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Effect of different microplastics on the mobilization of soil inorganic phosphorus by exomycorrhizal fungi

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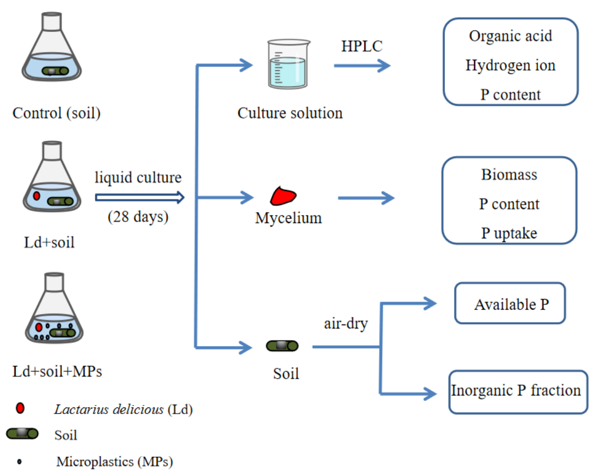
Graphical abstract



Abstract

Microplastics (MPs) can affect the phosphorus (P) cycle in soil by changing soil properties and the function of microbial communities, but their impact on soil P availability remains unclear. To explore the effects of different types of MPs on the mobilization of soil inorganic P by exomycorrhizal fungi, *Lactarius delicious* (Ld) was used as the test strain, and polystyrene microspheres (PS), polypropylene (PP) and poly (lactic acid) (PLA) were used as test materials. The soil was used as the only P source for the media. The effect of different types of MPs on the mobilization of soil inorganic P by exomycorrhizal fungi was studied using liquid culture method. The results showed that exposure to MPs at low to medium concentrations (10 or 100 mg L^{-1}) activated inorganic P in soil by stimulating the secretion of additional organic acids and hydrogen ions by ectomycorrhizal fungi, improving the available P in soil. The effect of mobilizing inorganic P was more significant in the treatment groups exposed to low and medium concentrations of biodegradable PLA. This may be related to the fact that PLA stimulated the ectomycorrhizal fungi to secrete more organic acids, and the release of a large amount of lactic acid after PLA was biodegraded. However, when the exposure concentration of MPs further increased, the growth of ectomycorrhizal fungal mycelium may be inhibited, the secretion of organic acids and hydrogen ions may be reduced, and the mobilization ability of soil inorganic P may be limited. In addition, it should be noted that high concentrations of non biodegradable MPs (PS and PP) had significant limiting effects on the mobilization of soil inorganic P by exomycorrhizal fungi. Therefore, our study results provide evidence that the presence of MPs may reduce the availability of soil P. Further research should focus on maintaining or improving P availability in plant-microbe systems in MP-polluted soil.

Keywords: Microplastics, ectomycorrhizal fungi, soil, inorganic phosphorus, phosphorus availability

1. Introduction

Plastics are shattered into smaller fragments under the action of environmental factors. These polymer fragments, which have a particle size less than 5 mm, have become an emerging pollutant in the environment - microplastics (MPs) (Wright and Kelly, 2017). In the ecosystem, the sources of MPs can be divided into two types: one is primary MPs, such as polyethylene microbeads contained in skin care creams, and directly synthesized polymers such as exfoliating agents in cleansers (Napper *et al.*, 2015). The other type is secondary MPs, which are produced by the further degradation of bulky plastics into small-sized plastic fragments under the actions of UV radiation, mechanical wear and biodegradation (Wang *et al.*, 2019). Due to the small diameter of MPs, they are easier to migrate, and pollution has become ubiquitous. There are a large amount of MPs in ecosystems such as lakes, oceans, and land, and even the human body develops MPs due to the laminar flow of the food chain (Li *et al.*, 2019). Compared with large plastic residues in the environment, MPs have a greater specific surface area and adsorption capacity, which are conducive to the adsorption of organic

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pollutants or heavy metal ions around the so-called “microplastisphere” (Yang *et al.*, 2022). It is a serious threat to soil health, biodiversity and the growth of plants and may even endanger human health and safety.

Over the previous two decades, studies on MPs have focused mainly on the source, distribution, migration and other characteristics of MPs and their ecotoxic effects in aquatic ecosystems, while few researchers have paid attention to their environmental behavior in terrestrial ecosystems (Guo *et al.*, 2023; Wang *et al.*, 2024). However, compared to the ocean, the annual release of MPs to soil may be 4-23 times greater than that in the oceans (Kumar *et al.*, 2020). Nizzetto *et al.* (2016) reported that approximately 300,000 tons of MPs accumulate in soil each year, which may exceed the total cumulative load of global ocean surface water. In addition, MPs entering the soil ecosystem not only directly affect the physicochemical properties of the soil but also have negative effects on soil nutrient cycling and the diversity and function of microorganisms (Guo *et al.*, 2020; Tang, 2023). It is important to evaluate the environmental risk of MPs to soil biogeochemical cycles.

Phosphorus (P) is an essential macroelement for the growth of all plants The forest soils in southern China are mainly distributed in mountainous areas, and the soil layers are thin, highly weathered, and severely P deficient. In addition, forest fertilization is generally not applied, so the mobilization and utilization of insoluble P in the soil is very important for tree nutrition (Zhang *et al.*, 2014). Ectomycorrhizal fungi are an important component of forest ecosystems. After mycorrhizae form with tree roots, they can promote the uptake of P by the host plant (Campos *et al.*, 2018). Several studies have shown that, due to the infection of mycorrhizal fungi, eucalyptus and pine plants form very thin mycelial sheaths on the root surface, which increase the surface area of the root system and can absorb extremely low concentrations of phosphate ions in the environment for plant uptake and utilization (De Oliveira *et al.*, 2022; Meeds *et al.*, 2021; Nworie *et al.*, 2017). MPs can further degrade to the micron or even nanolevel under the action of UV radiation, mechanical wear, and biodegradation (Sun *et al.*, 2022). Because the MP particles themselves strongly adhere, they are easily captured by secretions such as polysaccharide mucus, organic acids, and proteins produced by plant root - ectomycorrhizal fungal symbionts and more easily enter cells or organisms (Li *et al.*, 2022). Liu *et al.* (2023) reported that polystyrene MPs could significantly reduce the biomass of ectomycorrhizal fungi and respond to the stress of MPs by secreting oxalic acid and hydrogen ions into the culture medium. MP exposure can change the P cycle in the soil environment by directly providing a P source or increasing soil aggregation and thus enhancing soil enzyme activity and the function of microorganisms (Rillig and Lehmann, 2020). Other studies have shown that adding non degradable MPs (polypropylene: PP and polyethylene: PE) to soil inhibits soil alkaline phosphatase activity and reduces the copy number of microbial functional genes involved in organic P mineralization (*phoD* and *phoX*) and inorganic P dissolution (*ppk* and *pqqC*), resulting in a significant decrease in soil available P content (Zhang *et al.*, 2023a). In addition, a recent study reported that polyvinyl chloride (PVC) significantly reduce the available P content in rice soil by affecting the abundance of the P-soluble microorganism *Bacillus* (Yan *et al.*, 2021). However, the lactic acid produced by the degradation of poly(lactic acid) (PLA) is conducive to the dissolution of inorganic phosphate (Prabhu *et al.*, 2019).

At present, there have been no report on the effect of MPs on fungal mobilization of insoluble P in soil. Therefore, it is particularly important to explore the mechanisms by which different types of MPs affect the mobilization of soil ineffective P by ectomycorrhizal fungi. This experiment added soil as the sole P source to liquid culture medium and studied the effects of three types of MPs on the mobilization of soil insoluble P by ectomycorrhizal fungi, aiming to provide more useful information for the application of ectomycorrhizal fungi in forestry production.

2. Materials and methods

2.1. Test materials

The ectomycorrhizal fungus *Lactarius delicious* (Ld) was used as the test strain, which was originally isolated from a yellow soil (pH 4.0) in the Jinfu Mountain, Chongqing, southwestern China. The strain was cultured for 14 days in Pachlewsk solid medium without P source at 25°C. The composition of the medium was as follows: 0.5 g L^{-1} ammonium tartrate, 0.55 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4$, 20 g L^{-1} glucose, 0.1 g L^{-1} vitamin B_1, 1.0 ml L^{-1} trace element mixture (containing 8.45 mg H_3BO_3, 5 mg $MnSO_4$, 6 mg $FeSO_4$, 0.625 mg $CuSO_4$, 2.77 mg $ZnCl_2$ and 0.27 mg $(NH_4)_2MoO_4$ per L), agar 15~20 g. The vessel was shaken until the solute was completely dissolved, the pH was adjusted to 5.5, the volume was made up to 1 L with deionized water, and the culture medium was steam sterilized at 121°C for 30 min.

The test soil was red earth developed from acidic igneous and sedimentary rocks, collected from the bottom soil of the *Pinus massoniana* forest in Longwang Mountain Nature Reserve, Anji, Zhejiang Province, China. The soil pH was 4.2, the organic matter content was 19.8 g kg^{-1}, the total nitrogen content was 1.1 g kg^{-1}, the total P content was 0.6 g kg^{-1}, and the available P content was 11.4 mg kg^{-1}. The soil was air-dried, ground and passed through a 0.5 mm sieve. 1.0 g soil was placed into a plastic tube (2 cm length × 1 cm diameter) closed at the two ends by microporous membrane (0.22 µm aperture), and sterilized by steam at 121°C for 120 min. During the cultivation of the ectomycorrhizal fungus, a plastic tube filled with soil was placed into the liquid medium. Water molecules, MPs, inorganic ions, and organic acids can freely enter and exit, but the soil will not pass through the filter membrane and enter the solution.

Polystyrene (PS), PP and PLA microspheres with a particle size of 100 nm (concentration of 100 g L^{-1}) were purchased from Jiangsu Zhichuan Technology Co., Ltd, and three MP stock solutions were diluted into 10 g L^{-1} mother solution with sterile water and stored in a refrigerator at 4°C.

2.2. Design of the experiment

Under ultraclean bench conditions, a certain volume of the three MP stock solutions (at a concentration of 10 g L^{-1}) was added to sterilized phosphorus-deficient Pachlewsk liquid medium (KH_2PO_4 in the medium was replaced with KCl) and subjected to ultrasonic dispersion treatment before and after dilution to prepare MPs at various concentrations (0, 10, 100, and 1000 mg L^{-1} P-free Pachlewsk liquid medium, pH 4.2). Then, 20 mL of the abovementioned media was added to 150 mL Erlenmeyer flasks, and a sterile plastic tube filled with soil was added to each Erlenmeyer flask. Finally, a piece of activated solid mycelium blocks (6 mm in diameter) was inoculated in liquid medium. The liquid culture medium without inoculation and without adding MPs but with one soil plastic tube added was used as the control group, and the rest were treated with the same inoculation, with 5 replicates for each treatment. After inoculation, the culture medium was placed in a shaking table at 25°C and 120 r min^{-1} for 28 days to prepare for relevant indicators.

2.3. Sampling and sample analysis

At the end of the cultivation, the mycelia was filtered, and the filtrate was collected. The pH change in the filtrate was subsequently measured using a PHSJ-4A pH meter. The P content in the filtrate was determined by the molybdenum blue colorimetric method (Ames, 1966). The biomass was determined after the mycelia were dried (105°C), and then the mycelia was wet digested with concentrated sulfuric acid - hydrogen peroxide. The P content in the digestion solution was measured using molybdenum blue colorimetric method (Peng *et al*., 2021).

The soil in the plastic tube was removed, air-dried naturally, and subsequently extracted via the Olsen method. The available P content in the soil was determined by the molybdenum blue colorimetric method (Khan *et al*., 2018). Soil inorganic P components were determined by the method of Chang and Jackson (1957). In this method, soil inorganic P components were sequentially extracted with 0.5 mol L^{-1} NH_4F for Al-P, 0.1 mol L^{-1} NaOH for Fe-P, 0.5 mol L^{-1} H_2SO_4 for Ca-P and 0.3 mol L^{-1} $C_6H_5Na_3O_7$-1.0 g $Na_2S_2O_4$-0.5 mol L^{-1} NaOH for O-P (occluded P) by shaking at 150 rpm in 30°C for 30 min (soil: solution ratio = 1:50). Analysis of inorganic P components in extraction solution using molybdenum blue method.

The contents of oxalic acid, malic acid, acetic acid, succinic acid and citric acid in the filtrate were determined using high-performance liquid chromatography (Model 1260, Agilent company, USA). The standard substance was chromatographically pure. The chromatographic conditions were as follows: Hi-Plex H organic acid analytical column (300 mm × 7.7 mm, 8 μm), the mobile phase was water (dilute sulfuric acid was adjusted to pH 2.5), the flow rate was 0.6 mL Min^{-1}, the sample injection volume was 20 μL, the column temperature was 60°C, and the UV detection wavelength was 210 nm.

2.4. Data statistics and analysis

The data were calculated using Microsoft Excel 2007 software. The SPSS statistics 21.0 data processing system was used for the statistical analysis, Origin 2021b was used for drawing, and Duncan's multiple range test was used for the difference test at the level of $p \leq 0.05$.

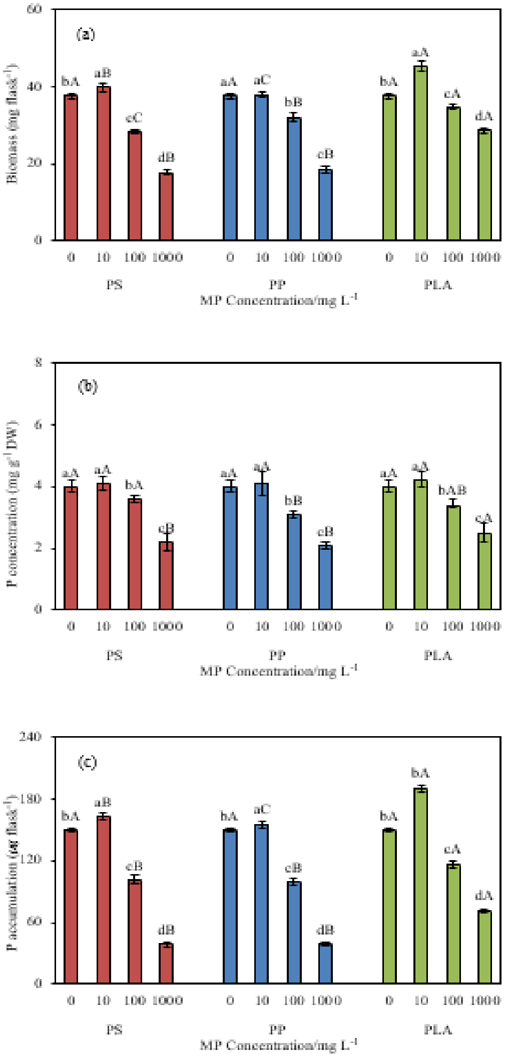

Figure 1. Effects of three MPs on the biomass (a), P content (b) and uptake capacity (c) of the Ld mycelium.

3. Results

3.1. Biomass, P content and P uptake

After 28 days of culture, the effects of the three types of MPs on the biomass of Ld are shown in Figure 1a. When exposed to low concentrations (10 mg L^{-1}) of PS and PLA, the biomass of Ld significantly increased. However, when exposed to MPs with medium to high concentrations, all three types of MPs significantly reduced the biomass of Ld mycelium. In addition, exposed to medium to high concentrations of MPs, all three types of MPs significantly reduced the P content of Ld mycelium (Figure 1b). When exposed to low concentrations of MPs, all three types of MPs significantly increased the P uptake of Ld mycelium, when exposed to MPs with medium to high concentrations, the P uptake by mycelium was significantly reduced (Figure 1c). Meanwhile, the biomass, P content, and P uptake of Ld mycelium showed significant differences due to different types of MPs, with the PLA treatment group significantly higher than the PS and PP treatment groups.

Note: Different lowercase letters indicate significant differences between different concentration treatment groups under the same microplastic exposure; Different capital letters indicate significant differences between different types treatment groups under exposure to the same concentration of microplastics ($p \le 0.05$), the same applies below.

3.2. pH and organic acids of the culture solution

As shown in Table 1, after 28 days of culture, four organic acids were detected, including oxalic acid, acetic acid, succinic acid and citric acid, with oxalic acid having the highest secretion. A small amount of oxalic acid was also detected in the cultures not inoculated with strain Ld (control group), but the oxalic acid content was so low that it could be ignored. After exposure to the three types of MPs, as the concentration of the MPs increased, the contents of oxalic acid and total organic acids in the cultures both increased first and then decreased. When exposed to a medium concentration (100 mg L^{-1}) of PLA, the content of oxalic acid and total organic acid is highest. The acetic acid content in the culture medium increases with the increase of MPs concentration. In addition, different concentrations of MPs have no significant effect on the secretion of succinic acid and citric acid by Ld. However, the content of oxalic acid and total organic acid in the culture medium showed significant differences due to different types of MPs. Under the same concentration conditions, the PLA treatment group had the highest content, followed by the PS treatment group, and the PP treatment group had the lowest content.

After exposure to the three types of MPs, the pH of the culture solutions all showed a trend of decreasing first and then increasing as the concentration of the MPs increased. In the PS treatment group exposed to medium concentration (100 mg L^{-1}), the pH in the culture medium decreased the most significantly, by 28.8% compared to the control group.

Table 1. The effect of three types of MPs on the secretion of organic acids by Ld (mg L^{-1})

Treatment	Concentration (mg L^{-1})	pH	Oxalic acid	Malic acid	Acetic acid	Succinic acid	Citric acid	Total organic acid
PS	Control	4.2aA	0.3eA	ND	ND	ND	ND	0.3eA
	0	3.7bA	78.3cA	ND	6.2bA	2.6abA	1.3aA	88.4cA
	10	3.1dB	95.4bB	ND	5.7cB	3.1aB	1.5aA	105.7bB
	100	3.0dA	152.6aB	ND	6.4bAB	3.4aB	1.4aA	163.8aB
	1000	3.3cB	65.2dB	ND	11.5aA	3.3aB	1.2aA	81.2dB
PP	Control	4.2aA	0.3dA	ND	ND	ND	ND	0.3dA
	0	3.7bA	78.3bA	ND	6.2cA	2.6bA	1.3aA	88.4bA
	10	3.6bcA	79.2bC	ND	6.7bA	4.1aA	1.3aA	91.3bC
	100	3.2dA	110.4aC	ND	6.8bA	4.4aA	1.5aA	123.1aC
	1000	3.5cA	54.2cC	ND	8.9aB	3.7abA	0.4bB	67.2cC
PLA	Control	4.2aA	0.3dA	ND	ND	ND	ND	0.3dA
	0	3.7bA	78.3cA	ND	6.2bA	2.6abA	1.3aA	88.4cA
	10	3.5cA	110.2bA	ND	5.8bB	2.4bC	0.8abB	119.2bA
	100	3.1dA	176.2aA	ND	6.9aA	3.2aB	1.1aA	187.4aA
	1000	3.5cA	80.4cA	ND	7.1aC	3.2aB	1.0aA	91.7cA

Note: Different lowercase letters indicate significant differences between different concentration treatment groups under the same microplastic exposure; Different capital letters indicate significant differences between different types treatment groups under exposure to the same concentration of microplastics ($p \le 0.05$). ND: not detected.

3.3. P content in the culture solution

In the culture solution, the control group had the lowest level of P, at 1.2 mg L^{-1} (Figure 2a). The group inoculated with strain Ld and without MP treatment had the highest P content (1.8 mg L^{-1}) in the culture solution, which was 1.5 times higher than the control group. Compared with the MPs treatment group exposed to 0 mg L^{-1}, the P content in the culture solution significantly decreased when exposed to three types of high concentrations (1000 mg L^{-1}) of MPs. However, there was no significant difference between different types of MPs.

Effects of three types and different concentrations of MPs on the soil available P content (Figure 2b). With increasing MPs concentration, the available P content in the soil first increased and then decreased. In addition, in the PLA treatment group, the available P content in the soil was significantly greater than that in the PS treatment group in general. In the PLA treatment group exposed to medium concentration (100 mg L^{-1}), the content of available P in the soil increased most significantly, with a 49.9% increase compared to the control group.

3.4. Soil inorganic P fractions

Variations in soil inorganic P fractions after 28 days of culture are shown in Figure 3. When the plants were inoculated with ectomycorrhizal fungi and supplemented with different concentrations of MPs, the soil Ca-P, Fe-P, Al-P and O-P concentrations decreased to varying degrees, and the total amount of inorganic P in the soil decreased significantly. The PLA treatment group exposed to 100 mg L^{-1} showed the greatest decrease in total soil inorganic P, while the PP treatment group exposed to 1000 mg L^{-1} showed the least decrease in total soil inorganic P, with a reduction rate of 8.8%.~ 28.0%.

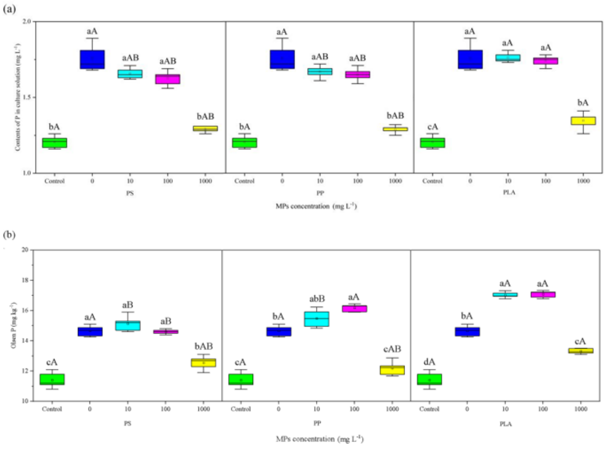

Figure 2. Contents of phosphorus (a) in the culture solution and available phosphorus (b) in the soil

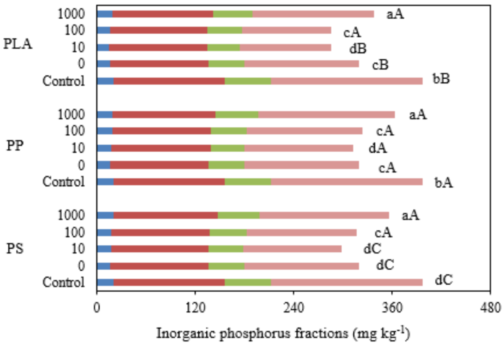

Figure 3. Variations of soil inorganic P fractions

After the inoculation of liquid media with ectomycorrhizal fungi and the addition of different concentrations of MPs, compared with the control group, except for the high concentration (1000 mg L^{-1}) PS and PP treatment group, all other treatments significantly reduced the Ca-P in the soil. Among them, the group exposed to 10 mg L^{-1} PLA treatment showed the greatest decrease in soil Ca-P, which was 25.1% lower than the control group (Figure 4a). In addition, exposed to three different types and concentrations of MPs, all treatment groups significantly reduced the content of Fe-P, Al-P, and O-P in the soil (Figure 4b, c, d). The PLA treatment group exposed to 100 mg L^{-1} showed the greatest reduction in soil Fe-P and O-P, with reductions of 12.7% and 41.4% compared to the control group, respectively. Moreover, exposed to the same concentration of MPs, the content of Ca-P and O-P in the soil showed significant differences due to different types of MPs. The group exposed to 10 mg L^{-1} of PLA showed the greatest reduction in soil Al-P, which decreased by 29.0% compared to the control group.

3.5. Relationships between soil available P (Olsen P) and inorganic P fractions, pH, and total organic acid

At the end of cultivation (after 28 days). There was a negative linear regression between the soil available P content and the inorganic P fractions (Fe-P and O-P) and pH in the culture solutions (Figure 5a, 5b). Moreover, There was a positive linear regression between the soil available P content and the total organic acid content in the culture solutions (Figure 5c).

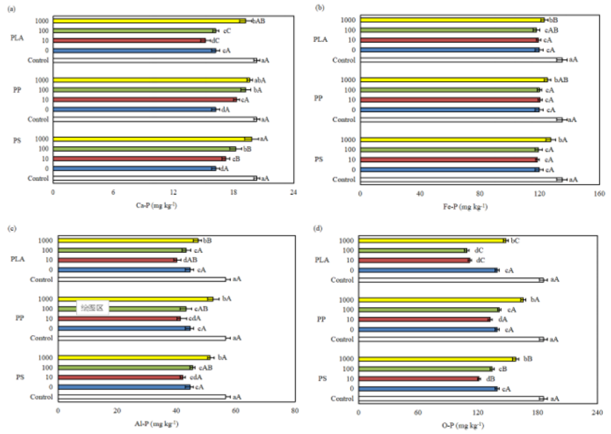

Figure 4. Changes in the Ca-P (a), Fe-P (b), Al-P (c) and O-P (d) contents in soils

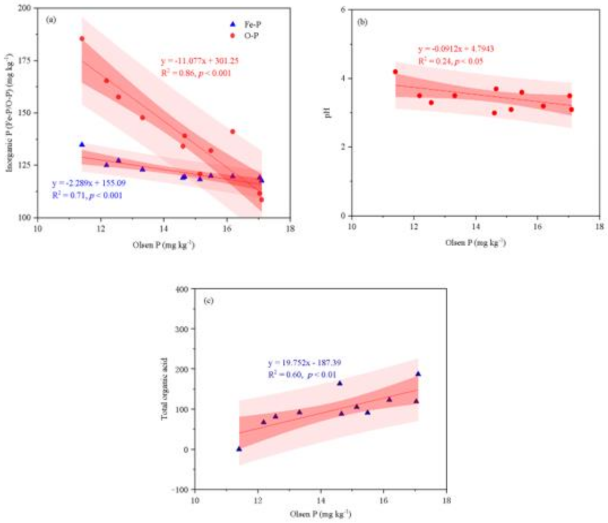

Figure 5. Relationships between soil available P (Olsen P) and Fe-P and O-P contents (a), pH (b) and total organic acid content (C)

A regression line was not drawn when the relationship was not significant ($p > 0.05$).

4. Discussion

Ectomycorrhizal fungi and tree roots can form symbionts, which are beneficial for plant resistance to adverse environments and the absorption of mineral elements (Campos *et al.*, 2018; Lehmann *et al.*, 2022). Li *et al.* (2022) reported that when exposed to high concentrations of polystyrene MPs, the biomass of ectomycorrhizal fungi was significantly inhibited, and the growth of fungi directly reflects their ability to withstand adverse environments. In the present study, Ld lived in phosphorus source-limited media and were exposed to a low concentration (10 mg L^{-1}) of MPs, compared with the control group, during which the biomass significantly increased. However, after exposure to medium and high concentrations of MPs, all three types of MPs significantly reduced the biomass of the Ld mycelium. Meanwhile, the P content and absorption in Ld mycelium also exhibit similar patterns. These results suggest that low concentrations of MPs can promote the uptake of P from the surrounding soil by ectomycorrhizal fungi. However, when the concentration of MPs exceeds a certain range, it will be unfavorable for fungi to absorb mineral elements from the environment, leading to poor mycelial growth.

The P in soil can be divided into inorganic P and organic P according to their existing forms. Inorganic P is the main component in soil, generally accounting for 60%-80% of the total P pool. It mainly exists as insoluble minerals, with low available P content, making it difficult for plants to utilize (Tang *et al*., 2023). P solubilizing microorganisms have multiple mechanisms that help dissolve fixed or unavailable P. The activation of insoluble inorganic P is produced by microorganisms through the secretion of organic acids, inorganic acids, extracellular polysaccharides, iron carrier proteins, and hydrogen ion efflux into the surrounding environment (Saeid *et al*., 2018; Wu *et al*., 2021; Remiszewski *et al*., 2016). Research has shown that ectomycorrhizal fungi (*Suillus* species) in pure culture can secrete oxalic acid and excrete hydrogen ions into the environment to mobilize insoluble phosphates in the soil (Peng *et al*., 2021). Other studies have shown that after plant infection, ectomycorrhizal fungi can prompt host plant roots to release hydrogen ions into the soil, dissolve insoluble phosphate through acid dissolution, and release phosphate and other important elements needed for plant growth (Meeds *et al*., 2021). A recent study revealed that, when exposed to low doses of MPs, buckwheat plants could resist the stress caused by MPs and even stimulate increased oxalic acid production in roots. The highest exposure dose (80 mg L^{-1}) decreased oxalic acid secretion from buckwheat roots (Zhang *et al*., 2023b). In this study, four organic acids, oxalic acid, acetic acid, succinic acid, and citric acid, were detected in liquid media among the treatment groups exposed to different concentrations of MPs. The group treated with MPs at medium concentrations (100 mg L^{-1}) showed the highest secretion of oxalic acid and total organic acids. In the treatment group exposed to high concentrations (1000 mg L^{-1}) of PS and PP, the secretion of oxalic acid was significantly lower than that of the group without adding MPs but inoculated with Ld. These findings indicate that low and medium concentrations of MPs can promote the secretion of organic acids by exomycorrhizal fungi, while exposure to high concentrations of MPs can inhibit the secretion of organic acids. This finding is basically consistent with previous study results.

Among the various low-molecular-weight organic acids, oxalic acid has the strongest ability to complex calcium, magnesium, iron, and aluminum, both $[Al(C_2O_4)_3]^{3-}$ and $[Fe(C_2O_4)_3]^{3-}$ have very high chelation constants of 16.3 and 20.2. Oxalic acid may therefore chelate Al^{3+} and Fe^{3+} in the insoluble phosphates such as Al-P, Fe-P and O-P, resulting in the solubilization of these phosphates and P release (Nworie *et al*., 2017; Ren *et al*., 2009). This study revealed that, compared with the treatment group without adding MPs but inoculated with Ld, the PLA treatment group exposed to 10 mg L^{-1} or 100 mg L^{-1} had a certain promoting effect on Ld mobilization of insoluble inorganic P in soil (Figure 4). This may be related to the fact that PLA stimulated the ectomycorrhizal fungi to secrete more organic acids, and the release of a large amount of lactic acid after PLA was decomposed by microorganisms. The high concentration (1000 mg L^{-1}) MP treatment group significantly inhibited the mobilization effect of Ld on soil inorganic P. Meanwhile, there was a negative linear regression between the soil available P content and the inorganic P fractions (Fe-P and O-P) (Figure 5a), while there was a positive correlation with the total organic acid content in the culture solutions (Figure 5c). This suggests that the organic acids secreted by ectomycorrhizal fungi are conducive to the mobilization of inorganic P in soil, thereby increasing the available P content in soil. However, exposure to high concentrations of MPs will weaken this mobilization effect. Additionally, in the present study, in the PS treatment group exposed to a medium concentration (100 mg L^{-1}), the reduction in the pH of the culture solution caused by ectomycorrhizal fungi was the most significant and was 1.2 units lower than that in the control group (i.e., the hydrogen ion concentration was 15.8 times higher than that of the control group), and there was a negative linear regression between the soil available P content and the pH of the culture solution (Figure 5b). Qi *et al*. (2022) reported that forest tree-mycorrhizal symbionts can effectively obtain inorganic P from soil through the release of organic acids and proton excretion. This indicates that exposure to low to medium concentrations of MPs is beneficial in stimulating ectomycorrhizal fungi to secrete more hydrogen ions into the soil, thereby mobilizing insoluble P in the soil and increasing the available P content.

Globally, increasing the availability of soil P by plants has been a major challenge (Zou *et al*., 2022; Mogollón *et al*., 2018). MPs are ubiquitous in soil ecosystems through different sources and migration, coupled with the scarcity of mineral nutrients in forest soil, which limits the growth of beneficial microorganisms around root system, thereby affecting the absorption of mineral elements such as P, potassium, calcium, and magnesium by the roots (de Souza Machado *et al*., 2019). After forming a symbiont with forest trees, ectomycorrhizal fungi help the dissolution and release of refractory P in soil and improve the efficiency of P uptake and utilization by plants. However, the effects of MPs on soil ecosystems and biogeochemical processes such as the P cycle are not well understood. Our results showed that MPs at medium and low concentrations stimulated the secretion of more organic acids and hydrogen ions by ectomycorrhizal fungi, which promoted the mobilization of soil inorganic P and improved the availability of P in the soil. However, when the exposure concentration of MPs further increased, the growth of ectomycorrhizal fungal mycelium may be inhibited, the secretion of organic acids and hydrogen ions may be reduced, and the mobilization ability of soil inorganic P may be limited. Moreover, the nanosized MPs used in this study had larger specific surface areas and greater electrostatic attraction, as well as various functional groups. This study result may underestimate the P adsorption capacity of MPs. In addition, in the future, the effect of different MPs on phosphorus availability in the plant–soil microbial system needs to be further investigated.

5. Conclusions

Our research findings emphasize that exposure to MPs at low to medium concentrations (10 or 100 mg L^{-1}) mobilizes inorganic P in soil by stimulating the secretion of additional organic acids and hydrogen ions by ectomycorrhizal fungi, improving the quality of soil P availability. Among them, the group treated with biodegradable MPs (PLA) exposed to medium to low concentrations significantly stimulated the mobilization of inorganic P in the soil by Ld. This may be related to the fact that PLA stimulated the ectomycorrhizal fungi to secrete more organic acids, and the release of a large amount of lactic acid after PLA was biodegraded. However, when the exposure concentration of MPs further increased, the growth of ectomycorrhizal fungal mycelium may be inhibited, the secretion of organic acids and hydrogen ions may be reduced, and the mobilization ability of soil inorganic P may be limited. In addition, it should be noted that high concentrations of nonbiodegradable MPs (PS and PP) had more obvious limiting effects on the mobilization of soil inorganic P by exomycorrhizal fungi. Overall, our research findings provide evidence that the presence of MPs may reduce the available P source for plant growth. Further research should focus on maintaining or improving the effect of abiodegradable MP pollution on plant–soil microbial system P availability.

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Not applicable.

Conflicts of interest

The authors declare no conflicts of interest.

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