processes

MDPI

Article

Effects of Polyethylene Microplastics and Phenanthrene on Soil Properties, Enzyme Activities and Bacterial Communities

Shasha Liu [1], Kaibo Huang [2], Guodong Yuan [1,*] and Chengfang Yang [3,*]

[1] Guangdong Provincial Key Laboratory of Environmental Health and Land Resource, School of Environmental and Chemical Engineering, Zhaoqing University, Zhaoqing 526061, China
[2] School of Ecology and Environment, Hainan University, Haikou 570228, China
[3] College of Environmental Engineering, Xuzhou University of Technology, Xuzhou 221018, China
* Correspondence: yuanguodong@zqu.edu.cn (G.Y.); ycf@xzit.edu.cn (C.Y.)

Abstract: Microplastics (MPs) or polycyclic aromatic hydrocarbons (PAHs) pollution has received increasing concern due to their ubiquitous distribution and potential risks in soils. However, nothing is known about the influences of PAHs-MPs combined pollution on soil ecosystems. To address the knowledge gap, a 1-year soil microcosm experiment was conducted to systematically investigate the single and combined effect of polyethylene (PE) /phenanthrene (PHE) on soil chemical properties, enzymatic activities and bacterial communities (i.e., diversity, composition and function). Results showed that PE and PHE-PE significantly decreased soil pH. The available phosphorus (AP) and neutral phosphatase activity were not considerably changed by PHE, PE and PHE-PE. Significant enhancement of dehydrogenase activity in a PHE-PE amended system might be due to the degradation of PHE by indigenous bacteria (i.e., *Sphingomonas*, *Sphingobium*), and PE could enhance this stimulative effect. PHE and PHE-PE led to a slight increase in soil organic matter (SOM) and fluorescein diacetate hydrolase (FDAse) activity but a decrease in available nitrogen (AN) and urease activity. PE significantly enhanced the functions of nitrogen cycle and metabolism, reducing SOM/AN contents but increasing urease/FDAse activities. There were insignificant impacts on overall community diversity and composition in treated samples, although some bacterial genera were significantly stimulated or attenuated with treatments. In conclusion, the addition of PHE and PE influenced the soil chemical properties, enzymatic activities and bacterial community diversity/composition to some extent. The significantly positive effect of PE on the nitrogen cycle and on metabolic function might lead to the conspicuous alterations in SOM/AN contents and urease/FDAse activities. This study may provide new basic information for understanding the ecological risk of PAHs-MPs combined pollution in soils.

Keywords: polyethylene; phenanthrene; microbial community; soil properties; soil enzyme

Citation: Liu, S.; Huang, K.; Yuan, G.; Yang, C. Effects of Polyethylene Microplastics and Phenanthrene on Soil Properties, Enzyme Activities and Bacterial Communities. *Processes* **2022**, *10*, 2128. https://doi.org/10.3390/pr10102128

Academic Editor: Flavia De Nicola

Received: 19 September 2022
Accepted: 17 October 2022
Published: 19 October 2022

1. Introduction

Plastics and their products are widely applied in production and peoples' lives because of their durability and low cost. Improper disposal of plastic waste causes severe environmental contamination. The fragmentation of plastics might occur in the environment by a physical, chemical and biological process to smaller sizes (<5 mm), defined as microplastics (MPs) [1]. Moreover, microparticles and microbeads used in industrial products, such as raw materials, drug delivery particles in medicines and personal care products, are the primary sources of MPs [2,3]. The terrestrial environment is an important reservoir of MPs [4]. Lv et al. found that the MPs abundance was 16.1 ± 3.5 items kg^{-1} in rice soils in Shanghai [5]. In the farmed soil of southwestern China, 95% of the sampled plastic particles were in the microplastic size (0.05~1 mm), with an average abundance of 18,760 items kg^{-1} [6]. In addition, the contents of polycyclic aromatic hydrocarbons (PAHs) increase dramatically in the environment as the demand and consumption of fossil fuels increase with the development of industry [7]. According to the survey, agricultural soils have

been contaminated by high concentrations of PAHs [8,9]. Chen et al. investigated the pollution levels of 16 priority PAHs in 32 farmland soil samples, and results showed that the total concentrations ranged from 0.602 to 1.271 mg kg^{-1}, with an average of 0.877 mg kg^{-1} [10]. Thus, MPs and PAHs pollutions in agricultural soils are of great concern.

PAHs can persist in the environment and have teratogenic, carcinogenic and mutagenic effects [11]. MPs are stable in properties with a large specific surface area and strong hydrophobicity [12], which may interact with the PAHs and then change their migration and distribution processes, resulting in combined toxic effect on the organisms [13]. Soil microorganisms are sensitive to the environmental changes induced by contaminants, playing an important role in soil nutrient cycling, fertility maintenance and soil function maintenance [14]. Soil microbial community composition, microbial activity, enzymatic activities and degradation genes were changed after PAHs contamination [15,16]. Picariello et al. [17] found that acute PAH contamination significantly affected the enzyme activities and microbial community structure. The three soil enzyme activities (hydrolase, laccase and peroxidase) showed different dynamics along the time (360 days) after spiking with PAHs, and each of them showed significant differences in relation to the time [18]. The addition of phenanthrene (PHE) and pyrene increased peroxidase/laccase activities a long time in forest soil, and the PAH concentrations were positively correlated with selected microbial groups (Gram+, Gram-, actinomycetes) [19]. Microorganisms have adaptive genetic mechanisms and will gradually form the dominant microflora that can degrade PAHs [20]. On the other hand, MPs may influence soil properties, consequently leading to changes in community structure, diversity and enzymatic activity [21,22]. The positive effects of MPs addition on pH, dissolved organic carbon, ammonia nitrogen and carbon nitrogen ratio in soil were proposed by the research [23,24]. The addition of polypropylene (PP, <180 μm) increased soil fluorescein diacetate hydrolase (FDAse) activity in Chinese Loess soils [21]. Gao et al. found that low density polyethylene (LDPE) decreased the microbial community diversity [23], while Ren et al. reported that polyethylene (PE) had a positive effect on the microbial community in fertilized soil [25].

Previous studies have indicated that PAHs or MPs could induce different effects on soil ecosystems. However, the impacts of PAHs-MPs combined pollution on soil properties and microbial communities have not been reported. Thus, a microcosm soil incubation experiment was conducted to systematically investigate the changes of soil chemical properties, enzymatic activities, bacterial community diversity/composition and potential functions (i.e., phenotypes, elemental cycles, metabolic pathways) with the addition of PHE, PE and PHE-PE. Results from this study will provide a novel theoretical basis for the ecological risk assessment of PAHs-MPs combined pollution in agricultural soils.

2. Materials and Methods

2.1. Chemicals

PHE (purity ≥ 98%) from Aldrich Chemical Company (Shanghai, China) was selected as the representative PAH. PE MPs (≤13 μm) was purchased from Guangzhou Baohui Biological Technology Co., LTD (Guangzhou, China). Other reagents were of analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Deionized water was used throughout the study.

2.2. Soil Incubation Experiment

Tested soil was collected from the surface (0–20 cm) of agricultural land in Zhaoqing (112°30′23″ E, 23°9′33.40″ N). The soil had been used for vegetable cultivation. Soil samples were randomly selected by "S" pattern form, and they were thoroughly mixed to form one composite sample. These soils were air-dried and sieved through a mesh (2 mm) to remove stones and gravel and then evenly mixed and stored in the dark at 4 °C before incubation. The microcosm experiments were performed in a climate-controlled chamber at Zhaoqing University, China. Soil, PHE-soil, PE-soil or PHE-PE-soil were mixed and then homogenized thoroughly to incubate in a glass container. The specific treatments are

as follows: (1) control soil (CK), no PE and PHE were added to the soils; (2) PHE, PHE was added into the soil with a content of 20 mg kg^{-1}; (3) PE, polyethylene was added into the soil at 5% dose (*w*/*w*); (4) PHE-PE, PHE (20 mg kg^{-1}) + PE (5%). Each treatment was performed in triplicate. The incubations were maintained at a consistent humidity (70%) and temperature (30 °C). After exposure for 1 year, soils were sampled for chemical properties, enzymatic activities and DNA extraction.

2.3. Soil Chemical Properties and Enzymatic Activities Analysis

Soil chemical properties were analyzed based on the method as described by Lu [26]. Briefly, soil pH was measured in water–soil (2.5:1) based on the potentiometric method. Available nitrogen (AN) was determined by the alkaline hydrolysis diffusion method. The available phosphorus (AP) was analyzed by sodium bicarbonate extraction molybdenum antimony resistance colorimetry. The content of soil organic matter (SOM) was determined by the hydrothermal potassium dichromate oxidation colorimetric method.

Soil enzymatic activities included urease, neutral phosphatase, fluorescein diacetate hydrolase (FDAse) and dehydrogenase, which were determined with the method described by Yi et al. [27] and Wallenstein et al. [28]. Urease activity was determined by the sodium phenol-sodium hypochlorite colorimetric method and expressed as the mass (mg) of NH_3-N in 1 g soil after 24 h. Benzene disodium phosphate colorimetric method was used for studying the neutral phosphatase activity and expressed as the mass (mg) of phenol released in 1 g soil after 24 h. The absorbance of the released fluorescein was used to determine FDAse activity and expressed as fluorescein (ug) measured in 1 g soil after 20 min. Dehydrogenase activity was determined by 2,3,5-triphenyltetrazolium chloride (TTC) reduction colorimetry and expressed as the production of 1,3,5-triphenylformazan (TPF) in 1 g of soil.

2.4. Soil DNA Extraction and 16S Amplicon Sequencing

DNA was extracted from 0.5 g of soil using the E.Z.N.A™ Mag-Bind Soil DNA Kit (Omega, Norcross, GA, USA) according to the manufacturer's instructions. The quality and concentration of the isolated DNA were evaluated. A Qubit3.0 DNA detection kit was used to accurately quantify genomic DNA, determining the amount of DNA to be added in the polymerase chain reaction (PCR) reaction. The primer pair 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R(5′-GACTACHVGGGTATCTAATCC-3′) were designed to amplify the V3-V4 hypervariable regions of bacterial 16S rDNA gene by polymerase chain reaction (PCR). The first round PCR reaction conditions were as follow: 94 °C for 3 min, 5 cycles at 94 °C for 30 s, 45 °C for 20 s and 65 °C for 30 s, followed by 20 cycles at 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s and a final extension at 72 °C for 5 min. A second round of PCR amplification was performed following 3 min at 95 °C, 5 cycles of 20 s at 94 °C, 20 s at 55 °C, 30 s at 72 °C and a final extension at 72 °C for 5 min.

The PCR products were purified and quantified and then sequenced using the Illumina Miseq platform by Sangong Bioengineering Co., LTD (Shanghai, China), and the raw reads were generated. After removing the primer connector sequence, the pairs of reads were merged into a sequence according to the overlap relationship between reads. Sequence quality control and filtering were carried out. The operational taxonomic units (OTUs) were selected at similar levels of 97% to perform statistical analysis by biological information.

2.5. Analysis of Microbial Community Diversity, Composition and Function

The diversity of the microbial communities in all treatments was presented as Shannon, Chao, Ace and Simpson. Chao and Ace are the indexes commonly used to estimate the total number of species. Shannon and Simpson are the indexes for estimating microbial diversity in samples. Statistical analysis was used to observe the community structure of the samples at the genus level. According to the full sequence of the 16S rDNA gene of the tested microbial genome, the gene function spectrum was deduced. The sequence data of 16S rDNA obtained by sequencing were compared with the Greengenes database to search for

reference sequences and classify them as reference OTU. The OTUs abundance matrix was normalized in accordance with the rDNA gene copy numbers. Function predictions were categorized into "Kyoto Encyclopedia of Genes and Genomes (KEGG)" and "Functional Annotation of Prokaryotic Taxa (FAPROTAX)" to determine the metabolic pathways and functions of soil microorganism, respectively, using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). The phenotypic traits of bacterial communities were predicted with BugBase.

3. Results

3.1. Soil Chemical Properties

Compared to the CK, soil pH was significantly decreased with the addition of PE and PHE-PE ($p < 0.05$). Moreover, PE addition significantly decreased the pH in the soil with PHE supplementation. The addition of PE significantly lowered the SOM content ($p < 0.05$), while PHE and PHE-PE exerted a slightly positive effect on SOM. Both PHE and PE decreased AN content singly or jointly, with a more pronounced effect by PE ($p < 0.05$), compared to CK. No significant changes in soil AP content were observed in all treatments (PE, PHE, PE-PHE) (Table 1).

Table 1. The soil chemical properties of all samples.

Soil Chemical Properties	CK	PHE	PE	PHE- PE
pH	7.427 ± 0.012	7.440 ± 0.017	7.270 ± 0.010 *	7.320 ± 0.036 *
SOM (g kg^{-1})	9.189 ± 0.439	9.358 ± 0.818	8.085 ± 0.147 *	9.869 ± 0.365
AN (mg kg^{-1})	37.157 ± 0.001	34.906 ± 3.906	30.402 ± 0.002 *	32.652 ± 3.894
AP (mg kg^{-1})	2.118 ± 0.066	2.018 ± 0.152	1.986 ± 0.095	2.024 ± 0.178

Significant differences ($p < 0.05$) between the treatments and CK are indicated with asterisk (*).

3.2. Soil Enzymatic Activities

Soil enzymes are mainly secreted by microorganisms and play crucial roles in catalyzing various biochemical reactions in the soil. Four soil enzymes, including urease, FDAse, dehydrogenase and neutral phosphatase, were measured in this study, as shown in Table 2. Compared to CK, a slight reduction or increase of the urease or FDAse activity was observed for PHE and PHE-PE treatments, PE amendment significantly increased the urease and FDAse activities in the soil. The presence of PHE/PE and PHE-PE slightly and considerably increased dehydrogenase activity, respectively. PHE, PE and PHE-PE had insignificant effect on the neutral phosphatase activity (Table 2).

Table 2. The soil enzymatic activities of all samples.

Soil Enzymatic Activities	CK	PHE	PE	PHE- PE
Urease (mg NH_3-N g^{-1} soil)	1.949 ± 0.179	1.712 ± 0.098	2.700 ± 0.302 *	1.918 ± 0.289
FDAse (μg FDA g^{-1} soil)	7.119 ± 1.351	7.224 ± 0.685	9.630 ± 0.293 *	8.555 ± 3.871
Dehydrogenase (μL H^+ 20 g^{-1} soil)	3.413 ± 0.451	3.613 ± 0.230	3.463 ± 0.087	5.618 ± 1.206 *
Phosphatase (mg phenol g^{-1} soil)	0.103 ± 0.006	0.089 ± 0.011	0.085 ± 0.016	0.089 ± 0.011

Significant differences ($p < 0.05$) between the treatments and CK are indicated with an asterisk (*).

3.3. Microbial Community Diversity and Composition

The effects of PHE, PE and PHE on bacteria diversity were assessed, as shown in Table 3. Compared with the CK, no significant difference in Shannon, Chao, Ace and Simpson indexes was observed in all treatments ($p > 0.05$).

Table 3. Effects of PHE and PE on the diversity of bacterial community in agricultural soils.

Treatments	Shannon	Chao	Ace	Simpson
CK	5.968 ± 0.076	2391.022 ± 116.673	2377.793 ± 88.873	0.008 ± 0.002
PHE	5.944 ± 0.071	2401.953 ± 120.764	2386.161 ± 91.593	0.010 ± 0.001
PE	6.017 ± 0.111	2378.052 ± 13.755	2363.966 ± 19.253	0.008 ± 0.001
PHE-PE	5.894 ± 0.206	2413.304 ± 44.935	2395.093 ± 35.803	0.011 ± 0.004

At the genus level, taxonomic profiling showed that the bacterial communities were dominated by *Sphingomonas*, *Gemmatimonas*, *Acidobacteria* (Gp4, Gp7 and Gp6), *Ohtaekwangia*, *Betaproteobacteria* and *Burkholderiales* in all treatments, as shown in Figure 1. The Venn diagram generated from genus is shown in Figure S1. CK shared a majority of the genus with PHE, PE and PHE-PE-treated soils. In terms of the bacterial community composition, no specific clustering pattern was observed between all samples based on non-metric multidimensional scaling (NMDS) (Figure 2). ANOSIM analysis confirmed that addition of PHE and PE had no significant effect on the overall bacterial community composition (CK vs. PHE, $p = 0.3$; CK vs. PE, $p = 0.2$; CK vs. PHE-PE, $p = 0.5$) (Table S3). Only minor changes of the *Sphingomonas*, *Gemmatimonas* and *Acidobacteria* (Gp3, Gp4, Gp6, Gp7 and Gp16) were observed (Figure 1). Statistical analysis of metagenomic profiles (STAMP) method was performed to determine whether the bacterial taxa significantly changed in specific treatment (Figure 3). In this study, we found some bacterial genera (i.e., *Azohydromonas*, *Sorangiineae*, *Sphingobium*, *Cupriavidus*, *Rhodospirillales*, *Devosia*, *Nannocystineae* and *Comamonadaceae*) were significantly influenced with the addition of PHE and PE solely or jointly. However, the changes in these genera were not sufficient to alter the bacterial community composition, as described below (Section 4.3.).

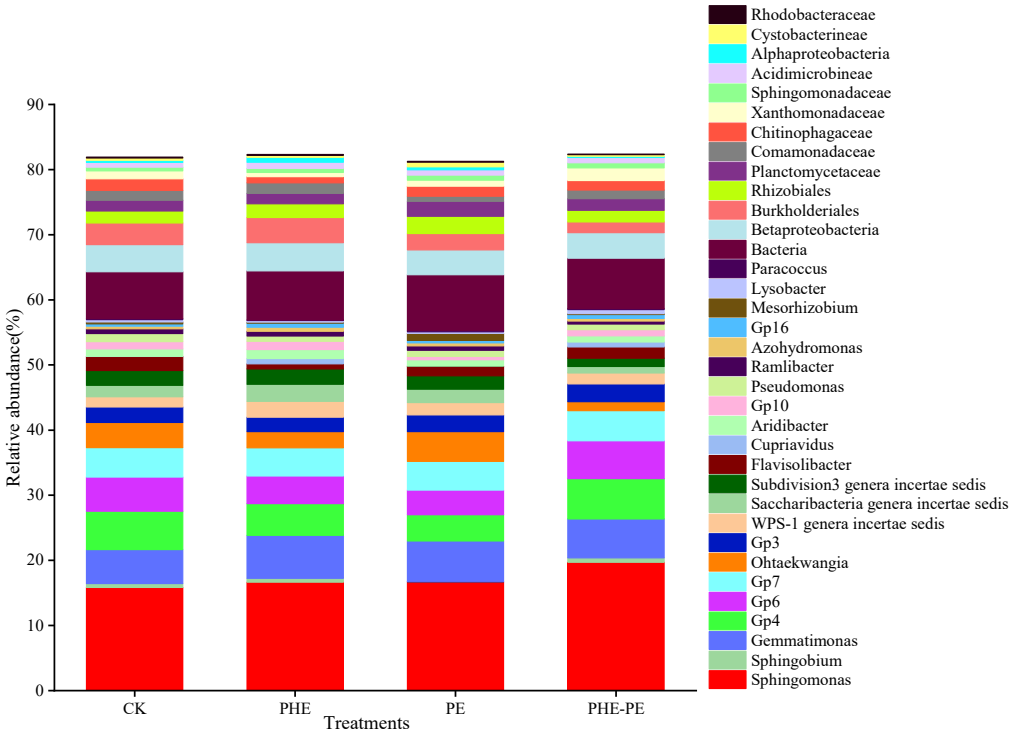


Figure 1. Relative abundance of the microbial communities in the different treatments at genus level.

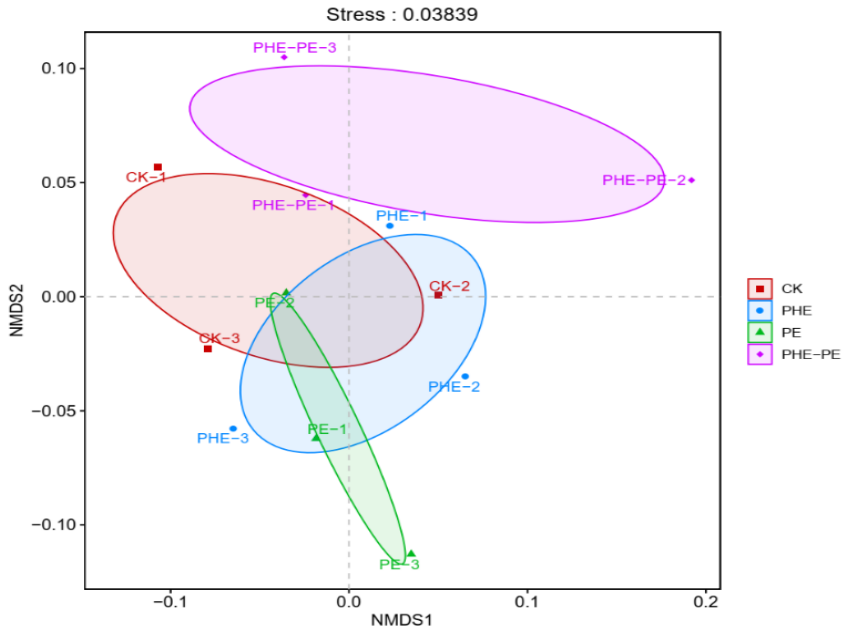
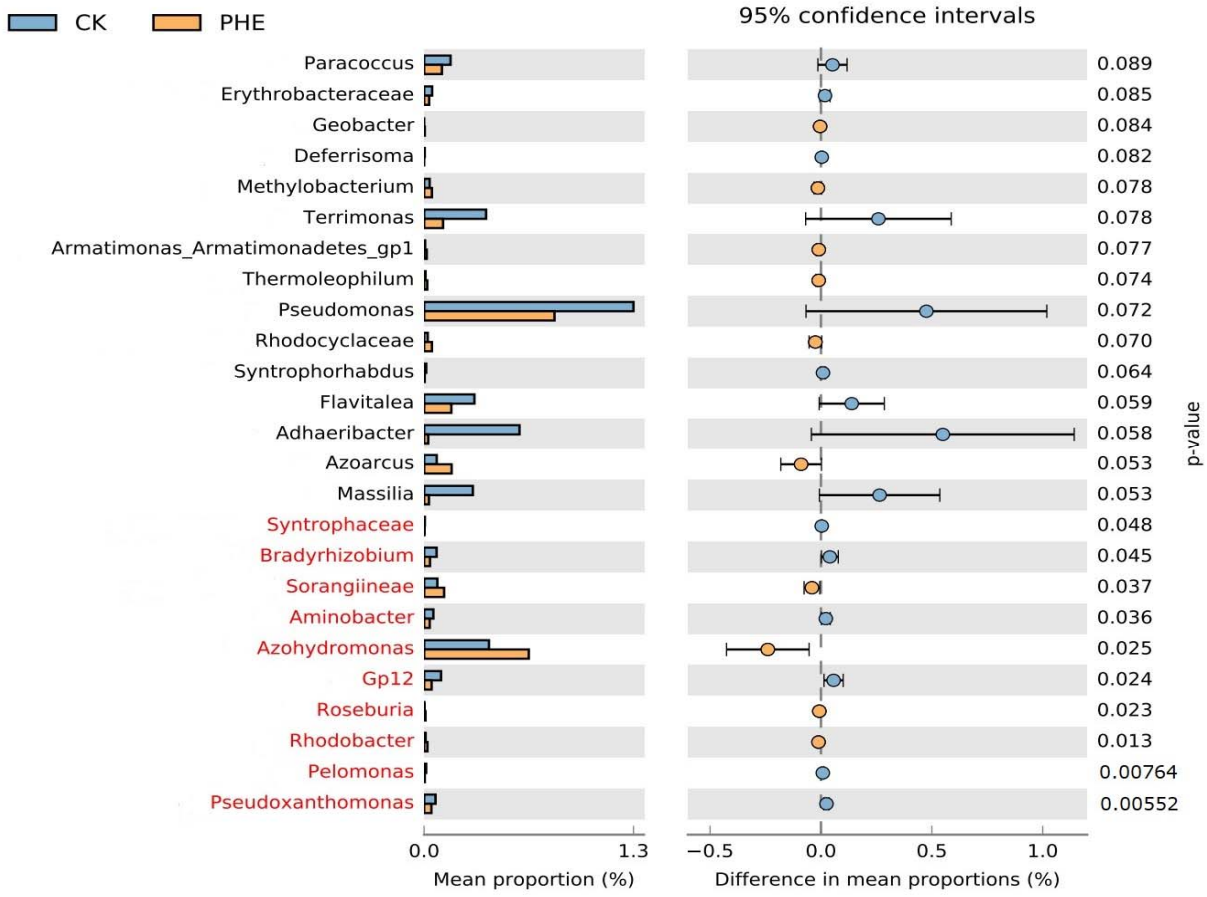
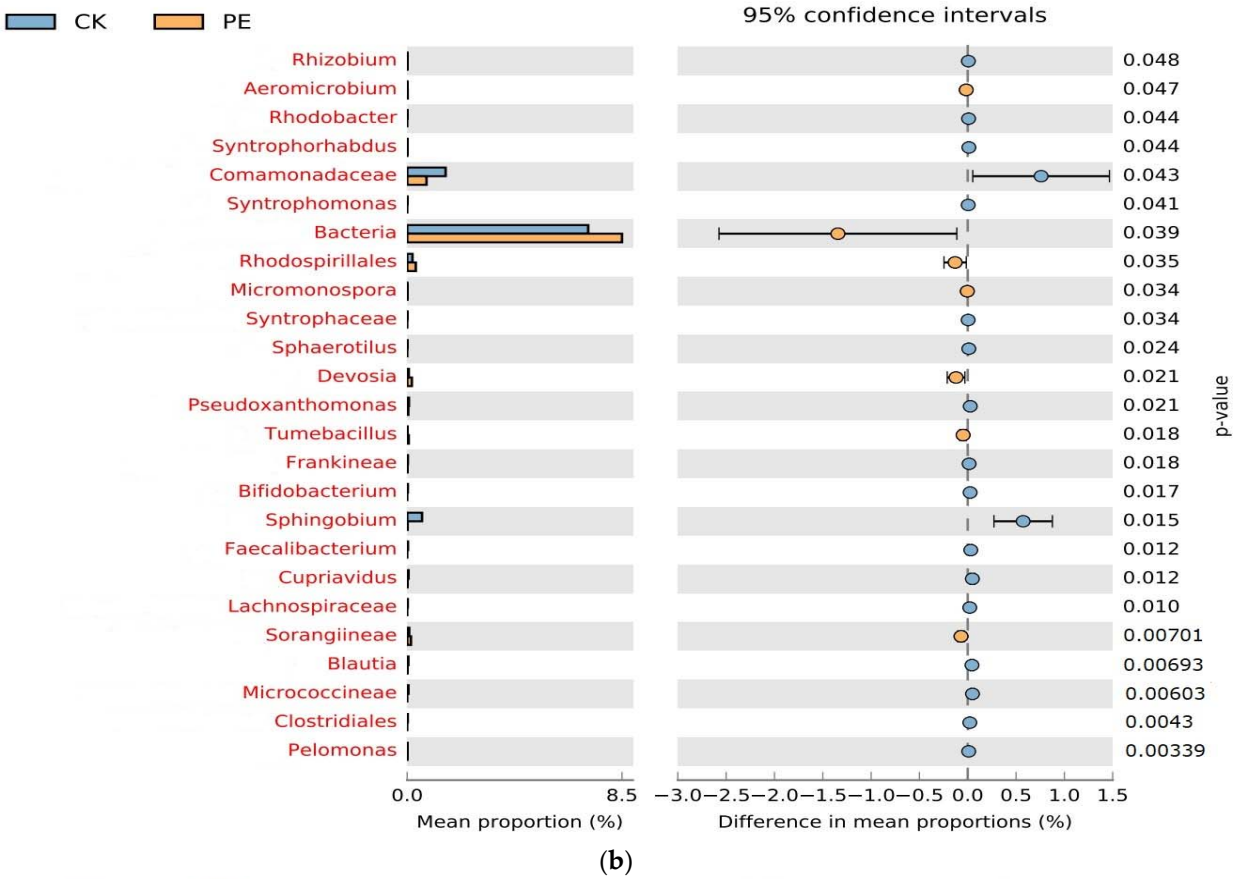


Figure 2. NMDS showing the distribution pattern of CK and different treated groups (PE, PHE and PHE-PE).

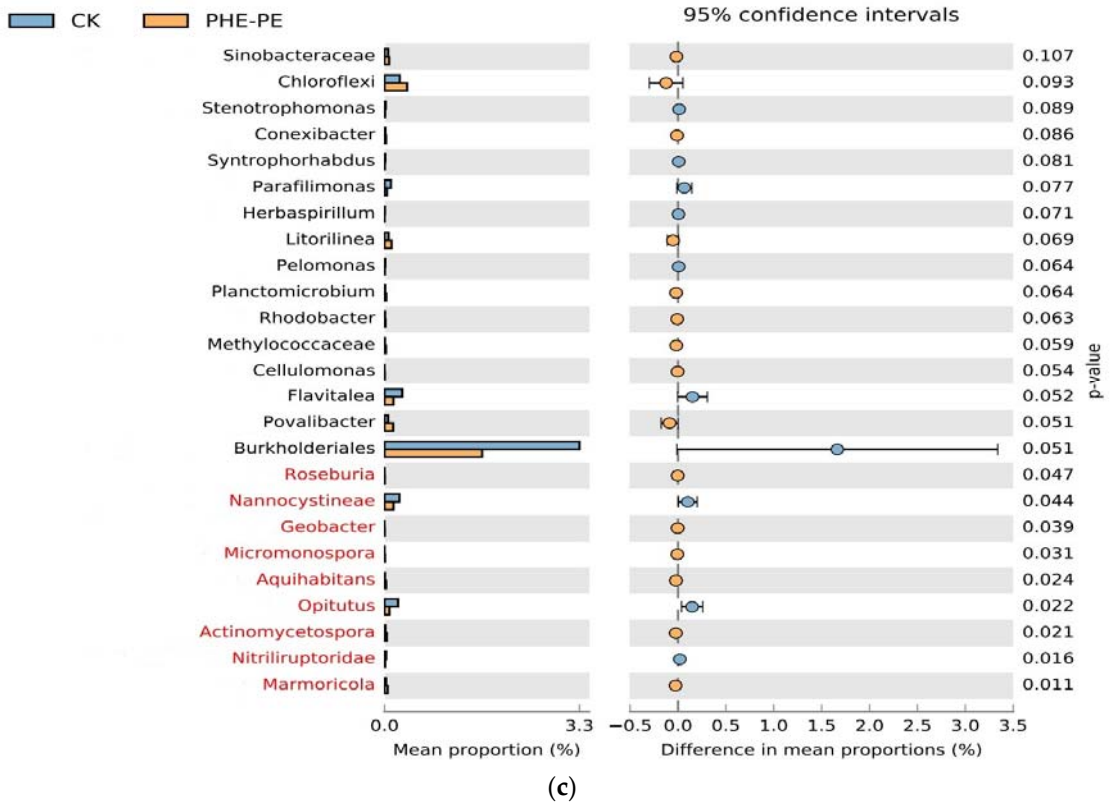


(**a**)

Figure 3. *Cont.*



(**b**)



(**c**)

Figure 3. STAMP plots of difference analysis between CK and soils treated with: (**a**) PHE; (**b**) PE; (**c**) PHE-PE. Only the 25 genera with the lowest p value are listed; $p < 0.05$ indicates significant difference, marked in red.

3.4. Functional Prediction of Soil Microbial Communities

3.4.1. BugBase

Based on 16S rDNA gene sequences, the phenotypic change of bacterial communities was investigated using BugBase (Figure 4). The bacterial phenotypes of all samples were mainly oxygen utilizing (i.e., aerobic, anaerobic) and Gram-negative. BugBase predictions indicated that PHE and PE exposure hardly altered the aforementioned phenotypes.

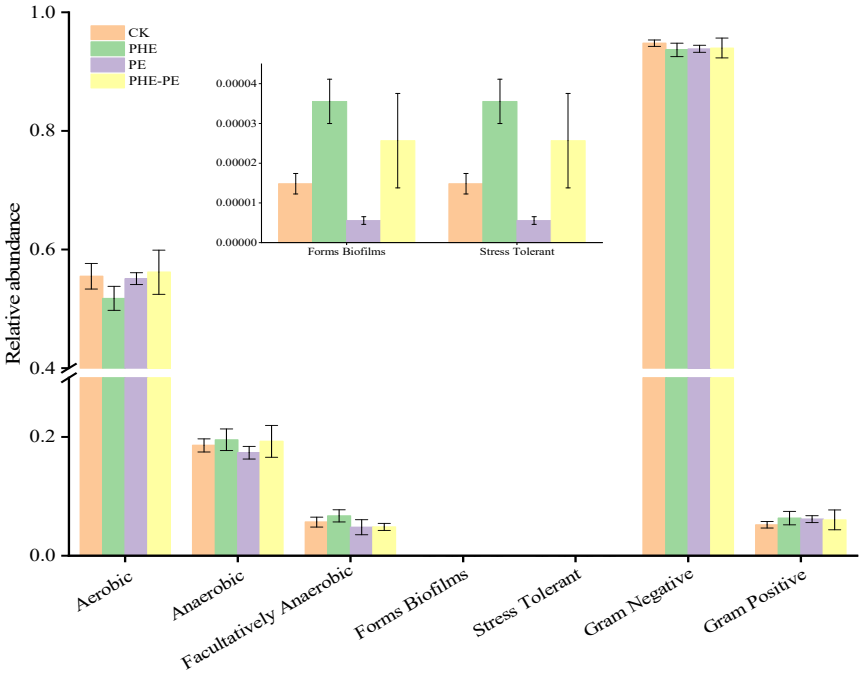


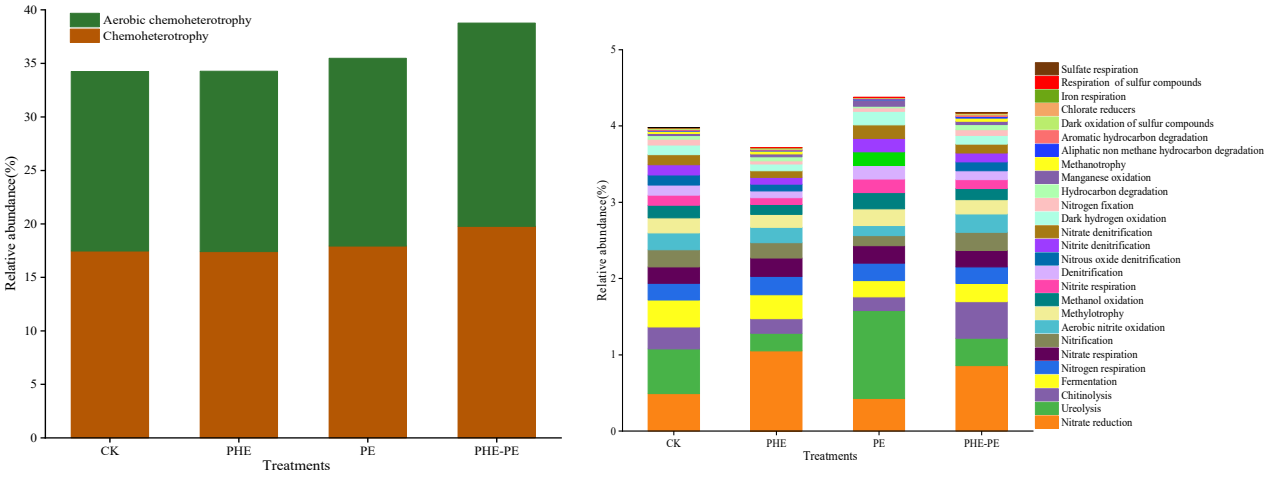
Figure 4. The prediction of bacterial community phenotypes based on BugBase.

3.4.2. FAPROTAX

FAPROTAX can annotate the functions of prokaryotes in elemental cycles (i.e., carbon, nitrogen, sulfur), nutritional types and degradation (Figure 5). Modifications in the relative abundance of the bacterial community associated with elemental cycles were observed for the treatment groups (PHE, PE, PHE-PE). Chemoheterotrophya and aerobic chemoheterotrophy were enhanced with the addition of PHE, PE and PHE-PE. The nitrate reduction, nitrification, nitrogen/nitrate/nitrite respiration, nitrite oxidation, denitrification and nitrogen fixation functions were mostly connected to the nitrogen cycle. PE showed a significantly influence on nitrogen cycle. Bacterial functions for sulfur cycles were dominantly composed of dark oxidation of sulfur compounds and sulfate/sulfur compounds respiration. Although the microbial functions according to FAPROTAX were stimulated by PHE-PE to some degree, the CK-, PHE- and PHE-PE-treated samples have a close relationship, as indicated by principal component analysis (PCA) (Figure S2). Whereas the treatment with PE clustered together at the right side of the PC1 axis and separated from the CK, PHE and PHE-PE.

3.4.3. KEGG

The distribution of the microbial functions assessed by KEGG pathway composition is shown in Table 4. The KEGG pathways at Level 2 annotations were mainly related to metabolism (e.g., amino acid, carbohydrate, lipid, nucleotide, xenobiotics), biosynthesis of secondary metabolites, membrane transport, replication and repair, translation, cell motility, nucleotide metabolism, genetic information processing, folding / sorting / degradation, transcription, signal transduction, enzyme families, cell growth and death, transport and catabolism, environmental adaptation and excretory system. The abundance of the aforementioned pathways in all treated soils (especially PE, $p < 0.05$) were higher than those in CK.



(**a**) (**b**)

Figure 5. FAPROTAX function prediction of bacterial community in all samples: (**a**) nutritional types; (**b**) elemental cycles and degradation.

Table 4. Metabolic pathway abundance based on KEGG database at level 2.

Pathways	CK	PHE	PE	PHE-PE
Amino acid metabolism	5,687,523 ± 1,106,040	6,008,889 ± 939,065	9,677,218 ± 1,179,844 *	6,773,054 ± 862,218
Carbohydrate metabolism	5,327,248 ± 1,022,491	5,593,268 ± 830,559	8987628 ± 1,112,622 *	6,320,786 ± 715,952
Membrane transport	4,823,873 ± 1,014,658	5,134,273 ± 814,056	8,333,681 ± 945,512 *	5,651,892 ± 713,316
Replication and repair	3,808,074 ± 734,029	3,998,707 ± 625,335	6,377,432 ± 797,193 *	4,478,773 ± 484,227
Energy metabolism	3,138,171 ± 627,139	3,325,013 ± 500,374	5,276,552 ± 642,955 *	3,686,084 ± 370,698
Lipid metabolism	2,099,475 ± 402,180	2,219,049 ± 354,419	3,577,720 ± 445,975 *	2,518,405 ± 332,718
Translation	2,377,536 ± 473,274	2,512,310 ± 383,629	3,992,523 ± 495,321 *	2,783,287 ± 282,147
Metabolism of cofactors and vitamins	2,267,936 ± 443,783	2,392,752 ± 370,863	3,831,557 ± 473,485 *	2,673,111 ± 314,202
Cellular processes and signaling	1,950,477 ± 363,800	2,031,076 ± 326,293	3,248,166 ± 388,559 *	2,296,525 ± 278,658
Xenobiotics biodegradation and metabolism	1,800,127 ± 337,033	1,938,425 ± 331,984	3,105,698 ± 359,437 *	2,240,873 ± 469,536
Cell motility	1,835,961 ± 312,990	1,957,392 ± 340,056	2,997,769 ± 368,876 *	2,227,334 ± 296,725
Nucleotide metabolism	1,727,368 ± 342,220	1,816,148 ± 279,130	2,905,554 ± 355,955 *	2,022,893 ± 209,472
Genetic information processing	1,282,896 ± 249,837	1,347,077 ± 206,965	2,175,475 ± 269,490 *	1,504,842 ± 161,464
Folding, sorting and degradation	1,270,334 ± 247,722	1,339,382 ± 206,626	2,123,214 ± 265,497 *	1,486,413 ± 155,618
Transcription	1,270,122 ± 236,126	1,327,185 ± 212,216	2,139,723 ± 277,642 *	1,504,305 ± 158,066
Glycan biosynthesis and metabolism	1,214,360 ± 240,003	1,247,544 ± 193,799	1,997,518 ± 283,128 *	1,393,183 ± 62,631
Signal transduction	1,208,537 ± 220,584	1,280,745 ± 221,183	1,998,523 ± 242,461 *	1,435,260 ± 178,339
Metabolism of terpenoids and polyketides	1,119,529 ± 217,912	1,192,808 ± 189,649	1,909,573 ± 235,880 *	1,340,870 ± 186,128
Enzyme families	1,082,715 ± 208,021	1,134,254 ± 192,093	1,813,880 ± 243,363 *	1,283,329 ± 109,820
Cell growth and death	311,845 ± 62,262	332,013 ± 51,409	541,832 ± 58,802 *	373,824 ± 53,605
Transport and catabolism	189,182 ± 34,903	195,612 ± 28,236	319,386 ± 42,214 *	224,248 ± 28,276
Signaling molecules and interaction	103,635 ± 18,364	103,044 ± 16,871	168,531 ± 25,056 *	1,22,624 ± 11,234
Environmental adaptation	85,022 ± 16,316	89,595 ± 13,882	140,888 ± 16,832 *	99,957 ± 9358
Immune system	21,286 ± 5060	22,507 ± 4475	36,422 ± 4323 *	23,761 ± 1891
Excretory system	20,762 ± 4345	21,552 ± 3309	35,427 ± 4640 *	23,473 ± 1343
Biosynthesis of secondary metabolites	589,242 ± 109,571	611,122 ± 94,164	991,848 ± 133,240 *	697,493 ± 54,578

Significant differences ($p < 0.05$) between the treatments and CK are indicated with asterisk (*).

4. Discussion

4.1. Effects of PHE and PE on Soil Chemical Properties

Soil pH is an important index of soil chemical variables, which plays a crucial role in the availability of nutrients [29]. We found that PE and PHE-PE significantly decreased soil pH, which is in accordance with previous findings that the agricultural soil exposed to HDPE is 0.62 units lower than the controls [30]. However, a different result has also been reported in which the MPs (i.e., HDPE, PLA) alone and jointly with multiwall carbon nanotubes (MWCNTs) could increase soil pH [31]. This may be attributed to the differences in MPs (type, dosage and size) and soil properties among these studies. Due to the strong adsorption capacity, MPs could change soil adsorption to regulate the acid–base balance. Various additives released from MPs during soil incubation might modify soil pH. Additionally, previous studies found that soil pH correlated negatively with the relative abundance of *Acidobacteria* [32]. Although PE and PHE-PE hardly affected the relative abundance of *Acidobacteria* (Figure S3, $p > 0.05$), the soil pH was markedly reduced. This suggested that soil microbes are not responsible for the changes in soil pH in our research. In sum, PE may singly or jointly with PHE decrease soil pH, while the underlying mechanisms need further investigation.

Due to its high organic carbon content, PHE can contribute to organic carbon accumulation in soil. Moreover, PHE can serve as carbon source for the growth of bacteria, such as *Sphingomonas* and *Sphingobium*, which suggested that PHE might be preferentially metabolized, thus reducing the soil original SOM decomposition in PHE and PHE-PE amended soil. PHE may consume soil oxygen (O_2) during its degradation process and further inhibit the mineralization of SOM. All of these probably resulted in a slight increase of SOM with the addition of PHE and PHE-PE. Previous studies have demonstrated that soil without additional carbon sources could decompose SOM for energy and carbon substrates [33]. Here, the energy required for microbial metabolism may be a key driver of native SOM decomposition in the PE-treated system, which showed significantly decreased SOM content compared with the PHE-PE ($p < 0.05$) (Table 1). SOM decomposition can be regulated by competition among bacterial populations [34]. The effects of MPs on SOM decomposition may be controlled by the balance between microbial anabolism and catabolism [32]. The "plastisphere" formed in the MPs surface could be a microbial activity hotspot that accelerated the decomposition of SOM [35]. These suggested that MPs induced the higher microbial activity and metabolic capability of probably availed SOM. As shown in Sections 3.4.3. and 4.4.3., the addition of PE significantly enhanced the metabolic processes of the bacterial community, which can explain the significant decrease in SOM content when exposed to PE. Consistent results were reported by Xiao et al., who found that low doses of PE (0.01%, w/w) strongly promote SOM decomposition in paddy soil [35]. The SOM decomposition was positively consistent with the carbon dioxide (CO_2) efflux rate in soils [33]. This indicated that the addition of PE would significantly induce the CO_2 efflux, while PHE and PHE-PE showed less influence.

Zhou et al. concluded that carbon source supply from biodegradable material could stimulate microbial biomass and intensify nitrogen immobilization [36]. This suggested that PHE as the carbon source of some bacteria in the soil could lead to the increase of biological nitrogen-fixation, resulting in the reduction of AN content in PHE and PHE-PE groups. Previous researcher pointed out that the decomposition of SOM could facilitate microbial growth [35]. We can speculate that increased nitrogen is required to support bacterial growth and thus accelerate AN demand from the soil, which can partly contribute to the significantly lowered AN with the addition of PE ($p < 0.05$). In another aspect, the accumulation of AN (including N-NH_4^+ and N-NO_3^-) are dependent on their balance between production and depletion, such as ammoniation, nitrification, denitrification and immobilization. The *Acidobacteria* and *Pseudomonas* have been reported to perform nitrate reduction, nitrification and denitrification [37,38]. Here, soil with PHE, PE and PHE-PE changed the abundance of this bacterial taxon, which might also lead to a decrease in AN. As shown in Sections 3.4.2. and 4.4.2., PE significantly influences the functions

related to nitrification, aerobic nitrite oxidation, denitrification, nitrite respiration and nitrite denitrification of the microbial communities, and this may be another reason why AN markedly decreased with the presence of PE.

A previous study demonstrated that the change of soil AP induced by MPs (i.e., PS, polytetrafluorethylene) was consistent with the change of phosphatase activity [39]. Similarly, we observed consistent (negligible) changes in AP content and neutral phosphatase activity in the present study (Table 2). The research of Satyaprakash et al. [40] and Qu et al. [41] found that the change of AP was related to the dissolution of inorganic phosphorus and organic phosphate mineralization mediated by microorganisms. Bergkemper et al. pointed out that *Acidobacteria* contributed significantly to phosphorus turnover and phosphorus availability in soils [42]. Here, PHE, PE and PHE-PE did not significantly change the relative abundance of *Acidobacteria* (Figure S3), which may partly explain the insignificant variation in AP content. However, Wang et al. discovered that 10% polylactic acid (PLA) significantly decreased the soil AP content, which might arise from its enhancement in soil pH [31]. Findings from Yan et al. confirmed that polyvinyl chloride (PVC) (0.1% and 1%) had no distinct effect on overall bacterial community diversity and composition while causing a significant change in soil AP content [43]. These controversial results may be attributed to the differences in MPs types and soil properties. The mechanisms of AP transformation in PE- and PHE-amended soils should be explored in the future.

4.2. Effects of PHE and PE on Enzymatic Activities

As the enzymes that catalyze the hydrolysis of nitrogen-containing organic matter (e.g., urea), urease plays an important role in regulating the nitrogen cycle. Previous studies confirmed that the urease activities were increased with the addition of PE [12,44], which was also observed in our current study. Compared to CK, a slight reduction of the urease activity was observed for PHE and PHE-PE treatments, whereas PE amendment significantly increased the urease activity in the soil, which was consistent with the results of FAPROTAX analysis (Section 3.4.2.) that the functions of ureolysis were stimulated or inhibited by PE or PHE/PHE-PE. Additionally, as a hydrolase, urease is important for transforming organic matter. Negative correlation between the urease activity and SOM content was found by Kompała-Bąb et al. [45], which was consistent with our findings in the present study. Wang et al. indicated that urease activity was correlated significantly with some bacterial genera (e.g., *Aeodermatophilus*, *Blastococcus*, *Bacillus*, *Marmoricola*, *Nitrospira*) [31], while these bacteria have not been found in our present study. Therefore, it is speculated that the microbial communities did not play an important role in regulating urease activity in this research.

FDAse activity can represent the overall metabolic activity of bacteria, and it is a good indicator of microbial activity [46]. FDAse activity was significantly promoted with the addition of HDPE and PP [21,31]. Here, we further observed higher FDAse activity in the presence of PHE, PE and PHE-PE (Table 2). PE addition dramatically increased FDAse activity relative to CK in the present study, indicating that PE could significantly enhance microbial metabolic activity, which was further verified in KEGG metabolic pathways determined by PICRUSt analysis (as shown in Sections 3.4.3. and 4.4.3.). This probably resulted in increased mineralization of SOM and assimilation of AN by bacteria with the PE treatment. The positive effect of PHE and PHE-PE on FDAse activity was weaker than that of PE addition. Previous studies showed that MPs might reduce soil bulk density and increase air circulation, resulting in the accumulation of aerobic microorganisms and then increased microbial activity [32,47]. Inversely, the decomposition of PHE by the soil bacteria (e.g., *Sphingomonas*, *Sphingobium*) would consume O_2, leading to the enrichment of anaerobic microorganisms with lower activity in PHE and PHE-PE systems than PE.

Dehydrogenase is directly involved in the degradation of PAHs (PHE, pyrene, naphthalene). Previous studies found that the removal percentage of PAHs is positively correlated with dehydrogenase activity in the soil [48,49]. The presence of PHE-PE considerably boosted dehydrogenase activity compared to the CK or PHE (Table 2), indicating that

increased natural biodegradation of PHE by indigenous bacteria in soil (i.e., *Sphingomonas*, *Sphingobium*) and PE could facilitate the degradation of PHE. A similar result was reported by Yi et al. [27] in which membranous PE improved the dehydrogenase activity in soil to the extent of 21%. On the other hand, PHE and PE had no discernible effect on the activity of neutral phosphatase singly or jointly (Table 2). Wang et al. pointed out that phosphatase activity was significantly associated with some bacterial genera, including *Phenylobacterium*, *Pseudonocardia*, *Ramlibacter*, *Marmoricola* and *Saccharimonadales* [31]. However, these aforementioned bacteria have not been found in our present study, leading to no discernible alterations in phosphatase activity. Previous research revealed that the inorganic phosphorus availability for microorganisms was related to the activity of phosphatase [50,51]. This indicated that the addition of PHE, PE and PHE-PE did not influence the demand for phosphorus by bacteria in the soil.

4.3. Effects of PHE and PE on Microbial Diversity and Community Composition

The high Chao and Ace values denote superior richness of the soil bacterial community. The greater Shannon or smaller Simpson values indicate a higher community diversity. The results of Section 3.3. indicated that PE, PHE and PHE-PE showed negligible effect on microbial community richness and diversity under our experimental conditions. Similarly, Sun et al. revealed that 1%PE (*w*/*w*) did not show a significant influence on soil bacterial diversity [52]. They conducted a thorough literature search and found that 11 of the 14 previous studies indicated that MPs amendment hardly influences the bacterial diversity in soil. The indices obtained for bacteria showed no significant differences between the CK and PHE (50 mg kg^{-1}) groups [53]. However, Fei et al. reported that the addition of PE (1% and 5%) declined the richness and diversity of the bacterial communities in an acid farmed soil [12]. A significant increase of microbial richness and diversity was observed with the addition of PHE [54]. Judy et al. reported that MPs-mixed waste organic output (MWOO) showed minimal effect on bacterial diversity [55], while polystyrene (PS) markedly alleviated sulfamethazine's adverse effects on bacterial diversity [56].

Sphingomonas was characterized by its ability to biodegrade organic compounds, such as PHE, petroleum, allethrin, phenols and dioxins [57,58]. *Acidobacteria* have appeared to tolerate or even degrade various pollutants (e.g., heavy metals, polychlorinated biphenyls, petroleum compounds, PAHs) [37]. Compared with PHE, PHE-PE increased the abundance of *Sphingomonas* and *Acidobacteria*, suggesting that the biodegradation of PHE might be enhanced with the addition of PE in our study. This deserves to be assayed in the future. In contrast, a significant reduction of *Sphingomonas* and *Acidobacteria* abundance with the addition of MPs (PE, PVC) was found by Fei et al. [12], which may be attributed to diverse soil properties and MPs types. Moreover, *Acidobacteria* plays significant ecological roles in soil, as evidenced by their active participation in carbon, nitrogen, phosphorus and sulfur cycling [59]. The abundance of *Gemmatimonas* was related to the organic carbon, nitrogen and phosphorus levels in soil [60]. Owing to insignificant changes in *Acidobacteria* and *Gemmatimonas*, they probably did not contribute to the variation in soil nutrients.

Compared to the CK, *Azohydromonas* and *Sorangiineae* showed a significantly positive response to PHE. The negative response group in PE comprised members affiliated to *Sphingobium*, *Cupriavidus* and *Comamonadaceae*, the positive response group was mostly from *Rhodospirillales*, *Devosia* and *Sorangiineae*. The relative abundances of *Nannocystineae* and *Opitutus* were significantly lower in the PHE-PE-treated sample than those in CK. *Azohydromonas*, *Rhodospirillales* and *Devosia* have the function of nitrogen fixation, which can transform atmospheric nitrogen into ammonium [61–63]. *Sorangiineae* are known to produce a number of protein clusters, including amino acid/ lipid/carbohydrate transport [64]. *Nannocystineae* is recognized for the production of long-chained polyunsaturated fatty acids and unusual steroids [65]. *Opitutus* might play a role in fermenting complex organics to simple ones [66]. The results potentially suggested that the addition of single or conjoint PE and PHE probably influenced elemental cycles and the metabolic process by affecting the abundance of some bacteria. However, these effects may not be pivotal in

regulating soil chemical properties and microbial community function, which is confirmed in Sections 3.1. and 3.4.

In summary, the PHE and PE addition influenced the microbial community to some extent. However, no crucial effects of single or co-contaminated PHE and PE were observed in altering bacterial community diversity and composition. This is not supported by the variational soil pH, AN, AP, SOM and enzymatic activities. Presumably, the variations of soil chemical properties incurred by PHE and PE were not enough to change soil bacterial diversity and composition. In addition, soils generally harbor a vast diversity of bacteria with an intrinsic capacity to cope with varied disturbances [20]. In addition to that, soil texture/structure/permeability are the important physical properties [67,68], and MPs could induce alterations of soil structure or permeability [69,70]. Such impacts may be associated with changes in bacterial communities [69]. Thus, the effects of PHE/PE on soil physical properties need to be determined to test these hypotheses in the future.

4.4. Effects of PHE and PE on Soil Microbial Community Function

4.4.1. Microbial Phenotype Prediction Based on BugBase

The bacterial cell membrane damage could induce the efflux of cytoplasmic substances and then facilitate biofilm formation, which was tolerant to the adverse environmental stresses. However, the relative abundance of biofilm-forming and stress tolerance were slightly changed in the presence of PHE, PE and PHE-PE, compared to the CK ($p > 0.05$) (Figure 4). In this regard, the relative abundance of Gram-negative phenotype (i.e., *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadete*, *Planctomycetes*, *Proteobacteria*) was significantly higher than Gram-positive (i.e., *Actinobacteria*, *Armatimonadetes*, *Chloroflexi*). Previous research pointed out that Gram-negative bacteria with an outer membrane were more resistant to membrane damage than the Gram-positive strains [71]. This demonstrated that PHE, PE and PHE-PE probably did not cause damage to the bacterial communities in our study.

4.4.2. Functional Prediction of Bacterial Community by FAPROTAX

Chemoheterotrophy, aerobic chemoheterotrophy and hydrocarbon degradation are important ecological function groups related to the carbon cycle [72]. Abundant chemoheterotrophy and aerobic chemoheterotrophy in soil suggest the bacteria mainly obtain carbon and energy by oxidizing organic compounds for their growth, thus increasing the emission of greenhouse gases [52]. The rise of these functions indicate that PHE and PE probably induced CO_2 emission singly and jointly in our study. Similarly, Gao et al. reported that PE addition (18%) significantly promoted soil CO_2 emissions [23]. It is noteworthy that PE exhibited a significant influence on the nitrogen cycle, which might lead to a significant decrease in AN content (Sections 3.1. and 4.1.) and an increase in carbohydrate/amino acid/lipid metabolism (Sections 3.4.3. and 4.4.3.). Sulfur cycles play an important role in maintaining the stability and health of the soil.

4.4.3. Predictive Metabolic Pathways Using KEGG

Amino acid metabolism and carbohydrate metabolism were the most abundant in all samples, followed by lipid metabolism, which was closely related to energy metabolism. Nucleotide metabolism is directly related to cell homeostasis, contributing to the production of energy suppliers (i.e., ATP and GTP) [73]. Biosynthesis of secondary metabolites could convert toxic substances that accumulate in the later growth phase, subsequently prolonging the survival of bacteria. In addition, secondary metabolites have been proven to act as antioxidants to protect organisms from oxidative stress [74]. PE showed a significantly impact on these metabolic pathways ($p < 0.05$), potentially suggesting that the bacterial community promotes their metabolic processes to generate energy for microbial life activities. Analogously, Sun et al. found that the carbohydrate/amino acid/lipid metabolism show significantly positive correlations with the microplastic concentrations (PP, PE, PS) in agricultural soil [52]. This may be due to the fact that MPs addition can

change the soil nutrient cycling [12]. As shown in Section 3.4.2., the observably enhanced nitrogen cycle after PE addition can confirm this point.

Membrane transport could mediate the uptake of basic substrates and assist in importing/exporting ions, small molecules and macromolecules, facilitating the growth and life activity of bacteria in soil [75,76]. Moreover, the excess metabolites can be excreted in a timely manner through membrane transport and thus prevent them from accumulating to toxic levels in bacterial cells [77]. PHE, PE and PHE-PE induced the functional response in membrane transport, and this may facilitate the bacterial communities to counteract the changing survival environment and then maintain homeostasis in soil. Replication and repair belonging to genetic information processing had higher abundances in treated samples compared to the CK. Replication is essential for the survival of species. However, the physical and chemical agents may attack DNA and lead to mutations. Bacteria possess the capability to repair damages and hold genetic stability. The increased replication and repair function may result in the stabilized bacterial community, which further verified that there was no significant effect on microbial diversity and composition in the presence of PHE, PE and PHE-PE.

In bacteria, the transcription and translation processes are concurrent for gene expression to accommodate the changing conditions [78,79]. Signal transduction is the biological response of microbial cells to the ever-changing environment, altering the bacterial transcriptome to mitigate the influence of contaminant stress [80]. Cell motility might provide a competitive advantage for the bacterial community to fit the changing environment and move towards nutrients or avoid toxic substances as well [81]. The abundance of these pathways was enhanced with the addition of PHE, PE and PHE-PE. This may be conducive to soil bacteria coping with PHE and PE pollution stress. A similar result that PE increased the cell motility of soil bacterial communities was observed by Fei et al. [12].

Previous studies indicated that the xenobiotics biodegradation and metabolism might include the decomposition of plastic polymers and pollutants (e.g., bisphenol, toluene, benzoate, heavy metals and PAHs) [52,53,82,83]. The increase in this metabolic pathway indicated that bacteria in treated soils could use PE or PHE as a carbon source to grow/reproduce and degrade them. Enzyme families are an extremely important class with catalytic activity [84]. Bacteria may contain enzyme families of functionally diverse members that are employed in various metabolic pathways [85], which would drive the cellular processes and result in cell growth and death. These exhibited increased abundances under PHE, PE and PHE-PE treatments. All of this may result in the higher resistance to PHE and PE, consistent with the enhanced function of environmental adaptation

5. Conclusions

The addition of PHE, PE and PHE-PE did not distinctly affect the soil AP content and neutral phosphatase activity. Soil pH was significantly decreased by PE and PHE-PE. PHE-PE considerably strengthened dehydrogenase activity in contrast to CK or PHE, which might arise from the degradation of PHE by indigenous bacteria. PHE and PHE-PE showed a slightly positive effect on SOM and FDAse activity but decreased AN and urease activity. PE exerted an observably negative effect on the functions of the nitrogen cycle as indicated by increasing the abundance of nitrate denitrification/ureolysis/nitrite respiration. PE also led to a significant increase of amino acid/carbohydrate/lipid/ nucleotide/xenobiotics metabolism. These may cause the decrease of SOM/AN contents and the enhancement of urease/ FDAse activities. Although PHE and PE solely or jointly exhibited insignificant influences on overall community diversity and composition, some bacterial genera were significantly reduced or enhanced in treated samples. However, the changes in soil chemical properties and enzymatic activities explained by bacterial community diversity and composition under all treatments were relatively small. Summarily, (1) there were some changes in soil chemical properties, enzymatic activities and bacterial communities (i.e., diversity, composition and function) with the addition of single or combined pollutants of PE and

PHE; (2) PE induced significant enhancements in nitrogen cycle and metabolic function, which contributed to obvious alterations of SOM/AN contents and urease/FDAse activities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10102128/s1, Figure S1: Venn diagrams showing the number of bacterial OTUs shared within and between groups of samples; Figure S2: PCA plots visualizing the distribution pattern among different treatments (CK, PHE, PE and PHE-PE); Figure S3: Relative abundance of the microbial communities in the different treatments at phylum level; Table S1: The chemical properties of the tested soil; Table S2: Enzymatic activities of the tested soil; Table S3: Similarity analysis of the Anosim group.

Author Contributions: Conceptualization, S.L.; methodology, S.L.; software, S.L.; validation, S.L.; formal analysis, C.Y. and K.H.; investigation, K.H.; resources, S.L. and G.Y.; data curation, S.L.; writing—original draft preparation, S.L.; writing—review and editing, S.L. and G.Y.; visualization, S.L.; supervision, C.Y.; project administration, S.L.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China (NSFC) (42007317), Guangdong Basic and Applied Basic Research Foundation (2019A1515110272), Zhaoqing Science and Technology Innovation Guidance Project (2022040302005), Zhaoqing University Excellent Young Teachers' Scientific Research Ability Improvement Program (YQ202105) and University-Industry Cooperation Research Project in Jiangsu (BY2021156).

Data Availability Statement: The data presented in this study are available on request from the Authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Isobe, A.; Uchiyama–Matsumoto, K.; Uchida, K.; Tokai, T. Microplastics in the Southern Ocean. *Mar. Pollut. Bull.* **2017**, *114*, 623–626. [CrossRef] [PubMed]
2. Napper, I.E.; Bakir, A.; Rowland, S.J.; Thompson, R.C. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar. Pollut. Bull.* **2015**, *99*, 178–185. [CrossRef] [PubMed]
3. Karbalaei, S.; Hanachi, P.; Walker, T.R.; Cole, M. Occurrence, sources, human health impacts and mitigation of microplastic pollution. *Environ. Sci. Pollut. Res.* **2018**, *25*, 36046–36063. [CrossRef] [PubMed]
4. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [CrossRef] [PubMed]
5. Lv, W.; Zhou, W.; Lu, S.; Huang, W.; Yuan, Q.; Tian, M.; Lv, W.; He, D. Microplastic pollution in rice–fish co–culture system: A report of three farmland stations in Shanghai, China. *Sci. Total Environ.* **2019**, *652*, 1209–1218. [CrossRef] [PubMed]
6. Zhang, G.; Liu, Y. The distribution of microplastics in soil aggregate fractions in southwestern China. *Sci. Total Environ.* **2018**, *642*, 12–20. [CrossRef] [PubMed]
7. Trilla-Prieto, N.; Vila-Costa, M.; Casas, G.; Jiménez, B.; Dachs, J. Dissolved black carbon and semivolatile aromatic hydrocarbons in the ocean: Two entangled biogeochemical cycles? *Environ. Sci. Technol. Lett.* **2021**, *8*, 918–923. [CrossRef]
8. Wang, J.; Liu, J.; Ling, W.; Huang, Q.; Gao, Y. Composite of PAH–degrading endophytic bacteria reduces contamination and health risks caused by PAHs in vegetables. *Sci. Total Environ.* **2017**, *598*, 471–478. [CrossRef]
9. Wang, J.; Zhang, X.; Ling, W.; Liu, R.; Liu, J.; Kang, F.; Gao, Y. Contamination and health risk assessment of PAHs in soils and crops in industrial areas of the Yangtze River Delta region, China. *Chemosphere* **2017**, *168*, 976–987. [CrossRef]
10. Chen, J.; Wu, Q.; Li, S.; Ge, J.; Liang, C.; Qin, H.; Xu, Q.; Fuhrmann, J.J. Diversity and function of soil bacterial communities in response to long–term intensive management in a subtropical bamboo forest. *Geoderma* **2019**, *354*, 113894. [CrossRef]
11. Zhang, P.; Chen, P.; Chen, Y. Polycyclic aromatic hydrocarbons contamination in surface soil of China: A review. *Sci. Total Environ.* **2017**, *605*, 1011–1020. [CrossRef] [PubMed]
12. Fei, Y.; Huang, S.; Zhang, H.; Tong, Y.; Barceló, D. Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. *Sci. Total Environ.* **2020**, *707*, 135634. [CrossRef] [PubMed]
13. Sørensen, L.; Rogers, E.; Altin, D.; Salaberria, I.; Booth, A.M. Sorption of PAHs to microplastic and their bioavailability and toxicity to marine copepods under co–exposure conditions. *Environ. Pollut.* **2020**, *258*, 113844. [CrossRef] [PubMed]
14. Delgado–Baquerizo, M.; Maestre, F.T.; Reich, P.B.; Jeffries, T.C.; Gaitan, J.J.; Encinar, D.; Berdugo, M.; Campbell, C.D.; Singh, B.K. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat. Commun.* **2016**, *7*, 10541. [CrossRef] [PubMed]
15. Yang, H.; Su, Y.; Zhu, Y.; Chen, M.; Chen, B.; Liu, Y. Influences of polycyclic aromatic hydrocarbons (PAHs) on soil microbial community composition with or without Vegetation. *J. Environ. Sci. Health Part A* **2007**, *42*, 65–72. [CrossRef]

16. Sutton, N.B.; Maphosa, F.; Morillo, J.A.; Al–Soud, W.A.; Langenhoff, A.A.M.; Grotenhuis, T.; Rijnaarts, H.H.M.; Smidt, H. Impact of long–term diesel contamination on soil microbial community structure. *Appl. Environ. Microb.* **2012**, *79*, 619–630. [CrossRef]
17. Picariello, E.; Baldantoni, D.; De Nicola, F. Acute effects of PAH contamination on microbial community of different forest soils. *Environ. Pollut.* **2020**, *262*, 114378. [CrossRef]
18. Picariello, E.; Baldantoni, D.; Muniategui-Lorenzo, S.; Concha-Grana, E.; De Nicola, F. A synthetic quality index to evaluate the functional stability of soil microbial communities after perturbations. *Ecol. Indic.* **2021**, *128*, 107844. [CrossRef]
19. Picariello, E.; Baldantoni, D.; De Nicola, F. Investigating natural attenuation of PAHs by soil microbial communities: Insights by a machine learning approach. *Restor. Ecol.* **2022**, *30*, e13655. [CrossRef]
20. Lee, D.W.; Lee, H.; Lee, A.H.; Kwon, B.; Khim, J.S.; Yim, U.H.; Kim, B.S.; Kima, J.J. Microbial community composition and PAHs removal potential of indigenous bacteria in oil contaminated sediment of Taean coast, Korea. *Environ. Pollut.* **2018**, *234*, 503–512. [CrossRef]
21. Liu, H.; Yang, X.; Liu, G.; Liang, C.; Xue, S.; Chen, H.; Ritsema, C.J.; Geissen, V. Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. *Chemosphere* **2017**, *185*, 907–917. [CrossRef] [PubMed]
22. Rillig, M.C. Microplastic disguising as soil carbon storage. *Environ. Sci. Technol.* **2018**, *52*, 6079–6080. [CrossRef] [PubMed]
23. Gao, B.; Yao, H.; Li, Y.; Zhu, Y. Microplastic addition alters the microbial community structure and stimulates soil carbon dioxide emissions in vegetable-growing soil. *Environ. Toxicol. Chem.* **2021**, *40*, 352–365. [CrossRef] [PubMed]
24. Qi, Y.; Ossowicki, A.; Yang, X.; Lwanga, E.H.; Dini–Andreote, F.; Geissen, V.; Garbeva, P. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. *J. Hazard. Mater.* **2020**, *387*, 121711. [CrossRef]
25. Ren, X.; Tang, J.; Liu, X.; Liu, Q. Effects of microplastics on greenhouse gas emissions and the microbial community in fertilized soil. *Environ. Pollut.* **2020**, *256*, 113347. [CrossRef]
26. Lu, R. *Soil and Agricultural Chemistry Analysis Methods*; China Agricultural Science and Technology Press: Beijing, China, 2000; pp. 146–187. (In Chinese)
27. Yi, M.; Zhou, S.; Zhang, L.; Ding, S. The effects of three different microplastics on enzyme activities and microbial communities in soil, Water. *Environ. Res.* **2020**, *93*, 24–32.
28. Wallenstein, M.D.; Burns, R.G. Ecology of extracellular enzyme activities and organic matter degradation in soil: A complex community-driven process. In *Methods of Soil Enzymology*; Dick, R.P., Ed.; Soil Science Society of America: Inc.: Madison, WI, USA, 2011; Volume 9, pp. 35–55.
29. Zeng, F.; Ali, S.; Zhang, H.; Ouyang, Y.; Qiu, B.; Wu, F.; Zhang, G.P. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environ. Pollut.* **2011**, *159*, 84–91. [CrossRef]
30. Boots, B.; Russell, C.W.; Green, D.S. Effects of microplastics in soil ecosystems: Above and below ground. *Environ. Sci. Technol.* **2019**, *53*, 11496–11506. [CrossRef]
31. Wang, F.; Wang, Q.; Adams, C.A.; Sun, Y.; Zhang, S. Effects of microplastics on soil properties: Current knowledge and future perspectives. *J. Hazard. Mater.* **2022**, *424*, 127531. [CrossRef]
32. Wang, Q.; Feng, X.; Liu, Y.; Cui, W.; Sun, Y.; Zhang, S.; Wang, F. Effects of microplastics and carbon nanotubes on soil geochemical properties and bacterial communities. *J. Hazard. Mater.* **2022**, *433*, 128826. [CrossRef]
33. Xiao, M.; Ding, J.; Luo, Y.; Zhang, H.; Yu, Y.; Yao, H.; Zhu, Z.; Chadwick, D.R.; Jones, D.; Chen, J. Microplastics shape microbial communities affecting soil organic matter decomposition in paddy soil. *J. Hazard. Mater.* **2022**, *431*, 128589. [CrossRef] [PubMed]
34. Coche, A.; Babey, T.; Rapaport, A.; Gonod, L.V.; Garnier, P.; Nunan, N.; de Dreuzy, J.R. Competition within low–density bacterial populations as an unexpected factor regulating carbon decomposition in bulk soil. *Soil Biol. Biochem.* **2022**, *164*, 108423. [CrossRef]
35. Xiao, M.; Shahbaz, M.; Liang, Y.; Yang, J.; Wang, S.; Chadwicka, D.R.; Jones, D.; Chen, J.; Ge, T. Effect of microplastics on organic matter decomposition in paddy soil amended with crop residues and labile C: A three–source–partitioning study. *J. Hazard. Mater.* **2021**, *416*, 126221. [CrossRef] [PubMed]
36. Zhou, J.; Gui, H.; Banfield, C.C.; Wen, Y.; Zang, H.; Dippold, M.A.; Charlton, A.; Jones, D.L. The microplastisphere: Biodegradable microplastics addition alters soil microbial community structure and function. *Soil Biol. Biochem.* **2021**, *156*, 108211. [CrossRef]
37. Kielak, A.M.; Barreto, C.C.; Kowalchuk, G.A.; van Veen, J.A.; Kuramae, E.E. The ecology of acidobacteria: Moving beyond genes and genomes. *Front. Microbiol.* **2016**, *7*, 744. [CrossRef]
38. He, T.; Li, Z.; Xie, D.; Sun, Q.; Xu, Y.; Ye, Q. Simultaneous nitrification and denitrification with different mixed nitrogen loads by a hypothermia aerobic bacterium. *Biodegradation* **2018**, *29*, 159–170. [CrossRef]
39. Dong, Y.; Gao, M.; Qiu, W.; Song, Z.G. Effect of microplastics and arsenic on nutrients and microorganisms in rice rhizosphere soil. *Ecotoxicol. Environ. Saf.* **2021**, *211*, 111899. [CrossRef]
40. Satyaprakash, M.; Nikitha, T.; Reddi, E.; Sadhana, B.; Vani, S.S. Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 2133–2144.
41. Qu, Q.; Zhang, Z.; Peijnenburg, W.; Liu, W.; Lu, T.; Hu, B.; Chen, J.; Chen, J.; Lin, Z.; Qian, H. Rhizosphere microbiome assembly and its impact on plant growth. *J. Agric. Food Chem.* **2020**, *68*, 5024–5038. [CrossRef]
42. Bergkemper, F.; Schöler, A.; Engel, M.; Lang, F.; Krüger, J.; Schloter, M. Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environ. Microbiol.* **2016**, *18*, 2767. [CrossRef]
43. Yan, Y.; Chen, Z.; Zhu, F.; Zhu, C.; Wang, C.; Gu, C. Effect of polyvinyl chloride microplastics on bacterial community and nutrient status in two agricultural soils. *Bull. Environ. Contam. Toxicol.* **2020**, *107*, 602–609. [CrossRef] [PubMed]

44. Huang, Y.; Zhao, Y.; Wang, J.; Zhang, M.; Jia, W.; Qin, X. LDPE microplastic films alter microbial community composition and enzymatic activities in soil. *Environ. Pollut.* **2019**, *254*, 112983. [CrossRef] [PubMed]
45. Kompaa–Bba, A.; Bierza, W.; Sierka, E.; Błońska, A.; Besenyei, L.; Woźniak, G. The role of plants and soil properties in the enzyme activities of substrates on hard coal mine spoil heaps. *Sci. Rep.* **2021**, *11*, 5155. [CrossRef] [PubMed]
46. Perucci, P. Enzyme–activity and microbial biomass in a field soil amended with municipal refuse. *Biol. Fertil. Soils* **1992**, *14*, 54–60. [CrossRef]
47. Zhang, F.; Li, M.; Qi, J.; Li, F.; Sun, G. Plastic film mulching increases soil respiration in ridge–furrow maize management. *Arid Land Res. Manag.* **2015**, *29*, 432–453. [CrossRef]
48. Rostami, S.; Azhdarpoor, A.; Baghapour, M.A.; Dehghani, M.; Samaei, M.R.; Jaskulak, M.; Jafarpour, S.; Samare–Najaf, M. The effects of exogenous application of melatonin on the degradation of polycyclic aromatic hydrocarbons in the rhizosphere of Festuca. *Environ. Pollut.* **2021**, *274*, 116559. [CrossRef]
49. Peng, D.; Ping, W.; Zhang, J.; Cui, M.; Liu, H. Damage of anodic biofilms by high salinity deteriorates PAHs degradation in single–chamber microbial electrolysis cell reactor. *Sci. Total. Environ.* **2021**, *777*, 145752.
50. Condron, L.M.; Turner, B.L.; Cade–Menun, B.J.; Sims, J.; Sharpley, A. Chemistry and dynamics of soil organic phosphorus. *Talnta* **2005**, *66*, 87–121.
51. Piotrowska–Dlugosz, A.; Charzynski, P. The impact of the soil sealing degree on microbial biomass, enzymatic activity, and physicochemical properties in the Ekranic Technosols of Toruń (Poland). *J. Soil. Sediment.* **2015**, *15*, 47–59. [CrossRef]
52. Sun, Y.; Duan, C.; Cao, N.; Li, X.; Li, X.; Chen, Y.; Huang, Y.; Wang, J. Effects of microplastics on soil microbiome: The impacts of polymer type, shape, and concentration. *Sci. Total Environ.* **2022**, *806*, 150516. [CrossRef]
53. Wang, Y.; Wang, M.; Chen, Y.; Li, C.; Zhou, Z. Microbial community structure and co–occurrence are essential for methanogenesis and its contribution to phenanthrene degradation in paddy soil. *J. Hazard. Mater.* **2021**, *417*, 126086. [CrossRef] [PubMed]
54. Li, J.; Luo, C.; Zhang, D.; Song, M.; Cai, X.; Jiang, L.; Zhang, G. Autochthonous bioaugmentation modified bacterial diversity of phenanthrene degraders in PAH–contaminated wastewater as revealed by DNA–stable isotope probing. *Environ. Sci. Technol.* **2018**, *52*, 2934–2944. [CrossRef]
55. Judy, J.D.; Williams, M.; Gregg, A.; Oliver, D.; Kumar, A.; Kookana, R.; Kirbya, J.K. Microplastics in municipal mixed–waste organic outputs induce minimal short to long–term toxicity in key terrestrial biota. *Environ. Pollut.* **2019**, *252*, 522–531. [CrossRef]
56. Xu, M.; Du, W.; Ai, F.; Xu, F.; Zhu, J.G.; Yin, Y.; Ji, R.; Guo, H. Polystyrene microplastics alleviate the effects of sulfamethazine on soil microbial communities at different CO_2 concentrations. *J. Hazard. Mater.* **2021**, *413*, 125286. [CrossRef] [PubMed]
57. Zhao, H.; Liang, S.; Yang, X. Isolation and characterization of catechol 2,3–dioxygenase genes from phenanthrene degraders *Sphingomonas* sp. ZP1 and *Pseudomonas* sp. ZP2. *Environ. Technol.* **2011**, *32*, 1895–1901. [CrossRef] [PubMed]
58. Bhatt, P.; Huang, Y.; Rene, E.R.; Kumar, A.J.; Chen, S. Mechanism of allethrin biodegradation by a newly isolated *Sphingomonas trueperi* strain CW3 from wastewater sludge. *Bioresour. Technol.* **2020**, *305*, 123074.
59. Kalam, S.; Basu, A.; Ahmad, I.; Kumar, A.J.; Chen, S. Recent understanding of soil acidobacteria and their ecological significance: A critical review. *Front. Microbiol.* **2020**, *11*, 1–15. [CrossRef]
60. Liu, M.; Lu, S.; Song, Y.; Lei, L.; Hu, J.; Lv, W.; Zhou, W.; Cao, C.; Shi, H.; Yang, X.; et al. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. *Environ. Pollut.* **2018**, *242*, 855–862. [CrossRef]
61. Dahal, R.H.; Chaudhary, D.K.; Kim, D.U.; Kim, J. *Azohydromonas caseinilytica* sp. nov. a nitrogen–fixing bacterium isolated from forest soil by using optimized culture method. *Front. Microbiol.* **2021**, *12*, 1–10. [CrossRef]
62. Flores–Núez, V.M.; Fonseca, C.; Desgarennes, D.; Eloe–Fadrosh, E.; Woyke, T.; Partida–Martínez, L.P. Functional signatures of the epiphytic prokaryotic microbiome of agaves and cacti. *Front. Microbiol.* **2020**, *10*, 3044. [CrossRef]
63. Negi, S.; Kumar, P.; Kumar, J.; Singh, A.; Dubey, R.C. Indigenous nitrogen fixing microbes engineer rhizosphere and enhance nutrient availability and plant growth. In *Rhizosphere Engineering*; Dubey, R.C., Pankaj, K., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; Volume 21, pp. 19–43.
64. Wang, W.; Wang, N.; Dang, K.; Dai, W.; Guan, L.; Wang, B.; Gao, J.; Cui, Z.; Dong, Y.; Wang, H. Long–term nitrogen application decreases the abundance and copy number of predatory myxobacteria and alters the myxobacterial community structure in the soil. *Sci. Total Environ.* **2019**, *708*, 135114. [CrossRef] [PubMed]
65. Garcia, R.; Pistorius, D.; Stadler, M.; Müller, R. Fatty acid–Related phylogeny of myxobacteria as an approach to discover polyunsaturated omega–3/6 fatty acids. *J. Bacteriol.* **2011**, *193*, 1930–1942. [CrossRef] [PubMed]
66. Feng, L.; Chen, K.; Han, D.; Zhao, J.; Lu, Y.; Yang, G.F.; Mu, J.; Zhao, X. Comparison of nitrogen removal and microbial properties in solid–phase denitrification systems for water purification with various pretreated lignocellulosic carriers. *Bioresour. Technol.* **2017**, *224*, 236–245. [CrossRef] [PubMed]
67. Sur, I.M.; Micle, V.; Polyak, E.T.; Gabor, T. Assessment of soil quality status and the ecological risk in the Baia Mare, Romania Area. *Sustainability* **2022**, *14*, 3739. [CrossRef]
68. Sur, I.M.; Micle, V.; Hegyi, A.; Lăzărescu, A.V. Extraction of metals from polluted soils by bioleaching in relation to environmental risk assessment. *Materials* **2022**, *15*, 3973. [CrossRef]
69. de Souza Machado, A.A.; Lau, C.W.; Till, J.; Kloas, W.; Lehmann, A.; Becker, R.; Rillig, M.C. Impacts of microplastics on the soil biophysical environment. *Environ. Sci. Technol.* **2018**, *52*, 9656–9665. [CrossRef]
70. Qi, Y.; Beriot, N.; Gort, G.; Huerta Lwanga, E.; Gooren, H.; Yang, X.; Geissen, V. Impact of plastic mulch film debris on soil physicochemical and hydrological properties. *Environ. Pollut.* **2020**, *266*, 115097. [CrossRef]

71. Akhavan, O.; Ghaderi, E. Toxicity of graphene and graphene oxide nanowalls against bacteria. *ACS Nano* **2010**, *4*, 5731–5736. [CrossRef]
72. Liang, S.; Deng, J.; Jiang, Y.; Wu, S. Functional distribution of bacterial community under different land use patterns based on FaProTax function prediction. *Pol. J. Environ. Stud.* **2020**, *29*, 1245–1261. [CrossRef]
73. Hubert, L.; Sutton, V.R. Disorders of purine and pyrimidine metabolism. In *Biomarkers in Inborn Errors of Metabolism*; Garg, U., Smith, L.D., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 283–299.
74. Rahmawati, S.I.; Izzati, F.N.; Hapsari, Y.; Septiana, E.; Rachman, F.; Bustanussalam, B.; Simanjuntak, P. Endophytic microbes and antioxidant activities of secondary metabolites from mangroves *Avicennia marina* and *Xylocarpus granatum*. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *278*, 012065. [CrossRef]
75. Rees, D.C.; Johnson, E.; Lewinson, O. ABC transporters: The power to change. *Nat. Rev. Mol. Cell. Biol.* **2009**, *10*, 218–227. [CrossRef] [PubMed]
76. Zhao, D. A nearest neighbor approach for automated transporter prediction and categorization from protein sequences. *Bioinformatics* **2008**, *24*, 1129–1136.
77. Hessel, S.; John, A.; Seidel, A.; Lampen, A. Multidrug resistance–associated proteins are involved in the transport of the glutathione conjugates of the ultimate carcinogen of benzo[a]pyrene in human Caco–2 cells. *Arch. Toxicol.* **2013**, *87*, 269–280. [CrossRef] [PubMed]
78. Gupta, S.; Pal, D. Clusters of hairpins induce intrinsic transcription termination in bacteria. *Sci. Rep.* **2021**, *11*, 16194. [CrossRef]
79. Xu, J.; Zhang, L.; Hou, J.; Wang, X.; Liu, H.; Zheng, D.; Liang, R. iTRAQ–based quantitative proteomic analysis of the global response to 17β–estradiol in estrogen–degradation strain *Pseudomonas putida* SJTE–1. *Sci. Rep.* **2017**, *7*, 41682. [CrossRef]
80. Piattelli, E.; Peltier, J.; Soutourina, O. Interplay between regulatory RNAs and signal transduction systems during bacterial infection. *Genes* **2020**, *11*, 1209. [CrossRef]
81. Kisand, V.; Valente, A.; Lahm, A.; Tanet, G.; Lettieri, T. Phylogenetic and functional metagenomic profiling for assessing microbial biodiversity in environmental monitoring. *PLoS ONE* **2012**, *7*, e43630. [CrossRef]
82. Sur, I.M.; Micle, V.; Damian, G.E. Assessment of heavy metal contamination and bioremediation potential of *Thiobacillus ferrooxidans* in soils around copper quarry. *J. Environ. Prot. Ecol.* **2020**, *21*, 56–62.
83. Micle, V.; Sur, I.M. Experimental investigation of a pilot-scale concerning ex-situ bioremediation of petroleum hydrocarbons contaminated soils. *Sustainability* **2021**, *13*, 8165. [CrossRef]
84. Evans, M.J.; Cravatt, B.F. Mechanism–based profiling of enzyme families. *Chem. Rev.* **2006**, *106*, 3279–3301. [CrossRef]
85. Kandlinger, F.; Plach, M.G.; Merkl, R. AGeNNT: Annotation of enzyme families by means of refined neighborhood networks. *BMC Bioinform.* **2017**, *18*, 274. [CrossRef] [PubMed]