

Article

Impacts of Various Microplastic Pollutants on the Functional Quality and Phytochemical Profile of Mulberry (*Morus alba* L.) Leaves

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Abstract

Mulberry (*Morus alba* L.) leaves are widely used as functional food and medicinal-food raw materials, yet the influence of soil microplastic contamination on their quality formation remains insufficiently understood. In this study, a 45-day pot experiment with 3–4 biological replicates was conducted to compare the effects of conventional polyethylene (PE) and biodegradable poly (butylene succinate) (PBS) microplastics at graded soil application rates on mulberry seedling growth, tissue ultrastructure, antioxidant-related quality attributes, and metabolic profiles. PE and PBS suppressed mulberry height, stem diameter and biomass in a dose-dependent way ($p < 0.05$). At 400 mg/kg, PE reduced plant height, stem diameter and fresh biomass by 34.7%, 30.8% and 31.4%, while PBS caused larger declines of 45.5%, 39.8% and 42.5%; total dry biomass dropped maximally by 34.2% under PE and 46.8% under PBS. Both treatments triggered 31.8% compensatory root elongation potentially for nutrient capture, yet PBS induced more severe root pore blockage and leaf cuticle damage than PE across tested concentrations. MP exposure raised leaf total flavonoids and phenolics markedly. At 400 mg/kg, PE and PBS boosted flavonoids by 82.8% and 90.9%, phenolics by 90.1% and 100.8%. Meanwhile, 400 mg/kg PE and PBS increased DPPH activity by 65.9% and 73.5%, ABTS activity by 66.3% and 74.4% relative to the control. Non-targeted metabolomics demonstrated pronounced shifts in the leaf metabolic profiles, characterized by the accumulation of coumaric acid, quercetin, and rutin, while glutathione levels declined. KEGG analysis identified phenylpropanoid and flavonoid biosynthesis as core responsive pathways; high PBS levels strongly disturbed lipid and stress metabolism. Overall, microplastic stress induced a trade-off between vegetative growth and antioxidant-oriented secondary metabolism in mulberry leaves. These findings suggest that biodegradable PBS residues can induce prominent physiological variations during the initial crop production stage, which highlights the need to closely track their temporal effects on functional raw materials.

Keywords: mulberry leaves; microplastics; functional quality; antioxidant activity; metabolomics

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1. Introduction

Agriculture faces a growing threat from microplastics (MPs) with particle sizes below 5 mm, which enter farmlands through irrigation, plastic mulching, and organic fertilization [1–6]. Because of their persistent nature and large specific surface area, MPs alter soil properties and potentially compromise plant primary metabolic performance [7, 8]. While research regarding MP phytotoxicity is expanding, the primary focus has centered on conventional staple crops, leaving a significant gap in our understanding of how these contaminants interact with high-value woody economic plants [9, 10].

Mulberry (*Morus alba* L.), a phylogenetically important perennial tree belonging to the Moraceae family, serves as the foundational pillar of the sericulture industry and possesses immense therapeutic and nutritional value. Recognized as a premier medicinal and food homology resource, its leaves are packed with a unique phytochemical profile rich in bioactive constituents, including specialized flavonoids, polyphenols, and alkaloids [11–13]. In this work, we define mulberry leaf functional quality by two indicators: the contents of key secondary metabolites (total flavonoids, total phenolics, rutin and quercetin), and antioxidant activity measured via DPPH and ABTS assays. These substances relieve inflammation and eliminate free radicals, so stable leaf chemical composition is essential for functional food production [14, 15]. Consequently, evaluating environmental stress in this species requires looking beyond vegetative biomass to assess specific variations within its active phytochemical matrix.

Accumulating evidence suggests that MPs can inhibit seed germination [16, 17], root development [18, 19], and photosynthetic efficiency [20, 21] through mechanisms such as physical clogging of root micropores [22], chemical toxicity from additive leaching [23], and the “Trojan horse” effect of carrying hazardous substances [24]. These environmental stressors frequently result in significant biomass reduction alongside a substantial redirection of plant metabolic pathways, prompting defensive accumulation of secondary metabolites to maintain cellular equilibrium under adverse conditions [25]. Notably, the impact of MPs extends beyond biomass changes to the intricate processes of quality formation and functional component accumulation [7, 26]. On one hand, MP stress can impair the synthesis of nutritional substances like soluble proteins and sugars by interfering with photosynthesis and primary metabolism. On the other hand, MPs may act as stress signals that activate secondary metabolic pathways, promoting the accumulation of antioxidant substances such as phenols and flavonoids [27]. Thus, the influence of MPs on plant quality is complex and dualistic. Although such effects have been reported in crops like rice [28], wheat [29, 30], maize [31, 32], and various fruits and vegetables [33–36], systematic research remains scarce for high-value woody plants—especially those like mulberry, where leaf-derived bioactive components are the primary basis for utilization.

PE is the most prevalent non-degradable agricultural plastic film in farmland, while PBS is the most commonly applied commercial biodegradable alternative. The two represent typical non-degradable and degradable plastic materials in modern agroecosystems, making them ideal candidates to compare the ecological risks of conventional and biodegradable microplastics. These two plastic types differ fundamentally in their polymer backbones and degradation pathways in agricultural soils. Conventional PE consists of inert, hydrophobic carbon–carbon (C–C) backbones that highly resist microbial cleavage, degrading extremely slowly primarily through abiotic ultraviolet (UV) photo-oxidation and physical weathering. In contrast, biodegradable PBS contains hydrolyzable ester linkages within its aliphatic polyester chains, which are susceptible to chemical abiotic hydrolysis and direct enzymatic cleavage by soil microorganisms. Although PBS can be mineralized by microorganisms under specific conditions, its degradation process still generates a substantial amount of secondary microplastics within a much shorter timeframe. [37]. Emerging evidence suggests that biodegradable MPs, due to their surface properties,

chemical reactivity, and the release of intermediate degradation products, may exhibit ecological effects distinct from traditional PE [38], sometimes resulting in higher acute toxicity [39]. Consequently, whether biodegradable plastics offer a true advantage in maintaining plant functional quality remains to be verified. Specifically, comparative studies regarding the differential impacts of PBS and PE on the quality formation of mulberry leaves are currently lacking.

In view of this, the present study utilized mulberry seedlings as experimental subjects to investigate the growth responses, tissue damage, and variations in leaf functional components under stress from PE and PBS microplastics at varying concentrations. Earlier research on medicinal and horticultural plants only tested single physiological or metabolic indicators, rarely linking metabolic shifts to functional quality data. This study combines untargeted metabolomics with targeted phytochemical and antioxidant assays. It connects pathway changes (phenylpropanoid, flavonoid synthesis) to quality-determining compounds like rutin and quercetin, and clarifies the growth-secondary metabolism trade-off under microplastic stress rarely discussed together in former work. By integrating growth metrics, transmission electron microscopy (TEM) observations, quality assessments, and non-targeted metabolomics, we aimed to clarify the effects of different MP materials on the physiological and metabolic processes of mulberry. This research provides a scientific basis for assessing the potential risks of microplastic pollution to the quality of mulberry leaves and evaluating the safety of biodegradable plastics in the production of edible plants.

2. Materials and Methods

2.1. Experimental Materials and Reagents

The mulberry cultivar “Yu 711” was provided by the Institute of Sericulture and Tea, Zhejiang Academy of Agricultural Sciences. Soil was collected from farmlands in Changxing, Huzhou, air-dried, cleaned and sieved through a 2 mm mesh. The soil was loam classified as a Fluvaquic Epiaquept under the USDA Soil Taxonomy (equivalent to a Hydric Anthrosol in the WRB classification system), with the following properties: pH 6.5, electrical conductivity (EC) $1.5 \text{ mS}\cdot\text{cm}^{-1}$, organic matter $2.64 \text{ g}\cdot\text{kg}^{-1}$, total nitrogen $1.56 \text{ g}\cdot\text{kg}^{-1}$, available phosphorus $0.89 \text{ g}\cdot\text{kg}^{-1}$, and available potassium $0.13 \text{ g}\cdot\text{kg}^{-1}$. Key traits including particle-size distribution, CEC and bulk density were not measured and were sourced from local geological literature [40], marking a clear limitation of this study. Two MP materials with a nominal particle size of $100 \mu\text{m}$ were used: biodegradable PBS and non-biodegradable PE, both purchased from Dongguan Changmutou Chengxing Plastic Products Co., Ltd. (Dongguan, China). As reported [11], microplastics fragmented from agricultural mulch films under field weathering are predominantly $10\text{--}100 \mu\text{m}$ and readily interact with plant–soil–root systems; we therefore chose particles with a nominal size of $100 \mu\text{m}$ for this study.

We characterized core physicochemical traits of PE and PBS before soil addition. SEM (GeminiSEM, Zeiss, Oberkochen, Germany) captured particle morphology, PBS averaged $130.5 \pm 38.2 \mu\text{m}$ and PE $132.9 \pm 36.5 \mu\text{m}$. XRD (Empyrean, Malvern Panalytical, Almelo, the Netherlands) tested polymer crystallinity, with relative crystallinities of 61.93% and 24.30% for PE and PBS, respectively. Detailed characterization results are provided in the Supplementary Material (Figure S1).

2.2. Experimental Design and Plant Cultivation

Uniform and healthy mulberry seedlings at the two-cotyledon stage were transplanted into ceramic pots (15 cm diameter, 12 cm height). A randomized complete block design was employed with two MP types (PE and PBS) and four concentration levels: 0

(CK), 100, 200, and 400 mg/kg, denoted as PE100, PE200, PE400, PBS100, PBS200, and PBS400, respectively. All pots were randomly arranged and rotated weekly inside the growth chamber to reduce boundary and microclimate biases. Three pots from each treatment were picked at random as biological replicates ($n = 3$) for growth trait measurements. We sampled four separate pots per treatment ($n = 4$) for leaf quality, antioxidant and metabolomic tests. Each treatment included six pots arranged in three biological replicates. Each pot contained 2.0 kg of dry soil that was uniformly and thoroughly mixed with the designated MP material throughout the entire soil profile before transplanting, ensuring homogenous exposure of the developing root system to the microplastics. Soil moisture was maintained at $75\% \pm 2\%$ of field water capacity using an LD-SS soil water potential monitor (Shandong Lainde Intelligent Technology Co., Ltd., Weifang, China). Plants were watered every two days, and seedlings were transplanted after a one-week soil stabilization period.

The cultivation was conducted from April to June 2025 in a controlled-environment chamber at the Agricultural Experiment Station of Zhejiang University. Environmental conditions were set to a light intensity of $700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a 14/10 h light/dark photoperiod, a 25/20 °C day/night temperature cycle, and a relative humidity of 65%.

2.3. Measurements and Analytical Methods

2.3.1. Growth Performance Indicators

After 45 days of cultivation, seedlings were harvested and roots were gently rinsed to remove adhering soil. We set a 45-day treatment duration to cover the key early vegetative phase of mulberry seedlings, when plants develop basic tissue structures and begin synthesizing large amounts of secondary metabolites [41]. Residual surface moisture was blotted with filter paper. Plant height, stem diameter, and root length were measured, and seedlings were separated into aboveground and root fractions. Fresh weight was recorded immediately, and samples were then freeze-dried to determine dry weight.

2.3.2. Determination of Total Phenols, Total Flavonoids, and Antioxidant Activity

Total phenolic (TP) content was determined using the Folin–Ciocalteu method [19]. Briefly, 0.20 g of mulberry leaf powder from each treatment was extracted and measured spectrophotometrically, with gallic acid used as the external standard.

Total flavonoid (TF) content was analyzed via the sodium nitrite–aluminum nitrate–sodium hydroxide colorimetric method [20]. Mulberry leaf samples (1.00 g) were extracted with 80 mL of boiling water in a 95 °C water bath for 6 min, and the filtrate was diluted to 100 mL. A 0.50 mL aliquot of the extract was mixed with 0.20 mL of 10% AlCl_3 solution, vortexed for 3 min, and adjusted to 5.00 mL with methanol. Absorbance was measured at 415 nm using rutin as the standard.

Antioxidant activity was evaluated using DPPH radical-scavenging activity, ABTS⁺ radical-scavenging capacity, and ferric reducing antioxidant power (FRAP). Freeze-dried leaf samples (0.10 g) were extracted with 2 mL of absolute ethanol in a 50 °C water bath for 2 h and centrifuged at 4000 r/min for 15 min. The DPPH assay was performed at 510 nm following a microplate method, and ABTS activity was measured at 734 nm using Trolox as the standard [21].

Strict quality control was implemented to ensure reliable and reproducible spectrophotometric measurements, with all calibrations based on high-purity analytical standards. Six-point standard curves were freshly established for total phenolics (gallic acid, 10–100 $\mu\text{g}/\text{mL}$, $R^2 > 0.995$) and flavonoids (rutin, 20–200 $\mu\text{g}/\text{mL}$, $R^2 > 0.995$). DPPH and ABTS antioxidant activities were determined as Trolox equivalents, with standard curves achieving $R^2 > 0.998$. All samples were analyzed in three technical replicates ($n = 3$). Pooled quality control (QC) samples were injected every 15 test samples to monitor microplate

reader signal drift. The intra- and inter-assay CVs were below 5.0% and 7.5%, respectively, confirming good assay repeatability.

2.3.3. Electron Microscopy Processing

Root and leaf tissues were cut into small sections and fixed in 2.5% glutaraldehyde under vacuum until the samples were fully submerged. Fixation was continued at 4 °C for 48–72 h. The samples were rinsed three times (15 min each) with 0.1 M phosphate buffer (pH 7.0) and post-fixed with 1% osmium tetroxide for 1.5 h in the dark. After rinsing, the tissues were dehydrated through graded ethanol (30–80%) and acetone (90–95%), followed by two treatments with pure acetone. Samples were infiltrated with Spurr resin/acetone mixtures (1:1 and 3:1, *v/v*), embedded in pure resin, and polymerized at 70 °C. Ultrathin sections (70–90 nm) were prepared using a LEICA EM UC7 ultramicrotome, stained with lead citrate and uranyl acetate, and observed using a Hitachi H-7650 transmission electron microscope.

2.3.4. Metabolomic Analysis

Metabolic profiling was performed using a Waters ACQUITY UPLC system equipped with an ACQUITY UPLC HSS T3 column (1.8 µm, 2.1 mm × 150 mm). The mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The column temperature was 45 °C, the injection volume was 3 µL, and the flow rate was 0.3 mL/min. The gradient was as follows: 0–1 min, 0% B; 1–12 min, 0–35% B; 12–21 min, 35–95% B; 21–25 min, 95% B; 25–26 min, 95–0% B; and 26–30 min, 0% B. Mass spectrometry was conducted on an AB SCIEX TripleTOF 5600 system with electrospray ionization in both positive and negative ion modes. Source parameters were as follows: ion spray voltage, 5000 V and −4000 V; GS1/GS2, 55 psi; curtain gas, 35 psi; and source temperature, 550 °C. Data-dependent acquisition was performed with a TOF MS scan range of 100–1200 Da and a product ion scan range of 50–1200 Da. Equal 10 µL aliquots of all leaf extracts were mixed to prepare pooled QC samples for evaluating UPLC-MS system stability and instrumental drift. The pooled QC was injected six times for instrument equilibration prior to sample batch analysis. One QC was inserted every 10 unknown samples to monitor shifts in mass accuracy, retention time and peak intensity, and these QC datasets were used for subsequent signal drift correction.

2.4. Data Processing and Statistical Analysis

Statistical significance was analyzed using SPSS 22.0, and figures were generated with GraphPad Prism 10.3.0. Metabolomic data were processed for peak extraction, alignment, and deconvolution using MS-DIAL (v5.5). Metabolite identification complied with MSI multi-tier confidence rules. Compounds matching retention time, precursor *m/z* and MS/MS fragments against our in-house library of over 800 plant standards were annotated at Level 1. Metabolites without authentic standards received Level 2 putative annotation via MS/MS matching against MassBank, METLIN and Mona databases in MS-DIAL v5.5 and SIRIUS v6.1.0, with a ±10 ppm precursor tolerance and >80% spectral similarity. Features lacking qualified MS/MS matches were classified as Level 3 or removed to guarantee data accuracy. Data underwent QC RLSC normalization and log₂ transformation. Differential expressed metabolites (DEMs) were screened based on T-tests (*p* < 0.05) and OPLS-DA models (VIP > 1, and Fold Change > 1.5 or <0.67). Metabolic pathways were annotated using the KEGG database.

3. Results

3.1. Effects of Microplastic Stress on the Growth Performance of Mulberry Seedlings

Both polyethylene (PE) and poly (butylene succinate) (PBS) microplastic treatments significantly affected the growth of mulberry seedlings (Figure 1). Compared to the control (CK), plant height and stem diameter exhibited an overall declining trend across all treatments (Figure 1A,B). This inhibitory effect intensified with increasing microplastic concentrations, indicating a concentration-dependent response in the aboveground growth of mulberry seedlings. The trends for fresh and dry weights were largely consistent (Figure 1D,E); with the exception of the 100 mg/kg PE treatment, all other treatments showed biomass levels significantly lower than the control, suggesting that microplastic exposure substantially suppressed matter accumulation. In contrast, root length increased in most treatments (Figure 1C), implying that mulberry seedlings may promote root elongation under stress to expand their resource acquisition range, potentially mitigating the adverse effects of restricted water and nutrient uptake.

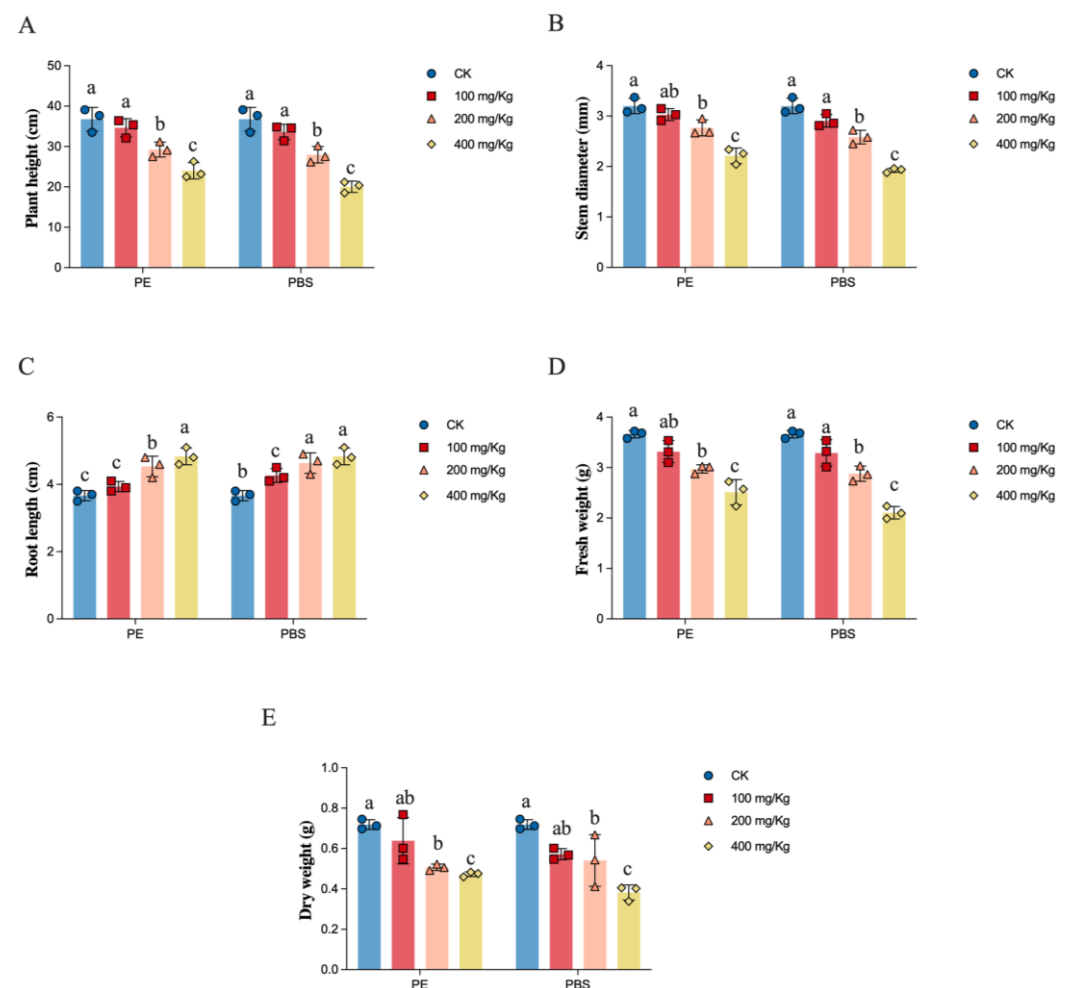


Figure 1. Effects of different microplastic treatments on the growth indicators of mulberry seedlings. Effects of polyethylene (PE) and poly (butylene succinate) (PBS) microplastics at concentrations of 0, 100, 200, and 400 mg/kg on the plant height (A), stem diameter (B), root length (C), total fresh weight (D), and total dry weight (E) of the seedlings. Error bars represent the SD ($n = 3$). The different lowercase letters indicate significant differences ($p < 0.05$) based on two-way ANOVA followed by Tukey's multiple comparisons test.

3.2. Physical Damage of Microplastics to the Micromorphology of Mulberry Seedlings

To further elucidate the structural basis for the growth inhibition observed in mulberry seedlings, transmission electron microscopy (TEM) was employed to characterize the surface morphology of roots and leaves (Figures 2 and 3). In the control group (CK), the root surface exhibited a continuous and smooth epidermal structure with clear cellular outlines and no detectable foreign matter attachment (Figure 2a,d). Correspondingly, the leaf cuticular layer remained intact and continuous, with well-defined stomatal morphology.

In contrast, both PE and PBS treatments induced varying degrees of particle adhesion, epidermal texture disorganization, and pore clogging on the root surfaces. In the PE treatment group, sporadic particle attachment and irregular epidermal textures began to emerge (Figure 2b,e). These physical alterations were particularly pronounced in the PBS treatment group; at 6000 $\times$ magnification, extensive particle aggregates were observed coating the root surface. Further magnification to 10,000 $\times$ revealed significant exfoliation of the epidermal tissue, with micropores deeply obstructed by microplastic particles and their degradation products (Figure 2c,f). Such physical blockages likely restrict the transmembrane transport efficiency of water and inorganic salts in the root system.

Leaf observations yielded similar results, showing that microplastic exposure led to cuticular damage and particle enrichment around the stomata. Notably, the degree of structural impairment was more severe under PBS treatment than PE treatment, suggesting that biodegradable microplastics may exert more intense physical stress on the microstructure of mulberry tissues.

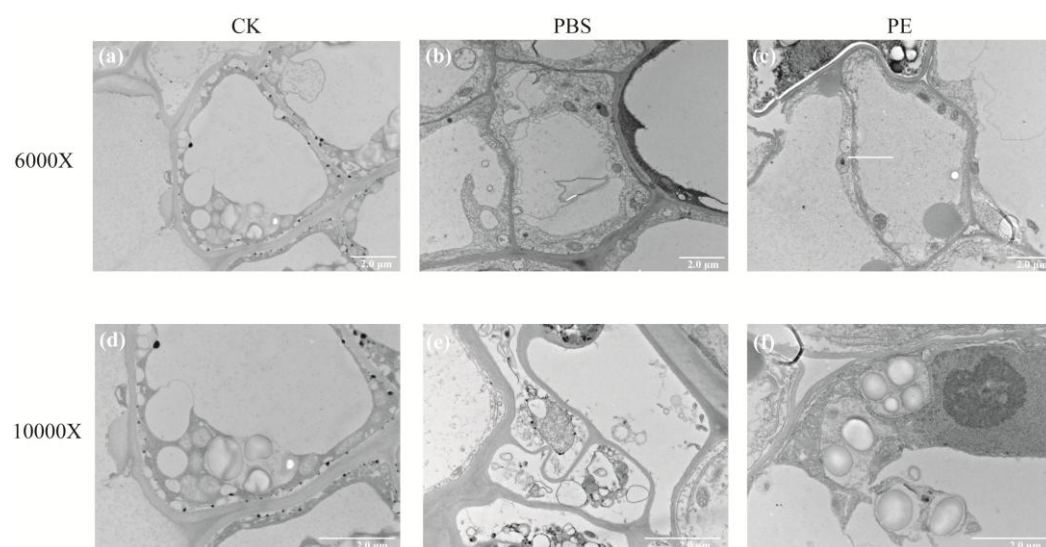


Figure 2. Impact of microplastic stress on the root system of mulberry seedlings under transmission electron microscopy (TEM). (a,d) Control group (CK); (b,e) PE group; (c,f) PBS group. Panels (a–c) are at 6000 $\times$ magnification; panels (d–f) are at 10,000 $\times$ magnification.

TEM observations of the leaf surfaces further revealed the detrimental effects of microplastic exposure (Figure 3). In the control group (CK), the leaves exhibited a dense and intact cuticular layer, with stomata showing normal morphology and uniform distribution (Figure 3a,d). PE exposure led to increased leaf surface roughness and distinct particle enrichment around the stomatal openings (Figure 3c,f). The PBS treatment group, however, displayed more pronounced features of cuticular impairment; under 30,000 $\times$ high-magnification, the originally smooth waxy layer appeared non-uniformly eroded, accompanied by substantial particle adhesion (Figure 3b,e).

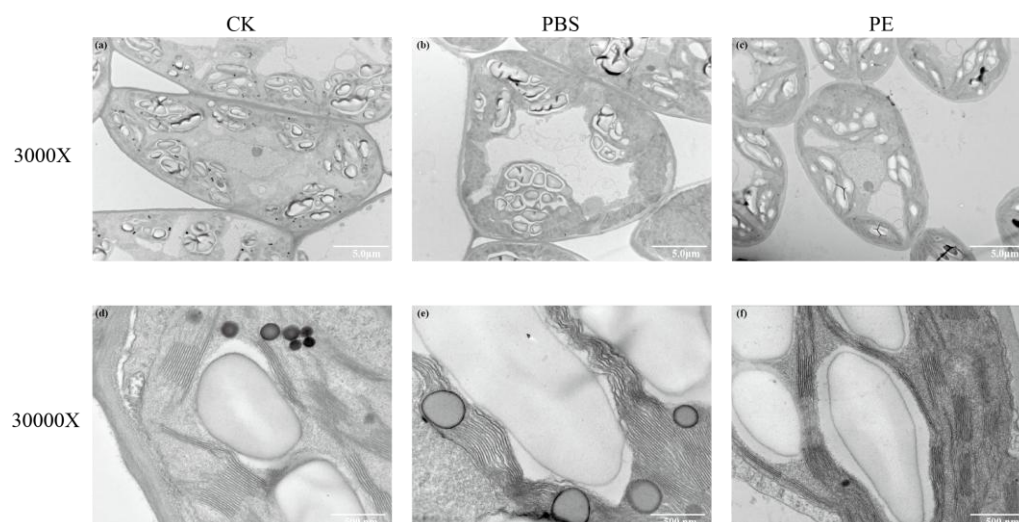


Figure 3. Impact of microplastic stress on the leaves of mulberry seedlings under transmission electron microscopy (TEM). (a,d) Control group (CK); (b,e) PE group; (c,f) PBS group. Panels (a–c) are at 3000× magnification; panels (d–f) are at 30,000× magnification.

3.3. Effects of Microplastic Stress on the Quality of Mulberry Leaves

Both PE and PBS treatments significantly altered the quality-related indicators of mulberry leaves (Figure 4). Regarding total flavonoid (TF) content (Figure 4A), with the exception of the 100 mg/kg PE treatment, all other microplastic treatment groups exhibited a significant impact on TF levels as the application rate increased ($p < 0.05$). Specifically, the TF content demonstrated a progressive upward trend with increasing treatment concentrations. For total phenolic (TP) content (Figure 4B), no significant difference was observed between the 100 mg/kg PE treatment and the control (CK); however, the 400 mg/kg treatment was significantly higher than all other groups ($p < 0.05$). In terms of DPPH radical scavenging rate and ABTS total antioxidant capacity (Figure 4C,D), both PE and PBS treatments yielded results significantly higher than the control ($p < 0.05$). Furthermore, both DPPH radical scavenging activity and ABTS total antioxidant capacity intensified gradually as the stress concentration increased.

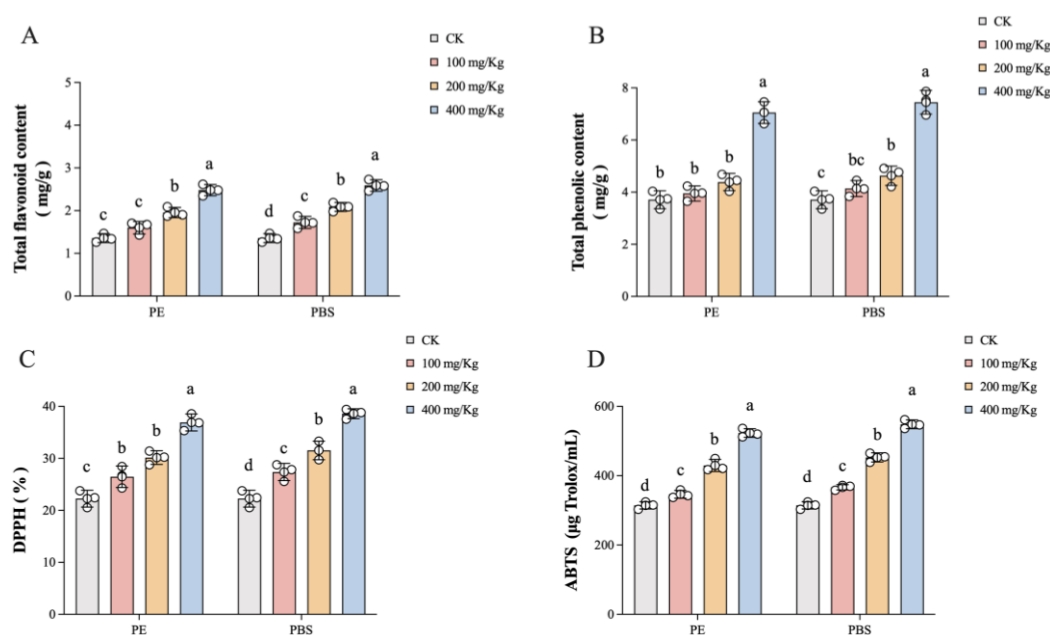


Figure 4. Effects of different microplastic treatments on the quality of mulberry leaves. Effects of polyethylene (PE) and poly (butylene succinate) (PBS) microplastics at concentrations of 0, 100, 200

and 400 milligrams per kilogram on (A) total flavonoid content, (B) total phenol content, (C) DPPH free radical scavenging activity, and (D) ABTS free radical scavenging activity. Error bars represent the SD ($n = 4$). The different lowercase letters indicate significant differences ($p < 0.05$) based on two-way ANOVA followed by Tukey's multiple comparisons test.

3.4. Metabolic Alterations in Mulberry Seedlings Under Microplastic Stress

To resolve the impact of microplastics on mulberry seedlings at the metabolic level, non-targeted metabolomic analysis was performed on tissues from different treatment groups (CK, PE100, PE200, PE400, PBS100, PBS200, and PBS400). PCA results showed distinct spatial separation between the control and the treatment groups (Figure 5A). Further analysis revealed that both PE and PBS induced a large number of differentially expressed metabolites (DEMs). Specifically, PE at concentrations of 100, 200, and 400 mg/kg induced 525, 696, and 585 DEMs, respectively; whereas PBS induced 711, 700, and 712 DEMs, respectively (Figure 5C). The overall higher number of DEMs in the PBS treatment groups reflects broader responsive fluctuations within the mulberry metabolite pool within the tested concentration range. This observation is in alignment with the growth indicators and TEM results, indicating that PBS elicits a more substantial responsive fluctuation across both phenotypic and metabolic levels under the current experimental framework. Hierarchical clustering analysis revealed that following microplastic treatment, primary metabolism-related substances were downregulated, while stress-related metabolites were upregulated. Furthermore, the high-concentration PE and PBS groups formed distinct characteristic metabolic clusters, indicating inherent differences in the toxicological effects of these two types of microplastics (Figure 5B). This suggests that under sustained stress, the metabolic activity of mulberry seedlings shifts away from maintaining normal growth toward a defense- and adaptation-oriented metabolic mode [42].

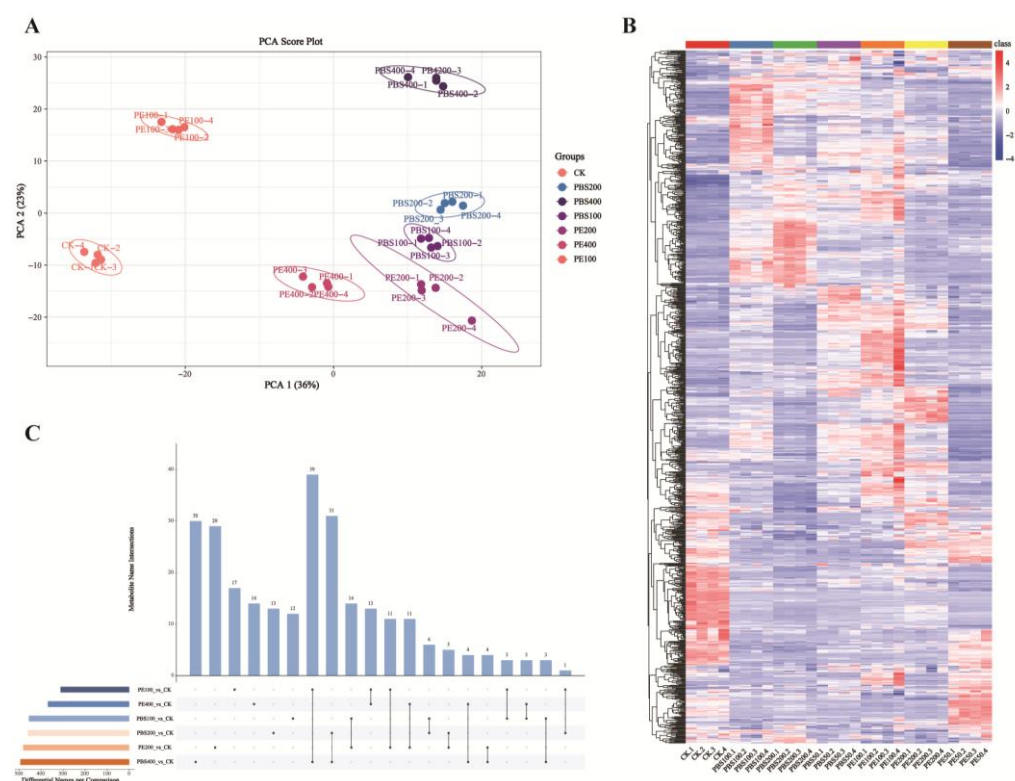


Figure 5. Global metabolic response profiles of mulberry seedlings under microplastic stress. (A) Principal component analysis (PCA) score plot of samples from all treatment groups and the control group; (B) Hierarchical clustering heatmap of global significantly differentially expressed metabolites (DEMs); (C) UpSet plot illustrating the intersection and specific distribution of DEMs across

various microplastic treatment groups. The vertical bar chart at the top represents the number of metabolites within specific intersections, while the horizontal bar chart on the left represents the total number of DEMs for each treatment group compared to the control (CK). Analysis was performed with $n = 4$ independent biological replicates per treatment group.

Analysis of key differentially expressed metabolites (DEMs) further demonstrated that coumaric acid, quercetin, and rutin accumulated significantly under microplastic stress (Figure 6A,B,D), while glutathione content markedly declined (Figure 6C). The accumulation of these metabolites was generally more pronounced under PBS treatment than under PE treatment.

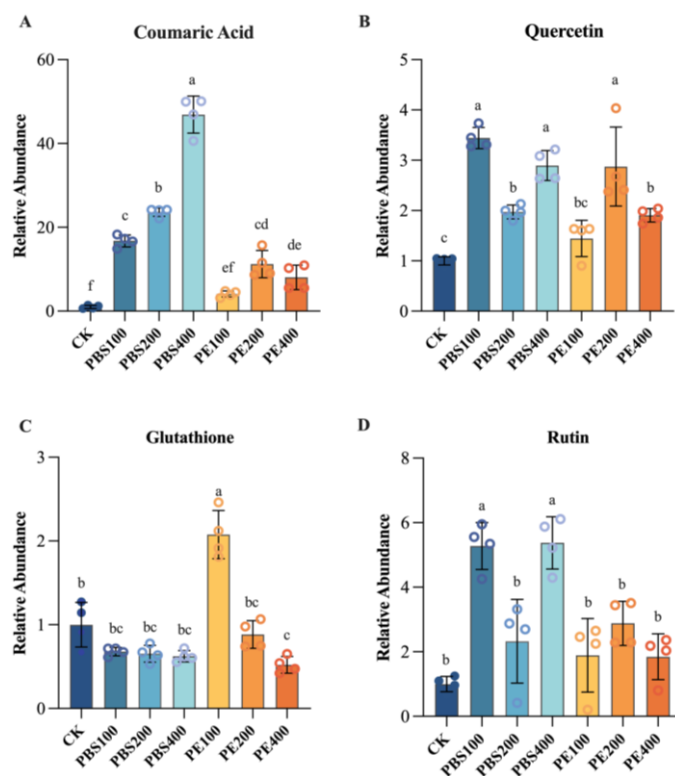


Figure 6. Relative abundance of core differentially expressed metabolites under different concentrations of microplastic stress. (A) Coumaric acid serves as a foundational precursor in the phenylpropanoid pathway; (B) quercetin and (D) rutin are major bioactive flavonoids in mulberry leaves that scavenge excess ROS. Their increased levels indicate stress-induced accumulation of functional metabolites under microplastic exposure; (C) glutathione acts as a key endogenous antioxidant. The vertical axis represents the relative abundance normalized to the mean of the control group (CK). Data are presented as Mean $\pm$ SD ($n = 4$). Different lowercase letters above the bars indicate significant differences between groups ($p < 0.05$, one-way ANOVA, Tukey's test).

3.5. Enrichment Analysis of Differential Metabolic Pathways Induced by Microplastic Stress

To further elucidate the metabolic mechanisms by which microplastics affect mulberry seedlings, KEGG pathway enrichment analysis was performed on the DEMs (Figure 7). The results showed that phenylpropanoid biosynthesis and flavonoid biosynthesis pathways were significantly enriched under all concentrations of both PE and PBS treatments. This is consistent with the accumulation trends of coumaric acid, quercetin, and rutin, indicating that the enhancement of secondary metabolism is a common characteristic of mulberry seedlings in response to microplastic stress. In addition to these pathways, primary metabolic pathways such as amino acid biosynthesis and the TCA cycle underwent significant changes, suggesting that microplastic exposure not only alters defensive

metabolism but also affects the plant's energy balance and basal metabolic homeostasis. Notably, the α -linolenic acid metabolism pathway was specifically enriched in the high-concentration PBS treatment.

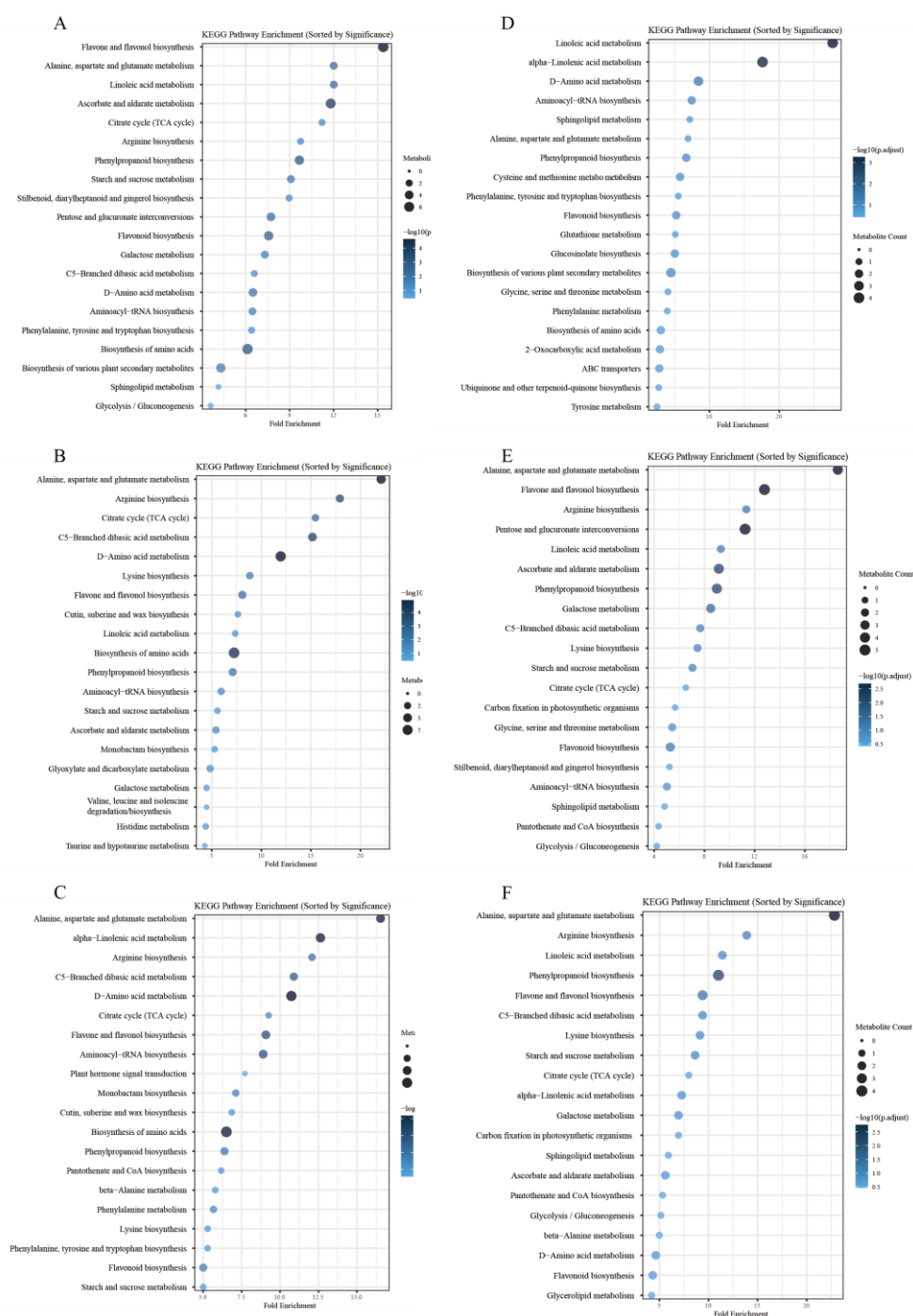


Figure 7. KEGG pathway enrichment analysis of differentially expressed metabolites (DEMs) in different treatment groups. (A–C) PBS treatment groups (100, 200, 400 mg/kg, respectively); (D–F) PE treatment groups (100, 200, 400 mg/kg, respectively). The horizontal axis represents the fold enrichment, and the vertical axis represents the significantly enriched metabolic pathways. The size of the bubbles represents the number of DEMs enriched in the pathway, and the bubble color intensity (from light to dark) indicates increasing levels of statistical significance.

4. Discussion

Plant phenotypic changes are intuitive indicators of environmental stress. Microplastic stress inhibited the aboveground growth of mulberry seedlings but induced adaptive root elongation, representing a typical organ-specific stress response [43]. Such morphological compensation failed to increase plant fresh and dry weight, suggesting it could not fully offset microplastic-induced growth inhibition. Consistent with previous findings that microplastics impair plant metabolism and rhizosphere environments reduce biomass accumulation [44–46], this study further confirms that microplastic damage covers both morphological variation and declined growth and accumulation capacity. Microplastics suppress plant growth through direct toxicity and indirect soil modification. They disrupt soil physical structure, reduce enzyme activity and adsorb mineral nutrients, limiting plant nutrient uptake [47]. Both PE and PBS microplastics also disturb rhizosphere microbial communities and trigger plant stress responses, jointly causing biomass reduction [39]. These microplastic-induced changes lower soil nutrient availability, as hydrophobic microplastics immobilize soil nitrogen and available phosphorus, while PBS degradation further enhances microbial nutrient fixation. To adapt to nutrient deficiency, mulberry seedlings elongate roots for nutrient foraging, though this strategy cannot reverse inhibited aboveground growth. The 100–400 mg/kg microplastic concentrations adopted in this study are environmentally realistic. Low and medium doses (100–200 mg/kg) match baseline levels in global farmlands with plastic mulching and biosolid application, while the high dose (400 mg/kg) simulates severely polluted agricultural soils [6, 39]. Nevertheless, it should be stressed that documented microplastic concentrations in agricultural soils vary drastically across the globe. Such wide divergence mainly arises from inconsistent sampling approaches (including different soil core depths and spatial sampling layouts), inconsistent extraction and quantification workflows (for instance, varied density separation solutions, alongside differing detection limits of FT-IR, Raman and Py-GC/MS), as well as region-specific field management practices, such as inconsistent mulch film service years, wastewater irrigation frequency and biosolid application rates. Our concentration gradient enables reliable assessment of present and future microplastic hazards in perennial planting systems. The applied microplastic dosages serve as stable, controlled references to offset the extreme variability of microplastic pollution reported globally.

TEM observations explain the structural mechanisms underlying suppressed mulberry growth. Microplastics adhere to roots and block surface pores, hindering water and nutrient uptake [24]; they also destroy leaf cuticles and accumulate around stomata to impair gas exchange and photosynthesis [48]. These tissue defects lower plant height, stem diameter and total biomass. Biomass decline is not driven solely by chemical toxicity; modified physical conditions at the root-soil interface also contribute. PE and PBS particles may cover root epidermis, potentially slowing water and ion transport and inducing physiological drought. Plants likely allocate more carbon to root elongation for water acquisition, which limits aboveground growth. We thus infer poor rhizosphere physical conditions are a key growth-limiting factor that amplifies chemical stress. Combined with growth metrics, microplastics restrain mulberry development via both physical tissue injury and metabolic disruption. PBS caused more severe root and leaf structural damage than PE, suggesting biodegradable microplastics are not safer before full degradation [49]. PE mainly forms physical barriers, whereas decomposing PBS release reactive oligomers and additives that disturb the rhizosphere and damage root cell walls. Consistent with earlier crop research, incompletely degraded biodegradable plastic residues carry clear risks for cash crops [50].

Microplastic exposure substantially alters the metabolic profile of mulberry leaves, increasing the accumulation of total flavonoids, total phenolics and key secondary metab-

olites. These compounds, together with antioxidant capacity, are critical indicators for evaluating the quality of mulberry leaf raw materials [51]. Metabolomic analysis revealed prominent enrichment of phenylpropanoid and flavonoid biosynthesis pathways, which serve as the core metabolic response to microplastic stress. The enhanced accumulation of coumaric acid, quercetin and rutin further verifies the upregulation of these antioxidant-related pathways under microplastic stress [52]. Notably, such metabolite enrichment reflects metabolic stress rather than active quality improvement. This explanation is supported by concurrent physiological damage, including inhibited growth, root pore blockage and impaired leaf cuticles [28]. Accumulated phenylpropanoid intermediates may alleviate microplastic-triggered oxidative stress in this coordinated response, while total antioxidant activity represents an integrated chemical trait. Microplastic-induced changes in metabolic profiles are closely linked to disrupted cellular redox homeostasis, and unstable antioxidant metabolism inevitably impairs the consistency of plant bioactive components [53]. Microplastic stress induces a typical growth-defense trade-off: it increases the relative content of secondary metabolites per unit dry weight but reduces overall plant biomass and breaks metabolic homeostasis. The elevated bioactive compounds therefore do not represent genuine quality promotion. For perennial medicinal plants that require stable raw material quality, microplastic pollution disrupts metabolite uniformity, highlighting the necessity of soil plastic monitoring and quality risk evaluation in agricultural production [35].

These physiological and biochemical adjustments are deeply rooted in global metabolic shifts and resource reallocation strategies within the plant. Under sustained stress, the metabolic activity of mulberry seedlings shifts away from maintaining normal growth toward a defense and adaptation-oriented metabolic mode [54, 55]. Phenylpropanoid biosynthesis, flavonoid biosynthesis, glutathione metabolism, and hormone signaling pathways collectively participate in this plant response [56]. The alterations in the TCA cycle and amino acid metabolism indicate a clear resource reallocation under stress conditions. To survive the stress, carbon sources, energy, and reducing power are diverted from normal growth processes toward defense-related secondary metabolism. Plants commonly shift metabolism to cope with abiotic stress by activating phenylpropanoid synthesis [27]. Untargeted metabolomics reveals stress like microplastics triggers oxidative signals, diverting carbon from growth to secondary metabolite production [20]. Increased coumaric acid and polyphenols strengthen antioxidant defenses, a typical metabolic response to environmental stress [56].

In this study, the α -linolenic acid metabolism pathway was specifically enriched in the high-concentration PBS treatment. Given that α -linolenic acid is a vital precursor for membrane lipid remodeling and jasmonic acid signaling, its enrichment is closely associated with wounding responses and severe membrane lipid stress [20, 41, 56]. This resource trade-off effectively explains the coexistence of biomass reduction and enhanced antioxidant-related metabolism observed in this study. This metabolic feature strongly correlates with the more severe root and leaf surface damage observed via TEM in the PBS treatments, confirming that PBS induces more intense structural impairment and stress signaling responses than PE [39].

The stronger biological effects of PBS may be related to its unique physicochemical characteristics as a biodegradable polyester. Compared with PE, PBS contains hydrolysable ester bonds that are more susceptible to environmental weathering and surface oxidation [57]. Yet PBS degradation rates, pathways and intermediate metabolites in farm soils are not constant; they shift greatly with soil pH, water content, clay proportion and indigenous microbial communities. Soil esterases and lipases break PBS ester bonds, but the quantity and activity of these degrading microbes vary with environmental conditions. Such variation further controls microplastic breakdown speed and the release of phyto-

toxic oligomers. These processes may increase particle surface roughness and alter surface chemical properties, thereby enhancing the interaction between PBS particles and plant tissues. Consequently, PBS particles may adhere more readily to root and leaf surfaces, intensifying physical obstruction and tissue damage. Moreover, during environmental aging and partial degradation, PBS may release low-molecular-weight degradation intermediates and additives that potentially impose additional physiological stress on plants.

Overall, the impact of microplastics on mulberry seedlings is a multi-dimensional process involving interconnected changes in tissue structure, physiological characteristics, and metabolic homeostasis. For agricultural applications, the finding that PBS exhibits a stronger perturbation effect than conventional PE across multiple structural and metabolic indicators highlights that biodegradable plastics require rigorous evaluation regarding raw material quality, biomass yield, and potential ecological risks prior to full degradation.

Our results extend beyond plant physiological responses and reveal major risks to medicinal crop quality, food safety and agricultural sustainability. Microplastics cause unstable levels of rutin, quercetin and coumaric acid, breaking consistent quality standards of mulberry medicinal materials and weakening uniform medicinal effects across batches. As mulberry leaves are made into food and herbal drinks, microplastics attached to leaf surfaces bring dietary pollution risks. Plastic particles on leaves, together with root-absorbed microplastics and additives, accumulate in silkworms and move up the human food chain. Both PE and PBS slow mulberry growth and reduce yields. Even biodegradable PBS mulch harms crops during degradation, so biodegradability alone cannot guarantee crop safety without accounting for temporary tissue damage and phytotoxicity.

We used metabolomics and DPPH/ABTS tests to observe enhanced antioxidant metabolism under microplastic stress, but did not measure oxidative stress markers including ROS, H₂O₂, MDA and key antioxidant enzymes (SOD, CAT, POD, APX). Without these data, we cannot fully explain the enzyme and cell pathways driving stress tolerance, which is a clear limitation of this study to be addressed in later experiments. Future work will combine multiple tests to uncover causal mechanisms. Long-term field trials with naturally aged microplastics and rhizosphere microbiome analysis will clarify soil-root-plastic interactions. Transcriptomics paired with complete antioxidant enzyme assays will further define molecular pathways and nutritional shifts under prolonged microplastic exposure.

5. Conclusions

In summary, this greenhouse pot experiment reveals how mulberry seedlings respond physiologically and metabolically to microplastic exposure. Both PE and PBS microplastics inhibited seedling growth and triggered tissue damage in a dose-dependent pattern, with PBS causing more evident morphological changes on roots and leaves. Untargeted metabolomics further identified clear metabolic shifts in leaves, alongside elevated levels of total flavonoids, phenolics, coumaric acid, quercetin and rutin. Our controlled pot trials lay a solid mechanistic basis for understanding microplastic stress on mulberry. Nevertheless, to better interpret our results for practical application, we stress that long-term field verification under natural soil weathering and dynamic rhizosphere environments is still urgently needed. Follow-up long-term field observations will help validate these results under natural farmland conditions, supporting balanced management strategies that secure both medicinal component quality and crop yield for sustainable mulberry cultivation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12080911/s1>, Figure S1: Physicochemical characterization of raw microplastics. (A) SEM images of PBS and PE at 100× magnification (scale bar = 100 µm, EHT = 3.00 kV, WD = 8.4 mm). PBS had irregular angular fragments (130.5 ± 38.2 µm), while PE formed rough cauliflower-like aggregates (132.9 ± 36.5 µm). (B) XRD patterns of PBS and PE powders recorded from 4° to 70° (2θ). PBS showed weak semi-crystalline peaks near 19.5°, 22.2°, 22.5° with crystallinity of $24.30 \pm 1.33\%$; PE exhibited sharp crystal peaks at 21.5° and 23.9°, with crystallinity of $61.93 \pm 0.69\%$. Both microplastics were pure additive-free resins from Dongguan Changmutou Chengxing Plastic Products Co., Ltd. No plasticizers or stabilizers were mixed during production, so plant toxicity observed in subsequent trials arises from physical particle effects instead of leached chemical pollutants.

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