EFFECTS OF MICROPLASTICS ON THE GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF MULBERRY

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(Received 24th Jul 2024; accepted 3rd Dec 2024)

Abstract. To comprehensively understand the toxic effects and ecological risks of microplastics on major economic tree species, a pot experiment was conducted using polylactic acid (mPLA) microplastics as the test object to explore the effects of different concentrations (0.1%, 0.5%, 1%, 5%, 10%, w/w, mass fraction) of microplastics on the growth and physiological characteristics of mulberry trees. The study results showed that, compared with the control group, the biomass, total chlorophyll content, and net photosynthetic rate of mulberry trees in the mPLA treatment group were significantly reduced under high concentration (10%) treatment; the activities of SOD and CAT and the MDA content were significantly increased by 50.00%, 47.83%, and 60.87%, respectively, at a 10% concentration. The results indicate that the toxic effects of microplastic addition on mulberry trees are related to the type and concentration of microplastics. High concentrations of mPLA can damage the photosynthetic system of plants, affecting photosynthesis, causing oxidative damage and thus inhibiting the growth of mulberry plants.
Keywords: *soil pollution, environmental stress, oxidative stress, antioxidant enzyme activity, mulberry physiology*

Introduction

Microplastics (tiny plastic fragments and particles) as a new type of environmental pollutant have attracted global attention (Bourioug et al., 2014; Akdogan et al., 2021). Their non-degradable nature leads to their accumulation in the environment and organisms, long-distance migration, and impacts on the ecological environment and human health. Especially in the soil environment, the presence of microplastics can be 4 to 23 times high than that in the marine environment. These microplastics mainly originate from agricultural mulch film fragmentation, organic fertilizer application, sewage irrigation, sludge application, atmospheric deposition, and surface runoff activities.

In China, due to the high utilization of plastic products and low recycling rates, a large amount of plastic waste enters the environment, causing pollution (Lebreton et al., 2017). These plastic wastes are broken down into microplastics in the soil through UV radiation, physical weathering, and biodegradation (Chi et al., 2014; Ma et al., 2016; Li et al., 2021). The annual accumulation of microplastics in the soil environment affects the physical and chemical properties and functions of the soil, thus harming the normal growth of plants. Microplastics have been shown to affect various aspects of plants, including germination, photosynthesis, and antioxidant systems (Franco-Otero et al., 2015; Arena et al., 2017; Rillig et al., 2017). For example, exposure to microplastics can reduce chlorophyll content in corn leaves and superoxide dismutase activity in wheat leaves, and may even accumulate through the food chain to harm human health, such as

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_13651376

inducing liver inflammation or crossing the blood-brain barrier and reaching into brain tissue. The selection of microplastic concentrations (0.1%, 0.5%, 1%, 5%, 10%) was based on observed pollution levels in highly polluted forest areas, as reported by studies on soil environments where microplastics accumulate due to anthropogenic activities (Liu et al., 2017; Cao et al., 2021). These concentrations simulate the potential environmental impacts under extreme conditions. The migration behavior of microplastics also poses a threat to plant growth. Microplastics in the soil environment can be absorbed by plants through the root system and migrate upwards to the edible parts of plants through transpiration, thus accumulating in the food chain. For example, larger-sized microplastics can enter watercress seeds and affect their germination ability (Qi et al., 2018; Sun et al., 2021).

In Northeast China, mulberry trees (Morus alba L.), as an important economic and ecological plant, play roles in silkworm breeding, windbreak and sand fixation, landscape beautification, and medicinal. However, research on the effects of microplastics on mulberry trees is still insufficient. Therefore, this study selected mulberry trees as the research subject and focused on two different types of microplastics—low-density polyethylene (LDPE) and polylactic acid (PLA)—to explore the effects of different concentrations and types of microplastics on the growth characteristics and physiological traits of mulberry trees. This study aims to deepen the understanding of the effects of microplastics on mulberry trees and their ecosystems, reveal the physiological response mechanisms of mulberry trees to microplastic pollution, and provide scientific basis and theoretical support for comprehensively understanding the ecological risks of microplastics to terrestrial ecosystems.

Materials and methods

Test materials and treatment

The mulberry seeds used in the experiment were “Long Sang No.1”, provided by Forestry and Grassland Bureau of Hegang. The type of microplastic used in the experiment was low-density polylactic acid (PLA), purchased from Hongchen Engineering Plastics Company, sieved through a 0.075 mm mesh without fluorescent dye. The test soil was loessal soil, collected from the experimental field at Harbin University. The soil had a pH of 7.87, organic carbon content of 9.45 $g \cdot kg^{-1}$, total nitrogen of 0.74 $g \cdot kg^{-1}$, nitrate nitrogen of 28.32 $mg \cdot kg^{-1}$, ammonium nitrogen of 1.84 $mg \cdot kg^{-1}$, available phosphorus of 5.32 $mg \cdot kg^{-1}$, and available potassium of 147.12 $mg \cdot kg^{-1}$.

A pot experiment was conducted using a completely randomized block design with six treatment groups: control group (CK), and microplastic mPLA treatments with concentrations of 0.1%, 0.5%, 1%, 5%, and 10% (w/w), designated as M1, M2, M3, M4, and M5, respectively, with four replicates per treatment. The collected soil was air-dried and sieved through a 2 mm mesh after removing plant roots, stones, and other impurities. Two types and different amounts of microplastics were added to the soil, mixed evenly, and then divided into pots with 1 kg of soil per pot. The control group used soil without added microplastic powder. The irrigation water level was maintained at 70% of the soil field capacity, as determined using a gravimetric method, and adjusted daily to ensure optimal soil moisture conditions.

The experiment was conducted from June to September 2021 in an artificial intelligence greenhouse (PB100, MoreBetter, Hangzhou) at the Experimental Station of

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the College of Resources and Environment, Northeast Agricultural University. The greenhouse by optimizing environmental parameters such as temperature, humidity and photoperiod based on real-time sensor feedback, the system provides more precise environmental control, thus ensuring more stable growth conditions for experimental plants. The temperature was maintained at 25°C/20°C (day/night), with a photoperiod of 14 h/10 h (light/dark), and a relative humidity of 60% to 70%. Uniformly growing one-year-old mulberry seedlings were selected and planted in the soil, with 10 seeds sown per pot, and the growth status of the mulberry trees was observed daily and watered regularly. After one week of cultivation, the seedlings were thinned to leave 6 plants per pot, and the plants were harvested after 60 days of continued cultivation.

Determination parameters and methods

The growth parameters were determined

Separate the above-ground and below-ground parts of the mulberry plants, wash them clean with tap water, rinse 2-3 times with deionized water, blot dry, then kill the enzymes at 105°C for 15 min, and dry at 75°C to a constant weight, then weigh the dry weight.

Measurement of root morphological indicators: Analyze and measure root morphological indicators such as total root length, average root diameter, and total root surface area using a root analysis system (Win RHIZO, Canada). Determination of total chlorophyll content: Measured using the spectrophotometric method (Hu et al., 2018). For the determination of chlorophyll content leaf samples were collected from the second fully expanded leaf from the top of three mulberry plants per pot, with five replications for each treatment. The number of root tips was determined by manually counting using a magnifying glass after thoroughly washing and separating the root systems. The process was repeated 5 times.

Photosynthetic gas exchange parameters were measured

The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of the second fully expanded functional leaf of mulberry were measured at 9:00-11:00 a.m. using Li-6400 photosynthetic measurement system (Licor company, USA). The penultimate leaf of mulberry seedlings was selected. The CO_2 concentration was fixed at 400 $\mu l \cdot L^{-1}$ in a CO_2 cylinder. The light intensity PFD was set to 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with the light source built in the instrument. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) of mulberry leaves under different treatments were measured. Repeat 5 times.

Determination of antioxidant enzyme activity

Superoxide dismutase (SOD) activity was measured using the nitrotetrazolium blue chloride (NBT) method, peroxidase (POD) activity was measured using the guaiacol method, and catalase (CAT) activity was measured using the ultraviolet spectrophotometric method. Determination of malondialdehyde (MDA) content: Measured using the thiobarbituric acid (TBA) method (Zhang et al., 2018). For the antioxidant enzyme activities, leaf samples were collected from the second fully expanded leaf from the top of three mulberry plants per pot, with five replications for each treatment (Lu, 1999).

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Data and analysis

Excel and SPSS (24.0) software were used for statistical analysis of the measured data. The data in the figure were the mean ± standard deviation (SE) of three repetitions. One way ANOVA and least significant difference (LSD) were used to compare the differences among different data groups.

Results and analysis

Effects of microplastics on mulberry biomass

High concentrations of mPLA treatment significantly reduced the above-ground and below-ground biomass of mulberry trees ($P < 0.05$) (*Figs. 1* and *2*). Compared with the control group, the above-ground biomass in M4 and M5 treatments and the below-ground biomass in M3, M4, and M5 treatments of the mPLA-treated group significantly decreased by 37.1%, 61.4%, and 26.1%, 38.4%, 59.7%, respectively, indicating that the inhibitory effect of mPLA treatment on mulberry biomass gradually increased with the concentration of microplastics.

Effects of microplastics on mulberry root morphology

Microplastic treatments inhibited various aspects of mulberry root morphology (root length, average root diameter, total root surface area, total root volume, and number of root tips) to varying degrees (*Table 1*). Compared with CK, mPLA treatments M2 and M3 promoted root length in mulberry trees, but the effects were not significant ($P > 0.05$); high concentration mPLA treatment (M5) significantly inhibited root length in mulberry trees ($P < 0.05$), reducing root length by 33.33%. Microplastic treatments significantly inhibited the total root surface area of mulberry trees ($P < 0.05$); the lowest value was observed in M5 treatment, reducing the total root surface area by 41.67%. The average root diameter showed a negative response to mPLA stress, with overall little variation, but the inhibitory effect was significant compared to the CK group ($P < 0.05$). mPLA significantly inhibited the total root volume of mulberry trees ($P < 0.05$). The number of root tips was also negatively affected, with the M5 treatment reducing the number of root tips by 28.57% compared to the CK group.

Table 1. *Effect of microplastics on root morphology of mulberry*

Treatment	Root length (cm)	Mean root diameter (cm)	Total root surface area (cm²)	Total root volume (cm³)	Root tip number
CK	1500.00 ± 30.00 ab	1.20 ± 0.05 a	600.00 ± 10.00 ab	20.00 ± 2.00 a	7000.00 ± 800.00 ab
mPLA M_1	1480.00 ± 60.00 abc	0.90 ± 0.04 bc	470.00 ± 15.00 c	12.00 ± 1.00 bcd	6800.00 ± 150.00 ab
mPLA M_2	1600.00 ± 50.00 ab	0.95 ± 0.03 bc	530.00 ± 30.00 abc	14.00 ± 1.00 bc	6850.00 ± 700.00 ab
mPLA M_3	1550.00 ± 250.00 ab	0.93 ± 0.08 bc	500.00 ± 35.00 bc	13.00 ± 2.00 bcd	8800.00 ± 2000.00 a
mPLA M_4	1300.00 ± 100.00 bcd	0.88 ± 0.03 c	400.00 ± 20.00 cd	10.00 ± 0.50 d	5500.00 ± 400.00 b
mPLA M_5	1000.00 ± 130.00 d	0.92 ± 0.03 bc	350.00 ± 40.00 d	9.00 ± 1.00 d	5000.00 ± 500.00 b

Effects of microplastics on mulberry chlorophyll

As shown in *Figure 3,* compared with the CK group, the total chlorophyll content in mulberry leaves responded differently to various concentrations of mPLA treatment. Specifically, the mPLA treatment showed a positive effect at low concentrations (M1

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and M2) and an inhibitory effect at moderate to high concentrations (M3, M4, and M5). The highest total chlorophyll content was observed in the M2 treatment group, with no significant difference from the CK group ($P > 0.05$). In contrast, the total chlorophyll content was significantly reduced in the M4 and M5 treatment groups, with M5 showing a reduction of 22.64% ($P < 0.05$).

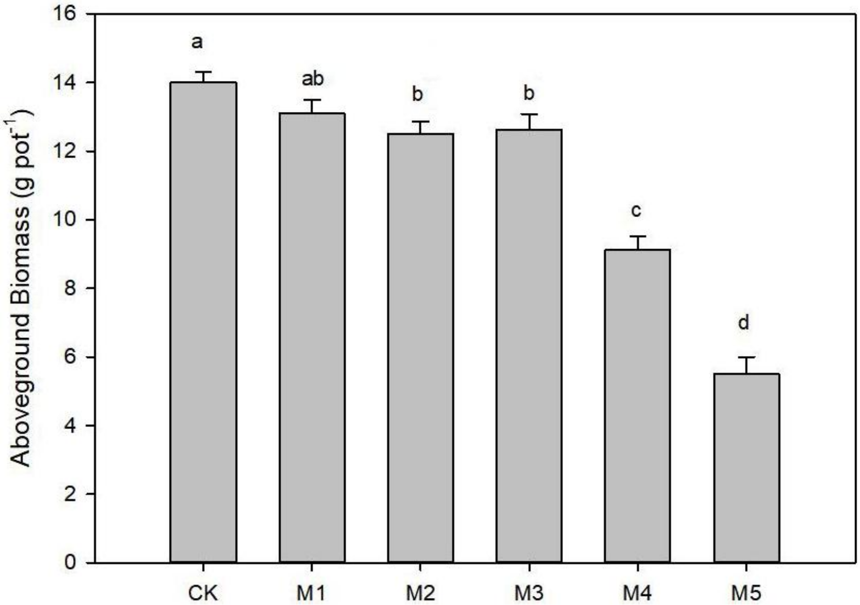

Figure 1. *Effect of microplastics on aboveground biomass of mulberry. CK: control group M1-M5: microplastic mPLA treatments with concentrations of 0.1%, 0.5%, 1%, 5%, and 10% (w/w). The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P <0.05)*

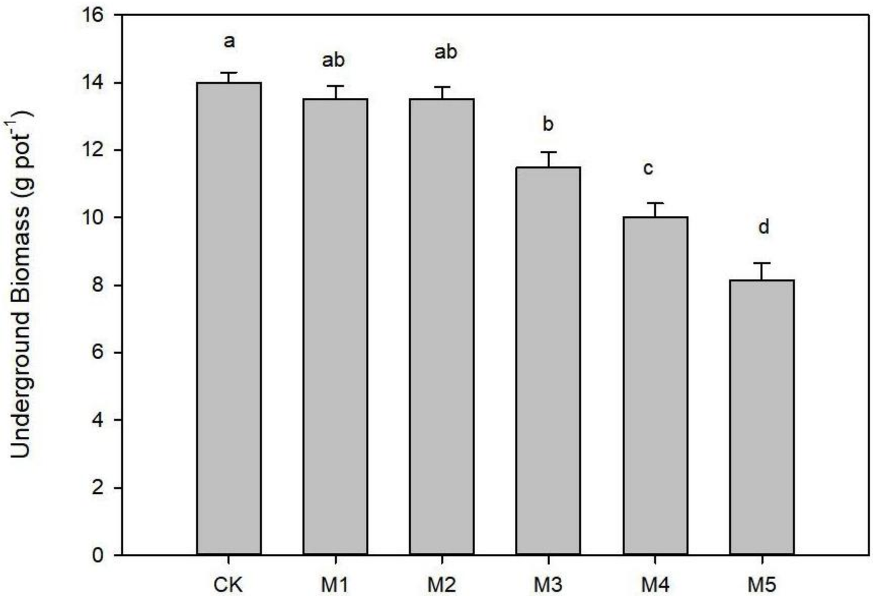

Figure 2. *Effect of microplastics on underground biomass of mulberry. The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P <0.05)*

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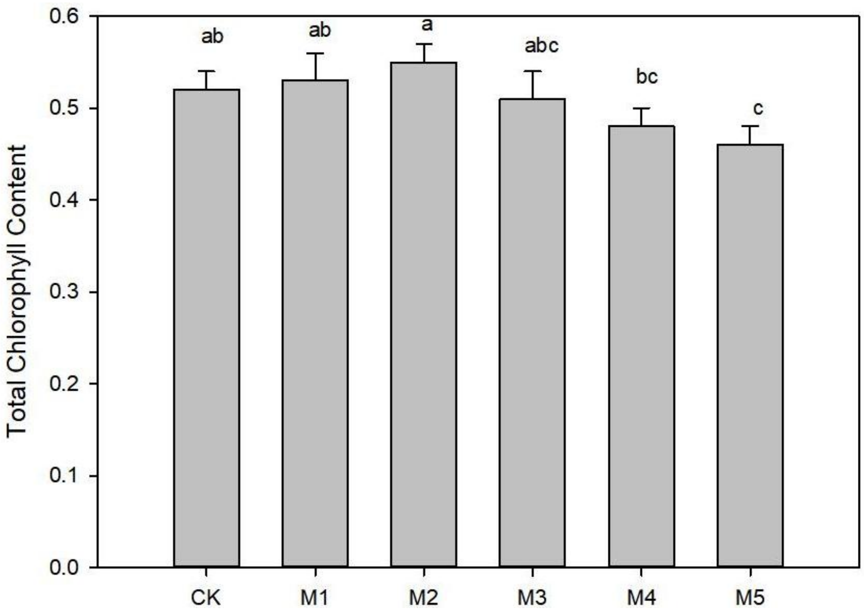

Figure 3. *Effect of microplastics on mulberry chlorophyll. The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P <0.05)*

Effects of microplastics on photosynthetic characteristics of mulberry trees

Figure 4 shows the effects of microplastic addition on photosynthetic gas exchange parameters in mulberry trees. Compared with the CK group, mPLA treatment overall increased the values of G_s, C_i, and T_r, showing an initial increase and then a decrease with increasing concentration. The highest values were observed in the M3 treatment, with significant differences in G_s and Tr compared to the CK group ($P < 0.05$). The stomatal conductance (G_s) showed significant increases in the M2 and M5 treatments, while the intercellular CO_2 concentration (C_i) was notably higher in the M2 and M3 treatments. The transpiration rate (T_r) displayed significant increases in the M3 and M5 treatments.

The net photosynthetic rate (P_n) showed an overall decreasing trend with increasing microplastic concentration, with significant inhibition at high concentrations ($P < 0.05$). Specifically, the P_n was significantly reduced in the M4 and M5 treatments, with reductions of 46.10% and 41.35%, respectively, compared to the CK group. This indicates that higher concentrations of microplastics have a detrimental effect on the photosynthetic efficiency of mulberry trees.

Effects of microplastics on the antioxidant properties of mulberry trees

Compared with CK, different concentrations of mPLA treatments showed a “low inhibition and high promotion” effect on the activities of SOD and CAT in mulberry leaves, and with the increase in concentration, enzyme activities continuously enhanced (*Fig. 5*). In M5, the promoting effect of mPLA on SOD and CAT activities was the strongest ($P < 0.05$), with enzyme activities increased by 50.00% and 47.83%, respectively. The effect of mPLA treatment on POD activity showed a promoting effect, but it was not significant ($P > 0.05$) (*Fig. 4II*). The effect of microplastic treatment on MDA content in mulberry leaves showed an overall promoting effect, with an increasing

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trend as the concentration of microplastics increased (*Fig. 5*), but MDA content was significantly increased only in the mPLA treatment group at M5, by 60.87% (P < 0.05).

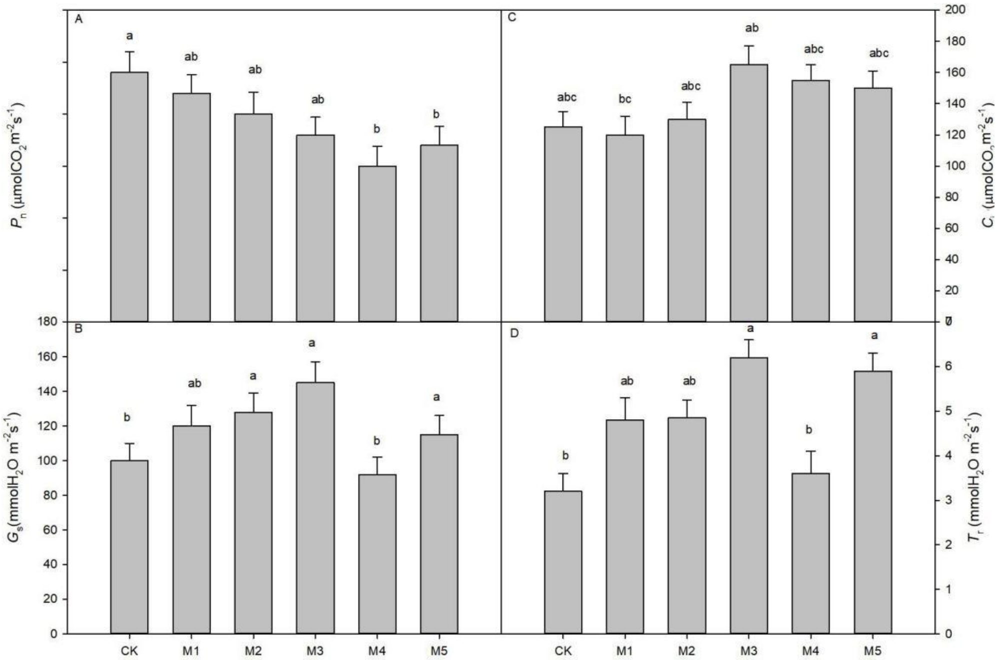

Figure 4. *Effects of microplastics on photosynthetic characteristics of mulberry. The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P <0.05)*

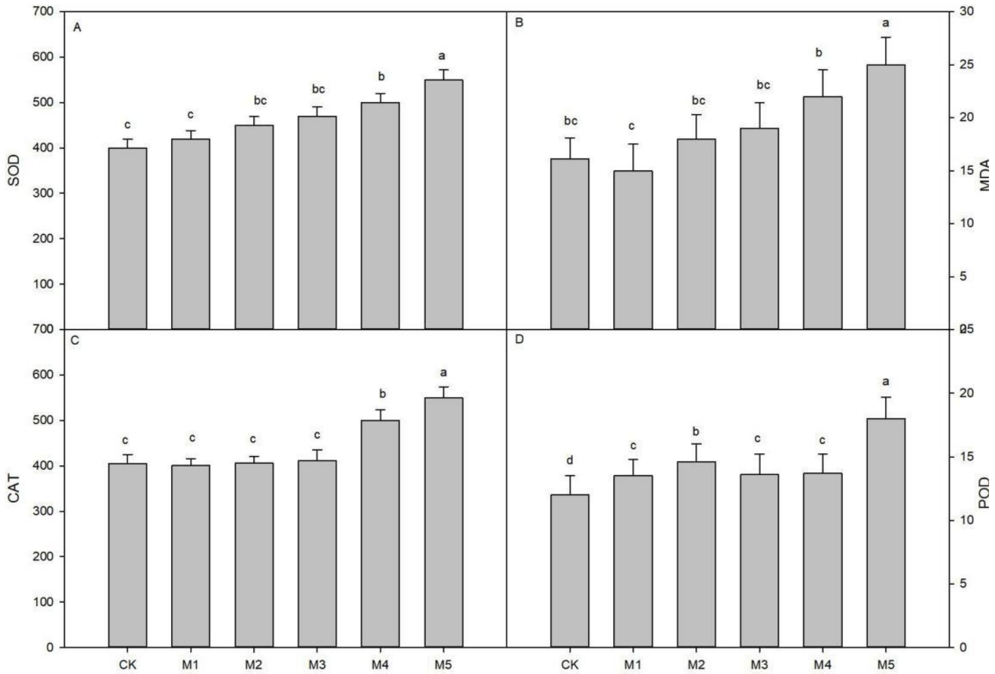

Figure 5. *Effects of microplastics on antioxidant properties of mulberry. The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences (P <0.05)*

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DOI: http://dx.doi.org/10.15666/aeer/2301_13651376

Discussion

Effects of microplastics on the growth characteristics of mulberry trees

Microplastics are an emerging pollutant, and currently, the impact of microplastics on higher plants has attracted widespread attention domestically and internationally, but there is little research on the effects of soil microplastics on economic forests (Cole et al., 2013; Liu et al., 2017; Cao et al., 2021). In this study, microplastic treatment affected the growth and development of mulberry trees, with higher concentrations of mPLA exerting stronger inhibitory effects on mulberry biomass. Similar to the trend of microplastic effects on mulberry biomass, the root system of mulberry trees in the mPLA treatment showed a clear negative response to microplastic stress. Microplastics can also adsorb pollutants from the soil and promote their transport to the above-ground parts of plants, enhancing the toxic effects of pollutants on plants, which may be one of the reasons for the reduced above-ground biomass of mulberry trees. Studies have shown that microplastics can be absorbed by plant roots and transported to the vascular bundles and leaves of the stem (Barnes et al., 2009; Asik et al., 2015). These microplastics within plants can be transferred and accumulated through the food chain, ultimately entering the human body and threatening human health.

Effects of microplastics on photosynthetic characteristics of mulberry trees

Chlorophyll can absorb, transfer, and convert light energy and is an essential substance for plants to perform photosynthesis (Abdulkhalid et al., 2008; Dabrowski et al., 2015; Cao et al., 2017). The results of this study showed that high concentrations of mPLA treatment significantly reduced the total chlorophyll content in mulberry trees, indicating that the damage to the photosynthetic system of mulberry leaves is related to the type and concentration of microplastics. In the mPLA treatments (*Fig. 3*), the net photosynthetic rate (Pn) of mulberry trees was significantly reduced, and the trend of increased inhibition with higher concentrations also supports this finding. Specifically, in the treatments with mPLA, the net photosynthetic rate (Pn) varied significantly, with a noticeable reduction observed in the M4 and M5 treatments.

In addition, the trends of stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were also affected. The Gs values in the treatments showed significant differences, with M3 and M5 treatments having the highest Gs values, indicating changes in stomatal function. The Ci values exhibited a similar trend, with higher concentrations of mPLA leading to variations in Ci, especially in the M2 and M3 treatments. The Tr values followed a pattern where the highest rates were observed in the M3 and M5 treatments, demonstrating the impact on water use efficiency and gas exchange.

Currently, many studies have examined the effects of microplastics on the photosynthetic system of plants, but fewer studies have focused on the mechanisms of their impact, mainly addressing the effects of microplastics on plant photosynthetic pigments (Oquist and Huner, 1993; Cao et al., 2007; Qin et al., 2017; Bellasio et al., 2018;). For example, some studies have found that the trend of SPAD value changes in soybean (*Glycine max*) seedlings treated with microplastics was consistent with the trend of Fe content changes in roots, suggesting that polyethylene microplastics affect the SPAD value of soybean seedlings by regulating amino acid metabolism and increasing Fe content in roots. Other research suggests that microplastic pollution leads to the accumulation of reactive oxygen species (ROS) in plant cells, causing damage to

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the chlorophyll structure and resulting in a decrease in chlorophyll content in plant leaves. It has been proposed that the benzene rings produced by the degradation of polystyrene nanoplastics in cucumber (*Cucumis sativus*) leaves may be a major factor affecting chlorophyll metabolism.

In this study, the net photosynthetic rate of mulberry trees was significantly reduced, while the trends of Gs, Ci, and Tr were different, indicating that the impact of microplastics on the photosynthesis of mulberry trees is due to non-stomatal limitations (Ahmed et al., 2009; Santos et al., 2015; Huang et al., 2019; Zhou et al., 2020). Based on the trend of the impact of microplastic treatments on the total chlorophyll content in leaves in this experiment, it is possible that aged and decomposed nanoplastics are absorbed and accumulated by mulberry trees, damaging the chloroplast structure and hindering the synthesis of photosynthetic pigments, thereby reducing the photosynthetic rate of plants (Hetherington et al., 2003; Song et al., 2018; Tie et al., 2020).

Effects of microplastics on antioxidant properties of mulberry trees

When plant cells are exposed to adverse environments, the antioxidant defense system can protect plant cells from oxidative damage by scavenging ROS (Li et al., 2021). SOD can convert excess oxygen free radicals in plants into H_2O_2 and O_2, while CAT and POD are involved in the removal of H_2O_2, converting it into harmless molecular oxygen and water, thus maintaining the steady-state level of oxygen free radicals in plants (Li et al., 2020; Gong et al., 2022). Studies have shown that high concentrations of PS-MPs can overcome the stress resistance of rice (Oryza sativa) seedlings, inhibit the synthesis of certain proteins in rice, and reduce the activities of SOD and CAT, leading to the accumulation of ROS in rice seedlings. In contrast, some studies found that the activities of SOD, POD, and CAT showed an increasing trend with higher microplastic concentrations to mitigate the toxic effects of microplastic pollution on soybeans, which is evidenced by the reduction in MDA content. Similarly, the results of this study showed that high concentrations of mPLA treatment enhanced the activities of SOD, POD, and CAT, indicating that mulberry plants produced a stress response due to oxidative damage, with the antioxidant system reducing ROS accumulation to mitigate the negative effects of microplastic addition. Notably, the MDA content was also elevated, indicating that the excessive production and accumulation of ROS led to a high degree of lipid peroxidation in the cell membranes of mulberry trees, causing cell membrane damage and thereby increasing MDA content (Andrady, 2013; Galloway et al., 2014). This indicates that the plant's antioxidant defense system could not eliminate the negative effects brought by high concentrations of microplastics, resulting in damage to the mulberry plants.

The observed changes in plant physiology, particularly in photosynthetic and antioxidant enzyme activities, are likely due to the direct interaction of microplastics with plant tissues, which either physically obstructs nutrient and water uptake or induces oxidative stress through the accumulation of reactive oxygen species (ROS). This conclusion aligns with findings by Liu et al. (2017), suggesting that high concentrations of microplastics may limit resource availability, thereby affecting physiological processes.

Conclusion

The addition of microplastics has certain stimulating and toxic effects on mulberry trees, and the impact varies depending on the concentration of the microplastics. High

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concentrations of polylactic acid reduced the total chlorophyll content and net photosynthetic rate of mulberry trees, damaging the photosynthetic system, affecting photosynthesis, thereby inhibiting the growth of mulberry plants, and the effect is stronger than that of low-density polyethylene. Mulberry trees can regulate their own antioxidant enzyme systems (SOD, POD, CAT) to alleviate oxidative damage caused by microplastics, but their effect is limited and cannot eliminate the negative impacts caused by the addition of high concentrations of microplastics.

Funding. Key Laboratory foundation of Heilongjiang province China (No. 2019-HXYKF-08).

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DOI: http://dx.doi.org/10.15666/aeer/2301_13651376

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