Original Research

Microbial Communities in Food Waste in Terms of Methanogenic and Residue Gob Remediation Potentials

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Abstract

Gobi beach management is a critical way to reduce carbon emissions but requires a large amount of nutrients to support in the early stages, while food waste degradation and resourcing can be used for production. The residue is also an excellent provider of nutrients. Therefore, in this study, the diversity of microorganisms in food waste was analyzed by high-throughput technology at different times to identify the common and core functional genera for potential resourcing. It was found that bacteria were mainly dominated by *Pseudomonas* (6.12%-12.16%) and *Acinetobacter* (20.71%-58.36%), but *Acinetobacter* accounted for a relatively low percentage at the Spring Festival. Mitochondria and chloroplasts, as independent carriers of genetic material, were extremely high in the Spring Festival, suggesting that fresh ingredients effectively reduced the number of opportunistic pathogens in it. For archaea, the methanogens *Methanosarcina* (15.07%-96.40%) and *Methanobacterium* (5.87%-98.55%) dominated, and the presence of *Nitrososphaeraceae* was also prevalent. The above genera were common in soil, which means these food wastes offer the possibility of landfill residues for saline soil improvement after methane production. Regarding diversity, festivals contribute significantly to the differences in microbe diversity in food waste, with archaea being more affected than bacteria. The results of this study show that food waste can be used as a methane-producing substrate,

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and the degraded residue can be used for Gobi management to enhance soil fertility after disinfection, which is very promising.

Keywords: food waste, traditional Chinese festivals, bacterial diversity, archaeal diversity, methanogens

Introduction

Food waste is the loss of quality and quantity in the supply chain during production, harvesting, and processing [1]. In 2019, China collectively classified kitchen waste and other food waste as food waste so that food waste can be viewed as a category of perishable, organic-containing household waste, including household food waste kitchens. Kitchen waste has four attributes: high oil and grease content, high water content, high organic matter content, and high salt content [1]. It contains many trace elements, making rotting easy if improperly handled. It produces a foul odor, pollutes the environment, and even spreads pathogens that can harm human health [2]. On the other hand, food waste with a high organic matter content suggests a high potential for microbial degradation, especially for methanogenesis [3]. At the same time, the large amount of non-degradable residue produced by the degradation of organic matter has good adsorption and permeability. It performs well in increasing the oxygen and nutrient content of the Gobi beach coat [4].

At this stage, more than half of household food waste is released into the natural environment without any disposal, which harms the ecosystem and human health. Food waste with high water content and low calorific value leads to high incineration costs [1]. In addition, it is difficult to solve the problem of secondary pollution, such as dioxins generated. In the United States, the amount of food waste generated in 2000 was 25.98 million tons, accounting for 11.2% of the total municipal solid waste, and the recycling rate of food waste was only 2.6%, which is much lower than the average recycling rate of municipal waste of 30.1%. Malaysia is located in the tropics with abundant rainfall, and the water content of food waste is pretty high. Due to the limited scope of food waste source separation and reduction projects implemented [5]. Currently, most of the food waste in Malaysia is disposed of through landfills, and some of the waste collected from segregation is generally used for anaerobic fermentation for gas production or vermicomposting. In Korea, food waste has not been allowed to enter landfills since 2005, and more stringent management measures have been applied to food waste management in Korea [3].

As cities continue to grow, the burden generated by food waste disposal is increasing and is strongly influenced by festivals. Three broad types of waste are generated in kitchens during festivals [6]: 1. Raw materials directly used in food preparation, e.g., melons, fruits, vegetables, flour, and cooking oils. 2. Residual waste from festival food, e.g. pastry crumbs, nut shells, fruit peelings, leftover beverages, etc. 3.

Packaging waste associated with festival food, e.g., food preservation bags, semi-finished boxes, etc. There are some differences in the microbial community structure among the three types of food waste [7], with more diverse microbial community structures in fruit and vegetable waste, and more superficial microbial community structures in food waste such as biscuits. There are minimal microbes in packaging, generally treated separately among these wastes. Therefore, food waste is dominated by high-fiber, micronutrient-rich fruits and vegetables and high-organic confectionery at this stage [6]. Its degraded residue is characterized by a slow degradation rate, environmental friendliness, good permeability, and nitrogen and phosphorus enrichment [4], which has good potential for Gobi beach coating improvement.

The Gobi is mainly desert and semi-desert [8], containing much coarse sand and gravel. The scarcity of plants leads to the inability of precipitation to be retained for a long time, and the evaporation is significant. Therefore, the initial stage of Gobi restoration mainly focuses on fixing sand and gravel, together with plant planting. However, due to the poor water capacity and lack of nutrients in the soil, it is often difficult for vegetation to survive in the initial planting stage [9]. The basic process of Gobi restoration starts with sand fixing and drought-resistant vegetation planting around the quadrat [10]. The composite ecological restoration matrix for restoration in the center requires farm manure to increase the nitrogen and phosphorus content, while the kitchen waste after methane production is suitable as a substitute for increasing soil fertility [3]. This process can reduce greenhouse gas emissions and economic expenditure in energy use and remediation, where the practice will be preferable to achieve desert restoration and environmental protection. Food waste from urban life can be degraded to produce methane for domestic and industrial feedstocks for vast land and safe distances with high temperatures [10]. The remaining residues can be used as substrate for the environmental transformation of the surrounding Gobi to improve the initial survival rate of vegetation and increase the water content of the soil [4], thus realizing the purpose of food waste resources and the restoration and transformation of the Gobi Desert.

Based on this, this study chose the most representative Spring Festival Meal in the north as one of the sampling times and also included Laba and New Year Day with daily meals as control groups. After the meals were prepared (Fig. 1), all food waste from the selected time points was mixed and collected, and the archaea and bacterial compositions were analyzed by high-throughput detection. The results of the study

Fig. 1. Food waste sample collection.

will provide data support for the efficient, low-cost, and environmentally friendly resource utilization of food waste and provide a theoretical basis for the improved utilization of degraded residues.

Material and Methods

Food Waste Collection

Wearing disposable gloves, food waste was placed in a large self-sealing bag for mixing and crushing in the Weifang home kitchen. The mixed solid kitchen waste samples were randomly collected. Then, the sample was fully placed in 1.5 mL sterile centrifuge tubes, with masks and disposable gloves worn to avoid contamination of the samples during the sampling process.

DNA Extraction and Sample Delivery

Food waste samples were collected into 1.5 mL sterile centrifuge tubes and labeled on the outside of the tubes with time information. Laba (L); New Year Day (Yuandan, Y); Spring Festival Dinner (Nianye Fan, N); Daily (Richang, R); and All Celebrating Day (Festival, F), in which the daily meal was collected on 4 January 2023.

The solid kitchen waste samples were transported to the laboratory by being held in a 4ºC warm box and frozen using a -80ºC refrigerator. High-throughput microbial screening: According to the standard protocol, sample DNA was extracted using the FastDNA™Spin Kit for Soil (MP Biomedicals, Santa Ana, CA). PCR was performed using archaeal primers AR109F/AR915R and bacterial primers BA27F/BA907R, targeting the V4-V5 region of 16S rRNA [11]. The reaction parameters were as follows: pre-denaturation at 94ºC for 2 min; 94ºC denaturation for 30 s; annealing at 55ºC for 30 s. The reaction lasted for 25 cycles at 72ºC for 1 min. PCR products were detected via gel electrophoresis and then cut and purified for high-throughput sequencing.

The Quantitative Insights Into Microbial Ecology (QIIME) version 1.7.0 pipeline (http://www. Qiime. org) was used to process raw sequencing data with the default parameters [12]. Briefly, the representative sequences from each OTU were defined by a 97% identity threshold level, after which chimeric and low-quality reads were removed. Using the Ribosomal Database Project (RDP) classifier [13], the taxonomic classification of each OTU was assigned. The average relative abundance (%) of the predominant genus-level taxonomy in each sample was assessed by comparing the assigned sequence number of a particular taxon to the total obtained sequence number. To clarify microbial community differences, alpha diversity, PCoA, and Venn were computed with R [11].

Results and Discussion

The Bacterial Community in Food Waste

There were large differences between the microbial communities of household food waste at different festival times (Fig. 2), with L samples mainly dominated by *Acinetobacter* (50.66%), *Pseudomonas* (12.16%), *Lactococcus* (7.19%), *Enterobacter* (4.82%), and *Vagococcus* (2.16%), but *Lactococcus* especially was less common in household food waste samples from other festival times. Y samples were dominated by *Acinetobacter* (58.36%), *Enterobacter* (6.91%), *Pseudomonas* (6.26%), *Weissella* (2.50%), and *Pantoea* (1.96%). N samples were dominated by *Pseudomonas* (10.91%), *Pectobacterium* (1.35%), and *Enterobacter* (1.06%). R samples were dominated by *Acinetobacter* (20.71%), *Pseudomonas* (6.12%), with *Aeromonas* (1.39%) featuring the most. The results showed that the microorganisms were closer in the food waste on New Year Day and Laba, which may be presumed to be due to the closer proximity of Laba (30 December 2022) and New Year Day in 2023. The proximity in terms of meals was accompanied by the lack of complete waste sterilization after removal, so inoculation and perpetuation might happen. Chronologically, daily and Nian Ye meals were far away from both. At the same time, mitochondria and chloroplast DNA comprised a more significant portion of the diets on both daily (37.30%, 3.06%) and Nian Ye meals (79.10%, 25.40%). It was hypothesized that there might be an increase in the use of vegetables and fresh produce after Laba, resulting in higher levels of chloroplasts and mitochondria, representing green vegetation, in the food waste.

The high-throughput results have revealed large differences in the bacterial communities of food waste samples from Laba, New Year Day, Spring Festival, and Daily Meal. Among them, *Pseudomonas* was the most common among all samples, with a relative abundance ranging from 6.12%-12.16%, and widely distributed in various environments as a common gram-negative
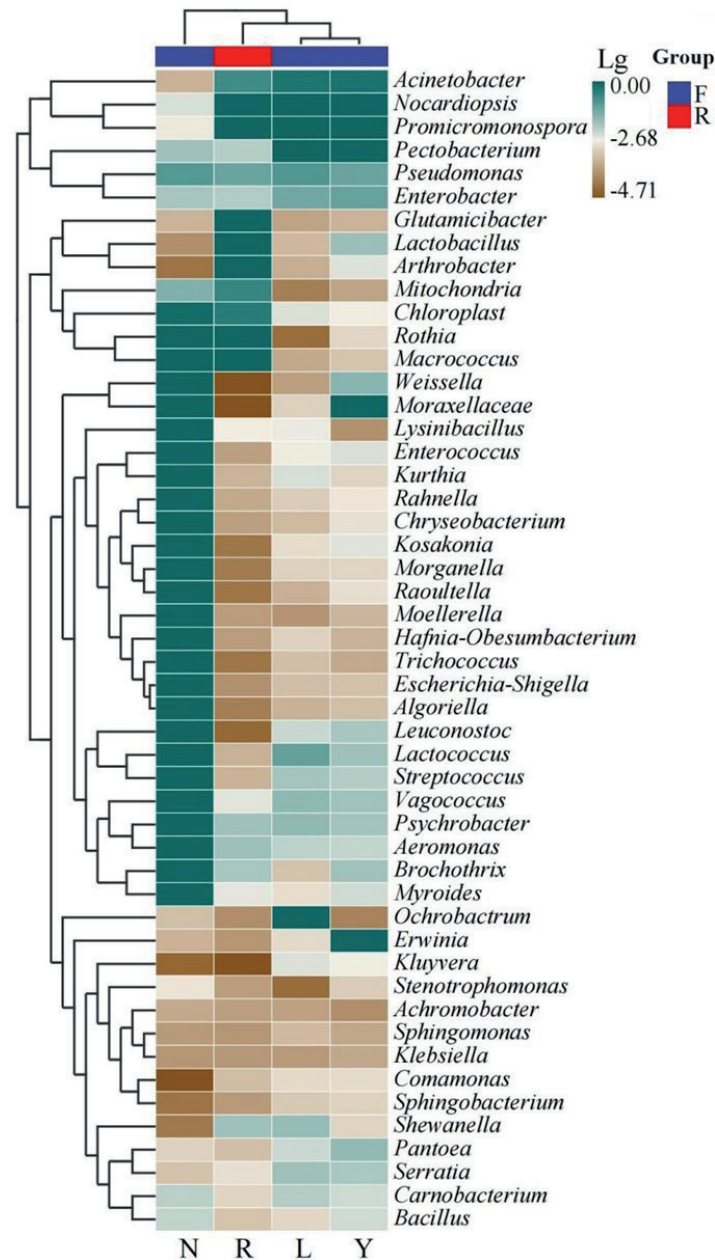
Fig. 2. Relative abundance of bacteria.
Note: The raw data is plotted by taking the logarithm of lg. Laba (L), New Year Day (Yuandan, Y), Spring Festival dinner (Nianye Fan, N), Daily (Richang, R), celebrating days (Festival, F).

opportunistic pathogen [14]. The genus has good drug resistance and mutation ability and grows well in anaerobic and other extremely harsh environments. *Acinetobacter*, a common saprophytic microorganism, is a Gram-negative opportunistic pathogen in all food waste samples except for the N sample. This phenomenon may be because Spring Festival Dinners are mostly made of fresh food, fruits, and vegetables, increasing mitochondrial and chloroplast content while reducing the living space for spoilage microorganisms and decreasing their relative abundance. It can be seen that fresh ingredients not only effectively provide us with abundant nutrients [15], but also help to reduce opportunistic pathogens in food waste, and timely removal and disinfection of food waste can effectively protect us from the possibility of opportunistic pathogens [16].

The Archaeal Community in Food Waste

The L sample was dominated by *Methanosarcina* (45.88%) and *Methanobacterium* (42.38%); the Y sample was dominated by *Methanosarcina* (15.07%) and *Methanobacterium* (5.87%); the R sample was dominated by *Methanosarcina* (96.40%) and *Nitrososphaeraceae* (3.31%); N was dominated by *Methanobacterium* (98.55%), of which, the relative abundance of *Methanosarcina* was higher in the L and

R samples, but was below the limit of detection in the N samples (Fig. 3). *Methanobacterium* dominated the N samples and had a higher relative abundance (98.55%) in the N sample. The results showed that methanogens were the dominant genus in food waste samples at different times. Still, the composition of methanogens in food waste at different times was inconsistent.

Regarding archaea (Fig. 3), the methanogenic bacteria *Methanosarcina* and *Methanobacterium*, both of which are strictly anaerobic, dominated. *Methanosarcina* can complete methane production through three pathways: hydrogen, methyl, and acetic acid [14], but it requires a high concentration. In contrast, *Methanobacterium* is a formate-type methanogenic bacterium with specificity [17]. It can be surmised by the variation in the relative abundance of both in different samples that the abundance of starch on Laba Day provides the possibility for diverse organic matter. New Year's Day is often not used as a major holiday. Therefore, the overall amount of litter may have been insufficient, leading to its low relative abundance of archaea. On the other hand, accumulating organic matter in daily life resulted in more diverse and abundant organic matter, providing better conditions for *Methanosarcina* to grow

and multiply. However, preparing the annual dinner may have caused a certain randomness in the abundance increase of a single genus. *Nitrososphaeraceae* were present in all the samples but in low abundance, only having a relative abundance of 3.31% in the daily diet. As anaerobic archaea can be involved in the reduction of Fe(III) while oxidizing NH_4^+ to produce nitrogen, nitrite, or nitrate [18], the greater variety and total amount of food waste generated from daily diets leads to the possibility of the existence of the above cycle in it, contributing to the degradation of food waste to form nitrogenous residues that can be used for restoration in the Gobi saline soil restoration [4].

The Analysis of the Bacterial Community in Food Waste

Principal Co-ordinates Analysis (PCoA) found (Fig. 4) that PCo1 and PCo2 explained 82.2% of the total sample variability, with 55.4% and 26.8%, respectively. In this case, the difference between bacteria in festive and daily food waste fell on the second principal axis, with an explanation of 26.8%. There were some differences between the bacterial communities in daily
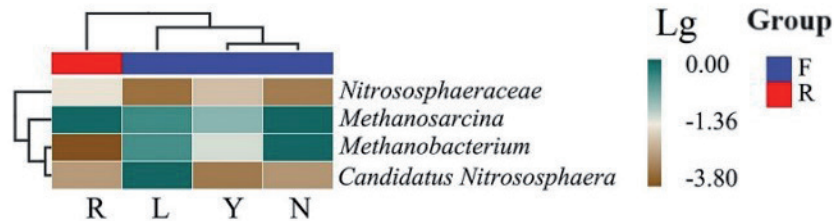

Fig. 3. Relative abundance of archaea.
Note: The raw data is plotted by taking the logarithm of lg. Laba (L), New Year Day (Yuandan, Y), Spring Festival dinner (Nianye Fan, N), Daily (Richang, R), celebrating days (Festival, F).
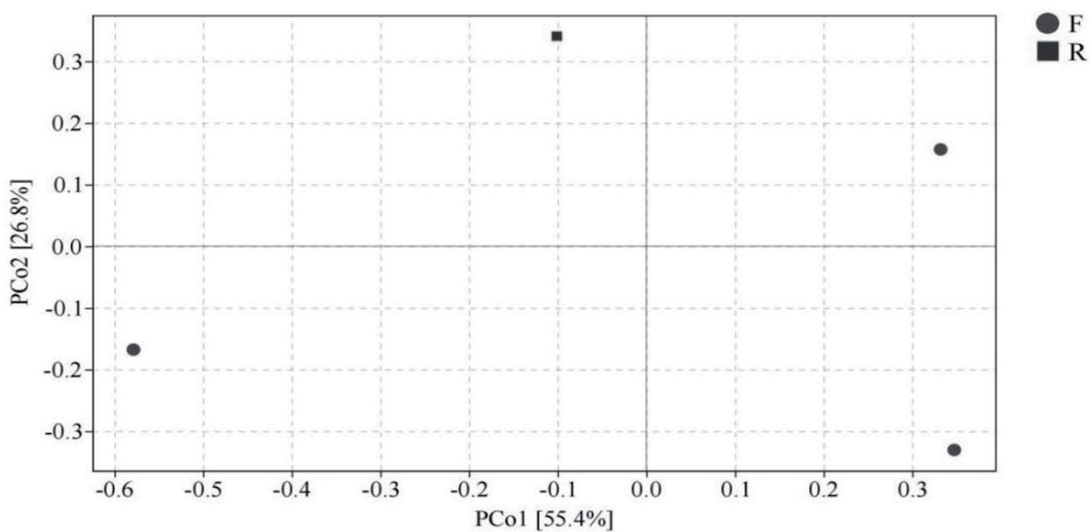

Fig. 4. PCoA analysis of bacterial community in food waste.
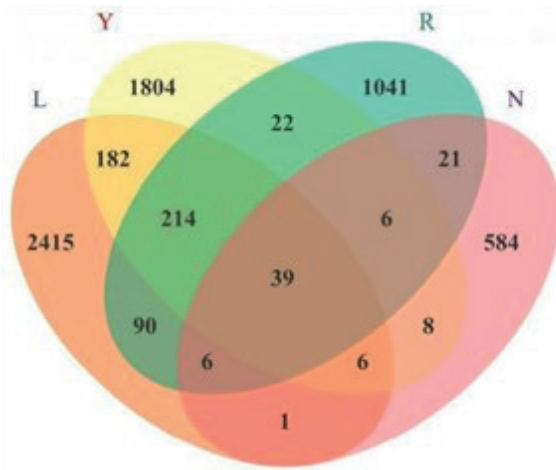Note: Daily (Richang, R), celebrating days (Festival, F).

Fig. 5. Venn analysis of bacterial community in food waste.
Note: Laba (L), New Year Day (Yuandan, Y), Spring Festival dinner (Nianye Fan, N), Daily (Richang, R).

food waste and those at festivals, but the differences were greater among festivals due to different dietary needs.

From the overall view of the Venn diagram (Venn) analysis (Fig. 5), the total number of OTUs measured in the four samples was 6,439, and the total number of shared OTUs was 39. Among them, the highest number of OTUs in the L sample was 2,953; the lowest number of OTUs in the N sample was 671; the highest number of shared OTUs happened in the L and R samples was 441; and the lowest number of shared OTUs happened in the L and N samples was 52. The results showed that the bacterial communities in the food waste samples on the day of the Laba were more unique. At the same time, the dietary habits of Laba might be closer to those

in daily routines, which resulted in more shared OTUs between samples that were further away from each other. Regarding bacterial colony diversity (Fig. 5), the bacterial communities of the festival and daily samples differed greatly, and the number of bacterial OTUs in the daily samples was lower than those in the samples of L and Y and higher than N.

Existing studies have found that the main purpose of food waste resources is to achieve organic energy and nutrient reuse while reducing environmental pollution and health and safety issues [3], consistent with the results of bacterial flora in this study. *Pseudomonas* and *Acinetobacter* are common opportunistic pathogens in food waste [14, 19], and the use of fresh ingredients can effectively provide more nutrients and reduce the proportion of pathogens in the flora, which can be reflected by the fact that *Acinetobacter* can degrade proteins rather than ferment sugars. The dietary structure of meat and eggs in China is more important. The composition of meat, eggs, and milk in our dietary structure is increasing significantly, providing conditions for opportunistic pathogenic bacteria [18]. Still, the timely removal of trash to ensure living safety has also become a noteworthy issue.

The Analysis of the Archaeal Community in Food Waste

PCoA (Fig. 6) analysis of the archaea revealed that the total explanation of inter-sample differences in both PCo1 and PCo2 axes was 85.9%, with the differences brought about by grouping being reflected in both PCo1 and PCo2 axes. This result suggests a significant difference in the archaeal community in food waste generated during holidays and usual diets, probably due to richer holiday diets.
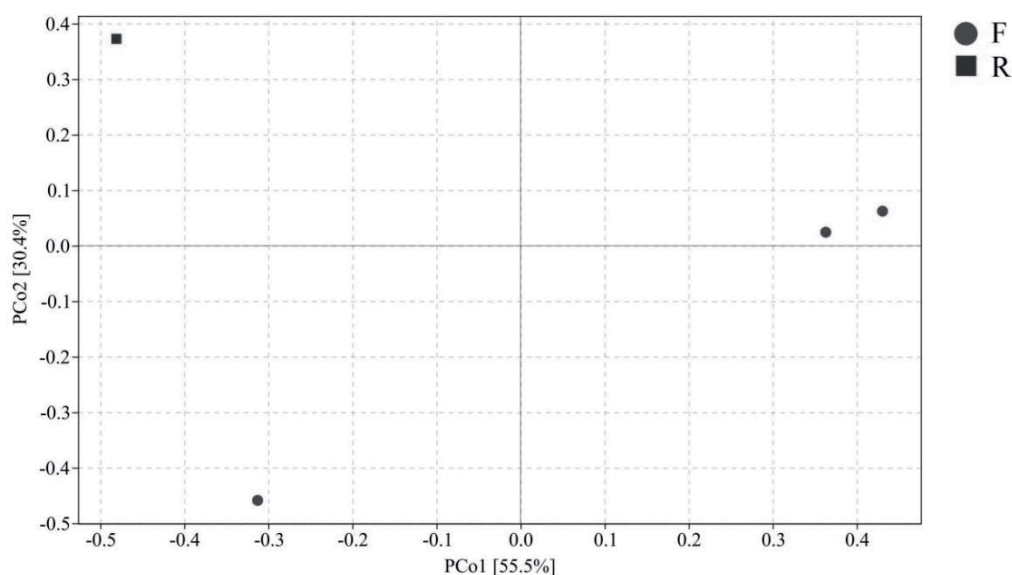

Fig. 6. PCoA analysis of archaeal community in food waste.
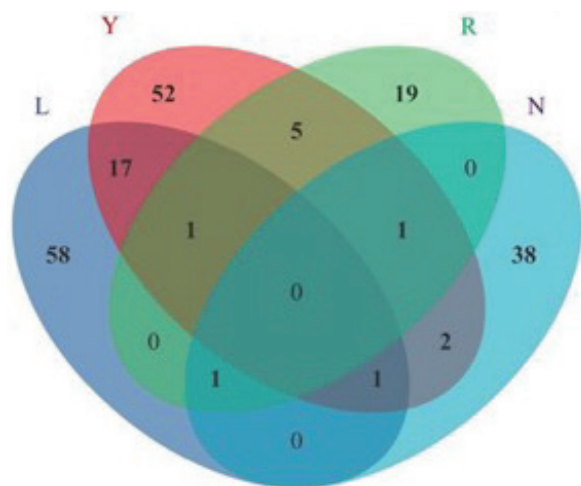Note: Daily (Richang, R), celebrating days (Festival, F).

Fig. 7. Venn analysis of archaeal community in food waste.
Note: Laba (L), New Year Day (Yuandan, Y), Spring Festival dinner (Nianye Fan, N), Daily (Richang, R).

Venn analysis of archaea revealed (Fig. 7) that the total number of OTUs measured in the four samples was 195, and there were no shared OTUs, but shared OTUs existed in the festival samples, which was only 1. The highest number of OTUs found in the Y samples was 79, and the lowest in the N samples was 43. The festival samples had the highest number of shared OTUs between L and Y at 19 and the lowest number of shared OTUs between L and N at 2. This result suggests that the number of OTUs in archaea is significantly smaller than that of bacteria and that temporal differences lead to greater variation in the archaeal community between samples.

The archaea are dominated by the methanogenic bacteria *Methanosarcina* and *Methanobacterium*, with *Nitrososphaeraceae* also prevalent. Thus, food waste can be cleaned with the help of methanogenic forms, and the waste can be resourced and rendered harmless by reducing its nitrogen content with the help of *Nitrososphaeraceae* [18]. In this progress, the nitrate-reducing bacteria coupled with anaerobic oxidation methanogens can use the methane produced by methanogens in kitchen waste, which realizes the simultaneous removal of nitrate and organic matter [17]. In this study, the open-ended collection of food waste in the bin did not facilitate the growth of anaerobic archaea. Still, rather than the growth of parthenogenetic opportunistic pathogens, a relatively anaerobic treatment environment would be more conducive to removing pathogenic microorganisms in future anaerobic food waste treatment to achieve harmless food waste recycling. However, the opportunistic pathogenic bacteria within food waste in real life are still a cause for concern, and the consumption of fresh vegetables, timely removal, and disinfection of waste are all key to ensuring human health [16].

Based on the results of the above studies, it was found that methanogens are important microorganisms

that can be used for resource utilization in food waste from northern diets, but also as opportunistic pathogens *Pseudomonas* and *Acinetobacter*, which are parthenogenetic anaerobic microorganisms, but are less likely to be found than the methanogens *Methanosarcina* and *Methanosarcina*. Although both *Pseudomonas* and *Acinetobacter* are parthenogenetic anaerobic microorganisms, their anaerobic survival ability is still insufficient compared to the methanogens *Methanosarcina* and *Methanobacterium*, as well as the *Nitrososphaeraceae*, so food waste resource utilization can be achieved through the direct anaerobic oxidation of the food waste. Meanwhile, the high temperature of the Gobi in the summer helps the anaerobic oxidation of food waste to produce methane [10], which can be stored for winter living or produced on a large scale for industrial production, and the remaining residue can be used for vegetation restoration in the spring of the following year [4]. In addition, both *Methanosarcina* and *Methanobacterium* can cooperate with other microorganisms for methane production through electron transfer, especially direct interspecies electron transfer [17, 20], and this process can be effectively facilitated with the help of conductors such as biogenic carbon, magnetite, and nanoconductors.

Conclusion

The study results revealed the methanogenic and nitrogen-cycling bacteria in the food waste, which provided the possibility of waste resourcing. However, in this study, the sample collection was conducted only in winter, which was not sufficient for detailed information. In the future, we will continue to explore the characteristics of the bacteria in food waste generated at other festivals and daily diets for subsequent food waste resources and residue utilization.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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