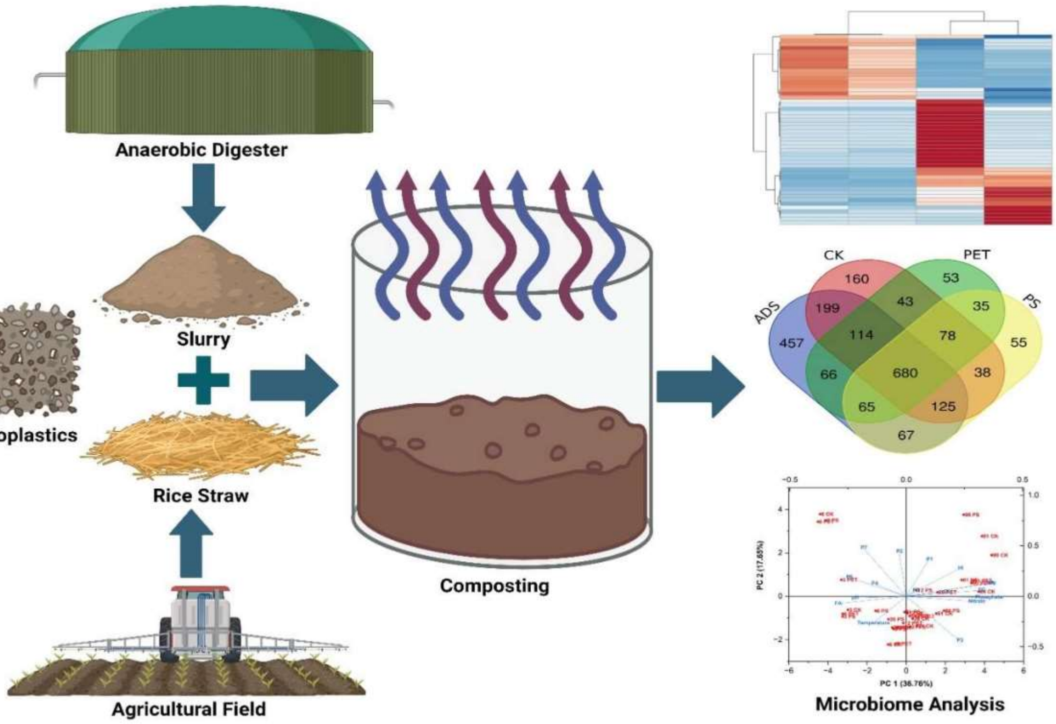
Chapter 6

Investigate the effect of polyethylene terephthalate and polystyrene microplastics on microbiome dynamics during rice straw composting



6.1 Background

MPs are plastic particles smaller than 5 mm and have become pervasive pollutants across various ecosystems, including terrestrial, freshwater, and marine environments [101,102]. While the presence of MPs in marine ecosystems has been widely recognized and studied, their impact on terrestrial environments, particularly soils, remains underexplored, revealing a significant gap in current research [103]. Recently, there has been a growing interest in understanding the presence and effects of MPs within soil ecosystems [104].

MPs ingested by soil-dwelling organisms or absorbed by plants can enter the food chain, posing risks to human health and disrupting soil ecosystems [105]. This concern has driven extensive research into the effects of MPs on soil physical properties, including bulk density, water-holding capacity, pore size distribution, and overall soil structure, all of which are critical for maintaining soil fertility and supporting plant growth [106]. Additionally, MPs can disrupt microbial activity essential for nutrient cycling and organic matter decomposition, interfere with plant rhizosphere processes, and pose potential risks to animal health [107]. These findings underscore the urgent need for strategies to mitigate the adverse effects of MPs on soil health and ecosystem functionality [108].

In composting systems, MPs can alter microbial communities by suppressing or stimulating bacteria involved in organic matter decomposition, potentially hindering the breakdown of RS, slowing the composting process, and leading to incomplete composting [109]. MPs may also adsorb and release chemicals, affecting nutrient availability, particularly nitrogen, phosphorus, and potassium (N, P, K), and altering the compost's physical properties, such as texture and porosity [110]. This can reduce water retention, making the compost less suitable for agricultural use [111]. Moreover, harmful additives or MP degradation products may leach into the compost, compromising its quality and safety for agricultural applications [112].

PET and polystyrene (PS) are MPs primarily used in packaging, textiles, and everyday products [113,114]. The lasting presence of these plastics in nature poses a risk to both ecological systems and human well-being, underscoring the pressing need to grasp their effects on living organisms and ecological functions [115]. Even agricultural soils have not escaped contamination by MPs, with increasing evidence suggesting their presence in production and waste-handling stages [116]. Composting is an essential practice for converting organic waste into beneficial soil amendments [127,132]. RS, an agricultural residue, is frequently composted due to its lignocellulosic content, which provides a valuable source of carbon and nutrients necessary for the composting process [118]. However, the introduction of MPs such as PET and PS into composting materials like RS presents significant challenges that can disrupt the composting process and degrade the quality of the final compost product [104]. The presence of PET and PS MPs in composting systems can significantly interfere with microbial activity and nutrient cycling, thereby hindering successful composting outcomes [119].

MPs in organic waste pose a notable challenge to composting. Composting relies on microorganisms to break down organic material, but these organisms often struggle to degrade plastics fully [120]. For example, hyperthermophilic bacteria can partially degrade plastic structures during sewage sludge composting, leading to a 43.7% reduction in MP content [131]. However, studies have shown that conventional composting methods do not significantly reduce the overall quantity of MPs, although they may cause plastics to fragment into smaller particles [133]. The interaction between MPs and the composting process is complex and dynamic. Certain plastics, including polyethylene (PE), polyvinyl chloride (PVC), and polyhydroxyalkanoates (PHA), can inhibit nitrification and delay compost maturity, thereby compromising the quality of the final product [138]. Similarly, it was observed that MPs disrupt nitrogen transformation and alter microbial community structures during composting [5]. Further reported that PE and PHA in compost destabilize fungal communities and increase the abundance of phytopathogenic fungi, potentially harming compost health [39]. Additionally, PE impedes organic matter degradation and reduces microbial diversity during the vermicomposting of sewage sludge [39]. These findings underscore the significant impact that MPs can have on microbial dynamics and nutrient processes during composting. Despite growing awareness of these issues, the effects of MPs on composting remain insufficiently studied. Each year, large quantities of organic waste containing MPs are processed into compost. Yet, the potential risks associated with MPs are not fully understood, posing challenges to the broader adoption and optimization of composting technology [101]. Understanding the effects of MPs on composting processes and compost quality was crucial for ensuring the sustainable application of composting. Without this knowledge, MPs could have undermined composting as an effective solution for organic waste management and soil fertility enhancement. This study investigated the influence of PET and PS MPs on RS composting, focusing on changes in microbial community composition through 16S rRNA metagenomic sequencing and flow cytometric analysis, physicochemical characteristics of the compost (including temperature, electrical conductivity, pH, and the seed germination index), nitrate and phosphate transformation, organic matter humification, and the impact on PET and PS after composting, assessed through SEM and FTIR analysis. Through controlled experiments, this research identified the mechanisms by which MPs impacted the composting process. The results provided valuable insights into the broader implications of MP pollution in compost and agriculture. These findings were essential for developing strategies to mitigate the adverse effects of MPs on soil health and agricultural sustainability.

6.2 Sample collection

Fresh ADS was collected from a nearby biogas facility situated at coordinates 25° 11' 33.95" N and 82° 51' 19.06" E in Varanasi, India. RS was harvested from an agricultural plot at Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India, after the growing season. Following harvesting, the RS underwent mechanical fragmentation using an electric fodder-cutting machine to reduce it into smaller segments. Immediately after that, the fragmented RS was transported to the laboratory and stored in zip-lock bags at ambient temperature. The RS underwent a two-step desiccation process to lower its moisture content. Initially, it underwent natural air drying to eliminate excess moisture. Subsequently, the RS was dried further at 50°C in a hot air oven (Equitron, Stream Series) until it attained a consistent weight. After drying, the RS was further processed into smaller dimensions (2 - 3 cm) using an electric grinder (Philips Supreme 550W). PET and PS granules were procured from Sigma Aldrich and processed similarly through the electric grinder (Philips Supreme 550W). All MPs were meticulously sieved to ensure their particle size when added to the compost ranged between 0.4 - 0.5 mm.

6.3 Experiment design

ADS and RS in a 25: 9 ratio were mixed to achieve an initial moisture content of approximately 60% and a carbon-to-nitrogen (C/N) ratio of about 25:1 [47]. Subsequently, 0.5% by dry weight of PET and PS MPs were incorporated into the mixture, designated as PET and PS treatments, respectively. A control (CK) was prepared without the addition of MPs. The mixtures were thoroughly homogenized, and aerobic composting was initiated using cylindrical reactors with dimensions of 35 cm in height, 32 cm in diameter, and a total volume of 28,148.67 cm^3. The reactors, with a capacity of 20 liters, were perforated with 35 evenly spaced holes, each 7 mm in diameter, positioned in the lower region to facilitate aeration. An air pump supplied a controlled airflow of 0.6 L/min for 10 minutes daily, promoting aerobic microbial activity. The compost mixtures were placed in 20-liter stainless steel reactors insulated with 2 cm of material, and composting proceeded for 90 days. Samples were systematically collected from each treatment on days 0, 3, 6, 9, 12, 21, 36, 51, 66, 81, and 90 to analyze physico-chemical properties, humification indices, and flow cytometric analysis. Additionally, microbial community structure and MPs were assessed using samples taken on the initial and final days of composting. Before each sampling, the compost mixtures were manually turned and homogenized to ensure uniformity [43].

6.4 Physicochemical analysis

Compost samples were systematically collected from three vertical sections: the upper layer (30 cm from the base), the middle layer (15 cm from the base), and the lower layer (5 cm from the base). These samples were thoroughly homogenized to ensure uniformity for subsequent physicochemical analyses. Temperature was measured at each vertical section using a calibrated digital thermometer to monitor thermal gradients within the compost reactor. Moisture content was determined by drying the samples in a hot air oven (Equitron, Stream Series) at a controlled temperature until a constant weight was achieved. Nitrate levels were quantified, and phosphate concentrations were determined using the stannous chloride method [51,35]. pH was measured with a portable digital pH meter (HI96107) from Hanna Instruments, India. The fresh samples' germination index (GI) was quantified to assess the phytotoxic potential of the composting materials. Ten Black Chickpea seeds were placed on filter paper saturated with compost extracts (w/v = 1:10). Following a 48-hour incubation at 25°C, the seed germination percentage and the resulting seedlings' root lengths were measured [121]. Deionized water was used as a control for comparison against the compost extracts. Compost samples were subjected to extraction using a 0.1 mol/L $Na_2P_4O_7$ and 0.1 mol/L NaOH solution at a ratio of 1:10 (w:v). The extraction process involved shaking the mixture at 180 rpm and 25°C for 24 hours, followed by centrifugation at 2000 rpm for 20 minutes. The supernatant containing the soluble humic substances (HS) derived from the compost was collected. The pH of the supernatant was adjusted to 1.0, and the solution was left undisturbed for 24 hours. Fulvic acids (FA) were then isolated from the supernatant by centrifugation. The precipitate was washed three times with 0.05 M H_2SO_4 and subsequently dissolved in 0.1 mol/L NaOH to obtain humic acids (HA). The potassium dichromate method quantified the organic carbon content in HS, HA, and FA [47]. The surface morphology of the samples was examined using a scanning electron microscope (SEM) (EVO - Scanning Electron Microscope MA15/18, Carl Zeiss Microscopy Ltd.) to visualize morphological features. A desiccated powder sample (5 g) was prepared by drying for three days at 105°C in a hot air oven. The sample was mounted on a holder and imaged at 5X, 20X, and 100X magnifications with software used for image analysis. A comprehensive elemental analysis, including carbon, nitrogen, hydrogen, and sulfur content, was conducted using a CHNS analyzer (EuroEA Elemental Analyser, EuroVector, Italy). The finely ground sample was placed in small tin capsules and precisely analyzed in the CHNS analyzer to determine its elemental composition.

6.5 Extraction of microplastics

MPs are extracted systematically from the compost samples collected on 0 and 90 days, using the protocol [133]. This method thoroughly homogenizes 20 gm of compost with 300 mL sodium chloride solution (NaCl). The mixture was continuously agitated for 15 minutes to ensure the effective dispersion of MPs in the solution. After stirring, the mixture settles for 2 hours and allows MP to be separated from the compost matrix. The supernatant containing suspended MPs was carefully removed and filtered under vacuum through a 37 m diameter vacuum sieve. This filtration step is carried out in three phases to maximize MP recovery, considering particles below 37 m may be lost during the process. The remaining material in the sieve is transferred to the beaker, oxidized, and removed by 100mL of 30% hydrogen peroxide (H_2O_2). The reaction was allowed to continue overnight, ensuring the complete digestion of organic materials. After digestion, the solution was diluted with 200 ml of distilled water and filtered by a GF/F glass fibre filter (Whatman) with a diameter of 0.8 m and 50 mm. The filters now contain isolated MPs and are carefully placed in Petri plates and dried at 50°C for three days to remove any remaining moisture. All procedures were carried out under strict conditions to prevent extraneous MP contamination, including clean cotton laboratories and nitrile gloves [47]. Each extraction was performed in three batches to ensure robustness and reproducibility in quantifying MP from the compost samples.

6.6 Flow cytometry analysis

Flow cytometry was used to analyze changes in microbial communities in compost samples, which were collected on days 0, 3, 6, 9, 12, 21, 36, 51, 66, 81, and 90. A 0.5 g sample was dissolved in 5 mL of phosphate-buffered saline (PBS-1X), centrifuged at 2000 rpm to remove debris, and filtered through a 35 μm nylon mesh. The analysis was conducted immediately using a Beckman CytoFlex Flow Cytometer equipped with blue and red lasers. The cytometer was calibrated using CytoFlex Daily QC Fluorospheres, and 100,000 events were captured with a threshold of 20,000 to distinguish individual cells within the heterogeneous population [122].

6.7 Result and discussion

6.7.1 Influence of microplastics on physicochemical characteristics

In *Figure 6.1. (a)*, the early stages of composting (Day 0 to Day 21), the CK demonstrated typical patterns of microbial activity, with temperature rising significantly by Day 3, indicating the onset of the thermophilic phase, where microbial activity was at its peak. The CK temperature increased from 30.26°C on Day 0 to 45.26°C by Day 3 and peaked at 60.26°C by Day 6, reflecting robust microbial degradation of organic matter [123]. In contrast, the PET and PS treatments showed even higher temperatures (47.23°C and 44.26°C on Day 3 and 62.36°C and 62.23°C on Day 6, respectively), suggesting that while microbial activity was initially enhanced, MPs likely altered the composting environment. However, despite the higher temperatures, the pH levels in the PET and PS treatments were slightly lower than in the CK, as shown in *Figure 6.1. (b)*.

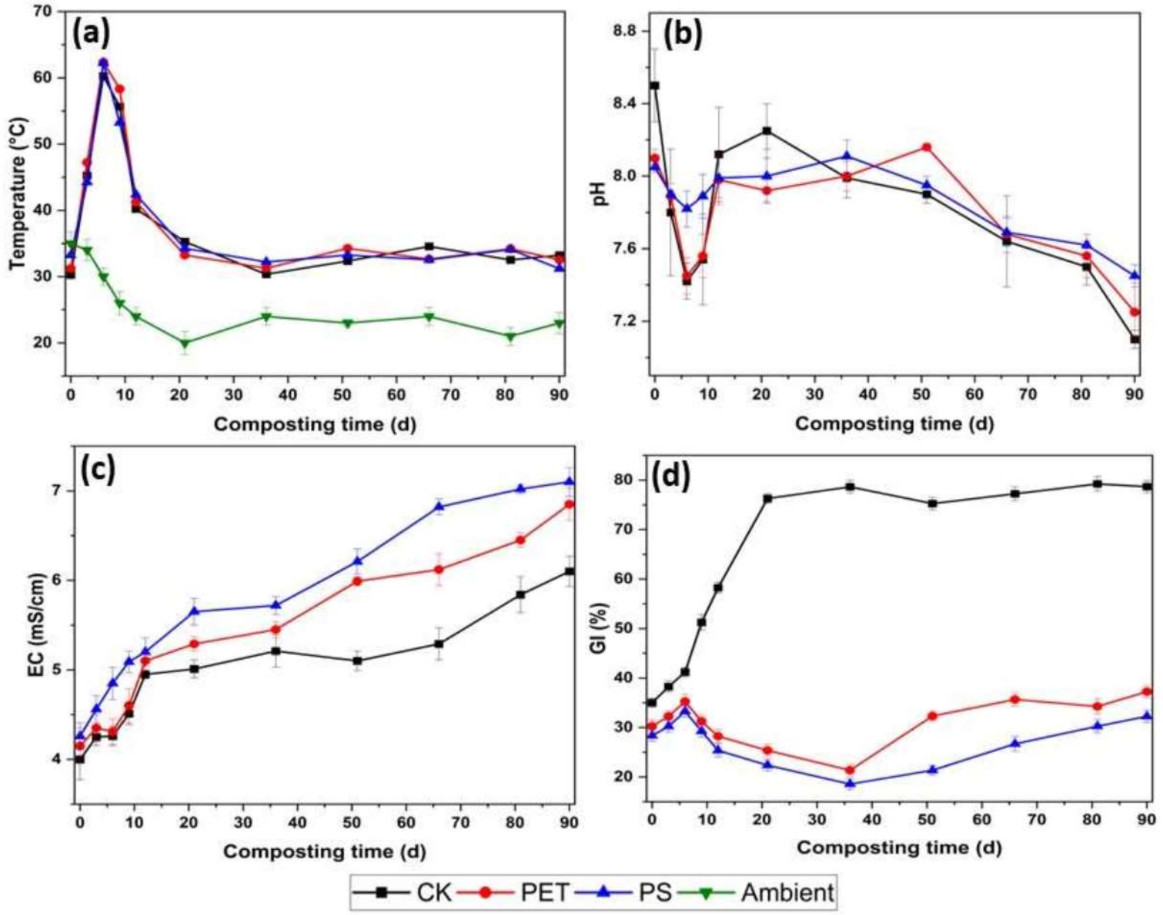


Figure 6.1. Variations in (a) Temperature, (b) pH, (c) Electrical Conductivity (EC), and (d) Germination Index (GI) throughout the composting process, where CK (control), PET (polyethylene terephthalate), and PS (polystyrene) are the treatments

The germination index (GI) was consistently lower across these treatments, indicating that MPs were already beginning to disrupt microbial processes responsible for transforming organic matter into stable compost [124]. As presented in *Figure 6.1. (d)*, lower GI in PET (32.25%) and PS (30.26%) by Day 3, compared to the CK (38.26%), suggested that the compost was less mature and possibly more phytotoxic in the presence of MPs. As the composting process progressed into the middle stage (Day 21 to Day 51), the CK continued to show typical composting progression, with the temperature gradually declining as the composting process stabilized and the microbial community transitioned to breaking down more complex organic materials [33]. The pH in the CK increased slightly to 8.25 by Day 21, reflecting the neutralization of organic acids produced during earlier stages. In contrast, the PET and PS treatments displayed slightly lower pH values. EC levels were higher, particularly in the PS treatment, indicating increased salt concentrations that could have inhibited microbial activity. The GI in the CK reached 76.28% by Day 21, signalling advanced compost maturity. However, the GI in PET (25.35%) and PS (22.36%) treatments remained significantly lower, indicating that MPs hindered microbial processes essential for compost maturity [47]. This stage also showed slower reductions in FA and lower HA levels in PET and PS treatments, suggesting that MPs disrupted the microbial conversion of labile organic matter into stable humic substances. [121]The study also suggested that adding MPs (polypropylene and polyethylene) significantly reduced compost quality. This was evidenced by lower seed GI values and reduced nitrate nitrogen content, indicating poorer plant nutrient availability. In the late stage of composting (Day 66 to Day 90), the CK exhibited advanced compost maturity, with the temperature stabilizing around 34.56°C by Day 66 and the pH remaining slightly alkaline, indicating that the compost had entered a more stable phase with reduced microbial activity. As shown in *Figure 6.1 (c)*, EC levels in the CK were stable, reflecting balanced nutrient availability. However, in the PET and PS treatments, the EC continued to rise, particularly in the PS treatment, reaching 6.82 mS/cm by Day 66, compared to 5.29 mS/cm in the CK. This increase in EC, coupled with the consistently lower GI in PET (35.65%) and PS (26.68%) treatments, suggested that MPs created a more saline environment that could have inhibited microbial activity and delayed compost maturity [45]. By Day 81, the GI in CK reached 79.24%, indicating highly mature compost, while in PET and PS treatments, the GI remained lower, reflecting the ongoing negative impact of MPs on compost quality and the microbial processes that drove compost stabilization. By the final stage of the composting process (Day 90), the CK had achieved complete stabilization of organic matter, with a temperature of 33.23°C, pH of 7.1, EC of 6.1 mS/cm, and a GI of 78.68%, indicating a highly mature and stable compost [125]. In contrast, the PET and PS treatments showed lower temperatures, higher EC levels, and significantly lower GI values (37.25% for PET and 32.26% for PS), suggesting that the compost in these treatments was less mature, less stable, and potentially less adequate for agricultural use. The continued lower GI and higher EC in PET and PS treatments indicated that MPs had a sustained adverse effect on the microbial processes essential for compost maturation, such as nitrification, humification, and forming stable organic polymers [45].

6.7.2 Impact on humification

Figure 6.2. demonstrates that MPs, specifically PET and PS, influenced the composting process by disrupting key physicochemical factors such as nitrate and phosphate levels, HA, FA, the humification index (HI), and the degree of polymerization (Dp) across various stages of the 90-day composting period. The intricate relationships between these factors and their progression over time underscored the critical role of microbial activity in composting and how it was adversely affected by the presence of MPs. As shown in *Figure 6.2.* (a) and (c), in the early stages of composting (Days 3 to 21), microbial colonization and the initial breakdown of organic matter were reflected in modest increases in nitrate and HA levels in the CK, with nitrate reaching 0.20 g/kg by Day 3 and HA rising to 34.25 g/kg [126]. These processes were crucial as they set the foundation for effective composting. However, in the PET and PS treatments, while nitrate levels were slightly higher than in the CK (0.21 g/kg for PET and 0.24 g/kg for PS), HA levels were lower, particularly in PET (31.21 g/kg), indicating an early disruption in humification [127]. Adding MPs (PP and PE) significantly compromised compost quality, as evidenced by lower seed germination indices and reduced nitrate nitrogen levels, indicating diminished nutrient availability for plants [141,142]. Furthermore, MPs hindered key composting processes, resulting in lower degrees of polymerization and humification, and disrupted the microbial community by reducing both the abundance and diversity of bacteria

essential for composting. These findings highlighted the detrimental long-term impacts of MPs on the composting process and the quality of the final compost product.

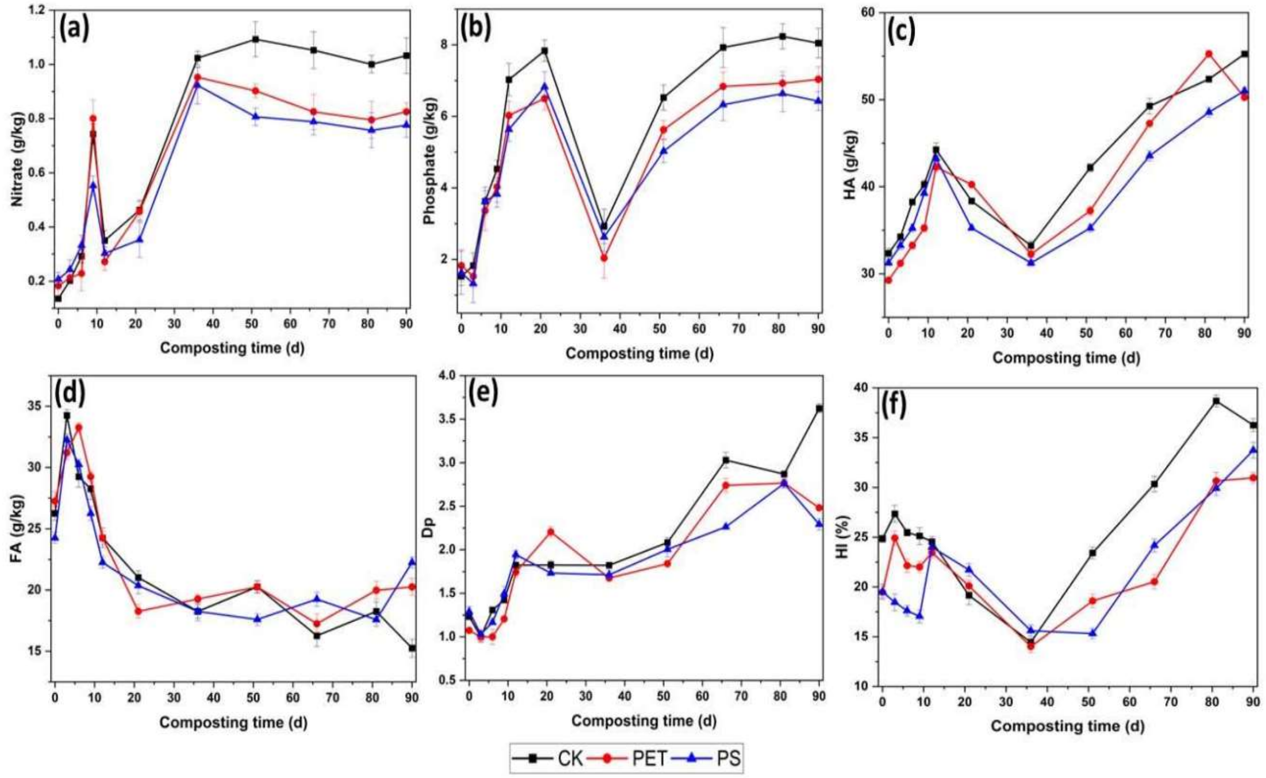


Figure 6.2. Variations in humification and nutrient levels during the composting process: (a) Nitrate (g/kg), (b) Phosphate (g/kg), (c) Humic acid concentration (HA), (d) Fulvic acid concentration (FA), (e) Degree of polymerization (Dp), and (f) Humification index (HI).

By Day 9, the CK showed a sharp increase in nitrate to 0.74 g/kg and HA to 40.25 g/kg, reflecting active microbial nitrification and humification. Conversely, in the PET and PS treatments, these increases were less pronounced, with HA levels at 35.25 g/kg for PET and 39.26 g/kg for PS, accompanied by lower HI values, suggesting that MPs were beginning to significantly impair microbial activity, particularly the processes responsible for transforming labile organic matter into stable humic substances. As presented in *Figure 6.2. (b)*, Phosphates in the CK treatment peaked at 7.5 g/kg by Day 21 and stabilized at around 6.5 g/kg by Day 90, reflecting efficient nutrient cycling. In contrast, PET and PS treatments exhibited lower peaks, at 6 g/kg and 5.5 g/kg, respectively, and consistently remained below CK levels throughout the composting process. This trend was consistent with lower nitrate levels and reduced HA formation observed in the PET and PS treatments. These findings suggested that MPs disrupted microbial processes critical for nutrient availability and the formation of humic substances, resulting in less mature and nutrient-deficient compost. As the composting process progressed into the middle stage (Days 21 to 51), the CK maintained higher HA levels and a steady decrease in FA, as presented in *Figure 6.2. (d)*, with nitrate peaking at 1.09 g/kg by Day 51, indicating ongoing and effective microbial activity. In contrast, the PET and PS treatments showed continued delays in humification, with lower HA levels (37.25 g/kg for PET and 35.26 g/kg for PS) and slower reductions in FA, particularly in PS, where FA remained at 17.59 g/kg by Day 51. The HI values in the PET and PS treatments were also significantly lower than in the CK (18.60% for PET and 15.31% for PS, compared to 23.41% in the CK), indicating less efficient humification. As presented in *Figure 6.2. (e)*, Dp values, which reflected the formation of stable organic polymers, also diverged. PET and PS treatments showed lower values than the CK, suggesting that MPs hindered the polymerization processes necessary for producing mature and stable compost. In the late stage (Days 66 to 81), the CK exhibited advanced compost maturity, with HA content peaking at 52.36 g/kg by Day 81. At the same time, FA continued to decrease, indicating significant organic matter stabilization. Nitrate levels remained relatively high, supporting ongoing microbial activity. However, the PET and PS treatments showed lower HA levels and higher FA levels, particularly in PS, where FA remained at 17.58 g/kg, suggesting incomplete humification. As shown in *Figure 6.2.* (e) and (f) HI and Dp values in the PET and PS treatments continued to lag behind those in the CK, with PET at 2.76 and PS at 2.76 for Dp, compared to 2.86 in the CK, indicating that the compost in these treatments was less polymerized and therefore less stable. By the final stage (Day 90), the CK had achieved complete stabilization of organic matter, with HA at 55.25 g/kg, FA at 15.25 g/kg, an HI of 36.26%, and a Dp of 3.62, indicating a highly mature and stable compost.

In contrast, the PET and PS treatments remained behind, with lower HA levels (50.26 g/kg for PET and 51.02 g/kg for PS) and higher FA levels, along with lower HI and Dp values, suggesting that the presence of MPs continued to hinder the complete transformation of organic matter into stable humic substances. PET and PS MPs disrupted composting, lowering nutrient levels, reducing HA content, and hindering maturation. This led to less mature and stable compost, negatively affecting its agricultural quality.

6.7.3 Alterations in the chemical composition and physical structure of microplastic

The Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analyses provided a detailed examination of the physical and chemical transformations that occurred in MPs (PS and PET) over the 90-day composting period. These findings were closely correlated with previously discussed microbial and physicochemical data. The SEM images revealed notable differences in the surface morphology of the MPs between Day 0 and Day 90, as shown in *Figure 6.3*.

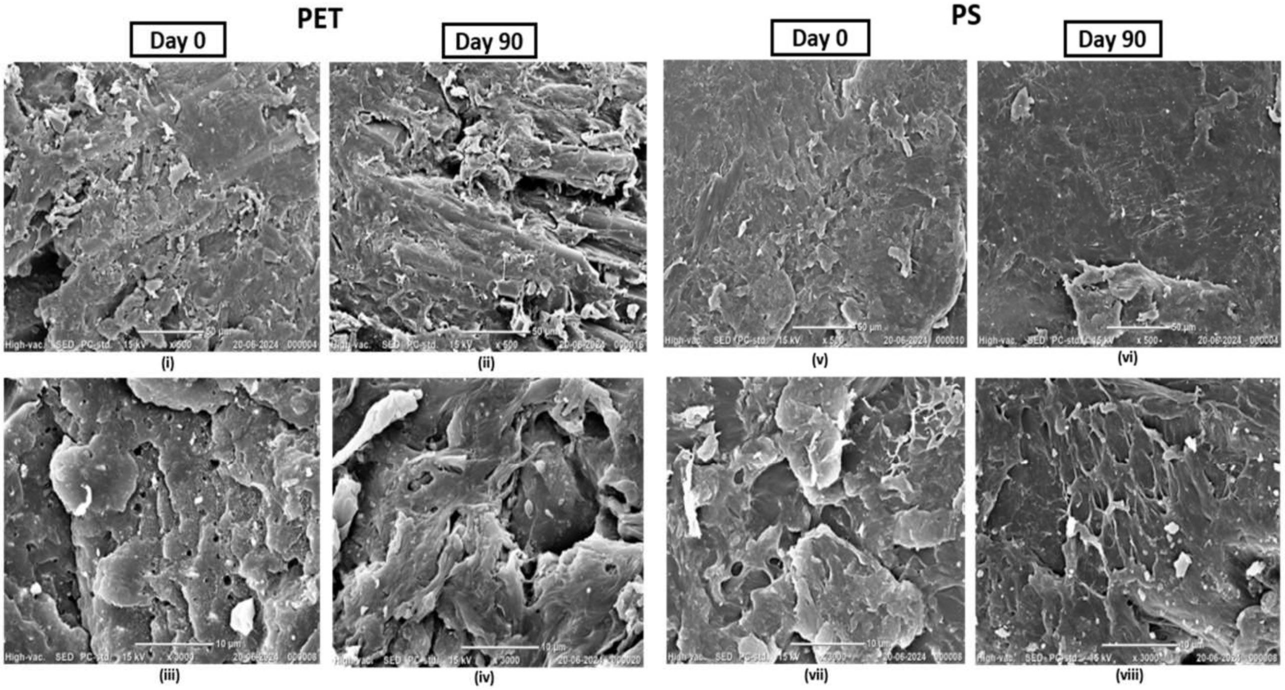


Figure 6.3. SEM micrographs of PET at Day 0 (i, iii) and Day 90 (ii, iv), and PS at Day 0 (v, vii) and Day 90 (vi, viii) at 50X and 10X magnifications

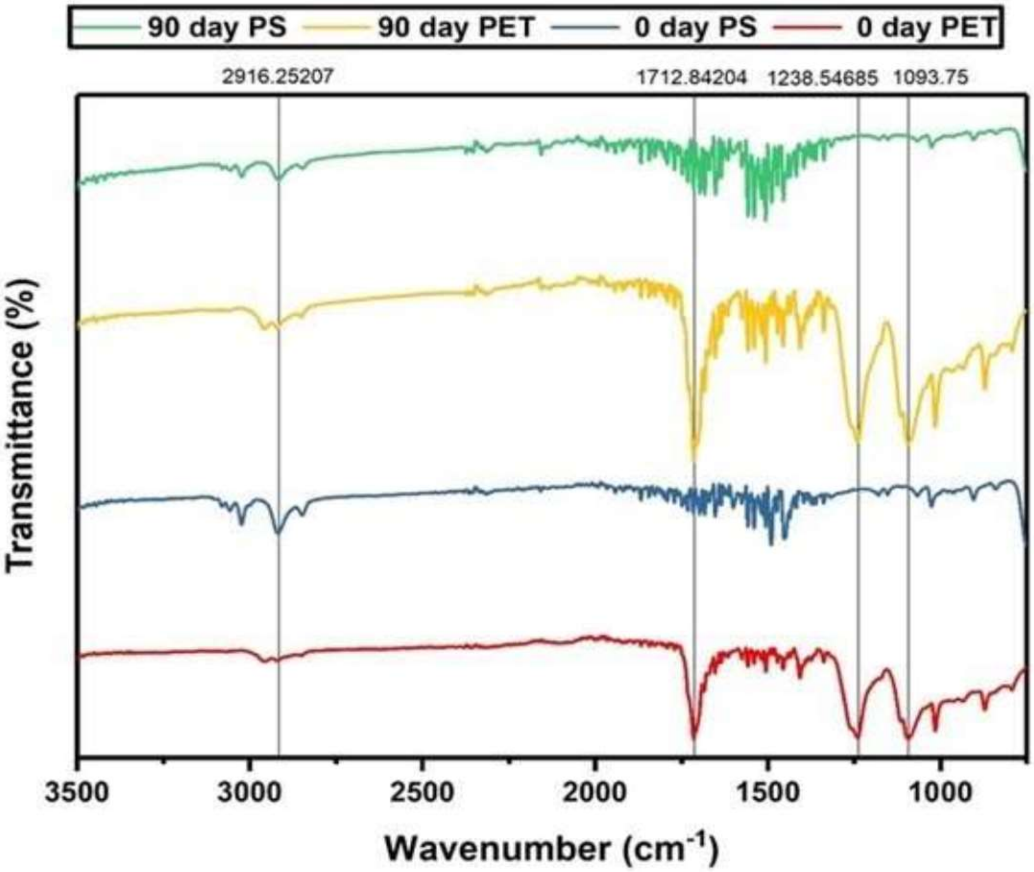


Figure 6.4. FTIR analysis of PS and PET Extracts at day 0 and day 90

Initially, at Day 0, both PS and PET MPs exhibited smooth, intact surfaces, characteristic of their pristine, undegraded states. These surfaces lacked significant features, indicating that the MPs had yet to undergo microbial or chemical degradation. This condition was consistent with the microbial diversity observed at the start of the composting process, where a diverse microbial community was present but had not significantly interacted with the MPs [128]. By Day 90, the SEM images displayed considerable surface degradation in both PS and PET MPs [134]. The surfaces became roughened, with visible cracks, pits, and evidence of material erosion. This degradation suggested active microbial colonization and enzymatic breakdown of the polymers, aligning with the microbial shifts observed during composting. PET and PS treatments, which exhibited reduced microbial diversity and less stable communities, showed less extensive degradation [128]. This observation was reflected in the SEM images, where the

PET and PS MPs retained more of their original structure, indicating that MPs in these treatments inhibited the microbial processes typically responsible for degrading these materials. The FTIR spectra provided further chemical insight into the changes in the MPs during composting. At Day 0, the PS and PET FTIR spectra displayed characteristic peaks corresponding to their polymeric structures. For PET, firm peaks were observed around 1712 cm^{-1} (C=O stretching in ester groups), 1238 cm^{-1} (C-O stretching), and 1093 cm^{-1} (O-H bending), which were indicative of the ester linkages within the polymer as shown in *Figure 6.4*. PS exhibited characteristic peaks around 2916 cm^{-1} (C-H stretching), reflective of the aromatic ring structures in its backbone. By Day 90, significant changes were evident in the FTIR spectra, particularly in the intensity and broadening of these peaks. In PET, the reduction in peak intensity at 1712 cm^{-1} suggested partial hydrolysis of ester bonds, likely due to microbial enzymatic activity [143]. Chemical changes in the PS spectra, such as reduced C-H stretching vibrations, indicated a microbial breakdown of aromatic structures. These alterations aligned with microbial data showing increased taxa capable of degrading complex polymers in PET and PS treatments by Day 90. Reduced peak intensities and new peaks correlated with earlier observed declines in nitrate levels, HA content, and germination indices. Rarefaction curves confirmed that PET and PS treatments had reduced microbial diversity compared to CK, reflecting poorer compost quality.

6.7.4 Impact on microbial communities

6.7.4.1 Microbial composition analysis

Figure 6.5. showed shifts in microbial and archaeal communities across composting treatments—ADS (inoculum, day 0), CK (control, day 90), PET (day 90), and PS (day 90)—emphasizing the significant impact of MPs on the composting process. At the onset of composting (ADS), the microbial community was highly diverse, dominated by genera such as *Pseudomonas*, *Streptococcus*, *Acinetobacter*, and *Clostridium,* as shown in *Figure 6.5 (a)*.

These microbes were critical in the initial stages of composting, particularly in breaking down simple organic compounds and rapidly decomposing easily degradable materials. For example, *Pseudomonas* was known for degrading a wide range of organic pollutants and contributing to nitrogen cycling through denitrification. At the same time, *Clostridium* played a key role in fermenting organic matter, producing organic acids and gases like CO_2, which set the stage for subsequent microbial activity. This high microbial diversity in ADS correlated with favorable initial physicochemical properties, such as a relatively high pH and moderate electrical conductivity (EC), essential for effective composting initiation. By Day 90, the CK treatment showed a more specialized and stable microbial community, predominantly featuring genera like *Bacillus*, *Paenibacillus*, *Streptomyces*, and *Pseudomonas*. These microbes were essential for the advanced stages of composting, particularly in the degradation of complex organic materials such as lignin, cellulose, and hemicellulose, which were crucial for forming humic substances. *Bacillus* and *Paenibacillus*, for instance, were known for their cellulolytic and ligninolytic activities, significantly contributing to the breakdown of plant materials. *Streptomyces*, a genus of actinobacteria, was particularly important for producing extracellular enzymes that degraded complex polymers and played a crucial role in HA synthesis, which is vital for soil fertility [144]. This microbial composition in CK was associated with advanced compost maturity, as reflected in the physicochemical data: high nitrate levels (1.032 g/kg), elevated HA content (55.25 g/kg), and a high GI (78.68%). These indicators suggested a well-stabilized compost with a balanced nutrient profile suitable for agricultural use. These microbes' efficient degradation of complex organic matter also led to a stable pH and lower EC, indicating a mature compost free from phytotoxicity and excess salts. In stark contrast, *Figure 6.5. (b)* showed PET and PS treatments by Day 90 displayed a significantly altered and less diverse microbial community, heavily dominated by anaerobic and halophilic genera such as *Methanosarcina*, *Methanobacterium*, *Halomonas*, and *Halorubrum*.

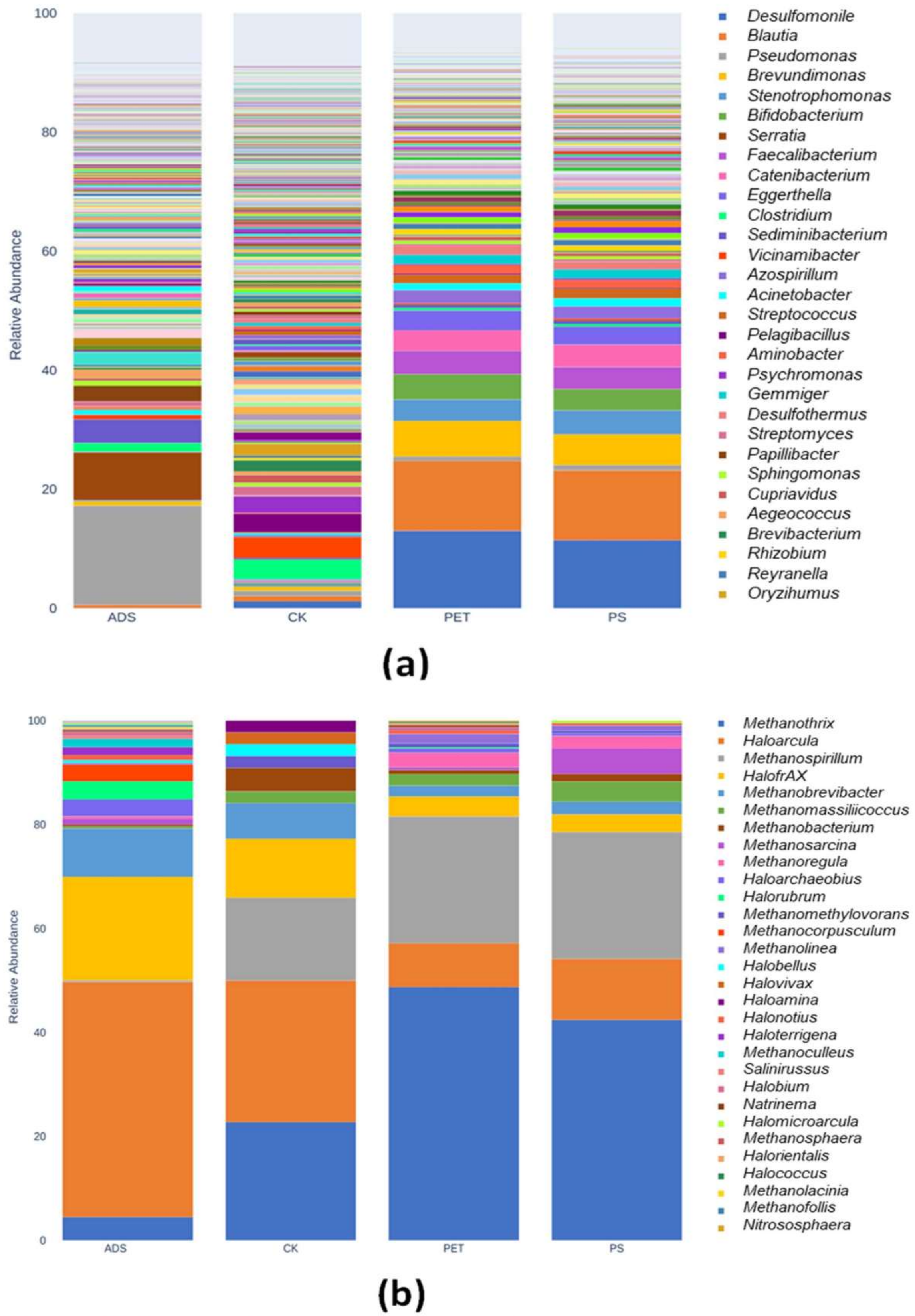


Figure 6.5 Relative abundance of different bacterial (a) and archaeal (b) genera at the genus level in the initial inoculum (Day 0, ADS) and at the final stage (Day 90, CK, PET, PS).

The dominance of methanogens like *Methanosarcina* and *Methanobacterium* suggested a shift towards anaerobic conditions, likely induced by the presence of MPs, which may have created microenvironments that limited oxygen diffusion. These conditions favored anaerobic pathways, leading to methane production rather than complete organic matter oxidation, which was less efficient in composting to produce humus-rich compost. *Halomonas* and *Halorubrum* indicated increased salinity, likely exacerbated by MPs, leading to higher EC levels. These halophilic microbes, typically found in high-salt environments, suggested that the composting process in PET and PS was less conducive to the growth of a broader range of beneficial composting microbes. This shift towards a less diverse microbial community dominated by stress-tolerant species correlated directly with the lower maturity of the compost in PET and PS treatments [144]. The physicochemical data supported this, showing reduced nitrate levels (0.82 g/kg in PET and 0.77 g/kg in PS), lower HA content (50.26 g/kg in PET and 51.02 g/kg in PS), and lower germination indices (37.25% in PET and 32.26% in PS). These indicators pointed to less mature and stable compost, which would be less effective in promoting plant growth and soil health. The archaeal communities in CK, PET, and PS treatments further reinforced these findings. In CK, the archaeal population was balanced, including *Methanothrix* (formerly *Methanosaeta*) and *Methanosarcina*, critical in methane production through acetoclastic and hydrogenotrophic methanogenesis, respectively. This balance between aerobic and anaerobic processes allowed for efficient organic matter degradation and compost stabilization [145]. In contrast, the PET and PS treatments exhibited a significant dominance of methanogens, suggesting a disruption in this balance. The overwhelming presence of methanogens in PET and PS likely led to the accumulation of intermediate organic compounds and a reduction in compost maturity, as indicated by the lower pH and higher EC observed in these treatments. The observed shifts in microbial and archaeal populations had direct implications for the overall physicochemical properties of the compost. CK's stable and diverse microbial community facilitated efficient nitrogen cycling, leading to higher nitrate concentrations critical for plant nutrition. The degradation of organic matter by *Bacillus*, *Paenibacillus*, and *Streptomyces* contributed to forming humic substances, enhancing soil fertility and structure. The lower EC in CK reflected the efficient breakdown of organic acids and salts, preventing the accumulation of phytotoxic substances and ensuring the compost's suitability for agricultural applications. In contrast, the PET and PS treatments, dominated by stress-tolerant anaerobes and halophiles, showed less efficient organic matter degradation, leading to lower nitrate production and humic substance formation [121]. The higher EC in these treatments suggested an accumulation of salts, possibly due to the reduced microbial diversity and altered metabolic pathways, hindering the composting process's effectiveness. MPs significantly altered the microbial and archaeal community dynamics during composting, leading to less efficient composting processes. The CK treatment, which maintained a more diverse and balanced microbial community, resulted in superior compost maturity and quality, as evidenced by higher nitrate levels, HA content, and GI.

6.7.4.2 Microbial composition and core microbiome

Figure 6.6. heatmaps provided an in-depth analysis of microbial community dynamics across different composting treatments—PET, PS, CK (control) (Day 90), and ADS (Day 0)—and their correlation with physicochemical properties. In the CK treatment, there was a high relative abundance of vital composting microbes such as *Paenibacillus*, *Brevibacillus*, *Methylobacterium*, and *Streptomyces*. These microbes played crucial roles in composting by participating in organic matter decomposition, nitrogen cycling, and humification. Their activities were essential for breaking down complex organic compounds into simpler forms and transforming labile organic matter into stable humic substances [133]. The dominance of these beneficial microbes in the CK treatment was directly associated with the production of

high-quality compost, as evidenced by the higher HA content (55.25 g/kg), elevated nitrate levels (1.03 g/kg), and superior GI (78.68%) observed by Day 90.

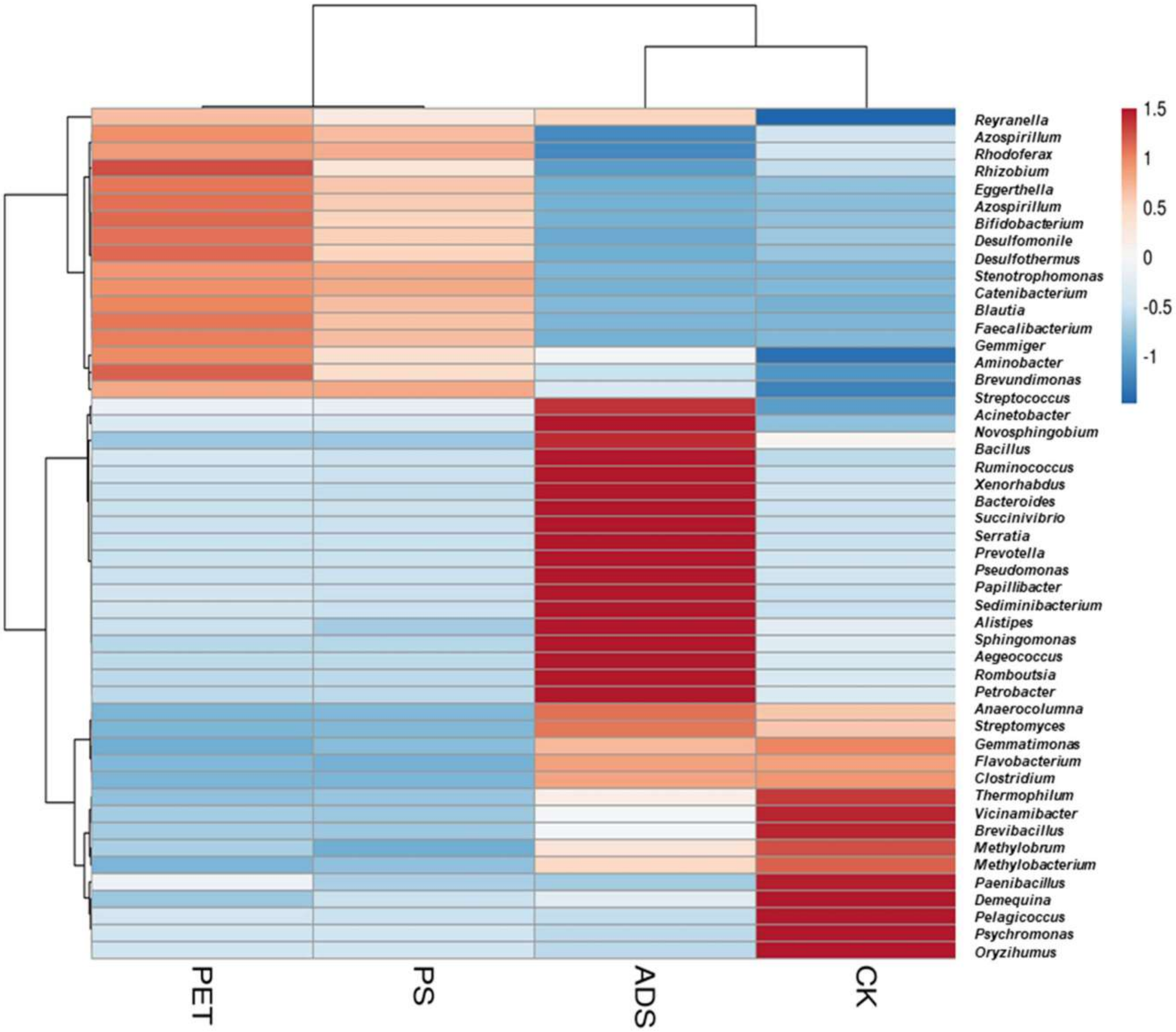


Figure 6.6. Heatmap shows the correlation of the top 50 genera within ADS, CK, PET

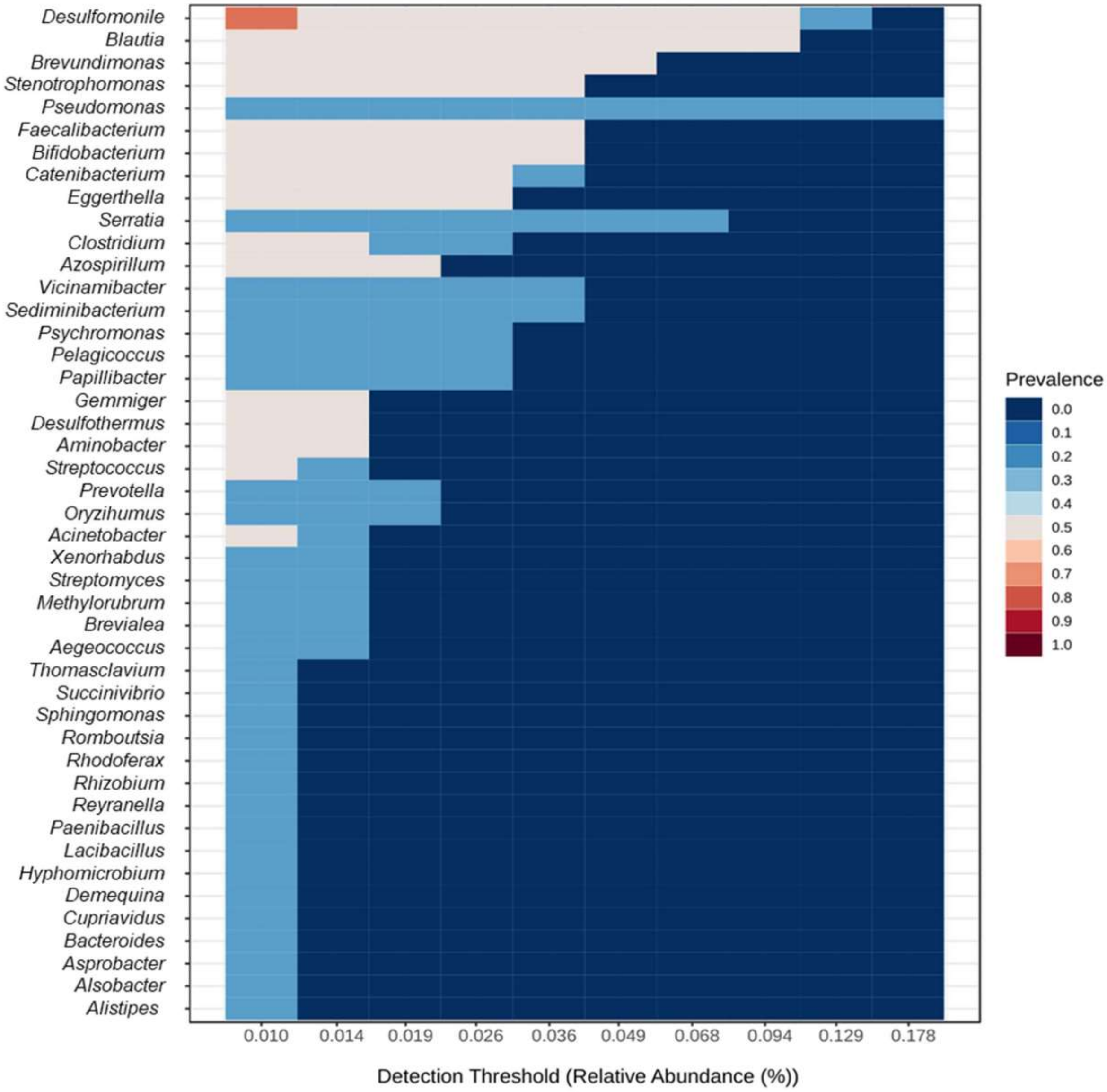


Figure 6.7. Heatmap represents the shared microbial genera across the core microbiomes.

The high alpha diversity indices (Chao1: 951.33, Shannon: 5.53) further supported the importance of maintaining a diverse and balanced microbial community, which ensured the efficiency and balance of all necessary biochemical processes, ultimately leading to stable compost maturation. In contrast, the PET and PS treatments, which contained MPs, showed significant alterations in microbial composition. There was a marked reduction in the relative abundance of beneficial composting microbes like *Paenibacillus* and *Streptomyces*. At the same time, opportunistic and stress-tolerant taxa such as *Reyranella*, *Rhodobacter*, and *Azospirillum* became more prevalent. These shifts in microbial community structure suggested that MPs created an unfavorable environment for critical composting microbes, potentially due to MPs' physical and chemical interference within the compost matrix. This disruption likely impaired critical microbial functions, such as cellulose degradation, nitrogen fixation, and humification, resulting in lower compost quality. This was reflected in the reduced HA levels (50.26 g/kg in PET and 51.02 g/kg in PS) and lower nitrate concentrations (0.82 g/kg in PET and 0.77 g/kg in PS) observed in these treatments. The lower alpha diversity indices (Chao1: 835.72 in PET, 840.79 in PS) and Shannon diversity (4.27 in PET, 4.40 in PS) indicated a decline in microbial richness and evenness, correlating with the less functional microbial communities observed. The *Figure 6.7.* heatmap illustrated the prevalence and relative abundance of various microbial OTUs (Operational Taxonomic Units) across different composting treatments, with darker shades indicating higher prevalence. Notably, microbes such as *Streptomyces* and *Azospirillum* exhibited high prevalence, particularly in the CK. These microbes were essential for the degradation of complex organic materials and the formation of humic substances, consistent with previous data highlighting their role in achieving mature, high-quality compost. In contrast, microbes like *Desulfomonile*, *Desulfothermus,* and *Romboutsia*, which are associated with anaerobic or stress conditions, were less prevalent overall but were more prominent in PET and PS treatments. This suggested that MPs created conditions favoring these stress-tolerant microbes. The heatmap also indicated that microbes such as *Reyranella* and *Lachnospiraceae* were more prevalent in PET and PS treatments, reflecting the selective pressures exerted by MPs. These observations corroborated earlier findings that MPs disrupted microbial stability, leading to a shift towards less diverse and less effective microbial communities [121]. This disruption was linked to reduced compost maturity and quality, as evidenced by lower nutrient levels and diminished HA content in PET and PS treatments. In contrast, with its higher prevalence of beneficial microbes like *Streptomyces*, the CK treatment supported more effective composting, underscoring the importance of maintaining a diverse and balanced microbial community for optimal composting outcomes [133]. The *Figure 6.8.* Venn diagram illustrated the distribution and overlap of microbial OTUs across ADS, CK, PET, and PS treatments, providing insight into microbial diversity and community structure evolved during composting, particularly in the presence of MPs. Initially, ADS had 457 unique OTUs, indicating a highly diverse microbial community at the start. By Day 90, CK retained 160 unique OTUs, reflecting substantial microbial diversity that supported effective composting. This was evidenced by higher nitrate levels (1.032 g/kg), HA content (55.25 g/kg), and a GI of 78.68%, all indicative of mature and stable compost. In contrast, the PET and PS treatments showed a marked reduction in microbial diversity, with only 53 and 55 unique OTUs, respectively, by Day 90.

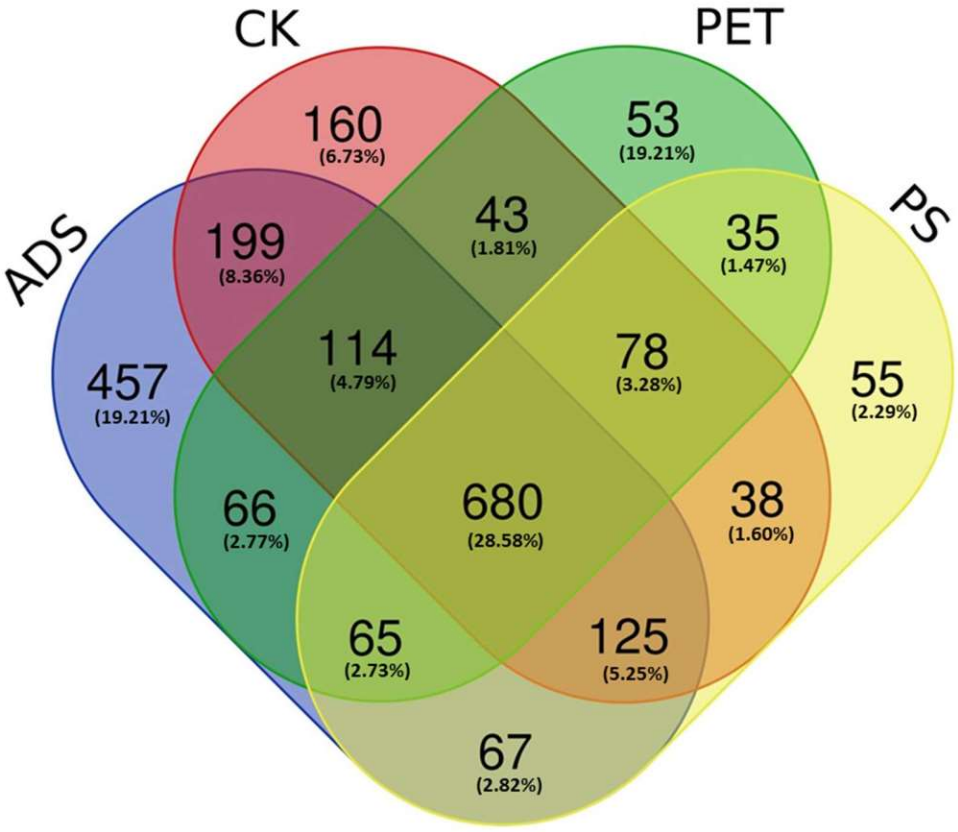


Figure 6.8. Venn diagram showing the distribution of shared bacterial OTUs across ADS (day 0), CK (day 90), PET (day 90), and PS (day 90)

This reduction was likely due to the selective pressures imposed by MPs, which favored stress-tolerant or opportunistic microbes while suppressing others. As a result, these treatments exhibited poorer compost quality, with lower nitrate levels (0.82 g/kg for PET and 0.77 g/kg for PS), reduced HA content (50.26 g/kg for PET and 51.02 g/kg for PS), and lower germination indices (37.25% for PET and 32.26% for PS).

Interestingly, PET and PS shared 125 OTUs, suggesting that MPs created similar selective environments, fostering the same microbial taxa in both treatments. However, this shared microbial community was less effective in promoting compost maturity and stability than CK. The central overlap of 680 OTUs across all treatments likely represented core microbial taxa essential for composting, demonstrating resilience across different environments, including those contaminated with MPs. Despite this resilience, the efficiency of these core microbes was reduced in MP-treated environments, leading to less mature compost in PET and PS treatments.

6.7.4.3 Alpha and beta diversity

The alpha diversity indices, such as Chao1, Shannon, and Simpson, revealed significant differences in microbial richness and evenness across the treatments shown in Table 6.1. The CK exhibited the highest microbial diversity, with a Chao1 index of 951.33, indicating a rich microbial community. The Shannon index for CK was 5.53, reflecting a balanced distribution of microbial species, while the Simpson index was near 0.99, suggesting that the community was not dominated by a few species but was well-distributed [134]. This high diversity in CK was critical for effective organic matter decomposition and compost stabilization, leading to a mature compost by Day 90, characterized by high nitrate levels (1.03 g/kg), elevated HA (55.25 g/kg), and a high GI (78.68%). In contrast, the PET and PS treatments, which included MPs, showed significantly lower microbial diversity.

Table 6.1. Analysis of alpha diversity metrics among the samples

Sample	Chao 1	Fisher	Shannon	Simpson
ADS	955.01	175.30	4.78	0.95
CK	951.33	170.38	5.53	0.99
PET	835.72	141.15	4.27	0.95
PS	840.79	144.44	4.40	0.95

The Chao1 index for PET and PS was 835.72 and 840.79, respectively, indicating reduced species richness compared to CK. The Shannon indices for PET (4.27) and PS (4.40) were also lower, reflecting both reduced richness and a less even distribution of species. This was further supported by the Simpson index (0.95 for both PET and PS), indicating a community structure more prone to domination by a few species, often a sign of environmental stress or disruption. This reduction in microbial diversity is directly correlated with poorer compost quality in these treatments. By Day 90, PET and PS treatments exhibited significantly lower nitrate levels (0.82 g/kg for PET and 0.77 g/kg for PS), lower HA content (50.26 g/kg for PET and 51.02 g/kg for PS), and lower germination indices (37.25% for PET and 32.26% for PS). These parameters indicated that the compost in PET and PS treatments was less mature, with slower organic matter stabilization and a higher likelihood of containing phytotoxic compounds [121].

Beta diversity analysis, visualized through PCoA, highlighted the separation between composting treatments over time. The PCoA plot showed that PC1 and PC2 explained 64.7% and 34.5% of the variance, respectively. The CK was positively positioned along PC1, indicating significant compost maturity by Day 90, with a well-adapted microbial community. In contrast, PET and PS treatments were negatively positioned along PC1. They showed greater variability along PC2, suggesting that MPs hindered microbial community development, resulting in less mature compost and ongoing disruptions in community structure [135]. The rarefaction curves and read count data provided additional evidence of these disruptions. CK and ADS had higher read counts and species richness, indicating robust and diverse microbial communities capable of sustaining efficient composting processes, as shown in *Figure 6.9.*

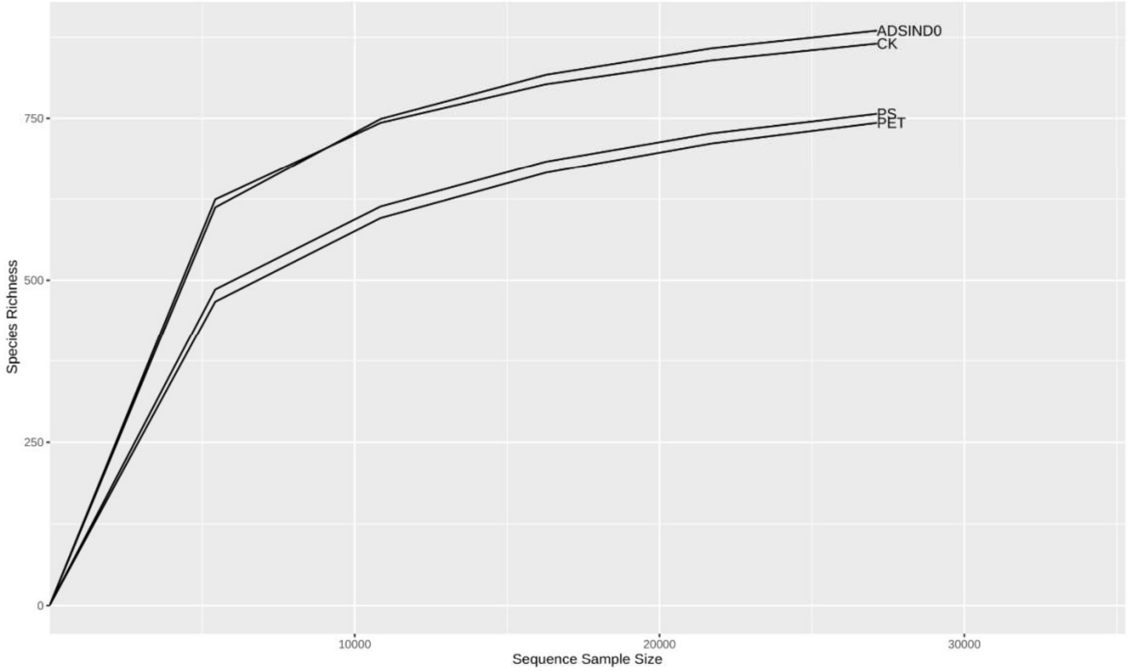


Figure 6.9. Rarefaction curves for samples ADS, CK, PET, and PS.

In contrast, PET and PS treatments exhibited lower read counts and species richness, consistent with the observed alpha diversity and compost quality reductions. The rarefaction curves for CK and ADS showed a steeper initial slope and higher asymptote, indicating that these treatments had more potential for microbial diversity. The flatter curves for PET and PS suggested that these treatments were limited in microbial diversity, likely due to the inhibitory effects of MPs. In conclusion, the data unequivocally demonstrated that MPs, mainly PET and PS, significantly disrupted the composting process by negatively impacting microbial diversity, community stability, and overall compost maturity.

6.7.5 Microbial flow cytometric fingerprint

Flow cytometric fingerprints revealed that MPs (PET and PS) disrupted microbial stability during composting. On Day 0, all treatments showed broad microbial diversity. By Day 3, CK exhibited tight microbial clustering, indicating effective adaptation, while PET and PS displayed broader distributions, signaling early disruptions. CK maintained stable, dense microbial fingerprints throughout, whereas PET and PS showed persistent instability and reduced composting efficiency. By Day 90, PET and PS still exhibited disrupted microbial fingerprints, indicating ongoing instability and compromised compost quality.

6.7.5.1 PCA of flow cytometry populations and physicochemical parameters

The Principal Component Analysis (PCA) *Figure 6.10.* provided critical insights into the temporal development of composting treatments—CK, PET, and PS—highlighting the influence of MPs on the composting process over different time points (Days 0, 6, 12, 36, 66, 81, and 90). The PCA results indicated that the first two principal components (PC1 and PC2) accounted for 54.4% of the total variance in the dataset, with PC1 explaining 36.76% and PC2 explaining 17.65%. These components effectively captured the differences in compost maturity and microbial community dynamics across the treatments, particularly when comparing the final stages of composting (Day 90) to the initial state (Day 0). The eigenvalues associated with PC1 and PC2 were 6.24 and 3.00, respectively, indicating that these components were the most significant in explaining the variability within the dataset. The high eigenvalue for PC1 emphasized its importance in reflecting the progression of compost maturity, while PC2 captured the secondary variations related to early-stage composting dynamics [135]. The eigenvectors further clarified the contribution of specific variables to these principal components. For instance, the positive coefficients for PC1 in variables such as nitrate (0.30), HA (0.35), GI (0.16), and Dp (0.36) suggested that higher values of these parameters were strongly associated with advanced compost maturity and stability.

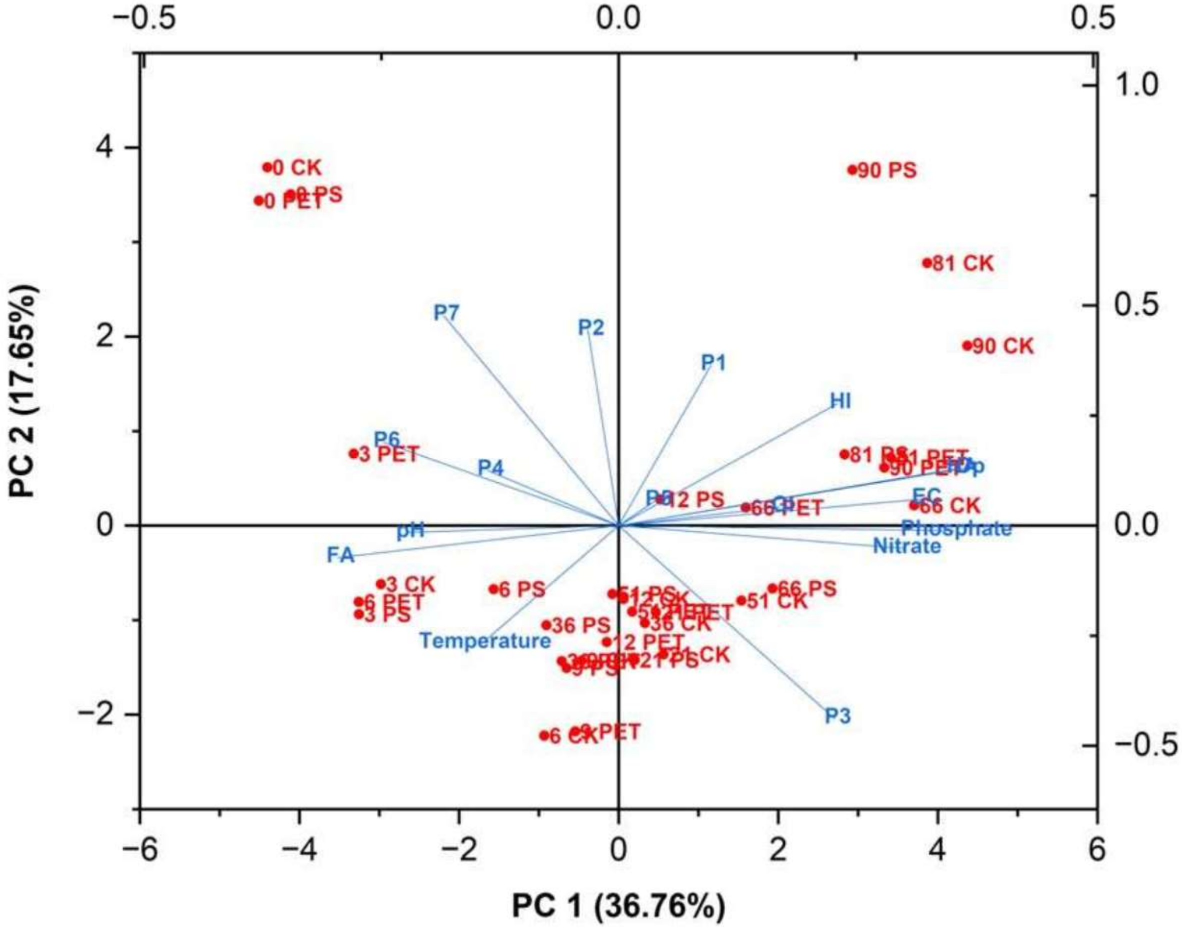


Figure 6.10. PCA analysis correlating microbial populations from flow cytometry with associated physicochemical parameters.

This trend was particularly evident in the CK treatment by Day 90, where the compost exhibited high nitrate levels (1.03 g/kg), elevated HA content (55.25 g/kg), and a high GI (78.68%). These results confirmed that the CK treatment successfully advanced toward a mature and stable composting state, as reflected in its positive positioning along PC1. PC2 captured variability related to early-stage composting dynamics, including factors such as temperature, pH, and specific microbial populations identified through flow cytometry (e.g., P1, P2). The eigenvector data for PC2 showed significant contributions from factors such as temperature (0.14) and certain microbial populations, reflecting the initial microbial activity and environmental conditions that influenced composting outcomes. On Day 0, all treatments

(ADS, CK, PET, PS) clustered near the origin of both PC1 and PC2, reflecting their initial similarity in physicochemical properties and microbial composition. The ADS treatment, representing the baseline (Day 0), was positioned near the origin, indicating lower levels of compost maturity and a more generalized microbial population before significant composting activity and microbial specialization occurred. As the composting process progressed, the CK treatment moved positively along PC1 by Day 90, indicating significant advancements in compost maturity, marked by increased nitrate, HA, and GI levels. The clustering of CK populations along PC1 also suggested the development of a stable and well-established microbial community essential for producing high-quality compost. This observation was consistent with previous data from flow cytometric analysis, where CK consistently maintained a more uniform and stable microbial population throughout the composting process. In contrast, the PET and PS treatments at Day 90 were positioned negatively along PC1, with greater variability along PC2. This positioning indicated that compost treated with MPs was associated with lower values of maturity indicators, such as reduced nitrate (PET: 0.82 g/kg, PS: 0.77 g/kg), HA (PET: 50.26 g/kg, PS: 51.02 g/kg), and GI (PET: 37.25%, PS: 32.26%). The eigenvectors for these variables showed lower contributions to PC1 in the PET and PS treatments, further underscoring the adverse impact of MPs on compost maturity. The broader spread of PET and PS along PC2 also indicated ongoing disruptions in microbial activity, likely due to the stress and selective pressures introduced by MPs, which were reflected in the more variable microbial community structures observed in flow cytometry.

6.8 Conclusion

Microplastics, specifically PET and PS, significantly impair the composting process. The presence of microplastics led to a notable reduction in microbial diversity, with Chao1 indices decreasing by 1.14-fold for PET and 1.13-fold for PS compared to the control. Additionally, the number of unique OTUs sharply declined from 457 on Day 0 to just 53 for PET and 55 for

PS treatments by Day 90. This reduction in microbial richness adversely affected compost quality, resulting in a 1.25-fold decrease in nitrate levels and a significant reduction in humic acid content in both PET and PS treatments compared to the control. Germination indices also dropped significantly to 37.25% for PET and 32.26% for PS, compared to 78.68% in the control, indicating increased phytotoxicity and diminished compost quality. Although some physical degradation of microplastics was observed, as evidenced by surface erosion seen through SEM, their chemical structures remained largely unchanged according to FTIR analysis, underscoring their persistent environmental impact. These findings highlight the urgent need for effective strategies to mitigate microplastics' negative effects and enhance the sustainability and quality of composting practices.