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Research Paper

Microplastics in soil can increase nutrient uptake by wheat

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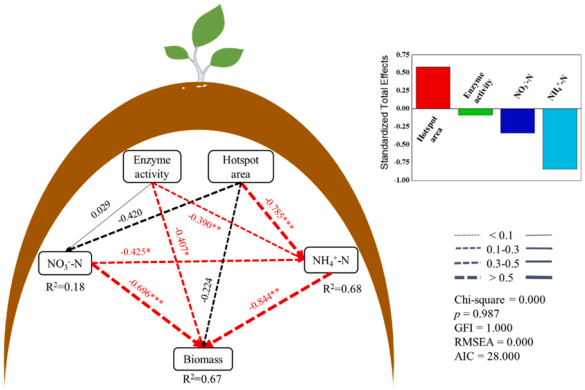
HIGHLIGHTS

- Different root resource-acquisition strategies disturb microbial nutrient turnover.
- Higher doses of polyethylene (5% (w/w)) enable roots to uptake additional N.
- PVC cannot lead to microbial SOM decomposition for N and P acquisition.
- PVC forced wheat to efficiently obtain available nutrients in the narrow root zone.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics can perturb microbial nutrient-mining strategies. However, the mechanism by which microplastics affect the resource-acquisition strategies of crops in agricultural systems remains unknown. The nutrient-acquisition potential of crops and microbes was investigated under treatments with two common microplastics (polyethylene [PE] and polyvinyl chloride [PVC]) at 0%, 1%, and 5% (w/w). Different root resource-acquisition strategies disturbed microbial nutrient turnover in the rhizosphere in response to microplastic addition. Specifically, the β-1,4-glucosidase (BG) hotspot expanded, whereas the rhizosphere expansion of BG activity decreased. A decrease of less than PE1% (w/w) and an expansion of less than PE5% (w/w) in the 1,4-N-acetyl-glucosaminidase (NAG) hotspot with wider rhizosphere expansion of NAG activity indicated that higher doses of PE allow roots to uptake additional N. The phosphomonoesterase (PHOS) hotspot decreased in PE1%

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(w/w) and expanded in PE5% (w/w), but rhizosphere expansion did not change under PE treatments. However, both NAG and PHOS hotspots expanded with decreasing rhizosphere expansion under PVC treatments, indicating that PVC limits the utilization of available N and P, forcing the crop to obtain nutrients from the narrow root zone. These results indicate that adding PE microplastics increases the demand for and consumption of NH_4^+-N and NO_3^--N by wheat.

1. Introduction

Microplastics (particles <5 mm in size), organic polymers synthesized from non-renewable resources (Othman et al., 2021), can enter agroecosystems through mulching film (Zhou et al., 2020; Xiao et al., 2021; Yu et al., 2021; Wang et al., 2022; Xu et al., 2022). Globally, approximately 5–35 kg of 4–8-μm thick plastic film per hectare per year is used as mulch (Liu et al., 2014), which is often fragmented into smaller particles (microplastics) by tillage and UV radiation after the harvest of crops and retained in the surface soil (0–20 cm) for long periods because of the low light and low oxygen conditions (Zhou et al., 2020; Xiao et al., 2021). Thus, residual plastic reaches the bottom soil at concentrations between 72 and 260 kg ha^{-1} (Liu et al., 2014; Zang et al., 2020). China uses the highest amount of plastic mulch film worldwide, with 19.8 million hectares of agricultural land covered by plastic mulch film (Liu et al., 2014), especially in upland soils (Qi et al., 2018). The main types of microplastics include polyethylene (PE) and polyvinyl chloride (PVC) (Zhou et al., 2020). Currently, the use of PVC has been prohibited in agricultural practices owing to its toxic and refractory nature (Fei et al., 2020; Li et al., 2020a, 2020b), but its legacy effects in agroecosystems cannot be ignored.

Microplastic accumulation has become a concern for the sustainable development of agricultural soil (Brodhagen et al., 2017; Yu et al., 2021). Microplastics can reduce soil bulk density and improve saturated hydraulic conductivity (De Souza MacHado et al., 2018a), forcing microorganisms to compete for available nutrients (Xiao et al., 2021). This will inevitably affect the microbial decomposition of organic matter in the soil (Nizzetto et al., 2016; Qi et al., 2018, 2020; Xiao et al., 2022; Zhang et al., 2022). However, microbes are more inclined to attach to the surface of microplastics during biodegradation (De Tender et al., 2017; Xie et al., 2021), forming oligomers, dimers, and monomers and mineralizing organic matter (Ganesh Kumar et al., 2020; Xiao et al., 2022; Zhang et al., 2022). Both these factors further influence C and nutrient cycle and turnover by affecting the microbial secretion of C, N, and P-related hydrolases (Allison and Vitousek, 2005; Ganesh Kumar et al., 2020; Zhou et al., 2020; Xiao et al., 2022). The activities of several carbohydrate hydrolases have been observed to increase (cellulase and laccase), decrease (β-glucosidase, cellobiohydrolase, and xylosidase), or remain unchanged (cellobiohydrolase), which is highly correlated with the type and dose of microplastics (Zang et al., 2020; Guo et al., 2021; Liang et al., 2021). Similarly, the activities of N- and P-degrading enzymes can be stimulated (urease and acid phosphatase), can be inhibited (N-acetyl-glucosaminidase and phosphatase), or remain unchanged (leucine aminopeptidase), regulating N or P cycling in response to the presence of microplastics (Fei et al., 2020; Zang et al., 2020; Liang et al., 2021). This inevitably leads to intense competition between plants and microorganisms for the available nutrients (Qi et al., 2020; Liu et al., 2021), which affects the two ecosystems in terms of nutrient acquisition during plant growth (Hofmann et al., 2016; Fu et al., 2020; Liu et al., 2021). The activities of hydrolases from microbes and plants were used to reflect nutrient acquisition by them (Liu et al., 2021; Schliemann, 1984; Wei et al., 2019b,c). Their potential activities and hotspots throughout the rhizosphere with the addition of microplastics become increasingly complex (Liu et al., 2021; Wei et al., 2019a; Wen et al., 2022). Results pertaining to the effects of microplastic contamination of agricultural soil on crop growth has been controversial (Liang et al., 2020), reporting increasing, decreasing, or not changing crop growth (Qi et al., 2020; Zang et al., 2020; Lozano et al., 2021).

However, nutrient acquisition under hydrolase activity in the rhizosphere is highly related to the shape of the roots (Wei et al., 2019b, c). Therefore, *in situ* soil zymography has been used to study the activities of soil hydrolases at the microscopic scale under continuous changes in two-dimensional space (Wallenstein and Weintraub, 2008). Conventional techniques used to measure soil enzyme activities only examine the potential maximum activity and therefore cannot reflect the actual activity of soil enzymes in the environment (Nannipieri et al., 2012; Wallenstein and Weintraub, 2008). Only a few studies have used *in situ* zymography to study the effects of microplastics on nutrient acquisition and the growth of crops and microbes in agricultural soil. Therefore, the strategies used by plant roots and rhizosphere microorganisms to obtain nutrients are studied by combining conventional with new *in situ* soil zymography techniques to measure the response of upland soils to the addition of microplastics.

The objective of this study was to clarify how microplastics affect both nutrient acquisition by and growth of plants. We hypothesized that (1) PE increases the demand for and consumption of NH_4^+-N and NO_3^--N by wheat by increasing the rhizosphere expansion of NAG activities and (2) PVC decreases the rhizosphere expansion of NAG and PHOS activities along with an extended root system to improve the use of available N and P, forcing the crop root to efficiently obtain nutrients from the narrow root zone.

2. Material and methods

2.1. Study site and soil sampling

Soil samples were collected from the plow layer (0–20 cm) of the upland soil in a wheat field in Longwo Village, Lunan County, Linyi City, Shandong Province, China (35°6.57′N, 118°38.14′E) in March 2021. The area has a warm sub-humid continental climate with a mean annual temperature of 14.1 °C and mean annual precipitation of 886 mm. The soil is aquic brown, and annual winter wheat/summer maize rotation has been performed in the area since 2012. The soil has no history of plastic pollution. Compound fertilizer is used at a rate of 750 kg ha^{-1} (nitrogen:phosphorus pentoxide:potassium oxide [$N:P_2O_5:K_2O$] = 14:7:9) before sowing (Wu et al., 2021). This experimental field has been used for the identification of resistance to wheat disease since 2013.

Soil was collected from the experimental area using a stainless-steel drill (diameter: 5 cm) and stored in air-permeable polyethylene bags for immediate transportation to the laboratory, where it was homogenized and sieved (<5 mm) under field-moist conditions. Fine roots and other plant residues were removed from the soil before use. Approximately 20 g of the soil was air-dried, passed through a 2-mm sieve, and homogenized for the measurement of its basic chemical properties. The properties of the soil were as follows: pH, 5.16; organic C, 24.60 g kg^{-1}; total N, 3.19 g kg^{-1}; nitrate nitrogen (NO_3^--N), 59.33 mg kg^{-1}; and ammonium nitrogen (NH_4^+-N), 40.30 mg kg^{-1}.

2.2. Experimental design and set-up

As the main source of plastic on farmland is mulch film, the two most common microplastics (PE and PVC; 125 μm; Sigma-Aldrich, St. Louis, MO, USA) (Zang et al., 2020) were selected for this study. The addition gradient was set at 0%, 1% (10,000 mg (w/w) kg^{-1} dry soil), and 5% (50,000 mg (w/w) kg^{-1} dry soil) in order to model the amount of

microplastic residues in agricultural soils in the future. Urea, potassium dihydrogen phosphate (KH_2PO_4), and potassium chloride (KCl) were added to the pre-incubated soil before the pot experiment as base fertilizers at 0.25, 0.15, and 0.04 g kg^{-1} dry soil, respectively (Feng et al., 2021). The soils and base fertilizers were then mixed evenly, divided into five groups for different treatments with three replicates: (i) no PE or PVC (CK), (ii) 1% PE (PE1% (w/w)), (iii) 5% PE (PE5% (w/w)), (iv) 1% PVC (PVC1% (w/w)), and (v) 5% PVC addition (PVC5% (w/w)). Specially, for treatments ii–v, PE and PVC were divided into multiple small parts to mix with the soil separately, and then mixed all small parts of the same PE and PVC concentration well, to ensure uniform mixing (Fei et al., 2020). Next, wet soil (approximately 0.7 kg dry) was evenly packed into a rhizobox (20.5 cm × 13.4 cm × 5.2 cm) with one removable side. Wheat seeds were germinated and the resulting seedlings were transplanted at the center of the applicable rhizobox for treatment. The plants were watered every 3 days and soil moisture was maintained at a gravimetric moisture content of 20% throughout the experiment (Zang et al., 2020). The experiment pots were then placed in a glasshouse under a day/night temperature regime of 25 ± 1/20 ± 1 °C for 54 days. The rhizoboxes were inclined at 60 °C, to ensure that the root system adhered to the glass wall, facilitating the distinction between the rhizosphere soil and bulk soil (Liu et al., 2021).

2.3. Sampling and soil analyses

2.3.1. Zymography image analysis

The wheat root systems was allowed to mature for 54 days after transplantation; thereafter, the rhizobox was opened and a soil zymography image was obtained after applying polyamide membranes (155 × 115 cm, 0.45 μm; Taoyuan, China) saturated with 4-methylumbelliferone (MUF)-labeled substrates to the soil surface (Wei et al., 2019b,c). Briefly, BG, MUF-β-1, 4-N-acetyl-glucosaminidase (NAG), and MUF-phosphate (PHOS) were dissolved in sodium morpholine-4-ethanesulfonate ($C_6H_{13}NO_4SNa_{0.5}$) buffer at a concentration of 10 mmol L^{-1}. The zymographic membranes for each enzyme were tightly adhered to the surface of the root soil in different rhizoboxes, incubated for 2 h in the dark, and carefully lifted off the surface of the root soil before gently removing any attached soil particles using a soft brush. Next, a zymographic image of each membrane was taken using a high definition camera (D7000, Nikon, Japan) under ultraviolet (UV) illumination with an extraction wavelength of 355 nm.

The spatial distribution of enzyme activities was quantified using Image J (1.8, National Institutes of Health, Wayne Rasband, USA). Briefly, enzyme activities were converted to 8-byte grayscale values to calculate mean and standard deviation (SD) according to a standard MUF curve of 0.01, 0.2, 0.5, 1, 2, 4, 6, and 10 mM. Hotspots and non-hotspots were defined according to the grayscale values indicating enzyme activity. Enzyme activity intensities greater than mean + 3 SD were considered hotspots (H), and intensities lower than mean + 3 SD were considered low enzyme activity (L) (Bilyera et al., 2020), and the rest was considered medium activity (M). The results are presented as a percentage of the total soil surface area (Wei et al., 2019b,c). Individual root systems were used as references to draw a perpendicular line to the root. Finally, the inter-rhizosphere was obtained by converting the horizontal units to millimeters according to the number of pixels and the image resolution.

2.3.2. Plant and soil samples

After obtaining the zymographic image, wheat shoot samples were collected and placed in paper bags. The rhizosphere was then collected using sterilized blades and a pair of tweezers to roughly delineate the area covered by the rhizosphere according to the growth of the root. The roots were removed carefully to avoid cross-contamination with bulk soil (Liu et al., 2021). Next, approximately 3 g of the rhizosphere and the bulk soil were placed in separate plastic zip-lock bags and stored at 4 °C to explore the potential activities of the extracellular enzymes (BG, NAG, and PHOS). Approximately 8 g of the rhizosphere and bulk soils was used to determine the dissolved organic C (DOC), NH_4^+-N, NO_3-N, and Olsen-P concentrations. Liquid comprising 0.5 M K_2SO_4 with 5 g fresh soil (1:4 w/v) was used to determine DOC content using a Shimadzu TOC-VCPH analyzer (Vwp, SHIMADZU, Germany) and NH_4^+-N and NO_3^--N concentrations were obtained using an auto analyzer (AA3, SEAL, Germany) (Liu et al., 2018; Liu et al., 2021). Olsen-P was determined from liquid comprising 0.5 M $NaHCO_3$ extract with 2.5 g of fresh soil (1:20 w/v) using a UV–Vis spectrophotometer (UV-2450, Agilent, Japan) (Olsen et al., 1954).

The potential activities of the soil extracellular enzymes (BG, NAG, and PHOS) were measured using a method used in previous studies (Yuan et al., 2017; Liu et al., 2020), with some modifications. Briefly, all assays were conducted by mixing 1 g of fresh soil with 250 mL of 50 mM sodium acetate buffer (pH 5.0) at 25 °C. The suspensions were stirred continuously and twelve 200-mL aliquots of the suspension were incubated in the dark at 25 °C for 4 h. BG, NAG, and PHOS activities were measured fluorometrically (excitation at 365 nm, emission at 450 nm) using a fluorescent tag (a 50-μL aliquot of 100 μM 4-methylumbelliferone in each sample well). The enzyme activities of all samples were then measured using an automated fluorometric plate reader (Victor3 1420–050 Multi-label Counter; PerkinElmer, Waltham, MA, USA) (Liu et al., 2020).

2.4. Calculations and statistics

Statistical analyses were performed using R software (4.0.0). One- and two-way analyses of variance were performed to test the effects of treatment and location using the aov function (Wei et al., 2019a, 2019b, 2019c). After performing the Levene Test for Homogeneity of Variance, the means of each treatment (CK, PE1%, PE5%, PVC1%, and PVC5% (w/w)) were compared using the least significant difference at the 5% level ($LSD_{0.05}$) with the "agricolae" package (Kabacoff, 2011; Liu et al., 2021). Amos 17.0 was used to perform structural equation modeling (SEM) and test the significance of the hypothesized causal relationships among the rhizosphere hotspot, potential enzyme activity (BG, NAG, and PHOS), NO_3^--N, NH_4^+-N, and wheat biomass (Xia et al., 2019; Liu et al., 2020). Before SEM analysis, the rhizosphere hotspot and potential activities of BG, NAG, and PHOS were downgraded using Canoco 5 software (Xia et al., 2019) and NO_3^--N, NH_4^+-N, and wheat biomass were standardized using SPSS 20.0 (Liu et al., 2020). The best-fit model was determined using a chi-square test, P-values, the goodness-of-fit index (GFI), root-mean-square errors of approximation (RMSEA), and Akaike information criteria (AIC) (Hooper et al., 2008). Relationships between the observed variables in prior models were added and removed until a final optimal model was built and the standardized total effects for wheat biomass were obtained. All other figures were produced using R software (4.0.0). Data are presented as mean ± standard error (n = 3).

3. Results

3.1. Wheat biomass

Compared to that in CK, the wheat shoot biomass was increased ($P < 0.05$) by 81.40–174.42% in response to the addition of PE5%, PVC1%, and PVC5% (w/w); the wheat root biomass increased be 196-fold ($P < 0.05$) in response to PVC5% (w/w) compared with that under CK; the shoot/root ratio (S/R) decreased ($P < 0.05$) by 83.34–98.81% in response to the addition of microplastics (Table 1).

3.2. Soil available nutrients

The soil DOC content was consistently higher ($P < 0.05$) in the rhizosphere than in the bulk soil under all treatments. The DOC content in the bulk soil was not affected by the addition of microplastics compared with that under CK; however, the DOC content was lower in

Table 1
Results of the one-way analysis of variance showing the effects of treatment on wheat shoot biomass, root biomass, and shoot/root ratio.

	Shoot biomass (g plot^{-1})	Root biomass	Shoot/root ratio
CK	0.43 ± 0.08c	0.01 ± 0.00b	55.24 ± 28.84a
PE1%	0.58 ± 0.26bc	0.07 ± 0.05b	9.77 ± 2.91b
PE5%	0.82 ± 0.18b	0.34 ± 0.21b	2.89 ± 1.19b
PVC1%	0.78 ± 0.13b	0.15 ± 0.17b	10.31 ± 6.66b
PVC5%	1.18 ± 0.18a	1.97 ± 0.92a	0.66 ± 0.21b

Note: Treatments were as follows: no polyethylene or polyvinyl chloride (CK), 1% polyethylene (PE1% (w/w)), 5% polyethylene (PE5% (w/w)), 1% polyvinyl chloride (PVC1% (w/w)), and 5% polyvinyl chloride addition (PVC5% (w/w)). Different English lowercase letters on the same horizon indicate significant differences among exudates at $P < 0.05$. All results are presented as mean ± standard deviation (n = 3).

the rhizosphere ($P < 0.05$) when exposed to PVC5% (w/w) compare to CK (Fig. 1a). The soil NH_4^+-N concentration was higher ($P < 0.001$) in the bulk soil than in the rhizosphere under treatment with PE5% (w/w). The NH_4^+-N concentration in the bulk soil was higher ($P < 0.05$) under the PE (1% and 5% (w/w)) treatments than under CK, whereas the NH_4^+-N concentration in the rhizosphere was high ($P < 0.05$) only under the PE1% (w/w) treatment (Fig. 1b). Similar to the NH_4^+-N concentration, the soil NO_3^--N concentration was higher ($P < 0.01$) in the bulk soil than in the rhizosphere soil under the PE5% (w/w) treatment. The NO_3^--N concentration in bulk soil was higher ($P < 0.05$) under the PE5% (w/w) treatment than under CK. The NO_3^--N concentration in the rhizosphere was low ($P < 0.05$) under all microplastic (PE and PVC) treatments compared with that under CK (Fig. 1c) and the soil Olsen-P concentration was higher ($P < 0.05$) in the rhizosphere than in bulk soil only when treated with PE1% (w/w). However, the Olsen-P concentration did not change under any other microplastic treatments (PE and PVC) (Fig. 1d).

3.3. Soil potential enzyme activities

The BG activity was higher ($P < 0.001$) in the rhizosphere than bulk soils under PVC1% treatment, whereas it was lower ($P < 0.05$) in PE5%, PVC1%, and PVC5% (w/w) -treated bulk soil than in CK. The BG activity was lower ($P < 0.05$) than CK only in the PE5%-treated rhizosphere (Fig. 2a). The NAG activity was lower ($P < 0.01$) in the rhizosphere than in bulk soil and was considerably lower ($P < 0.05$) in the bulk soil under PE5% (w/w) and PVC (1% and 5% (w/w)) treatments than under CK, whereas the NAG activity in the rhizosphere was lower ($P < 0.05$) under PE (1% and 5% (w/w)) and PVC5% (w/w) treatments than under CK (Fig. 2b). A lower ($P < 0.001$) PHOS activity was observed in the rhizosphere than in bulk soil under PE1% (w/w) treatment; however,

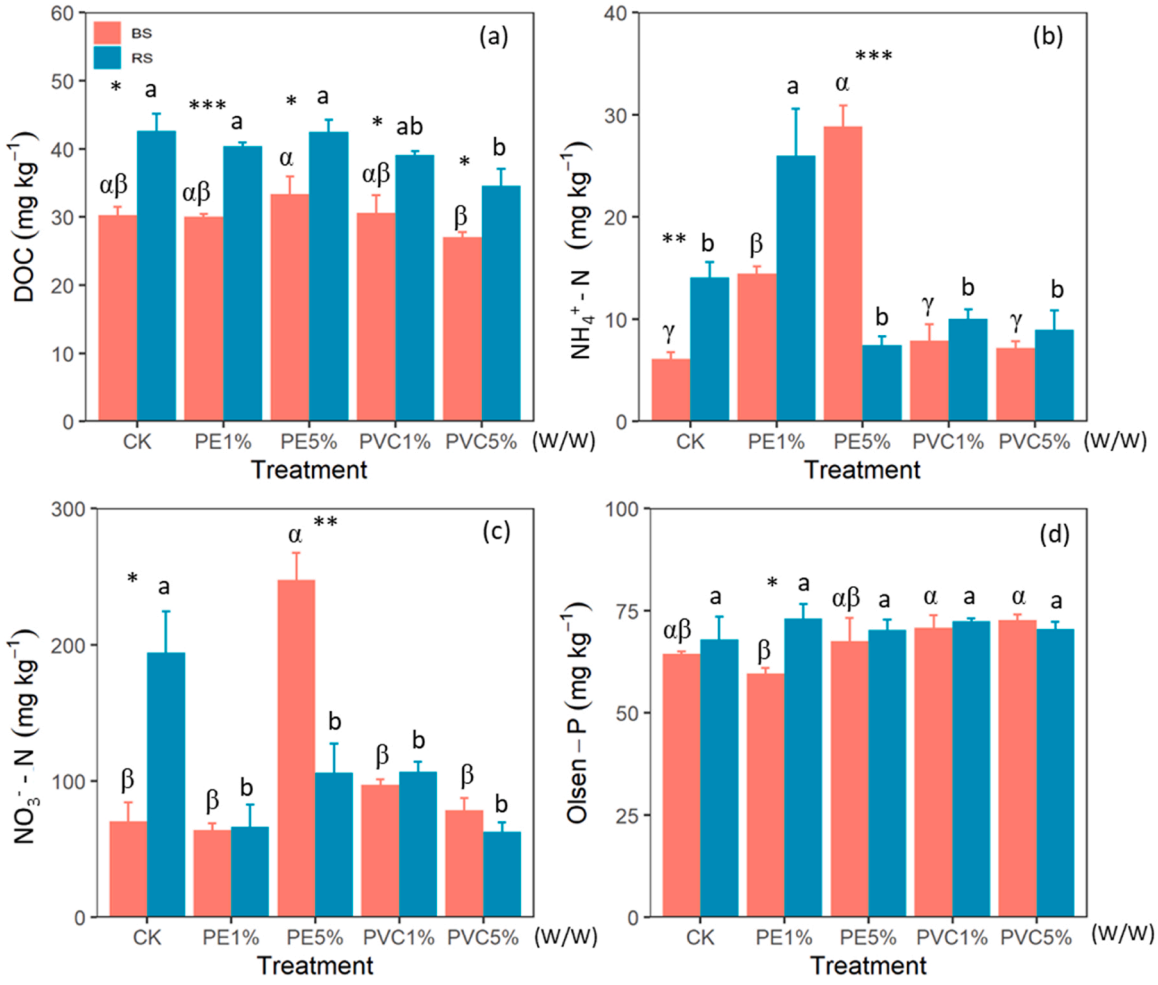

Fig. 1. Dissolved organic C (DOC, a), ammonal nitrogen (NH_4^+-N, b), nitrate nitrogen (NO_3^--N, c), Olsen-P (d) concentrations of bulk soil (BS), and rhizosphere soil (RS) 54 days after planting wheat under five treatments; no polyethylene or polyvinyl chloride (CK), 1% polyethylene (PE1% (w/w)), 5% polyethylene (PE5% (w/w)), 1% polyvinyl chloride (PVC1% (w/w)), and 5% polyvinyl chloride (PVC5% (w/w)). Different lowercase letters in English and Greek indicate significant differences ($P < 0.05$) between RS and BS, respectively. The symbols *, **, and *** represent significant differences between RS and BS at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. All results are presented as mean ± standard error (n = 3).

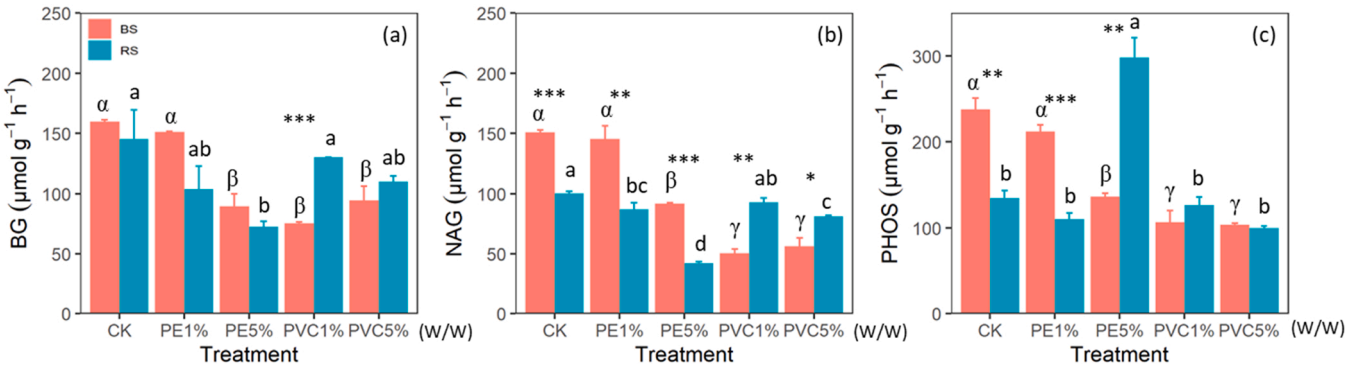

Fig. 2. Potential activities of BG (a), NAG (b), and PHOS (c) under BS and RS 54 days after planting wheat under five treatments; no polyethylene or polyvinyl chloride (CK), 1% polyethylene (PE1% (w/w)), 5% polyethylene (PE5% (w/w)), 1% polyvinyl chloride (PVC1% (w/w)), and 5% polyvinyl chloride (PVC5% (w/w)). Different lowercase letters in English and Greek indicate significant differences ($P < 0.05$) between RS and BS, respectively. The symbols *, **, and *** represent significant differences between RS and BS at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. All results are presented as mean ± standard error (n = 3).

this was higher (P<0.01) when treated with PE5%. The PHOS activity in bulk soil was lower ($P < 0.05$) under PE5% (w/w) and PVC (1% and 5% (w/w)) treatments than under CK. The PHOS activity in the rhizosphere was higher ($P < 0.01$) under the PE5% (w/w) treatment than under CK (Fig. 2c).

3.4. Hotspot area and rhizosphere expansion

The intensity of enzyme activities in the wheat roots under microplastic addition was also investigated (Fig. 3). Compared with that under CK, the BG hotspot areas increased ($P < 0.05$) by 92.47%, 213.3%, and 228.8% under the PE5%, PVC1%, and PVC5% (w/w) treatments, respectively (Figs. 4a and S1). Compared with that under CK, the NAG hotspot areas decreased ($P < 0.05$) by 39.73% under PE1% (w/w) treatment but increased ($P < 0.05$) by 48.8% under the PE5% treatment (Figs. 4b and S1). Compared with that under CK, the PHOS hotspot areas decreased by 28.13% under the PE1% (w/w) treatment; however, the PHOS hotspot increased by 36.66%, 56.56%, and 23.77% under the PE5%, PVC1%, and PVC5% (w/w) treatments (Figs. 4c and S1). The size of rhizospheric BG activity was reduced ($P < 0.05$) by 16.13%, 23.96%, 29.44%, and 35.27% under the PE1%, PE5%, PVC1%, and PVC5% (w/w) treatments compared with that under CK, respectively (Fig. 5a). The size of rhizospheric NAG activity increased ($P < 0.05$) by 12.27% in the rhizosphere under the PE5% (w/w) treatment and reduced ($P < 0.05$) by 33.02% and 32.43% under the PVC1% and PVC5% (w/w) treatment compared with that under CK (Fig. 5b). The size of rhizospheric PHOS activity in the rhizosphere decreased ($P < 0.05$) by 25.11% and 29.81% under the PVC1% and PVC5% (w/w) treatments compared with that under CK (Fig. 5c).

3.5. Line relationship and SEM

The linear regression analysis showed that the DOC content was positively ($R^2 = 0.2$, $P = 0.096$) correlated with the SR in the rhizosphere (Fig. S2a); the NH_4^+-N concentration in the rhizosphere was positively ($R^2 = 0.0092$, $P = 0.73$) correlated with the SR (Fig. S2b); the NO_3^--N concentration in the rhizosphere was positively ($R^2 = 0.54$, $P = 0.0019$) correlated with the SR (Fig. S2c); the Olsen-P concentration in the rhizosphere was negatively ($R^2 = 0.25$, $P = 0.055$) correlated with the SR (Fig. S2d); the potential activity of BG in the rhizosphere was positively ($R^2 = 0.32$, $P = 0.028$) correlated with the SR (Fig. S2e); the potential activity of NAG in the rhizosphere was positively ($R^2 = 0.23$, $P = 0.069$) correlated with the SR (Fig. S2f); and the potential activity of PHOS in rhizosphere was negatively ($R^2 = 0.029$, $P = 0.54$) correlated with the SR (Fig. S2g). The SEM (Chi-squared = 0.000, $p = 0.987$, GFI = 1.000, RMSEA = 0.000, AIC = 28.000) indicated that the interpretation

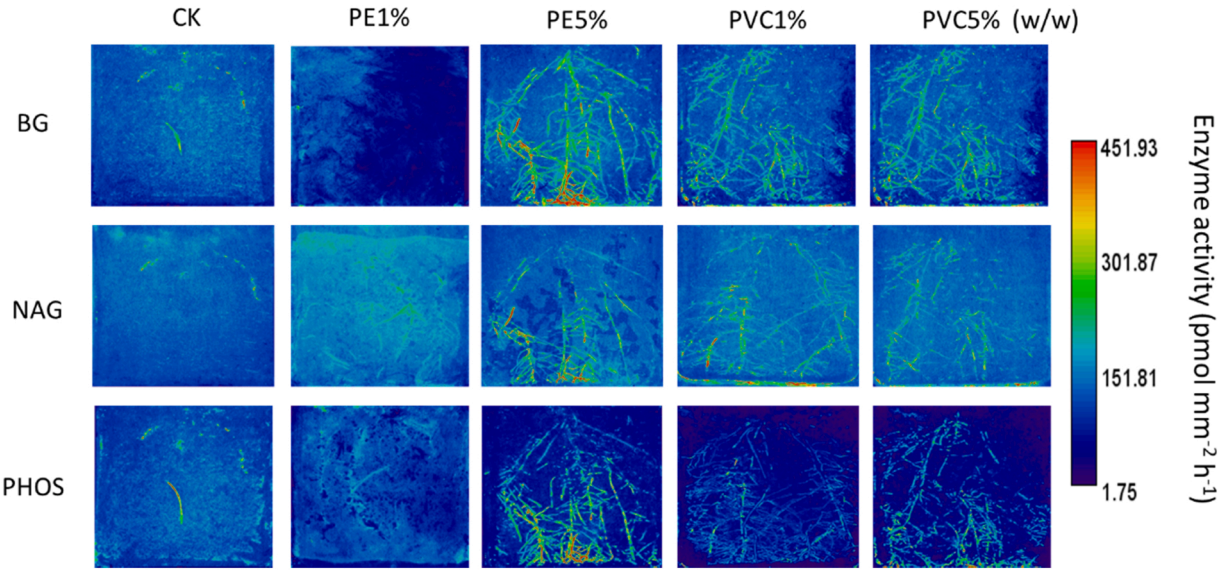

Fig. 3. Spatial distribution of BG, NAG, and PHOS 54 days after planting wheat under five treatments; polyethylene or polyvinyl chloride (CK), 1% polyethylene (PE1% (w/w)), 5% polyethylene (PE5% (w/w)), 1% polyvinyl chloride (PVC1% (w/w)), and 5% Polyvinyl chloride (PVC5% (w/w)).

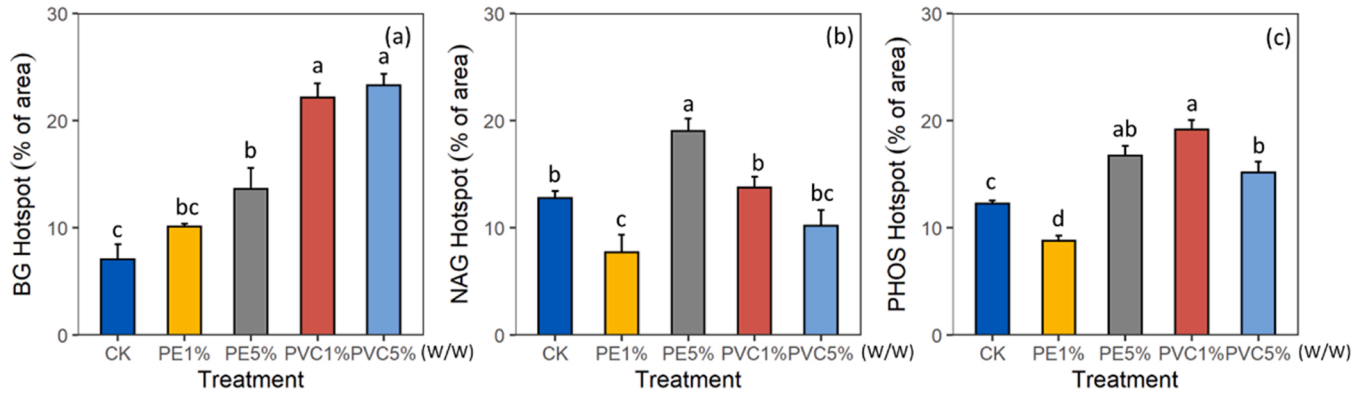

Fig. 4. Enzymatic hotspot areas for BG, NAG, and PHOS 54 days after planting wheat under five treatments; no polyethylene or polyvinyl chloride (CK), 1% polyethylene (PE1% (w/w)), 5% polyethylene (PE5% (w/w)), 1% polyvinyl chloride (PVC1% (w/w)), and 5% polyvinyl chloride (PVC5% (w/w)). All results are presented as mean ± standard error (n = 3).

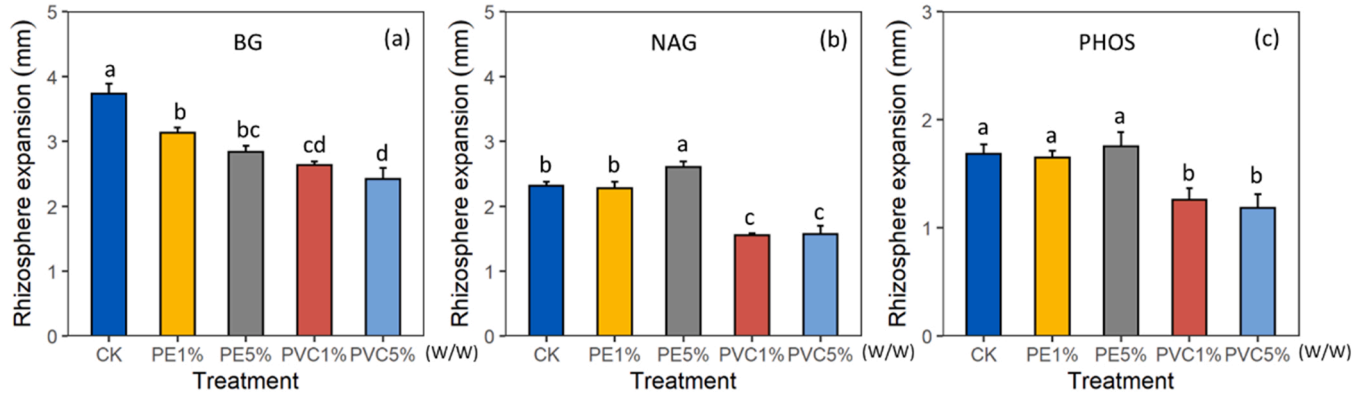

Fig. 5. Rhizosphere expansion of BG, NAG, and PHOS 54 days after planting wheat under five treatments; no polyethylene or polyvinyl chloride (CK), 1% polyethylene (PE1% (w/w)), 5% polyethylene (PE5% (w/w)), 1% polyvinyl chloride PVC1% (w/w)), and 5% polyvinyl chloride (PVC5% (w/w)). All results are presented as mean ± standard error (n = 3).

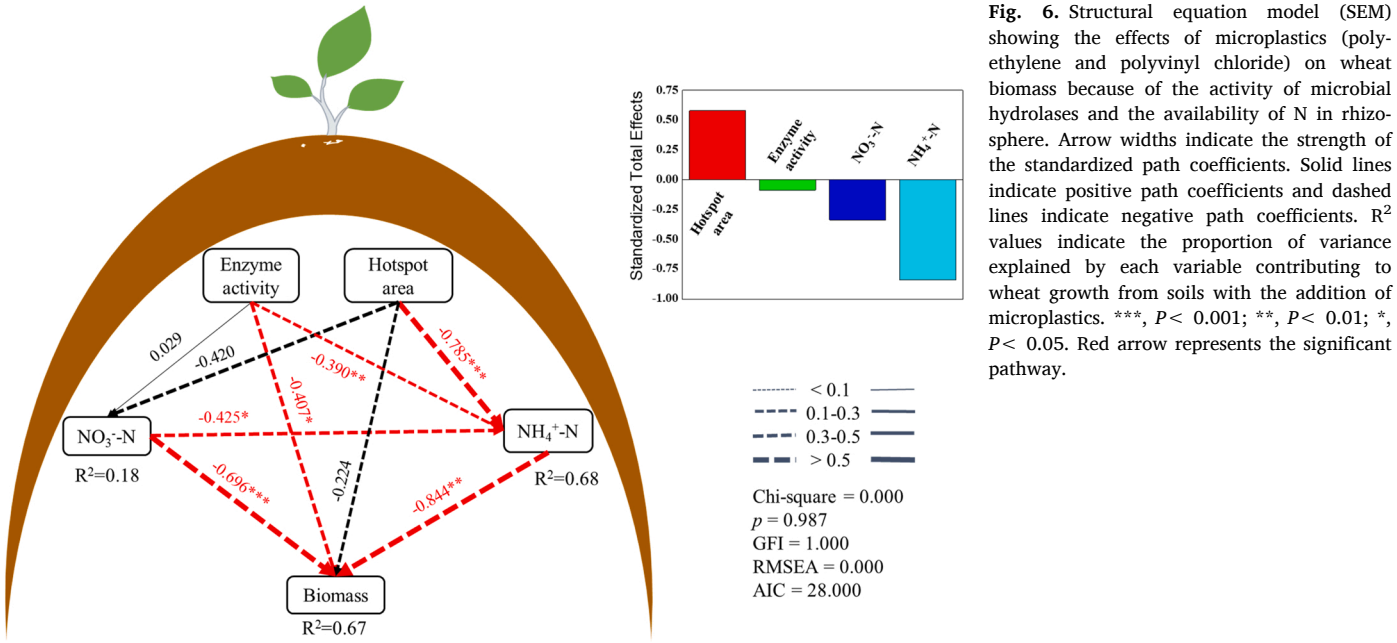

Fig. 6. Structural equation model (SEM) showing the effects of microplastics (polyethylene and polyvinyl chloride) on wheat biomass because of the activity of microbial hydrolases and the availability of N in rhizosphere. Arrow widths indicate the strength of the standardized path coefficients. Solid lines indicate positive path coefficients and dashed lines indicate negative path coefficients. R^2 values indicate the proportion of variance explained by each variable contributing to wheat growth from soils with the addition of microplastics. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Red arrow represents the significant pathway.

rate of all factors (biotic and abiotic) in the rhizosphere affecting the biomass of the wheat was 67%. The rhizosphere hotspot area of enzyme activity (0.58) had a positive effect on the biomass of wheat; however, the potential enzyme activity (−0.087), NO_3^--N concentration (−0.338), and NH_4^+-N concentration (−0.844) negatively affected the wheat biomass (Fig. 6).

4. Discussion

Microplastics are controversially correlated with shoot and root biomasses of crops in agriculture (Zang et al., 2020; Lozano et al., 2021; Wang et al., 2022). Our results showed an increase of 81.40–174.42% in the biomass of wheat shoots when the soil was treated with PE5%, PVC1%, and PVC5% (w/w), with a 196-fold increase in the biomass of the wheat root observed only under PVC5% (w/w) treatment. However, this result contradicts the findings of previous studies, which reported that the wheat shoot and root biomasses were suppressed by 13–53% at 1% and 5% (w/w) addition rates of these two microplastic types (Zang et al., 2020). We speculate this difference is related to the soil type and plastic mulch film usage in a region historically, and studies must consider toxin tolerance and adaptation of the soil. Microplastics could reduce the soil bulk density and water-stable aggregate formation (Lozano et al., 2021; Lozano and Rillig, 2020), which could directly reduce the penetration resistance of plant roots and improve soil aeration, increasing root growth and extension by changing its architecture and morphology (de Souza Machado et al., 2018b; Rillig et al., 2019; Wen et al., 2022). Root growth observed at the higher PVC5% (w/w) concentration facilitates water and nutrient uptake (Lozano et al., 2021; Ola et al., 2018). This result indicates that an increase in root biomass could improve water availability and promote rhizodeposition and mycorrhizal associations, ultimately contributing to an increase in shoot biomass (Ola et al., 2018; Shi et al., 2021a, 2021b; Li et al., 2022). The shoot biomass was greater than the root biomass, which resulted in a lower S/R than that under CK, indicating that more C was allocated to the shoot in response to microplastics (Zang et al., 2020). This increased the DOC content in the rhizosphere because the root exudates stimulate SOM mineralization (Liu et al., 2021; Wei et al., 2022). However, the DOC content in the rhizosphere soil was lower in the presence of PVC5% (w/w) than under CK, illustrating that higher doses of PVC limited microbial SOM decomposition (Zang et al., 2020; Gao et al., 2021). These results suggest that microplastics lead to a change in the resource-acquisition strategies of both roots and microbes by modifying the amount and composition of root exudates present to enhance the availability of nutrients in the rhizosphere (Kuzyakov and Domanski, 2002; Yin et al., 2013; Zang et al., 2020). Besides, the potential activities of BG in bulk soil were lower under the PE5% (w/w) and PVC treatments, but only in the rhizosphere, its activity under the PE5% (w/w) treatment was lower than that under CK. These results suggest that PE5% (w/w) decreases microbial C limitation and increases the energy of microorganisms (Rillig, 2018; Xiao et al., 2021), whereas fresh labile C from root exudates increased the need for higher amounts of C by microorganisms to synthesize carbohydrate hydrolase for SOM decomposition (Spohn and Kuzyakov, 2013; Liu et al., 2020; Li et al., 2020a, 2020b). Furthermore, microplastics are conducive to providing more habitats for soil microorganisms because of the close contact between the microplastics and soil (Gao et al., 2021). The generated microplastic-soil aggregates provide more growth sites for microorganisms, causing an increase in the number of cracks and voids on the surface morphology of the microplastics, which leads to biodegradation (Gao et al., 2021). Our results showed that the DOC content in the bulk soil was not affected by the addition of microplastics compared with that under CK, illustrating that alterations in the diversity and structure of the community do not necessarily involve changes in the decomposition rate of SOM due to microbial functional redundancy (Xiao et al., 2022). Wheat roots are forced to expand their growth range and area by facilitating symbiotic relationships with mycorrhizal partners to gain nutrients beyond the root depletion zone (Smith and Read, 2008), increasing the C turnover rate of rhizospheric microorganisms (Wei et al., 2019c). This leads to inconsistencies in the potential activities of BG in the rhizosphere and increase in the BG hotspot areas.

Microplastics affect N and P cycling (Chen et al., 2020; Yan et al., 2021). Here, compared with those under CK, the NH_4^+-N and NO_3^--N concentrations in bulk soil increased as the PE concentration increased, indicating that PE could absorb more NH_4^+ and NO_3^- ions at higher doses of PE (Li et al., 2021). However, the NH_4^+-N concentration in the rhizosphere was high under treatment with PE1% (w/w) and low under PE5% (w/w) compared with that under CK, and the NH_4^+-N concentration was low in the rhizosphere than in the bulk soil under PE5% (w/w) treatment, inferring that nitrification would be limited by the NH_4^+-N demand of growing wheat (Avrahami and Conrad, 2003; Liu et al., 2017). Similarly, the NO_3-N concentration under PE5% (w/w) was lower in the rhizosphere than in bulk soil, inferring that organic N is preferentially used by microorganisms exposed to higher levels of labile C, thus limiting denitrification (Ghani et al., 2013; Gao et al., 2021). Interestingly, Olsen-P was not significantly different in the rhizosphere and bulk soils in response to PE addition compared with that under CK, indicating that PE cannot stimulate microbial SOM mineralization for P acquisition as well as impede or increase plant P acquisition. This is inconsistent with the results of Shi et al. (2021b), who reported that sweet potato increased the adsorption of Olsen-P under PE addition, significantly reducing the Olsen-P concentration. This difference may be due to the lack of ability to adsorb P. However, it is interesting that the Olsen-P concentration was higher in the rhizosphere than in the bulk soil under PE1% (w/w) treatment, which suggests that lower doses of PE do not impede P acquisition by leading to an extension of the root system. Unexpectedly, PVC has no influence on NH_4^+-N, NO_3^--N, and Olsen-P in the bulk soil. This was also inconsistent with the results of previous similar research, which found that the NO_3^--N and Olsen-P concentration in the paddy soil (red) decreased under PVC1% due to decreased solubilization of inorganic P and mineralization of organic N and P (Satyaprakash, 2017; Yan et al., 2021). It can be inferred that in aquic brown soils, PVC cannot influence microorganism to mineralize SOM for acquire N and P. Here, similar results were obtained in the rhizosphere, illustrating that the rhizosphere (root exudates) does not influence microbial SOM mineralization under PVC addition.

Microplastics could directly increase the water-holding capacity and soil aggregation, thus stimulating root growth (Rillig et al., 2019). Wheat roots extend and secrete extracellular enzymes (reaching small distances from the root center) to acquire available nutrients for growth (Schliemann, 1984; Liu et al., 2021). Therefore, the three key extracellular enzymes can be characterized by their distribution along the root (Fig. 3). However, interestingly, different potential enzyme activities were observed in both rhizosphere and bulk soil in response to microplastics. The potential activity of BG was higher in the rhizosphere than in the bulk soil only when treated with PVC1% (w/w), illustrating that microorganisms preferentially consume energy (labile C) for SOM degradation (Qi et al., 2020; Zang et al., 2020; Xiao et al., 2021). The NAG hotspot observed under the PE1% (w/w) treatment increased when the soil was exposed to PE5% (w/w), stimulating wide rhizosphere expansion of NAG activity. It illustrates that higher doses of PE lead to an increase in the demand of the roots for available N. This competition between crops and microorganisms for N meant that NH_4^+-N and NO_3^--N were lower in the rhizosphere than in the bulk soil. The potential NAG activity was lower in the rhizosphere than in the bulk soil for PE, but higher in the rhizosphere for PVC. This may be because PVC improved denitrification by enhancing the anaerobic conditions or acting as a biocarrier for microbes, releasing toxic chemicals (Fei et al., 2020; Li et al., 2020a, 2020b). This led to the loss of N and a decrease in inorganic N (Li et al., 2020a, 2020b; Wei et al., 2019a, 2019b, 2019c), resulting in an insufficient N supply for crop roots and rhizospheric microorganisms and increased the activities of the N-degrading enzyme (NAG) to extract SOM for available N (Wen et al., 2022; Tong et al., 2022). Besides, the

addition of PE microplastics decreased the stability of bacterial populations and enhanced competition between different microbial communities, resulting in a higher temporal turnover and lower population numbers (Xiao et al., 2022). This inhabited the bacterial population and increased fungal activity due to the decreasing substrate availability. Root exudates can reduce pH in the rhizosphere (Liu et al., 2021), which produced an environment that favors fungal N acquisition by increasing the activities of NAG (Burns et al., 2013). More interestingly, compared with that under CK and PE1% (w/w), the potential activity of PHOS under PE5% (w/w) was low in bulk soil but high in the rhizosphere, inferring that higher doses of PE could lead to disintegration of the biofilms by secreting phosphatase, releasing P from its organic forms into the soil for the growth of crops (Chen et al., 2020). However, PVC treatments led to a narrower expansion of NAG and PHOS activities in the rhizosphere, indicating that PVC limited the ability of crops and microorganisms to use the available N and P, thus forcing the crop to obtain nutrients from the narrow root zone efficiently (Grossmann et al., 2011). All observed differences may be related to the microstructure and dose, crystallinity, reactivity, and/or toxicity of a plastic (Zang et al., 2020). Thus, PE and PVC have two slightly different pathways associated with the nutrient-acquisition strategies of crops. Higher doses PE (5% (w/w)) can increase the demand and utilization for NH_4^+-N and NO_3^--N by wheat by increasing the size of rhizosphere expansion of NAG activities. However, PVC decreased the size of rhizosphere expansion of NAG and PHOS activities along with an extended root system, forcing wheat root to efficiently obtain available nutrients in the narrow root zone.

5. Conclusions

This study revealed that the effects of adding different types and doses of microplastics to soil are inconsistent in terms of the turnover and acquisition strategies that crops use to obtain soil nutrients. The increase in NH_4^+-N and NO_3^--N under PE treatment is probably due to the lower labile C concentration in the bulk soil. Microplastics (except PE1% (w/w)) decrease the activities of BG, NAG, and PHOS in bulk soil, however, different root resource-acquisition strategies disturbed microbial nutrient turnover in the rhizosphere. It led to an inconsistent hotspot and size of key rhizospheric enzyme activities along with an extended root system, leading to an area of the rhizosphere with key enzymes. This suggests the potential effect on the nutrient-acquisition strategies of crops, resulting in crops obtaining more of the available nutrients than microorganisms. However, there are two slightly different pathways to the nutrient-acquisition strategies of crops for PE and PVC. At higher doses of PE (5% (w/w)), rhizospheric NH_4^+-N, and NO_3^--N were rapidly consumed by the growing wheat, by increasing the rhizosphere expansion of NAG activities. For PVC, the rhizosphere expansion of NAG and PHOS activities along with an extended root system decreased, forcing the wheat root to obtain available nutrients efficiently in the narrow root zone. Thus, the roots released larger amounts of labile C to stimulate microbial metabolism and N mining. This study provides important insights that link the resource-acquisition strategies of crops with microplastic addition during crop growth and improves our understanding of the mechanisms by which microbial nutrient turnover occurs in agricultural ecosystems.

CRediT authorship contribution statement

Yuhuai Liu: Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Mouliang Xiao**: Writing – review & editing. **Muhammad Shahbaz**: Writing – review & editing. **Zhi'e Hu**: Experimental analysis; **Zhenke Zhu**: Writing – review & editing. **Shunbao Lu**: Writing – review & editing. **Yongxiang Yu**: Writing – review & editing. **Huaiying Yao**: Writing – review & editing. **Jianping Chen**: Writing – review & editing. **Tida Ge**: Conceptualization, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data shown in graphs and tables, or contact corresponding author for data.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.129547.

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