Bacteroidaceae, *Paraprevotellaceae*, *Lachnospiraceae*, *Verrucomicrobiaceae*, *Veillonellaceae*, *Rikenellaceae* and *Clostridiaceae*, as well as those unclassified derived from *Bacteroidales* (order) and *Clostridiales* (order) (Fig. 2B). The relative abundance of the family *Bacteroidaceae*, *Rikenellaceae*, *Ruminococcaceae* and the unclassified *Bacteroidales* were more enriched in the gut microbiota of Group M; while *Paraprevotellaceae*, *Clostridiaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Verrucomicrobiaceae* and the unclassified *Clostridiales* were predominant in the gut microbiota of Group G.

At the genus level, the most predominant genera included 5-7N15, *Akkermansia*, *Oscillospira*, *Phascolarctobacterium*, *Clostridium*, *Prevotella* and *Dorea*, as well as those unclassified derived from *Ruminococcaceae* (family), *Bacteroidales* (order), *Clostridiales* (order), *Bacteroidaceae* (family), *Lachnospiraceae* (family) and *Rikenellaceae* (family) (Fig. 2C). The relative abundance of the unclassified *Rikenellaceae*, unclassified *Bacteroidales* and unclassified *Bacteroidaceae* were more enriched in the gut microbiota of Group M; while the relative abundance of the genera *Akkermansia*, *Treponema*, *Phascolarctobacterium*, *rc4-4*, the unclassified CF231 and the unclassified S24-7 were predominant in the gut microbiota of Group G.

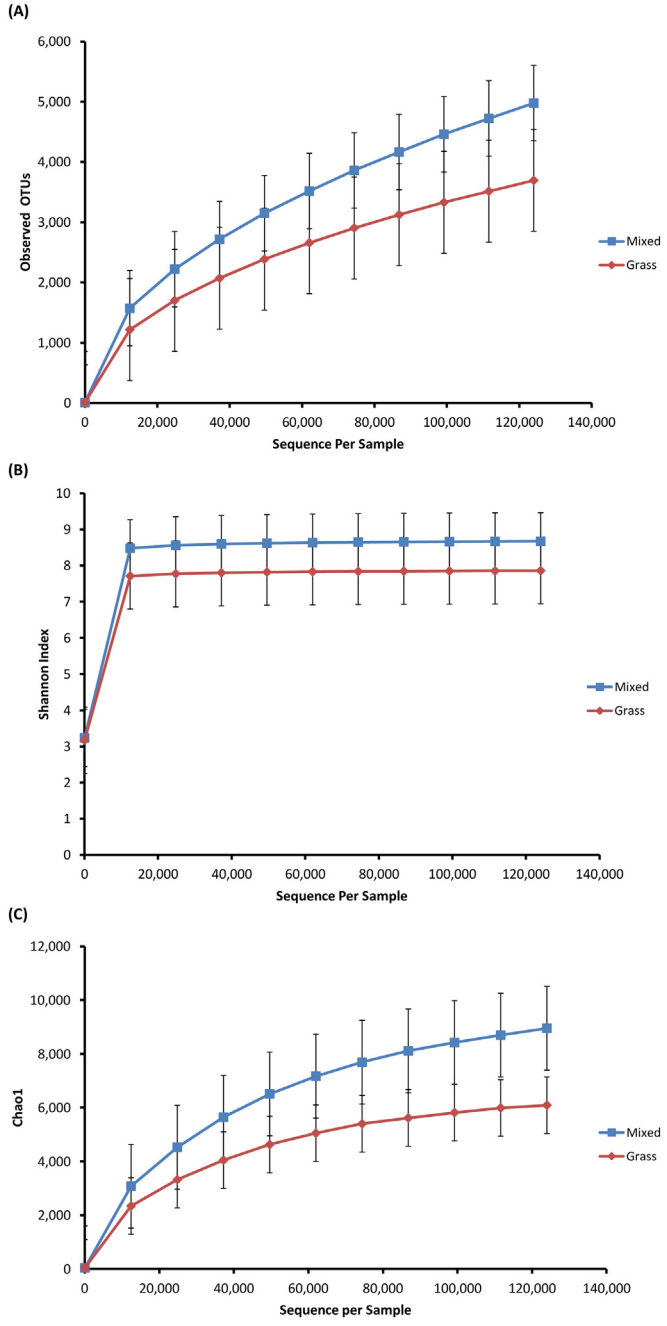

Fig. 3. Alpha diversity of fecal samples across the two dietary groups represented by relative changes of rarefaction depth in terms of (A) number of observed OTUs, (B) Shannon index (diversity) and (C) Chao1 (richness). Mixed, blue square, Group M; grass, red diamond, Group G.

3.3. Comparison of Bacterial Diversity

In order to give an unbiased comparison of impacts on the alpha diversity due to the different dietary consumption, the OTU table was rarefied to the number of reads of the sample with the lowest number of reads, which is 124,000 in such case. At the maximum sub-sample depth, Group M samples were found to consist of around 1282 more observed OTUs than Group G samples (Fig. 3A). Consistent differences in Shannon diversity index (evenness) and Chao1 values (richness) were also observed across the two sample groups at sub-sample depth point. The bacterial communities present in the microbiota of Group M ($H = 8.674$) had a higher Shannon index than that of Group G ($H = 7.860$), meaning an increase in OTU number as well as evenness of the distribution of individuals among the OTUs (Fig. 3B). Group M ($x = 0.991$) also had a higher Chao1 value than Group G ($x = 0.981$), indicating higher richness, i.e., increase in OTU counts per sample, in Group M (Fig. 3C). PCA was used to demonstrate the varieties of community structure of individual samples from the two dietary groups. Samples were found to be clustered together according to their dietary groups and a similar pattern was observed across PCA at all levels. Fig. 4 represent these clusters at the level of OTUs present.

3.4. Comparison of Bacteria Profiles at the Genus Level

Significant differences in abundance between the two dietary groups were observed for 15 bacterial populations at the genus level (Fig. 5). The relative abundance of *Anaeroplasma*, *Anaerovorax*, *Bacillus*, *Coprobacillus* and *Solibacillus* was significantly increased in Group M as compared to Group G. On the other hand, a significant reduction in abundance was observed for *Anaerofustis*, *Butyricimonas*, *Campylobacter*, *Coprococcus*, *Dehalobacterium*, *Phascolarctobacterium*, *rc4.4*, RFN20, *Succinivibrio* and *Turicibacter*. Overall, *Bacillus* had the most significant ($p < 0.001$) gain in abundance, while the abundance of *Turicibacter* decreased most significantly ($p < 0.001$) in Group M as compared to Group G. Furthermore, we detected that 369 (28.8%) of the 1282 observed OTUs unique to Group M were associated with these 15 genera which had significant differences in abundance among the two dietary groups. It was noted that the unique OTUs for *Anaerofustis*, *Butyricimonas* and *Campylobacter* were identified in Group G only.

4. Discussion

In the present study, we examined the potential effect of an environmentally-induced change of diet from herbivorous to omnivorous on the gut microbiota composition of cattle. As most of the feral cattle in Hong Kong roam freely in country parks and have access to a variety of food in addition to grass and vegetation, an herbivorous control group was not available locally. Instead, we sampled from cattle located on a free-grazing farm in Guangzhou, a city located just 119 km from Hong Kong, with a main diet of only grass and plants, which serves as an ideal herbivore gut microbiome control for comparison. Studies have also shown that while two cattle groups may be based in different geographical locations, when a comparable feed ration and management practice was used, the microbiome community between the two groups is observed to be highly similar in composition [17].

As our study examines free-living animals in their natural environment, there is no control over the type and quantity of food which is consumed, as opposed to animals housed under controlled experimental