



# Dose-dependent toxicity of polyethylene microplastics (PE-MPs) on physiological and biochemical response of blackgram and its associated rhizospheric soil properties

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## Abstract

Microplastic contamination in terrestrial ecosystem is emerging as a global threat due to rapid production of plastic waste and its mismanagement. It affects all living organisms including plants. Hence, the current study aims at understanding the effect of polyethylene microplastics (PE-MPs) at different concentrations (0, 0.25, 0.50, 0.75, and 1.00% w/w) on the plant growth and yield attributes. With blackgram as a test crop, results revealed that a maximum reduction in physiological traits like photosynthetic rate; chlorophyll *a*, *b*; and total chlorophyll by 5, 14, 10, and 13% at flowering stage; and an increase in biochemical traits like ascorbic acid, malondialdehyde, proline, superoxide dismutase, and catalase by 11, 29.7, 16, 22, and 30% during vegetative stage was observed with 1% PE-MP application. Moreover, a reduction in growth and yield attributes was also observed with increasing concentration of microplastics. Additionally, application of 1% PE-MPs decreased the soil bulk density, available phosphorus, and potassium, whereas the EC, organic carbon, microbial biomass carbon, NO<sub>3</sub>-N, and NH<sub>4</sub>-N significantly increased. Moreover, the presence of PE-MPs in soil also had a significant influence on the soil enzyme activities. Metagenomic analysis (16 s) reveals that at genus level, *Bacillus* (19%) was predominant in control, while in 1% PE-MPs, *Rubrobacter* (28%) genus was dominant. *Microvirga* was found exclusively in T<sub>5</sub>, while the relative abundance of *Gemmatimonas* declined from T<sub>1</sub> to T<sub>5</sub>. This study thus confirms that microplastics exert a dose-dependent effect on soil and plant characteristics.

**Keywords** Polyethylene microplastics · Blackgram · Growth and yield attributes · Soil properties

## Introduction

Plastics have now become an essential and inseparable component of human life. The exponential population growth exerts enormous pressure on plastic production, which is evident from the ever growing consumption of the plastics. As per the report of Plastics Europe 2021, there is a huge leap in the plastic production from 1950s to 2020 (1.5 to 367 million tons). According to estimates, 76% of plastic wastes is dumped in landfills or released into the soil, air, and water environment (Geyer 2020). These plastics are further broken down and enter into the environment as macroplastics (> 20 mm), mesoplastics (10–20 mm), and microplastics (< 5 mm) (Cole et al. 2011; Zhang et al. 2021a). With continuous breakdown of plastic particles, it is predicted that microplastic(MP) emission would increase by 1.3 to 2.5 times within 2040, amounting to roughly 3 million pieces (Lau et al. 2020).

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Effect of microplastics (MPs) on aquatic ecosystem was reported by many researchers (Auta et al. 2017; Lambert and Wagner 2018); however, the studies highlighting their effect on terrestrial ecosystem are negligible. In recent years, many studies validated the increasing occurrence of MPs in the terrestrial environment (Lohmann 2017; de Souza Machado et al. 2018). Modern agricultural technologies like greenhouse covers, plastic mulch films (van Schothorst et al. 2021; Zhang et al. 2021b), plastic seed coatings (Gündoğdu et al. 2018), wastewater irrigation (Gündoğdu et al. 2018; He et al. 2018), landfills and leachates from landfills (He et al. 2019; Su et al. 2019; Silva et al. 2021a, b), bio-solid application to agricultural fields (Nizzetto et al. 2016; Mahon et al. 2017; He et al. 2018), soil conditioner application (Zubris and Richards 2005), application of compost and organic fertilizer (Weithmann et al. 2018), and atmospheric deposition (Klein and Fischer 2019) are the potential sources of MP pollution in soil, and thereby significantly affecting the soil biodiversity (Rillig et al. 2017; He et al. 2018) and crop growth (De Silva et al. 2021a, b).

Amongst various agricultural sources, mulching film made of polyethylene (PE) forms the major source of MPs in agricultural soil. It has been estimated that the plastic demand for mulching, silage films, and green house would rise by 50%, i.e., from 6.1 million tons in 2018 to 9.5 million tons in 2030 (Geyer 2020). Owing to their tedious recovery from soil, these MPs buildup in soil, thereby leading to accumulation in the field. This MP accumulation in agricultural soils has undesirable effect on soil properties such as altered pH (Boots et al. 2019; Zhou et al. 2021b); changes in bulk density (de Souza Machado et al. 2018; Zhang et al. 2019a); nutrient mobility (Guo et al. 2020; Dong et al. 2021; Ya et al. 2021); rise in dissolved organic carbon content (Meng et al. 2022); release of the additives like dioxins and furans (Li et al. 2021a; Yan et al. 2021a); and the upregulation or downregulation of a particular group of microorganisms, which in turn causes changes in composition of microbial community (Zhang et al. 2019b; Rong et al. 2021; Yan et al. 2021b). As a consequence, the soil ecosystem and agricultural productivity gets severely affected.

As far as plants are concerned, MP interferes in number of ways including nutrient uptake by blocking pores on the cell surface or transport pathways between the cells (Ma et al. 2016; Qi et al. 2018; Bosker et al. 2019; Yu et al. 2021a), reducing or delaying seed germination, and altered root and shoot growth (Qi et al. 2018; Bosker et al. 2019; Yu et al. 2021b).

The presence of MPs on the surface of plant roots prevents other contaminants from physically reaching the roots; however, they are more likely to cause phytotoxicity. Blackgram, a predominantly cultivated crop in tropical countries contains 56.6% carbohydrate, 26.2% crude protein, and 1.2%

fat (Statista 2021). Although plastic mulch has not been used for blackgram cultivation, the left-over film residues from previous crop or the application of organic manures, sewage sludge, and littering of polyethylene plastic waste might be a source of MPs in soil (Dhevagi et al. 2022b). Alterations in the soil properties due to polyethylene microplastics may inhibit the growth and dry weight, since the crop is known for its sensitiveness to changes in the soil properties.

Although numerous researchers have examined the effect of MPs on cereals (Qi et al. 2018, Wang et al. 2020, Dong et al. 2021; Zhou et al. 2023; Iqbal et al. 2023), and horticultural crops (Mateos-Cárdenas et al. 2019; Bosker et al. 2019; Rillig et al. 2019; Yang et al. 2021a; Sahasa et al. 2023), only very few studies have been reported on the toxicity effect of microplastics on pulse crops, especially blackgram (In broad bean by Jiang et al. 2019; soybean by Wang et al. 2021 and mung bean by Soundarya and Sujatha 2023). Hence, the present study was formulated with the hypothesis that exposure to PE-MPs may influence the rhizospheric soil properties which in turn affect the growth and development of blackgram.

## Materials and methods

### Collection and characterization of materials

The polyethylene microplastics (PE-MPs) used for the experimental study were acquired from a private recycling business industry (M/s. Arunachal Polymer Industries (11.07469°N, 76.91207°E), located in Coimbatore, Tamil Nadu. Scanning electron microscope (SEM) was used to determine the size and shapes and EDAX (Quanta 250 (FEI, Netherlands)) to determine the composition of PE-MPs.

Blackgram (*Vigna mungo*) seeds (variety CO6) were obtained from NPRC (National Pulses Research Centre), Vamban. Viable seeds with a germination rate of 95% were chosen and steps have been taken to avoid microbial contamination. Uniform sized seeds were soaked in 2% sodium hypochlorite solution for 30 min and rinsed with sterile deionized water thrice before subjecting to various seed treatments.

The experimental soil (loam — top 0 to 20 cm) was collected from the crop field of wet land, Tamil Nadu Agricultural University, Coimbatore. The soils were shade dried, sieved through 2 mm to get rid of plant residues, large rocks, and gravel. Water holding capacity (Margesin and Schinner 2005), bulk density (Bashour and Sayegh 2007), porosity (Reynolds et al. 2008), pH and EC (Jackson 2005), soil organic carbon (Walkley and Black 1934), available nitrogen (Subbiah and Asija 1956), phosphorus (Olsen 1954), and potassium (Hanway and Heidal 1952) of the soil were characterized as per the standard procedures.

## Experimental details

The current study was carried out at Tamil Nadu Agricultural University farm land, namely, Eastern Block. The study was conducted under greenhouse conditions and temperature during the study period ranged from 33.3 to 36.2 °C, while the minimum temperature was 22.7 to 30.8 °C and an average precipitation of 22.7 mm. Ten kilograms of processed farm soil were taken in pots to which different concentrations of microplastics having the same size ( $T_1$  (0%),  $T_2$  (0.25%),  $T_3$  (0.50%),  $T_4$  (0.75%), and  $T_5$  (1.0%) on dry weight basis (w/w)) were added. The concentrations were fixed based on the previous studies that quantified microplastics in different soils (Fuller and Gautam 2016; Huang et al. 2019; Lian et al. 2021). Before being mixed with the soil, the polyethylene microplastic particles were sonicated at 25 °C for 2 h to avoid aggregation. Microplastics were uniformly mixed with soil and three replications for each treatment for three different stages of plant growth were fixed ( $t=5$ ,  $s=3$ ,  $r=3$ , and  $n=15$  for each stage of crop). Additionally, three plants per replication was maintained. The soil was maintained to 50% moisture and incubated 2 weeks for stabilization (de Souza Machado et al. 2019). Then, the blackgram seeds were sown, watered regularly, and steps were taken to maintain the plant density throughout the study period.

## Analysis

The plants were harvested through destructive method at the end of each growth stage; i.e., vegetative, flowering and harvest, and their physiological, biochemical, growth, and yield attributes were determined. Similarly, the changes in physicochemical properties of the rhizospheric soil were also examined at the end of each growth stage.

## Plant traits

With each treatment and growth stage, the third fully developed leaf from three randomly selected plants were subjected to physiological measurements through non-destructive method. Plant physiological parameters such as photosynthetic rate, transpiration rate, and stomatal conductance were measured using portable photosynthetic system (ADC Bio Scientific LCpro-SD System, UK) between 9.00 a.m. to 12.00 p.m. (Ramya et al. 2021). Plant biochemical traits like malondialdehyde (Heath and Packer 1968), proline (Bates et al. 1973), ascorbic acid (Keller and Schwager 1977), catalase, peroxidase, and superoxide dismutase (Kar and Mishra 1976) were quantified using standard procedures. Furthermore, plant height, root length, pod length, number of nodules, flowers, pods per plant, seeds per pod, total, and 100 grain weight were measured (Dhevagi et al. 2021). The changes in the root morphology were also assessed through

Gia Roots software. The roots of blackgram were imaged using a destructive approach, rotating for 360° and capturing 20 snap shots at 18° intervals. These images were then examined for phenotypic characteristics such as maximum number of roots (MNR), average root width (diameter) (ARW), network surface area (NSA), specific root length (SRL), network volume (NV), depth (ND), width (NW), and perimeter (NP) using the GiA Roots Software Framework (Galkovskiy et al. 2012). All the parameters except yield traits were measured at 30 (vegetative), 45 (flowering), and 75th (harvest) day after sowing.

## Soil properties

Similar to plant parameters, physico-chemical (water holding capacity, porosity, pH, EC, organic carbon, microbial biomass carbon,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , available P, and K) and biological properties ( $\beta$ -Glucosidase (Eivazi and Tabatabai 1988), dehydrogenase (Casida et al. 1964), urease, asparaginase (Hoffmann and Teicher 1961), and phosphatase (Tabatabai and Bremner 1972)) of rhizosphere soil were characterized at different plant growth stages using standard procedures.

Additionally, the rhizosphere soil samples (control and 1% PE-MPs) were collected at the flower initiation stage by following the method given by Chen et al. (2019) and subjected to 16 s meta-genomic analysis through destructive method. The DNA was extracted using QIAZEN kit, and the DNA was then tested using GEL check and Nano Drop (260/280 at ~ value of 1.8 to 2). Using primers (16sF:—5' AGAGTTTGATGMTGGCTCAG3' and 16sR:—5' TTA CCGCGGCMGCSGGCAC3') in a thermal cycler with initial denaturation temperature of 95 °C for 3 min, the purified DNA (40 ng) was amplified. Twenty-five cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s, elongation at 72 °C for 2 min, and final extension at 72 °C for 10 min were then performed followed by at 4 °C. Amplified DNA were purified using Ampure beads and quantified using a sensitivity assay kit (QubitdsDNA). Illumina Miseq with 2×300PE V3 sequencing kit was used to perform sequencing, and raw data QC was done using FASTQC and MULTIQC. Trimming of adapters and low-quality reads were done using TRIMGALORE. The trimmed reads were further taken for processing steps like merging of paired end reads; chimeria removal and OUT abundance calculation and estimation corrections were achieved by QIIME 2.

## Statistical analysis

Factorial CRD was performed for all the experiments, to analyze the differences between the factors and their interactions. The SPSS software was used for computation of results, and the OriginPro 2021 software (origin, Northampton, MA, USA) was used to draw the graphs. The results of each

treatment were documented and processed using Microsoft Excel 2016 and were represented as mean  $\pm$  SD (standard deviation). The differences and similarities among various treatment and their interaction were analyzed through principle component analysis (PCA) using the R software.

## Results and discussion

### Characteristics of experimental soil and PE-MPs

The experimental soil had a water holding capacity of 34%, while the bulk density was 1.08 g/cc. The porosity was 41.78% with a pH of 8.68 and an electrical conductivity of 0.34 dSm<sup>-1</sup>. The organic carbon, available nitrogen, phosphorus, and potassium were 0.32%, 267, 25.0, and 323 kg ha<sup>-1</sup>. Scanning electron microscope with EDAX was used to determine the particle size of MPs, and it was found that the microplastics under study was irregular in shape. The particle size ranged from 6 to 600  $\mu$ m with oxygen, carbon, potassium, and phosphorus content of 9.09, 90.88, 0.01, and 0.01%, respectively (Supplementary Figure S1). The concentrations and size were fixed based on the previous studies that quantified microplastics in different soils (Fuller and Gautam 2016; Huang et al. 2019; Lian et al. 2021; Sahasa et al. 2023).

### Effect on plant traits

The effects of application of PE-MPs at 0.25, 0.50, 0.75, and 1% on various plant parameters at vegetative, flowering, and harvest stages of blackgram are summarized in Table 1.

### Physiological traits

In the present study, physiological traits like photosynthetic rate, stomatal conductance, and chlorophyll content significantly declined upon exposure to different concentrations of microplastics. It was observed that compared to control, the maximum reduction in photosynthetic rate was observed at the rate of 8, 5, and 3% during vegetative, flowering, and harvest stages, respectively, when blackgram were grown in soil having 1% PE-MPs (Fig. 1a).

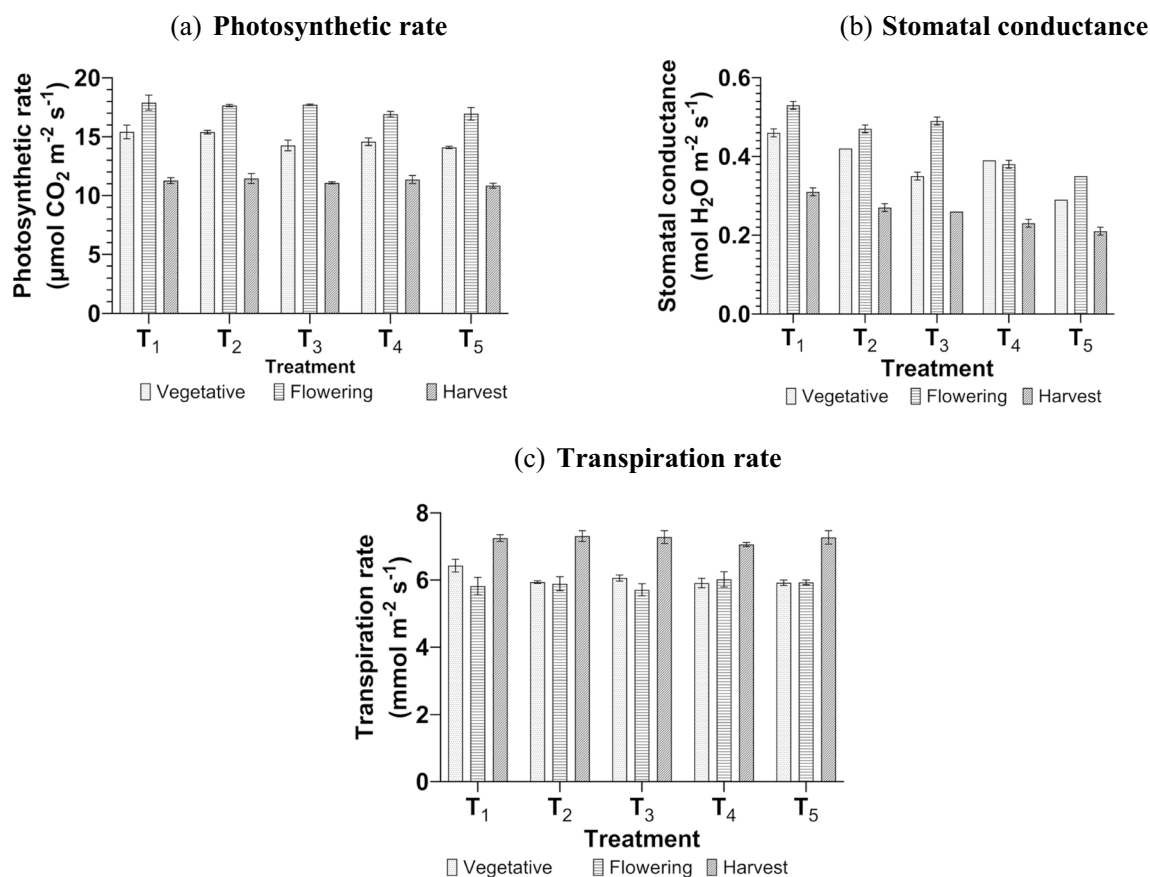
Similarly, the presence of PE-MPs had reduced stomatal conductance at all the growth stages of blackgram (Fig. 1b). The stomatal conductance was lowest during harvest stage, and irrespective of growth stages, the highest impact was observed in T<sub>5</sub> (1.00% PE-MPs) exhibiting 36, 33, and 34% reduction during vegetative, flowering, and harvest stages respectively. However, exposure to different concentrations of microplastics did not significantly influence transpiration rate of blackgram, though there were significant differences among various crop growth stages (Fig. 1c).

**Table 1** Effect of PE-MPs on growth, physiological and biochemical parameters of blackgram (CO 6)

Variables	Stages (S)		PE-MPs level (T)		S $\times$ T	
	F	sig	F	sig	F	sig
Root length	1377.8	***	11.60	***	2.510	*
Shoot length	4439.2	***	16.10	***	7.320	**
R/S ratio	485.07	***	9.487	**	10.17	**
Plant DW	4560.1	***	27.76	***	6.290	*
No. of branches	1460.6	***	30.18	***	6.437	*
No. of leaves	2766.9	***	91.38	***	11.45	**
Root nodules	1329.7	***	263.4	***	66.81	***
PR	1180.6	**	10.68	*	2.627	ns
SC	1352.8	***	280.7	***	29.65	***
TR	316.49	***	1.545	ns	3.369	ns
Ascorbic acid	13.701	**	20.18	***	4.216	*
MDA	315.96	***	57.21	***	14.24	**
Proline	365.66	***	35.60	***	6.506	*
SOD	273.33	***	76.59	***	7.640	**
POD	41.078	***	5.131	*	1.192	ns
CAT	1434.5	***	156.7	***	26.31	***
Total chlorophyll	1020.3	***	44.437	***	18.71	***
Carotenoids	1645.5	***	1.6929	ns	0.934	ns

DW - Dry weight; PR - Photosynthetic rate; SC - Stomatal conductance; TR - Transpiration rate; MDA - Malondialdehyde; SOD - Superoxide Dismutase; POD - Peroxidase; CAT - Catalase

\*\*\*  $p < 0.001$ , \*\*  $p < 0.005$ , \*  $p < 0.033$ , ns 0.12



**Fig. 1** Effect of PE-MPs on physiological traits of blackgram at different growth stages (T<sub>1</sub> — Control; T<sub>2</sub> — 0.25% PE-MPs; T<sub>3</sub> — 0.50% PE-MPs; T<sub>4</sub> — 0.75% PE-MPs; T<sub>5</sub> — 1.00% PE-MPs) (Mean ± standard deviation of three replicates presented by thin vertical bars)

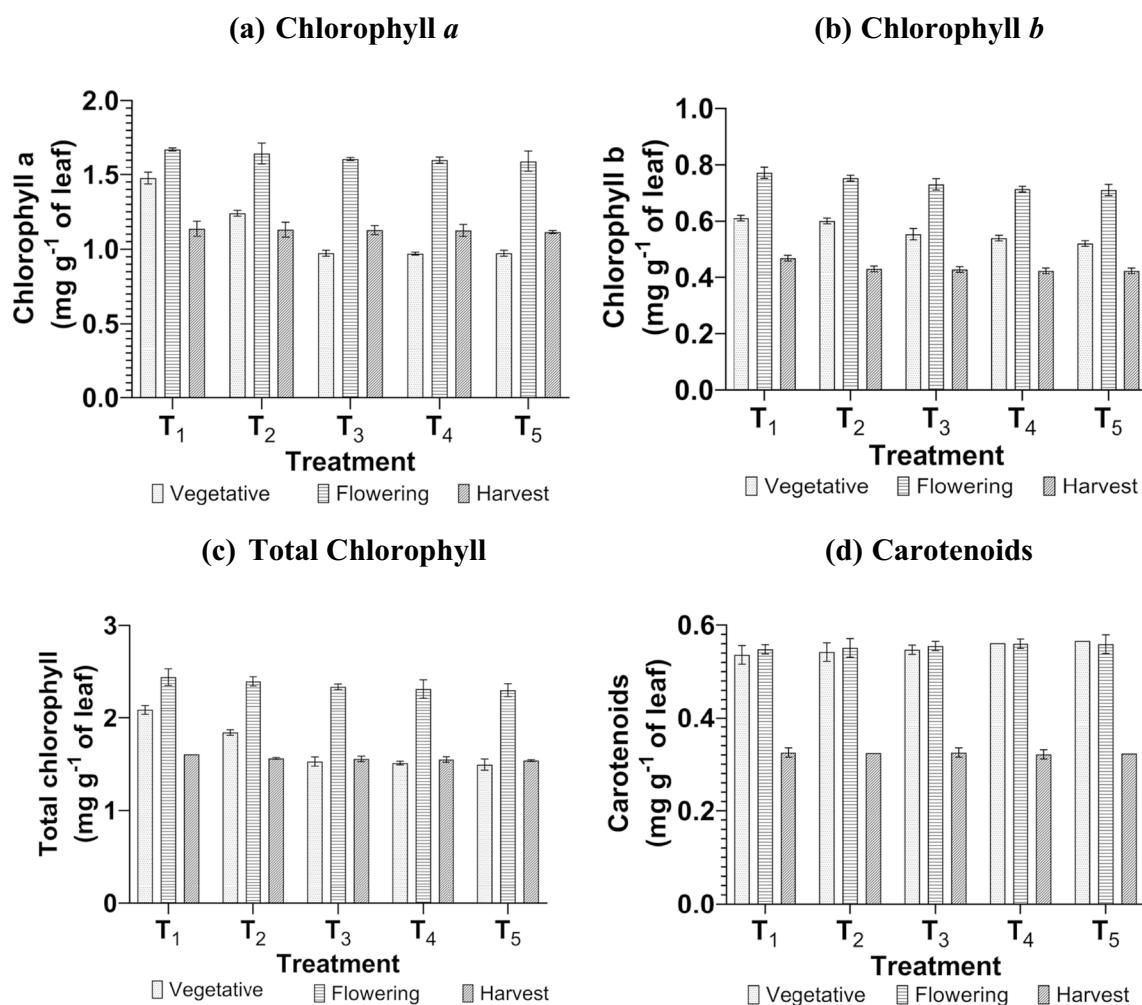
A significant impact on chlorophyll *a* content of blackgram leaves was observed at all stages of crop growth owing to PE-MP exposure. The maximum reduction in chlorophyll *a* and *b* content of 14 and 11%, respectively, was observed when blackgram was grown in soil having 1.00% PE-MPs compared to control (Fig. 2 a and b). Similar results as like chlorophyll *a* and *b* were also observed in total chlorophyll content, wherein a maximum of 13% reduction was observed in T<sub>5</sub> (1.00% PE-MPs) (Fig. 2c). In line with the present findings, a reduction in photosynthetic pigments with the addition of 0.3–1.0 g kg<sup>-1</sup> PS-NH<sub>2</sub> and PS-SO<sub>3</sub>H in *Arabidopsis thaliana* (Sun et al. 2020), 0.5% LDPE in common bean (*Phaseolus vulgaris* L.) (Meng et al. 2021), 0.1 and 0.01 mg L<sup>-1</sup> PSNPs in wheat (*Triticum aestivum* L.) (Zong et al. 2021), and 7% PE and PVC in soybean (Li et al. 2023) have also been reported. The reason for decrease in photosynthetic activity as observed in the present study might be due to decrease in the quantum yield of PS II reaction centres by MPs (Wu et al. 2019; Li et al. 2021b). Contradictory to the present study, MPs have also been shown to assist the plant photosynthesis by enhancing the alpha-amylase activity and breakdown of starch granules

thereby increasing the amount of soluble sugars in seedlings (Bosker et al. 2019; Pignattelli et al. 2020; Qi et al. 2018).

Carotenoids in leaves have recorded 3% increase in case of treatment added with 1.00% PE-MPs compared to control, though being non-significant (Fig. 2d). These results corroborate with the findings of Li et al. (2020), wherein PVC (0.5, 1 and 2% w/w; 100 nm–18 µm) exposure promotes the production of carotenoids in lettuce (*Lactuca sativa* L.). Upon MP exposure, the ROS accumulation in cells might have increased which in turn would have hindered the proteases activity involved in chlorophyll synthesis, thereby disturbing the photosynthetic electron transfer and uptake of nutrient and water by the plants (Gao et al. 2019).

### Biochemical traits

Exposure to microplastics in plants generates excess ROS like hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide anions, singlet oxygen (O<sup>-</sup>), and hydroxyl radicals (OH<sup>-</sup>), thereby causing a permanent damage to the plants (Li et al. 2021c; Maity et al. 2020; Zhang et al. 2021a). It has been established that the microplastic's polymer type and size are mostly responsible



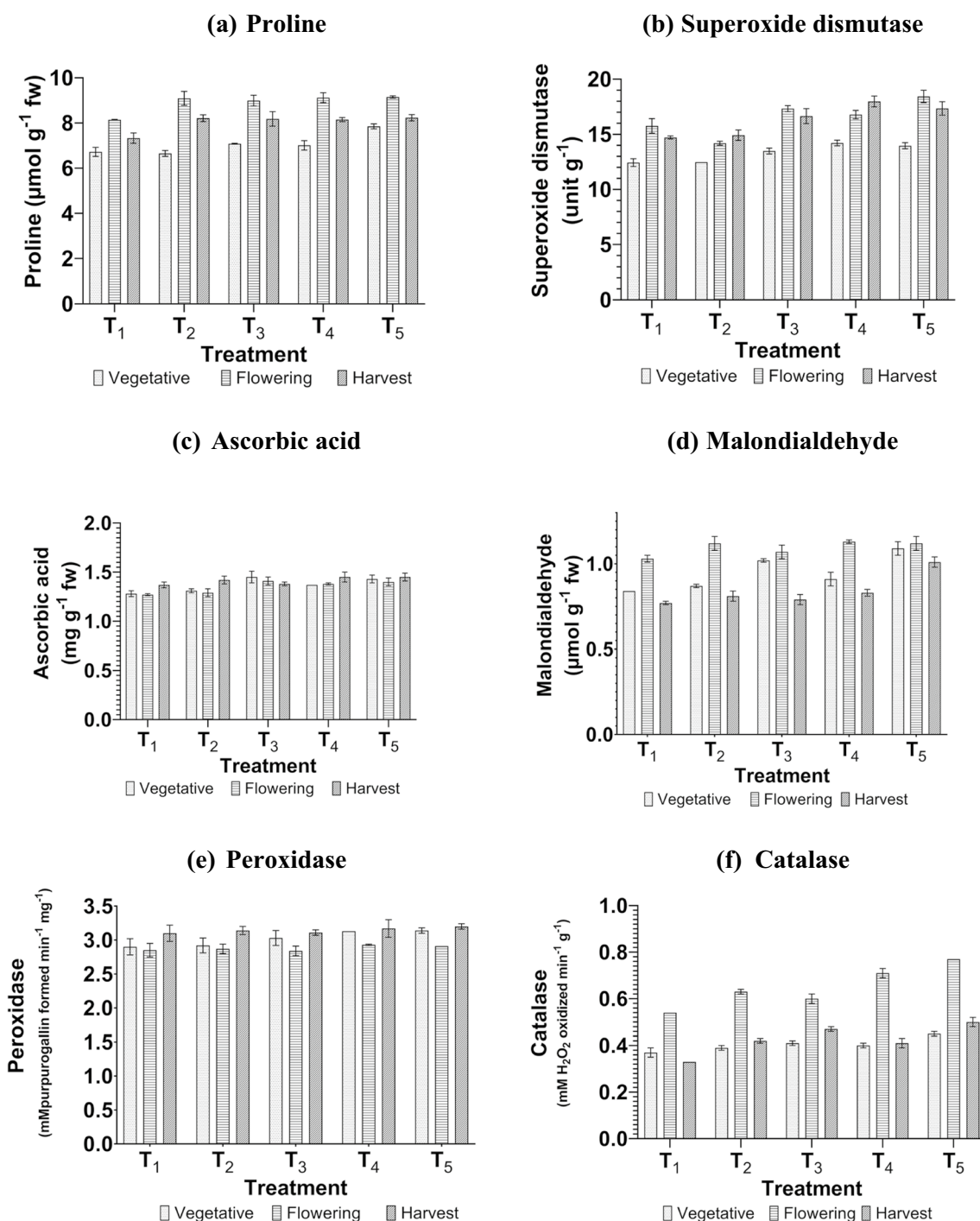
**Fig. 2** Effect of PE-MPs on photosynthetic pigments of blackgram at different growth stages ( $T_1$  — Control;  $T_2$  — 0.25% PE-MPs;  $T_3$  — 0.50% PE-MPs;  $T_4$  — 0.75% PE-MPs;  $T_5$  — 1.00% PE-MPs) (Mean  $\pm$  standard deviation of three replicates presented by thin vertical bars)

for the circumstances that lead to microplastic-induced ROS generation in blackgram.

Under abiotic stress condition, the essential amino acid, proline, is crucial for maintaining plant growth and metabolism. In the present study, a dose-dependent effect was observed in proline content at all the three growth stages (Fig. 3a). The presence of microplastics in soil increased the proline content in crop by approximately 16 and 12% during vegetative and flowering cum harvest stage, respectively, which might have been triggered due to abiotic stress caused by PE-MPs in soil (Rejeb et al. 2014). Superoxide dismutase (SOD), the first line of defence, catalyzes the conversion of harmful  $O_2^{\bullet-}$  into  $O_2$  and  $H_2O_2$ , thereby shielding cells from possible damage. Moreover, it has been reported that different MPs have different effects on SOD enzymes based on their concentration, size, and type. In the present study, SOD activity has increased at the rate of 14 ( $T_4$ ), 16 ( $T_5$ ), and 22% ( $T_4$ ) during vegetative, flowering, and harvest stage, respectively (Fig. 3b), and,

the highest activity was recorded in plants exposed to 0.75 and 1.00% PE-MPs. This significant increase is attributed as a result of the elevated ROS levels, which might upregulate the antioxidant producing genes (Li et al. 2013). The findings of the present study corroborate with the findings of Jiang et al. (2019), who described that *Vicia faba* root tips, compared to control, the SOD activity considerably increased after being exposed to 10, 50, and 100  $mg L^{-1}$  of 5  $\mu m$  and 100 nm PS-MPs. Additionally, PS-NPs at 50 and 100  $mg L^{-1}$  significantly boosted the SOD activity in rice (*Oryza sativa* L.) roots (Zhou et al. 2021a). On contrary, a dose-dependent reduction in SOD was also reported in rice (*Oryza sativa* L.) (Wu et al. 2021).

One of the most prevalent water soluble antioxidants in plants is ascorbic acid (AsA), or vitamin C (Shao et al. 2008). The presence of PE-MPs in the current study significantly increased the ascorbic acid production at all the stages. This variation accounted for 11, 10, and 5% increase in  $T_5$  (1.00% PE-MPs) during vegetative, flowering, and harvest stages, respectively.



**Fig. 3** Effect of PE-MPs on biochemical traits of blackgram at different growth stages (T<sub>1</sub> — control; T<sub>2</sub> — 0.25% PE-MPs; T<sub>3</sub> — 0.50% PE-MPs; T<sub>4</sub> — 0.75% PE-MPs; T<sub>5</sub> — 1.00% PE-MPs) (mean  $\pm$  standard deviation of three replicates presented by thin vertical bars)

The ascorbic acid production was highest during vegetative and flowering stages of crop in treatments with 0.50, 0.75, and 1.00% PE-MPs (Fig. 3c). Likewise, the increase in ascorbic acid due to MPs exposure have also been observed in other crops, including lettuce (*Lactuca sativa*) (Gao et al. 2019), broad bean (*Vicia faba*) (Jiang et al. 2019), spring barley (*Hordeum vulgare*) (Li et al.

2021c), cucumber (*Cucumis sativus*) (Li et al. 2020), and rice (*Oryza sativa* L.) (Zhou et al. 2021a).

Malondialdehyde formed during the lipid peroxidation is frequently used as an indicator to measure the cell damage caused by stress in plants. The MDA content in the present study significantly inclined with PE-MP exposure at all crop

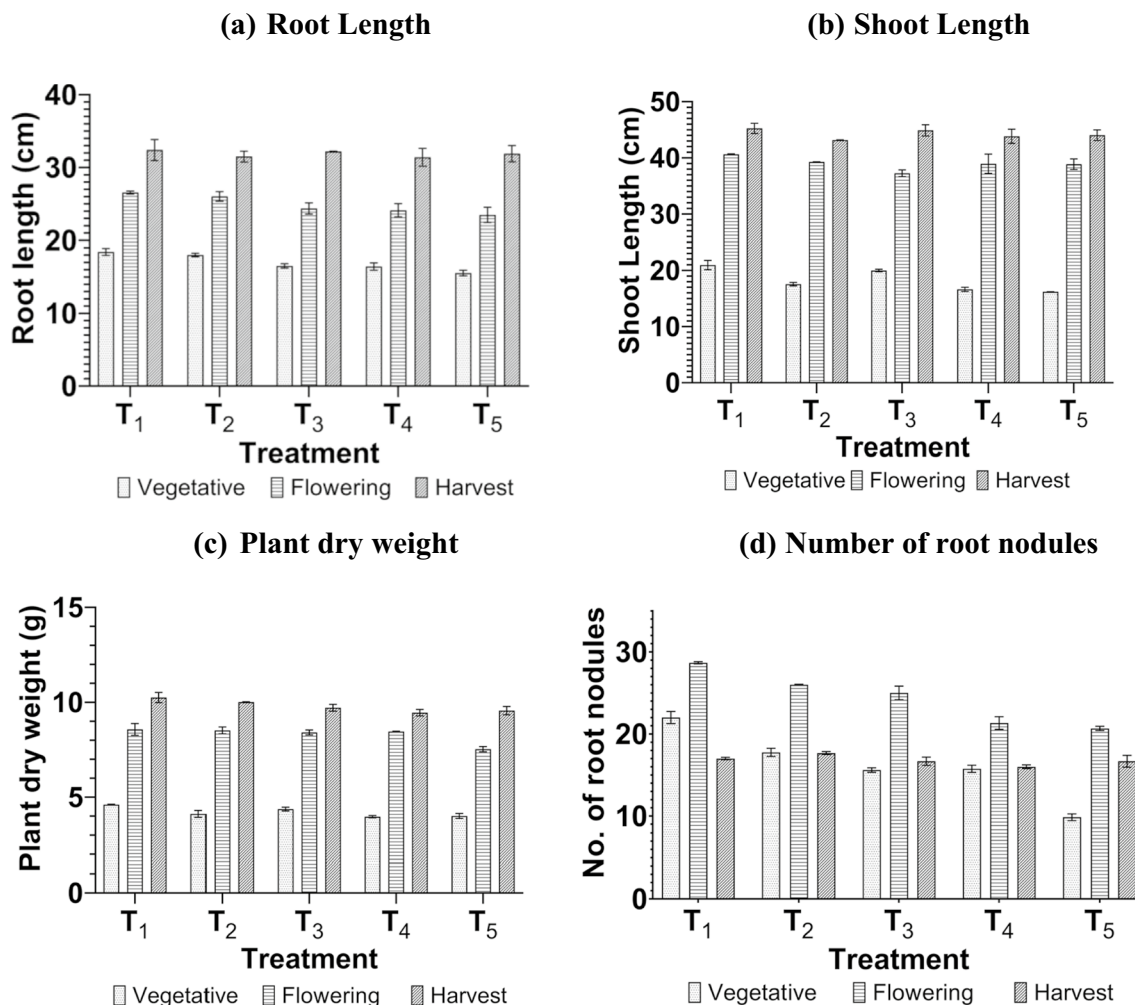
growth stages (Fig. 3d) with the maximum effect in 1% PE-MPs ( $T_5$ ). Moreover, The MDA content was recorded the highest during vegetative stage at a rate of 29% increase in  $T_5$ . This increase in MDA content is a sign of membrane lipid peroxidation in blackgram (Jiang et al. 2019; Dhevagi et al. 2022a) which might have been triggered by PE-MPs. Similar to the present findings, MDA was significantly affected in radish (*Raphanus sativus*) and broccoli (*Brassica oleracea* var. *italica*) grown with seven different levels of low-density polyethylene (LDPE) (Lopez et al. 2022).

Peroxidase, in the presence of  $H_2O_2$ , oxidizes a wide range of compounds by converting  $H_2O_2$  to oxygen and water. In the current study, PE-MP exposure had also increased the peroxidase (POD) activity in blackgram; however, the variations among the treatments were minimal (Fig. 3e). At all the three stages of crop growth, the overall increase in POD activity was observed to be 0.67 ( $T_2$ ), 1 ( $T_3$ ), and 4% ( $T_4$  and  $T_5$ ) compared to control. It was

also observed that the addition of PE-MPs in soil increased the activity of catalase (Fig. 3f), an important antioxidant enzyme which helps to reduce the ROS damage. The highest catalase activity was recorded during flowering stage, and irrespective of growth stage, highest catalase activity was observed in  $T_5$  (1.00% PE-MPs). In contrast to the present findings, Jiang et al. (2019) reported decreased CAT activity in *Vicia faba* root tips with increasing concentrations of PS microplastics (5  $\mu$ m).

**Growth and yield attributes**

It was observed that exposure to different concentrations of PE-MPs had a significant influence on the root length of blackgram at all the stages of crops (Fig. 4a). Maximum reduction in root length of about 15, 11, and 3% was observed during vegetative, flowering, and harvest stage. The root length was affected the most at vegetative stage



**Fig. 4** Effect of PE-MPs on growth traits of blackgram at different growth stages ( $T_1$  — control;  $T_2$  — 0.25% PE-MPs;  $T_3$  — 0.50% PE-MPs;  $T_4$  — 0.75% PE-MPs;  $T_5$  — 1.00% PE-MPs) (mean  $\pm$  standard deviation of three replicates presented by thin vertical bars)



due to microplastics and particularly in treatments with higher PE-MP concentrations (0.75% and 1.00% PE-MPs). The reduction in root length observed in the present study could be due to the adherence of microplastics which in turn inhibits the water imbibition as evidenced in Supplementary Figure S2 (Kalčíková et al. 2017). Similar results regarding altered root length were also reported in rye grass (*Lolium perenne*) (Boots et al. 2019), broad bean (*Vicia faba*) (Jiang et al. 2019), carrot (*Daucus carota*) (Lozano et al. 2021), wheat (*Triticum aestivum*) (Qi et al. 2018), onion (*Allium cepa*) (Maity et al. 2020), spring barley (*Hordeum vulgare*) (Li et al. 2021b), and soybean (*Glycine max*) (Lian et al. 2022). Furthermore, Spanò et al. (2022) also observed a gradual decrease in rice (*Oryza sativa* L.) root length with increasing PSNPs (0.1 to 1 g L<sup>-1</sup>) concentration.

The shoot growth is hampered by MPs because they can interfere with the nutrient transfer to the shoots thereby impeding the cell connection in roots (Jiang et al. 2019). Furthermore, MPs might negatively regulate the plant growth-related genes, restrict their expression (Zhou et al. 2021a), and limit the activity of specific enzymes involved in glucose metabolism thereby impairing plant growth and development. Application of various concentrations of PE-MPs had a significant influence on shoot length (Fig. 4b). A maximum reduction in shoot length of about 22, 8, and 3% from the control ( $T_1$ ) was observed during vegetative, flowering, and harvest stages when exposed to PE-MPs at 1%. Nevertheless, unlike the trend in root length, the pattern of shoot length decline was undefined. The ratio of root to shoot was higher in treatments with microplastics than in control during vegetative and harvest stages by 8 ( $T_5$  — 1.00% PE-MPs) and 2% ( $T_2$  — 0.25% PE-MPs) respectively, while at flowering stage, a decreasing trend was recorded (by 7% when exposed to 1.00% PE-MPs). These findings corroborate with other results wherein, in agricultural soil, the reduction in shoot growth under microplastic exposure has been observed in garden cress (*Lepidium sativum* L.) at 0.02% (PE) and mixture of PVC and PE (Pignattelli et al. 2020), wheat (*Triticum aestivum* L.) at 1% (w/w) Bio-MPs (Qi et al. 2018), and rice (*Oryza sativa* L.) at 50–500 mg L<sup>-1</sup> polystyrene (Wu et al. 2021). On contrary, MPs have also been reported to exhibit positive effects on plants like *Calama grostis* (Lozano and Rillig 2020), *Allium fistulosum* (De Souza Machado et al. 2019), and *Daucus carota* (Lozano et al. 2021). This indicates that since roots have direct contact with microplastics, they are more prone to being affected by microplastics than shoot.

Moreover, PE-MPs in soil had a significant influence on the dry weight at all stages. A maximum reduction of about 13, 9, and 7% was observed during vegetative, flowering, and harvest stage (Fig. 4c). Similar to the trend observed in root length, plant dry weight was also found to be most affected at vegetative stage, followed by flowering stage

particularly in treatments with higher PE-MP concentrations (0.75 and 1.00% PE-MPs). In line with the present findings, microplastics were also reported to reduce biomass in maize (*Zea mays* L.) (Lian et al. 2021), duckweed (*Lemna minor* L.) (Mateos-Cárdenas et al. 2019), plantain (*Plantago lanceolata*) (Van Kleunen et al. 2020), broad bean (*Vicia faba*) (Jiang et al. 2019), and cress (*Lepidium sativum* L.) (Pignattelli et al. 2021). The number of nodules per plant was significantly affected by the presence of PE-MPs in soil at various concentrations and in particular during vegetative stage (Fig. 4d). The root nodule declined by 29, 27, and 5% ( $T_5$  — 1% PE-MPs) during vegetative, flowering, and harvest stage of crop, and this was found to be consistent with the findings of Bouaicha et al. (2022). On contrary, Meng et al. (2021) reported that root nodule was enhanced at higher concentrations of LDPE-MPs.

The effect of microplastics on soil–plant system differs based on its size, shape, concentration, and polymer type (Bosker et al. 2019; de Souza Machado et al. 2019; Lozano et al. 2021; Wang et al. 2021, 2022). On the other hand, the presence of microplastics at different concentrations had no significant impact on number of flowers per plant, pod weight, and 100 grain weight of blackgram. However, pod length and number of seeds per pod were observed to decrease at higher doses of microplastics in soil (Table 2); nevertheless, the variations were unpredictable and irregular. The morphological traits of roots from all the treatments were imaged at different stages of blackgram and analysed through GiaRoots software. The initial results yielded 20 parameters, out of which 12 parameters (diameter,  $N_{\text{depth}}$ , MaxR, MedR, NwA, Perim, SRL,  $N_{\text{surf}}$ , NWDR,  $N_{\text{vol}}$ ,  $N_{\text{len}}$ , and  $N_{\text{width}}$ ) were selected to study the effect of polyethylene microplastics on root morphology. The presence of PE-MPs in soil had significant impact on all the 12 GiaRoots-derived root parameters except network depth (Table 3).

The principle component analysis (PCA) is used to determine the association in growth patterns among the treatments and its interactions. The PCA was performed for growth, physiological, biochemical, and root traits at vegetative, flowering, and harvest stage of blackgram to assess the variables which contributed to maximum variance. The analysis resulted in four principle components (PC<sub>1</sub>,

**Table 2** Effect of PE-MPs on yield parameters of blackgram (CO 6)

Variables	F	Sig
No. of flowers (NFP)	0.013	ns
No. of pods per plant (NPP)	0.910	ns
Pod weight	0.104	ns
Pod length	27.26	***
No. of seeds per pod	17.11	**
100 seed weight	0.497	ns

\*\*\*p < 0.001, \*\*p < 0.005, \*p < 0.033

**Table 3** Effect of PE–MPs on root morphological traits of blackgram (CO 6)

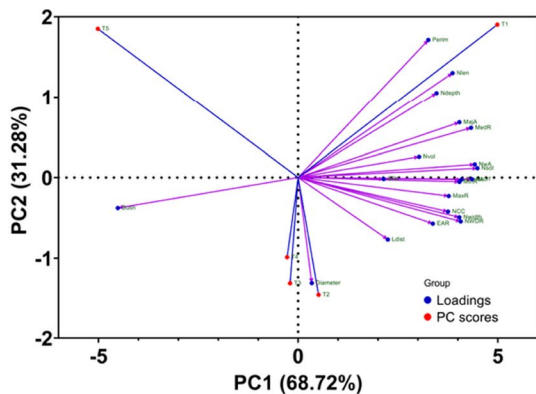
Variables	Stages ( <i>S</i> )		PE-MPs level ( <i>T</i> )		<i>S</i> × <i>T</i>	
	<i>F</i>	Sig	<i>F</i>	Sig	<i>F</i>	Sig
Diameter	366.00	***	150.03	***	27.683	***
<i>N</i> <sub>depth</sub>	1125.4	***	0.9317	ns	0.9677	ns
MaxR	1560.4	***	351.51	***	146.64	***
MedR	5771.8	***	434.80	***	220.71	***
NwA	2155.8	***	222.48	***	81.378	***
Perim	2182.5	***	820.00	***	139.03	***
SRL	1575.2	***	1089.6	***	180.92	***
<i>N</i> <sub>surf</sub>	2789.7	***	186.94	***	71.596	***
NWDR	3500.3	***	164.09	***	157.90	***
<i>N</i> <sub>vol</sub>	39256.3	***	26,315.1	***	26148.0	***
<i>N</i> <sub>len</sub>	1760.0	***	832.93	***	119.48	***
<i>N</i> <sub>width</sub>	446.21	***	39.94	***	24.978	***

\*\*\**p* < 0.001, \*\**p* < 0.005, \**p* < 0.033

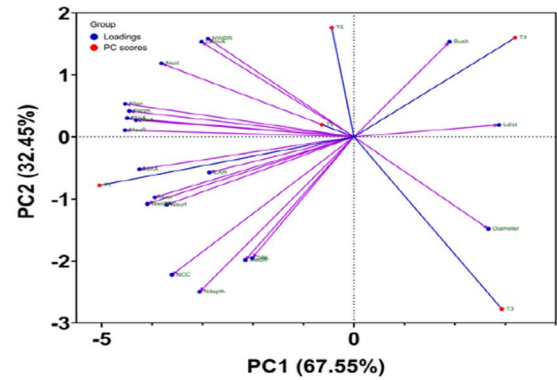
PC<sub>2</sub>, PC<sub>3</sub>, and PC<sub>4</sub>) and the PCs that contributed to > 70% variance was plotted in the biplot. During vegetative stage (Fig. 5a), the proportion of variance (%) of PC<sub>1</sub> (Eigenvalue 11.00), PC<sub>2</sub> (Eigenvalue 3.044), PC<sub>3</sub> (Eigenvalue 1.349), and PC<sub>4</sub> (eigenvalue 0.6118) was 68.72, 19.02, 8.43, and

3.82%, respectively. The variance (%) however was observed to be decreased during flowering stage (PC<sub>1</sub> (67.55%), PC<sub>2</sub> (16.67%), PC<sub>3</sub> (12.08%), and PC<sub>4</sub> (3.70%)) and harvest stage (PC<sub>1</sub> (55.12%), PC<sub>2</sub> (20.06%), PC<sub>3</sub> (16.38), and PC<sub>4</sub> (8.44%)). Treatments with 0.75 and 1% PE-MPs were

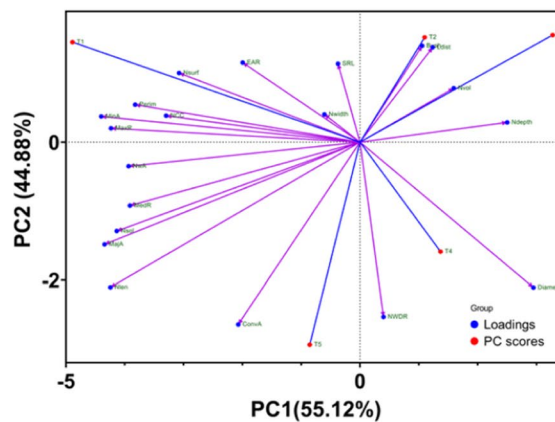
**a) Vegetative**



**b) Flowering**



**c) Harvest**



**Fig. 5** PCA depicting relationship between different root traits (GiaRoots) of blackgram at different growth stages

significantly different from other treatments during all the plant stages (Fig. 5a, b, c). From the results of PCA analysis, it can be observed that occurrence of microplastics in soil had induced stress regulating chemicals (catalase, peroxidase, SOD, MDA, ascorbic acid, proline, and carotenoids) in plants at 0.75 and 1% concentrations. Furthermore, at 0.75 and 1% PE-MPs levels, the growth and physiological parameters of plant are significantly influenced than at 0.25 and 0.50% PE-MPs. Regarding root traits,  $T_1$ ,  $T_2$ , and  $T_3$  contributed most of the weightage during vegetative stage as observed from the biplot graphs, while during flowering and harvest stage the differences among the treatments is lesser.

### Effect on soil characteristics

After entering the soil, MPs coalesce with soil organic matter (SOM) and microbial secretions and thereby become embedded in the soil microstructure (Rillig et al. 2017). This causes changes in the soil physicochemical properties through increasing the water-holding capacity and soil porosity, decreasing the moisture permeability, bulk density, altering soil pH, and thereby disturbing the integrity of soil structure (Lwanga et al. 2017; Zhao et al. 2021).

### Physico-chemical properties

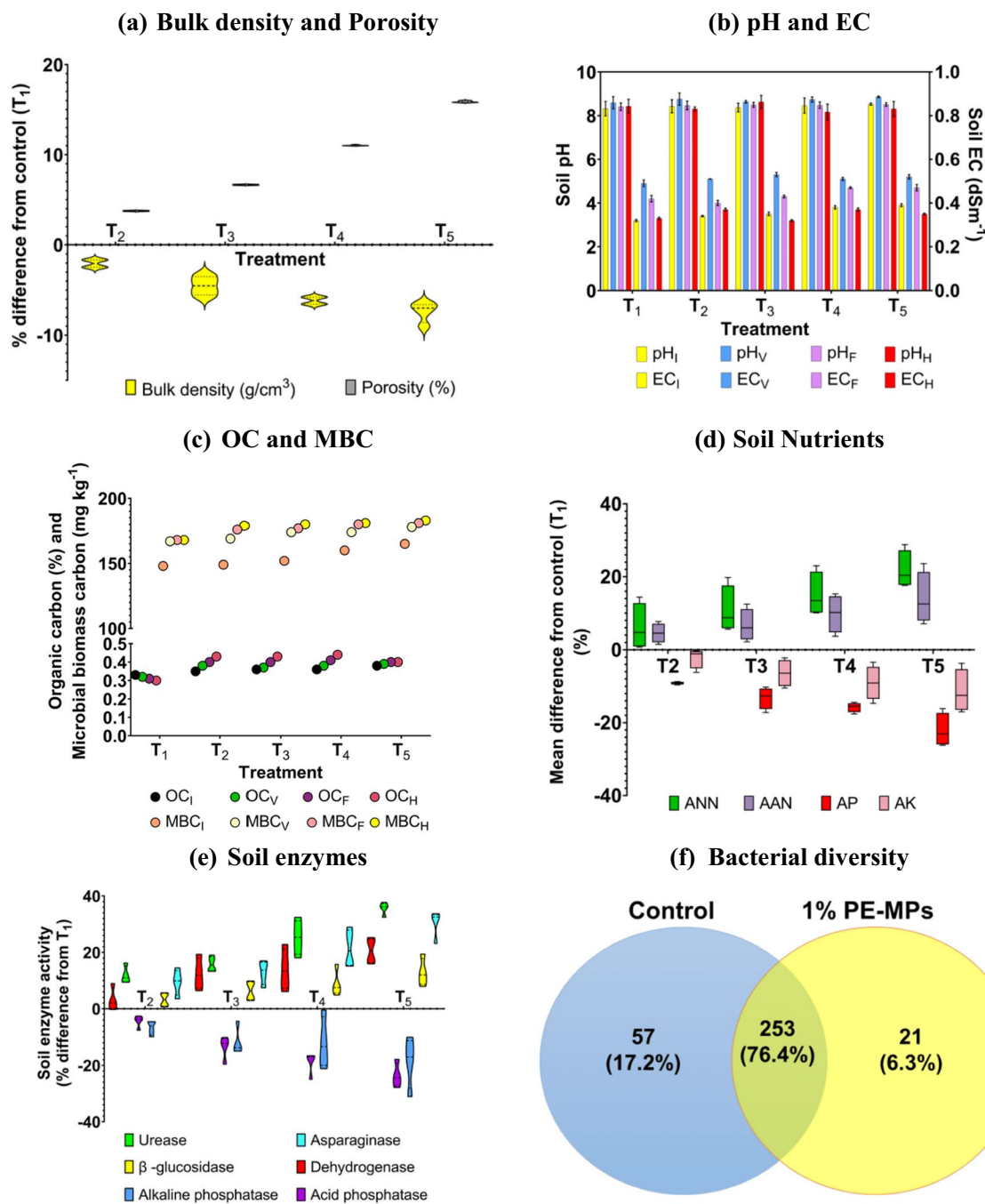
In the current study, the effect of PE-MPs on soil was examined at different growth stages. The effects of application of PE-MPs at 0.25, 0.50, 0.75, and 1% PE-MPs on physico-chemical and biological properties of rhizosphere soil at vegetative, flowering, and harvest stage of blackgram are given in Table 4. Bulk density and porosity of the soil are

inversely proportional to each other and the effect of different concentrations of polyethylene microplastics (PE-MPs) on bulk density and porosity is shown in Fig. 6a. The presence of microplastics in soil reduced the bulk density up to 7%, and as a consequence, the pore space percentage in soil increased up to 15%. de Souza Machado et al. (2019) reported that soil bulk density was decreased by PS, PP, PET, PES, and PEHD at 2.0% w/w. Similarly, Zhang et al. (2019a) indicated that higher concentration of MPs with more than 30  $\mu\text{m}$  size significantly decreased the bulk density. However, the PE-MPs had no significant effect on soil pH (Fig. 6b), though a maximum increase of 2% was recorded in  $T_5$  (1.00% PE-MPs) at different blackgram growth stages. These findings were in correspondence with the results of Jian et al. (2022) who reported an increase in soil pH at 0.1 and 1% microplastics and Zhao et al. (2021) who also reported a significant increase in pH with the addition of polyamide, polyester, polyurethane, and polycarbonate microplastics. Similarly, it was reported that PS-MPs and PTFE can lower soil pH (Dong et al. 2021), while PA-MPs and HDPE-MPs could increase it (Yang et al. 2021a). On contrary, Boots et al. (2019) indicated a decline in soil pH with the addition of synthetic fibers, polylactic acid, and high-density polyethylene. Meanwhile, the soil EC was also found to increase with increasing microplastics dosage and the increased was from 0.49, 0.42, and 0.33  $\text{dS m}^{-1}$  in control ( $T_1$ ) to 0.53, 0.47, and 0.37  $\text{dS m}^{-1}$  in the treatments with 1% PE-MPs during vegetative, flowering, and harvest stages of blackgram (Fig. 6b). The present findings are in accordance with the results of Jian et al. (2022), wherein an increase in soil EC was observed in the presence of 0.1 and 1% microplastics.

**Table 4** Effect of PE-MPs on physico-chemical and biological properties of blackgram (CO 6) rhizosphere soil

Variables	Stages (S)		PE-MPs level (T)		S×T	
	F	Sig	F	Sig	F	Sig
Bulk density	0.2868	ns	15.146	***	15.146	ns
Porosity	0.0065	ns	42.4703	***	42.4703	ns
pH	6.476	*	0.499	ns	0.721	ns
EC	1029.9	***	50.801	***	13.506	***
Organic carbon	53.177	***	145.775	***	13.330	***
MBC	85.0929	***	17.3192	***	0.8525	ns
NO <sub>3</sub> -N	8.9462	**	97.2768	***	2.4705	ns
NH <sub>4</sub> -N	12.6116	***	55.2639	***	3.6197	*
Available P	248.396	***	165.149	***	2.790	*
Available K	96.111	***	58.358	***	3.674	*
Dehydrogenase	122.569	***	84.432	***	2.7360	*
$\beta$ -glucosidase	193.1219	***	50.0552	***	3.9007	*
Urease	100.733	***	323.745	***	3.118	*
Asparaginase	51.5339	***	174.274	***	2.6827	*
Acid phosphatase	316.907	***	290.236	***	2.7787	*
Alkaline phosphatase	1804.383	***	89.052	***	12.642	***

\*\*\*p < 0.001, \*\*p < 0.005, \*p < 0.033



**Fig. 6** Effect of PE-MPs on physico-chemical and biological properties of blackgram rhizospheric soil ( $T_1$  — control;  $T_2$  — 0.25% PE-MPs;  $T_3$  — 0.50% PE-MPs;  $T_4$  — 0.75% PE-MPs;  $T_5$  — 1.00% PE-MPs) (subscript  $i$  — initial,  $v$  — vegetative,  $f$  — flowering, and  $h$  — harvest)

In addition, the soil organic carbon was observed to have increased after the addition of microplastics to the soil (Fig. 6c). The rate of increase in soil organic carbon ranged from 6 to 15, 18 to 21, and 23 to 26% in treatments with microplastics. Additionally, it was also recorded that the soil microbial biomass carbon (MBC) had increased with increase in microplastics concentrations in the soil (Fig. 6c). The soil MBC content increased at the rate of 6, 7, and

8% during vegetative, flowering, and harvest stages in  $T_5$  (1.00% PE-MPs). This might be attributed to the hike in soil organic carbon, which in turn promoted soil enzyme activities by triggering the microbial community growth. These results corroborate with the findings of Liu et al. (2017) who reported an increase in dissolved organic carbon with the addition of polypropylene powder. Similar findings were also reported by Rillig (2018), Zumstein et al. (2018), Zhang

et al. (2019a), and Ren et al. (2021). Contrastingly, Zhang et al. (2020) indicated that though there are no significant variations in soil total organic carbon due to the addition of polyester microfibers, the organic carbon content in the large macro-aggregates significantly reduced, while contrasting changes were observed in small macro-aggregates. Yu et al. (2021b) reported that after 150 days of incubation with polyethylene, the total organic carbon, and dissolved organic carbon significantly declined.

The changes in soil available nutrients due to PE-MPs at different growth stages of blackgram are graphically represented in Fig. 6d. During different growth stages of blackgram, the available  $\text{NO}_3\text{-N}$  was highest in  $T_5$  (1.00% PE-MPs) which accounted for 17, 22, and 22% increase at consecutive stages, while  $\text{NH}_4\text{-N}$  increased by 10, 14, and 23% in  $T_5$  at respective crop growth stages compared to control ( $T_1$ ). Contrastingly, the soil available P and K decreased with increasing concentrations of PE-MPs in soil. The maximum reduction in available P was recorded in  $T_5$  (1%) showing 20, 25, and 26% reduction during vegetative, flowering, and harvest stages, respectively. Likewise, the available K was lowest in treatments with high PE-MPs levels ( $T_4$  and  $T_5$ ) recording 10, 14, and 16% reduction in  $T_5$  at vegetative, flowering, and harvest stages, respectively. Chen et al. (2020) observed a significant reduction in  $\text{NH}_4\text{-N}$  with the exposure to polylactic acid, while the  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  increased significantly. Furthermore, Yan et al. (2021b) indicated a significant reduction in  $\text{NO}_3\text{-N}$  and available phosphorus in paddy soil with exposure to polyvinyl chloride microplastics. Several other studies have also reported similar results (Liu et al. 2017; Yu et al. 2020; Yang et al. 2021b).

## Biological properties

In soil, during the biochemical conversion of organic matter, soil enzymes play a vital role, and the activities of these enzymes are strongly correlated with soil organic matter (SOM), soil physicochemical characteristics, and microbial activity and/or biomass. Hence, studies on how soil microplastics influence soil enzymes are essential. In the present study, it was observed that the presence of PE-MPs in soil had positively influenced enzymes involved in C and N cycles like dehydrogenase (DHA),  $\beta$ -glucosidase (BG), urease (URE), and asparaginase (ASP) activity, while enzymes involved in P cycle like acid (ACP) and alkaline phosphatase (ALP) were negatively affected (Fig. 6e).

The soil dehydrogenase activity was highest during the vegetative stage (23% increase from control), while at flowering and harvest stages of blackgram, 15 and 17% increase was recorded with 1% PE-MP application. The  $\beta$ -glucosidase activity was also enhanced by PE-MPs by maximum of 7, 10, and 13% at vegetative, flowering, and

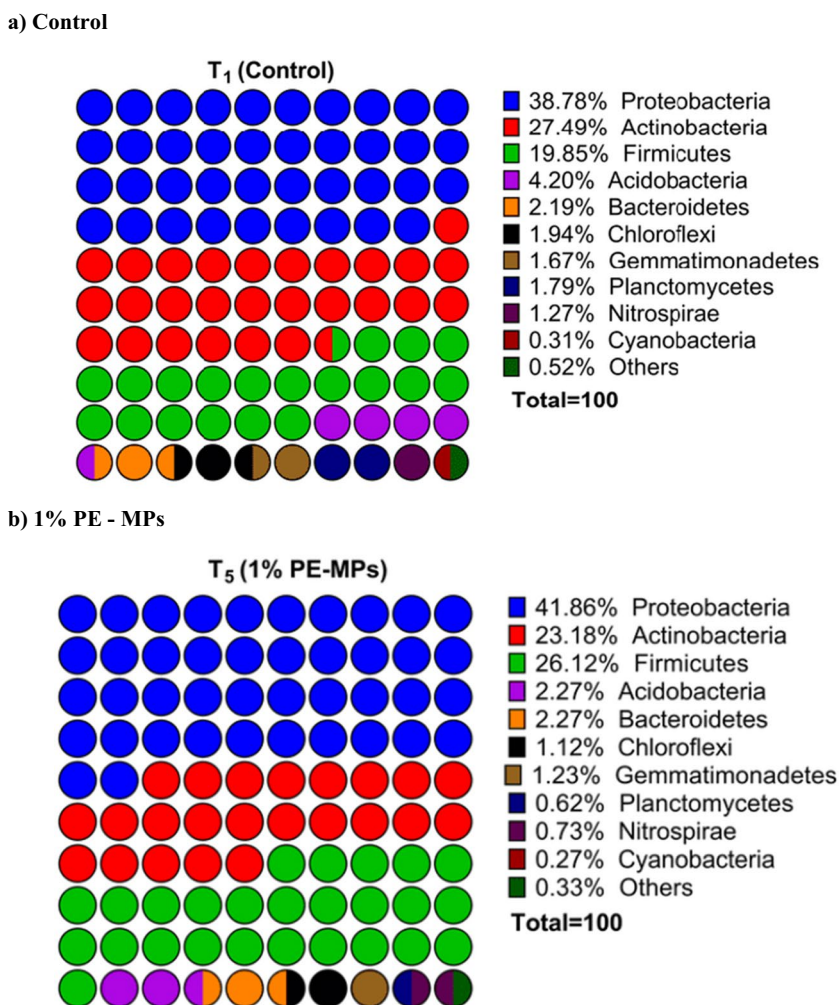
harvest stages of blackgram, respectively, with the application of 1% PE-MPs. The increase in soil organic carbon in treatment with microplastics might be due to MPs-enhanced dehydrogenase and  $\beta$ -glucosidase activities in soil. Furthermore, soil urease activity was highest at harvest stage (37%) with 1% PE-MP application. Similar to the current findings, Lian et al. (2021) observed 8% increase in the soil urease activity with maize as the test crop. Contrastingly, Fu et al. (2022) reported that PE-MPs (0.2%) had no significant effect on dehydrogenase and urease activity of maize rhizosphere under acidic soil ( $\text{pH } 5.17 \pm 0.03$ ) condition. The soil used in the current experimental study was alkaline, and PE-MP dosage was fixed up to 1% which would have attributed for the contrasting results. Similarly, asparaginase activity was also improved by PE-MPs up to 30% with 1% PE-MPs ( $T_5$ ) at all the crop growth stages. The end product of soil urease is ammonium and  $\text{CO}_2$  (Rao et al. 2014), and as discussed above, the increase in urease and asparaginase activity of soil triggered by PE-MPs might have led to increase in ammoniacal nitrogen in soil.

Contrasting to the effect of PE-MPs on other soil enzymes, acid and alkaline phosphatase activity in soil was observed to decrease from control ( $T_1$ ). The acid phosphatase activity in soil declined at the rate of 3–25, 2–23, and 7–27%, and as for alkaline phosphatase activity, the range of decline varied from 4 to 10, 9 to 14, and 7 to 31% at vegetative, flowering, and harvest stages, respectively. Parallel results were also reported by Rao et al. (2014), Lian et al. (2021), and Fu et al. (2022). The activities of four out of six enzymes analyzed were observed to have increased in soil with different microplastics levels, which might be the cause for enhanced soil microbial biomass carbon. Similarly, since the enzymes involved in P-cycling (acid and alkaline phosphatase) were down-regulated, available phosphorus in soil also decreased in the presence of PE-MPs. The decrease in acid and alkaline phosphatase enzyme activity could be a result of decrease in the abundance of *Bacillus* genus in soil where microplastics are employed as evidenced from the results of 16 s metagenomics analysis.

## Metagenomic analysis

The results of comparative analysis revealed that the soils had 253 common elements and 57 and 21 unique microbial groups were found in both  $T_1$  and  $T_5$  rhizosphere soil, respectively (Fig. 6f). The percent composition of each phylum in control ( $T_1$ ) and 1% PE-MPs ( $T_5$ ) is represented in Fig. 7. In both the soil samples, the dominant phylum was *Proteobacteria* (38.78–41.86%), followed by *Actinobacteria* (23.18–27.49%), *Firmicutes* (19.85–26.12%), *Acidobacteria* (2.27–4.20%), *Bacteroidetes* (2.19–2.27%), *Chloroflexi* (1.12–1.94%), *Gemmatimonadetes* (1.23–1.67%), *Plantomycetes* (0.62–1.79%), *Nitrospirae* (0.73–1.27%),

**Fig. 7** Phylum level abundance of microbes in rhizospheric soil exposed to PE-MPs



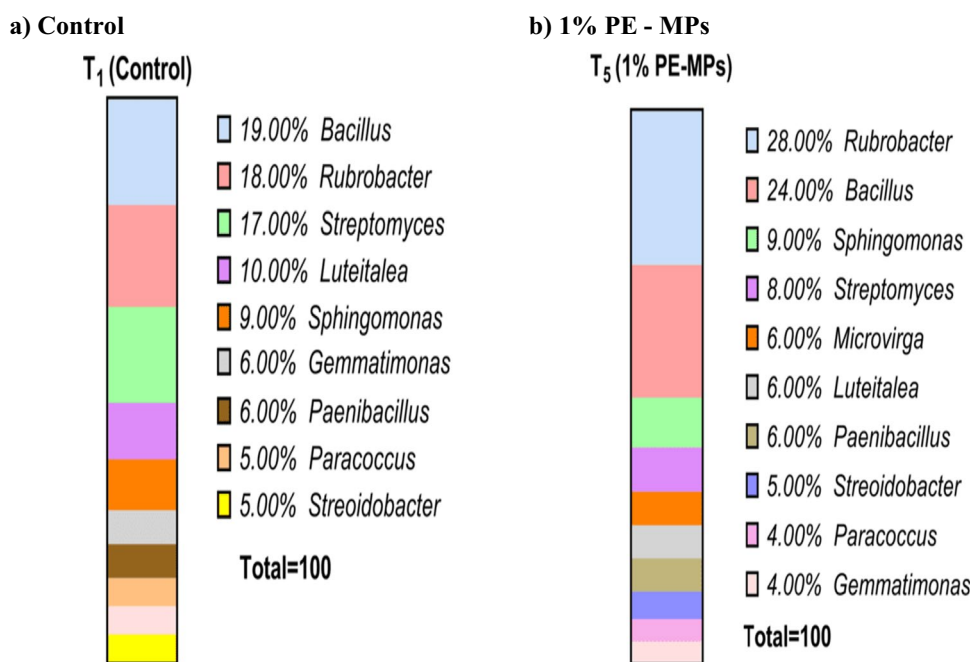
*Cyanobacteria* (0.27–0.31%), and others (0.33–0.52%). The addition of 1% PE-MPs resulted in higher abundance of *Proteobacteria*, *Actinobacteria*, and *Firmicutes* compared to control. The results of statistical analysis suggested no significant differences in the bacterial community composition at phylum level between control and soil with 1% PE-MPs, except *Proteobacteria* and *Firmicutes*.

At genus level, *Bacillus* (19%) was predominant in control (*T*<sub>1</sub>), while in 1% PE-MPs (*T*<sub>5</sub>), *Rubrobacter* (28%) community was dominant. *Rubrobacter* is a radio-tolerant group and is involved in organic matter decomposition. Similar results regarding its increase in abundance was also reported when exposed to polypropylene carbonate incorporated soil (Liang et al. 2022). However, the reason for increase in its abundance is not yet clearly known. *Microvirga* was found exclusively in *T*<sub>5</sub>, while the relative abundance of *Gemmatimonas* declined from *T*<sub>1</sub> to *T*<sub>5</sub> (Fig. 8). With *Microvirga* being root nodulating bacteria, the root nodulation frequency must have increased in plants grown in *T*<sub>5</sub> soil; however, the opposite effect was observed in the current study. The cause for this has to be explored further in future through studies

with specific focus on root nodulating microbial groups and microplastics. On further examination, it was observed that the relative abundance of *Streptomyces* genus also declined from *T*<sub>1</sub> to *T*<sub>5</sub>. This might be due to oxidative damage caused by microplastics in *Streptomyces* which is a gram-positive bacterium with no extracellular polymeric substances surrounding plasma membrane to protect against microplastics (Liu et al. 2021). The community heterogeneity ( $\alpha$ -diversity) in each treatment was measured using Shannon–Wiener *H* index and Simpson *D* index. The results suggest that control (*T*<sub>1</sub>) had higher relative genus richness ( $H=2.73$ ;  $D=0.243$ ), compared to rhizosphere soil with 1% PE-MPs ( $H=2.42$ ;  $D=0.277$ ). Whereas, the beta-diversity (measured by Sorenson’s co-efficient) was 0.867 which suggests that the communities overlap by more than 80% with slight differences.

Microplastics can influence soil microbial community by acting as a distinct habitat for microbial enrichment and colonization (Zhang et al. 2019b; Ren et al. 2020; Seeley et al. 2020; Qiang et al. 2023). In the current study, minor significant differences in alpha-diversity (Shannon index, *T*<sub>1</sub> — 2.72 and *T*<sub>5</sub> — 2.43) and community structure were observed between

**Fig. 8** Genus level abundance of microbes in rhizospheric soil exposed to PE-MPs



control and 1% PE-MPs as discussed above. These results were similar to the observations of Qi et al. (2020) who suggested that the effect of LDPE-MPs on bacterial community composition was relatively lower than bio-degradable microplastics. This is because bio-degradable microplastics after their degradation in soil act as carbon or in some cases nutrient sources for rhizosphere microbial communities (Qi et al. 2020); while PE-MPs although add to soil organic carbon, the carbon is inert and might not be available for microbes for their metabolism. The minor changes observed between control and 1% PE-MPs could be either as a result of changes in other soil parameters like soil bulk density and porosity brought about by PE-MPs or PE-MPs acting as habitat for specific community of bacteria (i.e., *Proteobacteria* and *Firmicutes*). Furthermore, rhizosphere soil from 1% PE-MPs was observed to have *Escherichia* and *Salmonella* which are pathogenic organisms as confirmed in previous studies (Wu et al. 2019; Kaur et al. 2021) which suggested that microplastics act as carrier of pathogenic microbes. Also, control soil had 57 unique microbial groups, while  $T_5$  had 21 exclusive microbial groups. Therefore, the hypothesis that microplastics in soil could alter microbial composition holds true as results of the metagenomics analysis suggest that relative richness of genus was affected by addition of PE-MPs, while the abundance of specific groups of microbes was observed to increase.

## Conclusion

Microplastics have been reported to significantly alter soil properties thereby affecting the growth and yield of agricultural crops. The outcomes of current study suggest that constant contact between plant and microplastics in soil would inevitably alter the plant growth either directly or indirectly. Amongst various concentrations under study, the maximum phytotoxic effect was observed when exposed to 1% PE-MPs. Hence, it is imperative to understand that the sensitivity of plants to microplastics might be dependent on crop growth stage as observed in the current study; wherein, higher impact was found during vegetative stage in most of the plant traits. Moreover, the effects of microplastics on different plant traits could be insignificant, mild, significant, or very severe. For instance, in this study, the impact of PE-MPs on root nodulation was significant, while the impact on other plant growth traits were mild and yield was unaffected. Although the yield was not affected as much as physiological and biochemical functions of blackgram, there could be compromise in the seed quality. Hence, in the future studies, along with plant growth traits, the changes in yield quality parameters due to microplastics have to be examined. Furthermore, it is also essential to compare the

sensitivity of every crop to microplastics in soil, establish sensitivity index which would aid in crop selection if an agriculture land is heavily polluted with microplastics.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11356-023-30550-4>.

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**Author contribution** Raveendra Gnana Keerthi Sahasa: writing original draft, conceptualization and editing; Periyasamy Dhevagi: conceptualization, methodology, supervision and editing; Ramesh Poornima: data curation, writing original draft and editing; Ambikapathi Ramya: conceptualization, data curation and editing; Subburamu Karthikeyan: conceptualization, supervision and editing; Sengottaiyan Priyadharshini: data curation and editing.

**Data availability** Not applicable.

## Declarations

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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