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RESEARCH ARTICLE

Comparative Analysis of Nasal Microbial Community between Tibetan Sheep with different Ages

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A B S T R A C T

Tibetan sheep is an important domestic animal inhabiting the Tibetan plateau, which is closely related to local economic development and national culture. Presently, there are many studies on the gut microbiota of Tibetan sheep, but little is known about the nasal microbial community. Here, we investigated the distributions and changes of the nasal microbial community in Tibetan sheep at different ages. Results of amplicon sequencing showed that 476,275 and 484,669 effective sequences were generated in the ASG (adult Tibetan sheep) and YSG (young Tibetan sheep) groups, respectively. These sequences were clustered into 6,817 OTUs and the ASG group and YSG group have 2,133 and 5,176 OTUs, respectively. Results of alpha diversity indicated that the ASG group had a significantly higher Chao1 index than the YSG, whereas Simpson and Shannon indices had no significant difference. Metastatistical analysis showed that compared with the ASG group, 8 bacterial phyla were significantly increased and 2 bacterial phyla were significantly decreased in the YSG group. At the genus level, 185 bacterial genera were significantly increased and 25 bacterial genera were significantly decreased in the YSG group compared with the ASG group. In conclusion, this study provided a preliminary analysis of the dynamic changes and distribution of nasal microbial community in Tibetan sheep. Results showed that the abundance and composition of the nasal microbial community of Tibetan sheep were significantly different at different ages.

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INTRODUCTION

Nasal cavity is one of the primary interfaces between the internal organism and external environment, which is also the important microbial barrier for preventing bacterial infections (Zeineldin *et al*., 2018). The continuous competition and interaction of microorganisms lead to the microbial community gradually change from simplicity to complicated and eventually form a complex ecosystem (Li *et al*., 2023). The normal microbial community play key roles in various physiological functions, whereas dysbacteriosis may cause many diseases (Khan *et al*., 2019). Constipation, colitis, diabetes, and obesity are closely related to gut microbial imbalance (Liu *et al*., 2022). Recent study on nasal microbial community has provided the evidence that the chronic rhinosinusitis may be the result of imbalance of nasal microbial community (Mahdavinia *et al*., 2018). The nasal mucosa also colonizes a large number of bacteria, which is same to the gastrointestinal tract. Increasing evidence revealed that microbial community play crucial roles in mucosal immunity, material metabolism, nutrient absorption and regulation. Moreover, multiple respiratory diseases are also associated with the changes in nasal microbial community (Lanaspa *et al*., 2017).

The altitude of the Tibetan plateau is between 3000-5000 meters, known as the spine of the world and the third pole (Lan *et al*., 2021; Chen *et al*., 2021). The environment of the Tibetan plateau is very harsh, including low temperature, lack of oxygen, and strong ultraviolet light due to its special geographical location (Liu *et al*., 2022). Tibetan sheep is an indigenous breed of the Tibetan plateau

characterized by strong anti-adversity, adapting hypoxic conditions and rough feed tolerance. This ancient breed has inhabited the Tibetan plateau for thousands of years and now it has strong adaptability to the local harsh ecological environment conditions (Liu *et al*., 2022; Lin *et al*., 2023). Sheep is an important source of meat and leather for local residents and is closely related to economic development and national culture (Oraby *et al.,* 2021; Zaki *et al.,* 2021; Al-Shammari and Sadoon, 2022). Given the importance of the Tibetan sheep on the Tibetan plateau, anything that endangers the health and development of this breed should be taken seriously. However, the health of Tibetan sheep is seriously threatened by extreme conditions, making them susceptible to respiratory diseases.

The microbial community and their functions of host could be affected by external environment (Naibaho *et al.,* 2021; Okeniyi *et al.,* 2022; Undugoda and Kannangara, 2022). Adak *et al*. (2013) reported that the short-term high-altitude exposure could significantly altered the gut microbial composition in mountaineers. Moreover, Li and Zhao also observed significant differences in the microbial community between human residing at high-altitude hypoxia region and lower mainland due to distinctive dietary habits and external circumstances (Li and Zhao, 2015). Therefore, Tibetan sheep may have evolved a specific microbial community that enables them to develop specific adaptations in terms of material metabolism and physiological functions. Presently, it has been demonstrated that Tibetan sheep possess special gut microbiota compared with other ruminants (Liu *et al*., 2021; Liu *et al*., 2022). However, little research has been performed on the nasal microbial community of Tibetan sheep. In this study, we explored the structural characteristics and differences of nasal microbial community in Tibetan sheep of different ages.

MATERIALS AND METHODS

Sample collection: Twelve Tibetan sheep obtained from Tibet, China, including 6 one-month and 6 one-year old populations. The ratio of females to males in both groups was 1:1. These Tibetan sheep were free-range on grassland and proved to be healthy. We swab the anterior nares with a sterile swab to obtain sample. Moreover, nasal swabs touching the nostril or upper lip skin were discarded to reduce contamination. The obtained nasal swabs were stored at -80°C for further study.

Illumine MiSeq sequencing: The specific steps and procedures of amplicon sequencing were performed based on the previous studies (Liao *et al*., 2022).

RESULTS

Sequences analyses: In this study, 12 samples collected from young and adult Tibetan sheep were subjected for amplicon sequencing. A total of 1,015,808 (ASG=504,287, YSG=511,521) raw sequence were obtained from ASG and YSG groups. After data processing, 960,944 (ASG=476,275, YSG=484,669) effective sequences were obtained, with an effective rate of about 94.59% (Table 1). The results of rarefaction and rank abundance curves showed that the current sequencing depth is enough for subsequent analysis (Fig. 1A, B, C). The effective sequences were clustered into 6,817 OTUs (ASG=2,133, YSG=5,176). Moreover, the unique OTUs of the ASG and YSG groups were 1,771 and 4,684, respectively (Fig. 1D).

Analysis of microbial community diversity in different groups: Good's coverage estimates of each sample varied from 99.85% to 99.93% and there is no significant difference between both groups (Fig. 2A). Moreover, the Chao index (505.83 ± 279.37 versus 1129.33 ± 441.77, $P = 0.02$) differed significantly between ASG and YSG groups, but the Simpson (0.90 ± 0.052 versus 0.84 ± 0.14, $P = 0.48$) and Shannon (5.00 ± 0.67 versus 5.51 ± 1.86, $P = 0.07$) indices were not dramatically different (Fig. 2B, C, D). These results showed that age can dramatically affect the nasal microbial community of Tibetan sheep. To further assess the effect of age on the nasal microbial community, we evaluated changes in the major microbial components of the ASG and YSG groups using beta analysis. The results showed that the both groups of samples showed a clear trend of separation, indicating significant changes in the main components of nasal microbial community (Fig. 3A. B).

Types and distribution of dominant bacterial phyla and genera: *Proteobacteria* (75.42% in ASG and 72.33% in YSG) and *Firmicutes* (13.04% in ASG and 14.93% in YSG) were the two most predominant phylum in all the samples, whereas *Bacteroidetes* (8.69%) and *Actinobacteria* (5.26%) were tertiary in the ASG and YSG, respectively (Fig. 4A). In addition, other phyla such as *Cyanobacteria* (0.15%, 0.61%), *Verrucomicrobia* (0.01%, 0.60%), *Acidobacteria* (0.13%, 0.42%), *Chloroflexi* (0.05%, 0.47%), *Tenericutes* (0.02%, 0.34%) and *Fusobacteria* (0.28%, 0.06%) in the ASG and YSG were represented with a lower abundance. At the level of genus, *Moraxella* (33.40%), *Mannheimia* (12.49%) and *Alysiella* (12.23%) were observed as the dominant in the ASG. Meanwhile, *Moraxella* (33.41%), *Mannheimia* (13.04%) and *Pseudomonas* (7.46%) were the predominant genera in the YSG (Fig. 4B). The heatmap can show the bacterial species and abundance distribution of each sample (Fig. 5).

The relative abundances of *Patescibacteria* and *Fusobacteria* in the ASG were significantly higher than the YSG, while the *Gemmatimonadetes*, *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Chloroflexi*, *Tenericutes*, *Deinococcus-Thermus* and *Kiritimatiellaeota* contents were lower (Table 2). In addition, 210 genera were significantly different between the ASG and YSG groups. Compared with the YSG group, 25 genera (*Rothia*, *Pasteurella*, *Granulicatella*, *Peptostreptococcus*, *Gracilibacteria_unclassified*, *Alysiella*, *Bergeyella*, *Bibersteinia*, *Enterococcus*, *Parvimonas*, *Acinetobacter*, *Succiniclasticum*, *Gemella*, *Porphyromonas*, *Streptococcus*, *Capnocytophaga*, *Prevotella_1*, *Absconditabacteriales_(SR1)_unclassified*, *probable_genus_10*, *Veillonellaceae_UCG-001*, *Caviibacter*, *Faucicola*, *Fusobacterium*, *Butyrivibrio_2* and *Neisseria*) were significantly increased and 185 genera (*Akkermansia*, *Methylobacterium*, *Chthoniobacter*, *Turicibacter*, *Modestobacter*, *Limnobacter*, *Acidiphilium*,

Table 1: Analysis of sequencing sequence.

Sample	Raw_Tags	Raw_Bases	Valid_Tags	Valid_Bases	Valid%	Q20%	Q30%	GC%
ASG1	84793	42.40M	80975	34.20M	95.50	97.78	93.33	51.98
ASG2	82291	41.15M	78115	33.25M	94.93	96.01	88.80	51.51
ASG3	80091	40.05M	74446	31.65M	92.95	97.54	92.55	50.93
ASG4	85550	42.77M	81815	34.72M	95.63	97.65	92.69	50.76
ASG5	84707	42.35M	77985	33.15M	92.06	90.80	78.38	52.17
ASG6	86855	43.43M	82939	35.14M	95.49	96.42	89.97	52.14
YSG1	87122	43.56M	83860	35.14M	96.26	97.90	93.66	52.09
YSG2	85956	42.98M	75327	32.01M	87.63	97.95	93.70	52.15
YSG3	85046	42.52M	80835	33.91M	95.05	94.35	85.04	52.56
YSG4	85662	42.83M	81980	33.25M	95.70	96.11	89.32	52.44
YSG5	87606	43.80M	84489	34.79M	96.44	97.79	93.34	52.07
YSG6	80129	40.06M	78178	33.05M	97.57	97.91	93.61	51.42

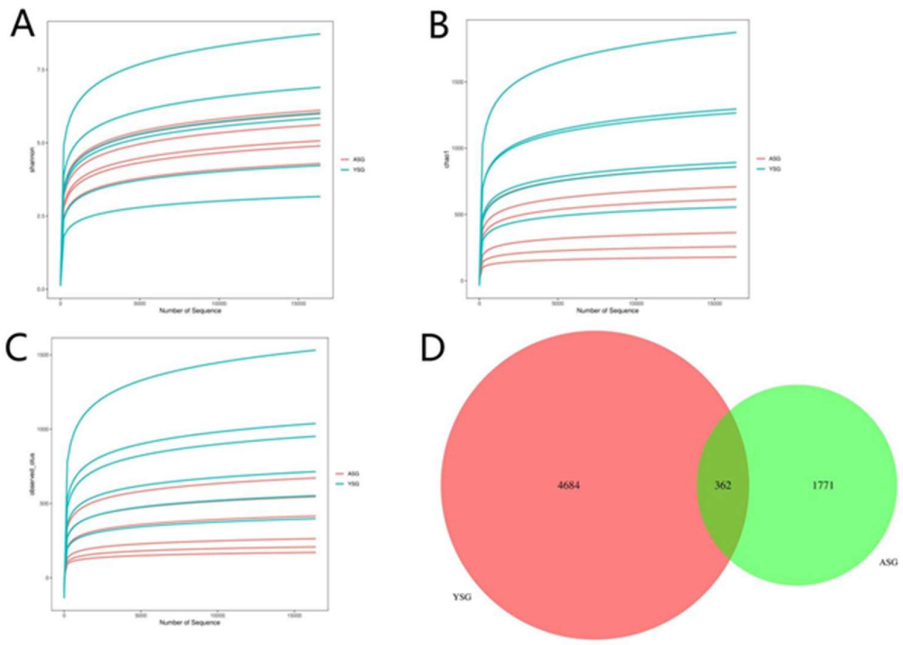

Fig. 1: Feasibility analysis (A, B, C) and Venn diagrams (D)

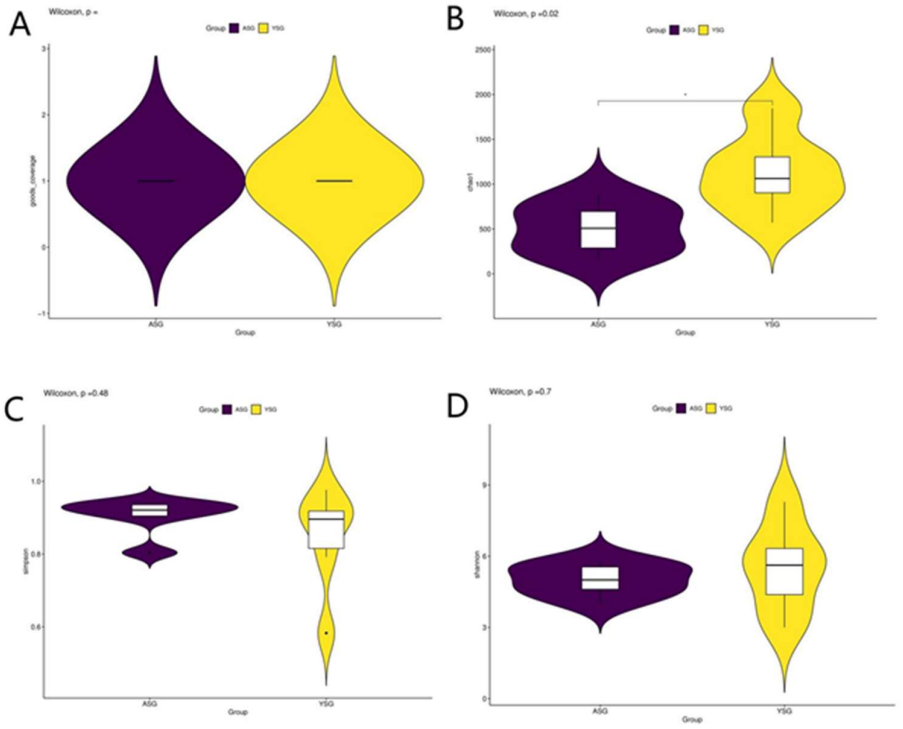

Fig. 2: Comparative analysis of alpha diversity of nasal microbial community

Table 2: Identification of differential bacteria at the phylum level

Taxa	ASG (%)	YSG (%)	P
Gemmatimonadetes	0.93	31.33	0.0037
Actinobacteria	48.94	525.58	0.0039
Patescibacteria	160.39	24.07	0.0039
Verrucomicrobia	1.03	60.21	0.0039
Planctomycetes	2.59	30.22	0.0039
Chloroflexi	5.34	46.53	0.0065
Tenericutes	1.72	33.95	0.0103
Deinococcus-Thermus	0	1.94	0.0222
Fusobacteria	28.32	5.79	0.025
Kiritimatiellaeota	0.05	1.55	0.0495

Table 3: Identification of differential bacteria at the genera level

Taxa	ASG (%)	YSG (%)	P
Akkermansia	0.00	41	0.0021
Rothia	21.56	0	0.0021
*BRC1*_unclassified	0.00	6	0.0021
*AKYG1722*_unclassified	0.00	5	0.0021
Methylobacterium	0.00	5	0.0021
Chthoniobacter	0.00	5	0.0021
Turicibacter	0.00	5	0.0021
Modestobacter	0.00	4	0.0021
*Chitinophagaceae*_unclassified	0.00	4	0.0021
67-14_unclassified	0.00	2	0.0021
Limnobacter	0.00	2	0.0021
Eubacterium] _hallii_group	0.00	2	0.0021
Acidiphilium	0.00	2	0.0021
Pasteurella	136.67	1	0.0028
Granulicatella	123.14	0	0.0028
Citricoccus	0.37	37	0.0028
Dietzia	0.16	17	0.0028
*Clostridiales_vadinBB60_group*_unclassified	0.08	17	0.0028
Kocuria	0.15	13	0.0028
Gemmatimonas	0.07	12	0.0028
Phascolarctobacterium	0.11	7	0.0028
*Acidobacteria*_unclassified	0.14	7	0.0028
Lysobacter	0.14	7	0.0028
Actinotalea	0.10	6	0.0028
Peptostreptococcus	5.86	0	0.0028
Pir4_lineage	0.10	5	0.0028
*Frankiales*_unclassified	0.07	4	0.0028
Iamia	0.07	4	0.0028
Marmoricola	0.07	3	0.0028
*Planctomycetales*_unclassified	0.19	3	0.0028
Rhodococcus	0.14	3	0.0028
*Gracilibacteria*_unclassified	129.65	0	0.0033
*JG30-KF-CM45*_unclassified	0.20	26	0.0033
Ornithinimicrobium	0.61	26	0.0033
*Longimicrobiaceae*_unclassified	0.43	9	0.0033
*Saccharimonadaceae*_unclassified	0.25	8	0.0033
Ornithinicoccus	0.15	8	0.0033
Sanguibacter	0.35	6	0.0033
*S0134*_terrestrial_group_unclassified	0.38	4	0.0033
Luteolibacter	0.38	4	0.0033
*Microscillaceae*_unclassified	0.16	3	0.0033
Planomicrobium	0.78	61	0.0037
Planococcus	0.98	46	0.0037
Paracoccus	1.37	23	0.0037
Georgenia	0.78	18	0.0037
*Betaproteobacteria*_unclassified	0.85	8	0.0037
Alysiella	1222.54	3	0.0039
Bergeyella	423.61	2	0.0039
Bibersteinia	235.05	4	0.0039
Corynebacterium_1	2.87	91	0.0039
Romboutsia	0.73	54	0.0039
Brachybacterium	2.24	50	0.0039
Jeotgalicoccus	1.50	46	0.0039
Ruminococcaceae_UCG-013	0.84	43	0.0039
Clostridium	1.43	24	0.0039
Altererythrobacter	0.86	16	0.0039
*Actinobacteria*_unclassified	1.29	13	0.0039
Psychrobacter	0.48	24	0.0048
Enterococcus	11.46	0	0.0048
*Gastranaerophilales*_unclassified	0.20	9	0.0048
*WD2101_soil_group*_unclassified	0.10	4	0.0048
olirubrobacter	0.08	2	0.0048
Parvimonas	7.87	0	0.0056
Bryobacter	0.22	3	0.0056
*Burkholderiales*_unclassified	0.59	6	0.0061
Acinetobacter	347.74	41	0.0065
Ruminococcaceae_UCG-005	5.39	105	0.0065
Luteimonas	1.49	30	0.0065
Succiniclasticum	30.89	0	0.0074
Gemella	7.59	0	0.0074
*dgA-11*_gut_group	0.00	7	0.0074
*R7C24*_unclassified	0.00	4	0.0074
Olsenella	0.00	4	0.0074
Dorea	0.00	4	0.0074
Ilumatobacter	0.00	4	0.0074
Agrococcus	0.00	3	0.0074
Jeotgalibaca	0.00	3	0.0074
Agathobacter	0.00	3	0.0074
Atopococcus	0.00	3	0.0074
Terrisporobacter	0.00	3	0.0074
Paeniclostridium	0.00	3	0.0074
*Gemmatimonadaceae*_unclassified	0.00	3	0.0074
Thermomonas	0.00	2	0.0074
Haliangium	0.00	2	0.0074
Aequorivita	0.00	2	0.0074
Pirellula	0.00	2	0.0074
Peredibacter	0.00	2	0.0074
Blastocatella	0.00	2	0.0074
Pontibacter	0.00	2	0.0074
*Rhodobacteraceae*_unclassified	0.00	2	0.0074
*Acetobacteraceae*_unclassified	0.00	2	0.0074
Verticia	0.00	2	0.0074
Facklamia	0.00	1	0.0074
Aminobacterium	0.00	1	0.0074
Noviherbaspirillum	0.00	1	0.0074
Family_XIII_AD3011_group	0.54	15	0.0091
Arthrobacter	0.99	14	0.0091
Caenimonas	0.61	5	0.0091
Fermentimonas	0.18	4	0.0091
Desemzia	0.60	24	0.0099
Nocardioides	0.78	19	0.0103
Flavobacterium	1.14	12	0.0103
Porphyromonas	172.50	13	0.0104
Sphingomonas	8.20	35	0.0104
Devosia	7.61	32	0.0104
Brevibacterium	0.35	8	0.0128
Corynebacterium	0.12	12	0.0132
Solibacillus	0.10	7	0.0132
*Sandaracinaceae*_unclassified	0.10	5	0.0132
Pseudoflavonifractor	0.12	4	0.0132
Tyzzerella_4	0.05	4	0.0132
Yaniella	0.13	3	0.0132
Gemmatirosa	0.05	2	0.0132
Bacillus	0.13	2	0.0132
Ruminococcus_2	0.61	6	0.0145
*Proteobacteria*_unclassified	0.50	4	0.0145
Allorhizobium-Neorhizobium-Pararhizobium-	0.39	4	0.0145
Rhizobium	1.42	18	0.0156
Chryseobacterium	0.63	6	0.0156
*Cyclobacteriaceae*_unclassified	2.93	27	0.0161
Prevotellaceae_UCG-004	0.69	5	0.0161
Ruminiclostridium_5	712.29	275	0.0163
Streptococcus	3.14	14	0.0163
Pedobacter	0.13	4	0.0201
Lachnoclostridium	0.32	3	0.0201
Comamonas	0.16	2	0.0201
Reyranella	0.38	3	0.021
Bifidobacterium	0.05	2	0.021
Adhaeribacter	19.36	0	0.0222
Capnocytophaga	13.16	0	0.0222
Prevotella_1	8.48	0	0.0222
*Absconditabacteriales_(SR1)*_unclassified	0.00	5	0.0222
Salinimicrobium	0.00	4	0.0222
Roseimaritima	0.00	3	0.0222
*Carnobacteriaceae*_unclassified	0.00	3	0.0222
*Rhodanobacteraceae*_unclassified	0.00	3	0.0222
*Izimaplasmatales*_unclassified	0.00	3	0.0222
Algoriphagus	2.51	0	0.0222
probable_genus_10	0.00	2	0.0222
Rhodopirellula	0.00	2	0.0222
*Ilumatobacteraceae*_unclassified	0.00	2	0.0222

Ellin6055	0.00	2	0.0222
KD4-96_unclassified	0.00	2	0.0222
Arenimonas	0.00	2	0.0222
Victivallis	0.00	2	0.0222
Lachnospiraceae_UCG-010	1.88	0	0.0222
Veillonellaceae_UCG-001	0.00	2	0.0222
Segetibacter	0.00	2	0.0222
Truepera	0.00	2	0.0222
Clostridium_sensu_stricto_1	0.00	2	0.0222
Cellvibrionaceae_unclassified	0.00	2	0.0222
Proteiniclasticum	0.00	2	0.0222
Hymenobacter	0.00	2	0.0222
Gemmata	0.00	2	0.0222
Parvibacter	0.00	2	0.0222
Paeniglutamicibacter	0.00	2	0.0222
Vitellibacter	0.00	1	0.0222
Microtrichaceae_unclassified	0.00	1	0.0222
Dokdonella	0.00	1	0.0222
Amaricoccus	0.00	1	0.0222
Barnesiellaceae_unclassified	0.00	1	0.0222
Coprococcus_2	0.00	1	0.0222
Nitrosospira	1.10	0	0.0222
Caviibacter	0.00	1	0.0222
Bdellovibrio	0.00	1	0.0222
Leifsonia	0.00	1	0.0222
Gemmataceae_unclassified	0.00	1	0.0222
Mumia	0.00	1	0.0222
Tessaracoccus	0.00	1	0.0222
Acidaminobacter	0.00	1	0.0222
Geminicoccus	0.00	1	0.0222
Gaiella	0.00	0	0.0222
Aeriscardovia	0.40	2	0.0225
Lautropia	2.15	11	0.024
Atopostipes	2.21	80	0.0247
Ruminococcaceae_UCG-010	4.85	55	0.025
Eubacterium]_coprostanoligenes_group	2.69	11	0.025
Stenotrophomonas	0.49	7	0.0309
Intestinimonas	0.42	4	0.0309
Blastococcus	0.12	5	0.0326
Pseudonocardia	2.65	0	0.0326
Faucicola	1.27	4	0.0341
Eubacterium]_nodatum_group	1.41	7	0.036
Mollicutes_RF39_unclassified	1.21	5	0.036
Aeromicrobium	0.79	5	0.036
Massilia	24.74	5	0.0374
Fusobacterium	2.31	22	0.0374
Alistipes	0.33	9	0.0416
Ruminococcaceae_UCG-009	0.95	4	0.0416
Qipengyuania	0.33	2	0.0416
Desulfovibrio	0.54	3	0.0463
Escherichia-Shigella	0.61	4	0.0493
Cellvibrio	0.29	4	0.0493
Candidatus_Soleaferrea	0.71	3	0.0493
Rhizobium	0.48	3	0.0493
Leucobacter	0.51	2	0.0493
Prolixibacteraceae_unclassified	0.37	1	0.0493
Pedosphaeraceae_unclassified	0.31	18	0.0495
Filobacterium	17.80	0	0.0495
Butyrivibrio_2	0.20	4	0.0495
Gillisia	0.34	4	0.0495
Bradyrhizobium	3.37	0	0.0495
Neisseria	0.12	3	0.0495
Carnobacterium	0.07	3	0.0495
Gaiellales_unclassified	0.10	3	0.0495
Microtrichales_unclassified	0.31	2	0.0495
Caldilineaceae_unclassified	0.19	2	0.0495
Ferruginibacter	0.08	2	0.0495
AKIW781_unclassified	0.05	2	0.0495
WCHB1-41_unclassified	0.12	1	0.0495
Porphyrobacter			

Citricoccus, *Dietzia*, *Kocuria*, *Gemmatimonas*, *Phascolarctobacterium*, *Lysobacter*, *Actinotalea*, *Marmoricola*, *Rhodococcus*, *Ornithinimicrobium*, *Ornithinicoccus*, *Sanguibacter*, *Luteolibacter*, *Planomicrobium*, *Planococcus*, *Paracoccus*, *Georgenia*, *Corynebacterium_1*, *Romboutsia*, *Brachybacterium*, *Jeotgalicoccus*, *Ruminococcaceae_UCG-013*, *Clostridium*, *Altererythrobacter*, *Psychrobacter*, *Agrococcus*, *Jeotgalibaca*, *Agathobacter*, *Atopococcus*, *Terrisporobacter*, *Paeniclostridium*, *Thermomonas*, *Haliangium*, *Aequorivita*, *Pirellula*, *Peredibacter*, *Blastocatella*, *Pontibacter*, *Verticia*, *Facklamia*, *Aminobacterium*, *Noviherbaspirillum*, *Arthrobacter*, *Caenimonas*, *Fermentimonas*, *Desemzia*, *Nocardioides,* *Flavobacterium,* etc.) were significantly decreased in the ASG (Table 3).

DISCUSSION

Increasing research showed that nasal microbial community play an important role in nasal health. Nasal microbial community is an important barrier against the invasion of pathogenic bacteria, indicating that the nasal microbial community plays an important role in disease development (Wang *et al*., 2019). Consequently, exploring the nasal microbial community is of great importance. To date, the structure and composition of gut microbial community in many species have been deeply investigated including chicken, pig, cattle and goat (Meale *et al*., 2017). However, little is known about the nasal microbial population in Tibetan sheep. The nasal cavity is the key portion of the upper respiratory mucosal system, and its microbial community composition can be influenced by the age. In the present study, we compared and analyzed the bacterial abundance and diversity of nasal cavity of Tibetan sheep with different ages.

Age has long been considered as a vital factor affecting the structure and composition of microbial community. Previous research has shown that the gastrointestinal and nasal microbiota are in a dynamic development process and reached a steady state at maturity. Bender *et al*. (2020) indicated that the nasal microbial abundance and diversity of cattle gradually improved and derived to maturity arrangement with age. In this study, the nasal microbial diversity of Tibetan sheep changed dramatically with age, which was in line with previous observations in ruminants (Liu *et al*., 2021). Interestingly, our results indicated that the adult Tibetan sheep possessed a higher number of OTUs as compared to the adult Tibetan sheep. Moreover, the average of Chao1 indices in the young Tibetan sheep was higher than that in the adult Tibetan sheep, suggesting that the young's possessed the higher microbial community richness. We speculated that the young Tibetan sheep evolved a richer nasal microbial community in order to resist harsh environments. To further validate our results, we also performed beta diversity analysis. The results showed that there were significant differences in the main components of nasal microbial community between young and adult Tibetan sheep. Although these Tibetan sheep lived in the same area and had the same diet, their nasal flora were significantly different. Therefore, we speculated that age is an important factor leading to nasal changes.

Studies have shown that microbial composition consistently changes with external factors such as age, diet and environment. In this research, results showed that *Firmicutes* and *Proteobacteria* were the main dominant phylum. Likewise, these bacterial phyla are also abundant in the nasal cavity of yaks (Liu *et al*., 2021). These results

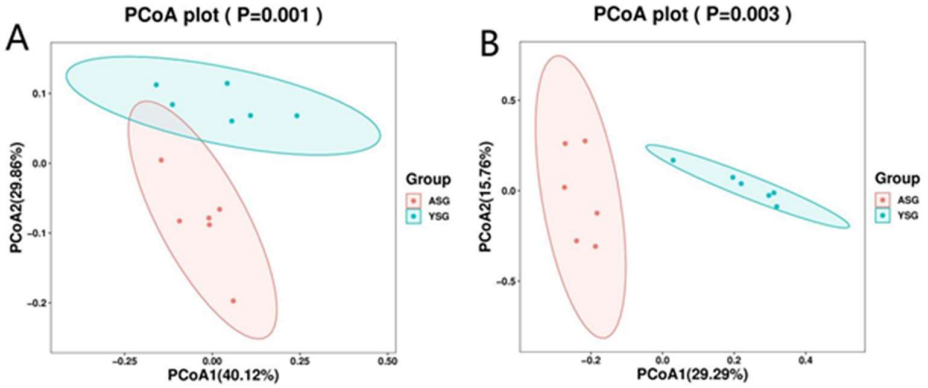

Fig. 3: Comparative analysis of beta diversity of nasal microbial community.

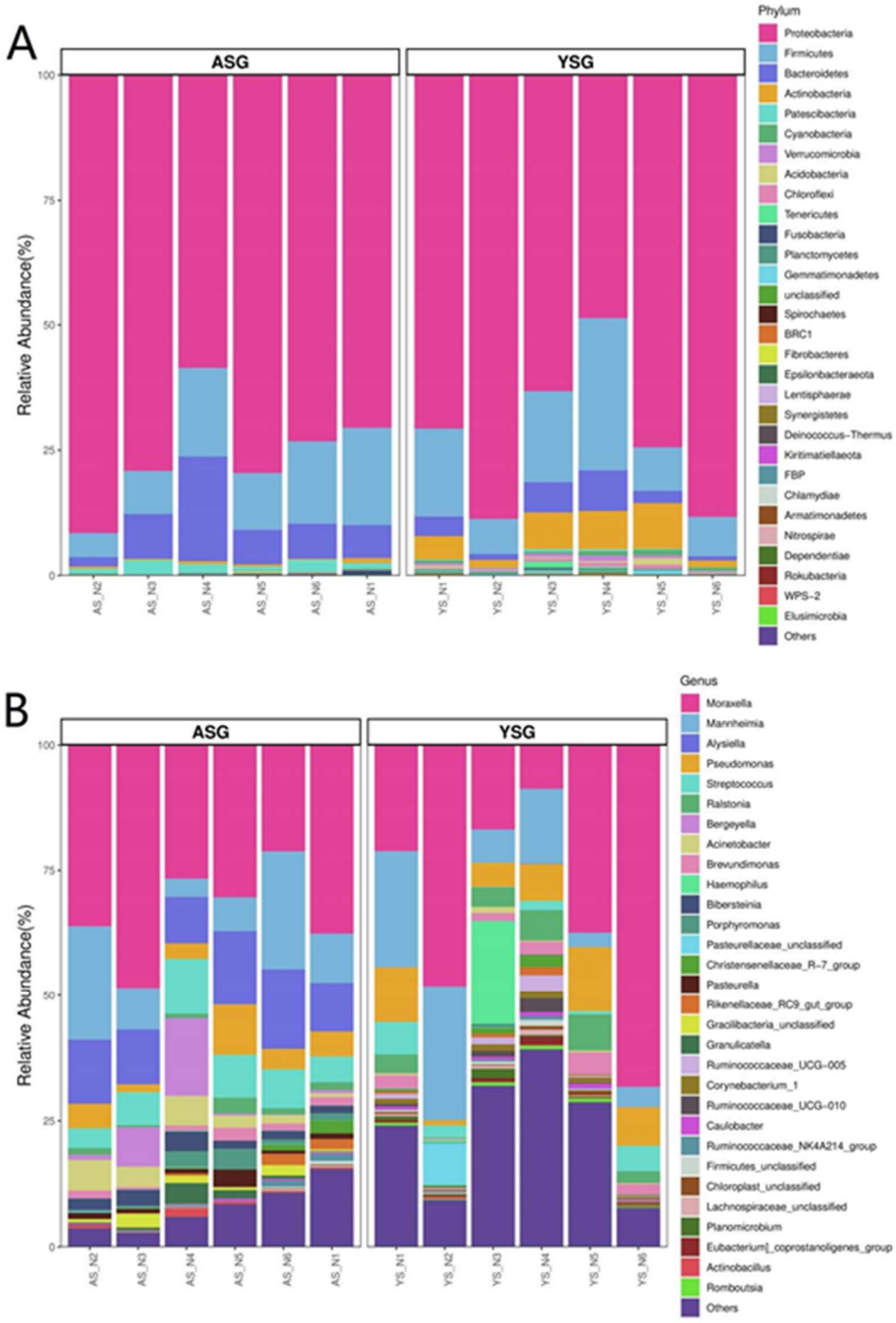

Fig. 4: Types and abundances of dominant bacterial phyla (A) and genera (B) in nasal microbial community.

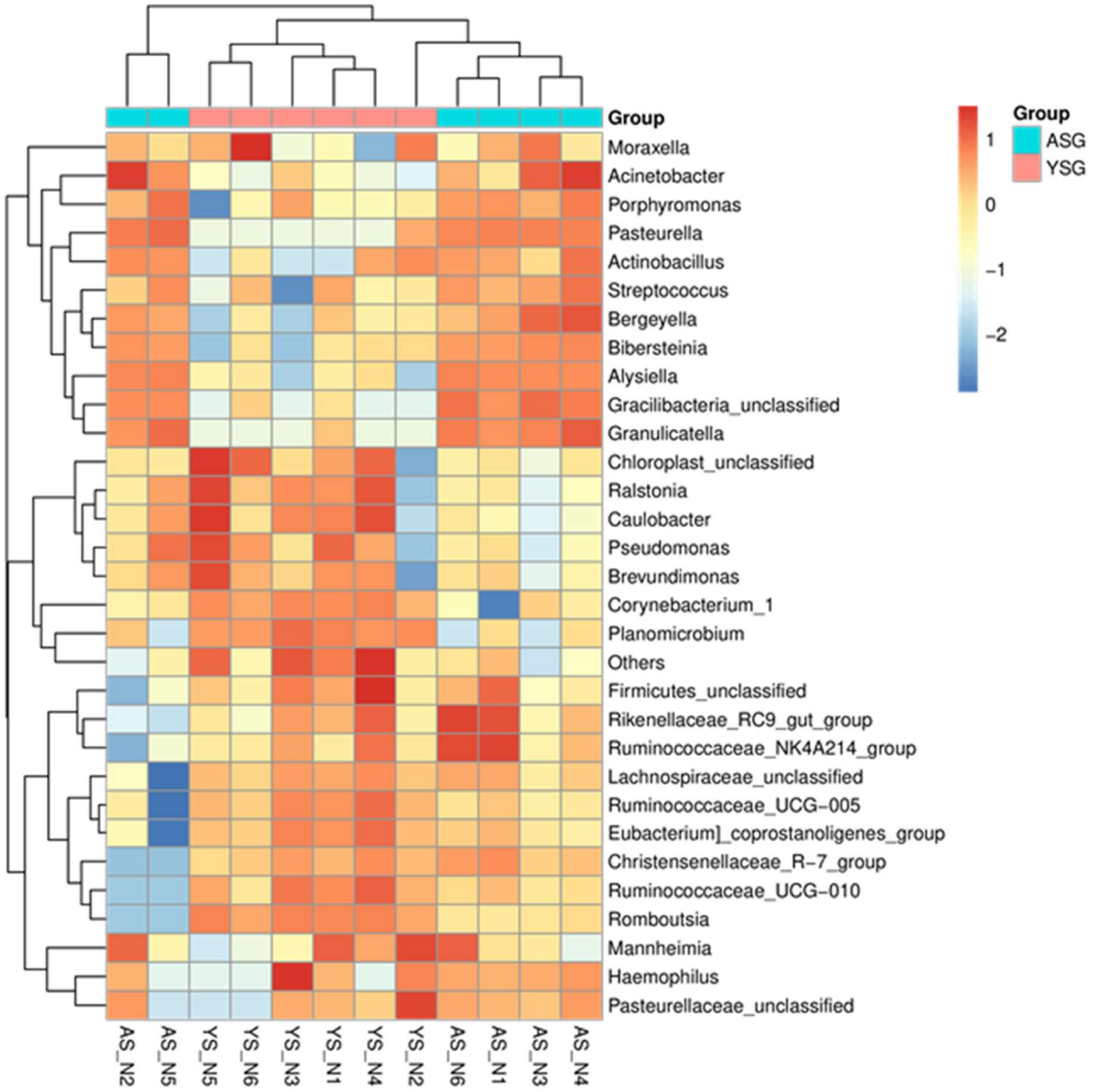

Fig. 5: Heat map at the bacterial genus level.

suggest an important role of *Firmicutes* and *Proteobacteria* in the nasal microbial community. The alterations in diversity and richness of microbial community can be used to detect host health status and determine various diseases. Therefore, we further calculated and analyzed the bacterial differences between the both groups of Tibetan sheep. The results showed that many bacteria varied significantly between the both groups. Notably, most bacteria showed a downward trend with age. *Akkermansia* is the potential beneficial bacterium in the intestine and show the positive regulation of the immune barrier function (Ansaldo *et al*., 2019). Additionally, its abundance of *Akkermansia* is negatively associated with obesity, inflammation and diabetes (Zhou *et al*., 2020). *Ruminococcus* has been shown to play a role in cellulose and starch degradation. *Psychrobacter* has been shown to be abundant in low-environments (Bakermans, 2018). In the current study, we observed many *Psychrobacter*, which is may be related to the the very cold environment of the Tibetan plateau. It has been reported that *Capnocytophaga* contributes to the occurrence of meningitis and septicaemia (Hannon *et al*., 2020). Early investigations showed that *Kocuria* could result in several diseases such as bacteremia and peritonitis (Dotis *et al*., 2015). It is well known that *Terrisporobacter* can lead to surgical site infection (Cheng *et al*., 2016). As a pathogenic bacterium, *Facklamia* has been demonstrate to septicemia and meningitis (Parvataneni *et al*., 2015). Previous research has shown that *Flavobacterium* could result in sepsis, pneumonia and meningitis during the immunity decline. *Brevundimonas* is closely related to the development of bacteremia. *Corynebacterium* is an acclimatized pathogen, which may lead to respiratory disease (Jaen-Luchoro *et al*., 2020). *Peptostreptococcus* was previously showed to result in spondylodiscitis and tumour formation (Diakakis *et al*., 2017). *Brachybacterium* may be related to bloodstream infection (Tamai *et al*., 2018). *Clostridium* may result in intestinal toxemia and diarrhea (Higashihara *et al*., 2018). Moreover, its toxins could also injury the host via multiple pathways. *Acinetobacter* has been shown to be widely present in the respiratory tract, skin and intestine. Moreover, it could lead to urinary infection, bacteremia and pneumonia (Liu *et al*.,

2019). As opportunistic pathogen, *Enterococcus* is associated with many diseases' development such as meningitis and sepsis (Mohanty *et al*., 2005). Although young yaks had a higher abundance of nasal microbial community, there were many pathogenic bacteria. We speculated that young yaks require a more abundant bacterial stable microbial structure to reduce disease.

Conclusions: In conclusion, this research showed that the abundance of nasal microbial community in young Tibetan sheep was significantly higher than that in adult Tibetan sheep. Moreover, age also caused significant changes in the main components of nasal microbial community. Meanwhile, we also observed significant changes in nasal microbial community among different ages. The abundances of *Patescibacteria* and *Fusobacteria* was increased in the ASG, whereas *Gemmatimonadetes*, *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Chloroflexi*, *Tenericutes*, *Deinococcus-Thermus* and *Kiritimatiellaeota* were reduced compared with YSG. Moreover, we also observed 210 genera were significantly different between the ASG and YSG groups. Our research has initially revealed the characteristics and changes of the nasal microbial community of Tibetan sheep at different ages, which may be helpful for the prevention and control of respiratory diseases in Tibetan sheep. However, this study has some limitations that need to be noted including small sample size and uncontrollable dietary factors and individual differences.

Authors contribution: ZL and XD conceived and designed the experiments. HJ, RZ, XC, HY, YZ, ST, XW, QW, WX contributed sample collection and reagents preparation. ZL analyzed the data. ZL wrote the manuscript. DF and MUS revised the manuscript. All authors reviewed the manuscript.

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