Environmental Research 204 (2022) 111938

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Effect of different polymers of microplastics on soil organic carbon and nitrogen – A mesocosm experiment

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ARTICLE INFO

Keywords:
Microplastics
Low-density polyethylene
PBAT+PLA
Soil nitrogen
Dissolved organic carbon (DOC)
Permanganate oxidizable carbon (POXC)

ABSTRACT

Agricultural microplastic pollution has become a growing concern. Unfortunately, the impacts of microplastics (MPs) on agricultural soil carbon and nitrogen dynamics have not been sufficiently reported. In an attempt to remedy this, we conducted a 105-day out-door mesocosm experiment in a soil-plant system using sandy soils amended with two types of MPs, low-density polyethylene (LDPE-MPs) and biodegradable (Bio-MPs), at concentrations of 0.0% (control), 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w, weight ratio of microplastics to air-dry soil). Soil organic matter (SOM), dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), available nitrogen (AN) of N-NH_4^+ and N-NO_3^-, and dissolved organic nitrogen (DON) were measured on day 46 (D46) and 105 (D105) of the experiment. SOM was also measured after microplastics were mixed into soils (D0). For LDPE-MPs treatments, SOM on D0, D46 and D105 showed no significant differences, while for Bio-MPs treatments, SOM significantly ($p < 0.05$) decreased from D0 to D46. Compared to the control, soil POXC was significantly ($p = 0.001$) lowered by 0.5%, 1.0% and 2.5% LDPE-MPs and $\geq$ 1.0% Bio-MPs on D105. LDPE-MPs showed no significant effects on soil DOC and nitrogen cycling. 2.0% and 2.5% Bio-MPs showed significantly higher ($p < 0.001$) DOC and DON (on D46 and D105) and $\geq$1.5% Bio-MPs showed significantly lower ($p = 0.02$) AN (on D46). Overall, Bio-MPs exerted stronger effects on the dynamics of soil carbon and nitrogen cycling. In conclusion, microplastics might pose serious threats to agroecosystems and further research is needed.

1. Introduction

Microplastics (MPs) are plastic particles with a diameter <5 mm. MPs pollution in the agroecosystem has received increasing attention globally (Andrady, 2017; Barnes et al., 2009; Qi et al., 2020a). Mounting evidence has shown that agricultural soils receive microplastics in various ways. For example, a field survey conducted in four different agricultural areas in southwestern China, where plastic mulching and sewage sludge was applied to agricultural fields, found MPs ranging between 7100 and 42,960 particles·kg^{-1} soil (Zhang and Liu, 2018). Corradini et al. (2019) found microplastic accumulation in agricultural fields that received sewage sludge irrigation. Inappropriate disposal of conventional plastic mulching films (low density polyethylene, LDPE) has been identified as one of the major contributors to agricultural microplastic pollution (Huang et al., 2020). To combat the growing plastic pollution caused by LDPE films used in agriculture, biodegradable (Bio) plastic mulches were developed as alternative solutions. However, recent research has suggested that most biodegradable materials currently available on the market tend to break down into smaller plastic particles rather than completely biodegrade, which leads to the accumulation of bio-microplastic in soils (de Souza Machado et al., 2018a; Li et al., 2014). Therefore, considering that agricultural microplastic pollution is likely to continue to be a problem in the future, uncovering the impacts of microplastics in agricultural soils deserves more attention.

The accumulation of microplastics in soil profiles could affect soil physical, chemical and biological processes (Iqbal et al., 2020; Ng et al., 2018). Numerous studies have shown that microplastics can significantly alter soil porosity, bulk density, water holding capacity and soil water repellency (de Souza Machado et al., 2018b; Qi et al., 2020b). In

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https://doi.org/10.1016/j.envres.2021.111938
Received 15 June 2021; Received in revised form 16 August 2021; Accepted 20 August 2021
Available online 31 August 2021
0013-9351/

addition, the small size and large specific area of microplastics allow them to interact with the soil microbiome, affecting the soil microbial community and nutrient dynamics (Fei et al., 2020; Torres et al., 2021). A study from Liu et al. (2017) found that 28% polypropylene (PP) MPs stimulated the soil microbial activity and enhanced decomposition of organic matter while also suppressing the accumulation of soil available nitrogen content. The suppressive effects of microplastics on nitrification and denitrification processes have also been observed in other ecosystems. Seeley et al. (2020) conducted an incubation experiment in a sedimentary system and found that polyvinylchloride (PVC)-MPs and PLA-MPs can alter the microbial community composition, inhibit sediment nitrification and denitrification processes and lower the content of available nitrogen. Although many efforts have been devoted to study the effects of microplastics on terrestrial ecosystems, the effects of microplastics on the dynamics of nitrogen in soil-plant systems remains largely unknown (de Graaff et al., 2010; Li et al., 2016).

Another concern is the effect of microplastics on the soil organic pool (Rillig, 2018; Rillig et al., 2021). Owning to the carbon-based composition, microplastics might have already made hidden contributions to current carbon storage (Rillig, 2018). Until now, however, the effects of microplastics on the soil organic matter (SOM) pool have only received limited attention (Zhang and Zhang, 2020). Soil labile organic carbon and nitrogen are sensitive and play important roles in soil ecosystem functions (Blanco-Moure et al., 2016; Muqaddas et al., 2019). For example, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) are more sensitive to soil microbial activity than total SOM (Bongiorno et al., 2019; Straathof et al., 2014). DOC and DON are small soluble fractions of SOM that mainly originate from the exudates of root and soil microorganisms. Soil permanganate oxidizable carbon (POXC) is mainly composed of polysaccharides and lignin originating from SOM decomposition and has been found to be closely related to soil microbial biomass and soil phospholipid fatty acid (Bongiorno et al., 2019; Jokela et al., 2009; Weil et al., 2003). As such, considering the current knowledge gaps in the effects of microplastics on soil fertility, a detailed study of the effects of microplastics on the dynamics of soil labile organic carbon and nitrogen cycling is necessary.

In our previous study, we observed that the occurrence of MPs in soil-plant systems alters the common bean growth (Meng et al., 2021). We speculated that the responses of common bean growth might be related to soil nutrient dynamics. Therefore, the general objective of this study was to investigate the effect of MPs on soil nutrient dynamics in a soil-plant system. Specifically, we measured soil labile C and N pools as indicated by (i) soil dissolved carbon and nitrogen (DOC, DON) and soil POXC; as well as (ii) available nitrogen content of soil N-NH_4^+ and N-NO_3^- in an outdoor mesocosm experiment that used two types of microplastic polymers: low-density polyethylene (LDPE-MPs) and biodegradable plastic of PBAT mixed with PLA (Bio-MPs). We hypothesized that both LDPE-MPs and Bio-MPs could affect the dynamics of soil labile carbon and nitrogen fractions, and that Bio-MPs would have stronger impacts than LDPE-MPs. The findings of this study will provide basic information for understanding the interactive effects of MPs and soil-plant systems.

2. Materials and methods

2.1. Experimental setup and soil sampling

2.1.1. Experiment setup

An outdoor net house mesocosm experiment was conducted (the side length of each square mesh was 0.25 mm) at Unifarm at Wageningen University & Research (WUR, the Netherlands) from the June 28, 2019 until the October 18, 2019. Sandy soil with 87% sand, 12% silt and 1% clay, and an organic matter content of 4% and an organic carbon content of 2% was used (more details on Supplementary Table S1). Common bean (*Phaseolus vulgaris* L.; *Cultivar: Bruine Noordhollandse, P.vulgaris*) was selected as the model plant. The microplastics used in the research were LDPE-MPs and Bio-MPs. LDPE-MPs is obtained from Agro-technolody & Food Science group of Wageningen University. Bio-MPs is 10 % Polylactic acid (PLA), 85 % polybutylene adipate terephthalate (PBAT), 5 % calcium. The raw pellets of LDPE and biodegradable materials were first frozen with liquid nitrogen and then ground using a grinding machine into microplastic particles (MPs). The resulting MPs were then categorised into 5 size groups of < 53 μm, 53–125 μm, 125–250 μm, 250–500 μm and 500–1000 μm by using steel sieves. The MPs used in this experiment were compromised of 250–500 μm (60 %) and 500–1000 μm (40%). These two size categories were chosen based on previous published research (Scheurer and Bigalke, 2018; Zhang and Liu, 2018). The ratio was chosen to simulate the heterogeneity of sizes of MPs in terrestrial ecosystems. The MPs used in current research have the shape of partly round (with edges and angular). The shape, particle number and flourier transform infrared spectroscopy (FTIR) is shown in supplementary files (Fig. S1). The mesocosm experiment consisted of 11 treatments including a control treatment (CON) with only sandy soil and sandy soils polluted with two types of microplastics in five different doses, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w, weight ratio of microplastic to air-dry soil). There were 8 replicates for each treatment.

To achieve the target doses of soil-MPs mixtures for each treatment, 50 kg of homogenized air-dried sandy soil was manually mixed with the target amount of MPs (0.25 kg, 0.50 kg, 0.75 kg, 1.00 kg and 0.25 kg) in an iron tank using a wooden stick for 10 min. 6 kg of the homogeneous soil-MPs mixture was then placed in a 7 L polypropylene (PP) pot (21 cm high, 16 cm bottom diameter and 21 cm top diameter). The rest of the soil-MPs mixtures were stored for initial soil sample measurements for the soil organic matter (SOM). The cultivation of the plants followed the same protocols as previously described (Meng et al., 2021). Two types of nutritive solutions were applied. At week 4 (26th of July) and 5 (2nd of August), 100 mL of Tomaat-N nutritive solution (Supplementary Table S2) was added to each pot. From the 6th to the 12th week, 100 mL of Hoagland 2.0 nutritive solution (Supplementary Table S2) was added to each pot once a week to ensure full development. Tomaat-N nutritive solution contained 1/3 of the nitrogen of the Hoagland 2.0, which served as a starter nutrient solution to initiate early growth of common bean (Chekanai et al., 2018). The nutritive solutions were prepared by Wageningen Unifarm. The PP pots used in the experiment were resistant and did not degrade during such a short time (105-day) of use. All treatments were treated in the same way. Hence, cross contamination could be ignored.

2.1.2. Soil sampling

Soil samples were collected twice. The first time was on the August 15, 2019, 46 days after seeding (D46), near the end of the vegetative stage when the plant roots and leaves completed the early development stage. The second sampling was carried out on the October 18, 2019 (105th days, D105), after plants were harvested. For each sampling time, four pots were harvested per treatment and plants were completely removed from the pots. Soil mass from each pot was thoroughly mixed. For each pot, 5 subsamples (50–60 g/per sample) of bulk soil were randomly collected and mixed to form a composite sample. The soil samples were air-dried and passed through a 2 mm steel sieve for measuring SOM, soil permanganate oxidizable carbon (POXC), available nitrogen (AN), including nitrate nitrogen (N-NO_3^-) and ammonium nitrogen (N-NH_4^+), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON) and DOC (All abbreviations are shown in Table 1).

2.2. Soil physiochemical parameter measurements

2.2.1. SOM content

SOM was measured following the loss on ignition (LOI) method. The method has long been used to estimate SOM content (Howard and Howard, 1990; Nakhli et al., 2019). First, the empty crucible cups were

Table 1
Abbreviations of measured soil parameters.

Abbreviation	Measured parameters
SOM	Soil organic matter
DOC	Dissolved organic matter
POXC	Soil permanganate oxidizable carbon
TDN	Soil total dissolved nitrogen
$N–NH_4^+$	Soil ammonium nitrogen
$N–NO_3^-$	Soil nitrate nitrogen
NO_3^-/NH_4^+	Ratio of nitrate nitrogen to ammonium nitrogen
AN	Available nitrogen, total amount of ammonium nitrogen and nitrate nitrogen
AN%	The proportion of AN to TDN
DON	Soil dissolved organic nitrogen
TC/TDN	Ratio of total dissolved carbon (TC) to TDN

placed into a 95 °C muffle furnace for 1 h and were cooled to room temperature and weighed (METTLER AE 200, METTLER AE 200, MARSHALL SCIENCE, accuracy of 0.1 mg). Then, 6.0 g of the air-dried soil samples were weighed into crucible cups and dried at 105 °C in a muffle furnace for 24 h to a constant weight. After oven-drying, the cups were then placed into a 550 °C muffle furnace for 4 h to combust the organic matter.

$$SOM = \frac{W2 - W3}{W2 - W1} \times 100\%$$

where: ***W1*** = the weight of each crucible cup; ***W2*** = total weight of crucible cup and soil after dried at 105 °C in a muffle furnace for 24 h; ***W3*** = total weight of crucible cup and soil after placed at 550 °C in a muffle furnace for 4 h.

2.2.2. SOM dynamics

SOM was measured 3 times, after initial mixing (0 day, D0) and at sampling times D46 and D105. Considering that there was no external organic matter added to the soil-plant systems during the growing period of *P.vulgaris* (apart from the decayed plant roots), we compared the SOM across D0, D46 and D105 for each treatment using one-way ANOVA (Detailed in data analysis section). For the treatments where SOM showed significant differences among D0, D46 and D105, the dynamics of SOM were calculated as follows:

$$SOM\ dynamic(D0\ to\ D46) = \frac{(SOM_{D46} - SOM_{D0})}{SOM_{D0}} \times 100\%$$

$$SOM\ dynamic(D46\ to\ D105) = \frac{(SOM_{D105} - SOM_{D46})}{SOM_{D46}} \times 100\%$$

$$SOM\ dynamic(D0\ to\ D105) = \frac{(SOM_{D105} - SOM_{D0})}{SOM_{D0}} \times 100\%$$

where: $\boldsymbol{SOM_{D0}}$ = SOM at day 0; $\boldsymbol{SOM_{D46}}$ = SOM at the end of vegetative stage; $\boldsymbol{SOM_{D105}}$ = SOM after harvest. The carbon dynamic was calculated by using the mean value of the replicates and is referred to as a minimum estimation thus, no statistical comparison was applied.

2.2.3. Soil permanganate oxidizable carbon (POXC)

Soil POXC was measured using the adaption method of Weil et al. (2003). The procedure followed Bongiorno et al. (2019). Specifically, 2.5 g (accuracy of 0.1 mg) of the air-dried soil sample was weighed into a 50 mL polypropylene tube. 18 ml of demineralized water and 2 ml of 0.2 mol·L^{-1} $KMnO_4$ was added to each tube. The tubes were vigorously shaken by hand for 30s and then shaken at 120 rpm for 2 min. After shaking, the tubes were placed in a dark cabinet to settle for 8 min while the $KMnO_4$ continued to react with the soil. Then, 0.5 ml of the supernatant solution from a tube was transferred into a second tube as soon as possible and diluted with 49.5 ml of demineralized water. The second tube was inverted to mix the final solution sample. Soil POXC was determined by measuring the absorbance of the sample solution at 550 nm (Abs) in a spectrophotometer (Abs, GENESYS 10S UV–VIS Spectrophotometer). Soil POXC was calculated using the following equation:

$$POXC\ mg{\cdot}kg^{-1} = \left[0.02mol{\cdot}L^{-1} - (a + b \times Abs)\right] \times \left(9000mg\ C{\cdot}mol^{-1}\right) \times \left(0.02\ L\ solution\ Wt^{-1}\right)$$

where: 0.02 mol·L^{-1} = initial concentration of the $KMnO_4$ solution; a = intercept of the standard calibration curve; b = the slope of the standard calibration curve; Abs = the absorbance of final sample solution; 9000 mg = the amount of carbon oxidized by 1 mol of MnO_4^- changing from Mn^{7+} to Mn^{2+}; 0.02 L = the volume of the $KMnO_4$ reacting with the samples; Wt = weight of air-dried soil sample (kg).

As a quality control measure, each set of soil samples (10) contained two blank samples of distilled water and two standard soil samples (ISE-989, International Soil-Analytical Exchange). This measurement was used to account for any contamination which could have occurred inside the lab.

2.2.4. Soil carbon and nitrogen analysis

Soil N-NH_4^+, N-NO_3^-, TDN, soil inorganic carbon (IC) and total dissolved carbon (TC) were measured using the Segmented flow analyser system (SKALAR). Quality control using blank samples of distilled water and a standard soil sample (ISE-989, International Soil-Analytical Exchange) was also included. Soil available nitrogen (AN) and its percentage of TDN (AN%), dissolved organic nitrogen (DON) and DOC were calculated as follows:

$$AN = \left(N - NO_3^-\right) + \left(N - NH_4^+\right)$$

$$AN\% = AN/TDN{*}100\%$$

$$DOC = TC - IC$$

$$DON = TDN - AN$$

2.2.5. Data and correlation analysis

All the collected data were checked for normality with Q-Q plots and the Shapiro-Wilk test and checked for homogeneity of variances with Levene's test to meet the assumptions for ANOVA. To meet the requirement of the assumptions for ANOVA, the transformation of some data was performed. Specifically, once the assumptions were met with the raw data, the difference in soil properties were tested with two independent one-way ANOVAs (LDPE-MPs and Bio-MPs) with the factor of microplastic concentration. When the significance level of $p < 0.05$ was met, a post-hoc test using the least significant difference method (LSD) at 95% confidence level was carried out. In the cases where the assumptions were not met, data were transformed using the square root and checked again following the method above. If the assumptions were not met after this transformation, a non-parameter analysis of Kruskal-Wallis *H* test with pairwise comparison was carried out. The results of one-way ANOVA are shown in Supplementary Table S3. Statistical analysis of current research was carried out using SPSS version 23.0 (SPSS Incorporated, USA) and results are presented as "mean ± standard deviation" (Supplementary Table S4). Comparisons between LDPE-MPs and Bio-MPs were performed using the Independent-Samples *t*-Test and Manny-Whitney *U* test (Supplementary Table S5). All figures were generated using Microsoft Excel 365.

To identify the relationships between the soil properties and microplastics, soil properties at the vegetative stage were subjected to correlation analysis (CA) and redundancy analysis (RDA). Firstly, a correlation analysis was performed to exclude the collinear soil properties (Pearson correlation > 0.9). According to the CA results (Supplementary Table S6), AN% had a high collinearity with DON%. Since AN% correlated strongly with other properties, it was used for the further analysis while DON% was removed. $N–NO_3/N–NH_4$, TC and TC/

TDN were removed for the same reason. For the remaining soil properties, we used RDA to identify the relationships among soil properties and experimental treatments (different microplastic types and doses). RDA was performed using CANOCO 5.

3. Results

3.1. Dynamics of soil organic matter

The SOM of all treatments were measured on D0, D46 and D105 (Table 2). Compared with the control treatment, for every measured time point, the addition of LDPE-MPs and Bio-MPs linearly increased SOM with the increasing MPs doses, significant differences ($p < 0.05$) were observed between each microplastic dose and the control treatment.

We also compared the SOM dynamics throughout D0, D46 and D105 for each treatment. For the control treatment, SOM on D0 and D46 showed no significant difference, which was significantly lower than on D105 ($p < 0.001$). For LDPE-MPs, SOM across D0, D46 and D105 showed no significant differences in treatments of 0.5%, 1.0%, 1.5% and 2.5% LDPE-MPs. Only for the 2.0 % LDPE-MPs treatment was SOM on D105 (57.6 mg kg^{-1}) significantly higher ($p < 0.05$) than on D0 (3.34%) and on D46 (3.54%). For Bio-MPs treatments, SOM on D0 was significantly higher ($p < 0.05$) than on D46, ranging between 2.96% and 4.41% (Table 2). From D46 to D105, SOM showed significant increments in 0.5%, 1.0% and 1.5 % Bio-MPs treatments, while no significant difference was observed for 2.0 % and 2.5 % Bio-MPs treatments.

Table 2
Soil organic matter content (mg·kg^{-1}) at different sampling time after expose to different types of microplastics with L. Phaseolus vulgaris.

Treatment	D0	D46	D105	C dynamic (%)	C dynamic (%)	C dynamic (%)
				D46-D0	D105-D46	D105-D0
CON	40.2 ± 1.35 f,B	39.3 ± 0.52 f, B	43.3 ± 0.69 f,A	nd	10.0	7.78
LDPE-0.5	45.5 ± 1.98 e	45.9 ± 1.33 e	45.4 ± 1.45 e	nd	nd	nd
LDPE-1.0	48.2 ± 1.16 d	48.1 ± 1.35 d	47.5 ± 0.77 d	nd	nd	nd
LDPE-1.5	52.2 ± 1.99 b	51.9 ± 1.02 c	52.3 ± 1.00 c	nd	nd	nd
LDPE-2.0	55.6 ± 1.15 b B	55.7 ± 1.90 b,B	57.6 ± 0.68 b, A	nd	3.34	3.54
LDPE-2.5	59.5 ± 4.50 a	60.5 ± 1.37 a	61.6 ± 1.90 a	nd	nd	nd
Bio-0.5	44.1 ± 1.33 e,A	42.6 ± 0.77 e,B	44.8 ± 0.94 e,A	−3.30	5.01	nd
Bio-1.0	46.4 ± 1.25 d,A	44.7 ± 0.54 d,B	46.4 ± 0.72 d, A	−3.72	3.86	nd
Bio-1.5	49.0 ± 1.98 c,B	47.4 ± 1.32 c, C	50.6 ± 0.98 c,A	−3.31	6.70	3.16
Bio-2.0	53.5 ± 1.82 b,A	51.9 ± 1.31 b,B	51.5 ± 1.16 b, B	−2.96	nd	−3.60
Bio-2.5	56.2 ± 2.46 a,A	53.7 ± 0.92 a,B	53.8 ± 2.18 a,B	−4.41	nd	−4.23

Note. Lowercase letters (a, b, c, d) within the same column mean significant differences among MPs doses in each sampling time; capital letters (A,B,C) within the same row mean significant differences in each individual treatment throughout D0, D46 and D105. **nd** means not detected.

3.2. Impacts of MPs on soil DOC and POXC

The effects of LDPE-MPs and Bio-MPs on soil DOC and POXC are shown in Fig. 1. For soil DOC, as compared to the control treatment (137 mg·kg^{-1} on D46 and 115 mg·kg^{-1} on D105), the addition of LDPE-MPs showed no significant effects on DOC on either D46 (Fig. 1A) or D105 (Fig. 1B, Supplementary Table S3 and Table S4). As for Bio-MPs, the addition of 2.0% and 2.5% Bio-MPs measured significantly higher ($p < 0.05$) DOC on D46 (153 mg·kg^{-1} and 159 mg·kg^{-1}) and D105 (137 mg·kg^{-1} and 148 mg·kg^{-1}) (Fig. 1A and B and Supplementary Table S4).

In terms of soil POXC, as compared to the control treatment (585 mg·kg^{-1} on D46 and 610 mg·kg^{-1} on D105), on D46, the addition of LDPE-MPs and Bio-MPs showed no significant effects on soil POXC (Fig. 1C and D). On D105, in general, the addition of LDPE-MPs and Bio-MPs led to lower POXC values, except for 0.5% Bio-MPs, which was slightly higher than the control but showed no significant difference (Fig. 1D). Significant differences ($p < 0.05$) were observed for LDPE-MPs treatments of 0.5% (570 mg·kg^{-1}), 1.0% (550 mg·kg^{-1}) and 2.5% (575 mg·kg^{-1}) and Bio-MPs treatments of 1.0% (552 mg·kg^{-1}), 1.5% Bio-MPs (572 mg·kg^{-1}), 2.0% Bio-MPs (540 mg·kg^{-1}), and 2.5% Bio-MPs (567 mg·kg^{-1}).

3.3. Impacts of MPs on soil nitrogen cycling and TC/TDN

Soil AN (including N-NH_4^+ and N-NO_3^-) and its proportion to TDN (AN%), DON, the ratio between nitrate and ammonium (NO_3^-/NH_4^+), and TC/TDN were measured in soil on D46 (Fig. 2) and D105 (Fig. 3). On D46, soil N-NH_4^+, N-NO_3^-, NO_3^-/NH_4^+, DON, AN% and TC/TDN in the control treatment were 3.55 mg·kg^{-1}, 1.30 mg·kg^{-1}, 0.37, 8.15 mg·kg^{-1}, 37.2% and 10.7, respectively. On D105, soil N-NH_4^+, N-NO_3^-, NO_3^-/NH_4^+, DON, AN% and TC/TDN in the control treatment were 8.43 mg·kg^{-1}, 5.70 mg·kg^{-1}, 0.83, 7.13 mg·kg^{-1}, 65.9% and 5.55, respectively (More details shown in Supplementary Table S4).

The addition of LDPE-MPs showed no significant ($p > 0.05$) effects on measured soil nitrogen cycling indicators (Figs. 2 and 3), except for soil N-NH_4^+ on D46 (Fig. 2A). However, on D46, we observed that the addition of LDPE-MPs led to a slight accumulation of N-NH_4^+, N-NO_3^- and NO_3^-/NH_4^+ from 0.5% to 1.0% and then dropped at > 1.0% LDPE-MPs doses (Fig. 2A), while there were no significant differences found.

As for Bio-MPs, on D46, the addition of Bio-MPs significantly ($p < 0.05$) affected all the measured indicators except for soil N-NH_4^+ (Fig. 2). Overall, as compared to the control, soil N-NO_3^- (Fig. 2B), NO_3^-/NH_4^+ (Fig. 2C) and AN% (Fig. 2E) showed a decreasing trend with the increasing Bio-MPs doses, while TC/TDN showed a rising trend. Significant differences ($p < 0.05$) were observed at 1.5% and 2.5% Bio-MPs for soil N-NO_3^-; 2.5 % Bio-MPs for soil N-NH_4^+, ≥ 1.5% for AN% and ≥ 1.5% for TC/TDN. DON was significantly higher in 2.5 % (Figs. 2 and 3 and Supplementary Table S4.4). While on D105, the addition of Bio-MPs only significantly ($p < 0.05$) affected soil N-NO_3^- and DON (Fig. 3). Significant differences ($p < 0.05$) were observed at 0.5%, 2.0% and 2.5% Bio-MPs for N-NO_3^- and 2.0% and 2.5% for DON (Fig. 3B and D).

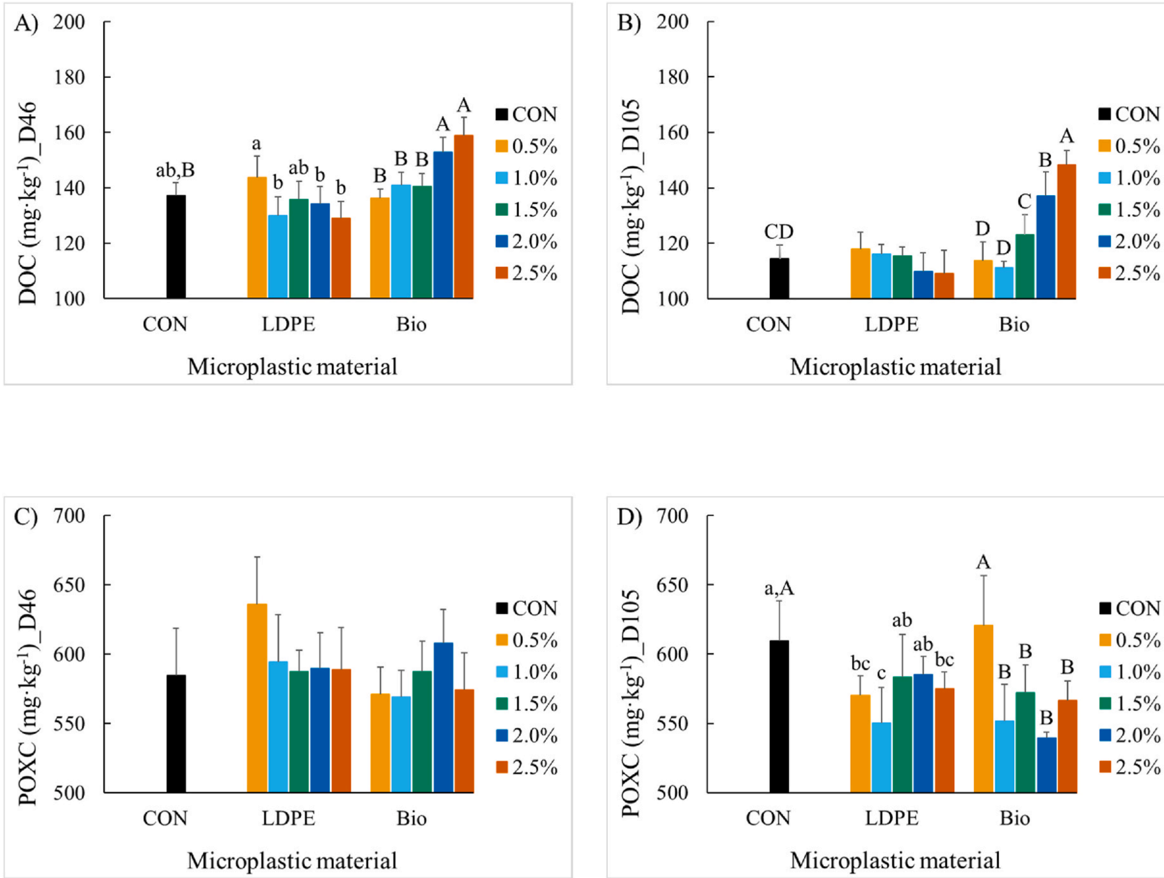

Fig. 1. Effects of LDPE-MPs and Bio-MPs at increasing concentrations on the labile carbon fraction on the end of the vegetative stage (D46) and fully mature stage (D105). (A) dissolved organic carbon (DOC) on D46; (B) dissolved organic carbon (DOC) on D105 (C) permanganate oxidizable carbon (POXC) on D46; (D) permanganate oxidizable carbon (POXC) on D105. Error bars are standard deviation (SD). CON (black column) is the control treatment, 0.5 (yellow column), 1.0 (light blue column), 1.5 (green column), 2.0 (blue column) and 2.5 (orange column) are the weight percentage of microplastic to dry soil weight. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP doses and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP doses and the control treatment. Data were plotted as "Mean ± SD". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Comparison of the effects of LDPE-MPs and Bio-MPs on soil labile carbon and nitrogen

The impacts of LDPE-MPs and Bio-MPs on soil physiochemical properties were compared using the Independent-Samples *t*-Test (Supplementary Table S5). Overall, as compared to LDPE-MPs, Bio-MPs showed significantly lower ($p < 0.05$) SOM and significantly higher ($p < 0.05$) soil DOC at 2.0% and 2.5% doses. LDPE-MPs and Bio-MPs showed no significant differences in terms of soil POXC, except on D46 where LDPE-MPs were significantly higher than Bio-MPs for the 0.5 % dose. On D105, the Bio-MPs were significantly higher than the LDPE at the 0.5% dose while LDPE-MPs were significantly higher than Bio at the 2.0% dose. For nitrogen cycling, as compared to LDPE-MPs, Bio-MPs showed significantly lower N-NH_4^+ and AN% for the 1.0 %–2.5% doses on D46, while it showed significantly higher DON at 2.0% (D46) and 2.5% (D46 and D105, more details showed in Supplementary Table S4).

3.5. Correlations of MPs to soil carbon and nitrogen

The relationships among the measured soil properties and common bean growth parameters are depicted in a redundancy analysis diagram (Fig. 4). The first four axes explain 52.4% of the variation according to the Monte Carlo permutation tests (Supplementary Table S7). In Fig. 4, soil AN, TDN and POXC values are on the left side of diagram while DOC, DON and DOC/DON are on the right side of the diagram. The treatments for the control, all LDPE doses and Bio-0.5 are found on the left side of the diagram while Bio-MPs treatments are on the right side. For LDPE-MPs, LDPE_1.5, LDPE_2.0 and LDPE_2.5 are close to each other and the control treatments, which can be found close to the origin point. LDPE_0.5 is positively correlated to POXC and LDPE_1.0 is positively correlated to AN%. Bio-MPs treatments, especially Bio_2.0 and Bio_2.5, lay in the positive direction of soil organic matter (DOC, DON and DOC/DON) and in the negative direction of AN (AN%, N-NO_3^- and N-NH_4^+) and TDN.

4. Discussion

4.1. Effects of microplastics on SOM

In the current study, the LOI method was applied to measure SOM. Because the carbon-base properties of the added LDPE-MPs and Bio-MPs, for all the treatments, the reported losses consisted of two fractions: SOM of the soil-plant system and added LDPE-MPs and Bio-MPs. According to our results, for each LDPE-MPs treatment, the loss mass across D0, D46 and D105 showed no significant difference among each other. This might be attributed to the chemical resistance of LDPE polymers. LDPE has a linear hydrocarbon structure, large molecular size, lack of functional groups and high hydrophobicity, which make this synthetic polymer quite resistant to degradation under natural field conditions (Contat-Rodrigo and Ribes Greus, 2002; Dilara and

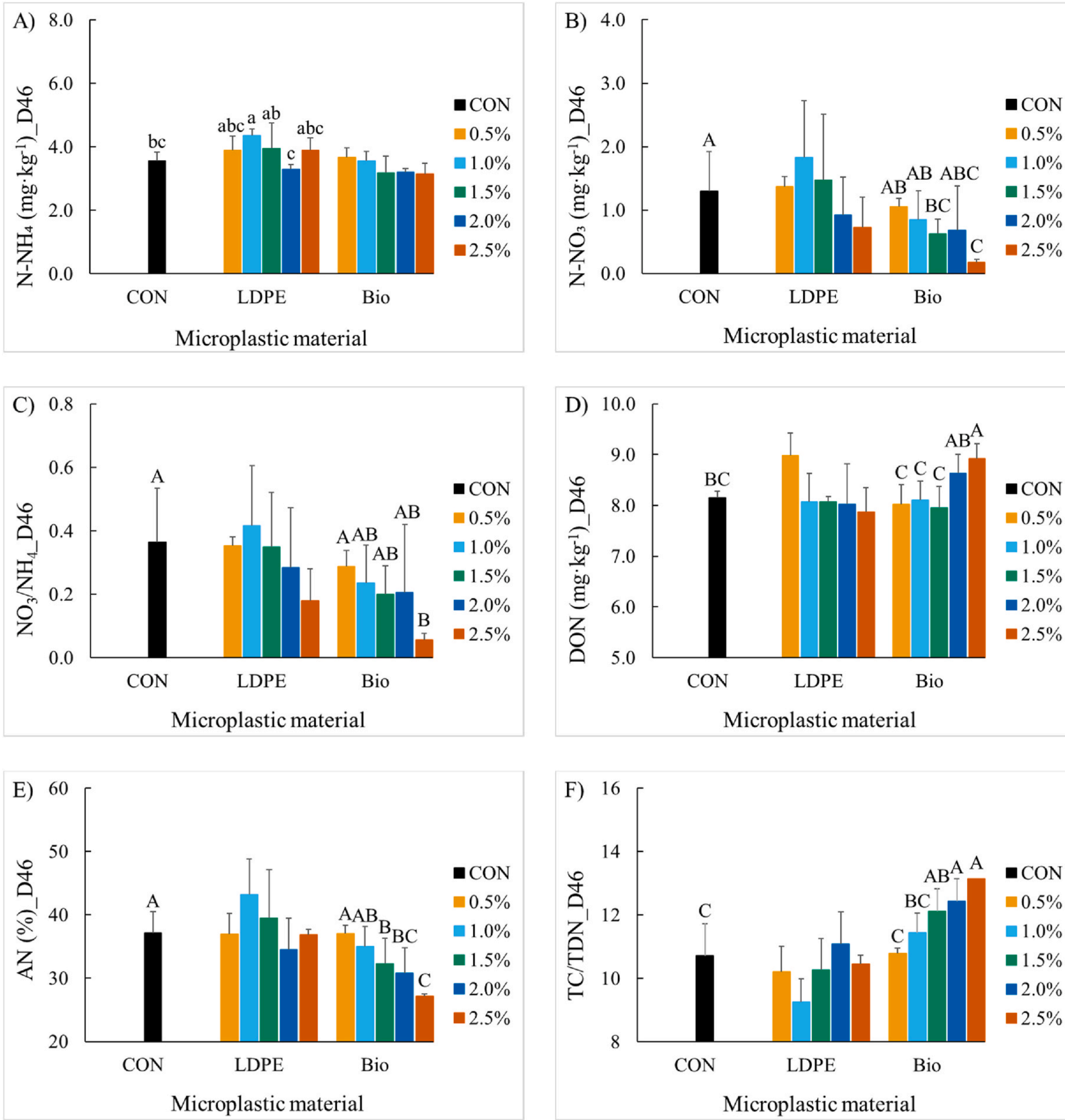

Fig. 2. Effects of LDPE-MPs and Bio-MPs at increasing concentrations on soil nitrogen and available phosphorus on the end of the vegetative stage (D46). (A) ammonium nitrogen (N-NH_4^+); (B) nitrate and nitrite nitrogen N-NO_3^-; (C) the ratio of nitrate and nitrite nitrogen to ammonium nitrogen NO_3^-/NH_4^+; (D) dissolved organic nitrogen (DON); (E) percentage of AN ($NO_3^- + NH_4^+$) to total dissolved nitrogen content (TDN); (F) ratio of total dissolved carbon (TC) to total dissolved nitrogen (TDN). Error bars are standard deviation (SD). CON (black column) is the control treatment, 0.5 (yellow column), 1.0 (light blue column), 1.5 (green column), 2.0 (blue column) and 2.5 (orange column) are the weight percentage of MPs to dry soil weight. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP doses and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP doses and the control treatment. No post-hoc was performed when $p > 0.05$ in ANOVA test. Data is shown as "Mean ± SD". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Briassoulis, 2000; Gajendiran et al., 2016; Miranda et al., 2020). As a result, the SOM of each LDPE-MPs treatment remained stable during the experiment. The mass loss for each Bio-MPs treatment significantly dropped from D0 to D46. This might be attributed to the biodegradation of Bio-MPs polymers. Bio-MPs applied in the current research contained heteroatomic polymers (i.e., PLA is an aliphatic polymer and PBAT is an aliphatic–aromatic polymer). Compared to LDPE, Bio-MPs presented low susceptibility to microbial attack and natural degradation (Palsikowski et al., 2017). This could account for the drop in mass losses between D0 and D46. However, considering the fact that the mass loss for each of the Bio-MPs treatments was still significantly higher compared to the control treatment on both D46 and D105, we assumed that the biodegradation of Bio-MPs was incomplete. In addition, we observed that mass losses of 0.5%, 1.0% and 1.5% Bio-MPs on D105 were significantly higher than on D46, while for 2.0% and 2.5% Bio-MPs, mass losses on D105 and D46 showed no significant differences. One possible explanation for this might be the decayed roots. Our previous paper showed that Bio-MPs on D46 led to significantly higher specific root length (SRL), while 0.5% and 1.0% showed higher root biomass and ≥ 1.5% showed lower root biomass (Meng et al., 2021). Our current results, coupled with previous findings of the effects of microplastics on plant growth, suggest that 0.5 %, 1.0 % and 1.5 % Bio-MPs might enhance the turnover of the roots, thus contributing the higher mass losses on D105 as compared to on D46. 2.0 % and 2.5% Bio-MPs

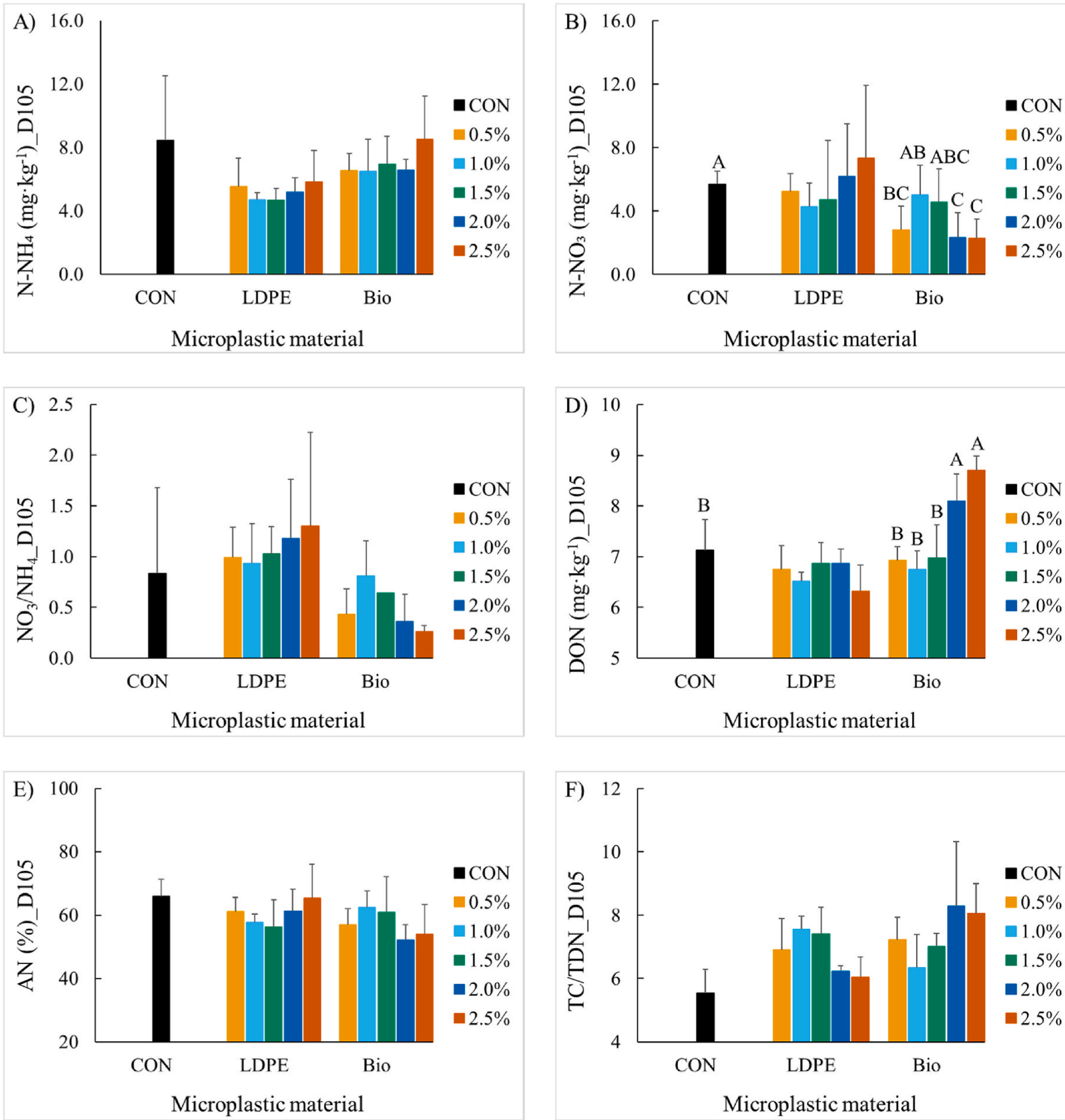

Fig. 3. Effects of LDPE-MPs and Bio-MPs at increasing concentrations on soil nitrogen and available phosphorus on the end of fully mature stage (D105). (A) ammonium nitrogen (N-NH_4^+); (B) nitrate and nitrite nitrogen N-NO_3^-; (C) the ratio of nitrate and nitrite nitrogen to ammonium nitrogen NO_3^-/NH_4^+; (D) dissolved organic nitrogen (DON); (E) percentage of AN ($NO_3^- + NH_4^+$) to total dissolved nitrogen content (TDN); (F) ratio of total dissolved carbon (TC) to total dissolved nitrogen (TDN). Error bars are standard deviation (SD). CON (black column) is the control treatment, 0.5 (yellow column), 1.0 (light blue column), 1.5 (green column), 2.0 (blue column) and 2.5 (orange column) are the weight percentages of MPs to dry soil weight. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP doses and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP doses and the control treatment. No post-hoc was performed when $p > 0.05$ in ANOVA test. Data is shown as "Mean ± SD". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exerted phytotoxicity to plants and supressed growth of common bean roots and as a result, roots failed to contribute the mass losses on D105 as compared to D46. These findings emphasize the importance of exploring the potential effects of microplastics on soil carbon cycling (Rillig, 2018; Rillig et al., 2021).

4.2. Response of labile fractions of soil organic matter to microplastics

According to our results, LDPE-MPs showed no significant effects on soil DOC and DON, while 2.0% and 2.5% Bio-MPs significantly increased soil DOC and DON. Previous research by Liu et al. (2017) found that 28% PP microplastic significantly stimulated the soil enzymatic activity and enhanced soil DOC concentration. However, the microplastic concentration used in Liu's study was much higher than our research, indicating that up to 2.5% LDPE-MPs were not strong enough to elicit soil DOC and nitrogen cycling. As for Bio-MPs treatments, we noticed that soil DOC and DON were significantly increased mainly by 2.0% and 2.5% Bio-MPs treatments. Our SOM results suggested that Bio-MPs could have experienced biodegradation. The Bio-MPs used in our study contained large amounts of labile carbon, which might account for the increased soil DOC in 1.5% (only on D105), 2.0% and 2.5% Bio-MPs treatments. However, the degradation did not significantly contribute to the DOC in 0.5% and 1.0% Bio-MPs treatments. Naturally, soil DOC and DON polymers were soluble fractions of decomposed SOM

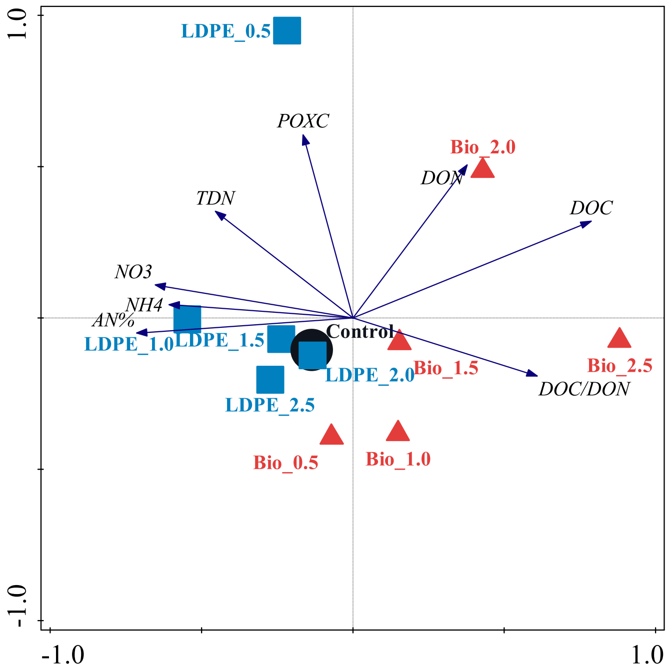

Fig. 4. Redundancy analysis ordination diagram of soil properties with treatment factors. LDPE-MPs treatments are indicated by the blue squares, Bio-MPs treatments are indicated by the red triangles, and the control treatment is indicated by the black circle. Soil properties are indicated by the arrows and the angles between the two arrows represent the correlations between each of the soil properties. The smaller the angle between two arrows, the stronger the correlation between the two corresponding parameters; the longer the arrow, the more important the corresponding properties are. The projected distances between the blue square/red triangles/black circle and the arrows represent the relative contribution of the treatment factors to the soil properties. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Pure soil: Control treatment;
LDPE_0.5: soil with LDPE microplastics of 0.5% w/w;
LDPE_1.0: soil with LDPE microplastics of 1.0% w/w;
LDPE_1.5: soil with LDPE microplastics of 1.5% w/w;
LDPE_2.0: soil with LDPE microplastics of 2.0% w/w;
LDPE_2.5: soil with LDPE microplastics of 2.5% w/w;
Bio_0.5: soil with biodegradable microplastics of 0.5% w/w;
Bio_1.0: soil with biodegradable microplastics of 1.0% w/w;
Bio_1.5: soil with biodegradable microplastics of 1.5% w/w;
Bio_2.0: soil with biodegradable microplastics of 2.0% w/w;
Bio_2.5: soil with biodegradable microplastics of 2.5% w/w;
Blue squares indicate LDPE-MPs;
Red triangles indicate Bio-MPs;
Black circle indicates control treatment.

as well as roots and microbial exudates (Bongiorno et al., 2019; Straathof et al., 2014). Carbon fractions from biodegradable materials have been reported could be utilized by microorganisms for increasing its biomass (Zhou et al., 2021). Hence, it could be that for 2.0 % and 2.5 % Bio-MPs, the overwhelming carbon fractions that leached/disintegrated from Bio-MPs promoted the growth of microorganisms, while for lower dose of Bio-MPs (0.5%, 1.0% and 1.5% Bio-MPs treatments), the biodegraded fraction from Bio-MPs polymers were totally catabolized by soil microorganisms and converted to microbial biomass, CO_2 and water (Bandopadhyay et al., 2018; Bettas Ardisson et al., 2014). So far, the effects of bio-microplastic polymers on the dynamics of DOC and DON fractions of SOM are rarely studied, and its impacts on and on soil-plant systems still needed more research.

Soil POXC is part of the labile fraction of SOM, which consists of mainly small-sized (53–250 μm), heavy organic particles (>1.7 g·cm^{-3}) and a portion of soil microbial biomass (Culman et al., 2012; Li et al., 2018). POXC has also been identified as a labile carbon fraction that is closely related to soil physical, chemical and biological processes (Bongiorno et al., 2019). Unfortunately, to our knowledge, no publications have reported the dynamic of soil POXC in microplastic-contaminated soil. In the current research, soil POXC was significantly lowered by LDPE-MPs and Bio-MPs on D105. The longer response time of POXC as compared to DOC suggested that the effects of microplastics on soil organic carbon pool cycling persist for a relative long period. Considering the composition of POXC, one explanation might be that the presence of LDPE-MPs and Bio-MPs altered soil biological processes, thus resulting in lower POXC content. Qi et al. (2020c) found that starch-based biodegradable MPs induced high amounts of decanal in the rhizosphere, which is known to have negative effects on fungal growth. Research by Cluzard et al. (2015) indicated that PE possessed antimicrobial additives and could regulate soil microbial taxa and affect soil microbial biomass. There were also studies showing that bioavailable carbon from biodegradable materials can increase microbial biomass (Zhou et al., 2021; Zumstein et al., 2018). In the current study, soil microbial biomass was not measured, as such, the decrease of soil POXC in microplastic polluted soil remains unexplained. Therefore, further studies related to soil microbial dynamics are needed to fully understand the effects of microplastics on the soil-plant system.

4.3. *Responses of available nitrogen to microplastics*

Nitrogen (N) is essential to manage agricultural soil health and crop productivity (LeBauer and Treseder, 2008). However, there are limited studies about the effects of microplastics on the dynamics of soil available nitrogen (AN) in soil-plant systems. Overall, in current study, LDPE-MPs exerted no significant effects on soil AN, while Bio-MPs significantly lowered the AN% and the ratio of N-NO_3^-/N-NH_4^+ with the increasing doses on D46. Previously, our findings showed that LDPE-MPs exerted no significant effects on root development, while 2.0% and 2.5% Bio-MPs resulted in higher specific root length (SRL, root length per gram of dry root weight) and specific root nodules (SRN, number per gram of dry root weight), but significant lower root biomass. We therefore hypothesized that soil available N content was greatly limited by addition of 2.0% and 2.5% Bio-MPs, but not by addition of LDPE-MPs (Meng et al., 2021). Here, we confirmed that indeed was the case.

The insignificant effects of PE-based microplastics on soil properties have also been observed in other studies. Previously, de Souza Machado et al. (2019) found that microplastics of polyethylene high density (PEHD-MPs) were less capable of triggering biogeochemical changes in the soil. They attributed the insignificant effects of PEHD-MPs to the resistant hydrocarbon structure. It should be mentioned that on D46, LDPE-MPs treatments showed an accumulating trend of AN from 0.5% to 1.0% and then a decreasing trend from 1.0% to 2.5%, even though there were no significant changes observed. LDPE-MPs have been reported to increase soil porosity and allow for greater diffusion of soil N-NH_4^+, thus facilitating the nitrification process (de Souza Machado et al., 2018b; Huang et al., 2019; Wan et al., 2019; Zhang et al., 2019). A research by Qi et al. (2020b) showed that as compared to control, 1.0% LDPE-MPs can significantly increase sandy soil (same as our soil matrix) porosity, soil porosity in 2.0% LDPE-MPs treatment was slightly higher (no significant difference) than control. However, the increased soil porosity could also allow more N leaching. Thus, our data suggest that LDPE-MPs might act as a dual-direction regulator in the soil-plant system depending on the concentrations of microplastics. This highlights the fact that robust investigations focusing on the effects of LDPE-MPs on soil nitrogen cycling are urgently needed.

For Bio-MPs, the decreasing trend of AN% and N-NO_3^-/N-NH_4^+ with the increasing doses of Bio-MPs on D46 indicated that Bio-MPs not only lowered nitrogen availability, but also suppressed the nitrification process of soil N-NH_4^+ to soil N-NO_3^-. This might be attributed to the sequestration of N-NH_4^+ by Bio-MPs polymers. According to our FTIR results (Fig. S4), the Bio-MPs used in current research showed a strong peak at wavelength 1711.44 cm^{-1}, this is within the characteristic peak range of carboxylic acids (-COOH) (Max and Chapados, 2004; Zain et al., 2017), indicated that our Bio-MPs also contained negative charge functional groups of -COOH. An incubation experiment conducted by Chen et al. (2019) observed a significant decrease in N-$NH_4^+$when soils were amended with 2% PLA-MPs. Another research in sediment system by Green et al. (2016) also showed that PLA-MPs reduced the N-NH_4^+concentration in pore water. Green et al. (2016) and Chen et al. (2019) ascribed the decrease of N-NH_4^+concentration to the potential adsorption of carboxyl (-COOH) of PLA structure to the cations of N-NH_4^+, however, this pathway is needed to be verify in the future research. An alternative explanation might be the microbial N immobilization. In our research, we have observed a clear increasing trend of soil TC:TDN ratio in Bio-MPs treatments on D46. This is in line with previous research by Qi et al. (2020c), who also reported that incorporating starch-based Bio-MPs into soils can substantially increase soil C:N ratio. Higher C:N ratio via microplastic addition can lead to soil nitrogen immobilization (Rillig et al., 2019). Another report by Zhou et al. (2021) concluded that carbon source supply from biodegradable material of PHBV (poly-(3-hydroxybutyrate-*co*-3-hydroxyvalerate)-$[COCH_2CH(CH_3)O]_m$ $[COCH_2CH(C_2H_5)–O]_n$)) can stimulate the growth of microbial biomass and intensify the nitrogen limitation. Thus, in our study, we suggested that the lower availability of nitrogen might have joint effects: 1). The absorption of Bio-MPs to cation N-NH_4^+ suppressed nitrification processes from N-NH_4^+ to N-NO_3^-; 2). The C supply from Bio-MPs to microorganisms stimulated the microbial N immobilization.

4.4. Limitation and implications

The wide range (0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/w dry soil weight) of LDPE-MPs and Bio-MPs used in current study was aimed to investigate actual environmental thresholds as well as to depict the subtle effects of microplastics on the soil-plant ecosystem (van Weert et al., 2019). RDA analysis showed LDPE-MPs and Bio-MPs were stand in the opposite directions of Y axis (except 0.5% Bio-MPs), LDPE-MPs were stand in the positive direction of soil available nitrogen, while Bio-MPs treatments were stand in the positive direction of soil DOC and DON (Fig. 4). Indicating they might affect soil C and N dynamics via different ways. Considering the stable C–C structure of LDPE-MPs, LDPE-MPs most likely affected soil nitrogen cycling by altering soil porosity (de Souza Machado et al., 2019). While the Bio-MPs, on the one hand, contained carbonyl (=O) and hydroxyl (-OH) groups that can absorb cation like N-NH_4^+, on the other hand, it can also provide more bioavailable C to microorganisms to increase microbial biomass and intensify soil nitrogen restriction (Boots et al., 2019; Chen et al., 2019; Wan et al., 2019; Yan et al., 2020; Zhou et al., 2021). However, the dynamics of microbial communities were not measured, lowering the connection between carbon and nitrogen cycling and soil microorganisms. As such, biological mechanisms affecting the decrease in soil nitrogen availability in microplastic-treated soil remain unexplained and require further study.

5. Conclusion

In this study, we verified our hypothesis that Bio-MPs exerted stronger effects on soil DOC, DON and soil available nitrogen (N-NH_4^+ and N-NO_3^-) than LDPE-MPs. Significant decreases of SOM in Bio-MPs treatments from D0 to D46 were observed, while the SOM of LDPE-MPs treatments on D0, D46 and D105 showed no significant differences. Exposure to LDPE-MPs (0.5%, 1.0% and 2.5%) and Bio-MPs ($\geq$ 1.0%) led to a reduction in soil POXC content on D105. LDPE-MPs showed no significant effects on soil labile organic carbon cycling, while Bio-MPs of 2.0% and 2.5% showed significantly higher soil DOC and DON (on D46 and D105) and lower soil available nitrogen (on D46). Even LDPE-MPs showed no significant effects on soil nitrogen cycling. The dynamics of carbon and nitrogen cycling in LDPE-MPs still showed deviations from the control treatment, indicating potential threats from LDPE-MPs to soil ecological function.

Declaration of competing interest

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

Acknowledgements

This work was supported by National Natural Science Foundation of China (41877072); State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (A314021402-2017); the SPRINT project (862568); the MINAGRIS project (101000407); China Scholarship Council (CSC: 201707720007). Many thanks to Andre Maassen, Sean Geurts, Bertus vander Laan and Gerard Derks from Wageningen Unifarm for helping manage the experiment in the net-house. We also thank Henny Gertsen and Ke Meng for their assistance in preparing the plastic materials.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111938.

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