# Odorous VOCs released from bio-decomposition and its interaction mechanism with bacteria: Compared inter-type with intra-type household garbage

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## ABSTRACT

Volatile organic compounds (VOCs) and pathogens that are generated during fermentation of food waste by microorganisms have caused odor annoyance and health risks. In this work, the evolution profiles of them during aerobic decomposition of inter-type and intra-type food waste were compared in laboratory-controlled reactors for three months. The emission fluxes of VOCs reached peak on the 10th day (except fish waste), then decreased sharply until leveled off. For vegetable waste, watercress emitted higher concentrations ($2.5 \times 10^4$–$2.4 \times 10^6$ $\mu g/m^3$) and emission fluxes of VOCs (5.4–508 $\mu g\ kg^{-1}\cdot h^{-1}$) than pepper and onion. While for meat waste, the emitted VOCs from fish ($1.4 \times 10^5$–$8.4 \times 10^6$ $\mu g/m^3$ and 68.4–$4.3 \times 10^3$ $\mu g\ kg^{-1}\cdot h^{-1}$) were higher than chicken and pork. Dimethyl trisulfide (DMTS) and ethyl acetate as well as DMTS and methyl methacrylate were dominant oxygenated VOCs and volatile organic sulfur compounds in vegetable and meat wastes, respectively. The odor from the leachate was attributed to the emitted sulfur-containing compounds. The main fermented bacteria in intra-type food waste were highly similar, but differed greatly between the inter-type food waste with *Lactobacillus* and *Comamonas* as well as *Peptoniphilus* and *Wohlfahrtiimonas* as the major fermented bacteria in vegetable and meat wastes, respectively. Significant correlations between microbial community and VOCs including ethyl acetate, DMDS and 2-butanone were observed. As predicted by functional annotation of prokaryotic taxa (FAPROTAX), *Lactobacillus* and *Bacteroides* were correlated with the respiration of sulfur-containing compounds and hydrocarbon degradation, respectively, suggesting that production of VOCs was contributed mainly by bacteria communities. Pathogenicity of food waste was mainly due to *Enterococcus*, *Proteobacteria*, *Mycobacterium* and *Salmonella*. Our results are conductive to develop countermeasures to reduce VOC emission during food waste decomposition.

## 1. Introduction

With the improvement of human living conditions and acceleration of urbanization, waste production increases sharply. According to the report of Statista, the production of worldwide municipal solid waste (MSW) was approximately $2.02 \times 10^9$ metric tons in 2016, and will reach $3.40 \times 10^9$ metric tons by 2050 (Triassi et al., 2023). Among them, food waste is the major component, accounting for $\geq 50\%$ in many countries including the United Kingdom, Sweden, Germany, Malaysia,

and India (Thi et al., 2015). In China, food waste typically amounted to 49.6% of total MSW (242 million tons) in 2019 (Ma et al., 2024). Nowadays, landfilling, incineration, anaerobic or aerobic fermentation are widely used to reduce the volume and quantity of food waste. Among them, fermentation has become one of the most widely used method as food waste has high moisture content and organic matter (Kastner et al., 2012). However, it may lead to the emission of odorous volatile organic compounds (VOCs) and adversely affect human health and air quality (Zheng et al., 2020). For example, direct exposure to VOCs may lead to

neurasthenia, chronic or acute effects on respiratory tract, sensory irritation and allergy, and even cancer (Tong et al., 2019).

Numerous works have demonstrated that odorous VOCs were mainly from the anaerobic or incomplete aerobic fermentation of organic matter in waste. For example, 18 VOCs belonging to 10 VOC families are detected during aerobic decomposition of banana peel and >50% of them are malodourous substances (Zhang et al., 2021). Sulfide and aldehydes ketones are the dominant odor contributors at initial decomposition stage of uncooked and cooked food waste, respectively (Nie et al., 2023). Among 15 volatile organic sulfur compounds (VOSCs) released during food waste aerobic decomposition, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), carbonyl sulfide, methyl 2-propenyl disulfide, and methyl 1-propenyl sulfide are the most abundant (Wu et al., 2010). Previously, we found that oxygenated VOCs (OVOCs) (methyl methacrylate, vinyl acetate, 2-butanone, ethyl acetate) and VOSCs (DMS, carbonyl sulfide, DMDS) are the main VOCs emitted from simulated mixed food waste fermentation (Zhang et al., 2020). Obviously, decomposition of different food wastes will result in diverse odorous gas emissions. Nevertheless, the comparison of VOCs emission profile between intra-type and inter-type food waste decomposition has long been ignored. Further investigation is essential to develop more appropriate control/remediation techniques to alleviate adverse impact of waste disposal.

The malodorous waste gases are produced during microorganisms decomposing organic matter including protein, lipid and sugar in food waste. Their emission potential mainly depends on the dominant microorganisms in the waste, and the microorganisms' ability to produce spoilage-associated compounds. For example, presence of *Actinomycetes*, *Bacillus* and *Pseudomonas* in biowastes is directly related to trimethylamine and DMDS emission (Mayrhofer et al., 2006). Proteobacteria and Bacteroidetes in soil are positively correlated to DMDS emission (Abis et al., 2020). Heterotrophic bacteria and/or archaea in water were known to emit isoprene (Ooki et al., 2022). Nevertheless, little is known about the emitted VOCs interaction mechanism with fermentation bacteria during food waste decomposition, especially from the view of microorganisms' function. The difference of bacterial community between intra-type and inter-type food wastes and how specific microorganism in food wastes affect VOCs emission were not well understood.

Furthermore, some environmental factors are also correlated with VOCs emission as they may influence microbial activity through promoting or inhibiting the survival and growth of a wide range of microorganisms. For example, the release of most VOCs increased with temperature at early decomposition stage of food waste (Cui et al., 2022). The VOCs concentration are low at aeration rate of $\leq 0.1$ $I_{air}$ $kg^{-1}$ $min^{-1}$, moisture of $\geq 55\%$ and C/N of 60–77 during MSW composition (Delgado-Rodríguez et al., 2010). Thus, revealing how factors including temperature and pH influence VOCs emission is quite important.

In this study, the fermentation of three different kinds of vegetable (watercress, pepper and onion) and meat waste (pork, chicken and fish) was simulated in the laboratory, respectively. The main purposes are: (1) to compare distinction of malodorous waste gas produced from inter-type and intra-type garbage fermentation; (2) to analyze response of bacterial community during whole bio-decomposition; (3) to uncover interaction mechanisms between odorous gases and bacterial community from the aspect of toxic effect of VOCs to bacteria or biotransformation of VOCs by the typical bacterial strains. The obtained data could provide fundamental knowledge for revealing the source and environmental fate of odorous VOCs during the bio-decomposition of household garbage, as well as advice for odor emission minimizing and related health risk reduction.

## 2. Materials and methods

### 2.1. Experimental design

To stimulate natural fermentation, fresh vegetable (watercress, pepper and onion) and meat (pork, chicken and fish) with inherent microorganisms were purchased from Tangde farmers' market (Guangzhou, China) and used for anaerobic fermentation in the self-made reactors (0.3 m i.d × 0.8 m height, 56.5 L), respectively, after shredded into approximately 2 cm pieces in size and well-mixed. These food wastes were selected because they are typical and commonly consumed food. Besides, for vegetable waste, they represent vegetables with edible bulbs, fruits, and leaves, respectively (Liang et al., 2022). The detailed components of meat and vegetable wastes as well as the design and operation of reactors have been described elsewhere (Liang et al., 2022; Yu et al., 2021). Briefly, the main body of reactor consisted of two cylindrical polymethyl methacrylate tanks that were used for waste fermentation and leachate collection, respectively. Each reactor has an air pump, an air outlet, and leachate recirculation system. Prior to loading waste, fiberglass and 5 cm gravel were placed at the bottom of the fermentation tank to filter leachate. Then, about 10.0–12.5 kg of shredded food waste was loaded into reactors to initiate the decomposition at room temperature (25 ± 0.5 °C). The internal temperature was monitored by a thermocouple probe inserted to half-depth of the waste. Leachate (50 mL) was sampled on days 0, 2, 4, 6, 10, 14, 19, 24, 30, 40, 50, 60, 75 and 90 for microbial analysis after mixing well with peristaltic pump with a rate of 2.5 L min⁻¹. After sampling, 100 mL sterilized deionized $H_2O$ was occasionally supplemented to reactor to maintain enough leachate.

### 2.2. VOC analysis

VOCs (emitted from the food waste and the leachate) in the headspace of food waste fermentation reactor were sampled in 2.7-L Summa canisters (ENTECH Instruments Inc, Silonite™) and analyzed qualitatively and quantitatively using an Entech 7200 pre-concentrator coupled with gas chromatography-mass spectrometer (7890B GC-5977B MS, Agilent Technology, USA). The target VOCs were identified according to retention time and mass peak of standard PAMS (Photochemical Assessment Monitoring Stations) and TO-15 (Linde Spectra Environment Gases, USA). Detailed information about sample analysis, quality assurance and quality control, and identification of each compound were referred to reference (An et al., 2014) and Supporting Information (SI). The calculation of emission fluxes (mg kg⁻¹ h⁻¹) was referred to reference (Wu and Wang, 2015). Briefly, it was calculated by the following equation:

$$Emission\ fluxes = Q \times (Co - Ci)/M_w$$

Where, $Q$ is the airflow rate of compressed air (L h⁻¹), $Co$ is the VOCs concentration in the outlet of reactor (µg·L⁻¹), $Ci$ is the VOCs concentration in the inlet of reactor (µg·L⁻¹), and $M_w$ is the wet weight of food waste (kg).

### 2.3. Odor analysis by electronic-nose (e-nose)

To identify which substance caused the odor, the respond intensity of malodorous gases emitted from leachate was detected using E-nose PEN3.5 (Schwerin, Germany). The substances and their threshold values that PEN3.5 could detect can be found in previous study (Xu et al., 2019). Briefly, leachate (10 mL) was sampled in a 40-mL glass vial and capped with a poly tetra fluoro ethylene septum. After incubated at 30 °C for 10 min, the headspace gases were injected into e-nose carried by zero gas (room air that had been filtered through standard activated carbon) at a constant flow rate of 400 mL/min for 60 s. Before sampling, E-nose was pre-warmed for 10 min and cleaned for 200 s with filtered air. Data were acquired at 1-s interval by the pattern recognition software (WinMuster, Airsense Analytics GmbH., Germany) and statistically analyzed based on the sensor responses using principal component analysis (PCA). The e-nose was reestablished by purging the sensor system with filtered air for 120 s before the next sample injection.

## 2.4. DNA extraction and sequencing

The leachate (1.5 mL) was used to isolate total genomic DNA by the Rapid Soil DNA Isolation Kit (Sangon Biotech, Shanghai, China). Its concentration and purity were determined by Nano-Drop (2000) spectrophotometer. Polymerase Chain Reaction (PCR) was performed using the extracted DNA as the template and the primers 515F: GTGCCAGCMGCCGCGG and 806R: GGACTACHVGGGTWTCTAAT targeting V4–V5 region of 16S rRNA. Details about PCR amplification are provided in the SI. Library preparation and sequencing on the Illumina PE300 platform were conducted by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA994654).

The sequences were divided into Operational Taxonomic Units (OTUs) by cluster analysis at 97% similarity. OTUs were divided into bacteria and pathogens by Ribosomal Database Project (RDP) Classifier (Wang et al., 2007), based on Silva 138 and Human pathogen database (HPB, https://www.cerl.org/resources/hpb/content). Alpha diversity and richness were estimated based on Shannon, Simpson, Ace, and Chao index (Yang et al., 2019).

## 2.5. Correlation analysis between environmental factors, VOCs and microbial community

Regarding temperature, pH and the emitted VOCs with high concentration and toxicity were factors that may affect bacterial community, and the correlations between VOCs, environmental factors and specific microbial communities were analyzed. The collected data of temperature and pH are shown in Table S1. Variance inflation factor (VIF) analysis was used to filter self-related factors. The factors with VIF value ≤ 10 were retained to conduct canonical correlation analysis (CCA).

## 2.6. Statistical analysis

The relevant data were analyzed by the statistical software (IBM SPSS Statistics 19.0) and $p < 0.05$ is considered statistically significant. R program (version 3.3.1) was used to draw Venn diagram, bar plot, pie diagram, heat map and conduct CCA. Canoco version 5.0 software was used to conduct PCA based on the Bray-Curtis distance. Non-metric multidimensional scaling (NMDS) analysis was run by Qiime (2020.2.0), R program (version 3.3.1) and vegan (vsesion 2.4.3). Qiime is used to calculate the beta diversity distance matrix, while R program and vegan are used to analysis and drawing. FAPROTAX was conducted by the software FAPROTAX 1.2.1.

## 3. Results and discussion

### 3.1. The composition and emission profiles of VOCs

#### 3.1.1. The evolution profiles of VOCs released from single vegetable waste fermentation

As Table S2 shows, among 47 VOCs characterized and quantified by GC-MS, 34, 39 and 28 VOCs were detected during onion, pepper, and watercress waste fermentation, respectively. Total of 22 VOCs included four halogenated hydrocarbons (HHs), four aliphatic hydrocarbons (AlHs), three aromatic hydrocarbons (AHs), six OVOCs and five VOSCs were shared by three vegetable wastes, suggesting that they are the common pollutants from vegetable fermentation (Fig. S1 and Table S2). OVOCs and VOSCs contributed to 59.4% and 26.6%, 8.6% and 86.4% as well as 51.4% and 41.9% of total VOCs (TVOCs) in onion, watercress and pepper waste, respectively (Figs. S2–S4).Previous study has pointed out that OVOCs and VOSCs could be released from vegetable waste

under incomplete aerobic and high initial moisture content conditions (He et al., 2020). Specifically, in pepper and onion waste, dimethyl trisulfide (DMTS, 60.9% and 64.9%) was the most abundant VOSC, followed by DMDS (23.5% and 27.0%) (Figs. S2c and S3c). Whereas, in watercress waste, the two most abundant VOSCs were DMTS (43.5%) and DMS (26.6%) (Fig. S4c). Lokke et al. found that volatile sulfur compounds, such as DMDS are the cause of odor from cut onion (Lokke et al., 2012). For OVOCs, ethyl acetate (62.9%, 3.2% and 34.7%) and methyl methacrylate (31.4%, 66.0% and 21.9%) were the most abundant in onion, watercress and pepper waste, respectively (Figs. S2b–S4b). Other VOCs including 1,1-dichloroethene, toluene, n-heptane were minor and shared less than 9.76%, 1.53% and 2.63% of TVOCs released, indicating that they are insignificant as compared to OVOCs and VOSCs.

Further comparing VOCs emission concentration and fluxes, we found that the changing trends were very similar in three kinds of vegetable waste (Fig. 1a–c). They increased smoothly in the first six days, reached a peak on day 10, and then sharply decreased until leveled off after 60 days of incubation. Watercress waste emitted higher concentrations and emission fluxes of VOCs ($2.5 \times 10^4$–$2.4 \times 10^6$ µg/m³ and 5.4–508 µg kg⁻¹·h⁻¹) than pepper ($1.6 \times 10^3$–$6.3 \times 10^5$ µg/m³ and 0.38–136 µg kg⁻¹·h⁻¹) and onion waste ($9.7 \times 10^3$–$3.5 \times 10^5$ µg/m³ and 3.2–134 µg kg⁻¹·h⁻¹). This might be attributed to the different microbial community structures in three kinds of vegetable waste regarding emission of VOCs is related to microorganisms (Wang et al., 2019). For watercress and pepper wastes, drastic increase of VOCs concentration and emission fluxes on day 10 was due to the increased emission of VOSCs (DMTS and DMS), while for onion waste, OVOCs (ethyl acetate and methyl methacrylate) emission was dominant. These differences may be because watercress is rich in sulfur-based glucosinolates (Kopsell et al., 2007), and pepper contains thiols and dithiolanes (Naef et al., 2008), while onion tissue cells contain diverse phenolic and sulfur-containing compounds (Zhao et al., 2021). Notably, emission fluxes of ethyl acetate and methyl methacrylate in onion waste were near zero on day 0, suggesting that they were secondarily formed during conversion of cellulose and other oxygen-containing organic matter. As reported, OVOCs would be secondarily formed during orange waste incubation (Wu and Wang, 2015). Comparatively, the emission rates of vinyl acetate (from onion and watercress) and DMS (from watercress) decreased sharply in the first four days, implying that they were mainly evaporated directly from waste. However, their emissions increased again to form one "peak emission window" as the fermentation time increased from days 6–10. Overall, VOCs were mainly emitted on days 2–60 and days 6–60 for onion and watercress wastes, respectively. While for pepper waste, their production in the first 10 days accounted for ≥77.0% of the total amount. These results indicated that VOCs especially OVOCs and VOSCs were mainly released at the initial stage of pepper waste decomposition, as well as at the early and middle stage of onion and watercress decomposition. Previous studies also revealed that relatively high generation of VOCs mainly occurred at initial stage of dewatered sewage sludge, digested MSW and untreated food waste decomposing (Schiavon et al., 2017; Wu and Wang, 2015), suggesting that the emission of odorant VOCs focused on the early stage of fermentation. For the remaining VOCs (HHs, AlHs, AHs listed in Table S2), their emissions were trivial and maintained low concentration during whole decomposition.

By analyzing the malodorous gases released from leachate using e-nose (Fig. 2a–c), we found that total response of odor from onion waste was higher than watercress, with pepper as the least. The variation of sensors' response intensity was similar between pepper and onion waste and different from watercress waste. However, they were all consistent with the trend of VOCs released by vegetable waste, suggesting that most VOCs emitted from vegetable waste were odor molecules. Besides, considering VOSCs were the main released VOCs and sulfur-compounds, aromatics, sulfur- and chlorine-containing organic sensors had higher signal response intensity, we concluded that sulfur-containing
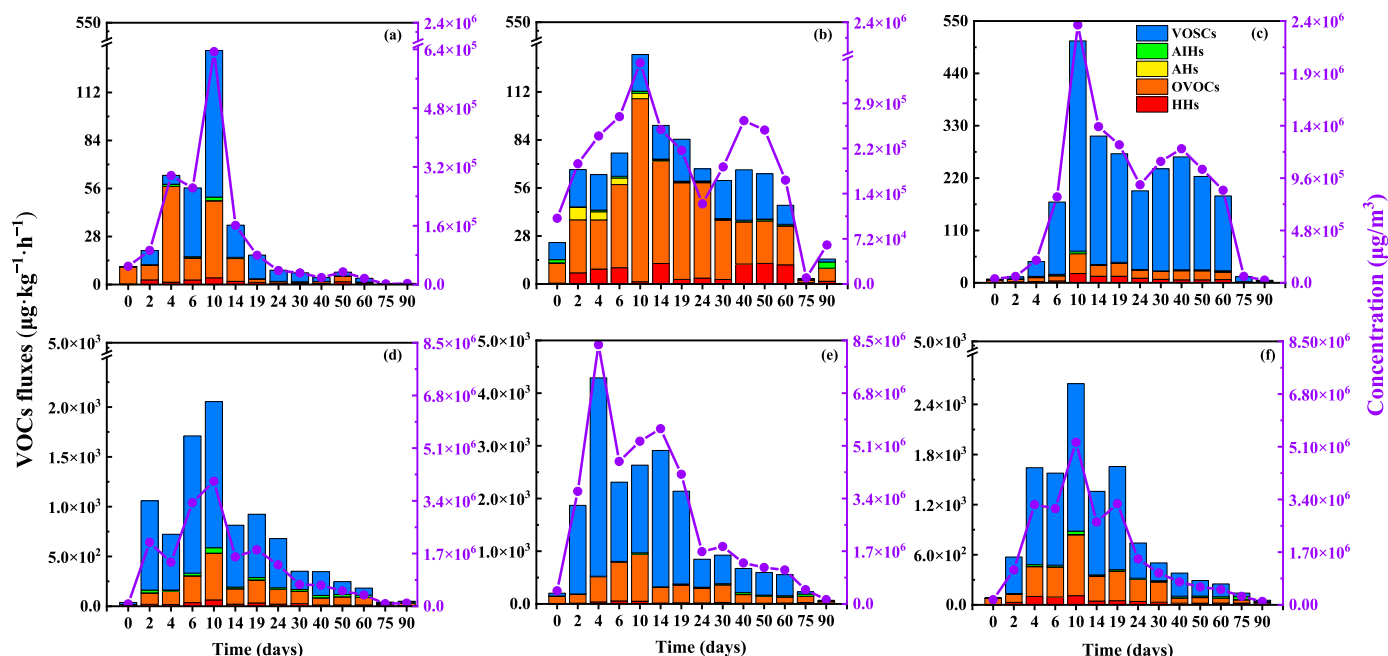
**Fig. 1.** The VOC emission fluxes and concentrations in pepper (a), onion (b), watercress (c), pork (d), fish (e), and chicken (f) waste.

compounds contribute greatly to the unpleasant odor. Furthermore, PCA was conducted to explain distinction of VOCs released by three kinds of vegetable waste. As Fig. S5a shows, principal component 1 (PC1) and 2 (PC2) together explained 90.4% of the variance. Pepper and onion waste were clustered together, but separated from watercress waste, suggesting that odor released by watercress was different from the other two kinds of waste. Overall, the trend of odor released from three kinds of vegetable waste was the same as that of VOCs.

*3.1.2. The evolution of VOCs released by single meat waste fermentation*

For meat waste, total of 41, 41 and 47 VOCs were detected from the headspace of chicken, pork, and fish waste fermentation, respectively, and 36 of them were shared by three kinds of meat waste (Fig. S1b). Similar to vegetable waste, OVOCs and VOSCs were the dominant VOCs, accounting for 67.4% and 25.4%, 20.9% and 72.8% as well as 20.9% and 76.2% in chicken, pork, and fish waste, respectively. While HHs, AHs and AIHs only accounted for less than 5.0%, 3.1% and 1.6% with dichloromethane, toluene, n-pentane as the dominant ones, respectively (Figs. S6–S8). This indicates that OVOCs and VOSCs were also the main odor nuisance in meat waste. Among VOSCs, DMTS accounted for 67.2%, 78.3% and 80.1% in chicken, pork and fish waste, respectively, followed by DMDS (Figs. S6c–S8c). These sulfur-containing compounds may originate from the microbial degradation of sulfur-containing amino acids/proteins in meat waste (Font et al., 2011). As reported, DMS could be emitted from naturally spoiling fish and sterile fish (Phan et al., 2012) and from microbial decarboxylation of sulfur-containing amino acids in meat (Li et al., 2021; Noseda et al., 2012). The percentage of OVOCs from chicken waste (25.4%) was higher than that in fish (20.9%) and pork (20.9%), with methyl methacrylate (65.4%, 56.7% and 63.0%) and vinyl acetate (18.2%, 22.9%, and 23.0% in chicken, pork and fish, respectively) as the two most abundant OVOCs (Figs. S6b–S8b). The different composition of meat might lead to the difference of the released VOCs. The relatively high concentration of OVOCs from chicken waste is possibly due to fermentation of its unsaturated fatty acids (Cartoni Mancinelli et al., 2021).

By analyzing the evolution trend of VOCs emitted from pork and chicken waste fermentation, we found that emission fluxes reached peak on the 10th day and then gradually decreased. However, for fish waste, the maximum emission flux appeared on the 4th day (Fig. 1d–f). It is

possibly because the intestinal microorganisms of fish accelerate decomposition of proteins and lipids. Besides, high level of myofibrillar proteins existing in soft fish muscle is also favorable for bacteria degradation (Delbarre-Ladrat et al., 2006). Thus, we can smell the odor immediately when fish is spoiled. The different emission concentrations in meat waste were mainly attributed to the produced methyl methacrylate and DMTS. The total concentrations of emitted VOCs followed the order of pork ($7.5 \times 10^4$–$4.0 \times 10^6$ μg/m$^3$) < chicken ($1.1 \times 10^5$–$5.2 \times 10^6$ μg/m$^3$) < fish ($1.4 \times 10^5$–$8.4 \times 10^6$ μg/m$^3$). Similarly, lower emission fluxes of VOCs were also found in pork (38.2–$2.1 \times 10^3$ μg kg$^{-1}$·h$^{-1}$) than chicken (55.3–$2.6 \times 10^3$ μg kg$^{-1}$·h$^{-1}$) and fish (68.4–$4.3 \times 10^3$ μg kg$^{-1}$·h$^{-1}$) (Fig. 1d–f). This may be due to that protein content per unit mass of fish and chicken was higher than that of pork. As reported, *Pseudomonas* spp. isolated from spoiled meat can decompose amino acids to sulphides, esters and amines (Casaburi et al., 2015). Very few VOCs were emitted on day 0, but about 79.0, 79.5 and 79.7% VOCs were released on days 2–19 in pork, chicken and fish waste, respectively, indicating that most VOCs were secondarily formed (Wu and Wang, 2015).

E-nose was further used to analyze the odorous VOCs emitted from leachate of meat waste (Fig. 2d–f). The total response of sensors increased rapidly in the first 30 days and then kept constant. Among the ten sensors, response of nitrogen oxides and sulfur compounds sensors was the highest. Specifically, in the first 6 days of fermentation, response of nitrogen oxides sensor was slightly higher than that of sulfur compounds sensor. However, with increasing incubation time, the response of sulfur compounds sensor was becoming dominant, suggesting that sulfur-containing odor was the main component of odor at later stage of meat waste fermentation. Moreover, the response trend of sulfur compounds sensor was consistent with concentration trend of VOSCs released from meat waste. As Fig. S5b shows, the discrimination of emitted odor between fish and the other two wastes was obvious, while the odor from chicken and pork wastes was clustered together, suggesting that they were similar. This is probably due to the similar chemical composition and fermented microorganisms between chicken and pork wastes.
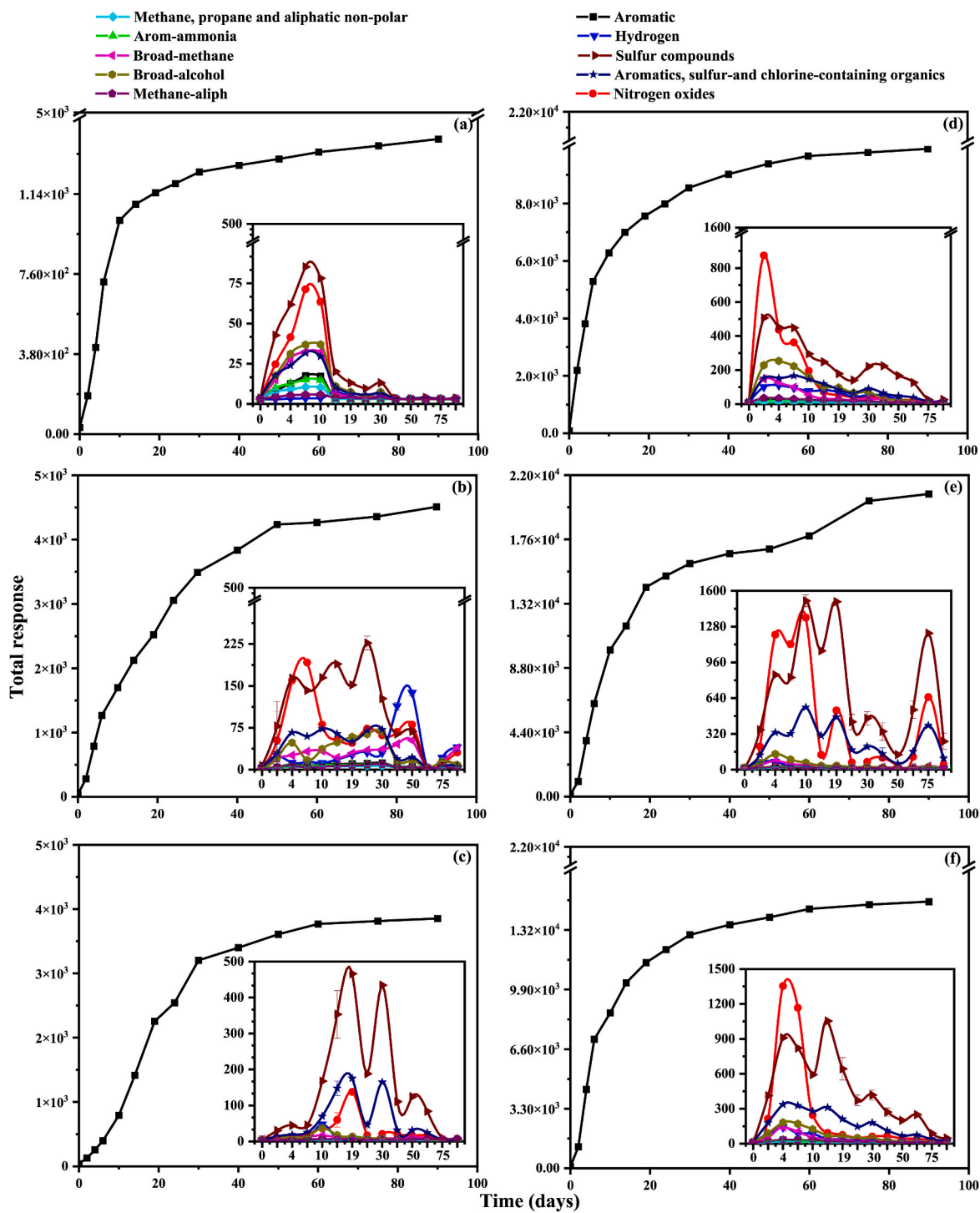
**Fig. 2.** The e-nose responds to the odor released from pepper (a), onion (b), watercress (c), pork (d), fish (e) and chicken (f) waste.

### 3.1.3. The comparison of VOC emission from inter-type food waste fermentation

By comparing VOCs emission from inter-type food waste, we found that vegetable waste fermentation produced slightly fewer types of VOCs (47 kinds) than meat waste (49 kinds), and up to 45 VOCs were shared (Fig. S9). The main VOCs were OVOCs and VOSCs. For vegetable waste fermentation, DMTS and ethyl acetate were the dominant VOSCs and OVOCs with total fluxes of $1.2 \times 10^3$ and $3.8 \times 10^2$ µg kg$^{-1}$·h$^{-1}$, respectively. Whereas for meat waste fermentation, the emissions of DMTS and methyl methacrylate were the highest with the total fluxes of $2.3 \times 10^4$ and $5.7 \times 10^3$ µg kg$^{-1}$·h$^{-1}$, respectively. Overall, the trend of

VOCs emission fluxes was similar, which increased first and then decreased with the maximum value detected around the 10th day. By further visualizing VOC dissimilarities between inter-type food waste by NMDS plots, we found that there is no significant separation at most fermentation time, except for days 2–6 of chicken and pork waste (Fig. 3a). The total amount of VOCs produced by meat waste was significantly higher than that of vegetable waste as they (such as chicken) contain more sulfur-containing protein and fat, while vegetable waste (such as watercress) contains more vitamins and cellulose (Gao et al., 2022). Besides, as the decomposer of food waste, different microbial community structures may also lead to the different VOCs
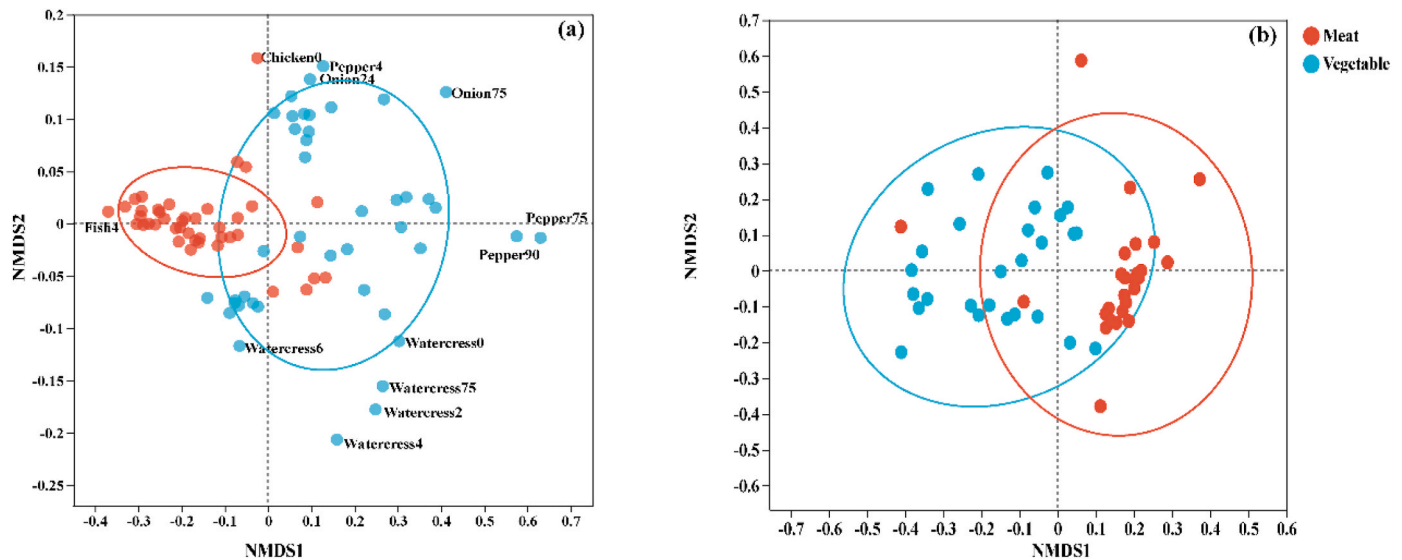
**Fig. 3.** NMDS analysis showing the overall distribution of VOCs (a) and pathogenic bacterial communities (b) produced during vegetable and meat waste fermentation.

pollution profiles between inter-type food waste.

### 3.2. Microbial community dynamics

#### 3.2.1. Composition and evolution of microbial community during vegetable waste fermentation

Microbial community plays a nonnegligible role during the whole process of waste fermentation. By Illumina sequencing of DNA from fermented microorganisms in leachate, total 282,091,9 reads were generated and clustered into 1753 OTUs. As Table S3 shows, the diversity of bacterial community in pepper and watercress waste showed a gradual increased trend with fermentation time, while for onion waste, the maximum and minimum bacterial diversity appeared on days 20 and 60, respectively. The average Shannon and Chao index in onion (1.79 and 163.10) and pepper (2.48 and 181.80) were lower than watercress (3.35 and 360.60), suggesting that watercress has higher microbial biodiversity and richness. Consistently, the concentrations and emission fluxes of VOCs emitted by watercress waste were also higher, indicating that VOCs emitted were relevant to microbial communities.

Based on the analysis of the microbial communities' structure, *Lactobacillus* (in onion and pepper) and *Comamonas* (in watercress) were the most dominant bacteria (Fig. S10). For onion waste, *Lactobacillus* gradually increased from 21.6% to 96.3% as the fermentation time increased from days 0–60 (except day 20); while for pepper waste, high abundance of *Lactobacillus* only found at early fermentation stage (days 2–30), with *Caproiciproducens* and *Lachnoclostridium* becoming dominant at late stage (days 45–90). Coincidently, a large amount of OVOCs were detected at whole stage of onion and early stage of pepper fermentation, suggesting that these VOCs may generate from waste decomposition by *Lactobacillus*. Previous study also revealed that *Lactobacillus* can convert oxygen-containing organic compounds such as sugars and xylose in waste into lactic acid, which can also be further converted to ethyl acetate and ketones (Cui et al., 2022; Sharma and Mishra, 2014). For watercress, although *Comamonas* only occupied ≤1% on days 0–2, it rapidly increased to 31.0% on the 10th day then decreased with average abundance of 17.8%. Notably, a similar changing trend was found for VOSCs emission fluxes in watercress, indicating that *Comamonas* may involve in the production of VOSCs. Previous study also confirmed that *Comamonas* could oxidize DMS to dimethyl sulfoxide (Horinouchi et al., 1999).

#### 3.2.2. Composition and evolution of microbial community during meat waste fermentation

By further analysis the microbial community in fermented meat waste, we found that the diversity on day 0 was the highest for chicken and pork waste, then decreased sharply on day 2, followed by a slow fluctuation till day 90. While for fish fermentation, a trend of decreasing diversity on the first 6 days and then gradually increasing to day 60 was found (Table S4). The Chao index of three kinds of meat waste was similar, which decreased first, then increased slowly and finally remained stable. Chicken waste had higher microorganisms' richness (215.23 on average) than pork (171.73 on average) and fish waste (145.44 on average).

The percentage of microbial community (Fig. S11) revealed that different from vegetable waste (*Lactobacillus* and *Comamonas*), *Peptoniphilus* and *Wohlfahrtiimonas* were the two most abundant bacteria in meat waste. Specifically, *Peptoniphilus* accounted for 37.6% and 27.0% of total bacterial 16S rRNA gene sequences on average in chicken and pork waste, respectively. They increased first then decreased sharply followed by gradually increased. The highest abundance occurred on days 20 (62.9% for chicken) and 90 (48.2% for pork). This is consistent with previous studies that *Peptoniphilus,* which can decompose peptone and amino acids (Aujoulat et al., 2021; Liang et al., 2020), was dominant in the initial phase of meat decomposition. For fish and pork waste, *Wohlfahrtiimonas* was dominant in the middle phase (days 10–60) with the maximum abundance detected on days 10 (44.8%) and 30 (44.0%), respectively. As *Wohlfahrtiimonas* is associated with sulfide formation (Li et al., 2019), we concluded that it may involve in the production of a large number of VOSCs in the middle phase of meat waste fermentation. Overall, *Peptoniphilus* and *Wohlfahrtiimonas* were the dominant microorganism influenced the VOCs emission during meat water fermentation.

#### 3.2.3. Comparison of microbial community between inter-type food waste

Fig. S12 shows the shared and unique genera identified in both meat and vegetable waste samples. Up to 36 genera were shared with *Enterococcus*, *Proteobacteria*, *Mycobacterium*, and *Salmonella* as the dominant bacteria. By further using Wilcoxon signed-rank test to investigate the difference of genera' relative abundance, we found that 15 genera had significant differences and the percentage of *Enterococcus, Salmonella* and *Bartonella* in vegetable waste was significantly (***$p <$ 0.001) higher than meat waste. Conversely, unclassified *Proteobacteria, Mycobacterium* and *Fusobacterium* were dominant in vegetable waste
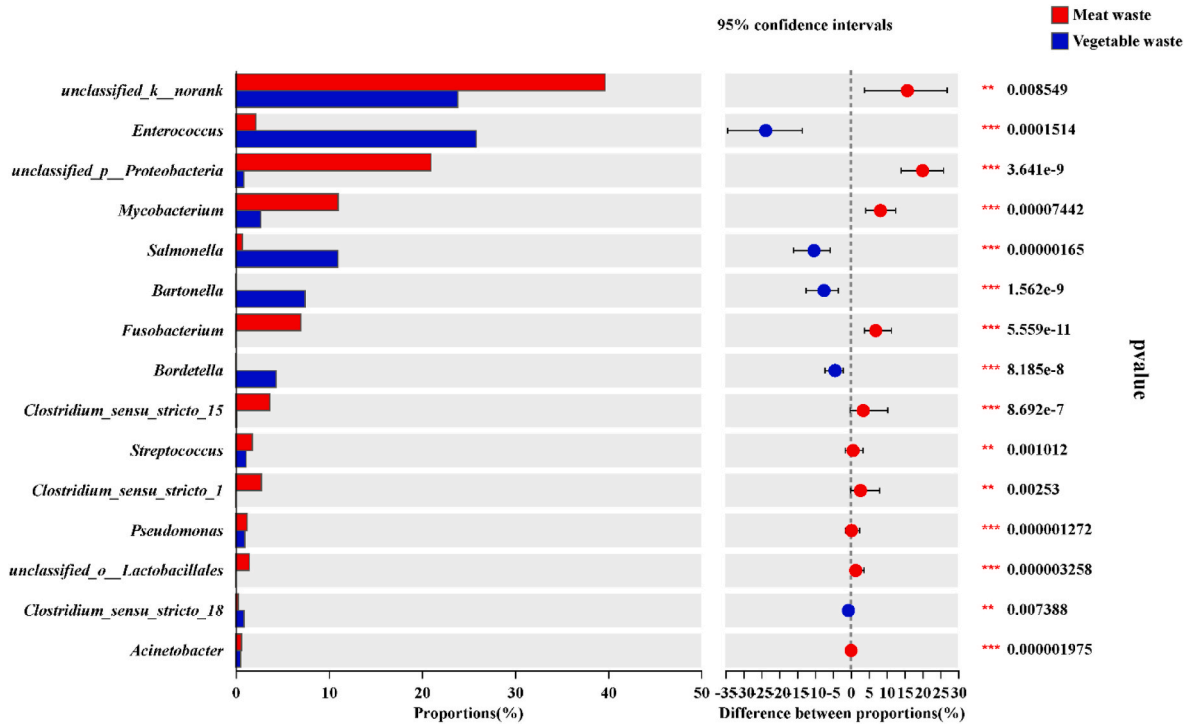
**Fig. 4.** Wilcoxon rank-sum test bar plot on genus level for meat and vegetable waste.

(Fig. 4). The NMDS analysis based on the Bray-Curtis distance was also used to assess the structural similarities of bacterial communities. As Fig. 3b shows, most bacterial communities in meat and vegetable waste were highly similar. Only a few samples from early stage of meat decomposition were separated from vegetable waste, indicating that bacterial communities in these samples are distinct from those in vegetable. Overall, microbial community composition between inter-type food waste was similar, indicating that they were typical residents in food waste responsible for VOC production.

*3.3. The relationship between fermented microorganisms and VOCs as well as environmental factors*

Regarding emitted VOCs and other environmental factors would affect the growth of fermented microorganisms, CCA was further used to reveal the relationship between these environmental factors and microorganisms. Firstly, multicollinearity, autocorrelation and heteroscedasticity of variables were checked by calculating the variance inflation factor (VIF). As Table S5 shows, 12 of 14 factors with VIF <10
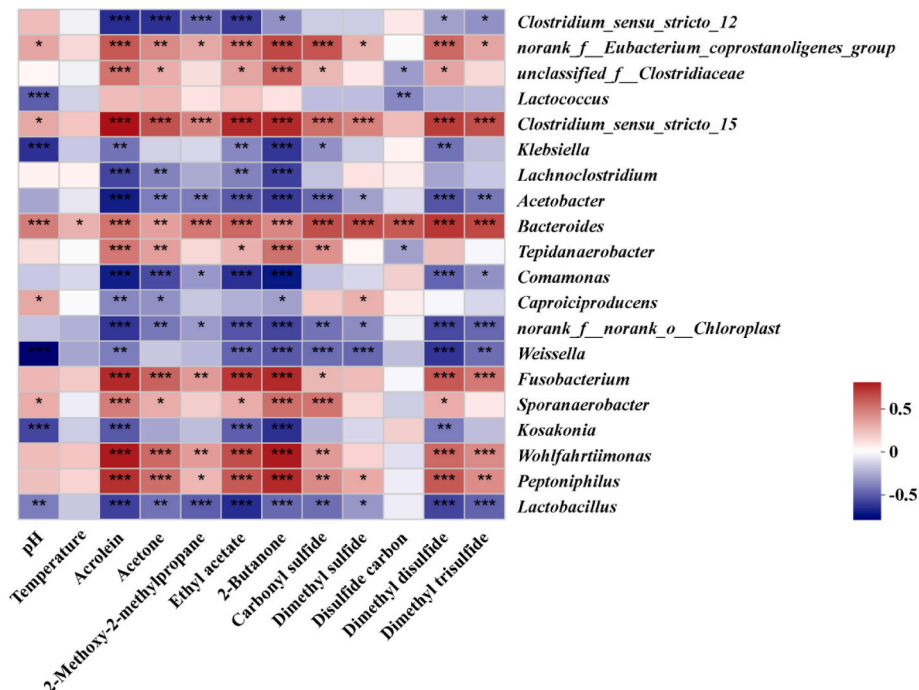


**Fig. 5.** Spearman correlation between bacterial community and environmental factors as well as VOCs.

was saved, meaning that the multicollinearity is less. Then, the relationship between these 12 factors and bacterial community was revealed by CCA, and the result showed that ethyl acetate, DMDS and 2-butanone were the top three factors that had significant effects on microbial communities (Fig. S13). DMS, carbonyl sulfide, and disulfide carbon mainly influenced the bacterial community from vegetable waste, while the rest factors had significant impact on the microbial community from meat waste. A correlation heat map (Fig. 5) showed that *Bacteroides*, *Clostridium sensu stricto 15*, *Eubacterium coprostanoligenes group*, *Wohlfahrtiimonas*, *Peptoniphilus* and *Fusobacterium* were positively correlated with these VOCs (except disulfide carbon), suggesting that the growth of these microorganisms would accelerate the release of these VOCs. As reported, *Bacteroidetes* from soil is positively correlated to the emitted VOCs such as DMDS (Abis et al., 2020). While *Lactobacillus*, *Chloroplast*, *Comamonas*, and *Acetobacter* were negatively related to VOCs in this study, indicating that these ten VOCs had an inhibitory effect on their growth. In addition, pH was negatively related to *Lactococcus*, *Klebsiella*, *Weissella*, *Kosakonia*, and *Lactobacillus*, and positively correlated with *Bacteroides*. This may be because *Lactobacillus* can decrease pH and increase acidity by decomposing large protein molecules into free amino acids and producing multiple organic acids (Shang et al., 2022). Overall, bacteria play an essential role in organic compounds decomposition and VOCs emission in food waste.

### 3.4. The function of fermented bacteria in food waste

To further explore the ecological function of fermented bacteria, the functional assemblage of microbiome was predicted by FAPROTAX (Yang et al., 2022). Nine of 69 functions related to VOC release was screened and used to construct the correlation heat map. For vegetable fermentation, *Lactobacillus, Weissella,* and *Klebsiella* were negatively correlated with respiration of sulfur-containing compounds (Fig. S14), meaning that they were related to sulfur cycling in food waste. *Acetobacter* and *comamonas* were positively correlated with hydrocarbon and aromatic compound degradation, leading to decrease of AHs and AIHs in food waste. This finding was inconsistent with previously studies, who found that *Comamonas* is one of the most common genera that is known to degrade hydrocarbons and aromatic compounds under oxic conditions (Fuchs et al., 2011; Parales, 2010), suggesting that *Comamonas* may degrade hydrocarbon and aromatic compounds under anaerobic–aerobic conditions. In contrast, *Bacteroides* and *Clostridium sensu stricto 15* were negatively related to hydrocarbon degradation in meat waste, suggesting that they may contribute to the emission of AHs and

AIHs (Fig. S15). However, further study also needs to demonstrate the relationship between VOCs and microorganisms.

### 3.5. The pathogenicity of food waste fermentation

Regarding pathogenic microbes can be aerosolized and transported to vicinities during waste disposal processes, the capacity of food waste decomposition to cause human infection was evaluated. As the composition of pathogenic bacteria shown in Fig. 6. *Enterococcus* was dominant in onion and at early stage of pepper fermentation. As a lactic acid bacterium, *Enterococcus* could cause invasive diseases in hospital patients and lead to high morbidity and mortality (Ali et al., 2022). Similar to *Lactobacillus*, in onion waste, *Enterococcus* showed an increased trend over time until reaching peak on the 60th day (90.4%) (except day 20), suggesting that onion fermentation may benefit its propagation. In pepper and watercress, the variation trend of *Enterococcus* was similar, which increased first and then decreased with the maximum abundance detected on day 6 (67.3% and 34.7%). *Salmonella* was found mainly at early stage of watercress and onion decomposition with the highest abundance found on day 2 (38.4% and 58.4% for onion and watercress waste). This indicates that early fermentation stage of watercress and onion waste may have higher health risk, considering the non-typhoidal *Salmonella* can cause acute enterocolitis and deaths (Marchello et al., 2022). Furthermore, *Burkholderia-Paraburkholderia* and *Bacteroides* that may cause melioidosis (Lafontaine et al., 2013) and periodontal diseases (van Steenbergen et al., 1989), respectively, were also dominant at later stage of watercress fermentation. Besides, high abundance of *Bartonella,* which may cause fever, lymphadenitis, endocarditis, myocarditis, and hepatitis (Buffet et al., 2013), was found at the mid and later decomposition stage of pepper waste. PCA analysis showed that the communities from onion and pepper waste were clustered together and separated from watercress waste, indicating that the pathogenic bacterial communities from pepper and onion have high similarity and were different from watercress (Fig. S16). This is in accordance with the above results that odor compositions emitted from pepper and onion were different from watercress, further demonstrating that these communities may involve in odor production by decomposing food waste. The dominance of *Enterococcus* and *Salmonella* suggested that vegetable waste fermentation may pose a serious threat or even life-threatening effect to human health, especially for the elderly, children, and other immunocompromised persons.

For meat waste decomposition, the main pathogenic bacteria were unclassified *Proteobacteria*, *Mycobacterium* and *Fusobacterium* (Fig. 7).
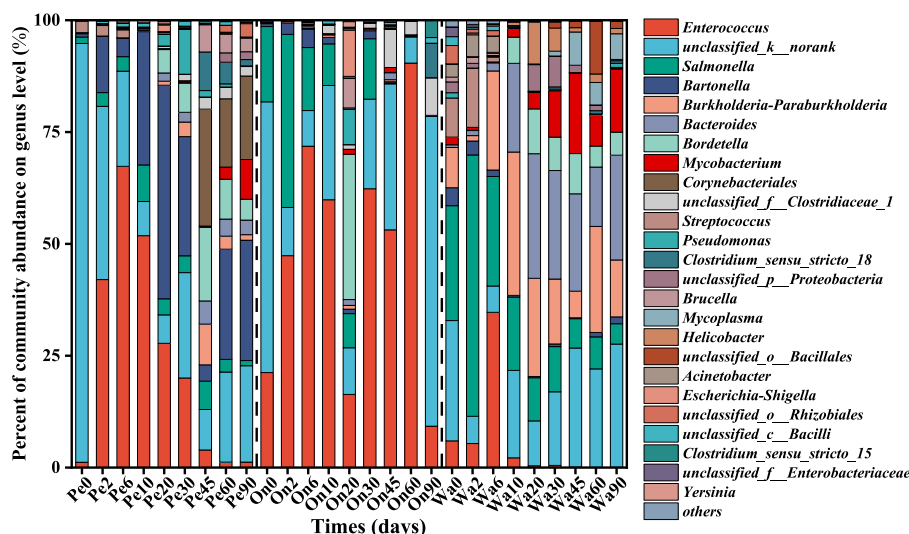


**Fig. 6.** The composition of pathogenic bacterial communities at genus level in different decomposition stage of pepper (Pe), onion (On) and watercress (Wa) waste.
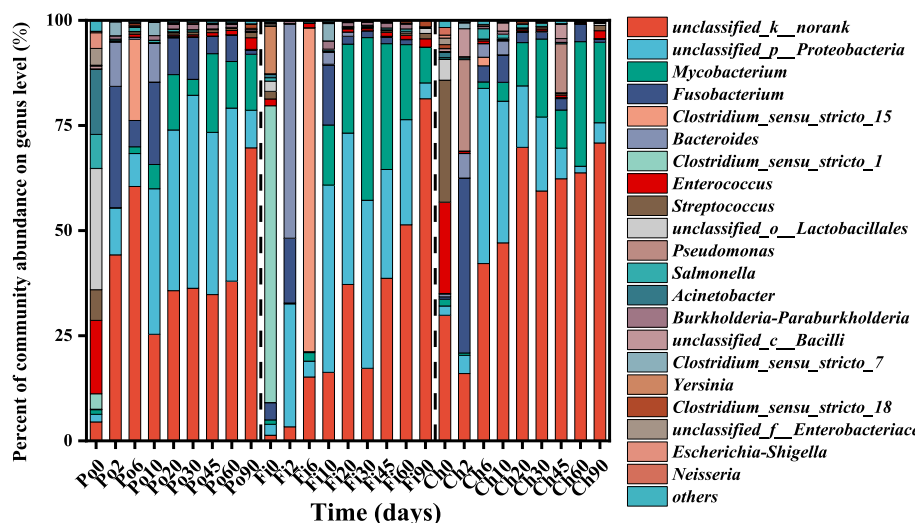
**Fig. 7.** The composition of pathogenic bacterial communities at genus level in different decomposition stage of pork (Po), fish (Fi) and chicken (Ch) waste.

Specifically, unclassified *Proteobacteria,* which function is decomposing simple polysaccharides or monosaccharaide (Bareither et al., 2013), was enriched in the middle phase and accounted for 14.2%, 23.4%, and 25.4% in the chicken, fish and pork wastes, respectively. *Mycobacterium* gradually increased before day 30 (day 20 for fish waste) then decreased, and it was dominant at later stage of meat fermentation. Regarding *Mycobacterium* may cause pneumonia or inflammation of other body organs (Zhao et al., 2022), attention should be paid at later decomposing stage. In contrast, *Fusobacterium*, which could cause upper respiratory infections, Lemierre's disease, and distant metastatic emboli (Lee et al., 2020; Thapa et al., 2022), was dominant at early stage of fermentation with the highest abundance on day 2. However, PCA showed that the pathogenic microbial communities in three kinds of meat waste were highly similar (Fig. S17). Overall, high abundance of pathogenic bacteria at the whole stage of meat fermentation suggests a serious threat to human health.

## 4. Conclusions

We measured VOCs and microbial communities during laboratory-controlled aerobic decomposition of inter-type and intra-type food waste. Vegetable waste fermentation produced slightly fewer types and concentrations of VOCs than meat waste with VOSCs (DMTS) and OVOCs (ethyl acetate and methyl methacrylate) being the most abundant. The emission fluxes were very low at day 0 and reached peak on day 10 (except fish waste which peaked on day 4), and then sharply decreased until leveling off after 60 days of incubation. Watercress and fish waste emitted higher concentrations and emission fluxes of VOCs than other vegetable and meat waste. The unpleasant odor was attributed to the sulfur-containing compounds emission. As predicted by FAPROTAX, the *Lactobacillus*, *Weissella*, and *Klebsiella* had the function of removing sulfur-containing compounds, while *Bacteroides* and *Clostridium sensu stricto 15* made positive contribution to the release of AHs and AIHs. *Enterococcus*, *Salmonella, Mycobacterium*, and *Fusobacterium* were the predominant pathogenic bacteria that may cause serious threat to human health. This research expounds the interaction mechanisms of microbial community to the malodorous VOCs during different food waste decomposition.

## CRediT authorship contribution statement

**Zhishu Liang:** Writing – original draft, Investigation, Formal analysis. **Qihao Feng:** Validation, Data curation. **Yuna Zhang:**

Methodology, Investigation. **Yun Yu:** Methodology, Investigation. **Wen Liao:** Validation, Data curation. **Guiying Li:** Writing – review & editing, Funding acquisition. **Taicheng An:** Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.141523.

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