA, USA), which included a bead-beating step, according to the manufacturer's specifications. Homogenization of the samples was performed using Precellys 24 (Bertin Corp, USA) at 6.5 m/sec, twice for 30 s. The purity and quantity of DNA were tested by electrophoresis in $0.5 \times$ TAE buffer on 1% agarose. DNA concentrations were measured at 260 nm using a SPECTROStar Nano (BMG LABTECH, Ortenberg, Germany). The average DNA yield was 2–5 µg DNA, with concentrations between 30 and 50 ng/µl. The purified DNA templates were amplified with universal multiplex primers F515 5′ - GTGCCAGCMGC-CGCGGTAA-3′ and R806 5′ -GGACTACVSGGGTATCTAAT-3′ (Bates et al. 2011) targeting the variable region V4 of bacterial and archaeal 16S rRNA genes.

Each multiplex primer contained the adapter, 4-bp key (TCAG), 10-bp barcode, and primer sequences. The expected length of the amplification product was 400 bp. Sequencing of the amplicon libraries was carried out using Illumina MiSeq in the Centrum 'Genomic Technologies, Proteomics and Cell Biology' (All-Russia Research Institute for Agricultural Microbiology). The raw sequences were processed using QIIME (Caporaso et al. 2010).

Preliminary processing of the raw reads was performed using TRIMMOMATIC software (Bolger et al. 2014). To reduce sequencing errors. The multiplexed reads were first filtered for quality and grouped according to barcode sequences. Sequences were omitted from the analysis if they were less than 200 bp, had a quality score of less than 25, contained uncorrectable barcodes, primers, ambiguous characters or a homopolymer length equal to or greater than 8 bp. All non-bacterial ribosomal sequences and chimeras were also removed from the libraries. Chimeras were removed by using the chimera_slayer.py script, incorporated in QIIME.

In total, 1,023,728 sequences were obtained with an average of 33,023 sequences per library. The dataset was subjected to the normalization procedure, resulting in 16,365 sequences per sample. Similar sequences were clustered into operational taxonomic units (OTUs) with a minimum identity of 97% using de novo and closed reference algorithms. A representative set of sequences was chosen by selecting the most abundant sequence from each OTU. Representative sequences from each OTU were subjected to an RDP naïve Bayesian rRNA Classifier (Wang et al. 2007) with a confidence level of 80% and

aligned using a PyNast algorithm and Greengenes database (DeSantis et al. 2006). Aligned sequences were used to build a distance matrix with a distance threshold of 0.1 and phylogenetic tree necessary for downstream analysis.

To compare the phylogenetic diversity of microbial communities, alpha and beta diversity analyses were performed. To estimate alpha diversity, the indices for richness (observed species, ChaoI) and phylogenetic diversity of communities (Faith's index, Shannon evenness) were calculated. The t-test was performed to verify the observed differences. For beta diversity, the weighted Unifrac metric (Lozupone and Knight 2005) was used to calculate the amount of dissimilarity (distance) between bacterial communities to be compared. Relative abundance of bacterial and fungal small subunit rRNA gene copies were analyzed by quantitative PCR (qPCR), as previously described (Pershina et al. 2015).

The abundances of OTUs were compared between samples by calculating the median relative change values for all groups of replicates. A positive median indicates an increase in abundance, whereas a negative median can be considered as evidence for a decline in abundance. A basic permutation test was used to infer significance, whereas a jackknife-like resampling approach was applied to test the stability of median estimates.

2.3 Metagenomic study of the rumen bacterial community

Molecular genetic analysis of the reindeer rumen bacterial community was performed in the laboratory of the BIOTROF + company (St. Petersburg) using NGS sequencing. Isolation of total DNA for molecular biological analyzes was carried out according to the method described in Maniatis with co-authors (Maniatis et al. 1982) in its own modification. Metagenomic sequencing was performed on a MiSeq genomic sequencer (Illumina, Inc., USA) with the MiSeq Reagent Kit v2 (Illumina, Inc., USA). The maximum length of the obtained sequences was 2 x 250 nt. Processing of the obtained reads, including overlapping, filtering by quality (Q30), and trimming of primers was performed using the Illumina bioinformatics platform. The determination of the taxonomic affiliation of microorganisms to the genus was carried out using the RDP Classifier program (https://rdp.cme.msu.edu/classifier/classifier.jsp).

Table 1: Alpha-diversity parameters of the vegetation materials

Sample replication	OTUs	PD_whole_tree	Chao1	Shannon
1	1678,5±39,0	14.19 ± 3.54 [a]	299,1±191,4	8,0±0,1
2	1401,5±87,2	23,11 ± 2,34 [a]	222,6±166,8,	8,9±0,2
3	1546,7±78,0	22.11 ± 2.01 [a]	287,5±144,0	7,7±0,3

Table 2: Alpha-diversity parameters of the rumen materials

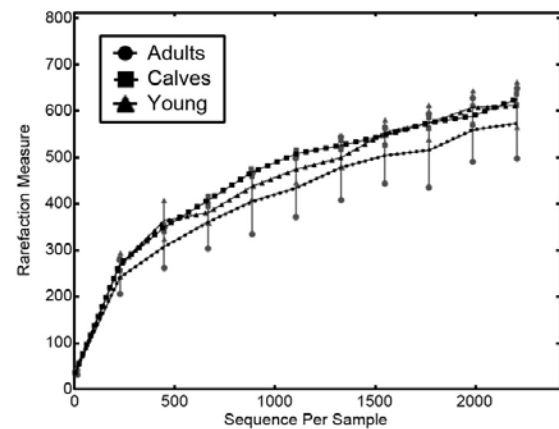| Sample type	OTUs	PD_whole_tree	Chao1	Shannon
Calves	3983.33±1705.21	24.18±2.49 [a]	707.10±78.15	4.07±0.03
Young	5676.67±1129.72	25,91±1.28 [a]	832.73.0±41.02	4.05±0.06
Adults	6765.50±1596.84	25.51±2.08[a]	865.43±154.55	4.14±0.10

a – statistically significant

3 Results

To evaluate the alpha diversity of the vegetation microbiomes, several indices for species richness and evenness were calculated (Table 1). The number of OTUs was essentially lower in vegetation materials (Table 1) than in rumen material (Table 2). The levels of phylogenetic diversity and Shannon indexes were also lower in vegetation materials than in rumen samples. This indicates that taxonomy diversity of rumen materials was higher than this parameter in the vegetation materials of the top soils investigated. The taxonomic analysis of the vegetation microbiomes revealed 43 bacterial and archaeal phyla, among which *Proteobacteria* (22% on average), *Actinobacteria* (18%), *Acidobacteria* (17%), *Chroloflexi* (11%), *Gemmatimonadetes* (6%), *Verrucomicrobia* (6%), *Planctomycetes* (5%), *Bacteroidetes* (3.9%), AD3 (3%) and *Nitrospirae* (2%) constituted the majority (more than 95% of sequences in the amplicon libraries). Archaea were represented by the phyla *Crenarchaeota* (0.3%), *Euryarchaeota* (0.2%) and *Parvarchaeota*.

As a result of reindeer rumen metagenomic community NGS, a library of reads was obtained, which included 270,430 sequences. The average number of analyzed sequences (reads) in 1 sample was 18,340, the minimum was 6,232, and the maximum was 27,581. The sequences were *de novo* clustered into OTU taxonomic units with a 97% identity threshold.

Table 2 presents the values of α-biodiversity parameters: operating taxonomic units or species (OTUs), Chao1 and Shannon indices. The number of OTU ranged from 3,983 to 6,765, the Shannon index varied within 4.00 - 4.27, depending on the sample. As can be seen from Table 2,

Figure 2: Chao1 index values of the reindeer rumen bacterial community

there is a tendency to increase the number of OTUs with the age of animals. Significant differences in the coefficients of biodiversity in animals of different ages were not found. In adult animals, compared with calves and young animals, there is a tendency to increase the value of the Chao1 index (Figure 2), the Shannon diversity index, and the level of phylogenetic diversity (PD whole tree).

The results of β-diversity assessment are presented in the form of a three-dimensional PCoA Emperor graph in Figure 2. As can be seen from Figure 2, the main component of PC1 described 52.33% of data, PC2 - 22.71%, and PC3 - 6.92%. The total application of this method allowed us to describe the changes occurring in the compositions of the microbiome, while maintaining 81.96% of the data information. Comparison of the rumen microbiota composition of reindeer from different subgroups by the method

of main components (Figure 3) showed that the greatest displacement along the axis of the first main component of PC1 was observed according to samples of rumen microbiomes from the adult's subgroup. Whereas the other subgroups placed closer to PC2 axis. The clustering in the calves and young subgroups was less expressed compared with the adult's subgroup.

In Figure 4, the diversity of the reindeer rumen metagenomic community, at the phylum level, is presented in the form of a histogram. As can be seen from Figure 3, 25 phyla of attributable microorganisms were represented in the metagenomic community of the reindeer rumen.
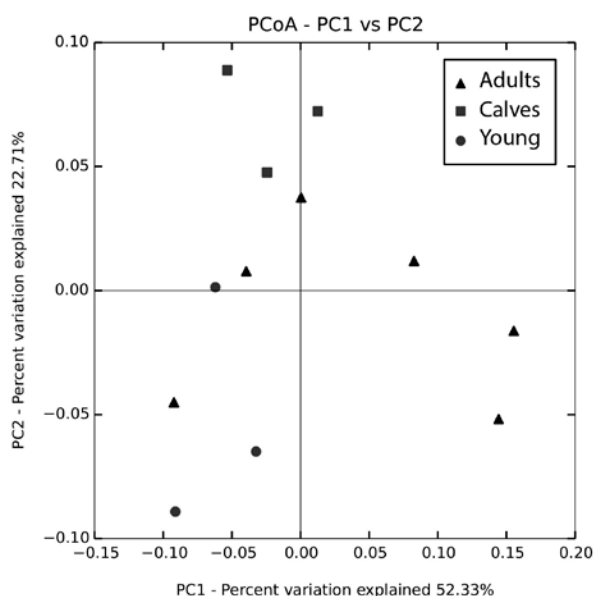

Figure 3: Comparative analysis of the bacterial community of the reindeer rumen using Principal Component Analysis using 3d map of PCoA EMPeror (one point for one sample) in subgroups: adults, calves, young

Firmicutes (up to 69.3%) and *Bacteroidetes* (up to 31.5%) dominated in the composition of microflora at the level of phylum, the total share of which did not differ significantly in the groups. Bacteria of the phylum *Proteobacteria* (up to 1.5%), *Euryarchaeota* (up to 4.9%), *Verrucomicrobia* (up to 4.3%), and TM7 (up to 3.2%) were found to a lesser extent in the rumen community. The percentage of other phylum representatives (*Spirochaetes, Cyanobacteria, Actinobacteria, Verrucomicrobia, Planctomycetes, Nitrospirae, Chloroflexi, Synergistetes, Fibrobacteres, Fusobacteria*, etc.) is less than 1% of the total bacterial community.

At the family level, in most samples, *Ruminococcaceae* prevailed (up to 34.3%) (Figure 3b). The dominant taxa with a relative amount of more than 5% included unclassified bacteria of the order *Bacteroidales* (up to 18.0%) and the order *Clostridiales* (up to 16.7%). There was a tendency to an increase in the relative amount of these microorganisms in the group of adults as compared with the calves' group.

The presence of microorganisms which traditionally belong to the causative agents of various mammalian diseases, including members of family *Campylobacteraceae* (up to 0.1%), *Enterobacteriaceae* (up to 0.1%), *Pasteurellaceae* (up to 0.1%), and *Mycoplasmataceae* (up to 0.3%), etc in some individuals was noted.

During ontogenesis, no significant changes in the number of microorganisms were observed at the phylum level (Figure 4a). At lower taxonomic levels in the rumen microbiome, significant changes in the composition ratio of the rumen microbiota were identified (Figure4b and 5). As can be seen in Figure 3a, among the bacteria, the most affected phylum was *Firmicutes*. In the composition of this phylum (Figure 5), the proportion of bacteria of the genera *Ruminococcus*, *Buturivibrio*, *Coprococcus* in calves was higher compared with the young and adults'
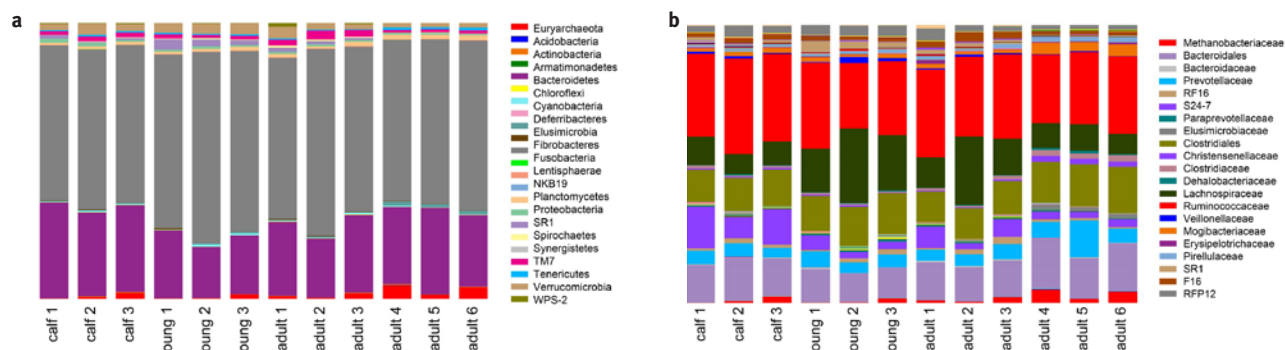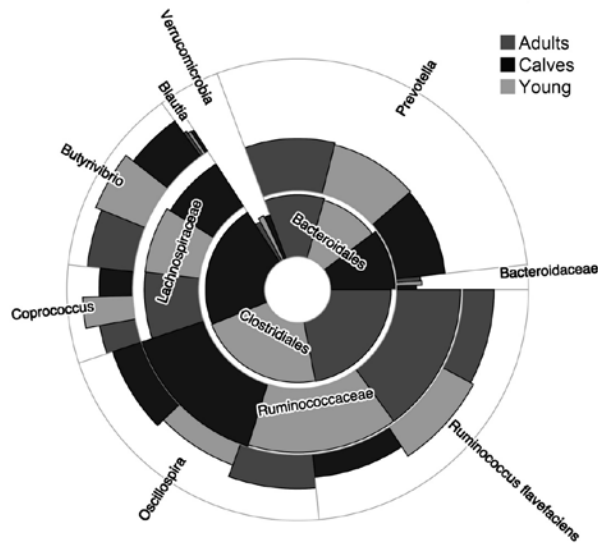

Figure 4: Biodiversity of the reindeer rumen microbiome at the level of phylum (a) and family (b): columns correspond to samples, rows to bacterial taxa

Figure 5: Biodiversity of reindeer rumen microbiome at low taxonomic levels: columns correspond to samples; strings correspond to bacterial taxas

groups (p <0.05). The proportion of acid-utilizing bacteria of the genera *Succiniclasticum* and *Selenomonas* in calves was lower compared with young and adult individuals (p <0.05).

4 Discussion

The vegetation microbiome was characterized by the dominance of *Proteobacteria, Actinobacteria, Acidobacteria, Chroloflexi, Gemmatimonadetes, Verrucomicrobia,* and *Planctomycetes*, while the rumen microbiome was characterized by the dominance of the following phyla of microorganisms: *Firmicutes* and *Bacteroidetes*, as well as the family *Ruminococcaceae*. *This indicates the complex transformation of the vegetation materials microbiological community during the food digestion.*

Previously, other authors, using cultural methods, showed significant limitations in studying the rumen's microbiota (Aagnes et al. 1995; Sundset et al. 2007; Sundset et al. 2009), which provided a far from complete picture. So, Sundset et al. (Sundset et al. 2007) showed a

limited number of species in the rumen microbial community of reindeer, including bacteria of the genus *Selenomonas*, class *Spirochetes*, species *Butyrivibrio fibrisolvens,* and *Streptococcus bovis*. However, it has been shown that the total number of some bacterial species in the reindeer rumen may vary depending on the sampling season. A change in the number of cellulolytic bacteria *Butyrivibrio fibrisolvens* (22% in summer and 30% in winter) and amylolytic bacteria *Streptococcus bovis* (17% in summer, 4% in winter) was noted.

In our study, using the NGS method in the rumen microbiota of reindeer, from 2,221 to 8,913 OTU were identified depending on the animal. The values of α-biodiversity parameters (Table 1) showed that animals tend to increase the number of OTUs with age. When assessing biodiversity, it is necessary to consider not only the qualitative composition of species (OTU), but also their relative amount or "evenness". The Shannon diversity index considers both the species richness and the OTU uniformity (Alimov 2000). The adult reindeer individuals showed a tendency to increase the Shannon diversity index and the level of phylogenetic diversity (PD whole tree). In addition, in adult animals, compared with calves and young animals, there is a tendency for their Chao1 index to increase (Figure 1), which, in addition to species richness, gives more weight to rare species. Thus, the obtained results indicate a greater heterogeneity of the rumen bacterial community in adults compared with calves and young individuals.

Comparison of the beta diversity of the rumen microbiota composition of different age groups using the principal component analysis (Figure 2) showed that the rumen microbiome compositions from the adults subgroup were separated into a segregated cluster and had the greatest displacement along the axis of the first main component PC1. This indicates a difference in the structure of the microflora in this age group compared with other subgroups (calves and young). This confirms the specificity of the rumen microbiome composition of adult reindeer individuals in comparison with other subgroups.

Previous studies have shown that specific microbial taxons can be presented in the digestive tract of the host organism. There may also be variations in the microbial community's composition of one species of animal or genotype (Smith et al. 2015). These observable intraspecific and interspecific variations of the microbiota composition can serve as indicators of the ecological processes that form the microbial community in interdependency with the host. The presence of changes in the indices of alpha diversity of the rumen microbiota in the reindeer specimens examined by us confirm the researchers' opinion

that the microbial community may also reflect the physiological state of the animals. In our opinion, changes in the rumen microbiota composition detected by us are logical, since it is known (Henderson et al. 2015) that ruminal digestion develops with age. However, in other ruminants, for example, in cattle, anatomical and functional changes characteristic of adult animals were noted already by the age of 6–8 months. In our studies rumen microbial communities of *Rangifer tarandus* showed the greatest changes in individuals of the adult group compared with the calves and young groups.

Based on taxonomic diversity, our results of the microbial communities assessment using the NGS method generally correspond to modern ideas about the rumen microbiota of both ruminants in general and reindeer (Salgado-Flores et al. 2016; Zielińska et al. 2016). In our study (Figure 3a), among the 25 phyla of identified bacteria, microorganisms of the phylum *Firmicutes* (up to 69.3%) and *Bacteroidetes* (up to 31.5%), which play an important role in food fermentation, dominated in reindeer rumen (Durso et al. 2010; Li et al. 2014; Delgado et al. 2017; Hu et al. 2017). Bacteria of other phylum (*Proteobacteria, Euryarchaeota, Verrucomicrobia*, TM7) were less represented in the reindeer rumen community. The percentage of other phylum representatives in the bacterial community is less than 1% (*Spirochaetes, Cyanobacteria, Actinobacteria, Verrucomicrobia, Planctomycetes, Nitrospirae, Chloroflexi, Synergistetes, Fibrobacteres, Fusobacteria*, etc.).

In other studies of ruminants, including reindeer, microorganisms of phylum *Firmicutes* and *Bacteroidetes* were also the most represented. For example, Pope et al. (2012) showed that the proportion of *Bacteroidetes* phylum was the highest (61%) in the reindeer rumen community and accounted for more than half of the entire community, and the proportion of *Firmicutes* phylum bacteria reached 30%. The remaining minor microorganisms were attributed to *Proteobacteria, Spirochetes* and *Chloroflexus*. In the fecal bacterial communities of reindeer, according to the results of Zielińska and co-authors (2016), more than 95% of the sequences among the 14 phyla were also represented by the *Firmicutes* and *Bacteroidetes*. In particular, the share of *Firmicutes* was 56.53%, and a and *Bacteroidetes* - 39.17% of the total number of sequences. The remaining 5% of the community was represented by *Tenericutes, Cyanobacteria*, TM7, *Actinobacteria, Proteobacteria, Verrucomicrobia, Elusimicrobia, Planctomycetes, Fibrobacteres, Spirochaetes, Chloroflexi*, and *Deferribacteres* (Zielińska et al. 2016).

Henderson et al. (2015) showed that the rumen microbiomes of various ruminants possess a core community that remained stable in all studied animals. The core community included such representatives of the phylum *Firmicutes* and *Bacteroidetes*, as bacteria of the genera *Prevotella, Butyrivibrio*, and *Ruminococcus*. Content in other members in microbial communities, such as the bacteria of the families *Lachnospiraceae, Ruminococcaceae, Bacteroidales* and *Clostridiales*, could vary depending on the diet and the environment, thereby determining the uniqueness of each type of ruminant (Henderson et al. 2015).

In this regard, it is interesting to consider the changes that are associated with the age of the animals. Our studies have shown the presence of statistically significant changes in the microbiota of the reindeer rumen in ontogenesis. Thus, according to Figure 4, during ontogenesis, the greatest changes were detected in the composition of the phylum *Firmicutes*. In the rumen of calves, the total content of cellulolytic bacteria of the genera *Ruminococcus, Buturivibrio*, and *Coprococcus*, which have the potential to hydrolyze the carbohydrates of vegetable feed to form volatile fatty acids (Hungate 1966), was higher for calves compared with the young and adults (p <0.05). The share of acid-utilizing bacteria of the genera *Succiniclasticum* and *Selenomonas* in the rumen of young and adult individuals was higher (p <0.05) compared with calves. This groups of microorganisms belong to the physiologically important group of microorganisms for ruminants, because they help to maintain the necessary acidity level in the rumen due to their ability to utilize monosaccharides, oligo-and polysaccharides of the acid (including acetic, propionic, and butyric), in dairy stock and others (Nocek et al. 1997),

In our study, microorganisms whose presence may be specific for reindeer compared with other subspecies of the ruminant family *Cervidae* were identified (Hofmann 1973; Hackmann et al. 2010; Henderson et al. 2015). First, the occurrence of *Cyanobacteria* microorganisms (up to 0.8%) in the rumen microbiota of the observed animals is of interest. Earlier, Zielińska et al. (2016) mentioned the presence of these microorganisms in the reindeer rumen, which seems to be quite natural, since *Cyanobacteria* belong to symbiotes of lichen, that appear to be one of the main reindeer feeding component, being up to 10-15% of the summer period ration for reindeer and up to 75% in the winter period ration (Mathiesen et al. 2000). According to Pankratov et al. (2017), the most common lichen cyanobionts are members of the genus *Nostoc*, and to a lesser extent, the genera *Calothrix, Scytonema* and *Fischerella* (Pankratov et al. 2017). We have identified cyanobacteria of the genera *Nostoc* and *Calothrix* in the *Rangifer tarandus* rumen.

Recent reports have shown that lichens are associated with a wide range of bacteria, among which are representatives of bacterial forms of *Actinobacteria, Firmicutes, Gammaproteobacteria, Bacteroidetes, Planctomycetes* and *Verrucomicrobia* (Bates et al. 2011; Sigurbjörnsdóttir et al. 2014). Thus, the question that lichens are related to symbiotic organisms, based on the interaction only between the fungus and photosynthetic algae (or cyanobacteria), remains controversial. Recent studies have led to the idea that microorganisms associated with lichens are a direct part of the lichen thallus. Interestingly, among lichen-associated microorganisms, many antagonistic bacteria have been identified. Lichen microbiome (*Lobaria pulmonaria* (L.) Hoffm) was studied in order to search for microorganisms with antagonistic activity to pathogens. Bacteria of the genera *Stenotrophomonas, Pseudomonas* and *Burkholderia* (Cernava et al. 2015) were dominant in the community.

In this context, the ability of some members of rumen microbial communities to detoxify the secondary metabolites of lichens, particularly usnic acid (Sundset et al. 2010), which was previously demonstrated by Sandset and co-authors, is also interesting. With the help of DGGE analysis, it was demonstrated that when adding usnic acid to the diet, there was no change in the rumen microbial community, which was represented by members of phylum *Firmicutes* (38.7%), *Bacteriodetes* (27.4%), *Verrucomicrobia* (14.5%), and *Proteobacteria* (1.6%) (Glad et al. 2014). On the other hand, Salgado-Flores et al. (2016) revealed the influence of toxic lichen substances on the reindeer rumen microbiome composition, reducing the concentration of microorganisms, which were sensitive to them (*Ruminococcus* sp.).

Thus, it can be concluded that several bacteria in the reindeer rumen, such as members of the phylum *Verrucomicrobia*, the genera *Stenotrophomonas*, *Pseudomonas* and other microorganisms, detected in our research, may have a pattern of their presence on various plants and lichens, which is partially confirmed by results of the investigation of the plant materials microbiome. The results we obtained are consistent with the notes of Cotillard et al. (2013) and Carmody et al. (2015), who believe that nutrition is the main part for the development of the microbial structure of the vertebrate intestinal tracts. According to the authors, the evolution of the intestinal microbiome of mammals is inextricably linked to the animal's nutrition. The animal, the intestinal microbiota and the type of feed exist in close relationship with each other (Ley et al. 2008). The inability of the first carnivorous mammals to digest cellulose from vegetable feed led to the formation of special organs and their associated microbial communities capable of converting inaccessible plant polysaccharides to accessible ones. This is clearly seen in the example of ruminant animals, which include reindeer. Hacquard et al. (2015) analyzed a large amount of metagenomic data to compare the microbial communities in the rhizosphere of various plants with the intestinal communities of vertebrates. It has been shown that bacteria belonging to the three main phylum (*Proteobacteria, Actinobacteria* and *Bacteroidetes*) dominate the rhizosphere of various plant species. At the same time, among the inhabitants of the intestines of animals, the dominant position is occupied by the phyla *Firmicutes* and *Bacteroidetes* representatives (Hacquard et al. 2015).

5 Conclusions

This study is the first report of the rumen microbial composition of vegetation materials and reindeer in different ages, living in the conditions of the Yamalo-Nenets Autonomous Region of the Russian Arctic. The results showed that the dominant position in microbial communities, like that of other ruminants, was occupied by representatives of the phyla *Firmicutes* and *Bacteroidetes*, whose total share between observed groups did not differ significantly. Digestion of vegetation by reindeers resulted in complex transformation in initial plant microbiome and in increasing biological diversity, which were expressed in increasing OTUs numbers and changes in indexes of alpha-diversity parameters. According to the results of alpha- and beta- diversity of the rumen microbial communities, the greatest uniqueness was revealed for the microbiomes of the adult in comparison with calves and young. In adults of reindeer there is a tendency towards an increase in the relative number of taxa dominating at a lower taxonomic level (with a relative abundance of more than 5%) of unclassified bacteria of the order *Bacteroidales* and the family of *Clostridiales*. The presence of changes in the biodiversity indices of the rumen microbiota in the reindeer examined by us, confirm the opinion of the researchers that the microbial community may also reflect the physiological state of the animals. It has also been demonstrated that the presence of the phylum *Verrucomicrobia*, the genera *Stenotrophomonas*, *Pseudomonas*, etc., may be specific to reindeer and have a pattern with their presence on various plants and lichens that are part of the reindeer diet. This is partially confirmed by data on microbiome taxonomic composition on the plants.

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