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Commentary

Growth of grasses and forbs, nutrient concentration, and microbial activity in soil treated with microbeads☆

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ABSTRACT

Microplastics have emerged as an important threat to terrestrial ecosystems. To date, little research has been conducted on investigating the effects of microplastics on ecosystem functions and multifunctionality. In this study, we conducted the pot experiments containing five plant communities consisting of *Phragmites australis*, *Cynanchum chinense*, *Setaria viridis*, *Glycine soja*, *Artemisia capillaris*, *Suaeda glauca*, and *Limonium sinense* and added polyethylene (PE) and polystyrene (PS) microbeads to the soil (contained a mixture of 1.5 kg loam and 3 kg sand) at two concentrations of 0.15 g/kg (lower concentration, hereinafter referred to as PE-L and PS-L) and 0.5 g/kg (higher concentration, hereinafter referred to as PE-H and PS-H) to explore the effects of microplastics on total plant biomass, microbial activity, nutrient supply, and multifunctionality. The results showed that PS-L significantly decreased the total plant biomass ($p = 0.034$), primarily by inhibiting the growth of the roots. β-glucosaminidase decreased with PS-L, PS-H, and PE-L ($p < 0.001$) while the phosphatase was noticeably augmented ($p < 0.001$). The observation suggests that the microplastics diminished the nitrogen requirements and increased the phosphorus requirements of the microbes. The decrease in β-glucosaminidase diminished ammonium content ($p < 0.001$). Moreover, PS-L, PS-H, and PE-H reduced the soil total nitrogen content ($p < 0.001$), and only PS-H considerably reduced the soil total phosphorus content ($p < 0.001$), affecting the ratio of N/P markedly ($p = 0.024$).

Of interest, the impacts of microplastics on total plant biomass, β-glucosaminidase, phosphatase, and ammonium content did not become larger at the higher concentration, and it is observable that microplastics conspicuously depressed the ecosystem multifunctionality, as microplastics depreciated single functions such as total plant biomass, β-glucosaminidase, and nutrient supply. In perspective, measures to counteract this new pollutant and eliminate its impact on ecosystem functions and multifunctionality are necessary.

1. Introduction

Microplastics refer to diverse suite of polymer particles that are <5 mm and occur in a rich set of morphologies including beads, fibers, fragments, and films (Law and Thompson, 2014). They are the by-products of broadly used plastics in industries and society because of their wide range of functions, low cost, and high durability (Gao et al., 2021; Jiang et al., 2019; Nava and Leoni, 2021). Large pieces of plastics in the environment are broken into microplastics with a diameter of less than 5 mm through many mechanisms, including ultraviolet irradiation, collision, and friction (Zheng et al., 2019), which leads to the potential accumulation in soils worldwide.

At present, information regarding the impact of microplastics on soil ecosystem functions is still limited but intensively pursued (Boots et al., 2019). Microplastics are artificially manufactured materials, which have abiotic components and structural properties that are distinguishable

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from natural matter (De Souza Machado et al., 2018b). After entering the soil, microplastics can change soil properties, such as aggregates and porosity, thus affecting soil permeability and water-holding capacity (Liu et al., 2022b; Lozano and Rillig, 2020; Lozano et al., 2021). In addition, the leaching of additives contained in microplastics (De Souza Machado et al., 2018b), the role of microplastics as "new habitats" for microbes (Lu et al., 2019; Wang et al., 2021), and the hydrophobicity of microplastics and their high specific surface area for the adsorption and release of organic pollutants are all factors and processes that affect the soil biota (Horton et al., 2017; Teuten et al., 2009).

Therefore, the existence of microplastics in soils may alter a variety of ecosystem functions related to soil nutrients by affecting soil properties and microbial communities (Huang et al., 2021). Only a few studies have focused on this theme, and no consistent conclusion can be drawn, if any (Rillig et al., 2018). Reportedly, microplastics regulate soil enzyme activities after entering the soil (Lozano et al., 2021). Experiments have shown that polyamide (PA), Polyester (PES) fibers, and polypropylene (PP) greatly enhance the activity of the fluorescein diacetate hydrolase (De Souza Machado et al., 2019; Liu et al., 2017). Comparatively, PE microplastics significantly increase the urease activity in the Lake Cinnamon soil (Huang et al., 2019). In contrast, PP and PE largely decrease the catalase activity on the 40th and 60th days after being added to the soil, respectively (Yu et al., 2021). However, the results of the effects of microplastics on key enzymes closely related to the carbon, nitrogen, and phosphorus cycles (such as the β-glucosidase in cellulose degradation, the β-glucosaminidase in chitin degradation, and the phosphatase) are limited (Liu et al., 2022a; Lozano et al., 2021), and more research is needed.

It is worthwhile to emphasize that microplastics can directly fine-tune nutrient cycling. For example, the effect of microplastics on soil inorganic nitrogen content obtained the opposite results. A study demonstrated that PP and rubber crumb (RC) could reduce soil inorganic nitrogen (Liu et al., 2023), whereas another study found that the polylactic acid (PLA) microplastics addition in soils significantly reduced soil NH_4^+ content, while the contents of NO_3^- and NO_2^- increased significantly (Chen et al., 2020). In addition, there are few and inconsistent findings on the effects of microplastics on soil phosphorus supply. As shown, PP and PLA microplastics showed positive and no impact on soil available phosphorus/inorganic phosphorus respectively (Chen et al., 2020; Liu et al., 2017).

Up to now, there have been no consistent results about the impact of microplastics on plant biomass, with both positive and negative reports. For example, a study found that polystyrene (PS) at 0.1–10% mass concentration reduced the plant biomass of *Phaseolus radiates* and *Oryza sativa* (Kim et al., 2019), whereas another inquiry showed that microplastics at a 2% mass concentration considerably increased the biomass of *Allium fistulosum* (De Souza Machado et al., 2019). Microplastics can inhibit the root growth of plants by attaching to the root surface and blocking ion channels in the root system, thereby hindering nutrient and water uptake (Gao et al., 2019), or by impeding cell connections or cell walls in the roots and obstructing nutrient transport (Jiang et al., 2019). In addition, microplastics indirectly spur plant growth by changing the physical and chemical properties of soil structure, nutrient contents, and microbial activity (Gao et al., 2019; Lozano et al., 2021). Besides, most studies on the effect of microplastics on plant biomass have been conducted in experiments with only one species. However, the plant community usually consists of more than one species in the real ecosystem. The effect of microplastics on plant biomass needs to be examined at higher community and ecosystem levels.

In this study, we set up pot experiments containing five plant communities consisting of three plant species (*Phragmites australis* and *Cynanchum chinense* as resident species, forming a community with one of *Setaria viridis*, *Glycine soja*, *Artemisia capillaris*, *Suaeda glauca*, and *Limonium sinense*, respectively), aiming to explore the effects of PE (lower and higher concentrations) and PS (lower and higher concentrations) on ecosystem functions and multifunctionality, which were related to total plant biomass, microbial activity, and nutrient supply. We expected that microplastics would have a positive or negative impact on single ecosystem functions and multifunctionality, depending on the type and concentration of microplastics.

2. Materials and methods

2.1. Study species

We selected seven species including grasses (*P. australis* and *S. viridis*) and forbs (*C. chinense*, *G. soja*, *A. capillaris*, *S. glauca*, and *L. sinense)*, which frequently co-occur in the wetland ecosystem of the Yellow River Delta in China. Distributed throughout the Yellow River Delta, *P. australis* is one of the most common native species in the Yellow River Delta, and thus set as the dominant species. The other six species frequently appear in the dominant community of *P. australis*, so they were set as secondary dominant species except for *C. chinense*. The abundance of *C. chinense* is usually rather low in the dominant community of *P. australis*, so it was chosen as the permanent marginal species. Some plant seeds were collected in the Yellow River Delta in 2019, and others were purchased from Jufeng Seed Industry Group Co., Ltd. (Guangzhou, China).

2.2. Microplastic particles

The microplastics we used were polyethylene (PE) and polystyrene (PS), which are among the most used thermoplastics (Esterhuizen and Kim, 2022), and among the plastic components with the highest environmental residues (Xiao et al., 2020). Purchased from Dongguan Zhangmutou Haobang Plastic Raw Materials Firm (Dongguan, China), PE and PS were spherically shaped (beads) and had an average diameter of ~150 μm. The size of the microplastics was selected from a field survey on microplastics, in which the proportion of 100–250 μm size microplastic particles in the soil was the highest (Zhou et al., 2018). The densities of PE and PS were 0.962 g/cm^3 and 1.05 g/cm^3, respectively.

2.3. Experimental setup

The experiment was conducted at Shandong University, Qingdao, China (36°22′8.4″N, 120°41′0.0″E). The region has a temperate monsoon climate with a mean annual temperature of ~13.3 °C and a mean annual precipitation of ~723 mm. In April 2021, we established the experiment in the man-made greenhouse with an average temperature of 30 °C, a relative humidity of 85%, and a light intensity of 50 klx. The plastic pots (17.5 cm in height and 25.5 cm in diameter) were purchased from Suzhou Zhonghan Service Outsourcing Co., Ltd. (Suzhou, China). Each pot contained a mixture of 1.5 kg loam (purchased from Shandong LIGO Technology Co., Ltd. (Qingdao, China) and 3 kg sandy soil (purchased from Qingdao Aoshanwei Sands Factory (Qingdao, China). The soil without microplastics was used as the control treatment. The soil was homogenized and mixed with the microplastic beads at two concentrations (in the ratio of microplastics to soil) of 0.15 g/kg (lower concentration, hereinafter referred to as PS-L and PE-L) and 0.5 g/kg (higher concentration, hereinafter referred to as PS-H and PE-H). We noted that the choice of microplastic concentrations was based on previous studies (Li et al., 2020; Sun et al., 2022), which can be considered environmentally relevant (Sun et al., 2022). Our study aimed to investigate the effects of microplastic concentrations on ecosystem functions and multifunctionality at the current stage.

On April 20, 2021, the seeds were planted in the pots. Five communities were set up with six replicates, each composed of three species. The species compositions of five plant communities contained 1) *P. australis* + *C. chinense* + *S. viridis*, 2) *P. australis* + *C. chinense* + *G. soja*, 3) *P. australis* + *C. chinense* + *A. capillaris*, 4) *P. australis* + *C. chinense* + *S. glauca*, 5) *P. australis* + *C. chinense* + *L. sinense*. The thinning started when the seedlings were about 10 cm high. The number of *P. australis*

and *C. chinense* in each pot was 12 and 2, respectively, while the number of the other five secondary dominant species was 6. Therefore, a total of 20 plants were contained in each pot. The individuals of each species and treatments were randomly distributed. Each pot was adequately watered in a regular manner to ensure the normal growth of the plants. In total, 150 experimental pots were set up, counting the two microplastic treatments (PE and PS) and the two concentrations (0.15 g/kg and 0.5 g/kg), and the control treatment as well as the five communities constituting three species, with six replicates each treatment (n = 6). All pots were arbitrarily placed in the greenhouse and swapped regularly to minimize the potential influence of the possible differences in the environmental conditions during the experiment. As no water drained out of the pots, it is anticipated that no microplastics added to the soil left the pots.

2.4. Measurements of ecosystem functions and properties

Before harvesting (October 2021), soil from the 0–10 cm soil layer was taken at four points in each pot, with a distance of 5 cm or more between each point. All the samples were sieved using a 2-mm sieve to remove plant materials and soil particles, which were used to determine the inorganic nitrogen content, enzymatic activities, and microbial biomass of the soil. The nitrate content of the soil was analyzed by the calcium chloride extraction method (Guo et al., 2014), and the ammonium content of the soil was analyzed by indophenol blue colorimetry (Tel and Heseltine, 1990). Ammonium was extracted by calcium chloride, and the spectrophotometry of the extract was measured at 550 nm. β-glucosidase was measured following the technique of Eivazi and Tabatabai (1988) and the β-glucosaminidase was measured using the method of Parham and Deng (2000). Phosphatase was quantified using the p-nitrophenyl phosphate method (Avidov et al., 1993), and the microbial biomass was analyzed by the arginine ammonification method (Lin, 1999). Specifically, after adding arginine solution to the soil sample and incubating at 37 °C for 4 h, the soil microbial biomass was calculated by measuring the ammonium content in the soil at 550 nm by UV spectrophotometer (UV-9000s, Metash, Shanghai, China).

On harvesting (November 2021), the aboveground and belowground plant parts were split, washed, and classified according to the species. The plant samples were then oven-dried at 80 °C for 48 h and weighed. The total plant biomass of all species in the same pot was taken as the total aboveground and belowground plant biomass. The soil was dried at 25 °C for three weeks, and then the soil total nitrogen and phosphorus content measurements were obtained. The total nitrogen content of the soil was determined by the Kjeldahl method (a Kjeldahl nitrogen analyzer, K9860, Hanon, Jinan, China), and the total phosphorus content of the soil was analyzed by the molybdenum antimony anticolorimetric technique (a UV–vis spectrophotometer, UV-9000s, Metash, Shanghai, China). The soil nitrogen-phosphorus ratio (N/P) was also calculated (Peters et al., 2019).

2.5. Assessment of ecosystem multifunctionality

We measured 10 ecosystem functions and properties: (1) total plant biomass, (2) β-glucosidase, (3) β-glucosaminidase, (4) phosphatase, (5) nitrate content (NO_3^-), (6) ammonium content (NH_4^+), (7) microbial biomass, (8) soil total nitrogen content, (9) soil total phosphorus content, (10) soil nitrogen phosphorus ratio (N/P). β-glucosidase, β-glucosaminidase, phosphatase, and microbial biomass were considered to represent microbial activity (Schuldt et al., 2018). The content of NO_3^-, NH_4^+, soil total nitrogen, soil total phosphorus, and N/P were described as soil nutrient supply (Li et al., 2021). These variables are ecosystem functions (such as total plant biomass) or related to key properties (such as soil total nitrogen and phosphorus content), which have been used in previous studies of ecosystem functions and multifunctionality (Berdugo et al., 2017; Maestre et al., 2012).

The quantitative outcomes of each of the 10 measurements (i = 1–10) of the ecosystem functions were scaled to range from 0 to 1 in terms of the formula $f(x) = (x_i - x_{min})/(x_{max} - x_{min})$, where x is the variable with its minimum (x_{min}) and maximum (x_{max}) values observed over all study pots (Schuldt et al., 2018). All scaled measurements of a given ecosystem function were then averaged per pot to acquire the corresponding ecosystem function variable that represents the mean of the various independent measurements, giving each function the same weight in the multifunctionality analyses.

We chose two of the most commonly used methods available to gauge multifunctionality, 1) averaging approach, and 2) the multiple threshold approach (Schuldt et al., 2018). The averaging approach takes the mean value across all standardized functions as an index of the multifunctionality for each pot under study (Schuldt et al., 2018), whereas the threshold approach measures how many ecosystem functions simultaneously exceed a predefined percentage of a maximum of the observed values for each ecosystem function (Lefcheck et al., 2015; Van Der Plas et al., 2016; Zavaleta et al., 2010). As the selection of a given threshold is arbitrary, analyzing multiple thresholds of maximal functioning is recommended (Schuldt et al., 2018). Consequently, we applied thresholds of 20%, 40%, 60%, and 80% to scrutinize how microplastics affect ecosystem multifunctionality at low, medium, and high thresholds, respectively. We proceeded with the mean of the five largest values of each ecosystem function as the observed maximum to reduce the impact of potential outliers (Allan et al., 2015).

2.6. Data analysis

One-way ANOVA was performed to test the difference of each ecosystem function and multifunctionality between the microplastic treatments and the control (Dytham, 2011). The variance homogeneity test was first carried out for each ecosystem function, and the data with uneven variance were then log-transformed (Zar, 1999). The Duncan test was introduced for post-hoc test $p \leq 0.05$. SPSS 26.0 was implemented for all statistical analyses.

3. Results

3.1. Response of plant biomass to microplastics

The total plant biomass was affected by PS-L ($p = 0.034$; Table 1; Fig. 1A), which led to a 19% reduction compared to the control, but insignificantly by PS-H (Table 1; Fig. 1A). In contrast, both concentrations of PE showed no effect on the total plant biomass (Table 1; Fig. 1A).

The belowground biomass decreased with both PE-L and PS-L ($p = 0.002$), which led to a 20% and 22% reduction compared to the control respectively, while PE-H and PS-H did not produce a significant effect (Table 1; Fig. 1C). The aboveground biomass was not affected by the microplastics (Table 1; Fig. 1B).

3.2. Response of soil microbial activity to microplastics

The β-glucosaminidase and the phosphatase were modified by the microplastics (Table 1). PS-L, PS-H, and PE-L decreased the β-glucosaminidase by 50%, 33%, and 39% compared to the control respectively ($p < 0.001$), while increasing the phosphatase by 177%, 180%, and 169% compared to the control respectively ($p < 0.001$; Table 1; Fig. 2B and C). In addition, the microplastics did not significantly change the β-glucosidase and the microbial biomass (Table 1; Fig. 2A and D).

3.3. Response of soil nutrient supply to microplastics

The soil total nitrogen content, the total phosphorus content, the ratio of N/P, and the ammonium content were significantly affected by the microplastics (Table 1). In detail, the soil total nitrogen content was depressed with PS-L, PS-H, and PE-H ($p < 0.001$; Table 1; Fig. 3A), which led to a 14%, 17%, and 9% reduction compared to the control

Table 1
Results from one-way ANOVA on ecosystem functions to polystyrene at lower concentration (PS-L), polystyrene at higher concentration (PS–H), polyethylene at lower concentration (PE-L), and polyethylene at higher concentration (PE-H). Data given with mean ± SE (n = 6). Different letters indicate significant differences (ANOVA, followed by a Duncan post-hoc test, with α = 0.05) between treatments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Ecosystem functions		Control	PS-L	PS-H	PE-L	PE-H	F-ratio
Primary productivity	Total plant biomass	26.78 ± 1.31^{b}	21.80 ± 0.72^{a}	25.73 ± 0.95^{b}	24.99 ± 1.26^{ab}	26.24 ± 1.57^{b}	2.686*
	Aboveground biomass	20.59 ± 1.28	16.99 ± 0.67	20.40 ± 0.80	20.05 ± 1.33	19.20 ± 1.14	1.877
	Underground biomass	6.19 ± 0.33^{b}	4.81 ± 0.35^{a}	5.33 ± 0.31^{ab}	4.94 ± 0.31^{a}	7.04 ± 0.72^{b}	4.692**
Microbial activity	β-glucosaminidase	0.18 ± 0.009^{c}	0.09 ± 0.005^{a}	0.12 ± 0.004^{b}	0.11 ± 0.007^{ab}	0.19 ± 0.007^{c}	45.207***
	β-glucosidase	0.15 ± 0.012	0.16 ± 0.015	0.18 ± 0.011	0.18 ± 0.015	0.15 ± 0.010	1.040
	Phosphatase	1.00 ± 0.176^{a}	2.77 ± 0.072^{b}	2.80 ± 0.082^{b}	2.69 ± 0.114^{b}	0.55 ± 0.025^{a}	106.402***
	Microbial biomass	0.45 ± 0.006	0.45 ± 0.009	0.48 ± 0.011	0.47 ± 0.005	0.46 ± 0.012	1.946
Nutrient supply	Soil nitrogen content	0.23 ± 0.008^{c}	0.20 ± 0.010^{a}	0.19 ± 0.008^{a}	0.23 ± 0.007^{bc}	0.21 ± 0.007^{ab}	5.337***
	Soil phosphorus content	0.14 ± 0.003^{b}	0.15 ± 0.005^{b}	0.11 ± 0.003^{a}	0.14 ± 0.005^{b}	0.13 ± 0.004^{b}	12.172***
	N/P	1.67 ± 0.051^{b}	1.42 ± 0.096^{a}	1.75 ± 0.075^{b}	1.72 ± 0.093^{b}	1.60 ± 0.065^{ab}	2.882*
	Nitrate content	0.20 ± 0.006	0.21 ± 0.017	0.23 ± 0.024	0.27 ± 0.033	0.19 ± 0.003	1.845
	Ammonium content	6.72 ± 0.561^{b}	1.98 ± 0.207^{a}	2.17 ± 0.334^{a}	2.99 ± 0.450^{a}	5.78 ± 0.689^{b}	20.583***

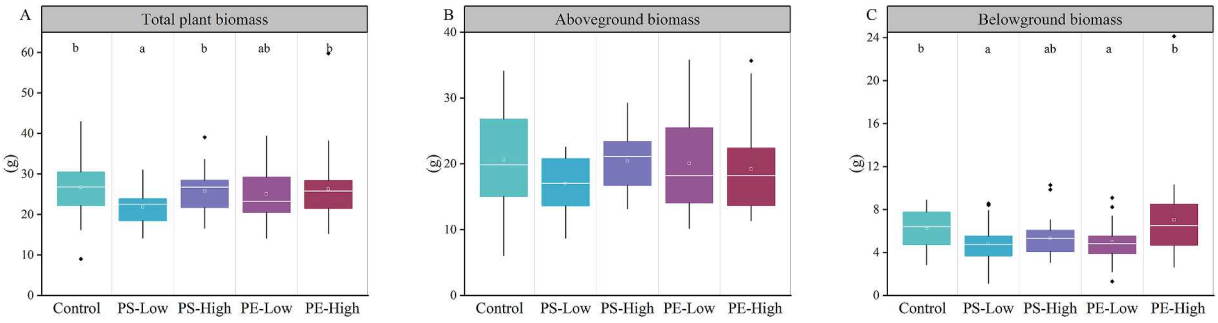

Fig. 1. Responses of total plant biomass (A), aboveground biomass (B), and belowground biomass (C) to low concentration of polystyrene (PS-Low), high concentration of polystyrene (PS–High), low concentration of polyethylene (PE-Low), and high concentration of polyethylene (PE-High) (n = 6). There were five plant communities in each treatment, and each community was composed of three species. The five plant communities were composed of *Phragmites australis*, *Cynanchum chinense* and one of the other five species (*Setaria viridis, Glycine soja, Artemisia capillaris, Suaeda glauca, and Limonium sinense*), respectively.

respectively, whereas the soil total phosphorus content dropped with PS-H ($p < 0.001$; Table 1; Fig. 3D), which led to a 21% reduction compared to the control. The ratio of N/P declined with PE-L ($p = 0.024$; Table 1; Fig. 3E), which led to a 15% reduction compared to the control, and PS-L, PS-H, and PE-L reduced the ammonium content by 71%, 68%, and 56% compared to the control ($p < 0.001$; Table 1; Fig. 3B). Furthermore, the microplastics gave negligible effect on the nitrate content (Table 1; Fig. 3C).

3.4. Response of ecosystem multifunctionality to microplastics

The average method showed that ecosystem multifunctionality scores were diminished only by PE-H ($p = 0.002$; Table 2; Fig. 4), which led to a 13% reduction compared to the control.

The thresholds adopted for calculating the ecosystem multifunctionality showed similar trends to the average multifunctionality (Fig. 5). In specific, the ecosystem multifunctionality decreased with PS-L at the 20% threshold ($p = 0.001$; Table 2; Fig. 5A), which led to an 18% reduction compared to the control. At the 40% threshold, PS-L, PS-H, and PE-H reduced the ecosystem multifunctionality by 23%, 29%, and 29% compared to the control, respectively ($p = 0.002$; Table 2; Fig. 5B). At the 60% threshold, the ecosystem multifunctionality went down a little bit with PE-H in comparison to the somehow increase with PE-L, but there was no significant difference between the two treatments and the control ($p = 0.004$; Table 2; Fig. 5C). The 80% threshold produced no significant impact on the ecosystem multifunctionality by the microplastics (Table 2; Fig. 5D).

4. Discussion

The microplastic treatments affected ecosystem functions associated with the total plant biomass, microbial activity, and nutrient supply as well as ecosystem multifunctionality. Overall, the microplastic treatments had a negative impact on the ecosystem functions and multifunctionality, but the degree of the effects was contingent on the types and concentrations of the microplastics.

4.1. Response of plant biomass to microplastics

Compared to the control, the total plant biomass and the belowground biomass were decreased with PS-L, but PS-H did not affect production. The decline of total plant biomass with PS-L might be caused by the inhibition of root growth. The size of the microplastics used in this study were 150 μm, which was an order of magnitude larger than the average pore diameter of the plant roots, so PE and PS used in this study shouldn't enter the roots in large quantities (Li et al., 2020). However, they could adhere to the root surface, physically thwarting the absorption of water and nutrients by the plants (Jiang et al., 2019; Li et al., 2020). As reported, an examination investigating the effects of three sizes of plastic particles (50, 500, and 4800 nm) on *Lepidium sativum* found that the microplastics blocked the pores in the seed capsule (Bosker et al., 2019). In contrast, total plant biomass was not reduced with PS-H. Understandably, a large number of microplastics could meaningfully alter the soil properties and promote the porosity and permeability of the soil after entering the soil, which would alleviate the inhibitory effect on root growth (De Souza Machado et al., 2018a; Lozano et al., 2021). PE did not influence total plant biomass, that is, PE had no effect on the total plant biomass. In comparison, an investigation discovered that PS depreciated the plant biomass of *P. radiates* and *O. sativa* (Kim et al., 2019), but it was not the case for high-density PE showing only a minute effect on the biomass of *Zea mays* (Wang et al., 2020). These results can be attributed to the following two reasons: (1)

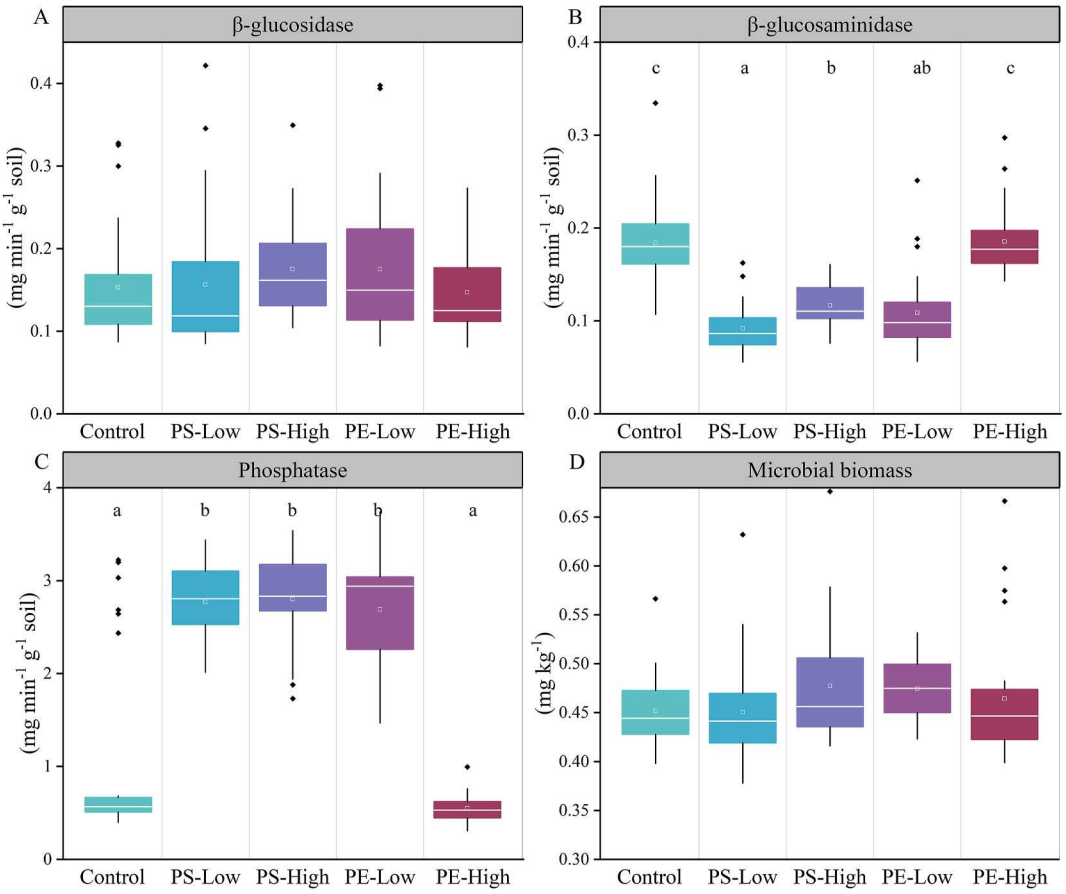

Fig. 2. Responses of β-glucosidase (A), β-glucosaminidase (B), phosphatase (C), and microbial biomass (D) to low concentration of polystyrene (PS-Low), high concentration of polystyrene (PS–High), low concentration of polyethylene (PE-Low), and high concentration of polyethylene (PE-High) (n = 6).

Additives contained in PE and PS are different (Do et al., 2022), influencing plant growth inconsistently; (2) With the same particle size (150 μm), the density of PE (0.962 g/cm^3) is smaller than that of PS (1.05 g/cm^3), indicating that the number of PE particles is higher and the volume occupied is larger under the same mass concentration, which means that the number of PE particles adhered to the root surface is more, but its impact on soil bulk density and porosity is greater, and the comprehensive effect of these factors makes that PE has little impact on the total plant biomass, even though PE-L reduced the belowground biomass. Hence, the response of the plant biomass to the microplastics relied on the types and concentrations of the microplastics. Notably, the reduction in the biomass was only observed for the belowground biomass but not for the aboveground biomass, indicating that the effect of the microplastics was not strong enough to exert influence on the aboveground biomass.

4.2. Response of soil microbial activity to microplastics

The results showed that the β-glucosaminidase and the phosphatase were affected by the microplastic treatments. The β-glucosaminidase decreased with PS-L, PS-H, and PE-L as the phosphatase increased with PS-L, PS-H, and PE-L. The activities of the β-glucosidase, the β-glucosaminidase, and the phosphatase reflect the lack of nutrients in the soil required by soil microbes (Sinsabaugh et al., 2011). Hence, the findings demonstrated that the supply capacity of the soil for available nitrogen decreased, but the supply capacity of the soil for available phosphorus increased. The phenomenon is attributable to three plausible reasons: (1) the impacts of microplastics on soil properties (such as pH) may increase the fixation of soil phosphorus, which reduced the bioavailability of soil phosphorus, thus stimulating the activity of phosphatase (Li and Liu, 2022); (2) alterations in surface morphologies of microplastics resulting from environmental exposure, including rougher surface morphologies, and negatively or positively charged, impacted bacterial colonization, shifting the relative abundance of microbial groups (Hossain et al., 2019; McCormick et al., 2014), which altered the demand for nitrogen and phosphorus nutrients by microbial groups; and (3) the microplastics released harmful contaminants into the soil, and their specific surface area could lead to the adsorption of organic pollutants, then migrate and diffuse in the soil to aggravate the toxic effect on specific microbial groups (Horton et al., 2017; Teuten et al., 2009), which also changed the nitrogen and phosphorus requirements of the microbial groups. We propose that our results suggest that the nitrogen demand of the microbial groups decreased, and as a result the excess nitrogen could be allocated to carbon-decomposing enzymes and phosphorus-decomposing enzymes, which would encourage the decomposition of high molecular weight organic matter (containing both carbon and phosphorus elements) that is difficult to degrade in the soil (Allison et al., 2006; Cenini et al., 2016). Nevertheless, PE-L affected both the β-glucosaminidase and the phosphatase, while PE-H had no impact on them, a similar finding to the impact on the total plant biomass. We remarked that numerous factors played a part in the impact of the microplastics on microbial activity, including but not limited to type, concentration, and density of microplastics, proving that the response of the soil microbial activity to the microplastics hinged on the types and concentrations of the microplastics and both their direct effects (e.g. inhibition of root growth) and indirect effects (e.g. changes in soil properties).

Both β-glucosidase and the microbial biomass were not changed by the microplastics. The results showed that the β-glucosidase had relatively stable activity and was less affected by environmental changes

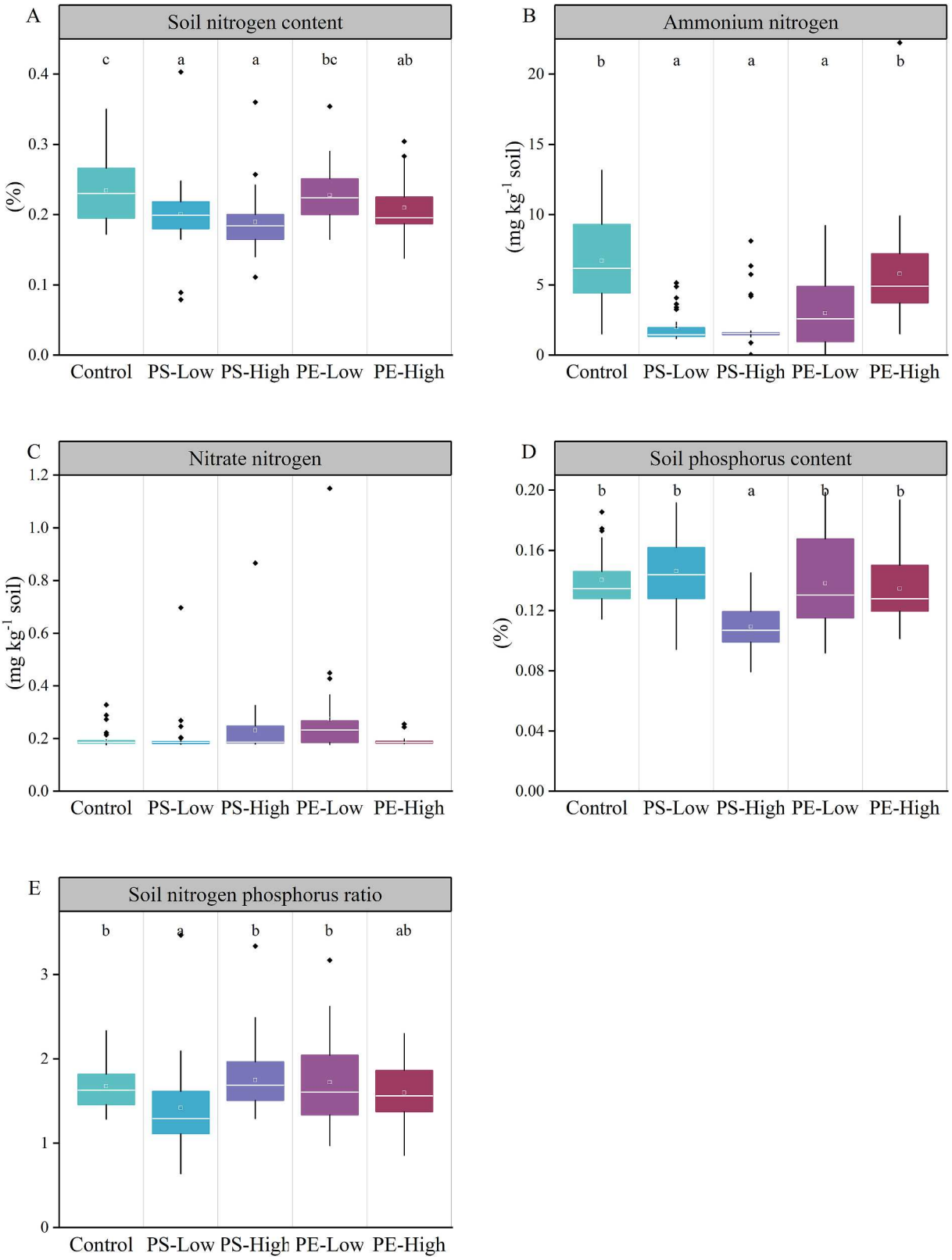

Fig. 3. Responses of total nitrogen content (A), ammonium content (B), nitrate content (C), total phosphorus content (D), and soil nitrogen phosphorus ratio (N/P) (E) to low concentration of polystyrene (PS-Low), high concentration of polystyrene (PS–High), low concentration of polyethylene (PE-Low), and high concentration of polyethylene (PE-High) (n = 6).

Table 2
Responses of ecosystem multifunctionality, by one-way ANOVA, to low concentration of polystyrene (PS-L), high concentration of polystyrene (PS–H), low concentration of polyethylene (PE-L), and high concentration of polyethylene (PE-H). Ecosystem multifunctionality was calculated based on the average approach and the threshold approach in which each function that exceeds 20%, 40% 60%, and 80% of the standardized maximum contributions to the multifunctionality scores. Data given mean ± SE (n = 6). Different letters indicate significant differences (ANOVA, followed by a Duncan post-hoc test, with α = 0.05) between treatments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

		Thresholds	Control	PS-L	PS-H	PE-L	PE-H	F-ratio
Multifunctionality	average approach		0.32 ± 0.013^{bc}	0.29 ± 0.013^{ab}	$0.30 \pm 0.009^{a-c}$	0.34 ± 0.010^{c}	0.28 ± 0.011^{a}	4.123**
	threshold approach	20%	0.67 ± 0.021^{b}	0.55 ± 0.028^{a}	0.64 ± 0.022^{b}	0.68 ± 0.022^{b}	0.61 ± 0.023^{ab}	5.205**
		40%	0.35 ± 0.024^{b}	0.27 ± 0.019^{a}	0.25 ± 0.023^{a}	0.34 ± 0.027^{b}	0.25 ± 0.020^{a}	4.489**
		60%	0.09 ± 0.023^{ab}	0.15 ± 0.017^{b}	0.12 ± 0.013^{ab}	0.14 ± 0.014^{b}	0.07 ± 0.015^{a}	3.977**
		80%	0.02 ± 0.007	0.06 ± 0.015	0.04 ± 0.011	0.05 ± 0.012	0.02 ± 0.009	1.794

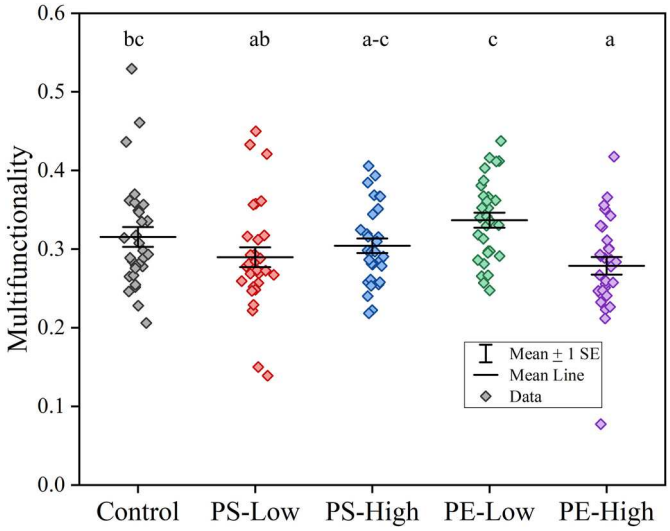

Fig. 4. Responses of ecosystem multifunctionality to low concentration of polystyrene (PS-Low), high concentration of polystyrene (PS–High), low concentration of polyethylene (PE-Low), and high concentration of polyethylene (PE-High). Ecosystem multifunctionality was calculated based on the average approach. Data points are shown as circles (n = 6).

owing to the binding and protective effect of soil colloids and aggregates (Moscatelli et al., 2012), avoiding the impact of the microplastics. Although the soil microbial biomass was not meaningfully varied by the microplastics, we couldn't guarantee that important changes in the microbial community structure and composition did not happen. Yet the microbial community structure and composition were not directly measured in our study though their importance was recognized, future studies shall give more attention to the exploration in this direction.

4.3. Response of soil nutrient supply to microplastics

As deliberated in the above, the results displayed that the total nitrogen content, the total phosphorus content, the ratio of N/P, and the ammonium in the soil were influenced by the microplastics. The soil total nitrogen content decreased with PS-L, PS-H, and PE-H, and the soil total phosphorus content decreased with PS-H. The microplastics decreased both the nitrogen supply and the phosphorus supply in the soil. The consequences of the soil aggregates by the microplastics have been confirmed in many studies, showing that the microplastics would bind to the soil aggregates and destroy the structure (De Souza Machado et al., 2018a, 2019; Lozano et al., 2021; Rillig et al., 2019; Zhang and Liu, 2018). As contended, the soil aggregates are closely related to the soil water holding capacity, and a decrease in the soil aggregates will reduce the soil water holding capacity (Lozano and Rillig, 2020; Lozano et al., 2021), resulting in accelerated water loss and reductions of nutrient availability. Furthermore, adding microplastics reduces the soil bulk density and increases the soil porosity after entering the soil (De Souza Machado et al., 2019; Lozano et al., 2021), which actually speeds up the soil water loss and leads to an increase the nutrient leaching. In addition, the decrease in soil bulk and the increase in saturated hydraulic conductivity caused by microplastics intensified the competition of microorganisms for soil nutrients, which will inevitably affect the microbial decomposition and mineralization of soil organic matter, thereby affecting the supply of soil nutrients (Liu et al., 2022a). These factors will lead to the decline of soil nutrient supply. As well, the ratio of N/P decreased with PE-L, which might indicate that the consequence of the microplastics on the nitrogen supply was greater than that of the phosphorus supply.

We found that the ammonium decreased with PS-L, PS-H, and PE-L, which was consistent with the change in β-glucosaminidase. A decrease in β-glucosaminidase reduced soil available nitrogen (Cluzard et al., 2015; Nasholm et al., 2009). The decrease in available nitrogen evidenced that the ammonium was affected, while the nitrate is less impacted than the ammonium due to its low content.

4.4. Response of ecosystem multifunctionality to microplastics

Our study found that microplastic treatments affected not only single ecosystem functions, but also the ecosystem multifunctionality. Results from both the averaging approach and the multiple threshold approach showed that microplastics reduced the ecosystem multifunctionality. Individual functions were affected by microplastics to varying degrees in this study, both positively (such as phosphatase) and negatively (such as total plant biomass, β-glucosaminidase, and soil nutrient supply), but the negative impact is more, which led to a negative impact of microplastics on the ecosystem multifunctionality. Similarly, a previous study found that the microplastic fibers reduced the ecosystem multifunctionality under well-watered conditions, but there was no significant impact under drought conditions, which suggested that microplastics in soils may negatively impact ecosystem multifunctionality as much as drought (Lozano et al., 2021). In addition, soil nutrient cycling is mainly driven by microbes (Ward and Jensen, 2014). These results proved the importance of the microbial community in maintaining soil functions (such as microbial activity and nutrient supply) and multifunctionality (Lozano et al., 2021), and emphasized the urgency of testing the impact of microplastics on the microbial community in order to maintain higher functions and multifunctionality.

Microplastics are notoriously difficult to degrade and will persist in soils over long periods (Qi et al., 2020; Ya et al., 2021). However, current studies emphasize a study period of just 1–3 months (De Souza Machado et al., 2018a, 2019; Deng et al., 2022; Yu et al., 2021). As a result, what is being explored is the short-term effects of microplastics. Our study lasted six months, to date one of the longest studies, and thus our results may offer greater representativeness of the general effects of microplastics at a longer time scale. Certainly, more research is needed to corroborate the findings.

Pertinently, a number of studies have reported the concentration distribution of microplastics in many regions, revealing the regional dependency over a wide range. For instance, microplastic

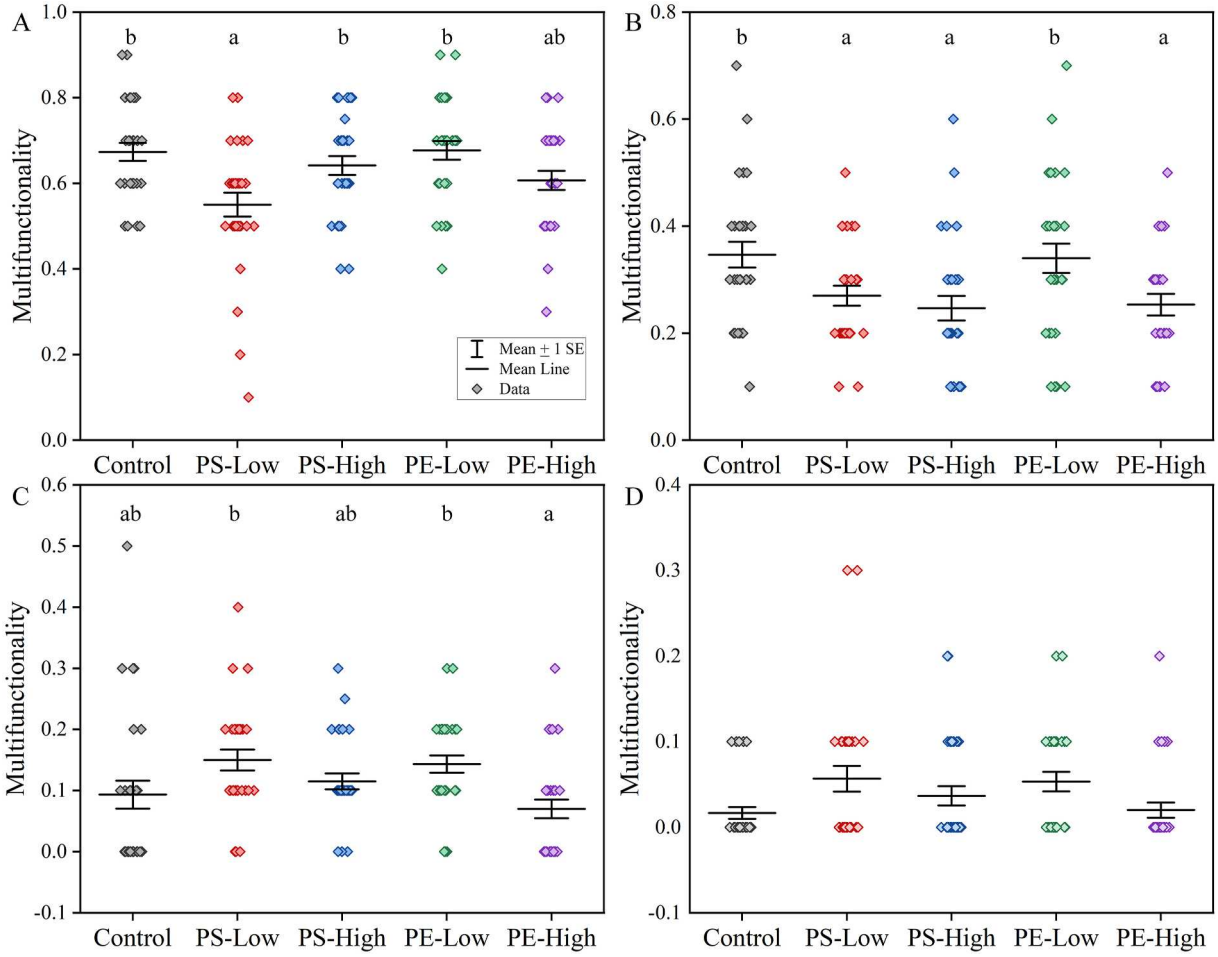

Fig. 5. Responses of ecosystem multifunctionality to low concentration of polystyrene (PS-Low), high concentration of polystyrene (PS–High), low concentration of polyethylene (PE-Low), and high concentration of polyethylene (PE-High). Ecosystem multifunctionality was calculated based on the threshold approach in which each function that exceeds 20% (A), 40% (B), 60% (C), and 80% (D) of the standardized maximum contributions to the multifunctionality scores. Data points are shown as circles (n = 6).

concentrations in flood plains and agricultural soils ranged from 0.0055% to 0.00129% at low and 0.022%–0.03% at moderate levels (Scheurer and Bigalke, 2018; Xu et al., 2019; Zhu et al., 2019), while microplastics concentrations up to 7% had been found in industrial soils (Fuller and Gautam, 2016). Accordingly, the concentrations of the microplastics used in this study might only represent the current microplastic concentrations. It should be noted that the dose-effect of microplastics may be nonmonotonic (De Souza Machado et al., 2018a). In other words, some of the lower concentration of microplastic seem to cause stronger effects than higher concentration compared to the control. However, this study cannot assess nonmonotonic dose-responses, because this study was not designed for that purpose. Nevertheless, this study could increase our understanding of the effect of microplastics on ecosystem functions and multifunctionality, suggesting the necessity for imminent management in reducing microplastic pollution.

5. Conclusion

With the aggravation of microplastic pollution, it is increasingly urgent to exploit its effect on ecosystem functions and multifunctionality. In this study, we performed experiments on a longer time scale and found that many of the ecosystem functions were depressed or altered, which influenced ecosystem multifunctionality. Microplastics reduced the total plant biomass by inhibiting the growth of plant roots, which was determined by the fact that microplastics inhibited the roots from absorbing nutrients and water, and improved soil permeability to promote root growth. Besides, there are many reasons for the influence of microplastics on β-glucosaminidase and phosphatase, including but not limited to changes in the relative abundance of microbial groups and the availability of soil nutrients. In addition, microplastics reduced soil total nitrogen and phosphorus supply by affecting soil properties, and the reduction in nitrogen supply was greater than the phosphorus supply. In perspective, such a new type of pollutant should be given adequate attention as microplastics may affect not only ecosystem functioning and multifunctionality but also ecosystem services and products which are crucial for the well-being of humans.

Author Statement

Jingfeng Wang: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing - Reviewing & Editing, Visualization **Xiao Guo:** Conceptualization, Methodology, Writing – original draft preparation, Writing - Reviewing & Editing, Supervision, Project administration **Zhenwei Xu:** Methodology, Investigation, Resources **Yi Hu:** Investigation **Wenyi Sheng:** Investigation **Yanni Chen:** Investigation **Mingyan Li:** Writing – original draft, Writing - Reviewing & Editing **Wei**hua **Guo:** Supervision, Project administration, Funding acquisition **Janice Brahney:** Formal analysis, Writing - Reviewing & Editing.

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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

The authors do not have permission to share data.

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