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# Research article

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# Manure enriched with nitrogen derived from high-protein food waste in a large dining facility

Mengjie Zhang<sup>a,b</sup>, Xiaoyan Zhang<sup>a</sup>, Hui Lin<sup>b,\*\*</sup>, Huabao Zheng<sup>c,\*\*\*</sup>, Qifa Zhou<sup>a,\*</sup>

<sup>a</sup> College of Life Sciences, Zhejiang University, Hangzhou, 310058, China

<sup>b</sup> State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Zhejiang Academy of Agricultural

Sciences, Hangzhou, 310021, China

<sup>c</sup> Zhejiang Province Key Laboratory of Soil Contamination Bioremediation, Zhejiang Agriculture and Forestry University, Hangzhou, 311300, China

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# ABSTRACT

Food waste (FW) from large dining facility has been a pressing environmental challenge in China recently. This study developed an innovative species-specific feeding strategy for producing pigeon meat and excellent manure from FW. Adding FW to the feed of pigeons significantly increased their feed intake and promoted their growth although the pigeons showed a strong aversion to the FW. We produced a "super manure" with exceptionally high nitrogen (N) content (mean = 10.77 % on a dry basis, 8.04–12.57 %, n = 264) by feeding slowly-growing pigeon species (*Columba livia vs.* and *Caoge Huzhou 11*) with protein-high commercial feed and FW. A significant negative relationship between the N and carbon (C) contents in the pigeon manure was found, with C depletion higher than N depletion. Furthermore, the N content in the anaerobic composting (AnC) manure was 29.16 % higher than that in the FW. Fourier transform infrared (FT-IR) analysis and stable isotopes  $\delta^{13}$ C and  $\delta^{15}$ N in the manure clearly identified the transformations of nutrients during pigeon feeding and the AnC process. This study opens a path for producing N-high manure using protein-high food waste.

# 1. Introduction

The annual global food waste (FW) generation amounts to 1.3 billion tons [1]. However, landfilling, the predominant method of waste disposal, is unsustainable. Consequently, FW management has recently emerged as one of the most pressing environmental challenges [2,3]. In China, the urban catering industry alone produces 17–18 million tons of FW, which is equivalent to the annual food consumption of 30–50 million individuals. In recent years, Chinese government departments such as the National Development and Reform Commission, the Ministry of Housing and Urban-Rural Development, the Ministry of Environmental Protection, and the Ministry of Agriculture have formulated a series of policies to promote the recycling of food waste. The per capita FW rate in the Chinese catering industry is 11.7 %, whereas large dining rooms exhibit a higher rate of 38 % [4]. Hence, the FW management of these establishments, known as large dining room food waste, requires urgent attention. In large dining rooms, customers typically consume nutrient-rich foods, indicating that the FW potentially contains valuable nutrients such as proteins. However, research on the nutrient

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<sup>\*</sup> Corresponding author.

<sup>\*\*</sup> Corresponding author.

<sup>\*\*\*</sup> Corresponding author.

E-mail addresses: linhui@zaas.ac.cn (H. Lin), zhenghuabao@zafu.edu.cn (H. Zheng), zzzqqq@zju.edu.cn (Q. Zhou).

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C and N analysis, stable isotope analysis and FT-IR analysis

Fig. 1. The flow chart of the experimental design in this study.

composition of this type of FW in China is scarce. Nitrogen (N)-rich manure is highly sought-after for crop production, particularly in organic farming. In crop production, the excessive use of chemical fertilisers has reduced the quality of arable land and environmental pollution [5]. The substitution of fertiliser with manure is beneficial for improving soil properties and crop yields [6,7]. Because of the low N content in organic manure, nitrogen deficiency poses a significant challenge to organic crop production. In addition, the low N content and slow release of many organic fertilisers make N a costly input for growers [8,9]. A rate as high as 15 t of farmyard manure per hectare per year is insufficient to maintain soil N levels and meet crop N requirements in the long term [10]. Therefore, N-high manure has great application potential for sustainable crop production, leading us to explore the feasibility of converting FW into nitrogen-rich manure.

FW is ideal for composting because of its rich organic matter content and minimal concerns regarding heavy metals and pathogens [11]. Composting has emerged as an effective technology for managing FW and transforming it into a stable and nutrient-enriched biofertiliser [12,13]. However, current composting practices are associated with significant N and carbon losses, resulting in environmental pollution and a decreased agronomic value of the final compost [14–16]. Aerobic composting of artificially synthesized N-rich FW leads to substantial N losses, resulting in lower N content in the final manure than in FW, which is primarily caused by nitrogen loss because of ammonia volatilisation [17]. Therefore, aerobic composting may not be a suitable method for obtaining nitrogen-rich (N-high) manure from FW. In contrast, anaerobic composting (AnC) successfully degrade kitchen waste [18]. AnC occurs in an oxygen-free environment; thus, it significantly reduces greenhouse gas emissions compared to aerobic composting [19]. Consequently, N loss is expected to be lower in AnC than in aerobic FW composting [20].

Feeding animals with FW is a longstanding global tradition. However, modern livestock production has reduced this practice due to concerns over foodborne biological hazards. Recently, a growing interest in transforming wasted food into safe and nutritious livestock feeds as a resourceful and climate-smart strategy to address sustainability challenges has been emerging. Among the options in FW recovery and recycling, feeding farm animals is the most favourable option for waste prevention. Innovative approaches, such as species-specific feeding strategies, can help societies improve food production with fewer unintended consequences while enhancing the circular and regenerative capacity of the agri-food system. Pigeon meat is highly consumed in China because of its taste. In addition, pigeons efficiently produce manure [21]. Pigeons have distinct digestion rates for different nutrients, with low protein and high carbohydrate and lipid digestion rates [22]. Therefore, pigeons can be effectively utilised to convert protein-rich feed into N-high manure by leveraging their digestive traits.

This study aimed to (1) investigate the nutrient composition of FW from a large dining room and (2) explore the feasibility of converting FW from a large dining room into N-high manure through pigeon feeding and AnC while characterising the resulting manure.

# 2. Materials and methods

A flow chart of the experimental design was shown in Fig. 1.

# 2.1. FW collections and sampling

Thirty L of FW were collected from the kitchen waste processed with a water flow kitchen waste treatment equipment(MEIKO AZP80, Meikeo Cleaning Technology Zhongshan Co., LTD, Zhongshan City, China) in the dining room at the Zijingang Campus, Zhejiang University, at lunchtime in the middle of each month from April 2022 to March 2023. Three hundred L of FW for the AnC and pigeon feeding experiments were collected in mid-November 2022. Zhejiang University is located in Hangzhou, a city in Zhejiang province that produces approximately 600 tons of food waste per day. The dining room at the Zijingang Campus is currently the largest

college dining room in China and can accommodate 15 000 individuals.

# 2.2. Anaerobic composting experiment

AnC experiments to obtain N-high compost were conducted at 30 °C in a 1.5 L beaker sealed with plastic film from November 2022 to March 2023. One kg of fresh FW was placed in each beaker. The AnC experiment was replicated four times.

# 2.3. Pigeon feeding experiment

Two months old pigeons (*Columba vs. Caoge Huzhou 11*) were obtained from a pigeon farm in Huzhou, Zhejiang Province, China. A flock of pigeons comprising five pigeons was raised in a pigeon house (Aoxing Cage Co., Shijiazhuang, China) in the Bio Clean Room for Animal Experiments, Zhejiang University. The room temperature was kept at  $25 \pm 1$  °C during the feeding period from November to December 2022. Four feeding treatments were employed: freshly milled FW, milled dry FW, commercial feed (Tan-Yilei Business Firm, Suyu, China) (designated as CFa), and a mixture of CF (70 %) and FW (30 %) (designated as MF). Each treatment was replicated four times.

# 2.4. Nutritional composition analysis

The nutritional composition of CF and FW was analysed according to the State Standards of the People's Republic of China: GB/T 5009.9–2008 (starch), GB/T 6432-2018 (protein), GB/T 6433-2006 (fat), GB/T 6434-2006 (fibre), GBT 6438-2007 (ash), GB/T 6436-2018 (calcium), and GB/T 6437-2018 (phosphorus).

### 2.5. Feed intake and manure yield

The daily feed intake for each pigeon house was determined, and the manure in each pigeon house was collected and weighed daily. The daily manure weight of each pigeon was regarded as the pigeon manure mass, and the manure yield was calculated as the manure dry weight from a unit dry CF or dry MF. The composting manure yield was calculated as dry manure weight per unit of dry FW.

# 2.6. Pigeon growth rate

The initial and final body weights were determined for each pigeon, and the growth rate was calculated as the increase in body weight divided by the feeding time (days).

## 2.7. C and N analysis

The C and N contents were determined from finely ground (<0.150 mm) subsamples using a Vario isotope cube analyser (Elementar Analysensysteme GmbH, Hanau, Germany). All analyses were performed in triplicates.

# 2.8. Stable isotope analysis

The manure sampled on the final feeding and composting day, the CF and FW-LDR sampled on the initial day, and the pigeon muscle sampled on the final day were freeze-dried, ground by a ball mill, and  $\delta^{13}$ C and  $\delta^{15}$ N were measured with an Elementar Vario MICRO cube elemental analyser (vario isotope cube, Hanau, Germany) coupled to the GV Isoprime 100 isotope ratio mass spectrometer (GV Instruments, Manchester, UK). All analyses were performed in triplicates.

# 2.9. N fractions in manure

The N fractions (organic N, ammonium N, nitrate N, and uric acid N) in the pigeon and the composting manure sampled on the final day were determined according to the methods of Chadwick et al. (2000) [23].Briefly, ammonium N, nitrate N were determined after shaking 40 g (fresh weight) of manure with 200 ml 2 M KCl for 2 h and uric acid N was determined using sodium acetate extracts and UV spectrophotometry. The organic N was calculated by total N subtracting ammonium N, nitrate N, and uric acid N.

# 2.10. Fourier transform infrared (FT-IR) analysis

The functional groups in the samples, similar to those identified in the stable isotope analysis, were characterised using FT-IR spectroscopy. Briefly, dry powder (<0.15 mm) samples were compressed with potassium bromide (KBr) into pellets at a ratio of 1:100. Next, FT-IR spectra were averaged total from 64 scans measured from 4000 to 400 cm<sup>-1</sup> with a 4-cm<sup>-1</sup>spectral resolution. The FT-IR analysis was performed using a Frontier MIR-FIR spectrometer (PerkinElmer, Waltham, MA, USA).

#### Table 1

Nutritional composition of the food waste (FW) and commercial feed (CF).Data are means  $\pm$  Sd (n = 3).

| Sample # | Sampling time | Starch (%)                         | Protein (%)                        | Fat (%)          | Fibre (%)                         | Ash (%)                           | Calcium (%)                       | Phosphorus (%) |
|----------|---------------|------------------------------------|------------------------------------|------------------|-----------------------------------|-----------------------------------|-----------------------------------|----------------|
| FW#1     | 04–2022       | $\textbf{28.48} \pm \textbf{0.24}$ | $24.54 \pm 0.02$                   | $12.69 \pm 0.04$ | $\textbf{1.79} \pm \textbf{0.04}$ | $\textbf{2.03} \pm \textbf{0.04}$ | $1.23\pm0.02$                     | 0.94±          |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.02           |
| FW#2     | 05-2022       | $\textbf{28.40} \pm \textbf{0.16}$ | $23.52\pm0.17$                     | $12.74\pm0.10$   | $1.97 \pm 0.10$                   | $2.39\pm0.04$                     | $1.41 \pm 0.01$                   | $0.96\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.01           |
| FW#3     | 06-2022       | $24.90\pm0.05$                     | $19.23\pm0.12$                     | $11.55\pm0.13$   | $1.45\pm0.05$                     | $1.92\pm0.04$                     | $1.13\pm0.05$                     | $0.66\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.04           |
| FW#4     | 07-2022       | $26.82 \pm 0.08$                   | $21.34\pm0.59$                     | $10.87\pm0.52$   | $2.55\pm0.16$                     | $2.31\pm0.08$                     | $1.33\pm0.05$                     | $0.65\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.04           |
| FW#5     | 08-2022       | $29.15\pm0.23$                     | $28.56 \pm 0.23$                   | $14.09\pm0.22$   | $2.61\pm0.02$                     | $\textbf{2.59} \pm \textbf{0.04}$ | $1.23\pm0.05$                     | $0.77\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.01           |
| FW#6     | 09–2022       | $28.74\pm0.05$                     | $19.02\pm0.34$                     | $12.2\pm0.04$    | $1.58\pm0.07$                     | $1.89\pm0.06$                     | $\textbf{0.88} \pm \textbf{0.04}$ | $0.65\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.03           |
| FW#7     | 10-2022       | $\textbf{26.44} \pm \textbf{0.42}$ | $16.98\pm0.27$                     | $14.31\pm0.33$   | $\textbf{2.03} \pm \textbf{0.23}$ | $1.78\pm0.13$                     | $\textbf{0.82} \pm \textbf{0.01}$ | $0.57\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.03           |
| FW#8     | 11-2022       | $25.73\pm0.14$                     | $17.88\pm0.17$                     | $13.25\pm0.19$   | $1.65\pm0.05$                     | $1.89\pm0.08$                     | $1.08\pm0.02$                     | $0.75\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.05           |
| FW#9     | 12-2022       | $27.08\pm0.05$                     | $\textbf{22.18} \pm \textbf{0.28}$ | $13.70\pm0.23$   | $\textbf{2.45} \pm \textbf{0.10}$ | $\textbf{2.19} \pm \textbf{0.07}$ | $\textbf{0.88} \pm \textbf{0.04}$ | $0.91\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.07           |
| FW#10    | 01-2023       | $\textbf{27.45} \pm \textbf{0.04}$ | $21.40\pm0.09$                     | $12.33\pm0.13$   | $1.87\pm0.17$                     | $1.69\pm0.07$                     | $0.91\pm0.05$                     | $0.58\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.06           |
| FW#11    | 03–2023       | $26.40\pm0.40$                     | $18.98\pm0.11$                     | $13.55\pm0.21$   | $1.56\pm0.07$                     | $1.92\pm0.06$                     | $1.12\pm0.08$                     | $0.80\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.06           |
| FW#12    | 03-2023       | $25.83 \pm 0.04$                   | $20.81 \pm 0.19$                   | $10.82\pm0.18$   | $1.34\pm0.11$                     | $1.58\pm0.03$                     | $\textbf{0.83} \pm \textbf{0.04}$ | $0.76\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.03           |
| CF#1     | 11-2022       | $31.45\pm0.07$                     | $16.48\pm0.29$                     | $5.09 \pm 0.23$  | $\textbf{2.87} \pm \textbf{0.05}$ | $2.59\pm0.09$                     | $0.91\pm0.04$                     | $0.77\pm$      |
|          |               |                                    |                                    |                  |                                   |                                   |                                   | 0.04           |

# 2.11. Composting manure DNA extraction and high-throughput sequencing

Five grams of composting manure from each beaker, sampled on days 20, 50, and 70, were merged into a mixture sample for analysis. A TIANNAMP Soil DNA Kit (TIANGEN, Beijing, China) was used to extract soil DNA. The quantity and quality of DNA were evaluated. The DNA was then stored at -80 °C in an ultra low temperature refrigerator (MDF-C8V1, Panasonic, Japan) until use. The 16S rRNA gene of bacteria (in the V3–V4 region) was amplified using the primers 515F and 806R and 528F and 706R. The fungal rRNA gene was amplified using the ITS1-F, ITS2-2043R, and ITS5-1737F primers. Bacterial and fungal DNA sequencing was performed using an Illumina HiSeq 2500 platform (BGI Co. Ltd., Shenzhen, China). Sequence analyses were conducted according to the methods described by Shi et al. (2022) [24].

#### 2.12. Statistical analysis

All statistical analyses were performed using Origin, version 9.0 (Originlab, Northampton, Massachusetts, USA). Differences between treatments were analysed using analysis of variance (ANOVA) with the least significant difference (LSD) as a post-hoc test. The significant levels were set as 0.05 and 0.01 in comparison of means and ANOVA, respectively.Correlation analysis was used to determine the relationship between the two variables. C (N) depletion was defined as the total C (N) per kilogram of feedstock subtracted from the total C (N) in the manure. The  $\delta_{\rm C}$  (N) enrichment was defined as the difference in C (N) values between the product and the feedstock.

# 3. Results and discussions

# 3.1. Nutritional composition in the FW

As shown in Table 1, the FW was consistently high in protein (16.98–28.56 %) across the sampling times. Compared to CF, FW was significantly higher in protein and fat, significantly lower in starch and fibre, and similar in ash, calcium, and phosphorus, which demonstrated that the FW was nutrient-rich.Proximate composition of FW in the canteens of Mulawarman University, Indonesia was 50.22 % carbohydrate, 10.47 % protein, 9.45 % fat and 7.44 % ash on a wet weight basis [25]. Therefore, the contents of protein and fat in the FW from the Chinese university are strikingly higher than that from the Indonesia university.

# 3.2. Feed intake and pigeon growth

During the entire feeding period, the pigeons exhibited active and frequent feeding behaviour, consuming and excreting throughout the day. Pigeons exhibited frequent movements and occasionally moved in circles. Interestingly, the pigeons showed a strong aversion to both freshly milled and dry-milled FW, indicating a potential dislike for the taste or flavour profile of the feed.



Fig. 2. The relationship between the food intake and manure mass during the pigeon feeding.



Fig. 3. The relationship between C and N contents in the pigeon manure.

Pigeons exhibited significantly higher feed intake in the FW-CF group than in the CFa group, and feed intake varied significantly across different feeding dates (feed type Fprob <0.0001, feeding date Fprob <0.0001, ANOVA, Table S1). The feed intake during the 33-day feeding period was  $30.28 \pm 0.54$  g per pigeon per day (n = 20) in the CFa group and  $36.24 \pm 0.43$  g per pigeon per day (n = 20) in the MF group. The pigeons in the MF group had a growth rate of  $2.28 \pm 0.27$  g/day (n = 20), which was significantly higher than that in the CFa group ( $1.65 \pm 0.27$  g/day; n = 20) (Fprob <0.01, ANOVA, Table S2), indicating that FW significantly enhanced the food intake and growth of pigeons, which could be primarily attributed to the higher nutrient content, particularly proteins and fats, present in the FW.

Furthermore, the safety of the dry FW powder obtained by heating fresh FW at 100 °C for 2 h was assured as upcycling nutrients through proper thermal processing of FW into animal feed offers biosecurity benefits compared to other disposal methods [26]. Compared to species with growth rates exceeding 20 g/day [22], the growth rate of the species investigated in this study was notably low. This finding further confirms that the species belongs to the slow-growing category.

# 3.3. Manure yield and characteristics

The manure yields were  $0.13 \pm 0.03$  kg/kg (n = 132) in CFa,  $0.17 \pm 0.02$  kg/kg (n = 132) in MF, and  $0.46 \pm 0.06$  kg/kg (n = 4) in AnC. A significantly higher manure yield was observed in the MF group than in the CFa group (Fprob <0.01, ANOVA, Table S3). As illustrated in Fig. 2, a significant positive correlation between the mass of pigeon manure and the feed intake was observed.

The manure nitrogen (N) contents on a dry basis were 10.29 % (8.04–11.41 %, Sd = 0.73 %, n = 132) in CFa, 11.28 % (10.08–12.57 %, Sd = 0.70 %, n = 132) in MF, and 3.72 % (3.68–3.76 %, Sd = 0.03 %, n = 4) in AnC. The N content in MF was significantly higher than that in CFa (Fprob <0.01, ANOVA, Table S4), which can be primarily attributed to the higher N concentration and different protein compositions in the MF and the increased food intake of the pigeons. Compared to the initial feedstock, the manure N content



Fig. 4. The relative bacterial and fungal abundance at phylum and genus level in the composting manure (CM). A- Fungal abundance at the phylum level, B- bacterial abundance at the phylum level, C- fungal abundance at the genus level, and D-bacterial abundance at the genus level. CM1, CM2, and CM3 represent CM on days 20, 50, and 70, respectively.

increased by 253.19 %, 286.98 %, and 29.16 % in CFa, MF, and AnC, respectively, indicating that the N content was enriched during the feed of pigeons and composting. Notably, the N content in pigeon manure was remarkably high and equivalent to that found in compound fertilisers [27]. Extensive studies have shown that the N content in organic manure is generally low. For instance, the N content in 86 fresh dairy manure and 91 poultry manure samples were 0.36 % and 1.45 % of fresh weight, respectively [28,29]. Similarly, the N content (on a dry basis) in 108 poultry manure samples from different locations ranged from 2.87 % to 3.58 % [30]. Chadwick et al. (2000) [23] analysed 50 different manure samples, including slurries, farmyard manure, and poultry manure, and found that the organic N content on a dry basis varied from 2.29 % to 5.09 %. Furthermore, Chen and Yu (2019) [31] collected 19 chicken, 21 pig, and 25 cow manure samples from six large-scale farms in the northern region of Shanxi Province, China, and found that the N content ranged from 1.62 to 4.37 % in chicken, 1.47–3.35 % in pig, and 0.71–2.47 % in cow manure. Composted manure generally has a lower N content owing to significant N losses during the composting process [14–17,32].

Herein, manure with nitrogen (N) content exceeding 10 % on a dry basis was first documented. Interestingly, we observed a significant and negative relationship between N content and C contents in the manure, as illustrated in Fig. 3.

Furthermore, we quantified the rate of N and C depletion in the manure. Specifically, the N depletion rate was 0.360 g/day/pigeon (0.090–0.673 g/day/pigeon, n = 132) in the CFa and 0.253 g/day/pigeon (0.089–0.423 g/day/pigeon, n = 132) in the MF groups. In contrast, the C depletion rate was 9.400 g/day/pigeon (4.562–16.622 g/day/pigeon, n = 132) in the CFa and 11.224 g/day/pigeon (8.767–15.38 g/day/pigeon) in MF groups. These findings shed light on the mechanism underlying the production of N-rich manure. The pigeons eliminated a significantly greater amount of C than N. Pigeons have a short intestinal system that allows only a brief period of food retention. Particularly, slow-growing and highly mobile species, such as pigeons, could have high energy requirements and relatively low N requirements. Consequently, pigeons engage in frequent feeding and excretion, rapidly digesting easily digestible nutrients such as carbohydrates and lipids while excreting undigested proteins, nitrogen metabolites (e.g. uric acid), and nitrogen-containing compounds transformed by microorganisms.

Composting of the four beakers was successfully completed by day 100, as evidenced by the transformation of the manure into a brown colour. The enrichment of N in the composted manure may be due to a greater depletion of C (66.08 g C/1000 g FW n = 4) compared to N depletion (33.69 g N/1000 g FW, n = 4). A previous study on the aerobic composting of N-rich FW resulted in manure with an N content that was 21.7 % lower than that of the initial FW, owing to significant N loss [17]. In contrast, the manure produced in this study through the AnC of protein-rich FW showed a remarkable increase in N content, which was approximately 29.16 % higher than that of the initial FW.

During composting, the fungal phylum *Ascomycota* was the dominant group, as depicted in Fig. 4A. These findings are consistent with those of previous studies [33,34]. The dominance of *Ascomycota* is associated with the presence of fungal communities that are adept at utilizing simple substrates, particularly those rich in sugars [35]. Regarding the specific fungal genera, *Candida* spp., *Pichia* spp., and *Kazachstania* spp. were dominant during composting, as shown in Fig. 4C. *Candida* spp. is effective in FW composting and efficiently degrade oils [36]. Therefore, *Candida* spp. may play a crucial role in composting FW-LDR by facilitating the breakdown of fats in the waste. Additionally, *Candida* spp. and *Kazachstania* spp. are the core fungal species that contribute to the decomposition of organic matter, including starch and protein, during FW composting processes [37]. The relative abundance of *Pichia* spp. exhibited a decreasing trend throughout the composting period. Previous studies have highlighted the ability of *Pichia* spp. to efficiently break down various types of organic matter, including organic acids, during the composting of FW [38]. *Firmicutes* and *Proteobacteria* were

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Fig. 5. FT-IR spectra of commercial feed (CF), food waste from large dining room (FW), manure of pigeons fed with CF (PM-CF), manure of pigeons fed with a mixture of CF and FW (PM-MF), and composting manure (CM).

| Table 2   |               |                |                |                       |          |
|---|---------------|----------------|----------------|-----------------------|----------|
| $\delta\text{-}C$ and $\delta\text{-}N$ in feed | , food waste, | pigeon manure, | pigeon muscle, | and composting manual | re (‰)ª. |

|       | δ <sup>13</sup> C (‰) | δ <sup>15</sup> N (‰) | $\delta^{13}$ C enrichment (‰) | $\delta^{15}$ N enrichment (‰) |
|-------|-----------------------|-----------------------|--------------------------------|--------------------------------|
| CF    | $-23.01\pm0.62$       | $-0.28\pm0.00$        | _                              | _                              |
| FW    | $-25.85\pm0.46$       | $0.70\pm0.03$         | -                              | -                              |
| PM-CF | $-24.18\pm0.45$       | $2.04\pm0.16$         | $-1.17\pm0.67$                 | $2.32\pm0.16$                  |
| PM-MF | $-23.55\pm0.21$       | $2.64\pm0.10$         | $0.31\pm0.39$                  | $2.62\pm0.11$                  |
| PMbm  | $-19.33\pm0.42$       | $3.89\pm0.26$         | $4.53\pm0.17$                  | $3.88\pm0.27$                  |
| CM    | $-27.64\pm0.21$       | $1.27\pm0.01$         | $3.26\pm0.95$                  | $2.22 \pm 0.39$                |

<sup>a</sup> Mean  $\pm$  standard deviation (n = 4). FD - commercial feed, FW - food waste from a large dining room, PM-CF - manure of pigeons fed with CF, PM-MF - manure of pigeons fed with a mixture of CF and FW, PMus - pigeon breast muscle, and CM - compost manure from FW.

the dominant bacterial phyla throughout the composting period (Fig. 4B), and their abundances were closely linked to carbohydrate and amino acid metabolisms [39]. Therefore, starch and protein were the dominant nutrients in the FW, and the composition of the bacterial communities was significantly influenced by these nutritional components. Specifically, the relative abundance of *Firmicutes* decreased from 89.8 % on day 20–61.5 % on day 70, whereas that of *Proteobacteria* increased from 8.5 % on day 20–31.7 % on day 70 (Fig. 4B). The sugar-consuming bacterium *Lactobacillus* [40] was the most dominant genus. However, the relative abundance of *Lactobacillus* declined from 78.3 % on day 20–30.1 % on day 70 (Fig. 4D). In contrast, the abundance of the cellulolytic bacterium *Acetobacter* [41] increased from 1.9 % on day 20–22.4 % on day 70. During FW composting, different constituents were degraded at varying rates, with starch exhibiting the highest degradation rate [37]. Therefore, observing a decrease in the starch-to-fibre ratio as composting progressed was reasonable, aligning with the observed changes in the *Lactobacillus* spp. and *Acetobacter* spp abundances. These results suggest a close association between the dominant fungal and bacterial species and the nutrient composition within the compost.

The manure yield achieved through AnC was twofold higher than that obtained from pigeon feeding, suggesting that AnC is an efficient method for producing N-rich manure. However, pigeon feeding resulted in a quicker production of manure than composting. Additionally, the pigeon manure exhibited N contents nearly three times higher than that of the AnC manure. Furthermore, pigeon feed yields marketable pigeon meat. In this study, conversion of 1000 kg of FW could derive 143 kg of pigeon manure and 27 kg of fresh pigeon in pigeon feeding and 460 kg of manure in AnC. The economic output values for conversion of 1000 kg of FW were estimated as 1108 Yuan RMB and 92 Yuan RMB in pigeon feeding and AnC, respectively. Consequently, from an economic standpoint, pigeon feeding is a more viable option for FW disposal than composting; however, further studies at the farm scale need to be conducted to confirm the findings of this study and system optimisation. Additionally, other species-specific feeding strategies can be developed for the sustainable management of the FW.

# 3.4. Manure characterization

In the FT-IR spectra (Fig. 5), the absorption intensity of the manures (PM-CF, PM-MF, and CM) was significantly lower than that of feedstocks (CF and FW-LDR) in a broad, with a weak band near 3404 cm<sup>-1</sup> typically associated with O–H stretching. This observation strongly indicates the depletion of –OH–rich carbohydrates in the manure. CF, FW, and CM exhibited a high intensity of narrow absorption peaks near 2925 cm<sup>-1</sup> and 2850 cm<sup>-1</sup>, which are attributed to asymmetric stretches of C–H bonds [42]. Interestingly, these peaks were absent in PM-CF and PM-MF, confirming the substantial depletion of C-rich nutrients such as starch and fat in these manure



Fig. 6. The nitrogen fractions in the different manure samples. PM-CF - manure of pigeons fed with CF, PM-MF - manure of pigeons fed with a mixture of CF and FW, CM - compost manure from FW.

samples. The PM-CF and PM-MF groups displayed higher absorption intensities than CF, FW, and CM groups, particularly in the narrow band around 1646  $\rm cm^{-1}$  associated with N–H bonds, which suggests that proteins were enriched in PM-CF and PM-MF, whereas their concentrations were diluted in the CM due to degradation, as compared to CF or FW. Therefore, the FT-IR analysis clearly demonstrated the transformation of nutrients from the feedstocks to manure regarding their composition and content.

The pigeon manure exhibited high  $\delta^{15}$ N values (>2 ‰), with the pigeon muscle displaying an exceptionally high  $\delta^{15}$ N value of 3.89 ‰ (Table 2). The  $\delta^{15}$ N values of FW-LDR were significantly higher than those of CF (Fprob <0.01, ANOVA, Table S5), which can be attributed to the higher  $\delta^{15}$ N values found in animal tissues compared to plant materials [43]. The  $\delta^{15}$ N enrichment was greater than the  $\delta^{13}$ C enrichment in PF-MF and PF-CF and lower than the  $\delta^{13}$ C enrichment in CM, suggesting that the differences between C and N depletion were more pronounced in PF-MF and PF-CF compared to CM. Compared to the feedstock, slight  $\delta^{13}$ C enrichment was observed in PM-MF, slight  $\delta^{13}$ C depletion was observed in PM-CF, and significant  $\delta^{13}$ C enrichment was observed in PMbm and CM. Lynch et al. (2006) [23] reported that bulk compost  $\delta^{13}$ C values showed slight depletion, whereas  $\delta^{15}$ N values exhibited significant enrichment. Similarly, Kim et al. (2008) [44] found that  $\delta^{15}$ N increased during composting. Notably, the pigeon muscle displayed a highly enriched  $\delta^{15}$ N signature, consistent with the  $\delta^{15}$ N pattern observed in small mammals [43]. These results indicate that  $\delta^{15}$ C and  $\delta^{15}$ N enrichment in the manure is closely associated with the depletion of C and N during pigeon feeding and composting.

In PM-CF, PM-MF, and CM, organic N accounted for 90.38 %, 89.65 %, and 91.53 % of the total N, respectively (Fig. 6). The lower percentages of organic N in PM-CF and PM-MF can be attributed to the presence of uric acid-N, which accounted for 0.59 % and 0.55 % in PM-CF and PM-MF, respectively. Additionally, the proportion of  $NH_4$ -N was significantly higher in the CM than in the PM-CF or PM-MF, indicating a stronger mineralisation facilitated by microbial activity in the CM. Notably, the content of  $NO_3$ -N was negligible in all manure samples. These N fractions suggest that manure is a suitable N source for crops [23].

# 4. Conclusions

Sustainable management of FW from large dining facilities is of particular concern in China. This study developed a pigeon-specific feeding strategy to produce pigeon meat and excellent manure using FW from large dining facilities. This type of FW is rich in starches, proteins, and fats. The pigeons showed a strong aversion to the FW.However, adding the FW to the feed of pigeons significantly increased their food intake and promoted their growth. Interestingly, pigeons that grew at a low rate produced manure with exceptionally high N content, with the AnC-produced manure containing 29.16 % more N than that of FW. Pigeons exhibited a remarkable depletion of C and N. The N content in pigeon manure was significantly negatively correlated with C content. FT-IR analysis effectively characterised the transformation of nutrients such as carbohydrates, fats, and proteins during pigeon feeding and AnC. The enrichment of  $\delta^{13}$ C and  $\delta^{15}$ N in the manure was closely associated with the depletion of C and N during pigeon feeding and composting. The dominant fungal and bacterial species were closely related to the nutritional composition of the compost. Further studies at a farm scale are needed to confirm the findings of this study and optimise the system.

# Data availability statement

There is no data deposited into a publicly available repository, hence it will be made available on request.

# **Ethics statement**

The authors declare that the animal protocol in this study was approved by Experimental Animal Welfare Ethics Review Committee, Zhejiang University (Animal Protocol No.: ZJU20231115).

### CRediT authorship contribution statement

Mengjie Zhang: Investigation. Xiaoyan Zhang: Data curation. Hui Lin: Methodology, Conceptualization. Huabao Zheng: Resources, Conceptualization. Qifa Zhou: Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Qifa Zhou reports financial support and administrative support were provided by Zhejiang Provincial Science and Technology Burea. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

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