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Original Research

Response of Tobacco (*Nicotiana tabacum* L.) Growth to Soil Microplastic Pollution

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Abstract:

Microplastics (MPs) have distributed in agricultural soil. However, the effects of MPs on the growth of tobacco remain unclear. In this study, a pot experiment was conducted to evaluate the effects of linear low-density polyethylene (LLDPE) MPs at four different concentrations (0 $mg \cdot kg^{-1}$ as control, 10 $mg \cdot kg^{-1}$, 100 $mg \cdot kg^{-1}$, and 1000 $mg \cdot kg^{-1}$) on soil enzyme activity, physiological characteristics, and tobacco growth. The results showed that compared with the control, the treatments of 100 and 1000 $mg \cdot kg^{-1}$ significantly inhibited the activities of soil catalase (S-CAT) and soil sucrase (S-SC). Compared with the control, the 1000 $mg \cdot kg^{-1}$ treatment significantly altered root morphology, inhibited tobacco growth, and water content, resulting in a significant decrease in chlorophyll *a* content, catalase (CAT) and superoxide dismutase (SOD) activities in tobacco leaves, thereby incited a significant increase in malondialdehyde (MDA) content and peroxidase (POD) activity. Interestingly, the 10 $mg \cdot kg^{-1}$ treatment stimulated the activity of soil urease (S-UE) and root biomass. Overall, this study highlights the significant impact of MPs on soil enzymes, oxidative damage to tobacco, and inhibition of tobacco growth and development. It emphasizes the environmental risks of MPs pollution in soil, particularly for commercial crops like tobacco, and provides insights for controlling MPs abundance in the environment. Further research is needed to investigate the underlying mechanisms of MPs' effects on metabolism and genes in the soil-tobacco-microbial system.

Keywords: microplastics; tobacco; root morphology; oxidative stress; soil enzymes

Introduction

Microplastics (MPs) are plastic particles smaller than 5 mm in size occurring in the environment and are an emerging global pollutant [1]. The leading causes of pollution in the terrestrial environment include anthropogenic activities such as aerial deposition [2], manufacturing [3], and use of agricultural plastic films [4]. It takes over 100 years for MPs in low-light and low-oxygen soils to decompose completely [5]. MPs have high hydrophobicity and a high specific surface area, which makes them susceptible to the adsorption

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of environmental pollutants and pathogens onto their surface [6]. MPs can be transported and enriched through the food chain [7], which lead to negative impacts on biota and ecosystem functions [8].

Agricultural soils are the main sites of long-term accumulation of MPs [9]. Studies have shown MPs can alter soil properties [10], leading to changes in soil enzyme activity [11], which affects the uptake of different substances by plant roots, impacting nutrient cycling and organic matter decomposition [12]. MPs adhere to the surface of roots and block their pores [13] and are also absorbed and accumulated by the roots and transferred to aboveground tissues, leading to changes in the physiological and biochemical properties of plants and affecting growth, photosynthetic pigments, metabolism, and enzyme activity [14]. MPs lead to reduced lettuce leaf area [15], maize root biomass [16], and chlorophyll content [17]. MPs induce oxidative stress in plants and disrupt the ultrastructure of organelles, affecting their overall shape and function [18]. MPs inhibit mitosis in onion root tips and induce chromosomal and nuclear aberrations leading to genomic instability and other toxic effects [19].

Tobacco is one of the main cash crops in China, and the production of quality tobacco leaves plays a crucial role in the country's economy [20]. Previous studies have mainly centered on grain and vegetable crops such as wheat [14], lettuce [15], corn [16], rice [21], and beans (*Phaseolus vulgaris* L.*)* [22], with most of them being experimental studies on hydroponics. However, there is still a lack of research on soil cultivation. Our study is based on the average concentration (40 mg kg^{-1}) of MPs in soils of various regions of China [23], combined with previous studies [24], in which significant changes in the plants were observed. We used MPs concentrations higher than the environmental reality to simulate possible acute toxicity in short-term trials, comparing differences in soil enzyme activity, tobacco root morphology, stress resistance enzymes, chlorophyll, and other physiological and growth parameters at different MP concentrations. The objectives of our study are as follows: (1) evaluate the effects of different concentrations of MPs on soil enzyme activity; (2) explore the effects of different MPs concentrations on tobacco root morphology and root activity; (3) investigate the effects of different MPs concentrations on tobacco physiological growth and stress resistance. We aimed to understand the effects of MP abundance in the environment on tobacco and soil, with the goal of providing a theoretical basis for identifying the environmental risk of MP contamination in tobacco field soils and controlling the abundance of MPs in the environment.

Materials and Methods

Experimental Materials

The experimental tobacco variety was *Nicotiana tabacum* L. variety Yunyan 87. The MP used was linear low-density polyethylene (LLDPE) powder with a particle size of 13 μm, purchased from Feihong Plastic Chemical Co. The test soil was obtained from the 0–20-cm cultivated layer of clean soil located in Mengba Village, Pingba District, Anshun City, Guizhou Province, China. No history of plastic mulch application was recorded, and no plastic pollution has been observed on site. The soil was naturally dried in a cool place. Then, stones, dead leaves, and other debris were removed, and the soil was sieved through a 20-mesh sieve and used for the experiment.

Experimental Site and Methods

This experiment was conducted from January to March 2023 and was located at the Guizhou Academy of Tobacco Science. In this experiment, the concentrations of MPs were designed as 0 mg kg-1 (control), 10 mg kg^{-1}, 100 mg kg^{-1}, and 1000 mg kg^{-1}. Among these, the concentration of 10 mg kg^{-1} was considered low, 100 mg kg^{-1} was considered medium, and 1000 mg kg^{-1} was considered high. The concentrations of MPs were chosen based on the average MPs concentration (40 mg kg^{-1}) in soils of various regions in China [23]. In addition, combined with previous studies [24] significant changes in the plants were observed. MPs concentration higher than environmental reality was used to better assess the possible acute toxicity of MPs in short-term trials. For each treatment, 5 kg of soil was mixed with MPs and placed in pots to stabilize for 7 days. The floating seedling method was used to cultivate tobacco. When the tobacco grew 2 leaves and 1 bud, the most robust and uniform tobacco was selected and transplanted into pots at one seedling per pot. Ten replicates of 30 plants were set up for each treatment and watered regularly every day. After 45 d of cultivation under natural light, we took samples of tobacco inter-root soil and plants, we washed the tobacco with ultrapure water to measure the soil enzyme activity, root morphology, biomass, stress tolerance, chlorophyll content and agronomic traits.

Measurement of Soil Enzyme Activities

Soil urease (S-UE) activity was measured by the indophenol blue colorimetric method, soil sucrase (S-SC) activity was measured by the 3,5-Dinitrosalicylic acid (DNS) method, and soil catalase (S-CAT) activity was measured by the UV absorption method [25]. These indicators were measured using a multifunctional enzyme labeller (Synergy H4, Biotek, Winooski, VT, USA).

Determination of Root Morphology and Root Activity

A scanner (EPSON Expression 1000XL, Canada) was used to scan the root samples, and the scans were then analyzed using WinRHIZO software (Regent Instruments Inc., Quebec) to determine the following root architecture parameters (including root length, root volume, root surface area, average root diameter, number of root tips, number of root forks and number of root intersections).

Root length density was calculated by the formula root length density = root length/root volume, and root activity was measured by the 2, 3, 5-Triphenyte-trazoliumchloride (TTC) method [26].

Measurement of Physiological Indicators

According to YC/T 142-2010 standard "Investigating and Measuring Methods of Agronomical Character of Tobacco", the plant height, stem circumference, number of leaves, leaf length and leaf width of tobacco were measured, and calculated the leaf area that is

leaf area = leaf length × leaf width × 0.6345
(leaf area index of tobacco).

The fresh weight of the aboveground and belowground parts of the tobacco was determined using an electronic balance (JCS-ZI, Harbin Zhong Hui Weighing Instrument Co., Ltd.). The samples were dried at 105 °C for 30 min to kill-green and then dried at 65 °C to constant weight and recorded. Functional leaves were selected to determine chlorophyll content, malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, catalase (CAT) activity, and peroxidase (POD) activity. Chlorophyll content was measured by the spectrophotometric method [27]. MDA content was measured by the thiobarbituric acid colorimetric method [28], SOD activity was measured by the nitrogen blue tetrazolium method [29], CAT activity was measured by the UV absorption method [30], and POD activity was measured by the guaiacol method [31]. These indicators were measured using a multifunctional enzyme labeller (Synergy H4, Biotek, Winooski, VT, USA).

Data Processing and Analysis

The data were processed using Excel 2016, and SPSS 22.0 was used to calculate the means and standard deviations for all treatment replicates. One-way ANOVA ($p < 0.05$) followed by Tukey's post hoc test was used to determine significant differences between treatments and control. GraphPad Prism 9.0.0 software was used for data visualization.

Results and Discussion

Results

Effects of Different MP Concentrations on Soil Enzyme Activities

The activities of S-CAT, S-SC and S-UE in soil were affected differently by MPs (Fig. 1). Compared with the control, the treatments of 100 mg kg^{-1} and 1000 mg kg^{-1} significantly ($p < 0.05$) inhibited the activities of S-CAT, S-SC, which were decreased by 18.44% and 25.12% (S-CAT), 18.15% and 29.30% (S-SC), respectively.

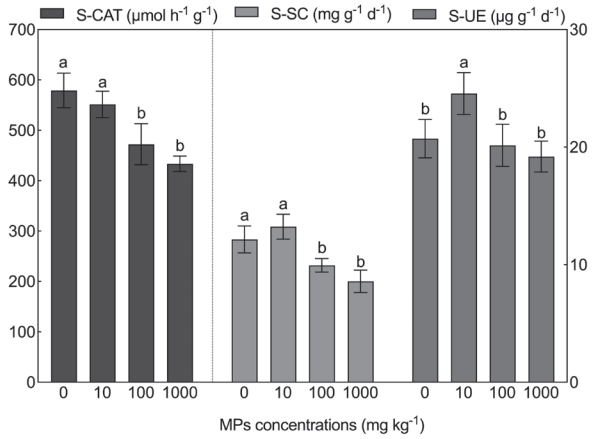

Fig. 1. Effects of different MP treatments on soil enzyme activities. Note: Different lowercase letters indicate significant ($P < 0.05$) differences between treatments, and the same applies below. MPs: Microplastics; S-UE: Soil urease; S-SC: soil sucrase; S-CAT: soil catalase.

Compared with the control, 10 mg kg^{-1} treatment significantly increased S-UE activity, the S-UE activity of 100 mg kg^{-1} and 1000 mg kg^{-1} of MPs treatment was inhibited, and significantly decreased by 17.93% and 21.85%.

Effects of Different MP Treatments on Root Morphology and Root Activity

The effects of different MPs concentrations on tobacco root morphology were shown in Figure 2. The results showed that compared to the control, 1000 mg kg^{-1} significantly inhibited root length density, root length, tips number, forks number, cross number, surface area, and average diameter of tobacco. Specifically, the treatment of 1000 mg kg^{-1} decreased these parameters by 22.95%, 43.87%, 64.96%, 46.83%, 33.44%, 44.72%, and 10.36%, respectively. The effects of different MPs treatments on tobacco root activity (Fig. 2) showed that the root activities of 10 mg kg^{-1}, 100 mg kg^{-1} and 1000 mg kg^{-1} of MPs were 0.14 μg min^{-1}ml^{-1}, 0.29 μg min^{-1}ml^{-1}, and 0.22 μg min^{-1}ml^{-1}, which were decreased by 52.49%, 2.51%, and 24.84%, compared with the control, respectively. The result indicated that both the growth and activity of roots were negatively affected by exposure to MPs.

Effects of Different MP Treatments on Biomass and Moisture Content of Tobacco

The effects on tobacco biomass under different MPs concentrations were shown in Figure 3. The shoot biomass, root biomass, and root-shoot ratio of tobacco reached the maximum value at 10 mg kg^{-1}. Compared with the control, root biomass, and root-shoot ratio of the 10 mg kg^{-1} treatment were significantly stimulated, increasing by 67.61% and 34.52%, respectively. However, there was no significant difference observed in the 100 mg kg^{-1} and 1000 mg kg^{-1} treatments.

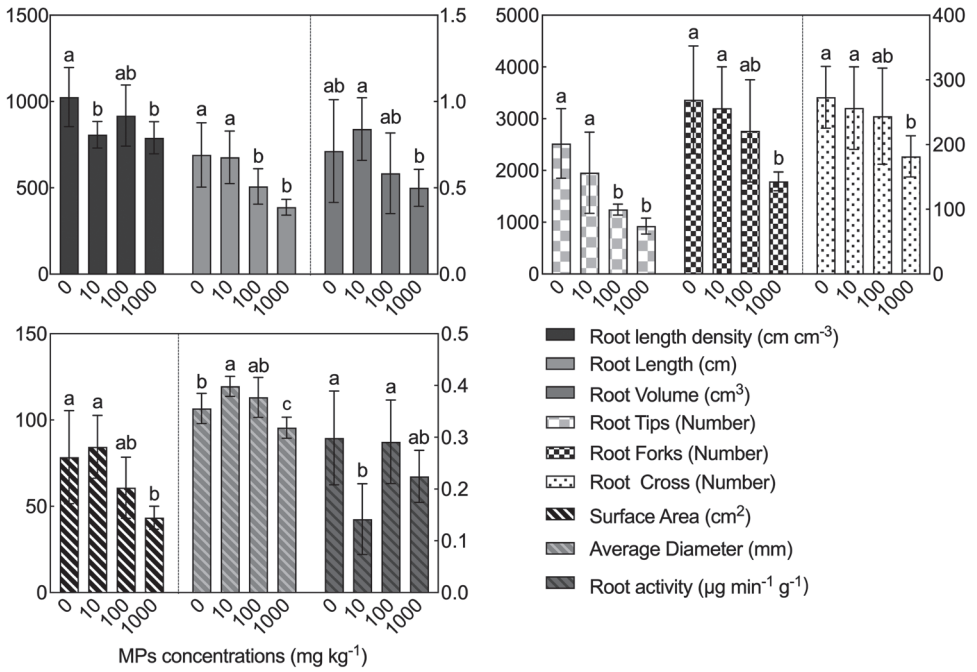

Fig. 2. Effects of different MP treatments on root morphology and root activity of tobacco. MPs: Microplastics.

As shown in Table 1, compared with the control, the shoot and root water content of 10 mg kg^{-1}, 100 mg kg^{-1}, and 1000 mg kg^{-1} were significantly reduced. The shoot water contents of 10 mg kg^{-1}, 100 mg kg^{-1}, and 1000 mg kg^{-1} of MPs were reduced by 1.38%, 2.61%, and 2.90%, respectively, while the root water contents were reduced by 2.37%, 4.43%, and 5.17%.

Effects of Different MP Treatments on Stress-Resistance Enzymes and Malondialdehyde

As shown in Figure 4, the CAT and SOD activities of tobacco were significantly inhibited, while the activity of POD was significantly increased at the concentration of 1000 mg kg^{-1} of MPs. The CAT activity of 1000 mg kg^{-1} treatment was reduced by 32.81% compared to the control. The SOD activity of 10 mg kg^{-1} treatment was 215.43 U^{-1}g^{-1}, which was significantly higher than that of other treatments. Compared with the 10 mg kg^{-1} treatment, the SOD activity of the 100 mg kg^{-1} and 1000 mg kg^{-1} treatments decreased sharply by 38.47% and 55.45%, respectively. In comparison to the control, the activity of POD treated with 1000 mg kg^{-1} of MPs increased by 39.79%, while the content of MDA of 10 mg kg^{-1}, 100 mg kg^{-1}, and 1000 mg kg^{-1} treatments increased by 6.53%, 16.26%, and 28.99%, respectively. Notably, the MDA content of the 1000 mg kg^{-1} treatment was significantly higher than that of the control.

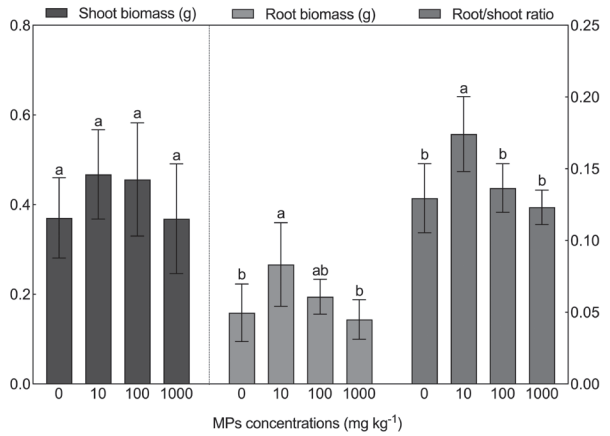

Fig. 3. Effect of different MP treatments on shoot and root biomass of tobacco. MPs: Microplastics.

Effects of Different MP Treatments on Chlorophyll in Tobacco

The effects of different MP treatments on tobacco chlorophyll were shown in Figure 5. The results showed that the contents of chlorophyll *a*, chlorophyll

Table 1. Effect of different MP treatments on the water content of tobacco.

Index	Control	10 mg kg^{-1}	100 mg kg^{-1}	1000 mg kg^{-1}
Shoot water content	0.93±0.01a	0.91±0.01bc	0.90±0.03c	0.90±0.01c
Root water content	0.94±0.01a	0.91±0.01b	0.89±0.02c	0.89.01c

Note: Different letters after the data in the same row indicate that the difference between different treatments reaches a significant level ($P < 0.05$) and the same applies below.

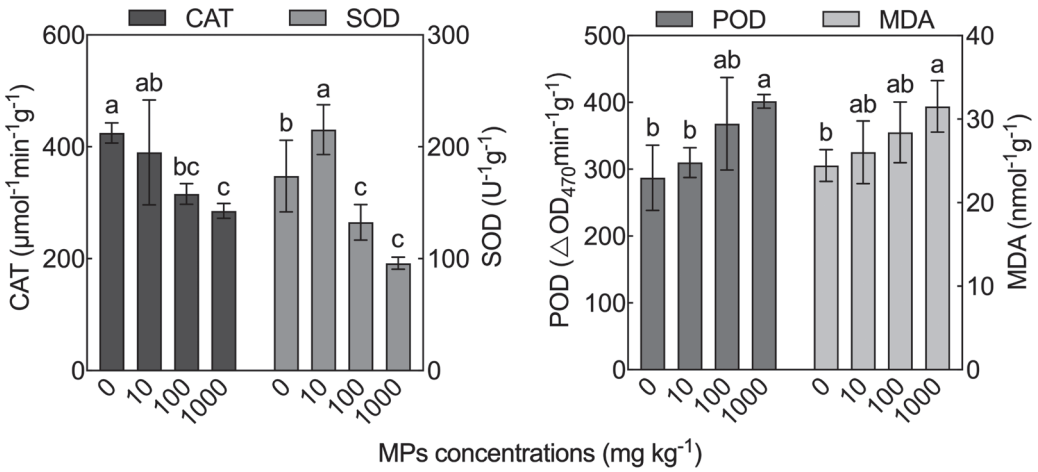

Fig. 4. Effects of different MP treatments on the activities of three antioxidant enzymes and content of MDA in tobacco leaves. MPs: Microplastics; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; MDA: malondialdehyde.

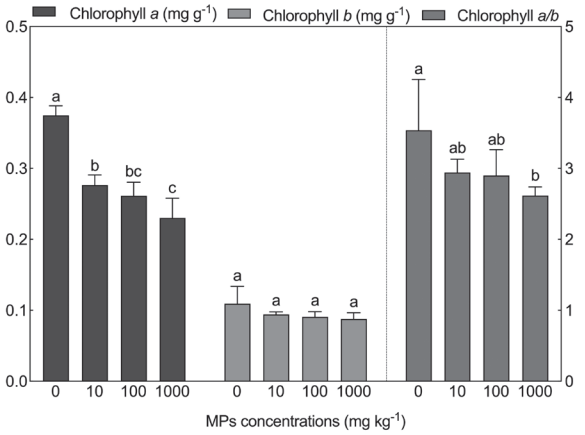

Fig. 5. Effects of different MP treatments on the chlorophyll in tobacco leaves. MPs: Microplastics.

b, and chlorophyll *a/b* of tobacco were as follows: control > 10 mg kg^{-1} > 100 mg kg^{-1} > 1000 mg kg^{-1}. The contents of chlorophyll *a* in 10 mg kg^{-1}, 100 mg kg^{-1}, and 1000 mg kg^{-1} treatments were significantly lower than control, and the content of chlorophyll *a/b* in 1000 mg kg^{-1} treatment was significantly lower than control, which indicated that the presence of MPs inhibited tobacco chlorophyll.

Effects of Different MP Treatments on Agronomic Traits of Tobacco

The effects of different MPs treatments on the agronomic traits of tobacco were shown in Table 2. The plant height, stem diameter, and the leaf area of the 1000 mg kg^{-1} treatment were all significantly lower than those of other treatments. Compared to the control, they decreased by 8.12%, 2.27% and 12.35%, respectively.

Discussion

Effects of MPs on Soil Enzymes and Tobacco Roots

Soil is the nutrient source for plant growth [32]. Microorganisms are crucial for soil nutrient turnover, and soil enzymes are essential players in microbial metabolism [33]. MPs have a direct effect on soil enzyme activity [34]. In our study, we observed that MPs disturbed soil enzyme activities. Compared to the control, the activities of S-CAT and S-SC were decreased under MPs treatment, particularly at the medium (100 mg kg^{-1}) and high (1000 mg kg^{-1}) concentrations. However, the S-UE activity was significantly increased under the treatment of low (10 mg kg^{-1}) concentration of MPs. Ma and Wang. [35] presented similar findings in the study of the impact of polypropylene (PP) MPs on soil enzyme activity. Their results indicated that presence of MPs inhibited S-CAT and S-SC activities while promoting S-UE activity. Fei et al. [12] also found that high-density polyethylene (HDPE) and polyvinyl chloride (PVC) MPs significantly promoted S-UE activity. A study done by Huang et al. [36] found that the addition of low-density polyethylene (LDPE) did not result in a significant change in S-SC activity throughout the experimental phase. While the activities of S-CAT and S-UE were significantly increased, different results may be due to the differences in the type of MPs concentration, soil conditions and exposure time. Studies have shown that MPs can change soil physical properties and nutrient conditions [37], resulting in changes in soil enzymes. The non-significant differences in S-CAT and S-SC activities at low concentrations of MPs may be due to the active or passive release of organic compounds by the tobacco root systems to remediate the contaminated soil [38], which maintains the S-CAT and S-SC activities at a steady state. In addition, the stress caused by medium and high concentrations of MPs exceeds the soil repair threshold, affecting the activity of enzymes related to the

Table 2. Effect of different MP treatments on agronomic traits of tobacco

Index	control	10 mg kg^{-1}	100 mg kg^{-1}	1000 mg kg^{-1}
Plant Height (cm)	5.77±0.56ab	6.24±0.86a	6.44±0.54a	5.20±0.89b
Stem Diameter (mm)	4.97±0.35a	5.08±0.42a	4.78±0.50a	4.10±0.28b
Number of Leaves	4.80±0.42a	4.80±0.42a	4.80±0.63a	4.80±0.42a
Leaf Area (cm^2)	82.69±12.12a	92.90±7.95a	82.06±14.19a	69.42±10.59b

nitrogen cycle and microbial genes [39], and reducing the content of nitrogen in soil, thus reducing the S-UE activity. The soil nutrient balance is disrupted, leading to a significant inhibition of the S-CAT and S-SC activities.

MPs reduce soil bulk weight, resulting in an increase in the number of aerobic microorganisms and a decrease in anaerobic microorganisms in the soil [40], and an increase in soil pore space and aeration, which in turn decreases rootability [41]. In this experiment, high concentrations of MPs significantly reduced the root length density of tobacco, limited the distribution of roots in the soil, and significantly inhibited the root length, root tips number, forks number, cross number, root surface area, and average root diameter of tobacco. S-UE activity was significantly correlated with soil organic carbon [42]. The release of dissolved organic carbon from PE-MP to the environment [43] resulted in a significant increase in S-UE activity at the MPs concentration of 10 mg kg^{-1}. Root volume, root average diameter and root surface area were stimulated, and root biomass increased, indicating that the root system adjusted to resist the stress brought by MPs. At this time, the root activity of tobacco was reduced, and the altered root metabolism affected the composition of root secretion, which further stimulated the activities of microorganisms around the root system, and promoted the root competitiveness [44]. S. Zhang et al. [45] showed that this may be due to the decreased nutrient status of the root environment at low concentrations of MPs, which stimulated a significant increase in the average root diameter. In our study, as the concentration of MPs increased, soil enzymes were inhibited, the root volume, root average diameter, and root surface area of tobacco were all suppressed, and the root biomass also decreased. It was possible that the strong adhesion of MPs led to a significant accumulation of MPs on the root surface, blocking the cell wall pores [46]. This hindered the growth of root hairs and restricted the entry of nutrients and water into the plant's internal tissues [41]. Consequently, the water content in tobacco decreased, further impacting changes in root tissue cells. Previous research found that increased concentrations of MPs resulted in a reduction in the length of meristematic tissues, thereby obstructing cell differentiation, division, and growth, leading to physiological disruption and tissue damage [47], ultimately inhibiting root growth and development.

In this study, the low concentrations of MPs significantly enhanced the root/shoot biomass ratio, indicating a greater impact of MPs on tobacco roots compared to shoots. Kleunen et al. [48] also confirmed that the roots of *Plantago lanceolata* L. were more susceptible to MP contamination in the soil compared to shoots due to direct contact between the roots and environmental MPs. Under exposure to MPs, the cell connections in V. *faba* roots are probably blocked, disrupting the transport of nutrients to the leaves [49]. The root system plays a crucial role in maintaining growth balance [50]. In response to individual competition, plants produce more roots to capture limited resources and reduce shoot growth [51]. Plants can adjust their morphological structure and allocate carbon assimilation products based on changes in environmental conditions, which reflect different survival strategies [52].

Effects of MPs on Chlorophyll Content and Stress Resistance of Tobacco Leaves

The results of the present study showed that compared to the control, CAT activity was significantly inhibited, POD activity and MDA content were significantly increased, and SOD activity was significantly increased at low concentrations and significantly decreased at medium and high concentrations. Jiang et al. [18] also found a decrease in CAT activity and an increase in POD activity in the study of the effect of 5 μm MPs treatment on *faba* beans. In comparison, [53] reported different results. They observed that CAT, POD and SOD activities, and MDA content of rice plants were stimulated in 10 μm treatment with polystyrene (PS) and polyvinyl chloride (PVC) MPs. Different results may be due to the differences in plants, incubation time, and type of MPs, etc. SOD, POD, and CAT belong to antioxidant systems [54] that play a synergistic role in protecting tobacco against oxidative stress. Among them, SOD and POD are responsible for scavenging excessive reactive oxygen species (ROS), while CAT helps in breaking down excess hydrogen peroxide, thereby preventing lipid peroxidation in plants [55]. The decrease in enzyme activity could be attributed to their involvement in antioxidant reactions aimed at scavenging free radicals. Conversely, the increase in enzyme activity may be a result of enhanced expression of genes encoding antioxidant enzymes in response to the excessive presence of free radicals. This regulatory response serves to mitigate the toxicity inflicted on plants by external stressors [56]. MDA is one of the products of membrane lipid peroxidation that occurs in plants under adverse conditions and can be used to indicate the degree of peroxidation [55].

In the present study, MDA content was consistently elevated compared to the control, indicating that oxidative stress on tobacco increased with the concentration of MPs. In addition, chlorophyll synthesis is a complex process involving multiple enzymes [57]. In this study, chlorophyll content was low. This finding aligns with the research conducted by Choudhury et al.[58], which demonstrated that under the stress of PE-MPs, the accumulated ROS in lettuce disrupted the structure of chlorophyll, leading to disturbances in chlorophyll metabolism and a subsequent decrease in chlorophyll content.

Taking the above into consideration, our study was consistent with the hypotheses that exposure to MPs had adverse effects on soil enzymes, inhibited root growth, and induced oxidative stress in tobacco. Previous studies have demonstrated that MPs alter soil microbial composition, inhibit lignin synthesis in roots, disrupt carbon fixation in leaves and impede ATP synthesis from ADP + Pi, resulting in changes in soil enzyme, obstruction of root cell wall formation, significant reductions in plants growth, development, oxidative stress, and impaired activation and absorption of nutrients [59]. However, due to the inadequate information available regarding the specific metabolic and gene expression responses of MPs in the soil-tobacco-microbial system, further studies are needed to explore the underlying mechanisms in greater detail.

Conclusions

The results of the study showed that MPs significantly affected soil enzyme activities, with S-CAT and S-SC activities significantly reduced compared to control, indicating that soil nutrient and microbial systems were disturbed. Consequently, the root system of tobacco experienced adverse effects, including a significant suppression of root length density, root length, number of tips, number of forks, number of crosses, root surface area, and average diameter. Additionally, the growth of root hairs was inhibited, leading to stress in root cells, which in turn limited the entry of nutrients and water into the internal tissues of the plant, and significantly reduced the water content of tobacco. The root system was significantly stimulated to resist the stress caused by MP at low concentrations, resulting in a significant increase in the average diameter of the root and root biomass. The root system and the aboveground part of the tobacco were interdependent and mutually restrictive. When the root system was stressed, the chlorophyll content of the tobacco was reduced, the MDA activity was elevated, and the activities of SOD, POD, and CAT were disturbed, which indicated that MPs damaged the oxidative system of the tobacco leaves and had a toxic effect on the tobacco.

In addition, MPs restricted root activity, indicating that the toxic effect of MPs on tobacco exceeded its own defense threshold, resulting in an imbalanced physiological and growth state of the tobacco. Therefore, we believe that MPs pose potential threats to the soil-tobacco system. In addition, MPs restricted root activity, indicating that when tobacco was stressed by MPs, defense mechanisms were turned on in the tobacco to reduce the harm. When the toxic effects on tobacco exceeded its own defense threshold, the physiological and growth status of tobacco was imbalanced, suggesting that MPs are a potential threat to the soil-tobacco system. However, despite our study demonstrating the adverse effects of MPs on soil enzymes and tobacco growth, significant challenges remain in identifying the ecological risks of MPs in soil-tobacco-microbial systems. Further research is needed to explore how MPs affect soil microbial community structure, microbial metabolic characteristics, and their regulation of metabolism and gene expression during tobacco growth.

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Conflict of Interest

The authors declare no conflict of interest.

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