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Mongolians core gut microbiota and its correlation with seasonal dietary changes

Jiachao Zhang[1]*, Zhuang Guo[1]*, Angela An Qi Lim[2]*, Yi Zheng[1], Eileen Y. Koh[2], Danliang Ho[2], Jianmin Qiao[1], Dongxue Huo[1], Qiangchuan Hou[1], Weiqiang Huang[1], Lifeng Wang[1], Chimedsuren Javzandulam[1], Choijilsuren Narangerel[1], Jirimutu[1], Menghebilige[1], Yuan-Kun Lee[2] & Heping Zhang[1]

[1]Key Laboratory of Dairy Biotechnology and Engineering, Education Ministry of P. R. China, Department of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot 010018, China, [2]Department of Microbiology, Yong Loo Ling School of Medicine, National University of Singapore, 5 Science Drive 2, Singapore 117597, Singapore.

Correspondence and requests for materials should be addressed to Y.-K.L. (yuan_kun_lee@nuhs. edu.sg) or H.P.Z. (hepingdd@vip.sina. com)

* These authors contributed equally to this work.

Historically, the Mongol Empire ranks among the world's largest contiguous empires, and the Mongolians developed their unique lifestyle and diet over thousands of years. In this study, the intestinal microbiota of Mongolians residing in Ulan Bator, TUW province and the Khentii pasturing area were studied using 454 pyrosequencing and q-PCR technology. We explored the impacts of lifestyle and seasonal dietary changes on the Mongolians' gut microbes. At the phylum level, the Mongolians's gut populations were marked by a dominance of *Bacteroidetes* (55.56%) and a low *Firmicutes* to *Bacteroidetes* ratio (0.71). Analysis based on the operational taxonomic unit (OTU) level revealed that the Mongolian core intestinal microbiota comprised the genera *Prevotella*, *Bacteroides*, *Faecalibacterium*, *Ruminococcus*, *Subdoligranulum* and *Coprococcus*. Urbanisation and life-style may have modified the compositions of the gut microbiota of Mongolians from Ulan Bator, TUW and Khentii. Based on a food frequency questionnaire, we found that the dietary structure was diverse and stable throughout the year in Ulan Bator and TUW, but was simple and varied during the year in Khentii. Accordingly, seasonal effects on intestinal microbiota were more distinct in Khentii residents than in TUW or Ulan Bator residents.

G astrointestinal (GI) microbiota play an important role in the health and wellbeing of the host[1]. Several studies have shown that the intestinal microbiota fluctuates in response to a variety of intrinsic and extrinsic factors, such as host health[2], genetic composition[3], age[4] and diet[5]. Among all factors, genotype and diet have been suggested to be the main components that exert a significant influence on the balance of GI microbiota.

Mongolian nationality originates from a tribe that was located in Northern China during the seventh century[6,7]. The Mongol Empire, one of the world's largest contiguous empires, exerted a major influence that greatly enhanced the cultural exchange between China and the occident that took place during the Middle Ages. In Mongolia today, more than 40% of the population lives in typical pasture areas (such as Khentii Province) and maintains a traditional nomadic lifestyle and diet. In contrast, many Mongolians living in Ulan Bator (the capital of Mongolia) and TUW Province (the suburbs of the capital) have adopted an urban lifestyle because of modernisation and economic development. However, little is known about the structure of Mongolian gut microbiota or how their microbial community is affected by such changes.

The typical Mongolian diet is characterised by a high and frequent consumption of fermented dairy products, red meat and liquor[6]. In the pastures of Khentii, locals exhibit a distinct seasonal variation in their food con-sumption. Meat and meat products are the main sources of energy during winter and spring (November to April), whereas dairy products are the main source during summer and autumn (May to October)[8]. However, in Ulan Bator, food is abundant and diverse; therefore, the diet in this city exhibited limited changes throughout the year. Given these divergent dietary lifestyles, Mongolians are excellent candidates to study the effects of seasonal dietary changes on intestinal microbiome compositions.

In a previous study, we described the profiles of the gut microbiota of Chinese Mongolians living in Inner Mongolia province by denaturing gradient gel electrophoresis (DGGE) and quantitative polymerase chain
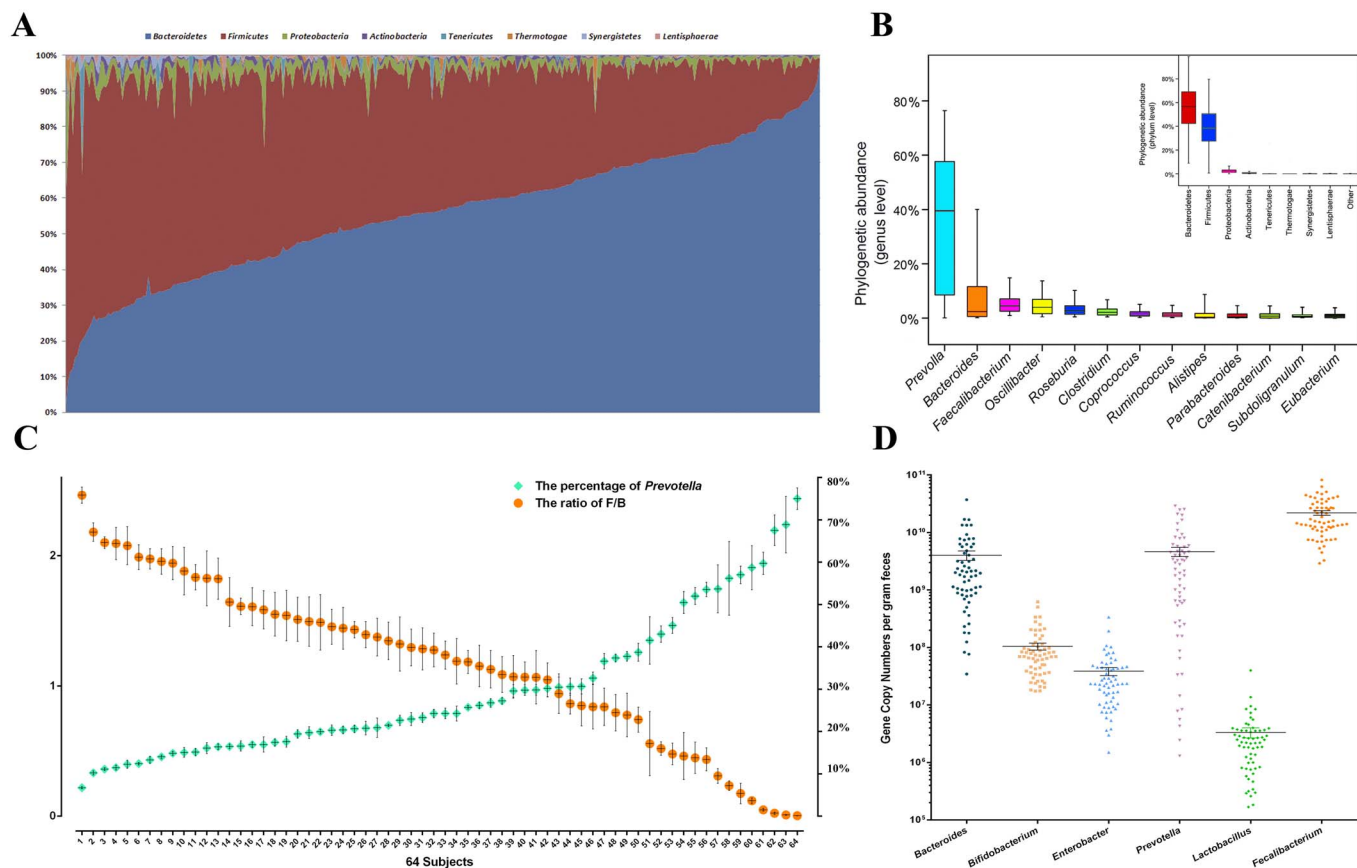
Figure 1 | The composition of intestinal microbiota of Mongolians. (A) Inter-individual variation in the proportion of major phyla. (B) Box-plots showing bacterial compositions at genus and phylum level; maximum and minimum values are indicated using whiskers. (C) Inter-individual variation in the proportion of the genus *Prevotella* and the ratio of *Firmicutes* to *Bacteroidetes* (F/B). (D) The amounts of *Bacteroides*, *Bifidobacterium*, *Enterobacter*, *Prevotella*, *Lactobacillus* and *Faecalibacterium* as quantified using q-PCR.

reaction (q-PCR) techniques[9]. However many Chinese Mongolians have inter-married with Han nationality race which is rare in Mongolia. Thus Mongolians in Mongolia were more authentic at the gene level. Moreover, the pyrosequencing has been suggested a more appropriate approach for intestinal microbiota diversity analysis than DGGE.

In the present study, 320 faecal samples were collected from 64 Mongolians distributed in three areas (Ulan Bator, TUW and Khentii) at five time points (January, March, June, September and November). 454 pyrosequencing combined with q-PCR technology were applied to explore the structure of Mongolians' gut microbiota and the effects of seasonal dietary changes on their intestinal microbiota.

Results

Sequencing coverage and estimation of bacterial diversity. In this study, the microbiotic compositions of the faecal samples were examined using a high-throughput 454 pyrosequencing technique. We generated a dataset consisting of 3,795,726 filtered high-quality and classifiable 16S rRNA gene sequences, and an average of 11,843 sequences was obtained for each individual (range: from 2,780 to 30,480). All sequences were clustered with representative sequences, and a 97% sequence identity cut-off was used. The number of OTUs per sample ranged between 118 and 1,815 (Table S3). The Simpson index, Chao1 index, Shannon index and observed number of species were estimated using the QIIME platform (Table S3).

The composition of intestinal microbiota in Mongolians. At the phylum level (Fig. 1A and 1B), *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* constituted the four most dominant

bacterial phyla (contributing 55.56%, 39.53%, 2.68% and 0.85% of the total amount of sequences, respectively). For all participants, the average ratio of *Firmicutes* to *Bacteroidetes* (F/B) was 0.711 (range: from 0.006 to 2.253, Fig. 1C). At the genus level (Fig. 1B), *Prevotella* of the *Firmicutes* phylum was the most abundant genus (contributing to 36.31% of the total number of sequences), and the amounts of *Bacteroides*, *Faecalibacterium*, *Oscillibacter*, *Roseburia*, *Clostridium*, *Coprococcus*, *Ruminococcus*, *Alistipes*, *Parabacteroides*, *Catenibacterium*, *Subdoligranulum* and *Eubacterium* all exceeded 1%. Correlations among the genera that contributed more than 0.1% of the total number of sequences in Mongolians were determined based on Spearman's rank correlation (Fig. S1A). A general negative correlation was found between *Prevotella* and other genera. Using genus-specific primers, we quantified the predominant microbiota in the human gut (Fig. 1D). The amounts of *Bacteroides*, *Bifidobacterium*, *Enterobacter*, *Prevotella*, *Lactobacillus* and *Faecalibacterium* genera were 9.61 ± 0.13, 8.02 ± 0.85, 7.59 ± 0.21, 9.66 ± 0.17, 6.53 ± 0.18 and 10.34 ± 0.71 in log-transformed 16S rDNA gene copy number per gram of sample, respectively (Fig. 1D).

The core intestinal microbiota of Mongolians. A major aim of the present study was to determine whether a common core microbiota is shared among all or the vast majority of the Mongolian participants. Using a detailed OTU analysis, we were able to assign 22 core OTU candidates (out of 19,451 OTUs identified in this study) (Fig. 2A); each of these candidates exhibited an average frequency of occurrence higher than 90% over all samples. These core OTUs primarily belonged to the genera *Faecalibacterium*, *Bacteroides*, *Dorea*, *Collinsella*, *Oscillibacter*, *Ruminococcus*, *Subdoligranulum*, *Coprococcus* and *Prevotella*. Correlations among these OTUs
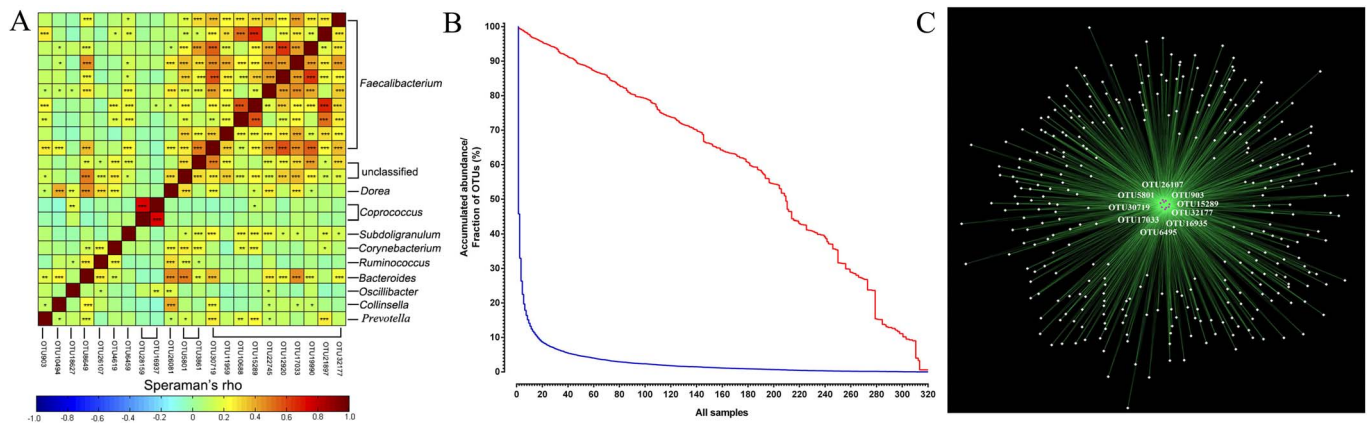
Figure 2 | The core intestinal microbiota of Mongolians. (A) Correlation matrix showing the Spearman's rank correlation among the 22 most abundant OTUs. The Spearman's rank correlation coefficient ranges from 1.0 to −1.0, corresponding to a strongly positive to a strongly negative correlation. (B) Fraction of OTUs shared across samples of the total OTUs within these samples (blue line) and the proportion that these OTUs represent of the total sequences obtained (red line) for the participants. (C) Bipartite network diagrams of evenly sampled bacterial 16S rRNA–derived top 9 core OTUs. Edges connect genus-level OTUs (purple points) to participant nodes (white points).

were determined based on Spearman's rank correlation (Fig. 2A). Additionally, 9 of the 22 core OTU candidates (OTU ID: 32177, 17033, 6459, 15289, 16937, 26107, 903, 5801 and 30719) primarily belonged to the genus *Prevotella*, *Bacteroides*, *Faecalibacterium*, *Ruminococcus*, *Subdoligranulum* and *Coprococcus*. These candidates were stably detected in almost every sample (Fig. 2B). Furthermore, a small proportion of OTUs (1.58%) that contributed 49.53% of the sequences were present in 78.26% of samples (Fig. 2B). In addition, the relationship between the 9 core OTU candidates and all samples was revealed by visualising a large network (Figs. 2C and S1B).

Differences in gut microbiota between Mongolians from the Khentii pasturing area, TUW province and Ulan Bator. A diversity analysis based on Simpson, Chao1, Shannon and observed species indices (Fig. S2A–S2D) revealed that the alpha diversity of the intestinal microbiota was greatest in the Khentii pasturing area but least in Ulan Bator. Additionally, we compared the composition of the intestinal microbiota of Mongolians from Khentii, TUW and Ulan Bator. A PCoA based on the unweighted (Fig. 3A and 3B) and weighted (Fig. S3A and S3B) Unifrac distances was performed using the obtained pyrosequencing data. An apparent clustering pattern was identified for the participants from different locations. Points representing the intestinal microbiota composition of Khentii, TUW and Ulan Bator residents clustered at the top right, the centre and the bottom left, respectively. The enterotype analysis provided a clear visualisation of the relationships among the different sample groups (Figs. 3D and 3E). The silhouette index was more than 0.6. All samples clustered into one of two groups. Cluster 1 primarily comprised Ulan Bator residents, and cluster 2 primarily comprised Khentii pasturing area and TUW province residents.

After establishing an intrinsic difference between the compositions of the gut microbiota of Mongolians living in different areas, we further identified differences in the specific bacteria of individuals that were principally responsible for the differences found using the Kruskal-Wallis test. The significant genera found ($p < 0.05$) are listed in Table 1, and the values were transformed into a heatmap (Fig. 3F). According to the heatmap, the genera *Prevotella*, *Solobacterium*, *Succinivibrio*, *Escherichia coli/Shigella group*, *Olsenella*, *Oribacterium* and *Lactobacillus* were abundant in Khentii residents, and the genera *Bacteroides*, *Oscillibacter*, *Roseburia*, *Alistipes*, *Coprococcus*, *Parabacteroides*, *Subdoligranulum*, *Barnesiella*, *Odoribacter*, *Parasutterella*, *Butyricimonas*, *Coprobacillus*, *Victivallis*, *Anaerosporobacter* and *Akkermansia* were abundant in Ulan Bator residents.

Seasonal changes in the Mongolians' intestinal microbiota. Based on Fig. 3B and 3C, we found that the changes in the range of intestinal microbiota of Mongolians from Khentii, TUW and Ulan Bator were discrepant and exhibited seasonal alternation. Combining the results from five sampling points (January, March, June, September and November), we noted that the seasonal changes in the range of intestinal microbiota were more distinct in Khentii residents than in TUW and Ulan Bator residents. We therefore analysed the data according to the sampling location.

For the Khentii residents, the results of a PCoA based on unweighted Unifrac and A partial least squares discriminant analysis (PLS-DA) (Fig. 4A–4C; weighted, Fig. S4) indicated that their intestinal microbiota compositions in June and September were similar and close to those observed in March but were significantly different from those observed in January and November. The changed genera representing more than 1% of the total number of sequences were listed in Table 2 and confirmed using the q-PCR data (Fig. S7). Further genus-level analysis revealed that *Faecalibacterium*, *Eubacterium*, *Dorea*, *Collinsella*, *Enterococcus*, *Solobacterium*, *Caldimonas*, *Escherichia coli/Shigella* group and *Subdoligranulum* levels were altered significantly ($p < 0.05$), exhibiting a lower contribution from March to September (Table 3); however, the abundance of *Prevotella*, *Bacteroides*, *Clostridium* and *Oscillibacter* remained stable throughout the year. The changes in the intestinal microbiota of the TUW residents were not as profound as those of the Khentii residents. The results shown in Fig. 4D–4F indicate that the intestinal microbiota compositions in June, March and January were similar to each other but distinct from those observed in September and November (weighted Unifrac distances and an enterotype analysis are listed in Fig. S5). At the genus level, *Faecalibacterium*, *Anaerosporobacter*, *Butyricimonas*, *Collinsella* and *Roseburia* changed significantly ($p < 0.05$) with season (Table 3), but the abundances of *Prevotella*, *Bacteroides*, *Clostridium* and *Oscillibacter* remained stable. However, for the Ulan Bator residents, little change was noted in their intestinal microbiota composition throughout the year (Fig. 4G–4I, weighted Unifrac distances and an enterotype analysis are listed in Fig. S6), and only the genera *Eubacterium*, *Dorea* and *Collinsella* differed among sampling points (Table 3).

Concordance of diet and intestinal microbiota. The traditional Mongolian diet is characterised by a high and frequent consumption of fermented dairy products, red meat and liquor. Currently, because of modernisation and economic development, many
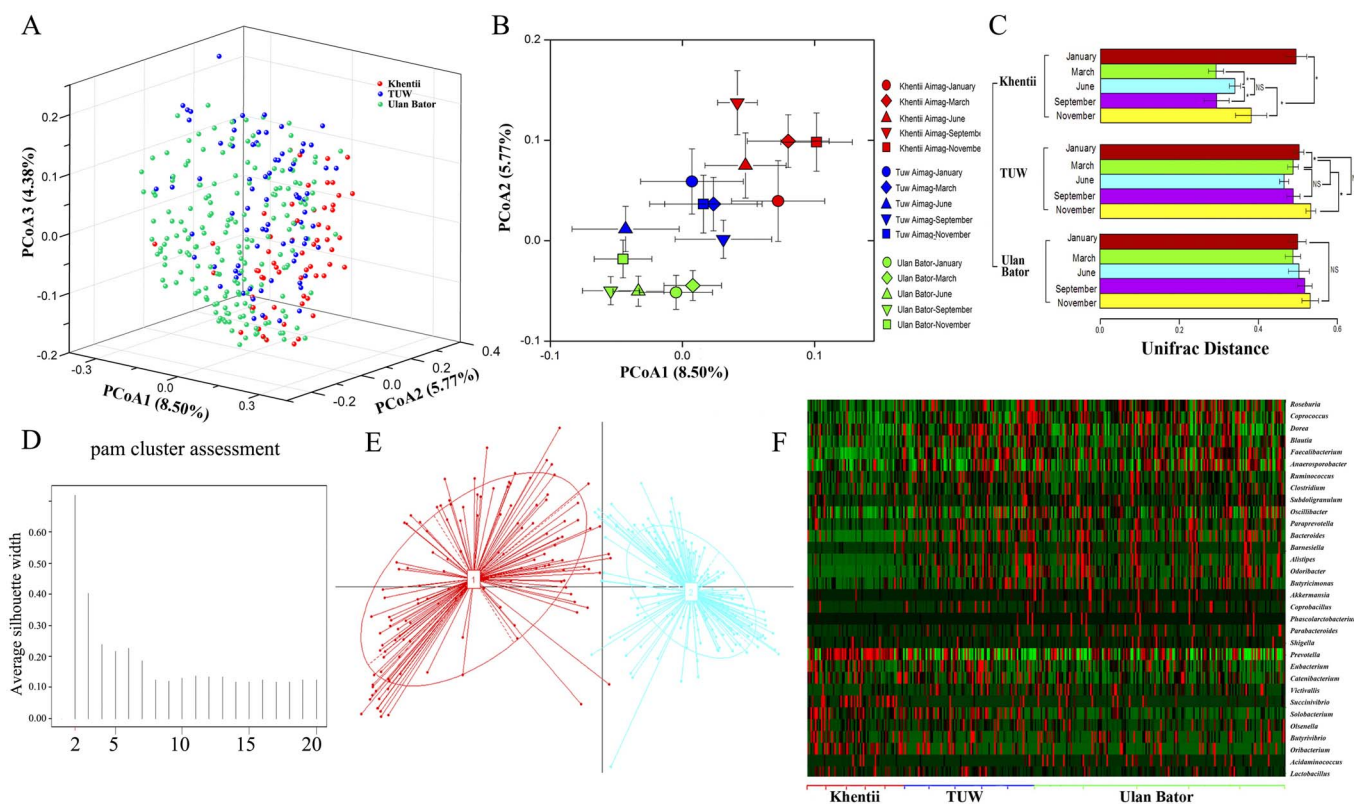
Figure 3 | Differences in gut microbiota among Mongolians from Khentii, TUW and Ulan Bator. (A) A principal component (PCoA) score plot based on unweighted UniFrac metrics for all participants. Each point represents the composition of the intestinal microbiota of one participant. (B) PCoA score plot based on unweighted UniFrac metrics. Each point represents the mean principal component scores of all volunteers at one location at one time point, and the error bar represents the standard deviation. (C) Sampling location and time point-driven unweighted UniFrac distances. (D) The silhouette index reflected the isolation degree of two enterotypes. (E) Enterotype analyses of the intestinal microbiota; cluster 1 contained participants primarily from Ulan Bator, and cluster 2 contained participants primarily from the Khentii pasturing area and TUW province. (F) Heatmap constructed using the amount of significantly different genera among participants in the Khentii pasturing area, TUW province and Ulan Bator city.

Mongolians living in Ulan Bator (the capital of Mongolia) and in TUW province (the suburbs of the capital) have gradually adopted an urban lifestyle, and only a few Mongolians, who mainly live in pasturing areas, retain the traditional diet. A partial least squares discriminant analysis (PLS-DA) based on participants' food types and weights also demonstrated this tendency (Fig. 5A). The red points in the figure represent the responses obtained from Khentii Mongolians using the food frequency questionnaire and are clustered at the right of the figure. To the left of this cluster are the responses obtained from TUW and Ulan Bator residents.

Based on the heatmap (Fig. 5B), we found differences in the food types chosen by Mongolians. Food type diversity was the greatest among Ulan Bator residents, and their dietary structure remained stable throughout the year. However, food type diversity was much less in TUW residents, and the dietary structure of these residents was not stable. Notably, Khentii residents lacked food type diversity, and their dietary structure changed significantly with season. Based on the results presented above, the food types that enabled the greatest discrimination were vegetables, fruit, red meat and kumiss, the consumption of which differed between the three groups of Mongolians.

To construct a concordance relationship between diet and intestinal microbiota, a procrustes analysis of the food frequency questionnaire and the microbiota β-diversity based on sampling locations was used to co-visualise the data (Fig. 5C–5E). Separations based on either diet or microbiota co-segregated along the first axis of both data sets (weighted UniFrac, Fig. S8A–S8C). Based on the figures (Fig. S8A–S8C), we observed a strong correspondence between diet

and intestinal microbiota (the p values for Khentii, TUW and Ulan Bator were < 0.001, 0.008 and 0.017 respectively Rev-4).

Discussion

The phyla Firmicutes and Bacteroidetes predominated, and together, represented an average of 91.6% of the sequences identified, in agreement with previous studies, which attributed the majority of human gut microbiota to these two phyla. A noteworthy feature of the faecal bacteria structure of Mongolians in our study was that the *Firmicutes* to *Bacteroidetes* (F/B) ratio was low, only 0.71. The F/B ratio relates to dietary habit and host physiology[10,11]. Those with high-fat western diets, the obese and young adults (versus the elderly) tend to exhibit higher F/B ratios. De Filippo et al.[5] concluded that a more westernised diet (higher fat and meat consumption and lower vegetable and legume consumption) causes a higher F/B ratio. Due to their nomadic lifestyle, some Mongolians have adapted to the classical diet including a high consumption of meat, alcohol and fermented milk, which more resembles typical western diets than that of rural African areas studied by De Filippo. Notably, the F/B ratio of Mongolian adult samples (0.71) calculated in this study was at the low end of the range obtained by De Filippo et al. (from 0.47 in rural Africa to 2.81 in urbanised Italian children). However, members of the Korean population were reported to have a high F/B ratio of 2.95, even though Korean diets contained a relatively high fibre content (19.8 g/day versus 15.1 g/day for Americans) primarily from kimchee and steamed rice[12]. The average age of our Mongolian participants was 34, which was closer to the adult group reported in Marion et al. Marion et al. reported adults to have a higher F/B ratio than the

Genus	Relative contribution (%)			Median, range (%)			Adjusted P-value
	Khentii	TUW	Ulan Bator	Khentii	TUW	Ulan Bator	
Prevotella	55.008	32.667	31.688	58.401,0.009–89.829	32.281,0–86.084	31.289,0–80.474	6.660000e-08
Bacteroides	2.928	9.849	11.243	0.372,0.017–39.389	3.203,0.109–57.94	5.489,0.019–62.345	5.899200e-12
Faecalibacterium	2.884	6.251	6.120	2.537,0.36–17.658	4.426,0.16–24.375	5.433,0–24.374	4.161920e-10
Oscillibacter	3.454	5.121	5.545	2.362,0.269–15.761	3.124,0.072–39.044	4.709,0.013–25.002	3.764706e-03
Roseburia	2.084	3.312	4.565	1.825,0.093–7.529	2.333,0.269–21.063	3.58,0–32.751	2.099200e-06
Clostridium	2.249	3.024	2.902	1.604,0.106–10.151	2.136,0.327–52.558	2.165,0.006–16.796	9.142857e-03
Ruminococcus	1.225	2.339	1.520	0.844,0.043–10.41	1.273,0.089–23.722	0.973,0–12.869	1.333333e-02
Catenibacterium	1.232	1.881	0.997	0.755,0–6.323	1.141,0–16.161	0.626,0–14.633	1.163636e-02
Alistipes	0.206	1.730	1.929	0.025,0–3.63	0.624,0–12.489	0.491,0–16.205	5.899200e-12
Coprococcus	1.211	1.439	2.316	1.075,0.073–3.858	1.223,0.096–7.292	1.787,0–15.642	1.344320e-06
Parabacteroides	0.653	1.266	1.530	0.252,0–6.66	0.508,0–11.401	0.635,0–25.699	2.000000e-03
Eubacterium	1.185	1.256	0.898	0.572,0–6.67	0.797,0–10.281	0.495,0–8.824	3.089655e-02
Subdoligranulum	0.776	0.993	1.520	0.529,0.038–4.963	0.564,0–12.473	0.769,0–32.147	9.142857e-03
Dorea	0.429	0.682	0.551	0.374,0.019–1.652	0.506,0.04–2.691	0.426,0–2.579	8.421053e-03
Blautia	0.169	0.345	0.300	0.108,0–0.759	0.208,0.01–1.929	0.21,0–2.061	2.000000e-03
Solobacterium	0.415	0.316	0.149	0.222,0–3.041	0.209,0–1.4	0.047,0–4.032	1.018971e-08
Barnesiella	0.109	0.316	0.324	0,0–4.539	0.041,0–5.649	0.011,0–8.824	8.421053e-03
Odoribacter	0.026	0.180	0.319	0.003,0–0.349	0.075,0–0.963	0.136,0–3.09	1.056107e-11
Parasutterella	0.023	0.158	0.211	0,0–1.217	0,0–2.487	0,0–5.689	3.210667e-04
Lactobacillus	0.173	0.133	0.067	0.024,0–5.214	0.047,0–1.993	0.021,0–1.082	2.251852e-02
Butyricimonas	0.045	0.101	0.113	0.014,0–0.462	0.063,0–0.581	0.041,0–1.226	1.333333e-02
Succinivibrio	1.358	0.097	0.283	0.161,0–20.844	0,0–2.274	0,0–5.832	2.294400e-10
Escherichia coli/Shigella group	0.232	0.084	0.186	0.022,0–4.813	0.022,0–1.595	0.034,0–7.771	6.660000e-08
Coprobacillus	0.035	0.084	0.135	0,0–1.387	0,0–1.629	0.015,0–3.755	5.899200e-12
Victivallis	0.034	0.082	0.090	0,0–0.602	0.013,0–1.21	0.023,0–2.136	4.161920e-10
Anaerosporobacter	0.056	0.080	0.082	0.048,0–0.249	0.07,0–0.278	0.065,0–0.283	3.764706e-03
Olsenella	0.150	0.079	0.068	0.024,0–2.104	0.041,0–0.776	0.014,0–0.956	2.099200e-06
Oribacterium	0.089	0.074	0.035	0.062,0–0.509	0,0–0.767	0,0–0.458	9.142857e-03
Butyrivibrio	0.107	0.063	0.076	0.04,0–1.109	0,0–0.561	0,0–1.655	1.333333e-02
Akkermansia	0.034	0.030	0.241	0,0–0.846	0,0–0.808	0,0–12.341	1.163636e-02
Acidaminococcus	0.055	0.002	0.077	0,0–1.314	0,0–0.066	0,0–1.701	5.899200e-12
Phascolarctobacterium	0.005	0.001	0.100	0,0–0.16	0,0–0.046	0,0–7.358	1.344320e-06

Table 1 | Significantly different genera among Mongolians in the Khentii pasturing area, TUW province and city of Ulan Bator

*Adjusted P values (P < 0.01) for the Kruskal-Wallis test are listed.

elderly (10.9 and 0.6, respectively). Our results and other findings suggest that the age, Westernised diet and lifestyle (rich in fat and meat, low in vegetables and legumes) of the participants may not be determining factors for gut microbiota composition indicators, such as the F/B ratio. Other dietary components and factors, such as host genetics, may exert considerable influence[3].

At the genus level, *Prevotella* and *Bacteroides* were found to predominate in the Mongolian samples, contributing 47.11% and 6.33% of the total sequences, respectively. This result was further supported by a high proportion of *Prevotella* and *Bacteroides* among the core OTUs (34/67, >50%). The genus *Prevotella* contains a wide array of carbohydrate- and protein-fermenting and acetate- and H_2-producing bacteria such as *Prevotella ruminicola*[13], and the genus *Bacteroides* has been mainly associated with the metabolism of animal proteins, a variety of amino acids and saturated fats[14]. The traditional Mongolian diet is characterised by a large amount of fried wheaten food, red meat and fermented dairy products with low quantities of vegetables and fruits. It is unsurprising, therefore, that these two genera dominated the microbiotic composition of Mongolian guts. In our previous study of Mongolians living in Inner Mongolia of China, the most abundant genus in intestinal tract was *Phascolarctobacterium*[9]. The Mongolians in Inner Mongolia of China usually live together with Han race which leads to a similar life-style and dietary habit with Han, but the Mongolians in Mongolia still keep the relatively traditional dietary habit, thus explains their differences at the genus level. Rev-1.

Nine OTUs, primarily belonging to *Bacteroides*, *Faecalibacterium*, *Ruminococcus*, *Subdoligranulum* and *Coprococcus*, were stably detected in nearly every Mongolian sample. Therefore, we defined these OTUs as core OTUs in Mongolians. Previous studies of the core microbiota in other nations have been widely reported. Martınez *et al.* characterised the faecal microbial communities of three young Americans over a one-year period by 454 pyrosequencing 16S rRNA tags to investigate the temporal characteristics of their bacterial communities[15]. The authors detected 16 stable core OTUs close to the genera of *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Ruminococcus*, *Blautia*, *Dorea*, *Eubacterium* and *Coprococcus*. Ling *et al.* investigated the faecal core microbiota of ten healthy Chinese undergraduates[16]. The dominant taxonomic groups in these faecal samples were *Faecalibacterium*, *Coprococcus*, *Blautia*, *Bacteroides*, *Roseburia*, *Ruminococcus*, *Subdoligranulum*, *Sporacetigenium*, *Oscillibacter*, *Dorea*, *Phascolarctobacterium* and *Prevotella*. Huse *et al.* explored the core faecal microbiota of more than 200 individuals from the NIH Common Fund Human Microbiome Project, and 7 OTUs representing the genera *Faecalibacterium*, *Oscillibacter* and *Bacteroides* were identified as core OTUs[17]. Based on these findings, a surprising consistency in core intestinal microbiota was found among the nations. Previous studies at the functional and metabolic levels indicated that these genera play a key role in the synthesis of basic metabolites in the human gastrointestinal tract. Therefore, the core intestinal microbiota in all humans might vary within a limited range.

Due to modernisation and economic development, many Mongolians living in Ulan Bator and TUW have gradually adopted an urban lifestyle, and only a few Mongolians, mainly in pasturing areas, maintain the traditional diet and lifestyle. Accordingly, the
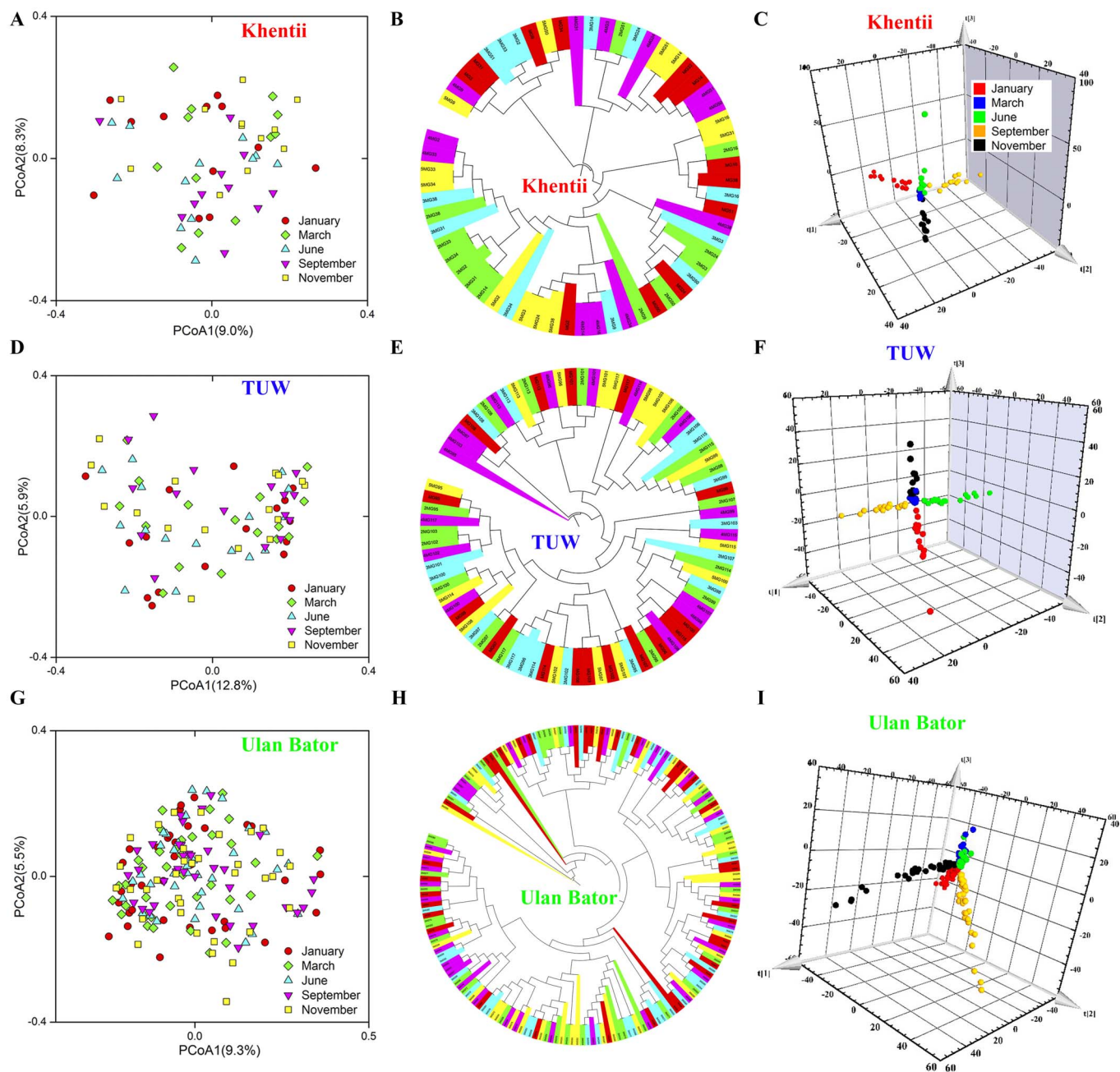
Figure 4 | The changed range of the intestinal microbiota of Mongolians from the Khentii pasturing area, TUW province and Ulan Bator city was discrepant with the seasonal alternation. (A, D and G) Principal component (PCoA) score plots based on unweighted UniFrac metrics of Mongolians in the three different locations. (B, E and H) The unweighted pair-group method with an arithmetic means (UPGMA) cluster analysis based on the distance metrics of Mongolians in the three different locations. (C, F and I) Partial least squares discriminant analysis (PLS-DA) based on the species abundance of Mongolians in the three different locations.

intestinal microbiotic compositions of Mongolians from the three areas differed. An analysis at the genus level revealed that the difference was primarily reflected in the populations of *Solobacterium*, *Olsenella*, *Oribacterium* and *Lactobacillus*, which were abundant in Khentii Mongolians. Previous reports indicated that *Oribacterium* and *Olsenella* are closely related to high incidences of periodontitis and gingivitis[18] and that *Solobacterium* is considered a major cause of bromopnea[19]. These distinctions were related to dental hygiene. The participants from Khentii brushed their teeth less frequently than did those from Ulan Bator and TUW and some herdsmen never brushed their teeth. *Lactobacillus* is widely distributed in fermented foods (such as fermented dairy products), which are habitually consumed by Mongolians residing in pastoral areas. The versatile adaptation

and remarkable colonisation ability of *Lactobacillus* in the human gut has been well demonstrated[20,21]. Therefore, it is not surprising that the faecal samples of the Khentii Mongolians consisted of a high amount of *Lactobacillus*.

Diet played an important role in shaping the intestinal microbiota of our subjects. In our research, we analysed the concordance of diet and intestinal microbiota by combining the data obtained using a food frequency questionnaire (FFQ) and pyrosequencing data at five sampling points (January, March, June, September and November). In Ulan Bator, food is plentiful and diverse, so limited seasonal changes were observed in the dietary structure of local residents. Accordingly, the composition of their intestinal microbiota was relatively stable. However, in the Khentii pasturing area, food is scant

Table 2 | The changed genera (relative amounts >1%) in Mongolians from Khentii, TUW and Ulan Bator due to seasonal change

Genus	Mean (%)					Range (25%–75%)					P-value
	January	March	June	September	November	January	March	June	September	November	
Khentii											
Prevotella	27.81	34.3	24.29	33.42	37.56	4.986–55.67	1.042–62.7	1.553–45.4	5.24–61.08	4.546–76.68	0.787
Bacteroides	9.522	13.09	12.64	13.87	9.145	1.53–9.51	1.191–25.76	0.9517–23.1	2.153–16.4	0.7746–19.58	0.877
Faecalibacterium	4.150	2.646	1.639	2.347	3.638	1.942–6.141	2.297–4.075	3.123–7.961	4.719–14.51	1.372–9.46	0.013
Oscillibacter	5.583	3.138	3.923	5.86	6.959	2.398–8.025	1.975–3.137	1.338–5.042	0.838–11.51	0.6962–7.938	0.605
Roseburia	2.445	2.052	2.196	1.708	2.757	1.946–8.387	1.102–2.713	2.372–8.373	0.8447–2.739	1.543–3.861	0.181
Ruminococcus	1.58	4.421	2.46	1.775	2.602	0.4895–1.701	0.6753–3.471	1.528–3.372	0.5725–3.045	0.4748–1.836	0.124
Clostridium	2.251	2.776	2.588	2.021	2.776	1.504–2.903	0.9375–4.552	1.219–3.615	0.9342–2.816	1.021–4.129	0.931
Alistipes	1.716	2.872	2.5	1.574	2.011	0.1659–1.767	0.367–4.097	0.4342–4.504	0.137–1.877	0.104–1.795	0.702
Catenibacterium	0.848	1.14	3.773	0.992	2.293	0.2934–1.606	0.003027–2.078	0.2263–7.377	0.002558–1.502	0.476–4.196	0.366
Parabacteroides	1.137	2.464	0.968	1.743	0.824	0.1625–1.93	0.478–4.993	0.189–1.471	0.3297–1.368	0.1494–0.8855	0.320
Coprococcus	1.107	1.101	1.48	1.043	1.097	1.17–2.486	0.639–1.435	0.7042–2.133	0.3101–1.363	0.6785–1.59	0.148
Eubacterium	2.039	0.745	1.760	1.094	0.288	0.472–2.049	0.09461–1.886	0.4482–3.197	0.07878–0.6949	0.06436–1.972	0.013
Subdoligranulum	1.397	0.563	0.685	0.381	0.851	0.2965–1.238	0.3877–1.1	0.5444–1.398	0.2005–0.9819	0.2467–0.5442	0.044
Streptococcus	1.467	1.006	1.455	0.760	0.802	0–2.02	0.03952–0.696	0.02347–3.591	0.008568–0.2995	0.03179–1.648	0.629
TUW											
Prevotella	56.9	52.69	50.35	56.99	54.55	47.13–70.79	37.99–67.08	49.42–79.49	54.87–70.62	5.314–49.39	0.791
Bacteroides	3.838	1.923	1.81	3.198	8.731	0.1602–8.535	0.1894–1.579	0.1915–1.379	0.3064–1.641	0.2139–9.483	0.708
Faecalibacterium	5.510	6.799	4.154	6.036	8.759	2.223–3.23	1.253–3.298	1.855–5.811	3.15–4.724	1.254–6.134	0.048
Oscillibacter	3.742	3.049	2.564	2.906	4.966	0.4957–6.943	1.122–4.238	0.8633–4.686	0.9728–4.436	0.8914–7.156	0.559
Roseburia	2.697	5.532	2.094	4.496	1.740	2.418–3.554	1.115–3.744	0.8549–3.495	0.7497–2.806	1.106–5.358	0.031
Clostridium	1.87	1.763	2.375	2.281	2.856	1.137–2.413	0.6798–1.993	0.4654–2.972	0.7352–2.516	0.8799–4.287	0.720
Coprococcus	1.355	1.639	1.079	1.902	1.634	0.7508–1.779	0.5193–2.382	0.276–1.477	0.6889–2.268	0.976–1.883	0.440
Catenibacterium	1.152	1.401	1.581	0.744	1.959	0.4274–1.854	0.253–1.599	0.6181–2.057	0.2497–0.9387	0.1644–3.223	0.422
Ruminococcus	1.131	1.436	0.942	0.758	1.491	0.3906–1.781	0.4827–1.128	0.5425–0.9025	0.2601–1.075	0.725–2.222	0.113
Succinivibrio	0.343	2.933	0.382	1.284	0.665	0–0.6054	0–4.367	0–0.4063	0–2.568	0–1.679	0.091
Megasphaera	0.805	1.587	1.231	0.716	1.133	0–1.849	0.0154–3.563	0–1.825	0–0.8674	0.003687–2.113	0.255
Ulan Bator											
Prevotella	32.48	37.84	26.09	26.09	29.53	5.386–49.39	13.71–64.41	5.955–46.56	0.8637–57.02	5.972–47.29	0.313
Bacteroides	11.49	9.038	13.23	13.23	9.155	1.108–17.91	1.029–15.55	0.9139–17.02	1.207–26.44	0.6411–14.16	0.906
Faecalibacterium	5.961	5.224	6.912	6.912	6.211	3.19–7.469	2.845–7.198	4.447–7.848	3.01–8.47	4.232–7.694	0.599
Oscillibacter	5.485	5.103	6.451	6.451	5.875	2.892–7.329	2.276–6.675	2.591–6.998	1.375–9.875	2.941–7.353	0.905
Roseburia	5.103	3.843	3.999	3.999	3.921	1.924–5.369	2.516–4.336	1.881–7.637	0.9767–5.816	1.57–5.748	0.623
Clostridium	2.369	3.145	3.251	3.251	2.709	1.428–2.819	1.326–4.267	1.878–4.004	1.238–4.205	1.092–3.505	0.419
Coprococcus	1.859	2.472	2.064	2.064	2.555	1.147–2.443	1.311–3.51	1.091–3.1	0.5922–3.131	0.9948–3.315	0.720
Alistipes	2.405	1.415	2.261	2.261	2.061	0.2045–2.545	0.1173–1.989	0.06741–1.197	0.05524–2.771	0.1239–3.815	0.320
Ruminococcus	1.384	2.034	1.341	1.341	1.487	0.4051–1.577	0.5826–2.428	0.9211–2.465	0.5605–1.732	0.4201–2.03	0.150
Parabacteroides	1.158	1.599	1.437	1.437	1.196	0.2105–1.578	0.1326–2.018	0.1973–2.267	0.08922–2.199	0.09452–2.035	0.957
Subdoligranulum	1.4	0.987	2.189	2.189	1.507	0.3866–1.408	0.3672–0.8275	0.4168–1.238	0.3632–1.972	0.4539–1.545	0.175
Catenibacterium	0.5889	0.919	0.751	0.7511	1.709	0–1.074	0–1.315	0.007768–1.658	0.00776–1.13	0–2.078	0.137
Dialister	0.9082	0.860	1.332	1.332	1.082	0.05808–1.288	0–1.513	0.04617–1.622	0.001862–2.823	0.009192–1.817	0.939

Note: Only genera representing more than 1% of the total number of sequences are included in the table.

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Table 3 | Significantly changed genera in Mongolians from Khentii, TUW and Ulan Bator due to seasonal change

Genus	Relative contribution (%)					Median, range (%)					P-value
	January	March	June	September	November	January	March	June	September	November	
Khentii											
Faecalibacterium	4.150	2.646	1.639	2.347	3.638	2.838,0.956–17.658	2.537,0.594–7.618	1.435,0.36–3.535	2.253,1.157–3.576	3.604,2.473–5.853	0.01275000
Eubacterium	2.039	0.745	1.760	1.094	0.288	1.634,0.028–5.125	0.482,0–1.724	0.993,0.075–6.67	0.694,0.061–2.692	0.214,0–1.366	0.01275000
Dorea	0.571	0.503	0.520	0.327	0.222	0.454,0.056–1.652	0.488,0.143–1.046	0.46,0.084–1.177	0.298,0.139–0.775	0.165,0.019–0.688	0.03075455
Collinsella	1.082	0.235	0.770	0.355	0.173	0.28,0.014–4.603	0.092,0–1.177	0.49,0.057–1.767	0.326,0.104–1.08	0.114,0–0.658	0.03075455
Enterococcus	0.001	0.000	0.816	0.082	0.002	0,0–0.009	0,0–0	0,0–6	0.005,0–0.798	0,0–0.023	0.03075455
Solobacterium	0.967	0.278	0.277	0.217	0.336	0.504,0–3.041	0.182,0.02–1.251	0.216,0–0.576	0.147,0–0.765	0.163,0–2.25	0.03075455
Caldimonas	0.067	0.161	0.043	0.234	0.306	0.012,0–0.549	0.06,0–0.781	0.024,0–0.133	0.126,0–0.755	0.128,0–1.528	0.03831538
Escherichia coli/Shigella	0.147	0.018	0.511	0.092	0.392	0.055,0–0.755	0,0–0.164	0.075,0–4.813	0.025,0–0.585	0.01,0–4.314	0.03831538
Subdoligranulum group	1.397	0.563	0.685	0.381	0.851	0.927,0.112–4.963	0.535,0.219–1.46	0.5,0.038–2.496	0.327,0.139–1.026	0.477,0.241–3.596	0.04383571
TUW											
Anaerosporobacter	0.068	0.095	0.036	0.109	0.091	0.069,0–0.147	0.084,0–0.229	0.021,0–0.133	0.099,0.027–0.278	0.088,0–0.217	0.03075455
Butyricimonas	0.052	0.080	0.058	0.150	0.166	0.026,0–0.305	0.052,0–0.323	0.032,0–0.279	0.122,0–0.498	0.093,0–0.581	0.04845000
Collinsella	0.739	0.469	0.176	0.975	0.412	0.189,0.04–3.612	0.139,0.067–4.05	0.112,0.012–0.657	0.599,0.161–5.139	0.227,0–1.644	0.04870000
Faecalibacterium	5.510	6.799	4.154	6.036	8.759	3.688,0.16–22.854	4.937,0.727–24.375	2.887,0.56–21.906	5.805,0.494–14.927	6.774,1.694–22.308	0.04828000
Roseburia	2.697	5.532	2.094	4.496	1.740	1.717,0.424–9.691	3.012,0.419–21.063	2.357,0.269–4.53	3.176,0.55–10.605	1.71,0.383–3.336	0.03075455
Ulan Bator											
Enterococcus	0.006	0.001	0.003	0.017	0.409	0,0–0.196	0,0–0.004	0,0–0.025	0,0–0.182	0,0–14.696	0.00031620
Collinsella	0.691	0.257	0.296	0.639	0.461	0.255,0–4.916	0.121,0–3.214	0.187,0–1.486	0.444,0–4.761	0.286,0–1.398	0.01275000
Dorea	0.658	0.493	0.619	0.619	0.365	0.466,0.078–2.493	0.364,0.012–1.864	0.435,0.075–1.823	0.553,0–2.579	0.231,0–1.89	0.03075455

*Only genera representing more than 0.05% of the total number of sequences are included in the comparison.
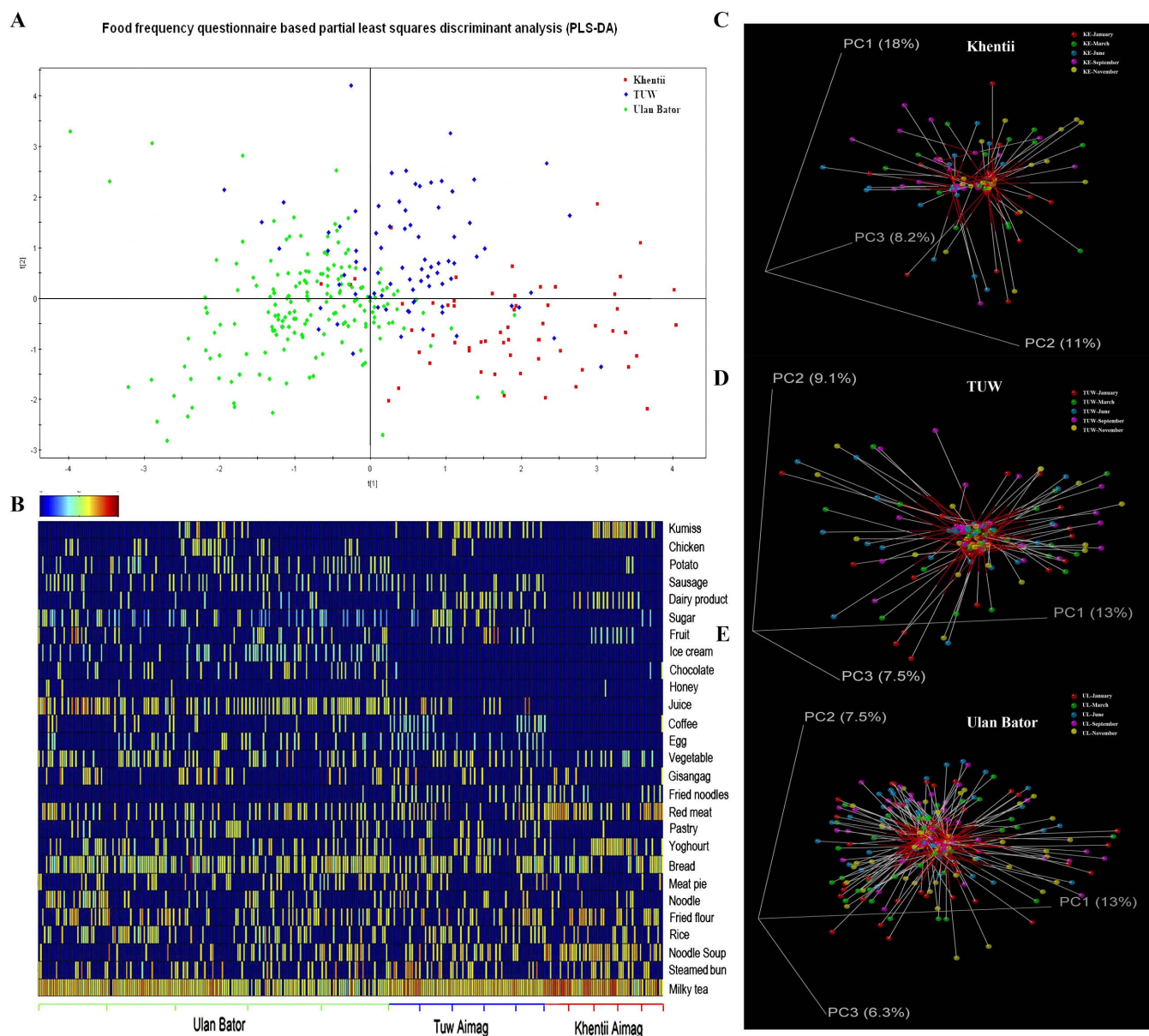
Figure 5 | Concordance of diet and intestinal microbiota. (A) Partial least squares discriminant analysis (PLS-DA) based on the participants' food types and weights. (B) Heatmap based on food frequency questionnaire data recording the food types and weights of all participants. (C–E) Procrustes analysis combining unweighted UniFrac PCoA of the microbiota, including a food type PCoA.

and simple, and the dietary structure of the local residents changed significantly with the season. Thus, the intestinal microbiota of local residents distinctly changed from season to season. This study suggests that seasonally different components of the Khentii diet, such as vegetables, fruits, red meat and/or kumiss, could directly or indirectly modulate the intestinal microbiota profile. Among the microbes that varied with season, *Faecalibacterium, Eubacterium* and *Subdoligranulum* produce butyrate[22–25] and may exert anti-inflammatory effects[26,27]. *Escherichia coli/Shigella group* is a potentially pathogenic bacterium that possesses pro-inflammatory properties. The seasonal variation of intestinal microbiota should therefore be further investigated due to its health implications.

Changes in the dietary composition have been associated with changes in the composition and metabolism of gut microbial populations. Long-term dietary intake influences the structure and activity of human intestinal microbiota, but it remains unclear how rapidly and reproducibly the human gut micro-biome responds to short-term macronutrient change. Recent research[28] confirmed that dietary interventions in humans can alter gut microbial communities

only 1 day. In addition, an animal-based diet had a greater effect on the microbiota than a plant-based diet. The study of Cotillard *et al.*[29] on diet-induced weight-loss and weight-stabilisation interventions on obese and overweight individuals concluded that dietary intervention improves low gene richness and clinical phenotypes but appears to be less effective at improving inflammation variables in individuals with lower gene richness.

In this study, 454 pyrosequencing combined with q-PCR technology was applied to examine the diversity of the intestinal microbiota of Mongolians at different phylogenetic levels. In addition, we explored the effects of the adoption of an urban lifestyle and seasonal dietary changes on Mongolians' intestinal microbiota. This basic research will bring a new understanding to the human gut microbiota of different countries and how they are affected by diet.

Methods

Participant recruitment. In this study, 64 healthy Mongolian adults with no history of gastrointestinal-related diseases were recruited (the participants' information is listed in Table S1). Among these participants, 36 volunteers lived a typical modern

lifestyle in Ulan Bator, the capital of Mongolia. Twelve volunteers were recruited from the Khentii pasturing area, a typical Mongolian grassland. The local residents maintain a traditional nomadic lifestyle and diet. Sixteen volunteers lived in the TUW province, which contains the suburbs of Ulan Bator. The living standards and experienced scale of urbanisation of these residents were lower than those of Ulan Bator residents but higher than those of Khentii pasture residents. Faecal samples were collected from these volunteers at five time points (January, March, June, September and November). After obtaining written and informed consent, we collected habitual long-term dietary information from all participants using a food frequency questionnaire (the dietary information is shown in a supplementary file). The study protocol was approved by the Ethical Committee of the Inner Mongolia Agriculture University (Hohhot, China).

Stool sample processing and DNA extraction. DNA was extracted from faecal samples using a QIAGEN DNA Stool Mini-Kit (QIAGEN, Hilden, Germany) in combination with a bead-beating method[30]. Isolated faecal DNA was then used as a template for further analyses.

PCR amplification, quantification, pooling and pyrosequencing. The V1–V3 region of 16S ribosomal RNA (rRNA) genes were amplified as described previously[31]. The PCR products were quantified using an Agilent DNA 1000 Kit using an Agilent 2100 Bioanalyser (Agilent Technologies, America) according to the manufacturer's instructions. The amplification products were pooled together in equimolar ratios with a final concentration of 100 nmol/L each. These pools sequenced using pyrosequencing with a Roche GS FLX.

Quantitative PCR analysis. Real-time quantitative PCR amplification was performed using an ABI Prism® 7500 Real Time PCR System (Applied Biosystems, California, USA) using the Maxima SYBR Green/ROX qPCR Master Mix (2×) (Thermo Scientific, Massachusetts, USA). The gene-targeted primer sequences, amplicon sizes and annealing temperatures used for each bacterial group are presented in Table S2.

Bioinformatic analyses. Low-quality sequences were removed based on the following criteria: a raw read shorter than 110 nucleotides, a sequence displaying an imperfect match to the barcode or a fuzzy match to at least one end of the 16S rRNA primers based on a standard BLAST search, a variable region shorter than 100 nucleotides, or more than 7% of the bases demonstrated a quality score of less than 20 in the raw read.

Bioinformatic analyses were performed using QIIME (v1.2.1)[32] on the extracted high-quality sequences. Briefly, the sequences were aligned using PyNAST[33] and clustered under 100% sequence identity using UCLUST[34] to obtain the unique V1–V3 sequence set. After representative sequences were selected, the unique sequence set was classified into operational taxonomic units (OTUs) with a 97% threshold identity using UCLUST. ChimeraSlayer[35] was employed to remove any potentially chimeric sequences in the representative set of OTUs. The taxonomy of each OTU representative sequence was assigned using the Ribosomal Database Project (RDP)[36] classifier with a minimum bootstrap threshold of 80%. OTUs that occurred only once or twice were discarded. A de novo taxonomic tree was constructed using a chimera-checked OTU representative set in FastTree[37] for downstream analyses, including alpha and beta diversity calculations. To evaluate alpha diversity, the Shannon-Wiener and Simpson's diversity indices and the Chao1 and rarefaction estimators were calculated. UniFrac[38] metrics were calculated to evaluate beta diversity. Both weighted and unweighted calculations were performed prior to a principal coordinate analysis (PCoA).

Statistical analyses. Differences in alpha diversity and the relative abundance of the families and genera in each sample were computed using Mann-Whitney and Kruskal-Wallis tests. The gut microbiota were clustered among the different groups using a multivariate analysis of variance (MANOVA) test on a PCoA based on weighted and unweighted Unifrac metrics. The aforementioned statistical analyses were conducted using Matlab® (The MathWorks, Natick, MA, USA). A partial least-squares discriminant analysis (PLS-DA) was used to identify any correlation between food intake and subjects location. The network was constructed using the software Cytoscape (version 2.6.0). Data from the food frequency questionnaire and the microbiota β-diversity were analysed using the procrustes routine in QIIME (V1.5).

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Author contributions

Additional information

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