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Traditional and biodegradable plastics improve pea (*Pisum sativum*) growth by promoting nutrient turnover in soil

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KEYWORDS

Biodegradable microplastics, microbial community, nutrient-acquisition strategies, standard microplastics

HIGHLIGHTS

- Polypropylene (PP) and polyethylene (PE) and polycaprolactone (PCL) microplastics can bond to soil aggregates.
- Polyadipate/butylene terephthalate (PBAT) microplastics can stimulate microbial growth, mining more available nutrients for pea root growth.
- Microplastics increased N uptake during pea growth with increasing *β*-1,4-N-acetylglucosaminidase activity.
- Increasing Shannon diversity was observed with 0.1% PP, PE and PCL, and with 0.1% and 1% PBAT at the seedling stage.
- Microplastics at 0.1% and 1% did not affect material exchange between soil bacteria and fungi but did negatively effect on ecosystem functions.

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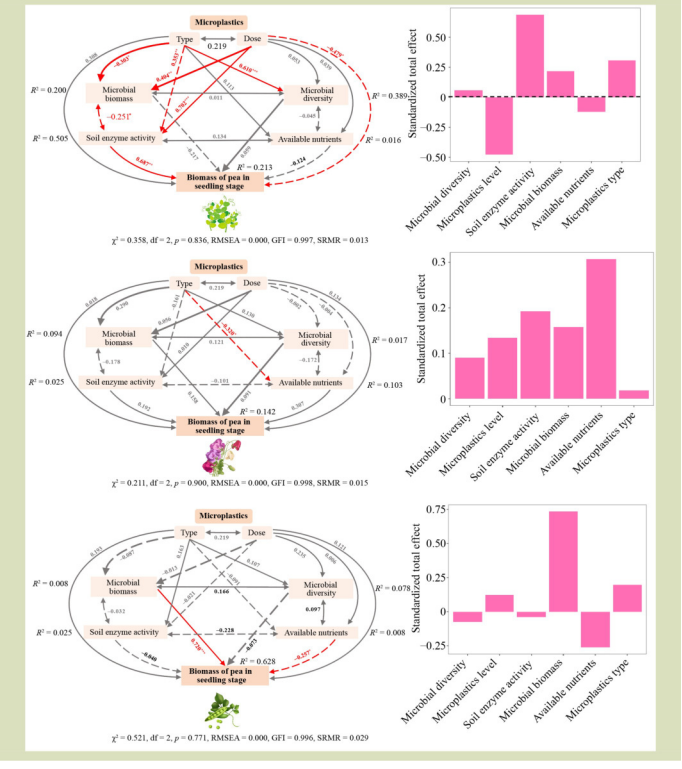
GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic accumulation caused by traditional plastic mulching can disturb plant nutrient-mining strategies. Biodegradable plastics may reduce these risks. However, the different effects of traditional and biodegradable microplastics on agroecosystems and optimal microplastic type for crop-soil systems remain largely unknown. A pot experiment was performed to identify the mechanisms underlying the effects of traditional [polypropylene (PP) and polyethylene (PE)] and biodegradable [polycaprolactone (PCL) and polyadipate/butylene terephthalate (PBAT)] microplastics at 0%, 0.1% and 1% (w/w) in a pea-soil ecosystem. Traditional microplastics caused greater carbon allocation to shoots, while PBAT did not significantly alter dissolved organic-carbon content. NH_4^+-N increased with 1% (w/w) PP whereas NO^{3-}-N decreased owing to enhanced *N*-acetylglucosaminidase activity with 0.1% and 1% PP and PE, and 1% PBAT during pea growth. Biodegradable microplastics enhanced microbial biomass carbon, nitrogen and phosphorus, whereas traditional microplastics gave inconsistent results. Microplastics increased the complexity of bacterial and fungal networks and impacted ecosystem functions because they may serve as labile carbon resources for soil microorganisms, stimulating organic matter decomposition. However, once labile carbon in native soils is depleted, inadequate fresh labile carbon from root exudates fails to alleviate microbial carbon limitations, resulting in peas competing with microorganisms for scarce nitrogen resources to promote its growth.

1 Introduction

Plastic pollution, particularly that associated with microplastics (< 5 mm diameter particles), has emerged as a pervasive threat to ecosystem health and safety[1]. Indeed, the extensive use of plastic mulch in agriculture has transformed agroecosystems into significant reservoirs of microplastic pollution. Once released from residual plastic films, microplastics readily interact with the surroundings in all ecosystems. As an exogenous input, microplastics have been shown to affect both abiotic and biotic soil processes, depending on particle characteristics and environmental conditions[2]. Numerous studies have documented the general detrimental effects of microplastics on soil properties, microbial communities and soil biota, with some of their effects being lethal for soil health[3,4]. Further, microplastic debris can contribute to the concentration of other environmental pollutants, such as heavy metals and persistent organic pollutants, altering their distribution patterns and bio-accessibility in soils because of their persistence and large specific surface area[5]. Additionally, microplastics can alter soil microbial community diversity and structure, thereby affecting ecosystem functionality and resilience[6]. These effects are contingent upon the type and dose of the specific microplastics present[7].

In agroecosystems, microplastics primarily originate from plastic films, both traditional and biodegradable types. Traditional microplastics, such as polypropylene (PP) and polyethylene (PE), pose emerging threats to soil health, plant growth and microbial diversity by altering soil structure, disrupting key nutrient cycles (e.g., carbon, nitrogen and phosphorus) and ultimately affecting ecosystem multifunctionality[8–10]. The different chemical structures of PE and PP result in distinct physicochemical and biological changes in the soil properties[6]. In response to these challenges, environmentally friendly agricultural films composed of biodegradable microplastics, such as polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT), have been developed as alternatives to mitigate such ecological damage. Thus, there is an ongoing trend to replace

non-degradable plastics with biodegradable ones[11]. In particular, biodegradable microplastics (PCL and PBAT) have been engineered to readily decompose in natural environments, potentially reducing their long-term ecological footprint. PCL, a biodegradable polyester, shows distinct advantages over other biopolymers, such as its hydrophobic and slow-degrading nature[12], while PBAT is an eco-friendly plastic with excellent heat resistance and ductility[13]. Yet there are downsides to these biodegradable microplastics, as they can impair agroecosystem multifunctionality by reducing bacterial diversity, fungal diversity, nutrient availability and enzymatic activity, thereby hindering plant growth[6]. PCL and PBAT may contain certain chemical additives that interfere with the biodegradation process and causes harm to the environment[14]. Small plastic particles can infiltrate the soil, thus posing potential risks to soil biodiversity and health. However, their effects on the soil, in terms of toxicity and environmental impacts, remain largely unexplored. Also, the different ecological effects of traditional and biodegradable microplastics on agroecosystems, as well as the optimal biodegradable plastic to use during critical crop growth stages, have not been determined. Finally, most studies looking at the effects of microplastics focus mainly on a single growth stage; few have considered the different key stages of plant growth (seedling, flowering and maturity)[10,15]. Therefore, the ecological safety of biodegradable plastics must be assessed across all growth stages before promoting their widespread commercial application in crop production.

The aim of this study was to examine the effects of both traditional and biodegradable microplastics on a crop-soil system across three key plant growth stages. Peas (*Pisum sativum*) were chosen as the experimental crop, being a nutritionally important and widely planted species. Numerous studies have reported that traditional microplastics (PE and PP) have significant negative effects on soil water and nutrient availability, microbial diversity, enzyme activity, photosynthesis and crop growth[16–18]. Considering the prevalence of microplastic pollution from traditional plastic film mulches in pea-soil systems, biodegradable plastic film mulches are currently under consideration as an alternative. However, whether the types and levels of degradable microplastics are superior to those of traditional microplastics during the critical plant growth stages in a pea-soil ecosystem remains an open question. Therefore, we hypothesized that biodegradable microplastics, as a C resource, included toxic and hazardous matter that is easily degradable during mineralize soil organic matter (SOM), releasing available nutrients for the growth of pea and offering a sustainable option for pea-soil systems. Therefore, these will have a better effect on a pea-soil system than traditional microplastics. This hypothesis was tested by a microcosm experiment in which traditional (PP and PE) and biodegradable (PCL and PBAT) microplastics were added at rates of 0.1% and 1% (w/w) in order to (1) assess soil nutrient availability, hydrolase activity and microbial diversity, (2) evaluate the effects of microplastics during the key pea growth stages and (3) examine the ecological effects of traditional and biodegradable microplastics on the pea-soil agroecosystem.

2 Materials and methods

2.1 Experimental design and setup

The experimental soil was collected from an edible bean-testing site in Haitong Times Agricultural Park, in Longshan Town, Cixi City, Zhejiang Province, China (30°03′ N, 121°28′ E). The region is characterized by a monsoon climate with an average annual temperature of 16.0 °C and average annual precipitation of 1273 mm. In November 2022, soil from the 0–20 cm tillage layer was sampled using a five-point sampling method. The test soil was cleared of impurities and plant residues, sieved through a 2-cm sieve and thoroughly mixed for the incubation experiments.

Four types of microplastics (powdered, pure polymer, 125 μm; Sigma-Aldrich, St. Louis, MO, USA) were selected for the experiment: PP, PE, PCL and PBAT. PP and PE represent traditional microplastics, and PCL and PBAT biodegradable microplastics. Microplastics were added to the soil at rates of 0%, 0.1% [1 g·kg^{-1} dry soil (w/w)] and 1% [10 g·kg^{-1} dry soil (w/w)] to simulate field conditions. The soil was mixed thoroughly with added nutrients and then divided into nine treatment groups with nine replicates each: (1) no microplastic addition (control), (2) 0.1% PP, (3) 1% PP, (4) 0.1% PE, (5) 1% PE, (6) 0.1% PCL, (7) 1% PCL, (8) 0.1% PBAT and (9) 1% PBAT (with all treatment concentrations expressed on a w/w basis). For treatments 2–9, PP, PE, PCL and PBAT were divided into multiple small particles and mixed separately with the soil to ensure a uniform distribution. Wet soil (~1.01 kg dry weight, about 60% water holding capacity) was added to PVC pots (14 cm × 11 cm). *Pisum sativum* cv. Zhong Qin No.1 seeds were germinated and three seedlings transplanted into each

pot. Subsequently, water-soluble fertilizer (0.50 g·L^{-1} urea, 0.30 g·L^{-1} potassium phosphate and 0.08 g·L^{-1} potassium chloride in deionized water) was added to each potted plant. The experimental pots were placed in an incubator with a day/night temperatures of 25/20 ± 1 °C and a relative humidity of 70%–80% until maturity. During the incubation, distilled water was added to maintain at 60% water holding capacity. Environmental factors were eliminated by arbitrarily rearranging the pots weekly. Two pea seeds of the same growth and health were sampled for each plot after 1 week.

2.2 Sampling and physicochemical properties for plants and soil

At the seedling (15 d after sowing), flowering (27 d after sowing) and maturity (41 d after sowing) stages of the pea growth (Fig. 1), shoot and root samples were carefully collected, initially oven-dried (FD 115, Binder GmbH, Tuttlingen, Germany) at 105 °C for 2 h, then at 65 °C for 24 h to constant mass before weighing.

At the three growth stages, the two soil samples from each treatment was collected and stored in separate plastic ziplock bags at 4 °C and in centrifuge tubes at −80 °C until measuring soil physical-chemical properties and extracting genomic DNA, respectively. Soil microbial biomass C and N were assessed using the chloroform fumigation-potassium sulfate extraction method[19]. Non-fumigated and fumigated extracts were analyzed for soil dissolved organic C (DOC) with a Multi N/C 2100 TOC/TN analyzer (Analytik Jena, Jena, Germany). Microbial biomass C (MBC) was calculated based on the difference in DOC between the non-fumigated and fumigated

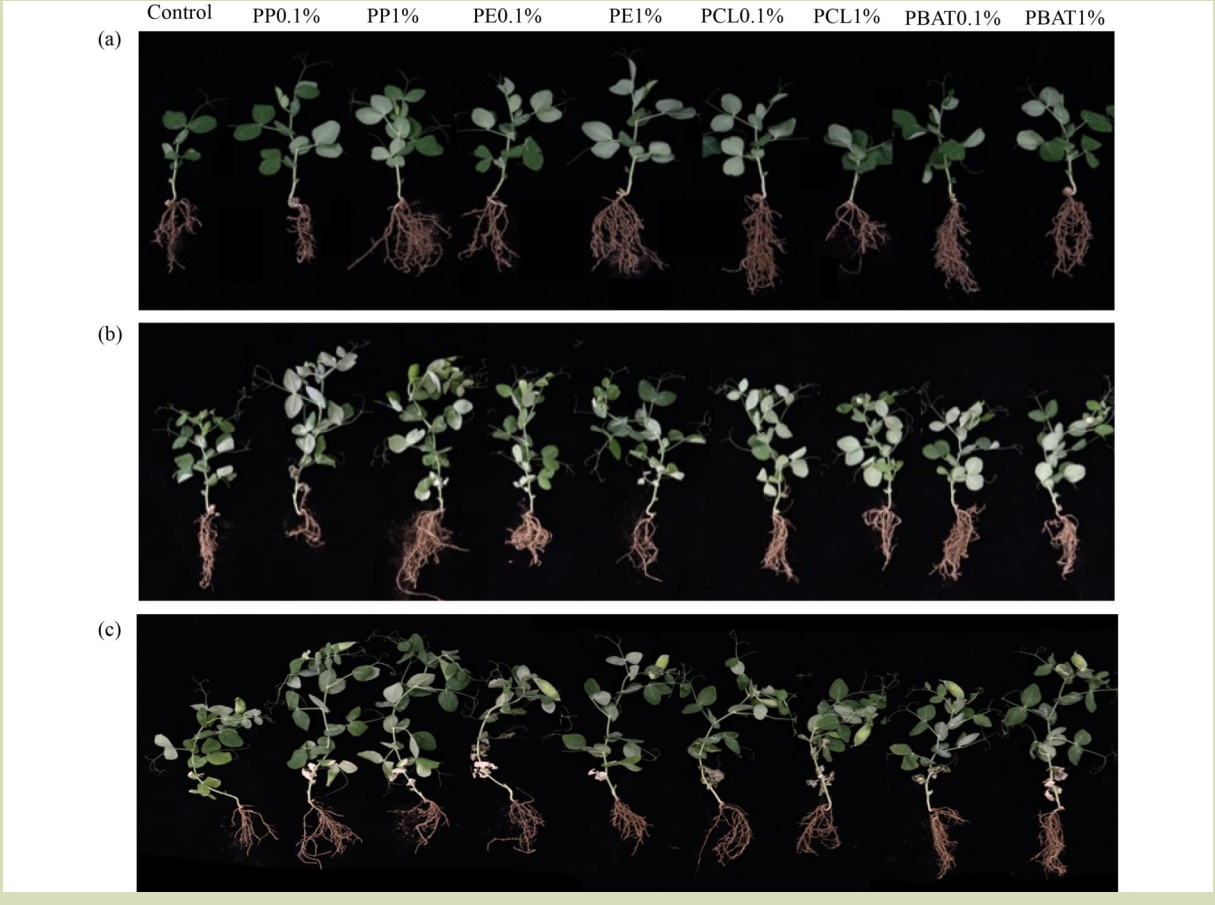

Fig. 1 The seedling (a), flowering (b), and maturity (c) stages of pea with polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT) additions of 0.1% and 1%.

extracts using a k_{ec} factor of 0.45[19]. In turn, microbial biomass N (MBN) was calculated to be similar to MBC using a k_{en} factor of 0.54[19]. Non-fumigated extracts were analyzed spectrophotometrically to determine ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations with a PowerWave XS microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). Soil microbial biomass phosphorus (MBP) was determined using chloroform fumigation-sodium bicarbonate extraction followed by applying the molybdenum antimony colorimetric method[19] with a UV-Vis spectrophotometer (UV-2450; Shimadzu, Kyoto, Japan). Briefly, two 5-g soil samples (non-fumigated and fumigated) were prepared, and a third 5-g fresh soil sample was treated with 0.5 mL of 250 μg·mL^{-1} KH_2PO_4 to calculate the recovery efficiency of P. The three soil samples were then extracted with 80 mL 0.5 M $NaHCO_3$ (pH 8.5). MBP was calculated using k_p = 0.4 [20] and Olsen-P was determined using the soil $NaHCO_3$ extract (1:20 w/v)[21].

The activities of the soil extracellular enzymes β-1,4-glucosidase (BG), β-cellobiohydrolase (CBH), β-1,4-*N*-acetylglucosaminidase (NAG), β-1,4-xylosidase (BX), polyphenol oxidase (PPO) and peroxidase (PER) were measured based on a modified version of a method used in previous studies[22]. Briefly, BG, CBH, BX and NAG activities were measured fluorometrically (excitation at 365 nm and emission at 450 nm) using substrate links to a fluorescent tag (50 μL aliquot of 100 μmol·L^{-1} 4-methylmbelliferone in each sample well)[23]. PPO and PER activities were measured colorimetrically (460 nm) using a 50 μL aliquot of 25 μmol·L^{-1} L-dihydroxyphenylalanine in each sample well[23]. Enzyme activities were quantified using an automated fluorometric plate reader (Victor3 1420–050 Multilabel Counter, PerkinElmer, Waltham, MA, USA).

2.3 DNA extraction, amplification, high-throughput sequencing and bioinformatic analysis

Total genomic DNA was extracted from 0.5 g of frozen soil (–80 °C) using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA concentration and quality were measured using a NanoDrop spectrophotometer (ND-100, Thermo Fisher Scientific, Waltham, MA, USA). The universal forward primer 341 F (CCTAYGGGRBGCASCAG) and the reverse primer 806 R (GGACTACNNGGGTATCTAAT) were used to amplify the bacterial hypervariable V3–V4 regions of the 16S rRNA gene[24]. For fungi, the ITS region was targeted by the primers ITS1F and ITS2[6]. Polymerase chain reaction conditions included an initial denaturation for 3 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 5 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. The polymerase chain reaction products were purified using a gel extraction kit (Qiagen) and sequenced on an Illumina MiSeq platform (Novogene Co., Ltd., Beijing, China). Sequencing libraries were generated using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) following manufacturer instructions. Index codes were then added. The library quality was assessed using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the library was sequenced on an Illumina platform and 250 bp paired-end reads were generated. The raw sequences were processed using Quantitative Insights Into Microbial Ecology (QIIME, version 1.7.0)[25]. In the first step, sample splitting was performed to determine the sample from which each sequence originated. Sequence denoising was performed to obtain amplicon sequence variants (ASVs) to reduce sequencing errors and redundancy. After obtaining the feature list (ASV list) and representative sequences, species annotation was performed for bacteria, fungi and protists. ASVs with total sequences length of < 20 were removed and the resulting bacterial, fungal and protist samples were individually flattened.

2.4 Statistical analysis

All figures were generated and statistical analysis was performed using R software (version 4.0.0). Levene's test for homogeneity of variance was performed and treatment means were compared using the R package "agricolae" with a least significant difference of 5% ($LSD_{0.05}$). FastSpar (version 1.0.0), which incorporates the SparCC algorithm, was used to infer microbial correlation networks. All treatment data were divided based on bacterial and fungal communities to create co-occurrence networks of bacterial and fungal communities. The abundance of ASVs was > 0.1% in each treatment. Subsequently, the correlations and p-values among the ASVs were inferred using 50 iterations and 1000 permutations. Significant ($p < 0.05$) correlations at > 0.7 and < 0.7 were used. The correlations were visualized and their topological features calculated using Gephi (version 0.9.2). Key network metrics, including the number of nodes and edges, were calculated by combining each treatment at the three key growth stages. Structural equation modeling (SEM) was performed using the

R package "lavaan" to test the significance of the hypothesized causal relationships among the type and content of microplastics, microbial biomass, soil enzyme activity, available nutrients, microbial diversity and plant growth. Before SEM analysis, DOC, DON and Olsen-P were downgraded to available nutrients, MBC, MBN and MBP to microbial biomass, BG, CBH, XYL, NAG, PPO and PER to soil enzyme activity, and the type and dose of microplastics and biomass of the pea plants were standardized. The best-fit model was determined using the chi-square test (χ^2), p-values, goodness-of-fit index (GFI), root mean square error of approximation (RMSEA) and standard root mean square residual (SRMR) methods. Based on the removal and addition relationships between the observed variables, an optimal model was finally built and the standardized estimate value (R^2), standardized total effects and effect of the addition of microplastics on the pea biomass were determined.

3 Results

3.1 Plant biomass

Pea shoot biomass decreased by 43.4% ($p < 0.05$) with 1% PP and by 10.8% with 0.1% PBAT. Pea root biomass increased by 35.3% ($p < 0.05$) with 0.1% PBAT at the seedling stage, compared to that of the control plants. The shoot:root ratio (S:R) increased by 36.8% ($p < 0.05$) with 0.1% PP (Table 1). Consistently, at flowering, shoot biomass increased by 126.1% with 0.1% PP, 88.0% and 68.7% with 0.1% and 1% PE, respectively, and 103.0% with 1% PBAT, compared to that of the control plants ($p < 0.05$); meanwhile, root biomass increased by 34.3% with 1% PP and 0.1% PBAT compared to that of the control ($p < 0.05$) at flowering. S:R increased by 110%, 60.7%, 57.3% and 50.1% with the 0.1% PP, 1% PE, 0.1% PCL and 1% PCL ($p < 0.05$), respectively, but decreased by 28.4% with 0.1% PBAT ($p < 0.05$) (Table 1). At maturity, shoot biomass increased by 72.5% to 193.0% ($p < 0.05$) with the addition of microplastics (except for 1% PCL), while root biomass increased by 66.4%, 110.0%, 103.4% and 104.9% ($p < 0.05$) with the 1% PP, 1% PE, 0.1% PBAT and 1% PBAT, respectively, compared to that of control plants. Consequently, S:R increased by 97.3% and 73.7% ($p < 0.05$) with the 0.1% PP and 0.1% PE, respectively (Table 1).

3.2 Soil available nutrients and microbial biomass

Soil DOC content was lower ($p < 0.05$) across all microplastics treatments (except for PBAT) than in the control at the seedling stage. At flowering and maturity, DOC content was not affected ($p > 0.05$) by microplastics addition relative to the control (Table 2). Similarly, soil NH_4^+-N content was not

Table 1 Results of two-way ANOVAs showing the effects of treatment, dose (0.1% and 1%) and treatment-dose interactions on shoot biomass, root biomass, and shoot:root ratio at the seedling, flowering and maturity stages of pea

Growth stage	Treatment	Dose	Shoot biomass (mg·pot^{-1})		Root biomass (mg·pot^{-1})		Shoot:root ratio	
Seedling	Control	0%	207.50 ± 81.80a		44.53 ± 15.75b		4.65 ± 0.91b	
	PP	0.1%	215.00 ± 65.57aAI		33.78 ± 7.62cBI		6.36 ± 1.53aAI	
		1%	117.50 ± 34.03cβII		37.23 ± 8.53cβI		3.19 ± 0.65cβII	
	PE	0.1%	152.50 ± 45.00aAI		41.63 ± 8.14cBI		3.65 ± 0.74cBI	
		1%	187.50 ± 3 8.62aαI		42.23 ± 8.37cβI		4.50 ± 1.01bαI	
	PCL	0.1%	140.00 ± 40.82bBI		46.20 ± 12.06aAI		3.18 ± 1.33cBI	
		1%	190.00 ± 36.51aαI		33.13 ± 5.49cβI		5.90 ± 1.75aαII	
	PBAT	0.1%	185.00 ± 36.97aAI		60.23 ± 13.86aAI		3.17 ± 0.76cBI	
		1%	215.00 ± 49.33aαI		59.10 ± 5.49aαI		3.71 ± 1.10cβI	
Factor (df)			F	p	F	p	F	p
Treatment (4)			1.03	0.41	6.72	***	1.71	0.18
Dose (1)			0.06	0.81	0.51	0.48	0.34	0.56
Treatment × Dose (3)			3.75	*	1.04	0.39	9.29	***

(Continued)

Growth stage	Treatment	Dose	Shoot biomass (mg·pot^{-1})		Root biomass (mg·pot^{-1})		Shoot:root ratio	
Flowering	Control	0%	335.00 ± 23.80e		42.23 ± 12.47b		8.35 ± 1.90c	
	PP	0.1%	757.50 ± 89.21aAI		44.35 ± 5.94aAI		17.50 ± 4.49aAI	
		1%	500.00 ± 106.14cαII		56.73 ± 13.24aαI		9.01 ± 1.96bβII	
	PE	0.1%	630.00 ± 119.72aAI		55.00 ± 11.32aAI		11.59 ± 2.19bBI	
		1%	565.00 ± 59.16bαI		42.48 ± 5.41aβI		13.42 ± 1.86aαI	
	PCL	0.1%	477.50 ± 65.51cBI		40.10 ± 12.76BI		13.13 ± 5.18aAI	
		1%	427.50 ± 93.23dβI		34.13 ± 7.29cγI		12.53 ± 1.06bαI	
	PBAT	0.1%	322.50 ± 66.02fCI		56.73 ± 11.16aAI		5.98 ± 2.37dCI	
		1%	680.00 ± 252.98aαII		54.70 ± 4.17aαI		12.36 ± 4.38bαII	
Factor (df)			F	*p*	F	*p*	F	*p*
Treatment (4)			6.03	**	4.09	*	3.30	*
Dose (1)			0.01	0.93	0.34	0.57	0.04	0.84
Treatment × Dose (3)			10.13	***	2.27	0.10	7.87	***
Maturity	Control	0%	427.50 ± 101.45d		30.75 ± 12.95c		16.37 ± 9.12c	
	PP	0.1%	1407.50 ± 102.10aAI		44.55 ± 6.93bBI		32.30 ± 6.56aAI	
		1%	1055.00 ± 239.79bαII		51.18 ± 11.88 aαI		21.03 ± 3.96 bαII	
	PE	0.1%	1252.50 ± 240.75aAI		45.18 ± 9.97bBI		28.44 ± 6.00aAI	
		1%	1062.50 ± 149.75bαI		64.58 ± 14.16aαI		17.14 ± 4.70cαII	
	PCL	0.1%	770.00 ± 178.33cBI		42.93 ± 4.78bcBI		17.86 ± 3.33cBI	
		1%	737.50 ± 57.37cβI		35.60 ± 10.88cβI		22.86 ± 9.70aαI	
	PBAT	0.1%	970.00 ± 41.63bBI		62.55 ± 10.10aAI		15.86 ± 2.90cBI	
		1%	1225.00 ± 546.90aαI		63.00 ± 4.34aαI		19.68 ± 9.59bαI	
Factor (df)			F	*p*	F	*p*	F	*p*
Treatment (4)			11.35	***	9.54	***	2.51	0.07
Dose (1)			0.93	0.34	1.80	0.19	2.10	0.16
Treatment × Dose (3)			2.44	0.09	2.50	0.08	3.66	*

Note: The treatments were: no microplastics addition (Control), polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT). The English lowercase letters represent significant differences in all treatments, different English uppercase letters and Greek letters indicate significant differences ($p < 0.05$) in traditional and biodegradable microplastics concentrations of 0.1% and 1%, respectively. The Roman letters represent significant differences between 0.1% and 1% of different microplastics type at $p < 0.05$. All results are means ± standard deviation ($n = 3$). *, ** and *** represent significant differences in the effects of treatment, sampling location and treatment-location interactions at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

affected ($p > 0.05$) by microplastics addition at the seedling and flowering stages, although at maturity it was higher ($p < 0.05$) with 1% PP than in the control. Soil NO_3^--N was higher ($p < 0.05$) with PCL (0.1% and 1% w/w) than in the control at the seedling stage, but was not affected at flowering ($p > 0.05$), and was lower ($p < 0.05$) at maturity with PP (0.1% and 1%), PE (0.1% and 1%) and PBAT (1% w/w) than in the control. Soil Olsen-P concentration was lower ($p < 0.05$) with 0.1% PP than in the control at the seedling stage, but remained unaffected at flowering and maturity ($p > 0.05$). Soil MBC content was higher ($p < 0.05$) with 1% PCL and 1% PBAT than in the control at the seedling stage. At flowering, MBC was higher ($p < 0.05$) with 0.1% PP, 1% PCL and 1% PBAT than in the control. At maturity, MBC was lower ($p < 0.05$) with 1% PE and higher ($p < 0.05$) with 0.1% and 1% PCL than in the control. Soil MBN content was higher ($p < 0.05$) with 0.1% PP, 0.1% PE, 0.1% PCL, 0.1% PBAT and 1% PBAT than in the control at the seedling stage. At flowering, MBN was higher

Table 2 Results of two-way ANOVAs showing the effects of treatment, dose (0.1% and 1%) and treatment-dose interactions on the available nutrients and microbial biomass in soil after the seedling, flowering and maturity stages of pea

Growth stage	Treatment	Dose	DOC (mg·kg^{-1})	NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	Olsen-P (mg·kg^{-1})	MBC (mg·kg^{-1})	MBN (mg·kg^{-1})	MBP (mg·kg^{-1})
Seedling	Control	0%	21.37 ± 3.23a	1.01 ± 0.44a	89.51 ± 29.43b	55.93 ± 7.45a	35.32 ± 2.07bc	16.64 ± 2.96c	7.45 ± 2.13e
	PP	0.1%	15.56 ± 0.57dBI	0.95 ± 0.50aAI	101.65 ± 16.59aAI	45.54 ± 2.45cAI	42.79 ± 6.89aAI	38.32 ± 10.13aAI	37.81 ± 5.57bBI
		1%	18.13 ± 0.96bαII	1.13 ± 0.27aαI	103.25 ± 16.91aαI	48.50 ± 0.97bαI	36.46 ± 5.88bβI	15.59 ± 2.84cβII	23.06 ± 8.07cβII
	PE	0.1%	17.03 ± 1.08bAI	1.15 ± 0.51aAI	104.62 ± 15.63aAI	48.53 ± 4.06bAI	36.43 ± 3.85bAI	39.50 ± 3.89aAI	11.67 ± 2.51dDI
		1%	16.83 ± 0.6bβI	1.28 ± 0.28aαI	109.04 ± 9.95aαI	51.29 ± 3.88aαI	34.61 ± 5.72bβI	13.33 ± 2.80cβII	35.51 ± 2.41bαII
	PCL	0.1%	17.62 ± 1.97bAI	1.00 ± 0.58aAI	110.29 ± 36.86aAI	48.80 ± 5.52bAI	42.79 ± 10.06aAI	27.71 ± 8.76bBI	24.08 ± 7.51cCI
		1%	16.21 ± 1.32cγI	1.19 ± 0.26aαI	120.55 ± 12.70aαI	48.87 ± 11.17bαI	47.14 ± 6.38aαI	10.10 ± 3.14cγII	8.89 ± 1.47dγII
	PBAT	0.1%	19.35 ± 3.46aAI	0.56 ± 0.36bAI	111.51 ± 8.81aAI	52.00 ± 5.43aAI	33.89 ± 3.32cAI	30.34 ± 4.07bAI	51.59 ± 9.37aAI
		1%	18.76 ± 1.73aαI	1.32 ± 0.16aαII	86.10 ± 19.84bβI	58.74 ± 7.19aαI	50.71 ± 4.97aαII	25.70 ± 4.12bαI	17.43 ± 7.76 cβII
Factor (df)			F *p*	F *p*	F *p*	F *p*	F *p*	F *p*	F *p*
Treatment (4)			5.52 **	0.52 0.72	1.31 0.29	2.88 *	3.53 *	5.73 **	19.46 ***
Dose (1)			0.02 0.89	4.97 *	0.10 0.76	2.14 0.16	2.44 0.13	86.77 ***	22.84 ***
Treatment × Dose (3)			1.59 0.22	1.12 0.36	1.20 0.33	0.41 0.75	5.83 **	6.12 **	33.39 ***
Flowering	Control	0%	16.45 ± 0.90a	1.12 ± 0.51a	96.70 ± 17.42a	45.68 ± 3.86a	30.78 ± 1.99c	20.98 ± 1.75d	13.23 ± 1.01c
	PP	0.1%	17.38 ± 1.97aAI	1.04 ± 0.52aAI	77.44 ± 22.62aAI	48.48 ± 4.41aAI	47.22 ± 6.17bAI	43.27 ± 4.15bAI	42.23 ± 14.01aAI
		1%	17.27 ± 1.60aαI	1.57 ± 0.44aαI	88.51 ± 23.17aαI	47.26 ± 3.68aαI	32.31 ± 4.53cγII	14.23 ± 1.79eγII	12.02 ± 1.95dβII
	PE	0.1%	17.24 ± 1.82aAI	1.18 ± 0.14aAI	76.82 ± 29.48aAI	39.37 ± 3.12bBI	28.27 ± 5.72cBI	22.85 ± 6.02dBI	24.16 ± 0.68bBI
		1%	16.09 ± 1.63aαI	1.35 ± 0.52aαI	84.76 ± 12.40aαI	46.15 ± 3.66aαII	30.38 ± 3.36cγI	31.46 ± 1.03cβII	33.74 ± 7.44aαII
	PCL	0.1%	17.35 ± 2.38aAI	1.19 ± 0.25aAI	107.28 ± 33.66aAI	47.04 ± 7.30aAI	27.51 ± 4.97cBI	44.61 ± 5.01bAI	9.28 ± 6.08dCI
		1%	14.34 ± 1.17bβI	0.85 ± 0.29aαI	97.90 ± 17.93aαI	47.69 ± 0.96aαI	65.33 ± 12.48aαII	56.60 ± 9.31aαI	11.23 ± 4.64dβI
	PBAT	0.1%	16.69 ± 2.34aAI	1.43 ± 1.10aAI	104.11 ± 11.01aAI	44.60 ± 3.05aAI	35.17 ± 3.74cBI	26.64 ± 2.01cBI	21.49 ± 1.84bBI
		1%	16.33 ± 1.65aαI	1.57 ± 0.61aαI	105.92 ± 11.96aαI	49.26 ± 7.93aαI	48.36 ± 9.62bβII	23.75 ± 4.29dβI	16.03 ± 2.07bβII
Factor (df)			F *p*	F *p*	F *p*	F *p*	F *p*	F *p*	F *p*
Treatment (4)			0.71 0.59	0.83 0.52	2.17 0.10	1.52 0.23	8.64 ***	44.89 ***	13.91 ***
Dose (1)			3.38 0.08	0.40 0.53	0.14 0.71	2.70 0.11	16.70 ***	2.96 0.10	8.12 **
Treatment × Dose (3)			1.08 0.37	0.84 0.48	0.36 0.78	1.22 0.32	22.36 ***	31.92 ***	16.57 ***
Maturity	Control	0%	14.97 ± 1.22a	0.67 ± 0.42b	100.32 ± 16.42a	44.27 ± 3.84a	41.69 ± 6.47b	22.77 ± 3.41e	36.70 ± 4.40a

(Continued)

Growth stage	Treatment	Dose	DOC (mg·kg^{-1})	NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	Olsen-P (mg·kg^{-1})	MBC (mg·kg^{-1})	MBN (mg·kg^{-1})	MBP (mg·kg^{-1})
	PP	0.1%	16.49 ± 1.81aAI	1.17 ± 0.39aAI	44.96 ± 13.71dBI	47.34 ± 4.43aAI	41.46 ± 4.16bBI	31.25 ± 1.74dCI	3.31 ± 0.72fDI
		1%	16.20 ± 0.60aαI	1.25 ± 0.51aαI	66.49 ± 17.17cβI	39.51 ± 1.35dγII	47.08 ± 1.82bβII	38.95 ± 6.19cαI	26.97 ± 7.69bβII
	PE	0.1%	15.37 ± 1.58aAI	1.09 ± 0.41aAI	67.48 ± 25.00cAI	46.05 ± 3.63aAI	37.45 ± 6.85cBI	40.95 ± 3.88bBI	15.71 ± 3.05dBI
		1%	14.53 ± 0.95bαI	0.53 ± 0.11dβII	72.11 ± 12.54bβI	44.74 ± 3.06aαI	32.74 ± 6.01dγI	43.36 ± 9.00aαI	33.40 ± 0.70aαII
	PCL	0.1%	14.84 ± 1.74aAI	0.42 ± 0.32eBI	92.49 ± 15.94aAI	41.52 ± 3.03cAI	65.83 ± 7.63aAI	30.47 ± 3.25dCI	21.07 ± 4.46cAI
		1%	13.57 ± 1.76cβI	0.97 ± 0.38aαI	95.31 ± 11.94aαI	42.16 ± 3.37bβI	66.69 ± 7.50aαI	50.11 ± 3.84aαII	7.86 ± 0.82eγII
	PBAT	0.1%	14.70 ± 0.91aAI	0.31 ± 0.13eBI	87.00 ± 18.31aAI	46.78 ± 7.22aAI	32.97 ± 2.28cBI	49.77 ± 8.27aAI	10.50 ± 1.92eCI
		1%	14.28 ± 0.99bβI	0.63 ± 0.31cβI	63.8 ± 12.76cβI	48.69 ± 3.45aαI	47.76 ± 8.60bβII	48.66 ± 9.78aαI	23.33 ± 1.36bβII
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			2.95	*	4.74	**	7.89	***	2.45	0.07	30.42	***	13.90	***	35.13	***
Dose (1)			2.19	0.15	0.60	0.44	0.06	0.81	1.37	0.25	3.63	0.07	10.91	**	65.82	***
Treatment × Dose (3)			0.22	0.88	3.65	*	2.53	0.08	2.36	0.09	3.62	*	4.38	*	41.40	***

Note: The treatments were: no microplastics addition (Control), polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT). The English lowercase letters represent significant differences in all treatments, different English lowercase letters and Greek letters indicate significant differences ($p < 0.05$) in traditional and biodegradable microplastics concentration at 0.1 and 1%, respectively. The Roman letters represent significant differences between 0.1% and 1% of different microplastics type at $p < 0.05$. All results are means ± standard deviation ($n = 3$). *, ** and *** represent significant differences in the effects of treatment, sampling location, and treatment–location interactions at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. MBC, microbial biomass carbon. MBN, microbial biomass nitrogen. MBP, microbial biomass phosphorus.

($p < 0.05$) with 0.1% PP, 1% PE, 0.1% PCL and 1% PCL than in the control. At maturity, MBN was higher ($p < 0.05$) across all microplastics treatments (except for 0.1% PP and PCL) than in the control. Soil MBP content was higher ($p < 0.05$) with all microplastics treatments (except for 0.1% PE and 1% PCL) than in the control at the seedling stage. At flowering, MBP was higher ($p < 0.05$) with 0.1% PP, 0.1% PE and 1% PE than in the control. At maturity, MBP was lower ($p < 0.05$) across all microplastics treatments (except for 1% PE) than in the control.

3.3 Soil potential enzyme activities

Soil BG activity was higher ($p < 0.05$) with 0.1% PE, 0.1% PCL and 0.1% PBAT, but lower ($p < 0.05$) with 1% PCL, than in the control at the seedling stage. At flowering, BG activity was lower ($p < 0.05$) with 0.1% PE and 1% PCL than in the control. At maturity, BG activity was not affected ($p > 0.05$) by the addition of microplastics compared to that in the control (Table 3). Soil CBH activity was higher ($p < 0.05$) with 0.1% PE, PCL and PBAT, and lower ($p < 0.05$) with 1% PCL, than in the control at the seedling stage. At flowering, CBH activity was not significantly affected ($p > 0.05$) by microplastic addition. At maturity, CBH activity was higher ($p < 0.05$) with 1% PE than in the control. Soil NAG activity was higher ($p < 0.05$) with 0.1% PP, 0.1% PE, 1% PE, 0.1% PCL and 0.1% PBAT than in the control at the seedling stage. At flowering, NAG activity was higher ($p < 0.05$) with 0.1% PP, PE, PCL and PBAT, as well as with 1% PBAT than in the control. At maturity, NAG activity was higher ($p < 0.05$) with 0.1% PP, 0.1% PE, 1% PE, 1% PCL, 0.1% PBAT and 1% PBAT than in the control. Soil BX activity was higher ($p < 0.05$) across all 0.1% microplastics treatments (except for PP) than in the control at the seedling stage. At flowering, BX activity was lower ($p < 0.05$) with 0.1% PE and 1% PLA than in the control. At maturity, BX activity was higher ($p < 0.05$) with 0.1% PE, PCL and PBAT, as well as 1% PBAT, than in the control. Soil PPO activity was lower ($p < 0.05$) with 1% PE but higher ($p < 0.05$) with 0.1% PCL, 1% PCL and 1% PBAT than in the control at the seedling stage. At

flowering, PPO activity was higher ($p < 0.05$) with all 0.1% microplastics treatments, and with 1% PP and PBAT than in the control. At maturity, PPO activity was lower ($p < 0.05$) with the addition of any microplastic (except for 0.1% PP and 1% PBAT) than in the control. Finally, soil PER activity was lower ($p < 0.05$) with 0.1% PP, 1% PCL and 1% PBAT, but higher ($p < 0.05$) with 0.1% PE, 0.1% PCL and 1% PE than in the control at the seedling stage. At flowering, PER activity was higher ($p < 0.05$) across all microplastics treatments, except for 1% PP, than in the control. At maturity, PER activity was lower ($p < 0.05$) with 0.1% PP, PCL and PBAT as well as 1% PCL than in the control.

Table 3 Results of two-way ANOVAs showing the effects of treatment, dose (0.1% and 1%) and treatment-dose interactions on the activities of hydrolases and oxidase in soil after the seedling, flowering and maturity stages of pea

Growth stage	Treatment	Dose	BG ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	CBH ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	NAG ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	BX ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	PPO ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	PER ($\mu mol \cdot g^{-1} \cdot h^{-1}$)
Seedling	Control	0%	16.67 ± 3.41b	2.02 ± 0.32b	1.93 ± 0.14c	7.01 ± 1.13d	1.25 ± 0.60c	4.49 ± 0.61c
	PP	0.1%	16.94 ± 1.62bBI	2.12 ± 0.33bBI	2.99 ± 0.53aAI	8.65 ± 1.57cBI	1.41 ± 0.21cAI	0.87 ± 0.47fCI
		1%	13.02 ± 3.07bαI	1.61 ± 0.25cαII	1.82 ± 0.31cβII	7.54 ± 1.22dαI	0.61 ± 0.55dβII	3.59 ± 0.65dβII
	PE	0.1%	23.43 ± 4.61aAI	3.90 ± 0.42aAI	1.74 ± 0.23cCI	10.54 ± 1.71aAI	0.64 ± 0.58dBI	5.64 ± 0.44bBI
		1%	16.72 ± 2.34bαII	1.84 ± 0.28bαII	2.79 ± 0.54aαII	8.16 ± 1.27dαI	0.38 ± 0.15eβI	5.59 ± 0.44bαI
	PCL	0.1%	21.76 ± 2.51aAI	3.63 ± 0.55aAI	2.59 ± 0.27aAI	10.71 ± 1.59aAI	2.11 ± 0.65bAI	7.17 ± 0.67aAI
		1%	11.66 ± 1.06cβII	1.33 ± 0.06dβII	1.54 ± 0.29cβII	5.83 ± 0.44eβII	3.15 ± 0.11aαII	2.50 ± 0.34eγII
	PBAT	0.1%	21.65 ± 2.95aAI	3.92 ± 0.22aAI	2.44 ± 0.22bBI	11.65 ± 1.69aAI	1.62 ± 0.52bAI	4.98 ± 0.60bBI
		1%	16.55 ± 4.59bαI	2.05 ± 0.44bαII	1.75 ± 0.14cβII	8.89 ± 1.45bαII	3.14 ± 0.35aαII	2.69 ± 0.16eγII
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			3.38	*	14.90	***	2.04	0.12	5.00	**	31.36	***	49.42	***
Dose (1)			34.26	***	189.77	***	16.11	***	31.99	***	5.46	*	35.26	***
Treatment × Dose (3)			1.48	0.24	10.73	***	19.87	***	2.53	0.08	11.34	***	76.14	***
Flowering	Control	0%	22.30 ± 10.79a		3.19 ± 0.73a		1.79 ± 0.14d		10.07 ± 1.60a		0.91 ± 0.49c		4.18 ± 0.18d	
	PP	0.1%	22.53 ± 2.92aAI		3.70 ± 0.72aAI		3.45 ± 0.58aAI		11.14 ± 0.59aAI		3.94 ± 0.84aAI		6.75 ± 2.19bBI	
		1%	22.27 ± 2.89aαI		3.31 ± 0.52aαI		1.98 ± 0.39dβII		10.76 ± 1.80aαI		0.61 ± 0.42cγII		5.44 ± 0.33cγI	
	PE	0.1%	15.79 ± 1.69bCI		2.69 ± 0.18bBI		2.66 ± 0.64bBI		7.33 ± 0.35cCI		3.39 ± 0.53aAI		9.78 ± 0.53aAI	
		1%	20.93 ± 4.30aαI		3.22 ± 0.51aαI		2.26 ± 0.16cβI		10.19 ± 0.58aαII		1.92 ± 0.37bβII		6.86 ± 0.47bβII	
	PCL	0.1%	17.89 ± 2.63aBI		3.75 ± 0.65aAI		2.88 ± 0.32aAI		9.44 ± 0.72bBI		3.37 ± 0.77aAI		5.59 ± 0.58bCI	
		1%	14.50 ± 0.79cβII		2.46 ± 0.51cβII		1.91 ± 0.39dβII		7.31 ± 1.21cβII		0.80 ± 0.25cγII		6.50 ± 0.86bβI	
	PBAT	0.1%	20.30 ± 3.13aAI		3.94 ± 0.28aAI		2.80 ± 0.39bAI		10.04 ± 1.16aAI		3.91 ± 0.68aAI		8.55 ± 0.99aAI	
		1%	22.81 ± 0.93aαI		3.47 ± 0.51aαI		3.42 ± 0.40aαI		9.46 ± 0.54bαI		4.21 ± 0.74aαI		8.47 ± 0.50aαI	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			2.91	*	2.56	0.06	7.90	***	7.27	***	22.18	***	22.44	***
Dose (1)			0.42	0.53	4.45	*	14.70	***	0.02	0.88	70.91	***	6.63	*
Treatment × Dose (3)			1.40	0.27	3.80	*	9.67	***	7.72	***	14.04	***	6.32	**
Maturity	Control	0%	25.12 ± 4.43a		3.73 ± 0.74b		2.43 ± 0.48c		7.74 ± 1.64d		4.84 ± 0.42a		6.71 ± 0.31a	
	PP	0.1%	29.24 ± 7.15aAI		4.56 ± 0.36aAI		3.88 ± 0.35aAI		10.01 ± 1.45cBI		4.48 ± 0.66aAI		1.43 ± 0.80fCI	
		1%	30.53 ± 10.15aαI		3.99 ± 1.10bαI		3.08 ± 0.64bαI		8.41 ± 1.64cαI		2.52 ± 1.02cβII		7.12 ± 0.90aαII	
	PE	0.1%	21.99 ± 2.09bAI		4.28 ± 0.63aAI		3.48 ± 0.49aAI		10.12 ± 1.06bBI		3.12 ± 0.96bAI		6.03 ± 1.00bAI	
		1%	31.51 ± 2.80aαII		5.12 ± 0.69aαI		3.97 ± 0.66aαI		9.88 ± 1.45cαI		1.66 ± 0.82dβI		5.85 ± 0.27bβI	

(Continued)

Growth stage	Treatment	Dose	BG (μmol·g^{-1}·h^{-1})	CBH (μmol·g^{-1}·h^{-1})	NAG (μmol·g^{-1}·h^{-1})	BX (μmol·g^{-1}·h^{-1})	PPO (μmol·g^{-1}·h^{-1})	PER (μmol·g^{-1}·h^{-1})
	PCL	0.1%	26.12 ± 7.62aAI	4.23 ± 0.99aAI	3.63 ± 0.42aAI	12.36 ± 2.29aAI	2.47 ± 0.90cBI	4.23 ± 0.28dBI
		1%	24.12 ± 3.59aαI	3.73 ± 0.65bβI	3.00 ± 0.64bαI	6.60 ± 0.64eβII	0.99 ± 1.11eγI	5.07 ± 0.93cβI
	PBAT	0.1%	28.62 ± 1.73aAI	4.71 ± 0.70aAI	3.48 ± 0.47aAI	12.79 ± 1.16aAI	1.83 ± 1.03cBI	3.56 ± 0.28eBI
		1%	27.31 ± 7.91aαI	4.81 ± 0.78aαI	3.24 ± 0.58aαI	10.24 ± 2.21bαI	4.15 ± 1.00aαII	5.74 ± 0.62bβII
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			0.81	0.53	2.16	0.10	4.04	*	4.41	**	9.51	***	13.74	***
Dose (1)			0.78	0.38	0.02	0.90	2.45	0.13	20.42	***	4.10	0.05	81.47	***
Treatment × Dose (3)			1.56	0.22	1.45	0.25	2.23	0.11	4.40	*	9.69	***	29.30	***

Note: The treatments were: no microplastics addition (Control), polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT). The English lowercase letters represent significant differences in all treatments, different English uppercase letters and Greek letters indicate significant differences ($p < 0.05$) in traditional and biodegradable microplastics concentration at 0.1 and 1%, respectively. The Roman letters represent significant differences between 0.1% and 1% of different microplastics type at $p < 0.05$. All results are means ± standard deviation ($n = 3$). *, ** and *** represent significant differences in the effects of treatment, sampling location, and treatment–location interactions at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

3.4 Microbial community diversity and co-occurrence network

None of the microplastics had any significant effect on bacterial richness, Chao1 and ACE at the seedling stage. However, an increasing trend in Shannon diversity ($p < 0.05$) was observed with 0.1% PP, PE and PCL, and with 0.1% and 1% PBAT (Table 4). In contrast, microplastics did not significantly affect bacterial richness, Chao1, Shannon and ACE at flowering and maturity (Table 4). In contrast, none of the microplastic treatments significantly affected fungal richness, Shannon, Chao1 and ACE across any of the three growth stages studied (Table 5). Nonetheless, all microplastic treatments enhanced the complexity of the bacterial and fungal networks by altering bacterial and fungal community structure, as well as increasing key network metrics (nodes and edges), although they did not change the bacterial communities dominated by Proteobacteria, Acidobacteriota and Crenarchaeota, nor fungal communities dominated by Ascomycota, Mortierellomycota and Chytridiomycota (Fig. 2).

3.5 Effect of microplastics on pea biomass

At the seedling stage, the structural equation model SEM (χ^2 = 0.358, df = 2, p = 0.836, GFI = 0.997, RMSEA = 0.000 and SRMR = 0.013) indicated that all factors (biotic and abiotic) accounted for 21.3% of the variance in pea biomass. Further, microplastic type (0.308), microbial biomass (0.216), microbial diversity (0.059) and soil enzyme activity (0.687) positively affected pea biomass, whereas microplastic concentration (−0.478) and available nutrients (−0.123) had negative effects on pea biomass (Fig. 3(a)). At flowering, SEM (χ^2 = 0.211, df = 2, p = 0.900, GFI = 0.998, RMSEA = 0.000 and SRMR = 0.015) revealed that all factors explained 14.2% of the variance in pea

Table 4 Results of two-way ANOVAs showing the effects of treatment, dose (0.1% and 1%) and treatment-dose interactions on α-diversity of soil bacterial after the seedling, flowering and maturity stages of pea

Growth stage	Treatment	Dose	Richness	Shannon	Chao1	ACE
Seedling	Control	0%	775.75 ± 237.49b	9.07 ± 0.52d	775.75 ± 237.49b	775.75 ± 237.49b
	PP	0.10%	1508 ± 279.42aAI	10.13 ± 0.37aAI	1508 ± 279.42aAI	1508 ± 279.42aAI
		1%	1071.75 ± 193.36aαII	9.53 ± 0.35bβI	1071.75 ± 193.36aαII	1071.78 ± 193.41aαII
	PE	0.10%	1529.25 ± 184.53aAI	10.19 ± 0.13aAI	1529.25 ± 184.53aAI	1529.25 ± 184.53aAI
		1%	792.5 ± 134.89bβII	9.19 ± 0.31cβII	792.5 ± 134.89bβII	792.5 ± 134.89bβII

(Continued)

Growth stage	Treatment	Dose	Richness		Shannon		Chao1		ACE	
	PCL	0.10%	1334 ± 385.32aAI		10.01 ± 0.38aAI		1334 ± 385.32aAI		1334 ± 385.32aAI	
		1%	1290.75 ± 575.33aαI		9.69 ± 0.38aαI		1290.75 ± 575.33aαI		1290.75 ± 575.33aαI	
	PBAT	0.10%	1458.25 ± 393.24aAI		10.16 ± 0.43aAI		1458.25 ± 393.24aAI		1458.25 ± 393.24aAI	
		1%	1449.75 ± 251.04aαI		10.12 ± 0.27aαI		1449.75 ± 251.04aαI		1449.75 ± 251.04aαI	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			3.27	*	6.02	**	3.27	*	3.27	*
Dose (1)			7.33	*	14.42	***	7.33	*	7.33	*
Treatment × Dose (3)			2.35	0.10	2.52	0.08	2.35	0.10	2.35	0.10
Flowering	Control	0%	1089.50 ± 249.04a		9.70 ± 0.32a		1089.50 ± 249.04a		1089.50 ± 249.04a	
	PP	0.10%	770.25 ± 449.48cBI		8.65 ± 1.79bAI		770.25 ± 449.48cBI		770.25 ± 449.48cBI	
		1%	1173.00 ± 278.68aαI		9.80 ± 0.37aαI		1173.00 ± 278.68aαI		1173.00 ± 278.68aαI	
	PE	0.10%	1279.50 ± 419.08aAI		9.89 ± 0.48aAI		1279.50 ± 419.08aAI		1279.53 ± 419.14aAI	
		1%	1338.75 ± 250.44aαI		9.92 ± 0.28aαI		1338.75 ± 250.44aαI		1338.75 ± 250.44aαI	
	PCL	0.10%	1273.50 ± 46.74aAI		9.95 ± 0.12aAI		1273.50 ± 46.74aAI		1273.50 ± 46.74aAI	
		1%	1406.25 ± 171.94aαI		10.09 ± 0.18aαI		1406.25 ± 171.94aαI		1406.25 ± 171.94aαI	
	PBAT	0.10%	893.00 ± 146.66bAI		9.36 ± 0.23aAI		893.00 ± 146.66bAI		893.00 ± 146.66bAI	
		1%	1063.25 ± 321.12aαI		9.66 ± 0.54aαI		1063.25 ± 321.12aαI		1063.25 ± 321.12aαI	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			3.04	*	1.75	0.35	3.04	*	3.04	*
Dose (1)			3.58	0.07	2.84	0.10	3.58	0.07	3.58	0.07
Treatment × Dose (3)			0.54	0.66	1.15	0.35	0.54	0.66	0.54	0.66
Maturity	Control	0%	1086.50 ± 80.52a		9.67 ± 0.07a		1086.50 ± 80.52a		1086.50 ± 80.52a	
	PP	0.10%	831.00 ± 88.92bAI		9.29 ± 0.20aAI		831.00 ± 88.92bAI		831.00 ± 88.92bAI	
		1%	950.00 ± 122.55aαI		9.48 ± 0.19aαI		950.00 ± 122.55aαI		950.00 ± 122.55aαI	
	PE	0.10%	957.25 ± 33.42aAI		9.53 ± 0.09aAI		957.25 ± 33.42aAI		957.28 ± 33.48aAI	
		1%	892.00 ± 99.79aαI		9.46 ± 0.13aαI		892.00 ± 99.79aαI		892.00 ± 99.79aαI	
	PCL	0.10%	1027.50 ± 70.50aAI		9.60 ± 0.07aAI		1027.50 ± 70.50aAI		1027.50 ± 70.50aAI	
		1%	937.75 ± 204.62aαI		9.37 ± 0.33aαI		937.75 ± 204.62aαI		937.75 ± 204.62aαI	
	PBAT	0.10%	797.25 ± 239.04cBI		9.13 ± 0.53bBI		797.25 ± 239.04cBI		797.25 ± 239.04cBI	
		1%	801.75 ± 232.85cαI		9.07 ± 0.74cαI		801.75 ± 232.85cαI		801.75 ± 232.85cαI	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			3.01	*	2.47	0.07	3.01	*	3.01	*
Dose (1)			0.02	0.88	0.12	0.74	0.02	0.88	0.02	0.88
Treatment × Dose (3)			0.79	0.51	0.53	0.67	0.79	0.51	0.79	0.51

Note: The treatments were: no microplastics addition (Control), polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT). The English lowercase letters represent significant differences in all treatments, different English uppercase letters and Greek letters indicate significant differences ($p < 0.05$) in traditional and biodegradable microplastics concentrations of 0.1% and 1%, respectively. The Roman letters represent significant differences between 0.1% and 1% of different microplastics type at $p < 0.05$. All results are means ± standard deviation ($n = 3$). *, ** and *** represent significant differences in the effects of treatment, sampling location, and treatment-location interactions at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 5 Results of two-way ANOVAs showing the effects of treatment, dose (0.1% and 1%) and treatment-dose interactions on α-diversity of soil fungal after the seedling, flowering and maturity stages of pea

Growth stage	Treatment	Dose	Richness		Shannon		Chao1		ACE	
Seedling	Control	0%	210.75 ± 32.48b		3.35 ± 0.82a		210.75 ± 32.48b		210.8 ± 32.53b	
	PP	0.10%	193.5 ± 36.67bBI		2.43 ± 0.94bBI		193.5 ± 36.67bBI		193.5 ± 36.67bBI	
		1%	265.5 ± 104.74aαI		4.23 ± 1.56aαI		265.5 ± 104.74aαI		265.5 ± 104.74aαI	
	PE	0.10%	270 ± 45.91aAI		3.54 ± 0.47aAI		270 ± 45.91aAI		270.05 ± 45.93aAI	
		1%	239.75 ± 21.33aαI		3.93 ± 0.56aαI		239.75 ± 21.33aαI		239.75 ± 21.33aαI	
	PCL	0.10%	249 ± 35.56aAI		3.94 ± 0.45aAI		249 ± 35.56aAI		249 ± 35.56aAI	
		1%	300.33 ± 55.86aαI		4.58 ± 1.09aαI		300.33 ± 55.86aαI		300.33 ± 55.86aαI	
	PBAT	0.10%	312.25 ± 85.53aAI		3.99 ± 1.11aAI		312.25 ± 85.53aAI		312.25 ± 85.53aAI	
		1%	235 ± 65.12aαI		3.98 ± 0.7aαI		235 ± 65.12aαI		235.06 ± 65.2aαI	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			1.32	0.29	1.37	0.27	1.32	0.29	1.32	0.29
Dose (1)			0.04	0.85	4.69	*	0.04	0.85	0.04	0.85
Treatment × Dose (3)			2.76	0.06	1.43	0.25	2.76	0.06	2.75	0.06
Flowering	Control	0%	278.75 ± 59.23b		3.83 ± 0.95a		278.75 ± 59.23b		278.75 ± 59.23b	
	PP	0.10%	273 ± 64.02bAI		4.48 ± 0.87aAI		273 ± 64.02bAI		273.12 ± 63.97bAI	
		1%	202.25 ± 44.46bγI		3.22 ± 0.94bβI		202.25 ± 44.46bγI		202.25 ± 44.46bγI	
	PE	0.10%	337.75 ± 79.83aAI		4.59 ± 0.6aAI		337.75 ± 79.83aAI		337.75 ± 79.83aAI	
		1%	231 ± 33.79bβII		3.46 ± 0.38bβII		231 ± 33.79bβII		231 ± 33.79bβII	
	PCL	0.10%	445.5 ± 243.3bAI		4.51 ± 2.14aAI		445.5 ± 243.3aAI		445.5 ± 243.3aAI	
		1%	287.25 ± 71.09aαI		4.39 ± 0.15aαI		287.25 ± 71.09bαI		287.25 ± 71.09bαI	
	PBAT	0.10%	215 ± 47.15bBI		4.02 ± 1.14aAI		215 ± 47.15bBI		215.06 ± 47.21bBI	
		1%	319.67 ± 59.7aαII		4.94 ± 0.36aαI		319.67 ± 59.7aαII		319.67 ± 59.7aαII	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			1.89	0.14	0.71	0.59	1.89	0.14	1.89	0.14
Dose (1)			2.76	0.11	1.26	0.27	2.76	0.11	2.76	0.11
Treatment × Dose (3)			2.69	0.07	2.04	0.13	2.69	0.07	2.69	0.07
Maturity	Control	0%	276.75 ± 34.01a		4.77 ± 0.41a		276.77 ± 34a		276.93 ± 33.84a	
	PP	0.10%	342.5 ± 95.29aAI		5.21 ± 0.72aAI		342.5 ± 95.29aAI		342.5 ± 95.29aAI	
		1%	311.25 ± 112.67aαI		4.92 ± 1.03aαI		311.25 ± 112.67aαI		311.3 ± 112.68aαI	
	PE	0.10%	284 ± 114.3aAI		4.38 ± 0.7aAI		284 ± 114.3aAI		284.05 ± 114.38aAI	
		1%	207.75 ± 51.93bαI		3.93 ± 0.97bαI		207.75 ± 51.93bαI		207.75 ± 51.93bαI	
	PCL	0.10%	197 ± 39.51cBI		3.78 ± 0.44cBI		197 ± 39.51cBI		197.07 ± 39.37cBI	
		1%	312.5 ± 72.84aαII		4.68 ± 0.66aαI		312.5 ± 72.84aαII		312.56 ± 72.93aαII	
	PBAT	0.10%	312 ± 95.57aAI		4.84 ± 0.86aAI		312 ± 95.57aAI		312.05 ± 95.61aAI	
		1%	258.25 ± 28.48aαI		4.6 ± 0.45aαI		258.25 ± 28.48aαI		258.25 ± 28.48aαI	
Factor (df)			F	*p*	F	*p*	F	*p*	F	*p*
Treatment (4)			1.30	0.29	2.18	0.10	1.30	0.29	1.30	0.29

(Continued)

Growth stage	Treatment	Dose	Richness		Shannon		Chao1		ACE	
Dose (1)			0.17	0.68	0.01	0.94	0.17	0.68	0.17	0.68
Treatment × Dose (3)			2.43	0.09	1.46	0.25	2.43	0.09	2.43	0.09

Note: The treatments were: no microplastics addition (Control), polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT). The English lowercase letters represent significant differences in all treatments, different English uppercase letters and Greek letters indicate significant differences ($p < 0.05$) in traditional and biodegradable microplastics concentrations of 0.1% and 1%, respectively. The Roman letters represent significant differences between 0.1% and 1% of different microplastics type at $p < 0.05$. All results are means ± standard deviation ($n = 3$). *, ** and *** represent significant differences in the effects of treatment, sampling location, and treatment-location interactions at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

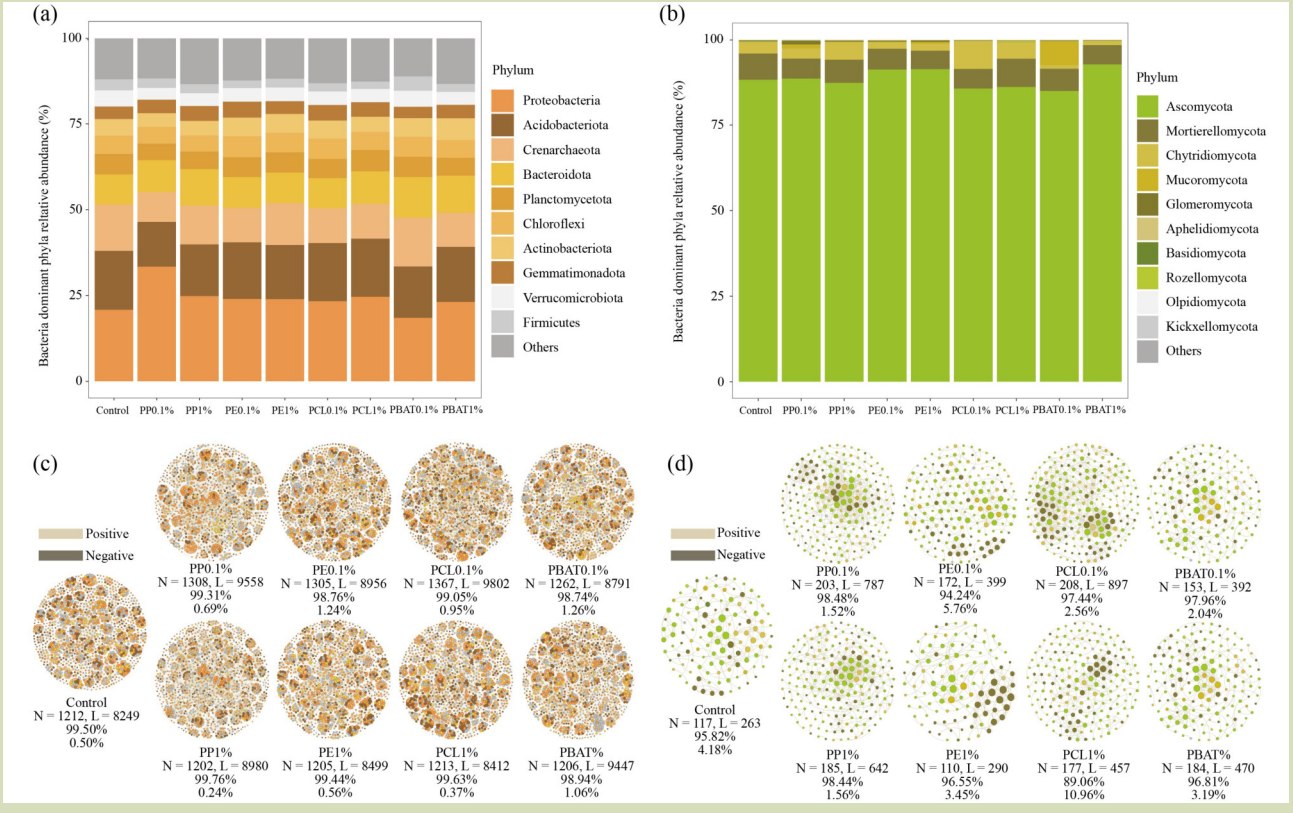

Fig. 2 Composition and structure of soil bacterial (a) and fungal (b) communities and co-occurrence networks of bacterial (c) and fungal (d) communities after the seedling, flowering and maturity stages of pea with polypropylene (PP) and polyethylene (PE), polycaprolactone (PCL) and polybutylene adipate terephthalate (PBAT) additions of 0.1% and 1%. N and L, number of nodes and links, respectively. Significant differences ($p < 0.05$) between amendments are indicated using different lowercase letters. Control, no microplastics addition; PP0.1%, 0.1% polypropylene; PP1%, 1% polypropylene, PE0.1% polypropylene; PE1%, 1% polypropylene; PLA0.1%, 0.1% polylactic acid; PLA1%, 1% polylactic acid; PBAT0.1%, 0.1% polybrene adipate; and PBAT1%, 1% polybrene adipate.

biomass. Microbial diversity (0.09), microplastics concentration (0.134), soil enzyme activity (0.192), microbial biomass (0.158) and available nutrients (0.307) all positively influenced the pea biomass (Fig. 3(b)). At maturity, SEM (χ^2 = 0.521, df = 2, p = 0.771, GFI = 0.996, RMSEA = 0.000 and SRMR = 0.029) revealed that all factors explained 62.8% of the variance in pea biomass. Further, microplastic concentration (0.124), microplastic type (0.196) and microbial biomass

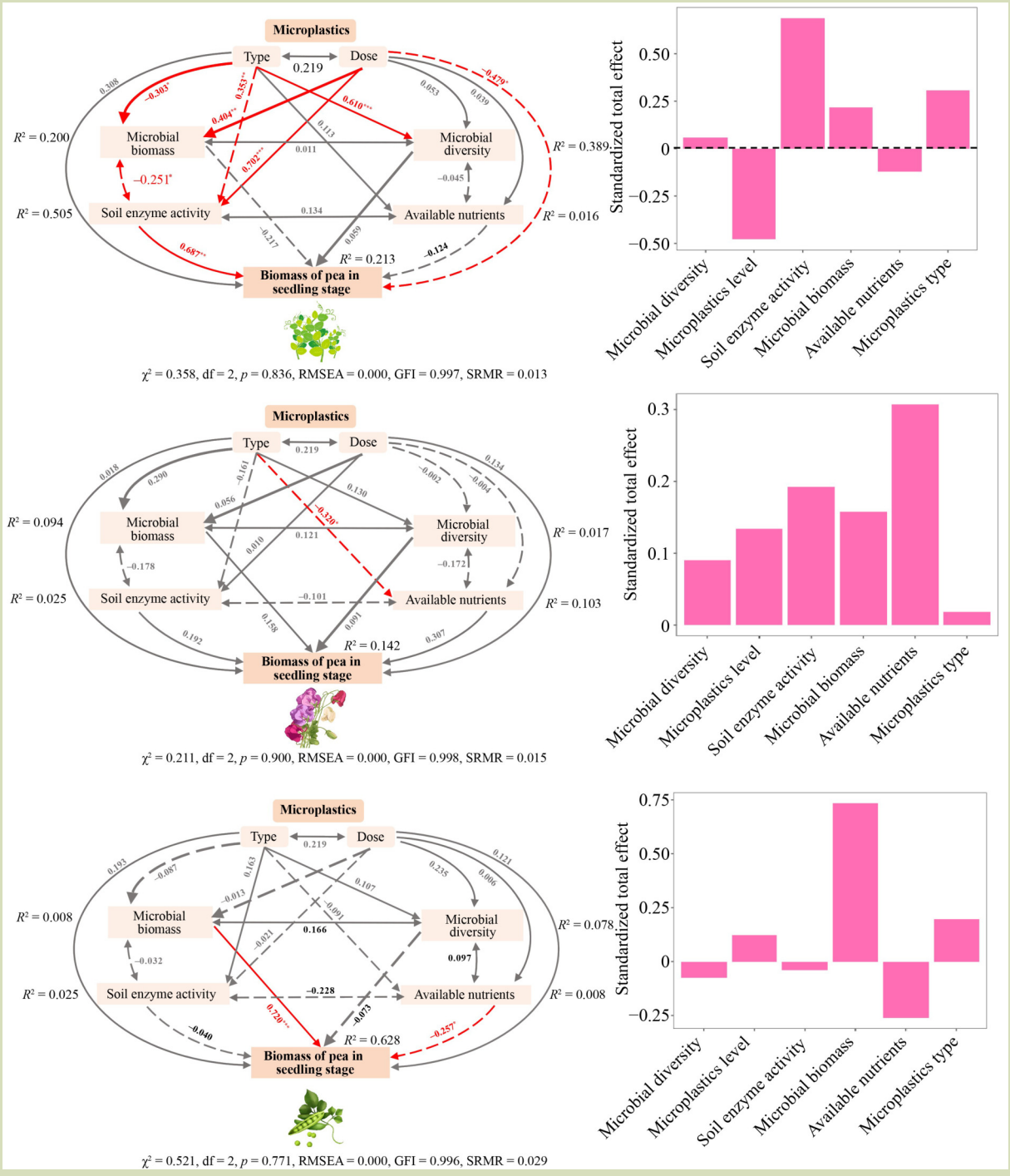

Fig. 3 Structural equation model (SEM) of the effects of microplastics addition on the biomass of pea and standardized total effects of the types and dose of microplastics, microbial biomass, soil enzyme activity, microbial diversity and available nutrients on the biomass of pea, as revealed by SEM at the seedling (a), flowering (b) and maturity (c) stages of pea. Arrow widths indicate the strength of the standardized path coefficients. Positive path coefficients are indicated using solid lines, and negative path coefficients are indicated using dashed lines. R^2 values indicate the proportion of variance explained by each variable contributing to the biomass of pea. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

(0.734) were positively related to pea biomass, whereas microbial diversity (–0.075), soil enzyme activity (–0.041) and available nutrients (–0.262) had a negative relationship to pea biomass (Fig. 3(c)).

4 Discussion

The impacts of microplastics on crop-soil systems in agriculture remain a contentious issue, largely because of the diverse types and doses of the specific microplastics involved[26,27]. The phytotoxicity of microplastics depends on characteristics such as concentration, size, shape, composition and charge, as well as on plant species and growth stage[28]. This study found inconsistent and contradictory results regarding the effects of four microplastics on pea roots and shoots at three stages of plant growth. Microplastics can adhere to plant surfaces, enter the plant through the root tips, affect root development, inhibit seed germination and induce oxidative stress responses, alter photosynthetic efficiency, and cause cytotoxicity and genotoxicity, thereby affecting plant metabolism and nutrient uptake[17,18,29]. Notably, the increase in root biomass observed at maturity in PBAT-amended soils indicates that PBAT, which shows high heat resistance and ductility, is relatively eco-friendly and supports pea root growth. PBAT microplastics can stimulate microbial growth and mine more available nutrients for pea root growth[6]. In contrast, our results showed variable impacts of standard microplastics (PP and PE) on plant growth. At the seedling stage, shoot biomass increased only in 1% PP-treated plants and, at flowering, shoot biomass increased with 0.1% PP, whereas root biomass increased only with 1% PP. Shoot biomass increased with both 0.1% and 1% standard plastics, while the root biomass increased only with 1% PP and PE at the maturity stage. These results indicate that different types and levels of standard microplastics have different effects on plant growth, potentially owing to their phytotoxicity, which depends on their characteristics and dose[10]. In addition, microplastics can reduce root penetration, improve soil aeration, and stimulate root growth and extension by altering root architecture and morphology[30,31]. S:R increased with 0.1% PP across all three growth stages; at flowering, the it increased with 1% PE and 0.1% PCL, and at maturity, with 0.1% PE. These findings indicate that more carbon was allocated to the shoots in the standard microplastics (PP and PE) treatments than in the PBAT treatment during pea growth.

Soil DOC content was not affected at flowering and maturity, indicating that at the seedling stage, PP, PE and PCL microplastics might have bonded with soil aggregates, protecting SOM from microbial decomposition[32]. This finding is consistent with a previous study which reported that PE did not affect the processes responsible for the degradation of organic matter[33]. Olsen-P decreased only in the 0.1% PP treatment at the seedling stage, indicating that PP microplastics present in the soil can reduce the number of adsorption sites[34]. Therefore, PP microplastics could cause peas to use more NO_3^--N than NH_4^+-N during growth. Notably, soil NO_3^--N increased at the seedling stage with the addition of 0.1% and 1% PCL but decreased upon addition of 0.1% PP, 1% PP, 1% PE and 1% PBAT compared to that in the control at maturity. This indicates that PCL caused cells to form aggregates entangled in exopolymers at the seedling stage due to these being immobilized by PCL in the soil as a biofilm[9,35]. Therefore, PP, PE and PBAT enhanced NO_3^--N uptake by pea roots[10,35]. However, this study did not analyze the microbiome, so it was not possible to determine the effect of microplastics on rhizospheric microorganism nutrient mineralization and root nutrient acquisition strategies during pea growth.

Microplastics can alter the resource acquisition strategies of both crop roots and microbes through their impact on the amount and composition of root exudates and subsequent effects on nutrient availability[27]. Our results demonstrated that biodegradable microplastics (0.1% PCL, 1% PCL and 1% PBAT) increased MBC, MBN and MBP at three growth stages (MBP decreased only at maturity), indicating that PCL and PBAT serve as biodegradable C sources that support SOM decomposition and nutrient cycling during pea growth owing to the altered metabolic status of the microbial community[36,37]. In this process, the highly biodegradable PCL was degraded by lipase and esterase activities, as Proteobacteria were the dominant phyla on the surface of the PCL polymer[38], resulting in more C being released for microbial activity, thus enhancing soil microbial network complexity[39,40], as PCL byproducts (linear and cyclic polymers and oligomers) can cause severe membrane depolarization by impairing membrane proton pumps and altering normal membrane ion-permeability[41]. PCL and PBAT microplastics increased the Shannon index of bacteria only at the seedling stage, illustrating that biodegradable microplastics promote the growth of bacterial taxa involved in their hydrolysis. This finding is inconsistent with a study that showed that

biodegradable microplastics have an adverse effect on other taxa[6], which is related to soil and crop plant types. Additionally, biodegradable microplastics did not have a strong toxic effect on bacterial interactions, but rather appeared to enhance bacterial network complexity[42]. Biodegradable microplastics create a resource-enriched environment in the soil that alleviates microbial resource competition and allows for mutualistic symbiosis[43]. Therefore, high auxiliary C metabolism in biodegradable microplastic-amended soils promoted microplastic-derived C into microbial biomass C, N and P. In contrast, traditional microplastics (PP and PE) introduced lysate and altered soil chemodiversity, thus facilitating metabolism with high C investment. However, pea root exudates can disrupt microbial nutrient acquisition when microplastics are added, leading to inconsistencies in MBC, MBN and MBP because traditional microplastics (PP and PE) are more resistant to degradation and form microplastic-soil aggregates that provide an additional habitat for soil microorganisms[16,44]. At the lower dose tested (0.1%), PE, PLA and PBAT showed higher activities of C-acquisition enzymes (i.e., BG, CBH and BX) compared to those with the higher dose (1%) at the seedling stage. A decline in BG and BX activity was observed with increasing PCL levels at seedling and flowering stages. Further, higher CBH activity was observed at the seedling stage with 0.1% PE whereas, at maturity, CBH was higher with 1% PE. In turn, at the maturity, BX activity was observed in 0.1% PE-, PCL- and PBAT-amended, and 1% PBAT-amended soil, but not in PP-amended soil. These findings indicate that increased labile C from root exudates did not alleviate microbial C limitations with microplastic addition. Also, NAG activity was higher with 0.1% PP, PCL and PBAT from the seedling to maturity stages, and with 1% PE and PBAT treatment at the seedling and maturity stages, respectively. This indicates that microplastics increased N uptake during pea growth, resulting in increased NAG activity.

The decomposition of biodegradable microplastics (PCL and PBAT) can produce water and labile C, thus providing energy for soil microorganisms[6,12] and promoting SOM decomposition through increased PPO activity in 0.1% PCL-, 1% PCL- and 1% PBAT-amended soil at the seedling stage and 0.1% PCL-, 0.1% PBAT- and 1% PBAT-amended soil at flowering, but decreased PPO activity in 0.1% PCL-, 1% PCL- and 0.1% PBAT-amended soil at maturity. In this process, PCL can degrade into nanoplastics and oligomers, which can be potentially toxic to surrounding organisms[44]. However, the cross-linked structure of PBAT restricts the access of water and microorganisms to the polymer chain, making it more resistant to degradation than PCL[39]. Once labile C is depleted, pea roots release root exudates (labile C), obviating the need for microbial decomposition of SOM until the maturity stage. However, PP-bonded soil aggregates are resistant to breakdown because of their higher malondialdehyde and hydrogen peroxide content, resulting in lower PER activity in 0.1% and 1% PP-amended soil at the seedling stage[45]. Conversely, higher PER activity may occur with 0.1% and 1% PE due to oxygen deficiency in PE-bonded soil aggregates by altering the antioxidant system, potentially causing the accelerated growth of pea plants[46]. However, PER activity also varied at flowering and maturity due to differing pea growth strategies. Microplastics can also alter the characteristics of plant–soil microbial communities, indirectly affecting crop growth[17,31]. Notably, an increasing trend in the Shannon diversity of bacteria was observed at the seedling stage in 0.1% PP-, PE-, PCL- and PBAT-amended soil, as well as in 1% PBAT-amended soil. However, the microplastic type and dose did not significantly affect the bacterial richness, Shannon, Chao1 and ACE indices across all growth stages, indicating that bacteria dominated microbial communication with increasing bacterial and fungal network complexity owing to microplastic addition at the seedling stage[47]. The reduction in bacterial dominance during flowering and maturity may have resulted from microplastics serving as a labile C source. However, when this source was depleted by microorganisms, the fresh labile C from root exudates was insufficient to sustain bacterial growth. Therefore, microplastics at the 0.1% and 1% doses did not affect material exchange among soil bacteria and fungi, but did impact ecosystem functions during microbial adaptation to the microplastic-contaminated environment[3,4]. Additionally, we conclude that, because of the short incubation time, the full toxicity of microplastics was not realized. Thus, these plastics represent a lower threat to soil microbial taxa and had a greater effect on nutrient cycling for pea growth. SEM analysis also demonstrated that the microplastic dose had a significantly negative direct effect on pea biomass whereas the microplastic types had a significantly positive indirect effect on pea biomass through microbial biomass and enzyme activities at the seedling stage. However, neither microplastic type nor dose significantly affected pea biomass, directly or indirectly at flowering and maturity, indicating that the accumulation of microplastics altered soil properties by serving as a C source for microbial growth, thereby influencing the size of related C, N and P pools which then affects pea growth.

Nevertheless, based on this short-term pot experiment, the differential ecological effects of traditional and biodegradable microplastics, and the safety of biodegradable plastics as replacements for traditional plastics in pea-soil ecosystems remain uncertain. Consequently, there are some uncertainties that need to be taken into account. The differential effects of traditional versus biodegradable microplastics in this study were mixed and thus there are no clear winner. However, given this was a short-term pot experiment, long-term field experiments with pea (or other legumes) that are fully dependent on biological-fixed N will be needed to fully evaluate whether these microplastics pose a threat to pea-soil ecosystems.

5 Conclusions

Our results showed that the type and dose of microplastics influenced the characteristics of the pea-soil system. Notably, PBAT appeared to be relatively eco-friendly and supported pea root growth, as it is a biodegradable C source that supports SOM decomposition and nutrient cycling during pea growth. Alternately, traditional microplastics (PP and PE) had varying effects on crop growth depending on their characteristics (e.g., dose, size, shape, composition and charge). However, once the labile C was depleted, fresh labile C from root exudates failed to alleviate microbial carbon limitations, forcing pea plants and microorganisms to compete for limited soil N and P resources. Consequently, at the seedling stage, easily degradable matter in microplastic-bonded soil aggregates may have broken down, leading to bacterial dominance in microbial communication. Bacterial dominance decreased as pea growth progressed because of the depletion of labile C from microplastics, thereby shifting plant resource acquisition strategies toward competition for limited N and P. However, whether traditional or biodegradable microplastics are preferable for pea growth remains to be clarified. Specifically, long-term field experiments are required to assess the potential ecotoxicological effects of microplastics on the pea-soil system.

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Compliance with ethics guidelines

Jialing Wu, Yuhuai Liu, Li Wang, Mouliang Xiao, Liang Wei, Jina Ding, Jianping Chen, Zhenke Zhu, and Tida Ge declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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