

RESEARCH ARTICLE

Microplastics affect activity and spatial distribution of C, N, and P hydrolases in rice rhizosphere

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HIGHLIGHTS

- Microplastics (MPs) increased activities of N and P hydrolases in paddy soil.
- MP amount increased nutrient acquisition ratio and total enzyme activity.
- MPs lead to soil nutrient decreased through microbial action.
- MPs impact nutrient availability and agricultural ecosystem functions.

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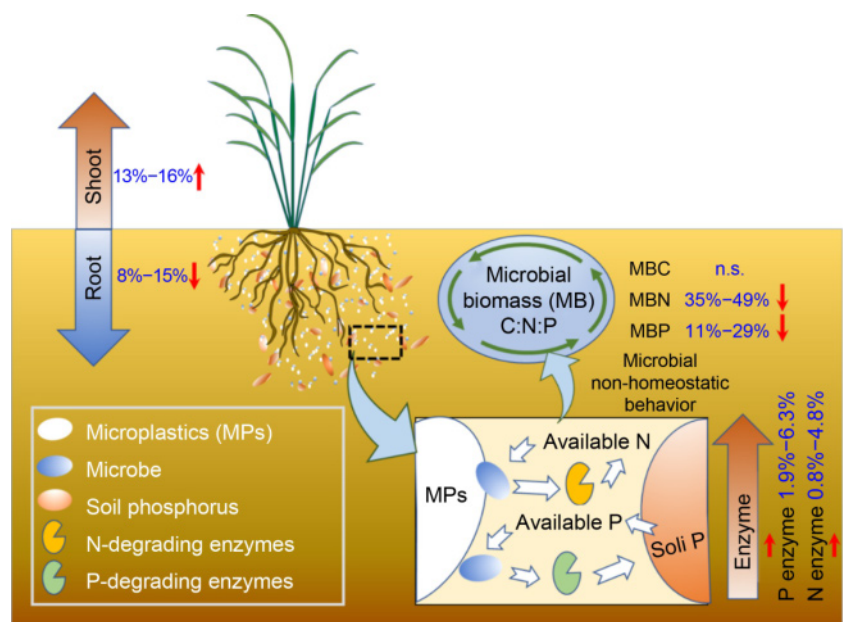
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GRAPHICAL ABSTRACT



ABSTRACT

Microplastics provide a new ecological niche for microorganisms, and the accumulation levels of microplastics (MPs) in terrestrial ecosystems are higher than those in marine ecosystems. Here, we applied the zymography to investigate how MPs – polyethylene [PE], and polyvinyl chloride [PVC]) at two levels (0.01% and 1% soil weight) impacted the spatial distribution of soil hydrolases, nutrient availability, and rice growth in paddy soil. MPs increased the above-ground biomass by 13.0%–15.5% and decreased the below-ground biomass by 8.0%–15.1%. Addition of 0.01% and 1% MPs reduced soil NH_4^+ content by 18.3%–63.2% and 52.2%–80.2%, respectively. The average activities of N- and P-hydrolases increased by 0.8%–4.8% and 1.9%–6.3% with addition of MPs,

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respectively. The nutrient uptake by rice plants and the enzyme activities in hotspots increased with MP content in soil. The accumulation of MPs in paddy soil could provide an ecological niche that facilitates microbial survival, alters the spatial distribution of soil hydrolases, and decreases nutrient availability.

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1 Introduction

The detrimental impact of microplastics (MPs) in marine ecosystems is well known (Cauwenberghe et al., 2014; Shivika et al., 2017), and the threats posed by them to terrestrial ecosystems are raising concerns (Liu et al., 2018; Ng et al., 2018; Rong et al., 2021). These concerns are supported by estimates that the accumulation of MPs in terrestrial ecosystems is far greater than that in the ocean (Luca et al., 2016; Horton et al., 2017; Alimi et al., 2018). In agroecosystems, compost, sludge, irrigation, and agro-plastics are the main pathways of MP input to farmland (Nizzetto et al., 2016; Steinmetz et al., 2016; Weithmann et al., 2018; Okoffo et al., 2021). For instance, plastic films are widely used on the soil surface of agricultural crops to improve productivity, with studies detecting a 2-fold increase in plastic fragments in soils with plastic mulching compared to soils without it (Zhou et al., 2020). Plastic film mulching practice is not commonly used in rice paddy soil. However, in paddy fields in water-stressed areas, the plastic film has been widely used to reduce water evaporation and to maintain grain yields (Qu et al., 2012; Liu et al., 2013; Yao et al., 2014). Lv et al. (2019) revealed that there are 12.1 ± 2.5 and 27.6 ± 5.9 items kg^{-1} of microplastics during non-rice and rice-planting periods in a rice-fish co-culture system, respectively. The polyethylene (PE) film and fiber, polypropylene (PP) fiber, and polyvinyl chloride (PVC) granules, which originate from the applications of plastic products, such as organic fertilizer and commercial fish diets, are the main sources for MP contamination into the rice-fish culture environment (Lv et al., 2019).

Polyethylene (PE) and polyvinyl chloride (PVC) are the most abundant types of MPs used in farmland ecosystems (Li et al., 2011; Zhao et al., 2017; Yang et al., 2015). Investigations of the impact of MPs on soil physical properties, the microbial community, and the plant nutrient ratio have mushroomed in farmland ecosystems in the last few years (Liu et al., 2017; Huang et al., 2019; Shin et al., 2021). However, few studies have linked the effects of MPs with soil nutrients and soil enzyme properties in farmland ecosystems. Microbes of specific communities (Feng et al., 2020; Tender et al., 2017) are inclined to attach to the surface of microplastics, which thus provide a new niche (Zettler et al., 2013). For instance, several fungi species with the potential to degrade PE have been found in soil contaminated by MPs (Sangale et al., 2019). In addition, Xie et al. (2021) reported that PE and PVC showed signs of biodegradation after three months of soil incubation. Fei et al. (2020) reported that MPs (PE and PVC) present in acidic soil stimulate phosphatase

activity. This is because microplastics increase the soil water holding capacity (De Souza Machado et al., 2018), which is positively correlated with soil acid phosphatase activity (Sardans and Peñuelas, 2005). The addition of 0.1% and 1% PVC MPs to paddy soil reduced soil available P content (Yan et al., 2020). Extracellular enzymes excreted by microbes provide an important medium for the cycling of soil nutrients (Schimel, 2003; Kujur et al., 2014; Cui et al., 2019). For instance, microorganisms accommodate nutrient uptake by allocating resources for C-, N-, and P-hydrolase secretion to achieve optimal growth (Allison et al., 2010). Soil hydrolase activity has been extensively measured for BG (β -glucosidase), XYL (xylanase), NAG (chitinase), and ACP (phosphatase), all of which are important for nutrient re-hydrolysis and maintaining soil fertility in paddy fields (Burns et al., 2013). Small changes to plant soil systems caused by MP addition might cause a series of impacts on ecosystem services over the long-term (Zang et al., 2020; Zhou et al., 2021). Therefore, it is important to explore how MPs affect soil hydrolases and soil nutrients.

Due to the characteristics of soil heterogeneity, conventional techniques used to analyze soil enzymes cannot provide accurate extracellular information (Wei et al., 2019a; Andrey et al., 2021). Soil *in situ* enzyme spectroscopy is an emerging method for studying the nutrient acquisition strategies of plant roots and rhizosphere microorganisms (Spohn et al., 2013). The fluorescence-labeled substrate reacts completely with extracellular enzymes at the soil-micro interface, and the released fluorescence-groups are retained *in situ* and can emit visible light at about 460 nm under excitation light of 355 nm (Vepsäläinen et al., 2001). The emitted fluorescence intensity shows a good regression relationship with the amount of fluorescein released, and the fluorescence-group participating in the enzymatic reaction is equal to the amount of the degraded fluorescent substrate. The intensity and distribution of the emitted light can be captured by a camera. A high resolution of *in situ* quantitative detection of enzyme activity can be realized. MPs serve as new substrates for the colonization and formation of assemblages of microorganisms, constituting a unique environment called the "plastisphere", which increases the heterogeneity of soil (Zettler et al., 2013; Harrison et al., 2014). Various studies have indicated that bacterial assemblages with distinct community structures, especially plastic-degrading bacteria, colonize PE (Huang et al., 2019). Zhou et al. (2021) reported that MPs increase the area of soil hotspots, these being locations where nutrient turnover is significantly faster than in the soil in general. Such studies have provided valuable insights into the impact of the "plastisphere" on microbial communities and nutrient conversion capacity. However, the

relationship between the expression of soil extracellular enzymes (such as BG, XYL, and ACP) and available nutrients under MP modification is not well known (Sinsabaugh et al., 2009; Sinsabaugh et al., 2015).

Here, we aimed to evaluate how MPs affect the spatial distribution of enzyme activity and rhizosphere properties. We focused on evaluating the two most common MPs in soil, and how they impact the activity of hydrolases responsible for dissolved organic carbon (DOC), NH_4^+ , and Olsen-P. We hypothesized that 1) MPs would provide more ecological sites for soil microbes and increase microbial activity, which would lead to accelerated soil nutrient consumption by both microbes and plants, and 2) the introduction of MPs to soil would cause N and P nutrient limitation, which would promote the extracellular enzyme secretion of N- and P-related hydrolases. To test these two hypotheses, the effects of the spatial distribution of C, N, and P hydrolases and soil nutrient content were determined based on C, N, and P hydrolase active hotspots in soil modified by MPs. Our results are expected to advance current understanding of the ecological risk presented by soil MPs to agricultural plants.

2 Materials and methods

2.1 Sample preparation

Soil samples were taken from the tillage layer (15% water content) at the Jinjing Agroecosystem Research Station, Hunan Province, China (28°155'N, 111°127'E). It has a humid subtropical monsoon climate with average air temperature of 16–18 °C, annual rainfall of 1200–1700 mm, and a frost-free period of 250–300 days. Soil was sifted through a 2 mm sieve to remove coarse plant residues, air-dried at 30 °C, and then pre-incubated at 25 °C for two weeks. Soil texture was of 4.0% clay, 40.4% silt, and 55.6% sand. We grew 30 rice plants, each in a separate rhizo-box with inner dimensions of 18.4 cm×11.4 cm×4.5 cm, as described by Ge et al. (2017).

2.2 Plant and soil sampling

Each rhizo-box soil was collected after 35 d of incubation, and one side of the root box was opened to remove the soil and plants. The above-ground plants were removed and allowed to dry, and the underground parts of the roots were separated from the soil. The soil within a distance of 2 mm from the roots surface was grouped as rhizosphere soil (RS), and the rest was grouped as bulk soil (BS). The basic physical and chemical indexes of soil were determined. DOC was extracted with K_2SO_4 (0.5 mol L^{-1}) and measured using a total organic carbon analyzer (Shimadzu, Kyoto, Japan). The same extracted solution was used to determine soil NH_4^+ using a continuous-flow auto-analyzer (Fiastar 5000; Foss Tecator AB, Höganäs, Sweden). Olsen-P was

extracted using 0.5 mol L^{-1} NaHCO_3 and measured as described by Olsen et al. (1982). The chloroform fumigation extraction method was used to measure soil microbial biomass C (MBC) and microbial biomass N (MBN) (Wu et al., 1990; Jenkinson et al., 2004). Soil microbial biomass P (MBP) was determined as described by Brookes et al. (1984).

2.3 Experimental setup and direct soil zymography

The contents of N ($\text{CO}(\text{NH}_2)_2$), P ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) and K (KCl) fertilizers were 100, 20 and 120 mg kg^{-1} dry weight (dw), respectively, and were used as a base fertilizer. Each rhizo-box contained 0.8 kg soil, and there were five treatments (control, 0.01%PE, 1%PE, 0.01%PVC, and 1%PVC; the MP percentage is the percentage dry weight of each treated soil), with six replicates per treatment. Rice was cultured for 35 days under a 3 cm submerged condition. The rhizo-box was covered with foil as described by Wei et al. (2019b). This experiment was conducted outdoors in Changsha (113°08924'E, 28°198715'N) in July.

Before measuring the activity of each enzyme assay, their respective enzyme substrate concentrations were measured in a pre-experiment. The contents of BG (4-methylumbelliferyl- β -D-glucoside), XYL (4-methylumbelliferyl- β -D-xylopyranoside), ACP (4-methylumbelliferyl-phosphate) and NAG (4-methylumbelliferyl-N-actyl- β -D-glucosaminide) were 1, 2, 4, and 10 mmol L^{-1} , respectively, determined after an incubation time of 1, 1.5, 1.5, and 2 h, respectively. After cultivating rice for 35 d, the spatial distribution of enzyme activity around the roots was measured by direct soil zymography. The visualization method of enzyme activity was the same as that detailed in Ge et al. (2017).

2.4 Image processing and analysis

The zymograms were quantified using ImageJ, as described by Razavi et al. (2016). Using a control group of different enzymes, the activity of enzymes related to C, N and P hydrolysis was greater than 106, 249, and 319 mol $\text{cm}^{-2} \text{h}^{-1}$, respectively, were defined as hotspots in the current study.

The extension of the rhizosphere was calculated by examining the enzyme activity distribution as a function of distance from the root center to the surrounding soil:

$$y = y_0 + \frac{a}{1 + e^{-\left(\frac{x-x_0}{b}\right)}} \quad (1)$$

where, a is the increase in enzyme activity from bulk soil to the rhizosphere; b is the steepness of the rhizosphere gradient, x_0 is the rhizosphere key zone extension, y_0 is the enzyme activity of bulk soil, and y_0+a is the maximum activity in the rhizosphere.

The average enzyme activity in hotspots (E_H) was calculated by fitting the grey values in hotspots to the calibration line (Fig. 2), and the total enzyme activity in hotspots (TE_H) was calculated using Eq. (2)

$$TE_H = E_H \times A_H \times S \quad (2)$$

where, TE_H is the total enzyme activity in hotspots, E_H is the average enzyme activity in hotspots, A_H is the percentage of hotspot area, and S is the area of the soil surface. The C/P, C/N, and N/P acquisition ratio in hotspots was calculated using Eq. (3) (Sinsabaugh et al., 2008):

$$\text{C/N acquisition ratio} = \ln(BG+XYL)/\ln(NAG)$$

$$\text{C/P acquisition ratio} = \ln(BG+XYL)/\ln(ACP)$$

$$\text{N/P acquisition ratio} = \ln(NAG)/\ln(ACP) \quad (3)$$

where BG, XYL, NAG and ACP indicate the TE_H of β -glucosidase, xylanase, N-acetyl-glucosidase and acid phosphatase activity, respectively.

Differences between treatments with and without MPs addition, and between rhizosphere and bulk soils were examined using an analysis of variance. Structural equation models were used to analyze the effects of MPs on resource stoichiometry and nutrient acquisition ratio. Statistical analyses were performed using SPSS 20.0. Least significant difference multiple comparisons ($p < 0.05$) tests were used to assess significant differences among the experimental treatments. Principal component analysis was used to determine differences between the MP treatments using R version 3.4.1.

3 Results

3.1 Effects of microplastics on plant and soil properties

The addition of either PE or PVC affected both rice shoot and root biomass. Relative to the unamended control soil, MPs increased shoot biomass by approximately 13.0%–15.5%. However, MPs reduced the biomass of rice roots by 9.2%, 15.1%, 8.0%, and 11.8% with 0.01% PE, 1% PE, 0.01% PVC, and 1% PVC, respectively (Fig. 1). Unlike the effect of MPs on rice shoot biomass, the reduction in rice root biomass was MP dose-dependent.

MP addition also reduced soil DOC content by 6.0%–17.7% and 11.0%–19.0% in RS and BS, respectively. In general, the Olsen-P content was higher in BS than in RS (Fig. S1). The Olsen-P content in RS decreased with increasing MP level by 1.0%, 4.8%, 11.5%, and 16.8% with 0.01% PE, 1% PE, 0.01% PVC, and 1% PE, respectively (Fig. S1). MBP content in RS decreased with the increasing MP levels by 15.6%, 29.0%, 18.8%, and 26.3% with 0.01% PE, 1% PE, 0.01% PVC, and 1% PE, respectively (Fig. S1). Compared to the value in the control, the RS NH_4^+ level significantly decreased by 18.3%, 56.8%, 35.6% and 52.2% with 0.01% PE, 1% PE, 0.01% PVC, and 1% PE, respectively, positively correlating with the MP levels (Fig. S1). The BS NH_4^+ levels followed the same pattern; with the exception that the decline was greater than that in the RS, decreasing by 19.0%, 70.0%, 63.2%, and 80.2% with 0.01% PE, 1% PE, 0.01% PVC, and 1% PE, respectively (Fig. S1).

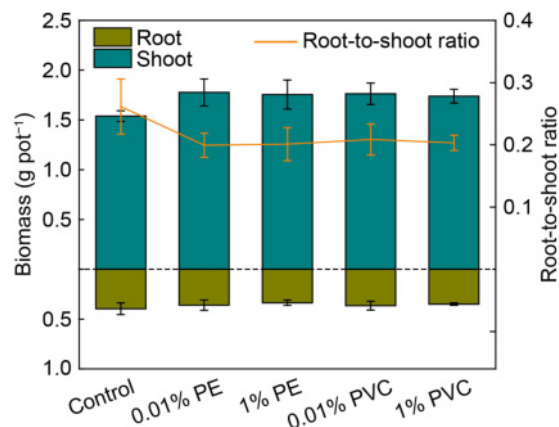


Fig. 1 Effects of microplastic (MP) amendment on shoot and root dry weight of rice and on the root-to-shoot ratio. Values represent the means and error bars represent standard deviation (\pm SD, $n = 3$). Plant biomass was measured after 35 days of rice growth under each treatment, Control; 0.01% PE; 1% PE; 0.01% PVC; 1% PVC.

3.2 Effects of microplastics in hotspot areas and spatial distribution of soil extracellular enzymes

The hotspots of C hydrolyzing enzymes (BG and XYL) were distributed along the roots, and enzyme activity was stimulated by PE addition, but inhibited by PVC addition (Fig. 2). Compared to the control, PE increased the average enzyme activity of BG and XYL in hotspot areas by 5.6%–8.4% and 1.5%–10.7%, respectively (Fig. 3). Phosphorus hotspots (ACP) were present in both RS and BS, with higher enzyme activity detected near the roots (Fig. 2). ACP average enzyme activity in hotspot areas with both PE- and PVC-MP-modified soil showed 2.4%–6.3% and 1.9%–2.4% increases, respectively, in comparison with the values in the control group (Fig. 3). NAG activity declined in RS in comparison with that in BS, particularly at the root surface (Fig. 2). Compared to the values in the control, the two levels of PE increased NAG average activity by 0.8%–4.8% in the hotspot areas (Fig. 3).

The rhizosphere hotspots of BG enzyme activity were stimulated by PE and inhibited by PVC, with both PE and PVC showing dose-dependent effects on BG enzyme activity (Figs. 4, S2). The rhizosphere hotspots of XYL enzyme activity were stimulated by both PE and PVC. However, a dose-dependent effect was not observed. There was no rhizosphere NAG enzyme activity effect. However, NAG was activated by PE around the root (Fig. 4). After 35 d of growth, BG activity (0.49–0.81 mm) in RS hotspots in the MP-modified group was lower than that in the control (0.94 mm). ACP activity increased with the increasing MP level, with 0.01% PE (0.60 mm) < 1% PE (0.67 mm) and 0.01% PVC (0.48 mm) < 1% PE (0.59 mm) (Fig. 4).

3.3 Effects of microplastics on resource stoichiometry and nutrient acquisition ratio

The soil C/N ratio (DOC/NH_4^+) and MBC/MBN ratio (Fig. 5)

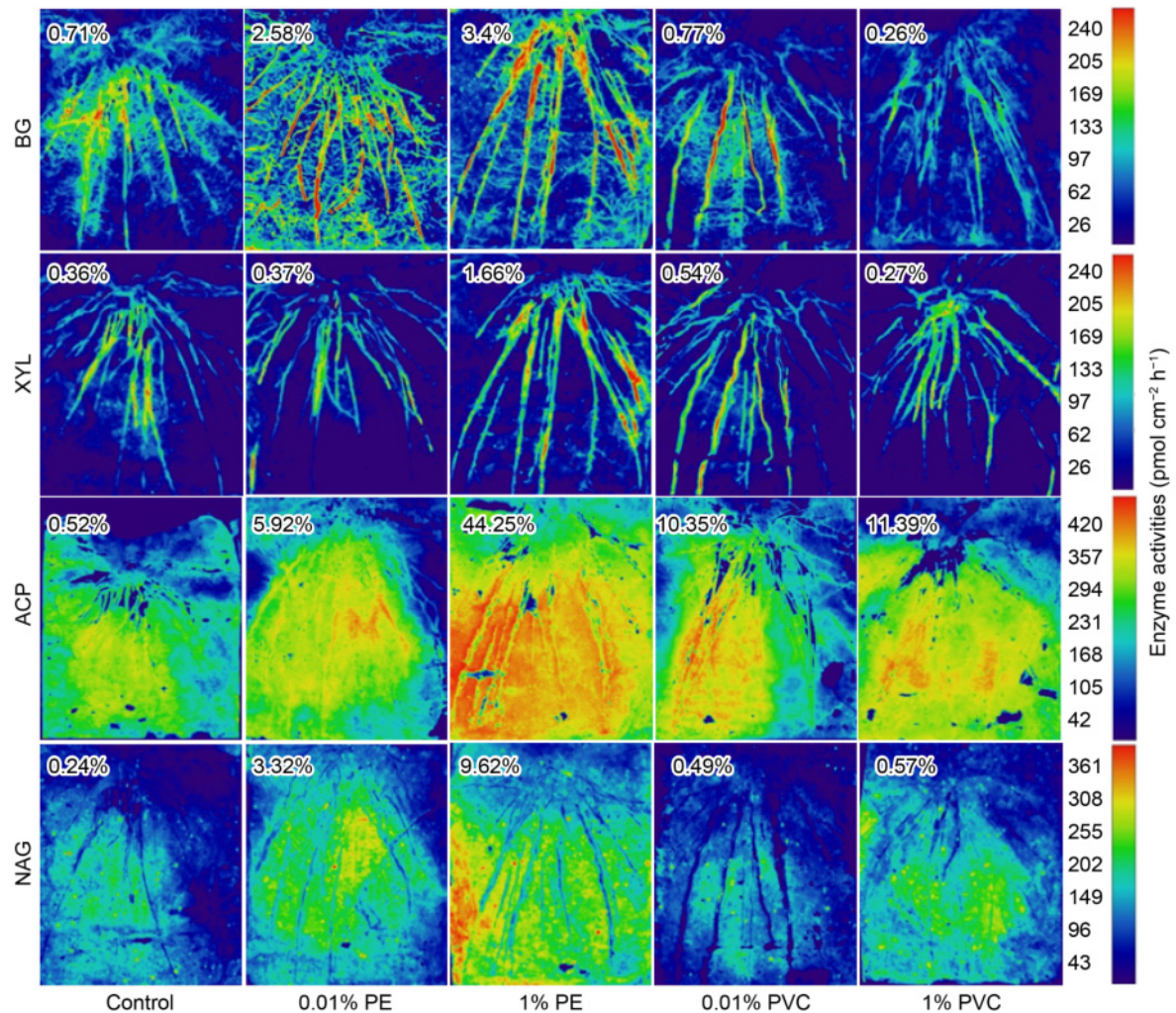


Fig. 2 Zymograms and hotspots of BG, XYL, ACP and NAG in control soil and soil to which PE and PVC were added at two levels (0.01% and 1% of soil dry weight). The color intensity is proportional to the enzyme activity ($\text{nmol cm}^{-2} \text{h}^{-1}$). Each zymogram is representative of six independent replicates. The numbers in the top left of each panel is the percentage of the total image area that belonging to a hotspots.

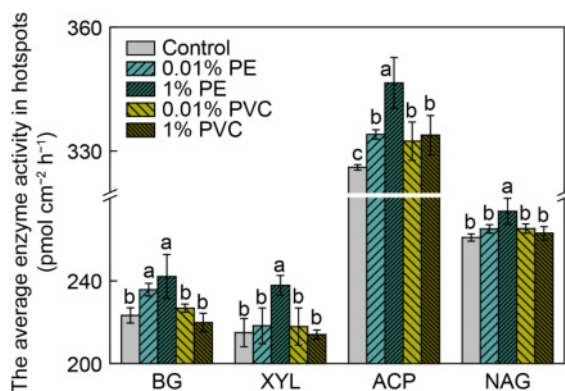


Fig. 3 The average enzyme activity of BG, XYL, ACP and NAG in hotspots (E_H , $\text{pmol cm}^{-2} \text{h}^{-1}$) under untreated soil (control) and soil to which the PE and PVC were added at two content levels (0.01% and 1%). Values are means (\pm SD, $n = 3$) of three replicates. Different lowercase letters (a, b, c) indicate significantly different means among the different content of PE and PVC ($p < 0.05$).

significantly increased with the increasing MP level ($p < 0.05$), except for the soil MBC/MBN of RS with PVC addition. Compared to the control, PE and PVC reduced soil available C/N ratio 1.1 to 2.9-fold and 1.3 to 4.5-fold, respectively. The MBC/MBN ratio after 1% MP addition decreased 1.5- to 1.8-fold (Fig. 5). The C/N acquisition ratio decreased 0.7- and 0.6-fold under 0.01% and 1% PE addition, respectively (Fig. 6). The soil available N/P ratio (NH_4^+ /Olsen-P, Fig. 5) in RS decreased 1.2- and 2.2-fold with 0.01% and 1% PE addition, respectively, and 1.4- and 1.7-fold with 0.01% and 1% PVC addition, respectively. The MBN/MBP ratio (Fig. 5) increased 1.4-fold with 1% PE addition in both RS and BS ($p < 0.05$). Compared to value in the control, the N/P acquisition ratio of soil with PE addition increased approximately 1.4- to 1.5-fold, whereas the ratio decreased 0.7- to 0.9-fold with PVC addition (Fig. 6). The soil available C/P ratio (DOC/Olsen-P, Fig. 5) in BS was lower than that in RS. The C/P acquisition ratio increased 1.06-, 0.90-, 0.86- and 0.68-fold with 0.01% PE, 1% PE,

0.01% PVC, and 1% PVC, respectively. Therefore, higher MP levels (1%) altered the ratio of available soil nutrients (C/N, C/P, and N/P; $p < 0.05$). Results of structural equation models of the effects of MPs showed a reasonable fit to our hypothesized causal relationships ($\chi^2 = 0.066$, $p = 0.996$, comparative fit index (CFI) = 0.999, root mean square error of approximation (RMSEA) = 0.000, and Akaike information criterion (AIC) = 50.066; Fig. 7), which indicated a significant effect of MPs on the soil nutrient ratio ($p < 0.001$) (Fig. 7).

4 Discussion

4.1 Effects of microplastics on plant growth and soil properties

Overall, MPs reduced the growth of roots and promoted the growth of shoots in paddy soils (Fig. 1). However, Zang et al. (2020) found that the growth of roots and shoots of wheat was reduced by the addition of 1% MPs (PE and PVC). This difference might be attributed to differences in the planting environment between rice and wheat. Flooding conditions are more conducive to the flow of nutrients and increased

nutrient uptake by plants (Chen et al., 2014). MPs may cause biogeochemical changes in the soil through the leaching of components on the surface and often through organic phosphite antioxidant additives in the bulk soil that are easily transformed to organic phosphates that may further possibly break down to phosphate (Machado et al., 2019). Soil properties were influenced by the type and content of MPs added; in particular, NH_4^+ levels showed a classic MP dose-dependent response in our study (Fig. S1). This phenomenon might be related to MPs stimulating soil niche nutrient competition, along with increased N-hydrolyzing gene expression (Rong et al., 2021).

The effect of microplastics on available N was greater than that on available C and P (Fig. S1). Microplastics form a new ecological niche and microbes from some specific communities are more inclined than other microbes to attach to microplastic surfaces (Feng et al., 2020; Tender et al., 2017; Zettler et al., 2013; Sangale et al., 2019). Therefore, the turnover of native SOM may be stimulated due to the altered metabolic status of the microbial community (Xiao et al., 2021; Kuzyakov, 2010; Zang et al., 2017; Li et al., 2020), and thus influence soil C and nutrient cycling. In ecological enzyme metrology, microorganisms secrete

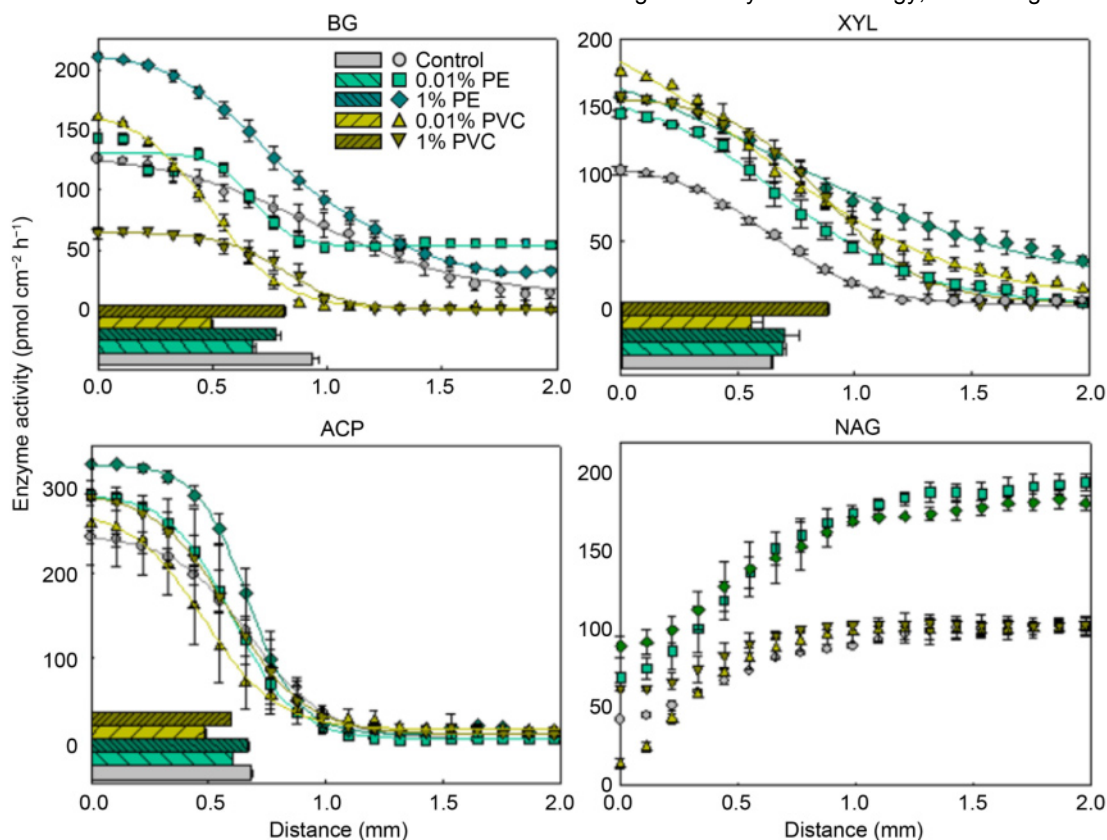


Fig. 4 Profiles of enzyme activity as a function of distance from the root center to the surrounding soil. Point values were obtained by analyzing three roots as replicates (see Fig. 1). The curves (continuous lines) represent the fitting of enzyme activity as a function of distance from the root tip by non-linear regression (Eq. (1) in Section 2.4). The bar graph at the bottom of each figure reflects the rhizosphere extensions for each enzyme from the fitted curves. Error bars represent standard deviation (\pm SD, $n = 3$). As chitinase activity lower more in the rhizosphere and at the root surface than in the bulk soil (in contrast to the other three enzymes). The presented bars reflect the rhizosphere extent of expansion of rhizosphere hydrolase activity. Consequently, there was no rhizosphere extension for the distribution of enzyme activity of NAG.

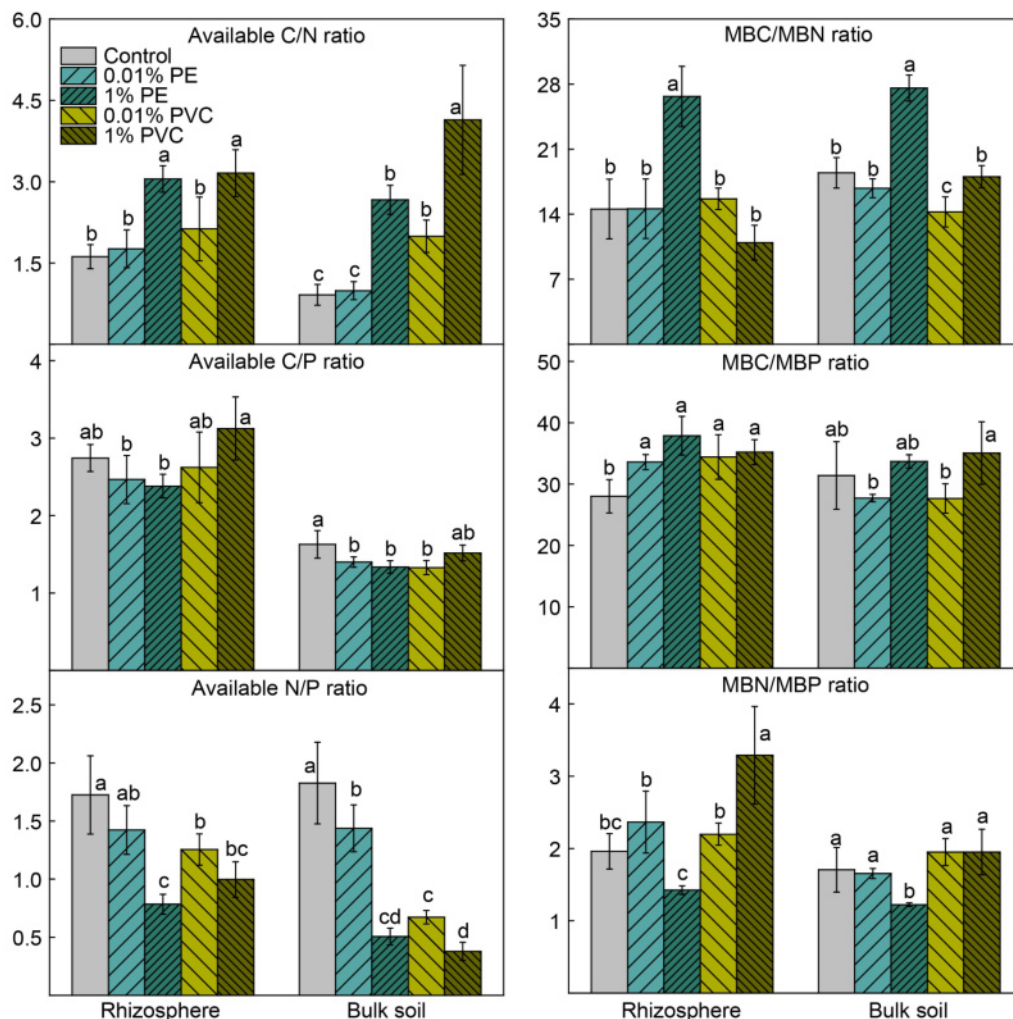


Fig. 5 Ratio of soil available C/P, C/N, N/P, MBC/MBP, MBC/MBN, and MBN/MBP in the rhizosphere and bulk soil in untreated soil (control) and soil to which PE and PVC were added at two levels (0.01% and 1%). Values are the means (\pm SD, $n = 3$) of three replicates. Lowercase letters (a, b, c and d) indicate that means were significantly different mean for PE and PVC levels ($p < 0.05$).

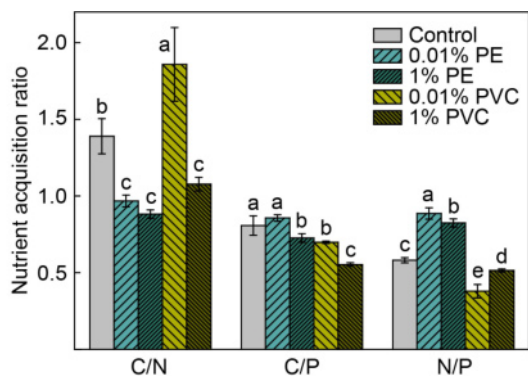


Fig. 6 C/N, C/P and N/P acquisition ratio (based on the activity of enzymes responsible for C, N and P hydrolysis) in untreated soil (control) and soil to which PE and PVC were added at two levels (0.01% and 1%) (Eqs. (2) and (3) in Section 2.4). Values are means (\pm SD, $n = 3$) of three replicates. Lowercase letters (a, b, c, d, and e) indicate significantly different means for PE and PVC levels ($p < 0.05$).

extracellular enzymes to maximize the decomposition of priority substrates that meet the requirements of restricted elements (Mooshammer et al., 2014). To maintain the balance of available P in soil (Richardson et al., 2011), phosphatase activity can be enhanced to increase P availability (Allison et al., 2005; Geisseler et al., 2010). The dynamic equilibrium of available P is maintained through microbe-mediated solubilization of inorganic P and the mineralization of organic P (Nikitha et al., 2017; Qu et al., 2020). Our findings are consistent with these previous findings on phosphatase hotspot areas (Fig. 2). Hence, MPs increase soil geochemical cycling in specific sites, providing an energy base for microbes and accelerating the depletion of soil nutrients. This result confirms our first hypothesis.

4.2 Effects of microplastics on the *in situ* distribution of soil enzyme activity

Consistent with our second hypothesis, the percentage of

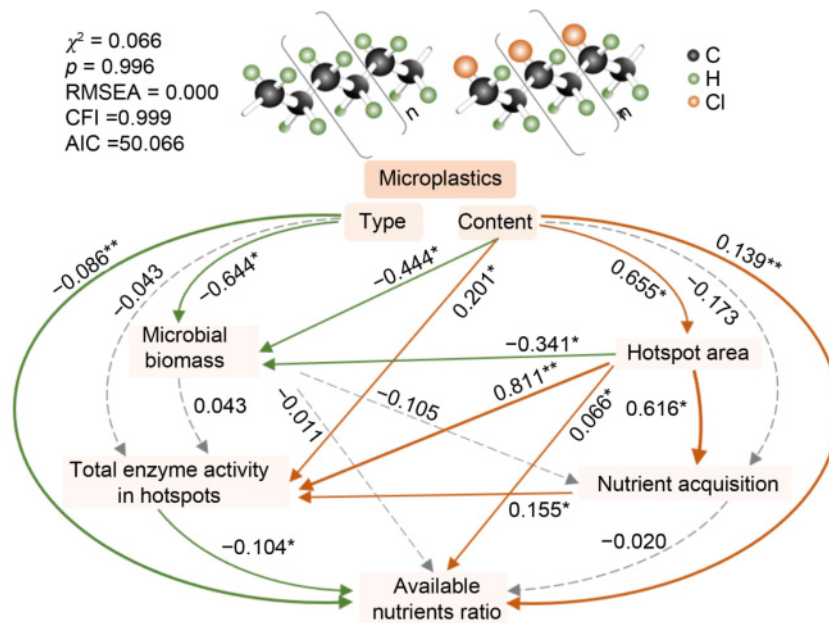


Fig. 7 Structural equation models (SEM) illustrating how the two types and levels of microplastics (MPs) impacted the soil's available nutrients ratio, nutrient acquisition, soil microbial biomass, and total enzyme activity in hotspot areas. Arrows represent effect path and direction; orange and green lines represent positive and negative effects, respectively; bold, thin, and dashed lines indicate highly significant (**), significant (*), and non-significant correlations, respectively. Top left values on the figure explain the variations by affecting factors; Chi Square (χ^2) represents the difference between estimated and observed values; "p" represents the significance level; root mean square error of approximation (RMSEA) represents the difference between the theoretical model and saturation model; comparative fit index (CFI) represents the degree of improvement of the model over the nihilistic model; and Akaike information criterion (AIC) metrics were used to assess the complexity of the models.

hydrolytic enzyme activity (ANG and ACP) hotspot area in soil was greater at higher MP levels than that at lower MP levels (PE and PVC) (Figs. 2, 3). The C hydrolyzing enzymes (BG and XYL) hotspot area in soil was similar to ANG and ACP under PE modification (Figs. 2, 3). Consequently, enzymes activity was enhanced in MP-amended soil. This phenomenon is important because several biogeochemical transformations affect the fate of nutrients, such as mineralization or denitrification (Bilyera et al., 2020; Fei et al., 2020; Ren et al., 2020; Seeley et al., 2020). In addition, MPs caused the active hotspot area of N and P hydrolases to increase by a much larger factor than that of C hydrolase. For instance, BG and XYL levels showed a 0.08- to 3.75-fold and 0.03- to 3.59-fold increase, respectively, whereas ACP and NAG levels showed a 10.48- to 84.76-fold and 1.09- to 39.71-fold increase, respectively (Fig. S3).

Consistent with the economics of soil extracellular enzyme secretion, MP input creates a C-rich environment (Rillig 2018; Rillig et al., 2019), in which rhizospheric soil secretases take precedence over other restrictive factors (e.g., N and P) (Figs. 2, 7). This dynamic nutrient balance provides optimal resources to meet the requirements of microbial elemental stoichiometry (Allison et al., 2010). Under PVC addition, the area of active soil hotspots of NAG and ACP did not increase as much as those under PE addition and even hindered the active hotspots of BG and XYL, supporting the findings of Zang et al. (2020). This

finding showed that PVC reduced the activity of BG and XYL average enzymes in hotspot areas at high level MP addition (Figs. 2, 3). The chlorine atoms in the PVC molecular chain might act as electron donors for metabolic hydrolyzing enzymes during component leaching; denitrification requires electron acceptors (Zou et al., 2009; Miao et al., 2017; Xie et al., 2021). Machado et al. (2019) obtained similar results, whereby polyamide-derived N was quickly metabolized by the microbiome directly on particle surfaces, thus increasing leaf N content. NAG enzyme concentrations in the root circumference of PE treatment exceeded those in the root PVC treatment circumference, supporting the identification of the significance of this phenomenon (Fig. 4). Thus, MP addition altered the spatial distribution of soil hydrolase activity, with the elements of MPs impacting hydrolase activity.

4.3 Effects of microplastics on the characteristics of ecological enzyme metrology

Soil microorganisms adjust their elemental balance in response to soil disturbances (Mooshammer et al., 2012; Sistla et al., 2012), exhibiting conservative stoichiometric responses (Moe et al., 2005; Sinsabaugh et al., 2012). Within the range of microbial capacity, microorganisms respond to changes in environmental nutrient resources by altering the ratio of C, N, and P extracellular hydrolysis enzymes (Allison et al., 2005).

There are two main mechanisms by which the non-homeostatic behavior of microbes reduces the stoichiometric imbalance of resources (Mooshammer et al., 2014). When C and P availability in the environment increases, microorganisms synthesize lipids, glucans, and polyphosphates, preserving C and P; however, there is no particular reservoir for storing N in microorganisms. The MBC/MBN ratio significantly increased when PE levels were high, and vice versa for MBN/MBP (Fig. 5). This phenomenon might be mediated by non-homeostatic behavior, which adjusts the ratio of microbial biomass (MBC/MBN and MBN/MBP) to maintain the imbalance in soil N and P resources (Mooshammer et al., 2014). Furthermore, the ratio of MBC/MBN was higher than the microbial community stoichiometric ratio (C/N = 60:7) (Cleveland et al., 2007), demonstrating a high utilization rate of soil N (Göran 2010). Alternatively, the non-homeostatic behavior of microbial biomass can possibly be attributed to shifts in community composition (Fanin et al., 2013; Mooshammer et al., 2014); however, shifts in community composition were not included in this study and can present a direction for future research.

Microbes maximize the mobilization of substrates that are rich in the limiting elements by adjusting extracellular enzyme production (Wallenstein et al., 2008; Burns et al., 2013; Mooshammer et al., 2014). Ecological enzyme stoichiometry can reflect the biogeochemical balance model involving microbial metabolism, nutrient acquisition, and nutrient availability in the environment (Waring et al., 2014; Fanin et al., 2016; Moorhead et al., 2016). The C/N and C/P acquisition ratios significantly declined when MP levels were high ($p < 0.05$) (Fig. 6). The C/P acquisition ratio value was smaller than one and declined with increasing MP concentration; however, there was no significant difference in the reduction of available P concentration when compared to that of the control group. Thus, in MP-modified soil, microorganisms may have to mine phosphate from organic matter (Zhang et al., 2015; Sinsabaugh, et al., 2009). Monoesters and diesters are the form of most organophosphorus compounds, which are released by phosphatases (Mcgill et al., 1981; Howarth, 1991). Therefore, MP addition may possibly lead to P mobilization from inorganic and organic sources. N and C are essential elements for the secretion of extracellular enzymes that may further exacerbate elemental limitation (Schimel et al., 2003). In an N-limited environment, the excretion of extracellular enzymes to mobilize N-containing substrates may not be an adequate strategy for microbes to regulate their N homeostasis (Schimel et al., 2003). This is possibly an explanation of why the C/N acquisition ratio did not significantly decline between the modification of PE by 0.01% and 1% modification (Fig. 6). Consequently, microorganisms must meet nutrient resource requirements by regulating resource utilization efficiency and their own microbial biomass composition. This phenomenon was confirmed by a decrease in NH_4^+ and MBN content in our study, which was significantly negatively relative to the MP levels (Figs. S1, 7).

5 Conclusions

MP contamination of soil differentially affects both the distribution of hydrolase activity and nutrient uptake by plants or microorganisms, depending on the level of MP contamination. Soil N (NAG) and P (ACP) hydrolase hotspot areas were stimulated by MP input, particularly due to PE and high levels of PVC. However, soil available N and P declined because of MP input. Soil NH_4^+ and Olsen-P content were 50% lower in the high-level MP modification group than those in the uncontaminated control soil. Compared to the values in the control, MP did not significantly increase the above-ground plant biomass but reduced the below-ground biomass. Consequently, MPs alter the spatial distribution of soil hydrolases and biogeochemical processes, limiting soil nutrients and retarding plant development, especially the root system. This study provides new insights into the short-term effects of common MPs regarding hydrolase distribution in paddy soils. However, the long-term effects of MPs on plant-soil-microbial interactions must be examined to obtain a holistic evaluation of their impact.

Author contributions

Yaoyao Tong: Writing-original draft. Jina Ding & Mouliang Xiao: Writing-review & editing, supervision. Muhammad Shahbaz: Writing-review & editing. Zhenke Zhu: Writing-review & editing. Ming Chen: Conceptualization, supervision, writing-review & editing. Yakov Kuzyakov: Resources, writing-review & editing. Yangwu Deng: Conceptualization, supervision, writing-review & editing. Jianping Chen: Methodology, resources, writing-review & editing. Tida Ge: Conceptualization, supervision, writing-review & editing.

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Abbreviations

MPs, microplastics; PE, polyethylene; PVC, polyvinyl chloride; BG, β -glucosidase; XYL, xylanase; NAG, chitinase; ACP, phosphatase; DOC, dissolved organic carbon; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P.

Electronic supplementary material

Supplementary material is available in the online version of this

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