reports allow us to understand the ways in which the intestinal microbiome interact and contribute to the wellbeing of the animal and how changes in the environment or conditions may impact their gut microbial ecosystem.

In Hong Kong, brown cattle were traditionally raised by local farmers for centuries as draft animals to plough rice fields. When the population gradually urbanized in the last few decades of the 20th century, the cattle were abandoned. Their descendants became wild cattle straying in the suburban areas of Hong Kong, such as the country parks. In recent years, these cattle surprisingly ate the food waste left by country park visitors at the barbeque sites and became omnivorous. Due to the change in diet, we hypothesized that the microbiomes of these omnivorous cattle may adapt to the new diet. As microbiome communities are vital for the breakdown and absorption of nutrients, which contributes to the health and well-being of the cattle, it is important to determine the effect of the dietary change on the gut microbiota. In this study, we characterized and compared the microbiome of omnivorous cattle in Hong Kong with that of the traditional herbivorous cattle, and explored the hypothesis that a change in diet from herbivorous to omnivorous significantly impacts the microbiota composition.

2. Materials and Methods

All animal experimental procedures were performed under protocols approved by the University of Hong Kong Committee on the Use of Live Animals in Teaching and Research (CULATR 3330-14).

2.1. Collection of Cattle Fecal Samples

Ten fecal samples (one from each cow) were collected from healthy wild cattle located at the Sai Kung Country Park in Hong Kong that consumed an omnivorous mixed diet of grass and barbeque food waste (Group M; mixed) (Table 1). The Sai Kung Country Park is a 3000 ha wild country park in the western part of Sai Kung Peninsula, Hong Kong [11]. The park is a mountainous terrain popular for hiking and has several trailside barbeque sites and picnic areas located within. The cattle roam these barbeque sites and feed on food waste left in rubbish bins or given to them by barbeque parties, thus their food consumption includes a mixed diet of grass and barbeque food waste including raw or partially cooked meat, such as beef, pork, chicken, or fish, as well as sweet potato, honey, corn and bread (Fig. 1). Another ten fecal samples (one from each cow) were collected from healthy cattle resided on a hill in a free-grazing farm located in Guangzhou, China, that consumed a main diet of grass and plants (Group G; grass) and served as the control herbivore group for microbiome comparison (Table 1). Fecal samples from cattle were collected immediately after natural defecation, stored immediately on ice, then transported to the laboratory and frozen at −80 °C prior to analyses. All samples were obtained from the inside of the feces using sterilized equipment, with no contact with soil or other pollution sources. PCR amplification and sequencing of the mitochondrial cytochrome-b gene was performed to validate the twenty cattle were of the species *Bos taurus* (data not shown).

Table 1
Characteristics and diet of cattle sampled in this study.

	Dietary group[a]	
	Mixed	Grass
Number of cattle sampled	10	10
Breed	*Bos taurus*	*Bos taurus*
Age	Adult	Adult
Status	Healthy	Healthy
Location	Sai Kung Country Park, Hong Kong	Free-grazing farm, Guangzhou, China
Diet composition	Forage and barbeque food waste (omnivorous)	Forage only (herbivorous)
Number of sample taken	1 fecal sample per cow	1 fecal sample per cow

[a] Mixed, Group M; grass, Group G.

2.2. DNA Extraction, PCR Amplification, and MiSeq Sequencing

Total genomic DNA was extracted from 200 mg of fecal sample using a QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturer's instructions. The genomic DNA and its quality were quantified and checked using Nanodrop spectrophotometer (ND1000; Thermo Fisher Scientific, Wilmington, DE, USA) and agarose gel electrophoresis, respectively. Primers that span the hypervariable regions V3–V4 of the bacterial 16S rRNA gene (Forward: 5′-CCTACGGGNGGCWGCAG-3′, Reverse: 5′-GGACTACHVGGGTAATCC-3′) were used for amplicon generation and sequencing using the MiSeq PE300 platform (Illumina) according to the manufacturer's instructions. Library preparation and sequencing were performed at the Centre for Genomic Sciences, The University of Hong Kong. Sequencing coverage was approximately 212,683 sequences per sample on average. Reads were submitted to the NCBI short-read archive (BioProject PRJNA371636 – Biosample accession numbers for individual animal samples sequencing data are SAMN06310326, SAMN06310355, SAMN06310356, SAMN06310361, SAMN06310362, SAMN06310375, SAMN06310392, SAMN06310393, SAMN06310399, SAMN06310428, SAMN06310429, and SAMN06310448–SAMN06310456).

2.3. Data Analysis

Raw sequence read data were assembled by fastq-join from ea-utils.1.1.2-537 [12] with all the unjoined reads filtered out. The joined paired-end reads were analyzed using the QIIME 1.8 pipeline [13] with default parameters. Operational taxonomic units (OTUs) were picked from the assembly paired-end reads via UCLUST [14] at 97% similarity, with OTUs fewer than 10 reads removed to avoid PCR sequencing errors, and representative sequences were selected from each OTU. Taxonomic assignments of OTUs were determined using UCLUST based on 16S rRNA gene reference sequences from GreenGenes taxonomy database (release 13_5) [15]. Alpha diversity of samples was calculated using a rarefaction curve from QIIME's alpha diversity pipeline [13]. Samples were rarefied to 124,000 reads (the least number of reads per sample), and diversity was calculated by the number of species per sequencing depth, Shannon index (estimation of the total diversity with both species richness and evenness taking into consideration) and Chao1 index (estimation of total species richness). Differences in the number of OTUs among the two dietary regimes were evaluated using an ANOVA. For multivariate analysis, Calypso [16] was used for the Statistical and Principle Components Analysis (PCA).

3. Results

3.1. Illumina Sequences

Raw reads were generated by Illumina MiSeq PE300 sequencing of the 20 fecal samples. 4,253,662 high quality joined reads were then obtained from the raw reads for downstream analyses via quality trimming, pair-end joining, and chimeric filtering. Individual samples that passed quality checking generated an average of 212,683 reads per sample. After clustering and taxonomic assignment at 97% similarity, OTUs with <10 observation counts were discarded, 68,181 unique OTUs were identified and the number of total OTUs for each individual sample ranged from 3955 to 12,251, with an average of 7983.

3.2. Composition of Bacterial Community at Phylum and Genus Level

At 97% similarity, alignments and phylogenetic assignments resulted in the identification of 26 phyla, 52 classes, 95 orders, 181 families, and 374 genera across the two bacterial domains.