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Effect of alkaline-thermal pretreatment on biodegradable plastics degradation and dissemination of antibiotic resistance genes in co-compost system

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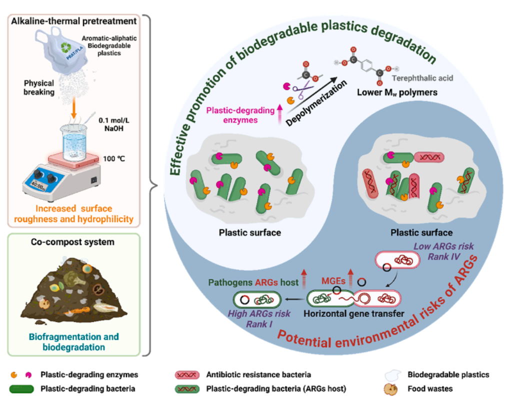
HIGHLIGHTS

- Pretreatment enhanced surface hydrophilicity and decreased BDPs' molecular weight.
- BDPs degradation aided compost heating and extended the thermophilic stage.
- Altered BDPs' surface properties promoted the enrichment of plastic-degrading bacteria.
- Produced microplastics notably increase ARGs abundance, often overlooked.
- Three plastic-degrading bacteria were identified as ARGs hosts.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradable plastics (BDPs) are an eco-friendly alternative to traditional plastics in organic waste, but their microbial degradation and impact on antibiotic resistance genes (ARGs) transmission during co-composting remain poorly understood. This study examines how alkaline-thermal pretreatment enhances BDPs degradation and influences the fate of ARGs and mobile genetic elements (MGEs) in co-composting. Pretreatment with 0.1 mol/L NaOH at 100℃ for 40 minutes increased the surface roughness and hydrophilicity of BDPs while reducing their molecular weight and thermal stability. Incorporating pretreated BDPs film (8 g/kg-TS) into the compost reduced the molecular weight of the BDPs by 59.70 % during the maturation stage, facilitating compost

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heating and prolonging the thermophilic stage. However, incomplete degradation of BDPs releases numerous smaller-sized microplastics, which can act as carriers for microorganisms, facilitating the dissemination of ARGs across environments and posing significant ecological and public health risks. Metagenomic analysis revealed that pretreatment enriched plastic-degrading bacteria, such as *Thermobifida fusca*, on BDPs surfaces and accelerated microbial plastic degradation during the thermophilic stage, but also increased ARGs abundance. Although pretreatment significantly reduced MGEs abundance (*tnpA*, *IS19*), the risk of ARGs dissemination remained. Three plastic-degrading bacteria (*Pigmentiphaga sp002188465*, *Bacillus clausii*, and *Bacillus altitudinis*) were identified as ARGs hosts, underscoring the need to address the risk of horizontal gene transfer of ARGs associated with pretreatment in organic waste management.

1. Introduction

Composting is a widely used method for managing the organic components of solid waste[1,2]. However, improper waste segregation practices have led to the inadvertent inclusion of plastics in compost[3], which then disperses into the environment and causes widespread pollution[4,5]. To address traditional plastics pollution in composting, alternative biodegradable plastics (BDPs) such as poly(lactic acid) (PLA) and poly(butylene adipate-co-terephthalate) (PBAT) have been introduced[6]. Although BDPs can degrade efficiently under industrial composting conditions at temperatures exceeding 55 ± 2℃[7,8], they often suffer from incompletely degradation due to factors such as temperature fluctuations, microbial activity, and inadequate composting times. Furthermore, BDPs characteristics such as polymer crystallinity, surface roughness, or hydrophilicity may influence the diversity of microbial communities and alter key physicochemical indicators of compost (e.g., carbon and nitrogen content)[9]. Our previous study demonstrated that during the co-composting of food waste with BDPs, thermophilic bacteria degrade the BDPs and form surface biofilms[10]. However, this process leads to increased nitrogen losses and the release of microplastics (MPs), emphasizing the need for effective strategies to ensure co-compost quality[11].

The depolymerization of ester bonds in aromatic-aliphatic BDPs is the primary bottleneck in their degradation process, prompting extensive research into pretreatment methods, including physical, chemical, and thermochemical approaches[3,12–15]. Among these methods, alkaline pretreatment is particularly notable for its cost-effectiveness, lower energy consumption, and ability to reduce polymer crystallinity [16,17]. For example, a 5 % w/v NaOH had been shown to significantly enhance the thermophilic anaerobic degradation performance and rate of BDPs such as PLA, and thermoplastic starch, although it is less effective for PBAT[18]. However, single pretreatment methods often require extended time periods and are less effective against more challenging BDPs like PBAT, which exhibit high resistance to solubility and hydrolysis. Combining alkaline pretreatment with thermal pretreatment has demonstrated superior performance compared to either method alone[17], due to the accelerated hydrolysis of ester bonds and enhanced biodegradation of BDPs when temperatures exceed their glass transition points[19,20]. For instance, Kriswantoro et al.[19] found that under conditions of 5 M NaOH and 70℃, PLA degraded by 99.99 % within a day, while PBAT required 18 days to reach 84.8 % degradation [21]. Similarly, Nie et al.[22] showed that thermal-alkaline pretreatment (48 h, 70℃, 1 % w/v NaOH) of PBAT and PLA improved methane production in anaerobic digestion. These studies suggest that alkaline-thermal pretreatment can break down polymers into more readily biodegradable oligomers, indicating good potential for application in co-composting degradation of BDPs.

While pretreatment methods have achieved promising results in improving degradation efficiency, there has been limited discussion on whether these methods increase the ecological and environmental risks of composting products, especially regarding the spread of antibiotic resistance genes (ARGs). Existing studies have primarily focused on UV pretreatment, which simulates natural aging to increase surface roughness and introduce oxygen-containing functional groups on plastics, thereby providing additional adsorption sites for ARGs[23,24]. However, the potential effects of more widely applied alkaline and thermal pretreatments remain underexplored. Additionally, biofilms on the surfaces of BDPs may harbor a higher diversity and abundance of ARGs compared to non-degradable plastics, potentially serving as reservoirs for these genes[25]. This implies that pretreatment could alter the characteristics of BDPs in ways that increase their ecological risks, particularly in relation to ARGs. In our previous study, we found that BDPs significantly enriched ARGs, with their abundance on plastisphere samples (0.682 ± 0.041 $\log_{10}$(copies/16S rRNA gene copies)) being notably higher than in compost piles[10]. ARGs present in compost can subsequently enter the soil, posing contamination risks and potential health hazards[26,27]. Nevertheless, within the context of co-composting food waste with BDPs, the mechanisms by which alkaline-thermal pretreatment influences BDPs degradation and ARGs dissemination remain insufficiently understood. Therefore, it is crucial not only to focus on the degradation efficiency of BDPs pretreatment but also to thoroughly investigate the potential environmental risks associated with these methods.

This study aims to investigate the effects of alkaline-thermal pretreatment on the co-composting of food waste and BDPs, focusing on three key areas: 1) Assessing the impact of pretreated BDPs on compost quality and humification; 2) Examining how pretreatment enhances the degradation of BDPs; and 3) Revealing the risk of ARGs propagation.

2. Materials and methods

2.1. Alkaline-thermal pretreatment experiment

The optimal conditions for alkaline-thermal pretreatment were determined using an orthogonal experiment. The BDPs used in the study, composed of poly(lactide acid)/poly(butylene adipate-co-terephthalate)/Starch (PLA/PBAT/St, 5/70/25 wt%), were purchased from Shanghai Xinqi Plastic Packaging Products Co.; Ltd. and cut into films with dimensions of 20 × 20 × 0.23 mm (L×W×H). A total of 4 g of BDPs were placed in a 100 mL beaker with 40 mL NaOH solution at a ratio of 1:10 (g/mL). The beaker was wrapped with aluminum foil to prevent light exposure and incubated in a constant-temperature water bath. The optimal pretreatment conditions (temperature and time, alkali concentration) were determined through an $L_9(3^3)$ orthogonal experiment (Table S1) and single-factor optimization experiments (Text S1).

2.2. Substrates and composting experiment setup

BDPs films and BDPs powders (< 150 μm) were treated under optimal alkaline-thermal pretreatment conditions (100℃, 40 min, 0.1 mol/L NaOH, as obtained in Figure S1-S3) (see Text S2 for details). Each compost heap consisted of 8 kg of food waste, 2 kg of straw, and either BDPs films or BDPs powders. The basic properties of the raw materials are presented in Table S3.

The simulated natural composting experiment was conducted in perforated foam boxes (32 L) to ensure that the compost could maintain well aeration and insulation. During the composting process, room temperature was maintained at 25 ± 2℃, the compost was turned and stirred every 3 days, and distilled water was added as needed to maintain moisture content at 50 % ± 10 %. This research included one control group and four BDPs-added groups: (1) without BDPs (CK); (2) 80 g/kg-TS BDPs powder non-pretreatment (NP); (3) 80 g/kg-TS BDPs powder with pretreatment (TP); (4) 8 g/kg-TS BDPs film non-pretreatment (NF); (5) 8 g/kg-TS BDPs film with pretreatment (TF). In the treatment group, BDPs powder was used to study its effect on composting, while BDPs film was used to study degradation. Based on temperature changes, the composting process can be divided into four stages: the calefactive stage, thermophilic stage, cooling stage, and maturation stage. Samples were collected on days 0, 1, 8, 19 and 35, representing these four stages.

2.3. Analysis methods

2.3.1. BDPs characterization analysis

The BDPs powder samples from the NP and TP groups could not be obtained because they could not be effectively separated from the compost. BDPs films were collected from orthogonal experiment and freeze-dried samples of compost (NF and TF) at each stage (BDPs films sizes > 1, 2, and 5 mm). The BDPs films were washed with ultrapure water and dried at low temperature at 40℃ to analyze the disintegration rate, mass loss rate, and recovery rate.

The pH, total organic carbon (TOC), and terephthalic acid concentrations in the liquid fraction after pretreatment were used to assess the hydrolysis efficiency of pretreatment on BDPs. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and changes in molecular weight distribution were determined by gel permeation chromatography (Viscoteck TDAmax, Malvern Panalytical, UK). The polymer dispersity index was calculated as $PDI=M_w/M_n$. The hydrophobicity of the BDPs was measured using a contact angle instrument with three randomly selected points on each sample (SDC-200S, Sindi, China). The surface structure of the BDPs was examined by scanning electron microscopy (SEM, Regulus8100, Hitachi, Japan). The thermal stability of BDPs was investigated by thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTA) using a thermal analysis-mass spectrometer (STA8000-MS, Switzerland). Functional groups of the BDPs were analyzed by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet™ iS50, Thermo Scientific, USA).

2.3.2. Microplastic analysis in co-compost

Microplastic fragments (MPs < 500 μm) were extracted from freeze-dried co-compost samples (15 mg) using the following procedure: the samples were oxidized overnight at 60℃ and 200 rpm in a solution of 30 % H_2O_2 mixed with Fenton's reagent (v:v = 1:1). After oxidation, the samples were filtered through a 5 μm alumina filter membrane (d = 20 mm) to recover all particles. The collected particles were then resuspended in a saturated $ZnBr_2$ solution (density = 1.2 g/cm^3) using sonication for 10 min. The suspension was then transferred to a density separation unit and allowed to settle overnight. The supernatant was subsequently filtered through a 5 μm alumina filter membrane to collect the MPs, which were then placed in a petri dish for μ-FTIR mapping analysis (Nicolet™ iN10,Thermo Fisher Scientific, USA). The specific of the μ-FTIR conditions are detailed in Table S4.

2.3.3. Co-compost indicators analysis

The temperature of at the center and along diagonal of the compost was recorded every 24 h using a probe-type digital thermometer. Volatile solids (VS), ammonia-nitrogen (NH_4^+–N), electrical conductivity (EC), pH and germination index (GI) of the compost were measured using fresh samples, following the methods described in our previous study[25]. Total carbon and total nitrogen were analyzed using an elemental analyzer (Vario Micro cube, Elementar, Germany), and the C/N ratio was calculated. Humic acid (HA) and fulvic acid (FA) concentrations were determined using a TOC analyzer (MiltoN/C3100, Analytik Jena, Germany). The characteristics of dissolved organic matter (DOM) were analyzed by an ultraviolet spectrometer (UV-1050, Techcomp, China), and $SUVA_{254}$ and $SUVA_{280}$ were calculated to evaluate the humification degree of DOM. All measurements were conducted in triplicate.

2.3.4. Metagenomic sequencing and binning analysis

To investigate the succession of bacterial communities during the composting process, microbial samples were collected from BDPs after washing with pure water, as well as from compost on days 0, 8, and 35. The DNA extracted from three samples were combined to create a homogenized DNA sample, which was then used for metagenomic sequencing on the Illumina Novaseq platform (150 bp paired-end strategy) at Shanghai Majorbio Co.; Ltd. (Shanghai, China). High-quality clean data were then subjected to metagenomic binning to construct metagenome-assembled genomes (MAGs) with a completeness > 50 % and contamination < 5 %. Functional genes associated with plastic degradation within the MAGs were annotated to evaluate their plastic degradation potential. Additionally, the functional genes in these reconstructed MAGs were analyzed for the presence of ARGs and mobile genetic elements (MGEs). MGEs play a critical role in horizontal gene transfer (HGT), accelerating the dissemination of ARGs across microbial communities[25]. The relative reads per kilobase per million mapped reads (rpkm) values of ARGs were calculated for each gene of bin via RRAP. A phylogenetic tree of the MAGs carrying ARGs was constructed to assess the associated risks posed by ARGs (see Text S3 for details). Additionally, the risk of ARGs in the MAGs was assessed by calculating the contribution of each ARGs risk rank using the formula: the average abundance of ARGs within a specific risk rank (I - IV) / the average abundance of all ARGs[28,29].

2.3.5. Statistical analysis

The performance of the different compost samples was compared with SPSS 25.0 (IBM SPSS Inc., USA) statistical software using the ANOVA test. A *P*-value of less than 0.05 ($P < 0.05$) was considered statistically significant. Phylogenetic trees were visualized using the R package ggplot (version 3.5.1) and ggtree (version 2.6). Correlation matrix networks were visualized using Gephi 0.10.1. Others graphs were drawn using Origin 2024.

3. Results and discussion

3.1. Impact of alkaline-thermal pretreatment on the maturity of compost containing BDPs

3.1.1. Changes of physicochemical indicators of composting

The changes in the physicochemical characteristics of the compost during the process are shown in Fig. 1. The temperature of all groups rapidly increased from ambient temperature to approximately 50℃ on day 4, driven by indigenous microorganisms that utilize easily degradable organic matter for metabolism and heat production[30]. The thermophilic stage lasted 9 days for CK, NF and TF, 10 days for NP and 11 days for TP. Meanwhile, a delayed thermophilic stage was observed in TF and NF groups. This result can be explained by the fact that pretreated BDPs powders are more readily metabolized by microorganisms compared to films. (Table S5). As shown in Fig. 1(a), the VS in each group also showed an overall decreasing trend throughout the composting process. The TP group had the lowest VS of 26.37 % ± 0.15 % at the end of composting, suggesting that BDPs may be converted into carbon sources.

During composting, the C/N ratio initially increased and then decreased (Fig. 1b). In the thermophilic stage, microbial metabolic activity was enhanced, leading to faster consumption of organic matter and the production of large amount of NH_4^+–N and NH_3 (Fig. 1c). In the maturation stages, the degradation rate of NH_4^+–N slowed down and the C/N ratio further decreased. At the end of composting, nitrogen losses in

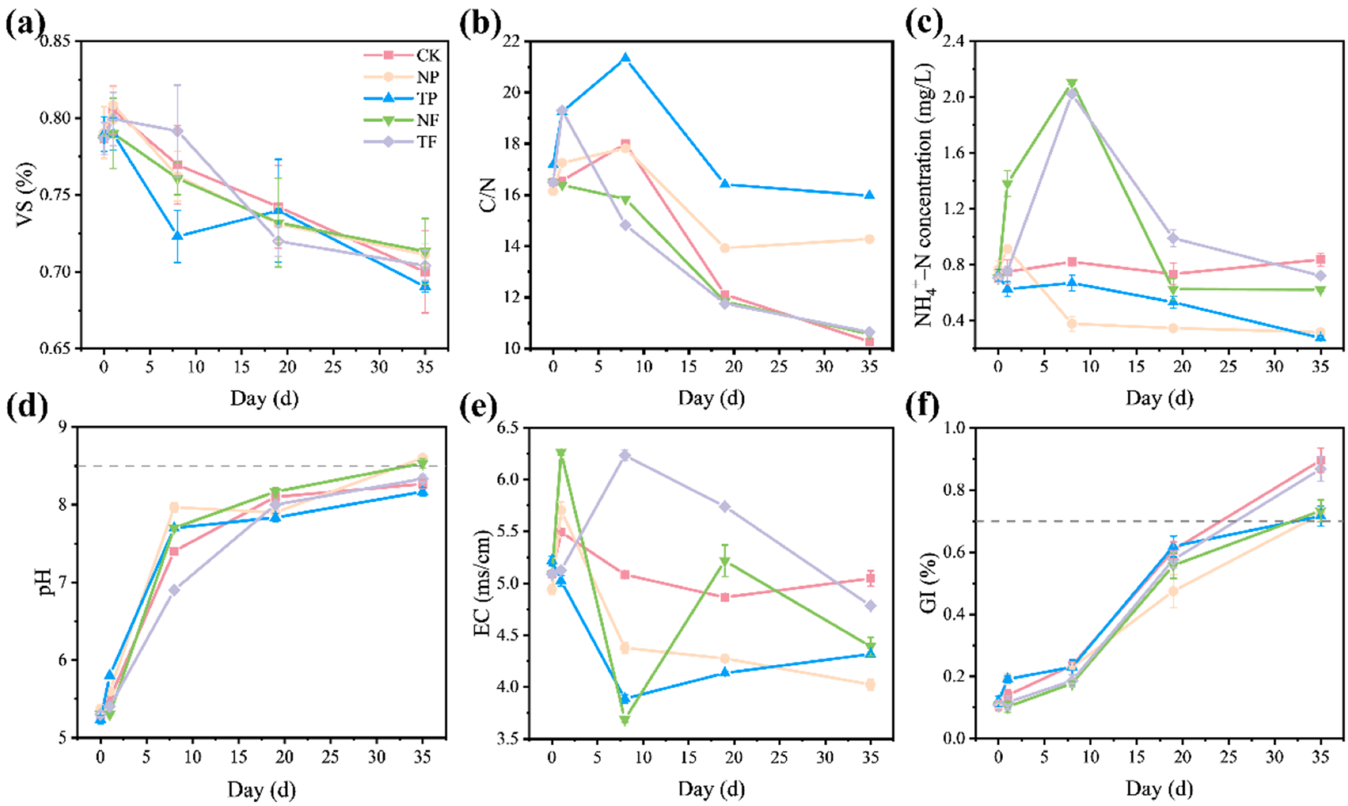

Fig. 1. Changes in VS (a), C/N (b), NH_4^+–N concentration (c), pH (d), EC (e) and GI (f) during co-composting.

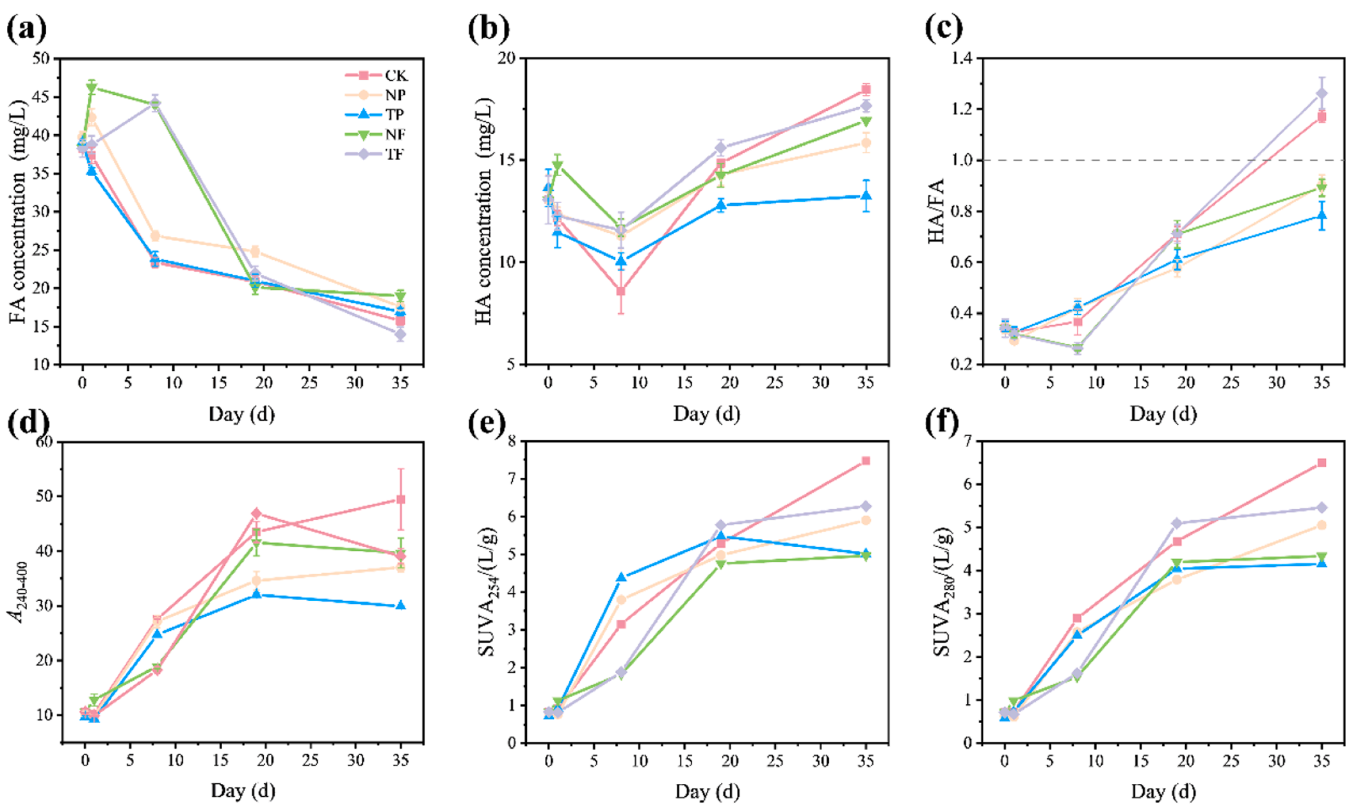

Fig. 2. Organic fraction and humification process changes in FA and HA concentration (a-b), HA/FA (c), $A_{240–400}$ (d), $SUVA_{254}$ (e) and $SUVA_{280}$ (f) during co-composting.

all BDPs-added groups were higher than those in the CK group. The pH values for all BDPs-added groups increased rapidly and then stabilized between 8.17 and 8.60, owing to the consumption of organic acid and the neutralization of NH_4^+–N (Fig. 1d). EC reflects the phytotoxicity and the concentration of soluble salts (e.g., NH_4^+, NO_3^-, Mg^{2+}, Ca^{2+}, Na^+) in the compost[31]. Throughout the composting process, complex organic substrates were biodegraded, causing fluctuations in EC in each BDPs-added group, which eventually stabilized between 4.02 and 5.05 mS/cm (Fig. 1e). The sharp decrease in EC observed in the TP group during the high-temperature stage may be due to the sustained elevated temperatures, which enhanced the emission of volatiles such as NH_3 and sulfides. In addition, prolonged high temperatures accelerated humification, resulting in ion immobilization and reduced solubility[32].

GI is commonly used to assess the maturity and phytotoxicity of compost products (Fig. 2f). The GI values of all groups gradually increased during the composting process and exceeded 70 % by the end. It is noteworthy that the GI values of the TF group at the end of composting (86.79 % ± 3.91 %) was significantly higher than those of the other BDPs-added groups ($P < 0.01$), and closer to that of the CK group (89.62 % ± 3.92 %). This indicates that the addition of a small amount (8 g/kg-TS) of pretreated BDPs film did not adversely affect compost maturity or phytotoxicity. On the contrary, both higher addition (80 g/kg-TS) and non-pretreatment of BDPs resulted in lower GI values, indicating reduced compost quality. In summary, the use of high amounts of BDPs (80 g/kg-TS) and the absence of alkaline-thermal pretreatment both led to nitrogen loss and increased phytotoxicity, ultimately diminishing the quality of the final compost product.

3.1.2. Organic fraction and humification process

The FA content (Fig. 2a) showed a decreasing trend during composting, reaching its lowest level in the TF group at the end of composting (at 14.01 ± 0.93 mg/L), with a reduction of more than 60 %. The HA content generally showed a slight decrease followed by an increase throughout the composting process, with the CK group having the highest HA content at the end (at 18.46 ± 0.29 mg/L), followed by the TF group at 17.66 ± 0.29 mg/L (Fig. 2b). The HA/FA ratio, often positively correlated with the degree of humification, is a key indicator of compost maturity, with a ratio greater than 1 typically signifying successful humification[33]. The HA/FA ratio increased consistently throughout the composting process, as shown in Fig. 2(c). By the end of the process, TF group demonstrated a significantly higher HA/FA ratio (1.26 ± 0.06) compared to the other BDPs-added groups ($P < 0.01$), and slightly exceeding that of the CK group (1.17 ± 0.02). This indicates that both the TF and CK groups achieved effective humification. In contrast, the TP group showed a considerably lower HA/FA ratio (0.78 ± 0.06), reflecting poor humification.

Additionally, Fig. 2(d) shows an overall increasing trend in $A_{240–400}$ during composting, likely due to the rise in aliphatic compounds containing hydroxyl and carboxyl groups[34]. $SUVA_{254}$, which primarily represents organic molecules with unsaturated C═C bonds (including aromatic compounds)[35], decreased in the groups with added BDPs during the maturation stage. Notably, the $SUVA_{254}$ of TF group exhibited the smallest reduction, suggesting that pretreatment could appropriately alleviate the inhibitory effect of BDPs on the formation of aromatic compounds during co-composting (Fig. 2e). The $SUVA_{280}$ values, which typically reflects the molecular weight of DOM, was highest in the TP group among the BDPs-added groups, increasing from 0.71 to 5.46 L/g. This indicates that soluble organic matter in the DOM increased during composting, contributing to the formation of more soluble humus and humus-like substances that microorganisms can utilize during the maturation stage (Fig. 2f). Overall, the results demonstrated that the addition of pretreated BDPs film (8 g/kg-TS) promotes compost humification, while adding pretreated BDPs powder (80 g/kg-TS) had the opposite effect. Therefore, selecting the appropriate form and amount of BDPs is crucial for practical composting applications.

3.2. BDPs degradation during composting

The macroscopic changes and residual mass of the BDPs film (in the NF and TF groups) over time during composting after pretreatment are shown in Figure S4. At the end of composting (day 35), the residual mass of BDPs per 100 g of compost was 1.7826 g for the NF group and 0.9471 g for the TF group. As the composting continued (at day 60 and day 90), the BDPs samples in the TF group showed significant fragmentation and a further decrease residual mass (0.7769 g and 0.6652 g, respectively), indicating that fragmentation and degradation of BDPs continued even after the composting period.

The changes and distribution of molecular weight are presented in Fig. 3(a) and (b). As composting progressed, the molecular weights of both the NF and TF groups continued to decrease, with a more substantial reduction occurring the cooling stage. Compared to the NF group, the decrease in M_n values of TF group during the calefactive, thermophilic, cooling, and maturation stages were 6.88 %, 11.90 %, 48.87 % and 44.64 %, respectively. Meanwhile, the decrease in M_w values of TF group were 7.51 %, 13.80 %, 28.85 %, and 42.35 %, respectively. The molecular weight distribution of BDPs showed the highest decrease rate during the thermophilic stage, while the lowest decrease rate was observed during the maturation stage, which is the longest stage. Lower molecular weight polymers are more susceptible to microbial degradation because they depolymerize more readily into oligomers and monomers[36]. Fig. 3(c) illustrated the thermogravimetric analysis of BDPs film during compost biodegradation. The weight loss of BDPs occurs in three distinct stages, corresponding to random main chain scission, ester bond cleavage, and autocatalytic bond cleavage facilitated by carboxyl groups[37]. The temperature at which the polymer experiences a 10 % mass loss is defined as the material's characteristic temperature, a key indicator of its low-temperature thermal performance. Pretreatment reduced the characteristic temperature from 360.19℃ (NF0) to 352.45℃ (TF0), indicating a decrease in thermal stability. After composting, the characteristic temperatures increased slightly to 376.47℃ (NF35) and 385.01℃ (TF35), likely due to cross-linking reactions between polymer chains induced and changed in crystallinity by microbial activity[38]. The first-order derivative of the TGA curve was processed to obtain the DTG curve (Fig. 3d). The DTG curve further revealed that the temperature corresponding to the maximum weight loss rate of BDPs was approximately 400℃. Three prominent peaks were observed, likely attributed to the decomposition of inorganic fillers, such as calcium carbonate and talc, aligning with the three stages of BDPs.

ATR-FTIR was performed to determine the transformation characteristic of functional groups in BDPs (Fig. 3e and f). The results showed that the TF0 exhibited two noticeable broad peaks corresponding to O–H bonds with hydroxyl functional function at the 3676 and 3300 cm^{-1} wavelength regions[39]. Moreover, as the compost matured, there was an increase in both height and width of the peak at 3300 cm^{-1} (TF35), indicating increased hydrophilicity of the BDPs surfaces. This result suggests that hydroxyl radicals formed by the breaking of ester bond and hydrolysis of BDPs after alkaline-thermal pretreatment contributed to the enhanced biodegradability of the plastics in compost. It was also observed that the pretreatment had a more pronounced effect on the characteristic peaks of BDPs at different composting stages. The absorption peak around 1711 cm^{-1} was due to the C═O stretching vibration of ester and carboxyl groups[40,41]. The peak at 1273 cm^{-1} could be attributed to C–O bonds of both aliphatic and aromatic compounds[42]. The peaks at 1142 cm^{-1} and 1074 cm^{-1} were associated with the stretching vibrations of the C–O–C bond, indicating intermolecular breakage in the chemical structure[43]. The C–H bending vibrations at 727 cm^{-1} could be the characteristic functional groups related to PBAT mainly involved in aromatic compounds[44]. These results clearly indicate that the BDPs changed from hydrophobic to hydrophilic properties after biodegradation, and PBAT is more difficult to degrade in BDPs compared to PLA.

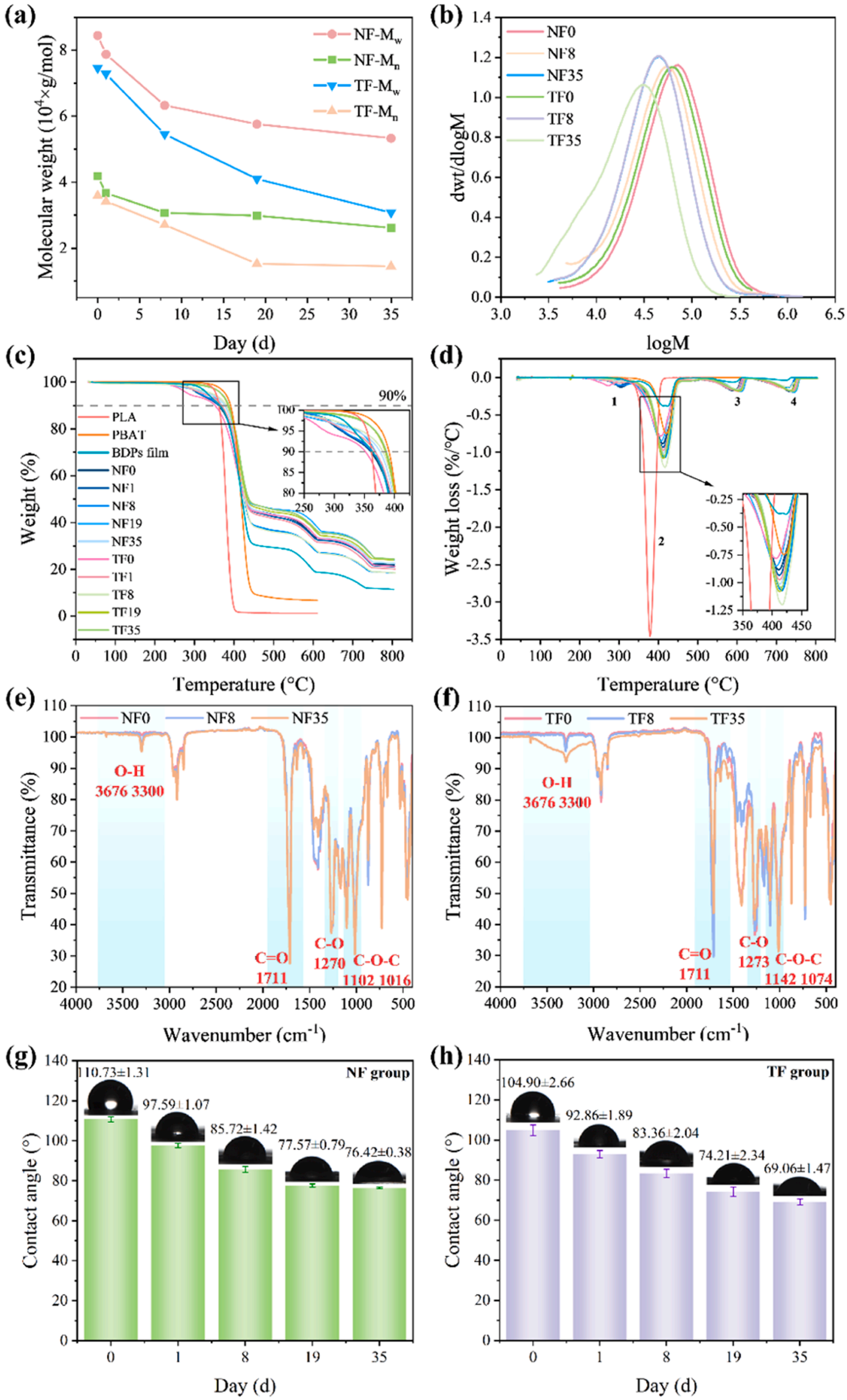

Fig. 3. Characteristic changes of BDPs in compost from TF and NF groups. molecular weight (a), molecular weight distribution (b), thermogravimetric analysis (c, d), ATR-FTIR spectra (e, f) and contact angle of NF and TF (g, h).

To further investigate the changes in hydrophilicity of BDPs after alkaline-thermal pretreatment, the contact angle was measured (Fig. 3g and h). Pretreatment significantly reduced the contact angle on the surface of BDPs film, which facilitates bacterial colonization on the plastic, thus accelerating BDPs biodegradation[17]. The contact angle of the TF group decreased rapidly to $83.36 \pm 2.04^{\circ}$ during the thermophilic stage of composting (TF8), suggesting a vigorous degradation reaction under high-temperature conditions. Moreover, the contact angle of the TF group decreased significantly faster than that of the NF group throughout the composting process, indicating that the pretreatment promoted the degradation of BDPs during composting. These results suggest that alkaline-thermal pretreatment can effectively promote the degradation of BDPs, particularly in the thermophilic stage.

Despite the significant degradation of BDPs during the thermophilic composting stage, generating MPs remains a concern. At the end of composting, μ-FTIR mapping revealed the release potential of MPs from pretreatment (TF) and non-pretreatment BDPs film (NF) to be 31.74 ± 3.46 (56.57 μm to 332.80 μm) and 39.13 ± 5.75 (115.55 μm to 332.80 μm) particles/mg, respectively. Similarly, the release potential of BDPs powder (TP and NP) was 249.11 ± 50.27 (20.66 μm to 278.30 μm) and 125.93 ± 33.722 (25.54 μm to 332.80 μm) particles/mg, respectively (Figure S6). These findings clearly demonstrate that pretreatment promotes the formation of smaller-sized MPs. While the biodegradation of BDPs primarily occurs at elevated temperatures, the MPs generated from incomplete degradation may resist further breakdown at ambient conditions, leading to their persistence in the environment[45]. These MPs may serve as carriers for microorganisms, potentially facilitating the widespread dissemination of ARGs.

3.3. Role of microorganisms carrying plastic-degrading genes

Alpha diversity indices showed that BDPs powder increased the diversity and abundance of microbial communities, suggesting that BDPs powder is more readily available for microbial utilization. In addition, the compost Chao1 index was slightly higher in the TF group than in the NF group, revealing that BDPs film was more likely to enrich microorganisms after pretreatment (Fig. 4a). However, the diversity and abundance of bacterial communities may affect the abundance of ARGs and HGT[46] (Section 3.4).

The succession of microbial communities during composting is further investigated by analyzing changes at species level (Figure S7). It can be seen that the microbial communities in all groups clustered into two distinct groups. In the CK group, *Limosilactobacillus fermentum* was the dominant species in the early stage of composting, whereas *Therobifida fuusca* and *Saccharomonospora viridis* became dominant during thermophilic and maturation stages. Compost was an important source of potential plastic-degrading bacteria, and pretreatment selectively enriched BDPs with degrading bacteria, mainly *Saccharomonospora viridis* and *Thermobifida fusca*, during the thermophilic and maturation stages[47,48]. The co-occurrence relationships of dominant microbes were explored through networks analysis (Fig. 4b-f). The network in NF and NP had relatively fewer links compared to those in TF and TP, indicating a smaller network size in the alkaline-thermal pretreatment. Across all networks, the majority of nodes tended to be positively linked (red lines) rather than negatively linked (green lines), especially in the CK, TF, and TP groups. This suggests that the addition of BDPs reduces the proportion of positive microbial correlations and that pretreatment reduces the detrimental effects of BDPs on microorganisms. Keystone species in the TF and TP group networks (e.g., *Novibacillus thermophilus*, *Alcaligenaceae bacterium* and *Thermobifida alba*) were mainly affiliated with the phylum *Firmicutes* and *Proteobacteria*, underscoring their pivotal roles in maintaining the stability of microbial networks. The species *Thermobifida alba* has been reported to significantly degrade polyethylene terephthalate copolymer as well as reduce polymer particle sizes, yielding terephthalic acid[49]. These results demonstrated that potential plastic-degrading bacteria were more likely to persist stably in the TF and TP groups over an extended period, proving the alkaline-thermal pretreatment effectively promoted the biodegradation of BDPs in composting.

Genome binning analysis was performed to identify the host bins of potential plastic-degrading genes (Fig. 4g). A total of 30 MAGs contained at least one plastic-degrading gene with an identity higher than 70 %. These MAGs were associated with *Proteobacteria* (6 MAGs), *Firmicutes* (14 MAGs) and *Actinobacteriota* (10 MAGs) phylum. The predominant bacteria in the CK group were *Pseudomonas formosensis* and *Thermobifida fusca*, which carried genes encoding PETase, lipase, polyester hydrolase, PBAT hydrolase, esterase, and cutinase. These enzymes are capable of hydrolyzing ester bonds, and the presence of these bacteria in the composting is essential for BDPs degradation[50]. In the TF and TP groups, pretreated BDPs significantly enriched *AK92 sp003399765*, *Saccharomonospora viridis* and *Thermobifida fusca* compared to the NF and NP groups. *Thermobifida fusca* is one of the most dominant bacteria in compost environments and contributes to the degradation of macromolecules such as lignocellulosic materials[51]. During the thermophilic stage of composting, the compost pile and the surface of BDPs (TF8 and TP8(Plastic)) were heavily enriched with *Thermobifida fusca*, which hydrolyzes ester bonds and converts polymers into oligomers through PETase, polyester hydrolase, serine hydrolase, PBAT hydrolase, and cutinase[52]. It is noteworthy that all bacteria belonging to the *Firmicutes* phylum possess genes encoding PLA depolymerase. Previous studies have shown that bacteria from the *Firmicutes* (e.g., *Paenibacillales*) containing PLA depolymerase have the capability to degrade PLA[53].

In conclusion, pretreatment and high additions of BDPs powder increased microbial diversity and abundance, particularly enriching plastic-degrading bacteria such as *Thermobifida fusca* and *Saccharomonospora viridis* during the thermophilic and maturation stages. Alkaline-thermal pretreatment improved surface roughness and reduced M_n, making BDPs more accessible to microorganisms, thereby promoting plastic degradation during composting. Additionally, the ester bond cleavage ability of indigenous microorganisms in the composting system plays a crucial role in the degradation of BDPs.

3.4. The effects of pretreatment with BDPs on the ARGs-host bacteria

A total of 36 high-quality non-redundant MAGs carrying ARGs were identified as ARGs-host bacteria, with an identity higher than 90 % (Fig. 5a). These ARGs hosts were primarily affiliated with the *Firmicutes* (18 MAGs), *Actinobacteriota* (6 MAGs), *Proteobacteria* (6 MAGs), *Bacteroidota* (4 MAGs), *Patescibacteria* (1 MAGs), and *Chloroflexota* (1 MAGs) phyla. The 36 MAGs predominantly carried 15 ARGs types, including multidrug, aminoglycoside, fluoroquinolone, glycopeptide, and bacitracin resistance genes. These MAGs mainly belonged to *DSM-21159* (3.76 Log_{10}(rpkm)), *JACDFO01* (3.29 Log_{10}(rpkm)), *Mycobacterium thermoresistibile* (2.25 Log_{10}(rpkm)), and *Thermoactinomyces vulgaris* (1.95 Log_{10}(rpkm)). A particularly concerning finding was the identification of *Lactococcus lactis*, *Bacillus clausii* and *Paenibacillus lactis* from *Firmicutes* phylum carried up to four ARGs, which was much higher than other MAGs. Furthermore, 26 MAGs carrying ARGs were identified as potential pathogenic ARGs hosts. Among them, *Pigmentiphaga sp002188465*, *Bacillus clausii*, and *Bacillus altitudinis* also carried potential plastic-degrading genes. The ARGs types associated with these plastic-degrading genes included peptide, aminoglycoside, multidrug, and bacitracin, mainly found in the NP8 and NP35 groups. It is worth noting that the addition of BDPs increased the abundance of MAGs carrying ARGs, especially during the thermophilic stage of composting (Fig. 5a).

In addition, a comprehensive profile of ARGs subtypes was constructed to reveal the diversity of resistance genes (Fig. 3b). Among them, *bacA*, *FosB*, *ugd*, *lnuH* and *sul1* were identified as the most abundant subtypes, shared across all samples of compost and surface of BDPs. *bacA* was the most prevalent ARGs subtype at the onset of composting,

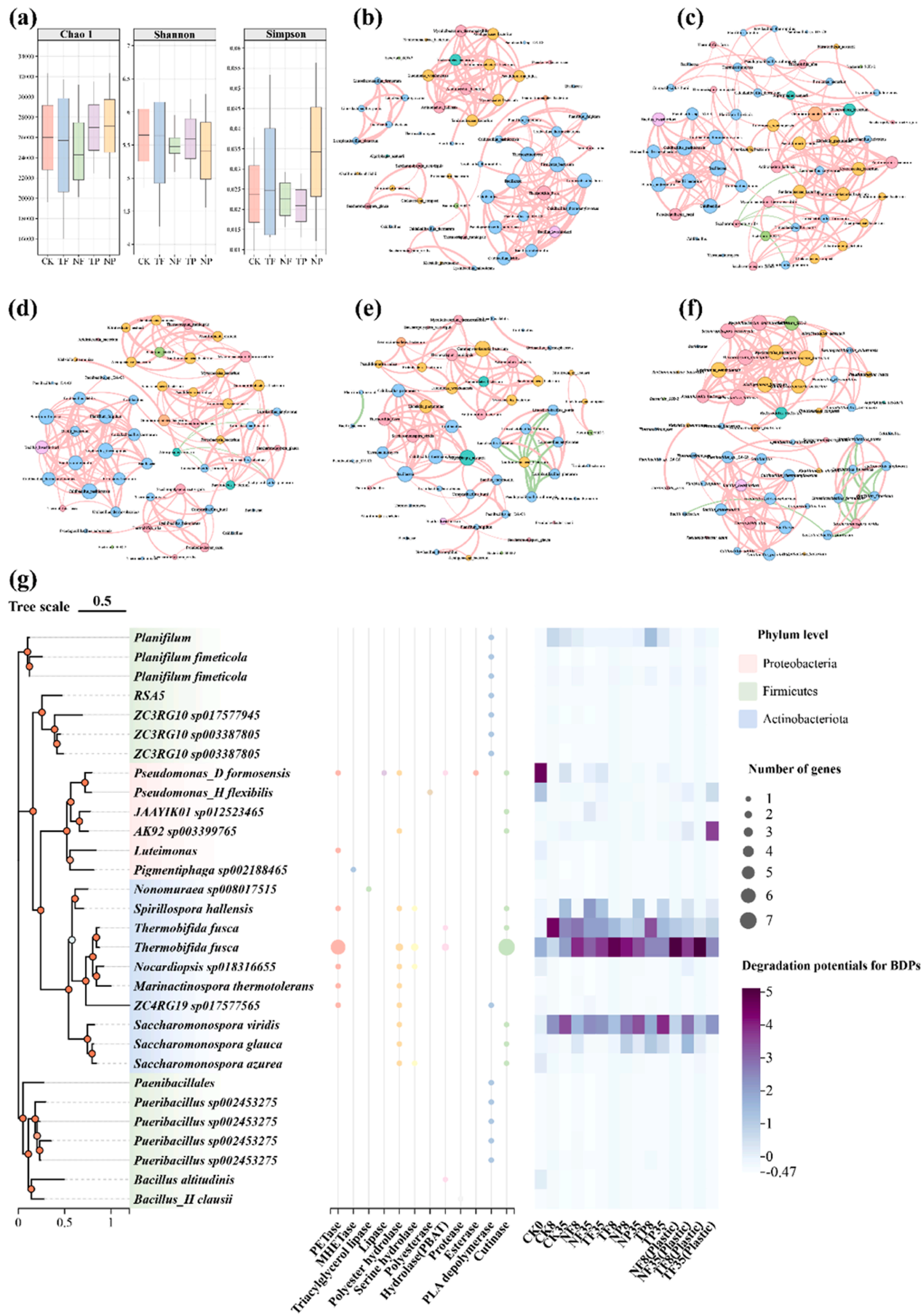

Fig. 4. Microbial alpha diversity (a), co-occurrence relationships of species level microbial communities in four BDPs-added groups (r > 0.8, $P < 0.05$) (b-f) are CK, NF, TF, NP, TP, respectively, and phylogenetic tree, number of degradative enzymes and BDPs degradation potential of potentially degrading bacteria (g).

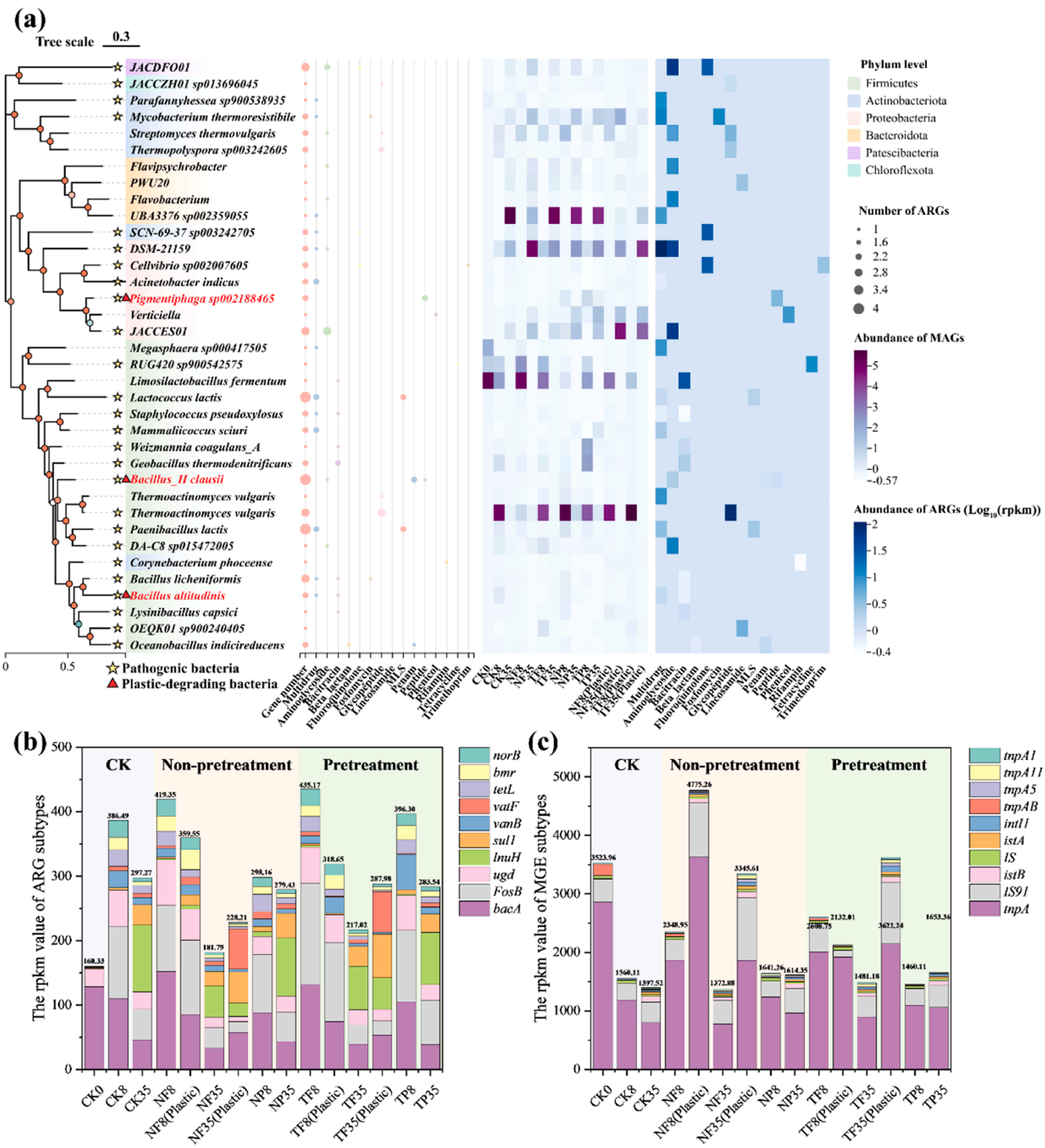

Fig. 5. Phylogenetic tree of MAGs carrying ARGs, the types of ARGs, the relative abundances of MAGs and rpkm values of ARGs types in MAGs (a), rpkm values of ARGs subtypes (b) and rpkm values of MGEs subtypes (c) in each group.

accounting for 79.96 %, followed by ugd at 17.20 %. As co-composting progressed, particularly during the thermophilic stage, the abundance of ARGs subtypes increased. After co-composting with pretreated BDPs, the rpkm values of ARGs subtypes in the TF8 and TP8 groups reached 435.17 and 396.30, respectively, significantly higher than those in the NF8 (419.35) and NP8 (298.16) groups, representing increases of 3.63 % and 23.76 %. Notably, the abundance of ARGs in the NP8 group (co-composted with BDPs powder) was 29.62 % lower than that in CK8, whereas pretreatment resulted in a 2.47 % increased in ARGs abundance. In contrast, co-composting with BDPs films led to an overall increase in ARGs abundance. During the maturation stage, although the rpkm values of ARGs in co-composted groups with BDPs were lower than in CK35 (297.27), the TF35 group (217.01) and TP35 group (283.54) exhibited higher values than the NF35 group (181.79) and NP35 group (279.43). This trend was consistent with observations on BDP film surfaces. Overall, the maturation stage reduces ARGs abundance overall, pretreated BDPs still exhibit higher ARGs levels compared to untreated samples, underscoring the potential long-term ecological risks. ARGs are often located on MGEs, which play a crucial role in the HGT of ARGs, facilitating bacterial adaptation to antibiotic selection pressure[54]. To better understand and control the spread of ARGs in BDPs co-composting, it is essential to consider the trends of MGEs (Fig. 5c). The *tnpA* (rpkm values of 24318.96), *IS91* (7133.36), and *istB* (674.52) were the dominant MGEs subtypes in composting. In the thermophilic stage, the rpkm values of MGEs in TF8 (2608.75) and NF8 (2348.95) were higher than those in TP8 (1460.11) and NP8 (1641.26), respectively. The highest values were found on the surface of BDP membranes, with NF8(Plastic) and TF8(Plastic) reaching 4775.26 and 2132.01, respectively. This indicates that ARGs have a greater HGT potential on the surface of BDPs film despite the low addition levels. In addition, in the maturation stage, TP35 (1653.36) still exhibited elevated MGEs compared to other groups, suggesting that the pretreatment promotes the production of smaller MPs, which provide carriers for the HGT of ARGs. These findings suggest that while pretreatment improves BDPs degradation, it inadvertently facilitates ARGs dissemination by increasing MGEs levels and producing smaller MPs. The high HGT potential observed on BDPs surfaces, particularly films, highlights the need for strategies to mitigate MGEs-mediated risks in co-composting systems. Addressing these challenges will be essential for minimizing ARGs spread while promoting effective plastic degradation.

Dynamic changes in the relative risks of ARGs during co-composting were evaluated based on their classification into different ranks (Figure S8). The results showed that that as composting progressed, the risks associated with Rank I, II, and IV ARGs increased. Notably, in non-pretreament groups, Rank I and II ARGs contributed more significantly to the overall ARGs risk compared to pretreated groups. Specifically, samples NP35 and NF35 exhibited the highest Rank I values, at 0.11 and 0.096, respectively. Although the thermophilic stage can reduce the abundance of most microorganisms carrying ARGs, thermophilic microorganisms carrying MGEs and already present in pathogenic bacteria still maintain a high abundance[55], resulting in the persistence or even an increase in the risk associated with Rank I. Due to their broad host range and niche adaptability, Rank I ARGs are already present in pathogenic bacteria and may further facilitate the spread of multidrug resistance through MGEs.

The Mantel analysis revealed that various abiotic factors and BDPs characteristics in the composting process influenced the frequency of MGEs-mediated HGT of ARGs (Figure S9). Specifically, environmental factors such as pH, HA, FA, and the HA/FA ratio, along with the Chao1 index, showed a significant positive correlation with ARGs types and plastic-degrading bacteria ($P < 0.05$). Furthermore, key BDPs characteristics, including PDI and hydrophilicity, were significantly positively correlated with the types of MGEs ($P < 0.01$). These findings indicate that the surface characteristics of BDPs, along with surrounding environmental factors, collectively influence the formation and evolution of microbial community composition[30,56].

Overall, microbial interactions play a pivotal role in both ARGs dissemination and BDPs degradation during co-composting. As the co-composting process progresses, dominant microorganisms gradually stabilize and enrich the surface of the BDPs. This proximity among microbial species enhances the HGT of the ARGs through the MGEs, increasing the potential for ARGs dissemination. For instance, biofilm-associated bacteria, including *Thermobifida fusca* and *Saccharomonospora viridis*, enriched in the thermophilic and maturation stages, exhibit plastic-degrading enzyme activity while also as ARGs-host. During the maturation stage, microbial communities diversified, with *Bacillus altitudinis* and *Pigmentiphaga sp002188465* playing a larger role. This diversification was accompanied by a decline in ARGs abundance and further degradation of BDPs, suggesting that microbial succession contributes to both the degradation process and the mitigation of ARGs risks. However, due to the uniform particle size (< 150 μm) of the BDPs powder used in this study, the effect of particle size on HGT of ARGs remains unclear. As BDPs degrade, especially after pretreatment, they are more likely to form MPs, and both particle size-dependent effects and contact angle play significant roles in ARGs distribution[57]. Additionally, this study did not account for the fact that MPs provide a unique ecological niche for viruses, which may influence the rapid dissemination of ARGs through virus-host interactions[58,59]. These limitations hinder a comprehensive understanding of the effects of BDPs pretreatment on plastic-degrading bacteria, ARGs bacterial hosts in co-composting, and the underlying mechanisms of their assembly. Further research should consider the impact of MPs in real-world composting systems affected by seasonal fluctuations, which would provide more reliable and practical insights.

4. Conclusions

The study demonstrates that alkaline-thermal pretreatment significantly enhanced the degradation of BDPs and extended the duration of the heating and thermophilic phases during co-composting. This improvement in degradation during the thermophilic stage can be attributed to the reduction in molecular weight and the increased surface hydrophilicity of BDPs, which facilitated the selective enrichment of bacteria harboring plastic-degrading genes. While the alkaline-thermal pretreatment effectively enhanced the degradation performance of aromatic-aliphatic BDPs, it also inadvertently promoted the enrichment of ARGs within the compost, highlighting a dual environmental challenge. Although the pretreatment process partially reduced the abundance of MGEs on the BDPs surfaces, there was still a high risk of ARGs. Additionally, three plastic-degrading bacterial strains were identified as ARGs hosts, emphasizing the need for further investigation of the metabolic pathways of other potential hosts bacteria and even viral-mediated HGT. Given that pretreatment alters the characteristics of BDPs and releases substantial quantities of MPs, which could contribute to the dissemination of ARGs, posing risks to microbial ecosystems and public health. Therefore, the need for optimized pretreatment strategies and composting protocols to balance the benefits of improved BDPs degradation with the mitigation of ARGs dissemination risks. Future research should focus on developing novel pretreatment technologies or optimizing the composting process (extending the composting duration, adjusting composting conditions, and addition of specific amendments) that can promote ARGs degradation and reduce MGEs-mediated HGT to effectively control environmental risks associated with ARGs induced by pretreatment BDPs.

Abbreviations

BDPs, biodegradable plastics; PBAT, poly(butylene adipate-co-terephthalate); PLA, poly(lactic acid); MPs, microplastics; ARGs, antibiotic resistance genes; MGEs, mobile genetic elements; HGT, horizontal gene transfer; TOC, total organic carbon; M_n, number-average molecular weight; M_w, weight-average molecular weight; PDI, polymer dispersity index; MAGs, metagenome-assembled genomes; SEM, scanning electron microscopy; TGA, thermogravimetric analysis; DTA, differential thermogravimetric analysis; VS, volatile solids; NH_4^+–N, ammonia-nitrogen; EC, electrical conductivity; GI, germination index; HA, humic acid; FA, fulvic acid; DOM, dissolved organic matter; MAGs, metagenome-assembled genomes; rpkm, relative reads per kilobase per million mapped reads.

Environmental implications

Alkaline-thermal pretreatment enhances biodegradable plastics (BDPs) degradation during co-composting but raises environmental concerns by increasing antibiotic resistance genes (ARGs) abundance and releasing microplastics. The enrichment of plastic-degrading bacteria that host ARGs highlights the potential for horizontal gene transfer, posing risks to microbial ecosystems and public health. While pretreatment reduces mobile genetic elements (MGEs) abundance, its unintended effects on ARGs dissemination necessitate careful evaluation. Future advancements should focus on optimizing pretreatment methods and composting processes to balance effective BDPs degradation with minimizing ARGs-related risks, ensuring sustainable waste management practices and mitigating environmental impacts.

CRediT authorship contribution statement

Hong Seungkwan: Writing – review & editing. **Kim Hyunook:** Writing – review & editing. **Zhan Min:** Writing – review & editing, Supervision. **Xie Bing:** Writing – review & editing, Methodology, Conceptualization. **Zhang Yuchen:** Writing – review & editing. **Su**

Yinglong: Writing – review & editing, Supervision. **Li Weiying:** Writing – review & editing. **Wang Wenyue:** Writing – original draft, Validation, Investigation, Data curation. **Tao Jianping:** Investigation, Data curation. **Pang Ruirui:** Data curation. **Zhang Linjie:** Data curation.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.137644.

Data Availability

Data will be made available on request.

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