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Responses of ammonia-oxidizing bacteria community composition to temporal changes in physicochemical parameters during food waste composting

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Abstract

Chemoautotrophic ammonia-oxidizing bacteria (AOB) serve an important function in ecological nitrogen transformation because of their great potential to alleviate ammonia emissions during aerobic composting. However, studies on the influence of specific environmental factors on AOB community dynamics in the food waste composting field are scarce. Hence, this study aimed to identify and prioritize some environmental parameters that affect AOB community composition during food waste composting. The composition and diversity of the AOB community were determined using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Relationships between the obtained parameters and AOB community composition were simultaneously evaluated by multivariate analysis. Phylogenetic analysis indicated that large amounts of *Nitrosomonas*-like and *Nitrosospira*-like lineages existed in different periods. The *Nitrosomonas* europaea/eutropha were the most dominant AOB species in the thermophilic stage. Redundancy analysis revealed that the dynamics of AOB community was mainly attributed to temporal changes in nitrate and pH of the compost material ($p < 0.05$). Variations (54.7% for AOB species data) were statistically explained by nitrate and pH, suggesting that these parameters were the most likely to influence, or be influenced by AOB community composition, and may further influence nitrogen cycle in the food waste composting ecosystem.

Keywords: Food waste composting; Ammonia-oxidizing bacteria (AOB); Community composition; Multivariate analysis

1. Introduction

Food waste is the largest component of household waste in China, more than 60 million tons of which are being produced each year, accounting for about 40%–50% weight of the total household waste. Food waste is usually transported to a landfill site for disposal.[1] However, various problems, such as putrid smell and leachate pollution of underground waters, are encountered. Incineration is another method for disposal but is not suitable for use because the low calorific value and high water content of food waste require high energy input. In addition, incineration causes air and environmental pollution.[2]

To date, composting is a promising alternative treatment technique that enables the reuse of valuable organic contents of food waste.[3, 4] However, high concentration of organic nitrogen in food waste is readily converted into ammonia-N (NH_4^+-N) by microorganisms. In addition, an alkaline pH may lead to substantial losses of nitrogen as gaseous ammonia (NH_3) during the composting process. The emission of NH_3 contributes to air pollution and reduces the fertilizing value of the compost.[5] Ammonia-oxidizing bacteria (AOB) serve an important role in nitrification during composting because it can oxidize ammonia and reduce the emission of gaseous ammonia (NH_3).[6] Moreover, the function of AOB to the nitrogen cycle has gained increasing attention in research on the basis of the fact that ammonia oxidation might be the rate-limiting step of nitrification.[7] Therefore, the underlying community succession of AOB and their responses to composting conditions need to be deeply understood.

AOB are now widely accepted as the major agents of nitrification. A number of AOB populations have been detected in various types of composting materials including commercial biofertilizer products. Several clusters including the genera *Nitrosospira* and *Nitrosomonas* are present among different kinds of composting materials, such as mushroom cultivation and pig or chicken manure.[8] Jarvis et al. detected *Nitrosomonas* in the theromophilic stage and *Nitrosospira* in the maturation phase of household waste composting.[9] Maeda et al. also detected *Nitrosomonas* throughout the process, especially from the surface

layer of a cattle manure composting pile.[10] Innerebner et al. found that the clone library from the sewage sludge compost was dominated by *Nitrosospira*-like sequences.[11] Food waste as raw material is now widely used in composting; however, few studies on the composition and diversity of AOB community have been reported.

AOB community dynamics may be influenced by various environmental parameters, including temperature, pH, ammonium concentration, and organic matter. Yamamoto et al. reported that pile temperature affected the AOB community structure.[12] They reported that a member of the *Nitrosomonas europaea* cluster dominated the community at high-temperature stage. Another study showed that different ammonia oxidizer phylotypes were selected in soils with different pH.[13] Furthermore, another study demonstrated that AOB population size was significantly greater in annually fertilized soil than that in unfertilized, suggesting that ammonium fertilization has a long-term effect on AOB population size.[14] Other researchers observed that the organic matter affected the nitrification rate and AOB community composition in the wastewater treatment reactor.[15] These reports helped us further understand that environmental parameters cause the actual composition of AOB community. However, few studies on the effects of temporal changes in physicochemical parameters on the community structure of AOB in the food waste composting field have been conducted.

An improved understanding of the different environmental parameters and their combinations that affect community composition of AOB, as well as their responses to environmental change is essential for the prediction and control of the ecosystem functions in food waste composting. Therefore, the objectives of this research were two-fold: firstly, investigated the diversity and community structure of AOB during different phases of the food waste composting by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE); and secondly, the influence of the physicochemical parameters, such as temperature, pH, ammonium concentration and organic matter which would significantly affect the AOB

community structure, were prioritized using multivariate approach. We suppose that our study will play a guiding role for the practical application of food composting.

2. Material and methods

2.1 Lab-scale composting reactor

A schematic of the lab-scale composting reactor is illustrated in Fig. 1. A 75 L stainless steel cylindrical reactor (inner diameter: 30 cm, length: 50 cm, net volume: 75 L, filing height of the compost: 45 L) with insulation was used for the composting study. High density polyurethane was employed as the outer layer of the thermal insulation materials to prevent heat loss. An agitator shaft with agitating blades was horizontally mounted inside the reactor for intermittent mixing. An aeration tube was also installed at the bottom of the composting reactor to maintain aerobic condition. The air was supplied using an air pump at a flow rate of 0.1 $m^3 \cdot min^{-1} \cdot m^{-3}$. On top of the reactor, an exhaust air pipe was connected to a water-cooling device where water vapor can be condensed, collected, and sent back to the reactor to maintain the moisture content of the composts. A thermocouple was also placed at the middle-level of the reactor to continuously monitor the temperature variation using a controller. Ammonia gas released during composting was captured by an ammonia sensor. The exhaust air passed through a sodium hydroxide solution for the absorption of carbon dioxide before the final emission.

2.2 Raw material and composting process

Food waste from the dining room of the University of Science and Technology of Beijing, Beijing City, China was used as raw material. The mushroom residue used as a bulking agent because of its high porosity and low moisture content was collected from a local edible mushroom factory in Fangshan District in Beijing. These materials were cut into pieces of approximately 1 cm. The physicochemical characteristics of the initial raw materials are listed in Table 1.

Carbon (C), and nitrogen (N) are the primary nutrients required by the microorganisms involved in

composting. Microorganisms use carbon for both energy and growth, while nitrogen is essential for protein production and reproduction. The C/N ratio (i.e., the ratio of carbon to nitrogen) of 25:1 to 30:1 is considered the ideal range for active composting, since the microorganisms require 30 parts of C per unit of N.[16] High C/N ratios make the process very slow as there is an excess of degradable substrate for the microorganisms. But with a low C/N ratio there is an excess of N per degradable C and inorganic N is produced in excess and can be lost by ammonia volatilization or by leaching from the composting mass. As shown in Table 1, food waste had relative low C/N ratios, but high moisture content. Therefore, addition of mushroom residue with high C/N ratio as a bulking agent was beneficial for adjusting the initial C/N ration to the range of 25:1 to 30:1. The initial pH was adjusted to about 6.5 and moisture content was adjusted to 60% using deionized water; 45 L of the mixture was then placed into the 75 L lab-scale composting reactor. Moisture content was maintained at 50% using the condensed water sent back to the reactor. Deionized water was supplemented when the moisture content can no longer be maintained by the water being sent back to the reactor. Composting was carried out under aerobic conditions for 15 days. Subsamples were randomly collected at three different depths. Samples were pooled, mixed, and then divided into three parts, where one part was stored at −80 °C and the other two were air-dried immediately and stored at 4 °C.

2.3 Physicochemical analysis of the composting samples

Temperature was continuously recorded by a computer connected to the reactor through a sensor inserted into the middle of the composting materials; environmental temperature was also measured at the same time. The pH level was measured by a compound electrode (PE-10, Sar-torious, Germany) dipped into a solution of 5 g of fresh sample and 50 mL of deionized water. Moisture content was determined by drying the fresh sample in a drying oven at 105 °C until constant weight was achieved. Organic matter content (OM) was quantified by weight loss after ignition in a furnace at 550 °C.[17] Concentrations of nitrate (NO_3^--N) and ammonium (NH_4^+-N) were extracted with 2 M KCl and measured using an AutoAnalyzer (AA3, Bran and

Luebbe, Germany).

2.4 DNA extraction and PCR-DGGE

Total genomic DNA was extracted according to the method described previously by Yang et al..[18] DNA extracts were 10-fold diluted before PCR to overcome the possible inhibition by humic acids. The extracted DNA was purified, dissolved in 100 μL of TE buffer, and then stored at −20 °C before use.

A nested PCR approach was used to amplify ammonia-oxidizer specific16S rRNA for DGGE.[19] The first-round of PCR was conducted using the AOB-specific primer pair CTO189f-GC and CTO654r, which was amplified as a 465 bp fragment.[20] The product from this round of PCR was then used as template DNA for the second-round of PCR carried out using universal primers F338-GC and R518.[21] PCR mixtures diluted to a final volume of 25 μL contained 12.5 μL 2 × *EasyTaq* PCR SuperMix (TransGen Biotech, Beijing, China), 0.5 μM of each primer, and 1 μL of 10-fold diluted DNA. PCR reactions were performed on a MyCycler Thermal Cycler (Bio-Rad, USA) as previously described.[19] All PCR amplicons were examined by electrophoresis in 1.0% (wt/vol) agarose with ethidium bromide staining to confirm the product size.

The nested PCR amplicons were separated by DGGE using a DCodeTM Universal Detection System (Bio-Rad, USA) according to the instructions of the manufacturer. Approximately 20 μL of each PCR product was loaded onto an 8% (w/v) polyacrylamide gel (acrylamide : bisacrylamide = 37.5 : 1) with a denaturant gradient of 30%–60% for AOB (100% denaturant contains 7M urea and 40% deionized formamide). Electrophoresis was then conducted at 60 °C in 1 × Tris-acetate-EDTA buffer at 110 V for 12 h. After DGGE, the gels were stained with 1:10,000 SYBR green I for 30 min, and then scanned with a Bio-Rad image scanner. Band intensity and position data were analyzed using the software Quality One v4.6 (Bio-Rad, USA).

2.5 Sequencing and phylogenetic analysis

Prominent DGGE bands were excised using sterilized cutter blades and transferred into 40 μL Milli-Q

water, and then incubated overnight at 4 °C. DNA was recovered from the gel by freeze-thawing three times. The eluted DNA from excised DGGE bands were re-amplified with the primer set F338-GC/R518, and the products again were subjected to DGGE to check their migration. The target DNA fragments were then excised and re-amplified using the primer set F338-GC/R518 without the GC-clamp, thus obtaining a pure sample for the cloning and sequencing step. The purified PCR products were cloned into the pGM-T vector (Tiangen Biotech, Beijing, China) and transformed into *Escherichia coli* TOP10 (Tiangen Biotech, Beijing, China). The plasmids of positive colonies were extracted and sequenced.

The sequences of the DGGE bands were compared with those available in the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi). GenBank database using the BLAST algorithm. The nucleotides generated in this study and those from the NCBI GenBank database were aligned. A phylogenetic tree was constructed by the neighbor-joining method using Kimura 2-parameter distance, as implemented in MEGA version 5.0. Bootstrap support (> 50%) from 1000 replications is shown at the nodes of the tree.

2.6 Statistical analysis

Statistical analyses were performed using SPSS version 20.0. Three replicates were used in all parameter analysis. Data presented as the mean values of triplicates and the maximum difference among triplicate results was below 5%. DGGE profiles were converted into matrix data based on the number of bands and their relative intensities among the individual samples using the software Quality One v4.6 (Bio-Rad, USA).

Shannon diversity index *(H)* was calculated by the following equation: $H = -\Sigma\,(ni/N)\log(ni/N)$, where ni/N is the community proportion made up by species i (brightness of the band i/total brightness of all bands in the lane).[22]

The correlation between environmental factors and the AOB community was evaluated by multivariate analysis using Canoco 4.5 software.[23]

2.7 Nucleotide sequence accession numbers

The sequences obtained from the DGGE bands in this study were submitted to GenBank under the accession numbers KJ890593 to KJ890606.

3. Results and discussion

3.1 Temporal changes in the properties of material during composting

Variation of pile temperature during food waste composting is illustrated in Fig. 2a, including the mesophilic phase (days 1–2), thermophilic phase (days 3–9), and cooling phase (days 10–15). Pile temperature rapidly increased in less than three days and reached a thermophilic level (>50 °C), indicating that indigenous microorganisms easily utilize the food waste organic matter. The thermophilic phase that lasted for seven days was necessary to attain proper disinfection of waste materials from animal and plant pathogens.[24] After the sharp breakdown of organic matter on the ninth day, the pile temperature decreased gradually and went back to ambient, indicating the end of the composting process. The pH slightly dropped to 6.50 starting on the second day because of the produced organic acids as intermediate by-products of easily degradable organic matter in food waste.[25] Following the increase in temperature, the pH value reached its peak on the sixth day as a result of the production of ammonia from the degradation of organic decomposition (Fig. 2b and Fig. 2c). The pH then decreased slightly to 8.2 at the end of the process. Process performance regarding pH is similar to that reported in other runs using the same compost reactor.[3] Moisture content was maintained at 50% by periodic watering (Fig. 1b) because it was suitable for aerobic microbial activity.[26] Organic matter content decreased from 87.7% to 67.5% during the composting process (Fig. 1b) because labile fractions of the organic matter were mineralized into stable compounds by microbial activities. The increase in ammonium (NH_4^+-N) concentration from initial 528.1 mg kg^{-1} to the maximal 1142.7 mg kg^{-1} on the sixth day indicates the decomposition of nitrogenous organic compound into ammonia (NH_3). The NH_4^+-N concentration decreased because of the NH_3 volatilization and the immobilization by

204 microorganisms. Nitrate (NO_3^--N) concentration showed a slight increase on the third day and kept a
205 downward trend to 125.2 mg kg^{-1} on the ninth day as high temperature and excessive amount of ammonia
206 inhibited the activity and growth of nitrifying organisms. Nitrate concentration then gradually increased to as
207 high as 445.6 mg kg^{-1}.

208 **3.2 Temporal dynamics of AOB community during food waste composting**

209 DGGE analysis was used to investigate the community structure and identify certain AOB groups present
210 during the different stages of food waste composting. The distribution of the 14 bands (Fig. 3) detected in the
211 DGGE profiles during different phases indicated that AOB composition was dynamic during food waste
212 composting. Most of the bands were ubiquitous but had different relative abundances in different stages, due
213 to the composting environments of different stage was unique, affecting the intrinsic AOB community. The
214 diversity of the AOB community was evaluated using the Shannon diversity index since it is a
215 comprehensive parameter used to evaluate microbial diversity, and considers both numbers and relative
216 intensity of bands. As shown in Table 2, the indices and band number in the samples collected from the
217 mesophilic (days 1–2) and cooling phases (days 10–15) were higher than that of the thermophilic phase
218 (days 3–9). Indigenous ammonia-oxidizing population proliferated in the food waste in ambient temperature
219 because of the relative abundance of easily degradable organic compounds at the beginning of composting.
220 However, the diversity of AOB decreased with increasing temperature because the sensitivity of AOB in
221 these conditions varies from species to species.[27] Finally, the diversity indices of AOB increased after the
222 thermophilic phase and reached its peak value on the 12th day of the cooling phase. These results indicate
223 that AOB communities are active in both the mesophilic and cooling phases. This phenomenon is similar to
224 the result of Zhang et al., who reported that AOB were related to ammonia oxidation in the mesophilic and
225 maturation phases.[23] Furthermore, this finding confirmed the reasons for the increase in nitrate concentration
226 during the cooling phase (Fig. 1c).

DGGE fragments were carefully excised and sequenced from the DGGE gel to better visualize the temporal dynamics of the AOB communities. Nucleotide sequences obtained were compared with those available in the NCBI database using BLAST. Phylogenetic tree based on AOB nucleic acid sequences is shown in Fig. 4. The majority of the sequences were closely related to ammonia-oxidizing lineages belonging to the β-subclass of the *Proteobacteria*. Nine out of 14 sequences were most similar to the genus *Nitrosomonas*, and five practically belonged to the genus *Nitrosospira*.

In the thermophilic phase, majority of the sequences (band a, b, c, d, f, and g in Fig. 4) were correlated with those recovered from the sludge, municipal solid waste, and saline rhizospheric soil and were grouped into the *Nitrosomonas europaea/eutropha* cluster.[28,29] This result was consistent with previous reports showing that the *Nitrosomonas europaea/eutropha* preferred environments with high ammonium and pH.[9,12] Our results also confirmed that the *N. europaea/eutropha* can tolerate high temperature (Fig. 2a) and high ammonium concentration (Fig. 2c), which may serve an important function in the ammoxidation of food waste composting. On the other hand, bands a, h, i, j, l, and m in the mesophilic phase were affiliated with the *Nitrosomonas* and *Nitrosospira* lineage (Fig. 4). Bands a and m particularly dominated over the entire composting period, while other bands appeared only in specific period. It might be because that, as aforementioned, different composting period contains a unique environment, affecting the intrinsic AOB community. The species of band m can better adapt to changing environmental conditions, compared with other species. And this flexibility is important in determining the dynamics of ammonia oxidation during composting.[9] Furthermore, band m fell within the *Nitrosospira* cluster 3, which was also detected in the initial stage of mushroom cultivation composting.[8] However, this result was inconsistent with the report of Yamamoto where he stated that the difference in species dominance may be attributed to the chemical properties of raw materials.[12] Most of the bands during the cooling phase (bands a, e, f, g, j, k, l, and m in Fig. 4) fell into the *Nitrosomonas* cluster 7 and *Nitrosospira* cluster 3, suggesting that they co-migrated with the

predominant bands from the mesophilic and thermophilic phases. Therefore, these data showed that *Nitrosomonas*-like and *Nitrosospira*-like sequences abundantly existed during the different periods of the process and acted as ammonia oxidizers. Furthermore, dominant AOB species shifted with various environmental parameters.

3.3 Correlations of the environmental parameters with community structures of AOB

Detrended correspondence analysis was performed first to choose between linear or unimodal response models for AOB species.[30] In this study, the length of the first ordination axis was 1.915, showing that linear species response models are well-suited for the data analysis. Therefore, the influence of the environmental factors on AOB community (Fig. 5) was investigated by redundancy analysis. The analysis result is shown in Table 3. The first two canonical axes for the AOB DGGE fingerprints explained 41.1% and 28.8% of the variation during the species data, respectively. The 91.2% increase in variation in the species data was explained by all canonical axes. Monte-Carlo permutation tests demonstrated that both the first axis and all axes combined explained the significant amount of variability in the AOB community structure ($p < 0.05$), indicating that environmental variables may have an important role in explaining the variability of the AOB community.

This research aimed to identify which among the environmental variables affect AOB community composition. Forward selection was performed to identify the variables that best describe the most influential gradients. Explanatory variables were added until the addition of further parameters failed to significantly improve the model explanatory power ($p < 0.05$). In this procedure, nitrate and pH statistically explained the variation ($p < 0.05$) on the distribution of AOB species data; whereas, the other parameters did not statistically explain the variation ($p > 0.05$). Furthermore, the percentages of variation explained by each of the significant parameters in Table 4 were those without shared variation. Nitrate solely explained 27.3% ($p = 0.012$) of the variation on the AOB species data, whereas pH explained 21.7% ($p = 0.024$). Meanwhile, the

RDA model (i.e., nitrate and pH) statistically explained 54.7% of the variation (p = 0.002). These results showed that environmental parameters (i.e., nitrate and pH) and their interactions predominantly affect AOB community composition.

Multivariate statistical analysis suggested that nitrate and pH have predominant effect on AOB community composition ($p < 0.05$). The importance of nitrate variation in the AOB community had been highlighted by Zhang et al..[23] Moreover, AOB may serve an important function in nitrification during composting.[12] In our study, *Nitrosomonas*-like and *Nitrosospira*-like ammonia oxidizers of the β-subdivision of class *Proteobacteria* had been detected (Fig. 4). Betaproteobacterial AOB are chemoautotrophic and generate energy from the hydroxylamine oxidation step, the ATP produced is used to fix CO_2 as a carbon source. Therefore, the presence of these AOB indicates that these bacteria oxidize ammonia in the composting process. Accumulation of nitrate in the cooling phase of the composting process (Fig. 2c) reveals that ammonia oxidizers are more active in the food waste compost pile. Environmental conditions are favorable for the AOB activity may affect the net nitrogen balance during the composting process.[9]

Another important factor affecting AOB community structure is pH. The presence of low pH values usually coincide with reduced microbial activities.[25] In addition, pH affects the chemical form, concentration, and availability of substrates, as well as cell growth and activity.[31] For example, rates of nitrification and, in particular, ammonia oxidation are significantly reduced in acid soils, whereas higher nitrification rate is found in the alkaline environment.[32,33] The reduced growth and activity of ammonia oxidizers at low pH is attributed to the exponential reduction in NH_3 availability with decreasing pH, through ionization to NH_4^+, decreasing NH_3 diffusion and increasing the requirement for energy-dependent transport of NH_4^+.[34] Furthermore, pH affects the AOB community structure. Jarvis and Nugroho reported a selection for the *Nitrosospira* clusters 2 and 4 strains in the acidic compost and neutral soil.[9,33] Yamamoto et al. indicated that the *N. europaea/eutropha* (cluster 7) have been shown to be favored by high pH and high ammonia

conditions.[12] The pH of the food waste composting in this study is basic. Therefore, strong correlations between pH and AOB community activity were observed in our work. The pH of the food waste composting in this study is critical. Strong correlations between pH and AOB community activity were observed in our work. This result revealed that the activity of the AOB could be encouraged through proper control of the pH of the compost during the fermentation process. Consequently, the negative effect to environment due to ammonia emission and nutrient (i.e., fertilization) elevation of the compost by nitrogen conservation could be gained. However, more convincible result could be obtained after demonstrating it in the scale-up composting plant. On the other hand, not with standing, no significant relationship was observed between the other environmental parameters and AOB community composition in this work. This result does not imply that those parameters are of no importance in determining the AOB community composition. It can be only concluded by statistical analysis in this research. Future experimental studies should be conducted to verify the influence of these factors on the AOB diversity index.

4. Conclusions

AOB community dynamics in food waste composting was monitored by PCR-DGGE combined with clone library. The results showed that higher diversity indices of AOB appeared during the mesophilic and cooling phases. In addition, both *Nitrosomonas*-like and *Nitrosospira*-like lineages existed in large amounts in different periods. *Nitrosomonas europaea/eutropha* dominated the thermophilic stage and probably represents a group of bacterium that can adapt to high temperature. Multivariate statistical analysis suggested that nitrate and pH have a predominant effect on AOB community composition in the composting ecosystem. These findings therefore enrich the theory that the relationship between AOB community dynamics and environmental parameters covary. The findings also offer insight into the parameters that control the AOB community dynamics in food waste composting. The results obtained in this study may help lay the foundation to better understand and manage nitrogen cycle in the food waste composting ecosystem.

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376 **Figure legends:**

377

378 **Fig. 1** Schematic of the lab-scale composting reactor

379

380 **Fig. 2** Variation of (a) temperature and pH, (b) organic matter content and moisture content, and (c)
381 ammonium and nitrate in the food waste composting for 15 days. The bars represent the standard deviations
382 of the mean values (n = 3).

383

384 **Fig. 3** DGGE profile of AOB generated by nested PCR-DGGE. Labels along the bottom indicate the
385 composting days; (A) DGGE profile; (B) diagram of sample lanes.

386

387 **Fig. 4** Neighbor-joining tree with Kimura 2-parameter substitution of partial 16S rRNA gene sequences of
388 AOB DGGE bands in this study and from NCBI GenBank database. Bootstrap values (> 50%) are indicated
389 at branch points. The scale bar represents 1% sequence divergence.

390

391 **Fig. 5** Distance triplot of the redundancy analysis on the AOB community composition and environmental
392 parameters during food waste composting. Composting environmental parameters are indicated by solid lines
393 with filled arrows. AOB communities are shown using gray dotted lines with unfilled arrows. Samples are
394 represented by solid circles and sample numbers refer to the sampling days.

Table 1 Physicochemical characteristics of the initial raw materials with standard deviation

Parameter	Food waste	Mushroom residue
Moisture content (%, *WW*)	74.06 ± 2.81	11.87 ± 0.34
pH	6.08 ± 0.01	7.63 ± 0.02
EC (mS cm^{-1})	8.90 ± 0.34	2.50 ± 0.51
Organic matter (%, *DW*)	91.43 ± 0.09	82.97 ± 0.52
Total C (%, *DW*)	47.11 ± 0.04	42.79 ± 0.26
Total N (%, *DW*)	2.73 ± 0.11	0.23 ± 0.01
C/N ratio	17.26	186.04

Mean and standard error are shown (n = 3), *WW* wet weight, *DW* dry weight

Table 2 AOB band number and Shannon diversity index (*H*) of the DGGE profiles for each compost sample

Index	Days of composting					
	1 d	3 d	6 d	9 d	12 d	15 d
Band number	6	6	4	3	8	4
Shannon diversity index (*H*)	1.63	1.49	1.32	0.89	1.85	1.04

Table 3 Redundancy analysis results of the AOB DGGE profiles

Axes	Axis 1	Axis 2	Axis 3	Axis 4	Total variance
Eigenvalues	0.411	0.288	0.170	0.042	1.000
Species-environment correlations	0.997	0.998	0.999	0.777	
Cumulative percentage variance of species data	41.1	69.9	86.8	91.0	
Cumulative percentage variation of species-environment relation	45.1	76.8	95.4	100.0	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.910

Monte Carlo significance tests for AOB data: sum of all Eigen values, 1.000; significance of first canonical axis, F value = 4.880, p = 0.002; significance of all canonical axes, F value = 17.688, p = 0.002. F and p values were estimated using Monte Carlo permutations.

Table 4 Eigenvalues, *F* values, and *P* values obtained from the partial RDA testing the influence of the significant parameters on the AOB community

Parameters included in the model	Eigenvalues	% Variation explains solely	*F* value	*p* value
Nitrate	0.273	27.3	3.751	0.012
pH	0.217	21.7	2.774	0.024
All the above together	0.547	54.7	5.439	0.002

Partial RDA based on Monte Carlo permutation (n = 499) maintained only the significant parameters in the models. For each partial model, the other significant parameter was used as a covariable. *F* and *p* values were estimated using Monte Carlo permutations. The sum of all eigenvalues for the partial RDA was 1.000.

Fig. 1

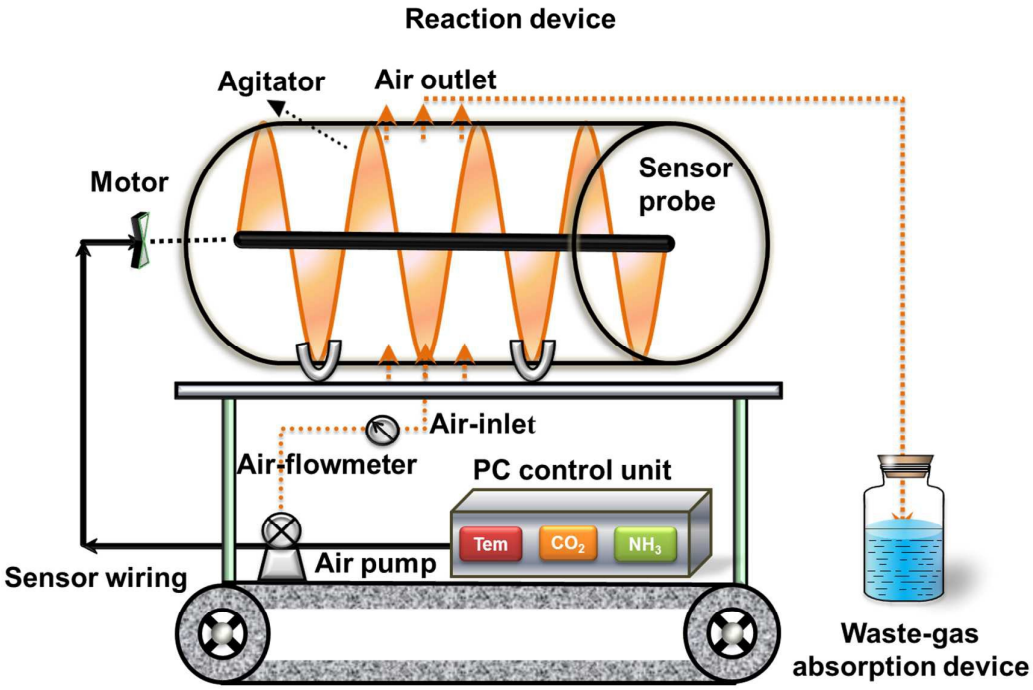


Fig. 2

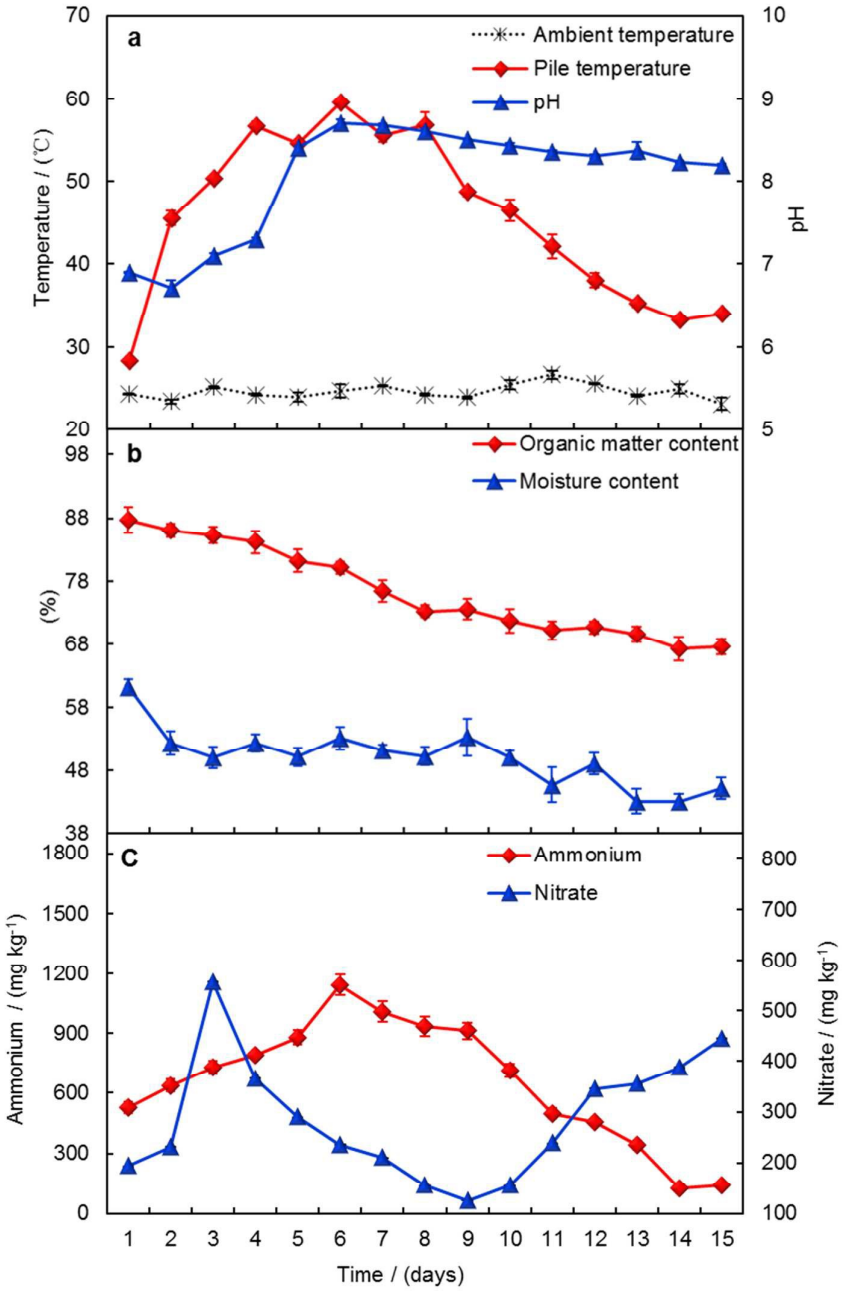


Fig. 3

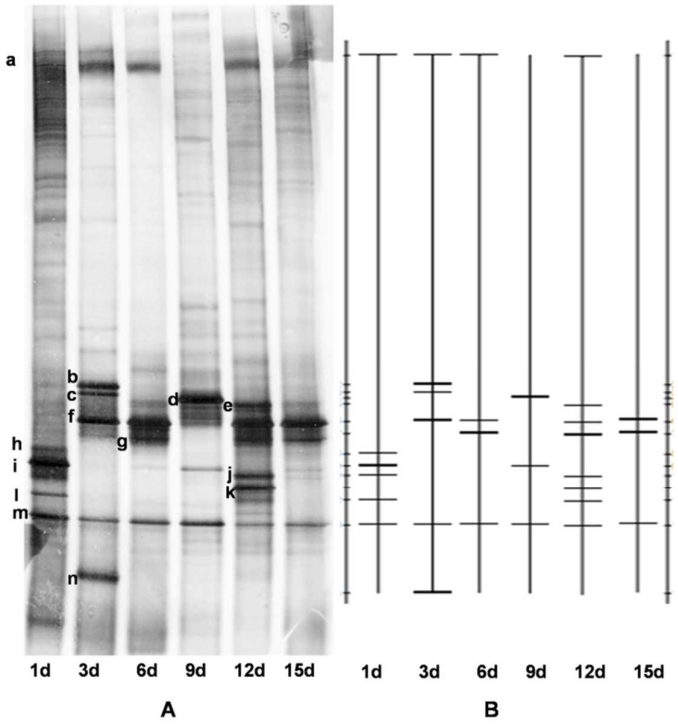


Fig. 4

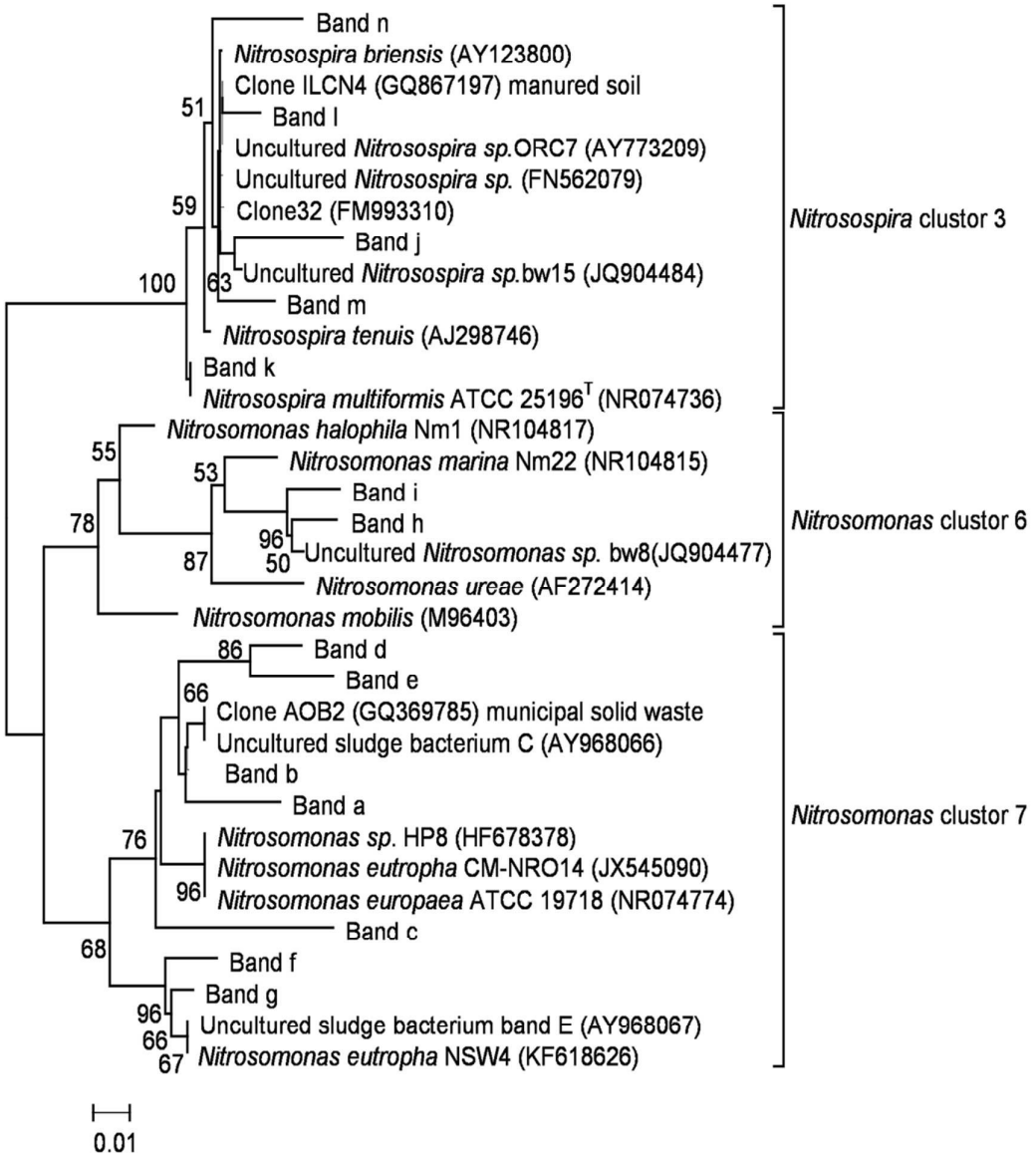
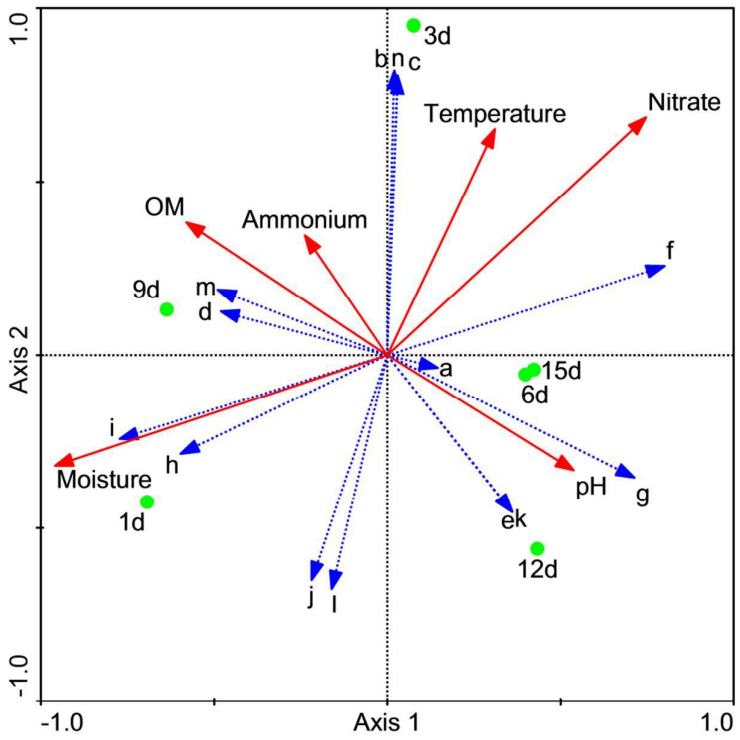


Fig. 5



Graphical abstract

This paper aimed to identify and prioritize some environmental parameters that affect AOB community composition during food waste composting.