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Microplastics in the agroecosystem: Are they an emerging threat to the plant-soil system?

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ABSTRACT

Despite plastics providing great benefits to our daily life, plastics accumulating in the environment, especially microplastics (MPs; defined as particles <5 mm), can lead to a range of problems and potential loss of ecosystem services. Current research has demonstrated the significant impact of MPs on aquatic systems, but little is known about their effects on the terrestrial environment, especially within agroecosystems. Hereby, we investigated the effect of MPs type and amount on plant growth, soil microorganisms, and photoassimilate carbon (C) allocation. MPs had a negative, dose-dependent impact on plant growth affecting both above- and below-ground productivity (−22.9% and −8.4%). MPs also influenced assimilated ^{14}C allocation in soil (+70.6%) and CO_2 emission (+43.9%). Although the activity of β-glucosidase was suppressed by MPs, other C- and N-cycling related enzyme activities were not affected. The type and amount of MPs in soil greatly altered C flow through the plant-soil system, highlighting that MPs negatively affect a range of C-dependent soil functions. Moreover, MPs increased the soil microbial biomass (+43.6%; indicated by PLFAs), and changed the structure and metabolic status of the microbial community. The evidence presented here suggests that MPs can have a significant impact on key pools and fluxes within the terrestrial C cycle with the response being both dose-dependent and MPs specific. We conclude that MPs in soil are not benign and therefore every step should be made to minimise their entry into the soil ecosystem and potential to transfer into the food chain.

1. Introduction

The use of plastic within modern society is endemic with global plastic consumption, and subsequent disposal, now exceeding 280 million tonnes annually (Thompson et al., 2009; Duis and Coors, 2016; Machado et al., 2018a). Despite the remarkable benefit of plastics to society, there are increasing concerns associated with the vast amount of plastic entering our environment and its subsequent resistance to degradation (Rochman, 2018). These concerns are supported by estimates that >30% of the world's plastic waste is disposed of inappropriately, with most ultimately entering the soil ecosystem (Jambeck et al., 2015; Weithmann et al., 2018). It is likely that most agricultural and urban soils are now contaminated by plastics. Of these, microplastics (MPs; particles <5 mm in size), typically formed from the disintegration of larger plastic debris, are thought to be the most environmentally damaging (Rillig, 2012; Huerta Lwanga et al., 2016).

Microplastics in aquatic environments have been widely studied and are now recognized as having negative impacts on organisms which can lead to a loss of marine and freshwater ecosystem functioning (Syberg et al., 2005; Cole et al., 2011; Wright et al., 2013; Van Cauwenberghe and Janssen, 2014; Sharma and Chatterjee, 2017). The potential effect of MPs in terrestrial ecosystems, however, remains largely unexplored (Machado et al., 2018a), despite the fact that more than 80% of plastic pollution arriving in the oceans was produced, used, and often disposed of on land (Rochman, 2018). In terrestrial ecosystems, MPs contamination might be 4–23 fold larger than in the ocean (Horton et al., 2017), and soil alone may store more MPs debris than oceanic basins (Nizzetto et al., 2016). MPs can enter the soil environment in numerous ways. Of

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these, the addition of organic wastes to soil (e.g., composts, biosolids) which are potentially contaminated with plastics and the use of plastic mulch covers are the most common inputs to soil (Huerta Lwanga et al., 2016; Mahon et al., 2017). Thin plastic mulch films (4–8 μm thick) are widely used in agricultural systems to improve plant growth and water use efficiency (Ekebafe et al., 2011; Qi et al., 2018). Although extremely effective, after crop harvest they are notoriously difficult to recover from soil and are rarely recycled. Consequently, plastic films are often incorporated into the soil where fragmentation is facilitated by tillage and exposure to UV radiation (Liu et al., 2014). Although MPs may confer some benefits to agricultural soils (e.g. enhanced C storage, structure, aeration), it is thought that these will be far outweighed by the potential disbenefits (Qi et al., 2020).

Microplastics present in soil can be ingested and transferred to larger soil organisms, leading to uncertain effects on soil fauna and microorganisms (Nizzetto et al., 2016). Several taxa including plastic-degrading bacteria and pathogens were more abundant on MPs polluted soil, indicating that MPs can act as a distinct microbial habitat, potentially altering the ecological functions of soil ecosystems (Huang et al., 2019). Additionally, the exposure of soil to MPs can change the association between microbial activity and water stable aggregates (Machado et al., 2018b), since the diversity and interactions of soil microbes are significant causal factors in soil aggregation (Lehmann et al., 2017). Although MPs pollution on soil macroorganisms has received great attention, research investigations of their effects on the microbial community are rare, especially the interactions among plant, soil and microbes. As small changes in plant-soil-microbe systems by MPs addition may lead to significant long-term impacts on a range of soil ecosystem services (e.g., C storage, nutrient cycling, pollutant attenuation) (Zang et al., 2018; Zhou et al., 2020), it is vital to understand how MPs affect microbial communities and below-ground C flow (Zang et al., 2019; Qi et al., 2020).

Low molecular weight organic substances (LMWOS) appear to dominate the total CO_2 flux from soil (up to 30%) and strongly affect nitrogen (N) cycling at a global scale (Wen et al., 2019a). CO_2 fluxes originating from soil organic matter (SOM) mineralization are also controlled by how the microbial community partitions the LMWOS between catabolic (i.e. energy yield processes associated with CO_2 production) and anabolic (i.e. microbial biomass growth) pathways (Jones et al., 2018; Zang et al., 2020). Carbon use efficiency (CUE) is commonly used to quantify the proportion of C incorporated into new microbial biomass, which could have significant influences in response to MPs pollution (Wen et al., 2019a, b). However, it remains unclear how LMWOS mineralization and CUE respond to the type and amount of MPs pollution.

It is now expected that the negative impacts of plastics on ecosystem health will worsen over time as their decomposition rates are extremely slow relative to the rate of entry to the system, leading to a progressive accumulation within soil (Rillig, 2012; Rillig et al., 2017). It has been observed that topsoil near roads in an industrial area might contain up to 7% of MPs by weight (Fuller and Gautam, 2016), while some researchers argue that levels up to 60% by weight may be realistic in highly contaminated areas (Huerta Lwanga et al., 2016). In agricultural areas, farmers undertaking plasticulture typically use between 5 and 35 kg plastic film ha^{-1} yr^{-1}, with residual plastic present in the underlying soil varying from 72 to 260 kg ha^{-1}, depending on the number of years of use, percentage of ground covered and film thickness (Liu et al., 2014). Due to our poor understanding of plastic behaviour in soil, it is currently not possible to make informed decisions on future policies relating to the use and disposal of agricultural plastics. The severity of impact, however, is likely to depend on the type and amount of plastic entering the soil. Polyvinyl chloride (PVC) and Polyethylene (PE) are the two most common plastics found in soil (Kasirajan and Ngouajio, 2012; Liu et al., 2014). The aim of this study was, therefore, to explore how different types and amounts (1, 5, 10, 20% w/w) of MPs would influence: 1) plant growth; 2) assimilated C allocation in the plant-soil system; 3) soil microbial community and exoenzyme activity; and 4) the dynamics and turnover of LMWOS (glucose and amino acids) in soil.

2. Materials and methods

2.1. Soil sampling and preparation

Soil was collected from the upper layer (0–20 cm) of a lowland (15 m altitude) freely-draining *Lolium perenne* L. dominated grassland field at the Henfaes Agricultural Research Station located in Abergwyngregyn, Gwynedd, North Wales (53°14′N, 4°01′W). The soil is classified as a Eutric Cambisol with silty clay loam texture. The mean annual soil surface temperature varies from 8 to 10 °C and the annual rainfall is 1050 mm. The soil has no previous history of plastic pollution. This site is an experimental site owned by Bangor University, which was established more than 50 years ago. The farming history of the site is well known. No plastic mulch was applied, and no plastic pollution was recorded for the site. The soil was stored in air-permeable polyethylene bags after collection and immediately transported back to the laboratory. The soil was homogenized and sieved (<5 mm) in a field-moist condition and the fine roots and other plant residues removed before use. The basic characteristics of the soil were as follows: pH (H_2O), 5.7; organic C, 35.0 g kg^{-1}; total N, 2.6 g kg^{-1}. Further information about the experimental site is presented in Jones et al. (2004).

2.2. Experimental design and set-up

A pot experiment with completely randomized design and four replicates per treatment was set up in an unheated glasshouse. Five hundred grams of soil (dry weight) was placed in pots (11 cm × 8 cm surface area, and 17 cm height, $n = 36$). Two common types of MPs (PVC and PE; 125 μm; Sigma-Aldrich, St. Louis, USA) were manually incorporated into the soil to give concentrations of 1%, 5%, 10%, and 20% of the soil dry weight. An additional control treatment was included, with no plastic addition but with the equivalent amount of soil disturbance. Subsequently, the pots were pre-incubated under field-moist conditions in a greenhouse for 2 weeks (Song et al., 2020). After pre-incubation, six wheat (*Triticum aestivum* L.) seeds were sown in each pot and thinned to four seedlings per pot after 5 days of growth. Plants were watered every three days and the soil moisture was maintained at a gravimetric moisture content of 20% throughout the experiment.

2.3. $^{14}CO_2$ labeling of plants

After 30 days, the patterns of C allocation in plant tissues under the different treatments were determined using the $^{14}CO_2$ labeling approach described in Hill et al. (2007). Briefly, the pots were put in air-tight plastic chambers (1 m × 1 m × 80 cm) before labeling. The chamber consisted of a transparent polyethylene film, which was hung from a wooden frame and sealed using adhesive tape to avoid gas leakage. $^{14}CO_2$ gas was generated by the reaction of $Na_2^{14}CO_3$ and excess HCl. Using a syringe, HCl was carefully added to a glass beaker inside the chamber containing the $Na_2^{14}CO_3$ solution. Puncture holes caused by the syringes were sealed with tape and three fans (5–12 V) in the chamber were used to ensure a uniform distribution of $^{14}CO_2$. Assimilation took place within 2 h after the addition of $^{14}CO_2$ (Hill et al., 2007; Zang et al., 2019), after which the chamber was removed.

2.4. Plant, soil and gas sampling

The plants and soil were sampled 5 days after labeling. Shoots were cut off at the base of the stem and the roots and soil were collected separately. Plant roots were removed from the soil by washing with tap water. Shoots, roots, and soil were oven-dried (60 °C, 24 h), weighed, homogenized and then ball-milled before further analysis (Wang et al., 2020). Soil microbial biomass (^{14}C-MBC) was determined on fresh soil

by the chloroform fumigation extraction method (Vance et al., 1987; Wu et al., 1990). Given that chloroform may degrade plastic particulates and thus result in overestimate of microbial C, we only quantified ^{14}C-MBC using this method. For the total microbial biomass, we estimated it as the sum of all extracted PLFAs (see below). After destructive sampling, the fresh soil was carefully mixed and a 5 g subsample directly extracted using 20 ml of 0.05 M K_2SO_4 (Zang et al., 2016; Wen et al., 2020). Another 5 g portion of soil was fumigated with chloroform for 24 h and then extracted in the same manner. The non-fumigated extractions were used to measure ^{14}C in the dissolved organic C fraction (^{14}C-DOC). The ^{14}C in the microbial biomass was estimated as the difference in K_2SO_4-extractable ^{14}C between fumigated and non-fumigated soils without a correction factor (Glanville et al., 2016). $^{14}CO_2$ evolved from the soil and wheat root compartment was trapped in a 1 M NaOH solution after labeling following the method of Hill et al. (2007).

The ^{14}C content of the oven-dried plant and soil materials were determined by oxidization in an OX400 Biological Oxidiser (RJ Harvey Instrument Corp., Hillsdale, USA), with $^{14}CO_2$ collected in Oxosol scintillant (National Diagnostics, Atlanta, USA). ^{14}C activity was measured by liquid scintillation counting using a Wallac 1404 liquid scintillation counter with automated quench correction. ^{14}C activities of CO_2 in the NaOH traps and K_2SO_4 extracts were measured by mixing 1 ml of this solution with 4 ml of HiSafe 3 scintillant (Fisher Scientific, Loughborough, UK) and then measured with the Wallac 1404 scintillation counter.

2.5. Measurements

2.5.1. Phospholipid fatty acid analysis of microbial communities

After plant harvest, the whole soil from each pot was mixed, homogenized, and fine roots and residues were carefully removed manually. Then, 20 g of soil was stored at −80 °C for microbial community analysis. Given that phospholipid fatty acids (PLFA) are the main component of the cell membrane of all microbes, PLFA analysis was used to quantify total microbial biomass and provide a general profile of the microbial community (Kim et al., 2018). PLFA was undertaken according to the method of Bartelt-Ryser et al. (2005) with taxonomic groups ascribed to individual PLFAs using the Sherlock® PLFA Method and Tools Package (PLFAD1; Microbial ID Inc., Newark, USA). The soils were suspended in a solution of methanol-chloroform-phosphate buffer. After filtration, the chloroform phase was separated and the phospholipids were separated from glycolipids and neutral lipids by solid-phase extraction. The phospholipids were saponified and methylated to fatty acid methyl esters by using an Agilent 6890 gas chromatograph equipped with a flame ionization detector and an Ultra-2 column (Kim et al., 2018). A total of 70 fatty acids were found in our soil samples but we only chose those that represented more than 0.5% of the total PLFAs for biomarker and taxonomic group annotation. The fatty acids considered to establish the different taxonomic groups are shown in Table S1.

2.5.2. Soil exoenzyme activity

Five enzymes related to soil C (β-glucosidase, cellobiohydrolase, and xylosidase) and N (leucine aminopeptidases and chitinase) cycling were selected as indicators for changes in enzyme activities under MPs pollution. Enzyme activities were measured using fluorogenically labeled substrates (Wen et al., 2019b; Zhou et al., 2020; Zhang et al., 2020). Briefly, 1 g of fresh soil was collected from the wheat mesocosms at harvest and suspended in 50 ml of sterile water by shaking for 30 min, and dispersing for 2 min using low-energy sonication (50 J s^{-1}). A 50 μl aliquot of the soil suspension was pipetted into 96-well black microplates. Afterwards, 50 μl of buffer and 100 μl of the corresponding substrates at concentrations of 200 μmol substrate g^{-1} soil were added. At 0, 30, 60 and 120 min after substrate addition, the microplates were measured fluorometrically at an excitation wavelength of 360 nm and an emission wavelength of 450 nm (Victor^3 1420-050 Multi-label Counter, PerkinElmer, USA).

2.5.3. Low molecular weight organic substrate (LMWOS) mineralization

A separate incubation experiment was established to evaluate the LMWOS mineralization in response to MPs pollution. The mineralization rate of LMWOS was investigated following the methods of Boddy et al. (2007) and Wen et al. (2019a). Briefly, fresh soil (2 g) was collected from the previous pot experiment under different type and amount of microplastics pollution. The soil was placed in a 50 ml centrifuge tube and equilibrated at 20 °C for 3 days prior to substrate addition. Subsequently, 200 μl of either ^{14}C-glucose or a ^{14}C-amino acid mixture (<10 nM; 16 kBq ml^{-1}; Amersham Biosciences UK Ltd, Chalfont St. Giles, UK) was injected into the soil. The amino acid mixture was an equimolar mixture of 15 uniformly ^{14}C-labeled L-amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine; pH 5.60). Subsequently, a $^{14}CO_2$ trap (1 M NaOH, 1 ml) was placed into the closed container to capture evolved CO_2. The NaOH traps were changed at 1, 3, 5, 7, 10, 24, 32, 48, 72, 108, 169 h after LMWOS addition to measure the production of $^{14}CO_2$. The ^{14}C content of the NaOH traps was determined as described above.

2.6. Calculations

^{14}C data for each replicate were expressed as percentages of total ^{14}C recovered in the plant-soil system (Sanaullah et al., 2012; Zang et al., 2019). The total ^{14}C recovered was calculated as the sum of ^{14}C amount in CO_2, shoots, roots, and soil as follows:

$$\text{Total } ^{14}\text{C recovered} = ^{14}\text{C–CO}_2 + ^{14}\text{C-shoot} + ^{14}\text{C-root} + ^{14}\text{C-soil} \quad (1)$$

The ^{14}C incorporation (% of ^{14}C recovered) into CO_2, shoots, roots, soil, DOC, and microbial biomass were calculated as follows:

$$^{14}\text{C incorporation} = ^{14}\text{C activity in X / total } ^{14}\text{C recovered} \times 100\% \quad (2)$$

where X is the particular pool within the plant-soil system.

To characterise the rate of LMWOS turnover in soil, a model was fitted to the experimental data. Within the model, $^{14}CO_2$ production was apportioned into two pools: 1) the fast pool, where the substrate is immediately used for catabolic processes, rapidly influencing CO_2 flux; 2) the slow pool, constitutes the remaining ^{14}C immobilized within the microbial biomass (i.e. used for cell growth, maintenance, and ultimately necromass turnover), which is only later broken down and respired (Glanville et al., 2012, 2016; Wen et al., 2019a,b). Therefore, substrate mineralization was described by a two-step process, double first order decay model as follows:

$$S = a_1\, e^{-k_1 t} + a_2\, e^{-k_2 t} \quad (3)$$

where S is the ^{14}C remaining in the soil (% of total added ^{14}C), a_1 and a_2 are pool sizes for the fast and slow mineralization phases (% of total added ^{14}C), respectively. Here, a_1 represents the fast pool immediately used for catabolic processes, whereas a_2 represents the slow pool immobilized within the microbial biomass. Additionally, k_1 and k_2 are the rate constants (% of total added ^{14}C per hour) for a_1 and a_2, respectively, and t is time (h) (Glanville et al., 2016; Wen et al., 2019a). Microbial substrates C use efficiency (SCUE) for each C substrate was calculated according to Jones et al. (2018) where:

$$\text{Substrate CUE} = a_2 / (a_1 + a_2) \quad (4)$$

Thus, substrate CUE was calculated based on the proportion of added C incorporated into new microbial biomass. It should be noted that the CUE values here are only for the C within the glucose or amino acids added (i.e. substrate C use efficiency) and do not account for other C compounds also used by the microbial biomass. A full description of the model is provided in the supporting references (Glanville et al., 2012, 2016; Wen et al., 2019a; Jones et al., 2018) should the reader need more information.

2.7. Statistical analysis

Each variable was first tested for normality using the Shapiro-Wilk's test, and for equality of variance using Levene's test. Variables with non-normal distributions (PLFAs) or unequal variances (^{14}C incorporation into DOC and CUE of amino acid) were logarithmically transformed. Differences in plant and soil properties among treatments (MPs amounts: 0%, 1%, 5% 10%, 20%; MPs types: PVC and PE) were then analysed using one-way ANOVA with Fisher's least significant difference (LSD) test. All the differences were considered significant at the $P < 0.05$ level. The PLFA profiles were analysed based on principal component analysis (PCA). They were Gram-negative, Gram-positive, actinomycetes, putative AM fungi, eukaryote, and fungi, and all of them were used for PCA analysis. The mole percentages of individual PLFAs were used in the PCA to determine if the PLFA signatures of microbial communities varied with treatments. We selected the two main principal components (PC) 1 and 2 with an explanation of 56.2% and 29.2%, respectively. Statistical analyses were carried out using SPSS v20.0 (IBM Inc., Armonk, USA).

3. Results

3.1. Shoot and root biomass

Both PVC and PE affected wheat shoot and root biomass, with the magnitude of the response strongly dependent on the amount of MP added to the soil ($P < 0.05$; Fig. 1; Table S2). In addition, wheat growth was affected by the type of plastic, albeit to much lesser extent. Relative to the unamended control treatment, shoot and root biomass were suppressed by 13–53% at low rates of MP addition (i.e. 1% and 5%), whereas plant biomass increased by 50–80% at higher MP addition rates ($P < 0.05$; Fig. 1a and b; Table S2). The root-to-shoot ratio was doubled with the 20% PVC and 10% PE MP treatments ($P < 0.05$; Table S2), but was unaffected by the low MP doses (Fig. 1c).

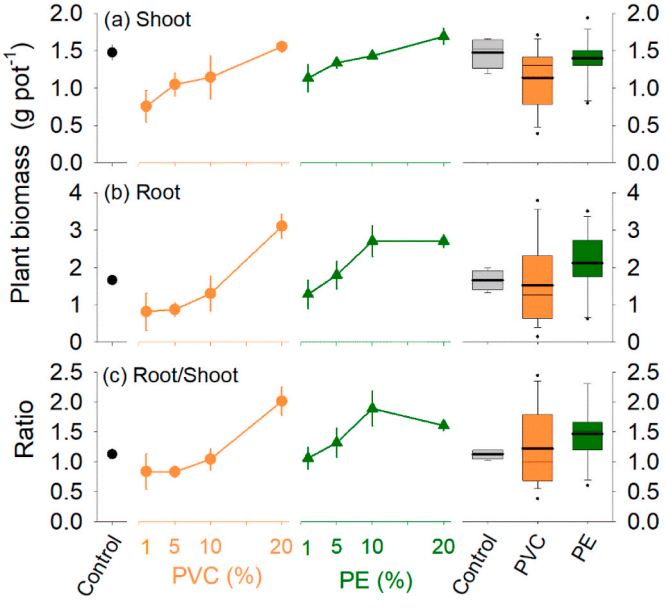

Fig. 1. Effects of microplastics type and amount on wheat shoot (a) and root biomass (b) and root-to-shoot ratio (c). The microplastics used here were polyvinyl chloride (PVC) and polyethylene (PE) at soil addition rates of 1%, 5%, 10%, and 20% by soil dry weight. Left parts: Impact of microplastics on plant performance over the range of amounts added. Values are means ± standard errors ($n = 4$). Right parts: Summary of the effect of microplastic types on plant performance combining the amounts added. The black points are outliers beyond the 10th and 90th percentiles; the boxes are 25th and 75th percentiles; the central thin horizontal line represents the median, and the central bold horizontal line represents the mean. The black, orange, and green represent the microplastic types as control, PVC, and PE, respectively. Note the different scales on the y-axis in the figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Assimilated ^{14}C allocation in the plant-soil system

Regardless of MP pollution, plant shoots were the main sinks for assimilated CO_2, incorporating more than 50% of the total ^{14}C recovered (Fig. 2a). ^{14}C incorporation into the shoots was increased by PVC addition compared to the unamended control, especially at low rates of MP addition (i.e. 1% and 5%; Fig. 2a). Low PVC additions decreased ^{14}C incorporation into the roots from 36% to 20% of the total ^{14}C recovered (Fig. 2b), but no effect was observed at 20% PVC addition. The ^{14}C remaining in soil was 2–3 times higher with 1% and 5% of PVC treatments compared to the control (Fig. 2c), while the lower $^{14}CO_2$ emissions were observed in the treatments with low PVC addition than high PVC addition (Fig. 2d). ^{14}C incorporation into the soil microbial biomass and DOC pool represented <3% of the total $^{14}CO_2$ assimilated, regardless of the MP type and amount. Compared to the control, low amounts of PVC addition (i.e. 1% and 5%) increased ^{14}C incorporation into the microbial biomass, but it was decreased in the high PVC treatments (i.e. 10% and 20%; $P < 0.001$; Fig. 2e). Similarly, ^{14}C incorporation into DOC increased with PVC concentration compared to the control ($P < 0.05$; Fig. 2f). However, PE addition had no effect on ^{14}C incorporation into these pools in the plant-soil system ($P > 0.05$).

3.3. Soil microbial community

Total soil microbial PLFAs increased with MP addition, regardless of the added amount (Fig. 3). Specifically, total PLFAs for the control soil was 95 nmol g^{-1} and this increased by 2.0, 1.3 and 1.6 times with 5%, 10% and 20% of PVC addition ($P < 0.001$; Table S3), respectively. However, the total PLFAs was only slightly enhanced (17–45%) by PE addition and did not show strong variation across the different addition rates (Fig. 3). MP addition slightly increased Gram-negative bacteria, but decreased Gram-positive bacteria (Fig. 4c,e). Actinomycetes increased with PVC addition, whereas PE addition decreased actinomycetes (Fig. 4g). PE addition did not affect the amount of putative AM fungi and total fungi, however, PVC addition stimulated AM fungi but suppressed the general fungal community (Fig. 4d, f). Eukaryotes increased with PE addition but was not affected by PVC addition. The PCA calculated from six variables (PLFA fingerprint) explained 85% of the total variance (Fig. 4). The six variables were correlated with principal components (PC) 1 and 2 in different directions (Fig. 4). The Gram-negative and Gram-positive bacteria were most strongly correlated with PC2, which separated the MPs addition from the control soil. The actinomycetes, putative AM fungi, eukaryote, and fungi were more related to PC1, which separated the responses of microbial communities to PVC and PE addition. The different amount of PVC addition showed small within-treatment (i.e. same MP concentration) variance and showed separation from each other, indicating a dose effect. However, the different amount of PE inputs showed large within-group variance along PC2 and were overlapping.

3.4. Soil exoenzyme activity

As an overall indicator of microbial status, exoenzyme activity was only slightly altered by MP addition. The activity of β-glucosidase and xylosidase were reduced by 16–43% with PVC input compared to that of the control ($P < 0.05$; Fig. 5a and b). This was supported by the depressed microbial activity as evidenced by the lower soil respiration with PVC addition (Fig. S1). Moreover, PVC input had no effect on cellobiohydrolase ($P > 0.05$) and N-related leucine aminopeptidase activities ($P > 0.05$; Fig. 5c–e; Table S3). However, PE addition did not affect these C- and N-cycling related enzyme activities ($P > 0.05$;

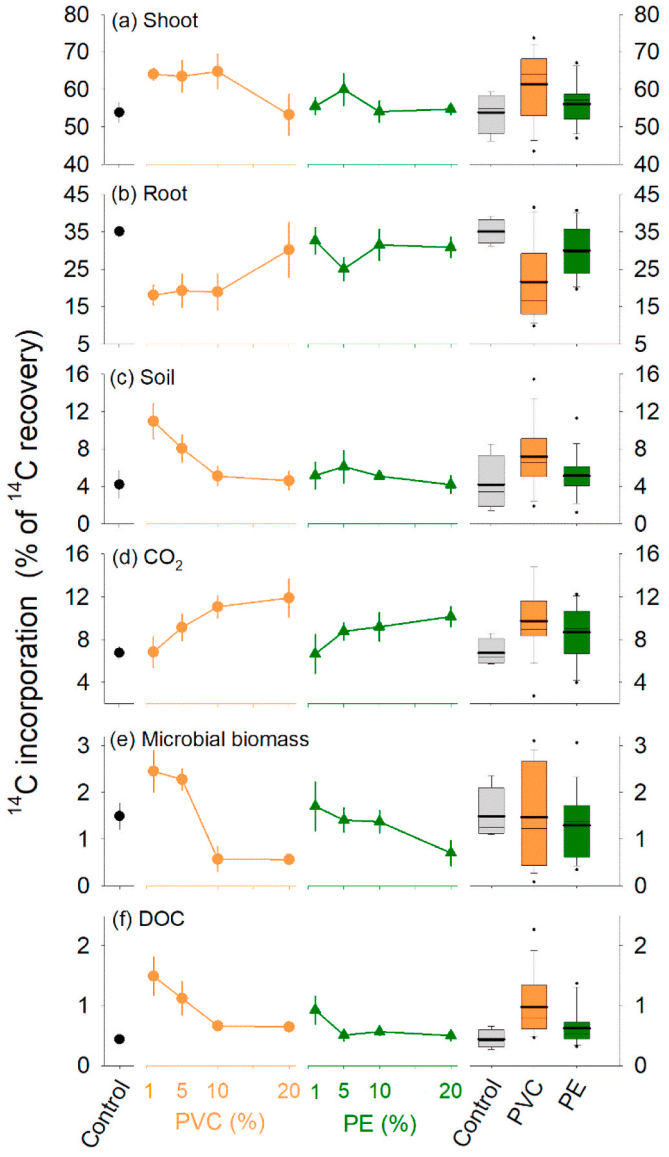

Fig. 2. Effects of microplastics type and amount on the allocation of photosynthetically-fixed C, as a percentage of the total ^{14}C recovered, to shoots (a), roots (b), soil (c), CO_2 emission (d), microbial biomass (e), and dissolved organic C (f). The microplastics used here were polyvinyl chloride (PVC) and polyethylene (PE) with increasing concentrations of 1%, 5%, 10%, and 20% by soil dry weight. Left parts: Impact of microplastics on ^{14}C incorporation over the range of amounts added. Values are means ± standard errors ($n = 4$). Right parts: Summary of the effect of microplastic type on ^{14}C incorporation combining all treatments. The black points are outliers beyond the 10th and 90th percentiles; the boxes are 25th and 75th percentiles; the central thin horizontal line represents the median, and the central bold horizontal line represents the mean. The black, orange, and green represent the microplastic types as control, PVC, and PE, respectively. Note the different scales on the y-axis in the figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table S2).

3.5. Dynamics and turnover of low molecular weight organic substrates

The total loss of $^{14}CO_2$ from the soil decreased with increasing PVC addition ($P < 0.05$; Fig. S2), regardless of LMWOS type and amount. However, no clear trend was observed with PE addition. Both types of MP resulted in changes in microbial CUE for glucose (Fig. 6). PVC addition significantly stimulated microbial substrates CUE, showing a dose-dependent effect (Fig. 6a and b). Although the trend of PE addition was not as clear as PVC addition, it also slightly increased CUE for amino acids ($P < 0.05$).

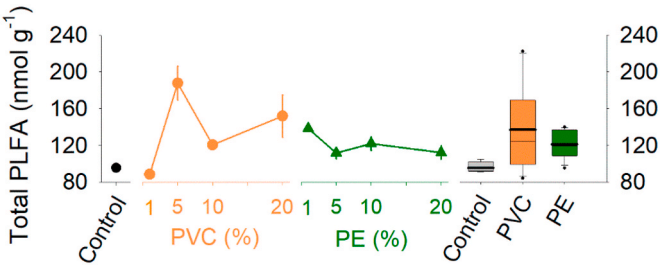

Fig. 3. Effects of microplastics type and amount on total PLFAs. The microplastics used here were polyvinyl chloride (PVC) and polyethylene (PE) at soil addition rates of 1%, 5%, 10%, and 20% by soil dry weight. Left parts: Impact of microplastics on PLFAs over the range of concentration added. Values are means ± standard errors ($n = 4$). Right parts: Summary of the effect of microplastic types on PLFAs combining the amounts added. The black points are outliers beyond the 10th and 90th percentiles; the boxes are 25th and 75th percentiles; the central thin horizontal line represents the median, and the central bold horizontal line represents the mean. The black, orange, and green represent the microplastic types as control, PVC, and PE, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. Microplastics effects on plant growth

Microplastics contamination of soil alters plant growth, which was evidenced by plastic-induced changes in C allocation and above- and below-ground biomass production (Figs. 1 and 7). The effect of MPs on plant growth can be ascribed to a range of factors including: 1) direct toxicity of the plastic on the plant (mainly by nanoparticles); 2) direct toxicity from contaminants present in the plastic (e.g. metals, plasticisers); and 3) indirect effects on plant growth via changes in soil properties and microbial communities (Saarma et al., 2003; Gu et al., 2017; Rillig et al., 2019 Qi et al., 2020). Our results showed that the response was dependent on both the type and amount of MPs within the soil (Fig. 7), however, it did not show a classic dose-dependent response (e.g. S-shaped curve described by a multi-parameter log-logistic equation) as seen for many soil-borne xenobiotics (i.e. reduced growth as dose rate increases; An et al., 2004; Dimkpa et al., 2013). Given that the PVC and PE used in the current study were pure, the different impact of MPs type on the plant-soil system may be attributed to the material itself. However, as we still possess limited information about the different responses to PVC and PE, further studies are needed to explore the underlying mechanisms in more depth. It could be that the differences relate to the microstructure of the plastics, their crystallinity and/or reactivity. It should be noted that similar differences in ecological response to PVC and PE have been noted in marine systems (Rochaab et al., 2020). We observed that the reduction in plant biomass was most acute at low MPs additions. This could be attributed to a direct toxic effect by nanoparticles existing in the added MPs. It is accepted that nanoparticles can be taken up by plants and can induce damage to tissues (Navarro et al., 2008), although the mechanisms still remain poorly understood (Yang et al., 2017). The MPs used in this study contained no plasticisers, however, we cannot completely eliminate the presence of other contaminants which might have affected plant development (e.g. nonylphenol; Bokern and Harms, 1997). Currently, we do not know if MPs size will have an effect on plant and soil microbial biomass (Machado et al., 2018a), however, it is generally hypothesized that the toxic effects on biota will increase with decreasing MPs size (Yang et al., 2017; Machado et al., 2018a). The plastic particles tested here were

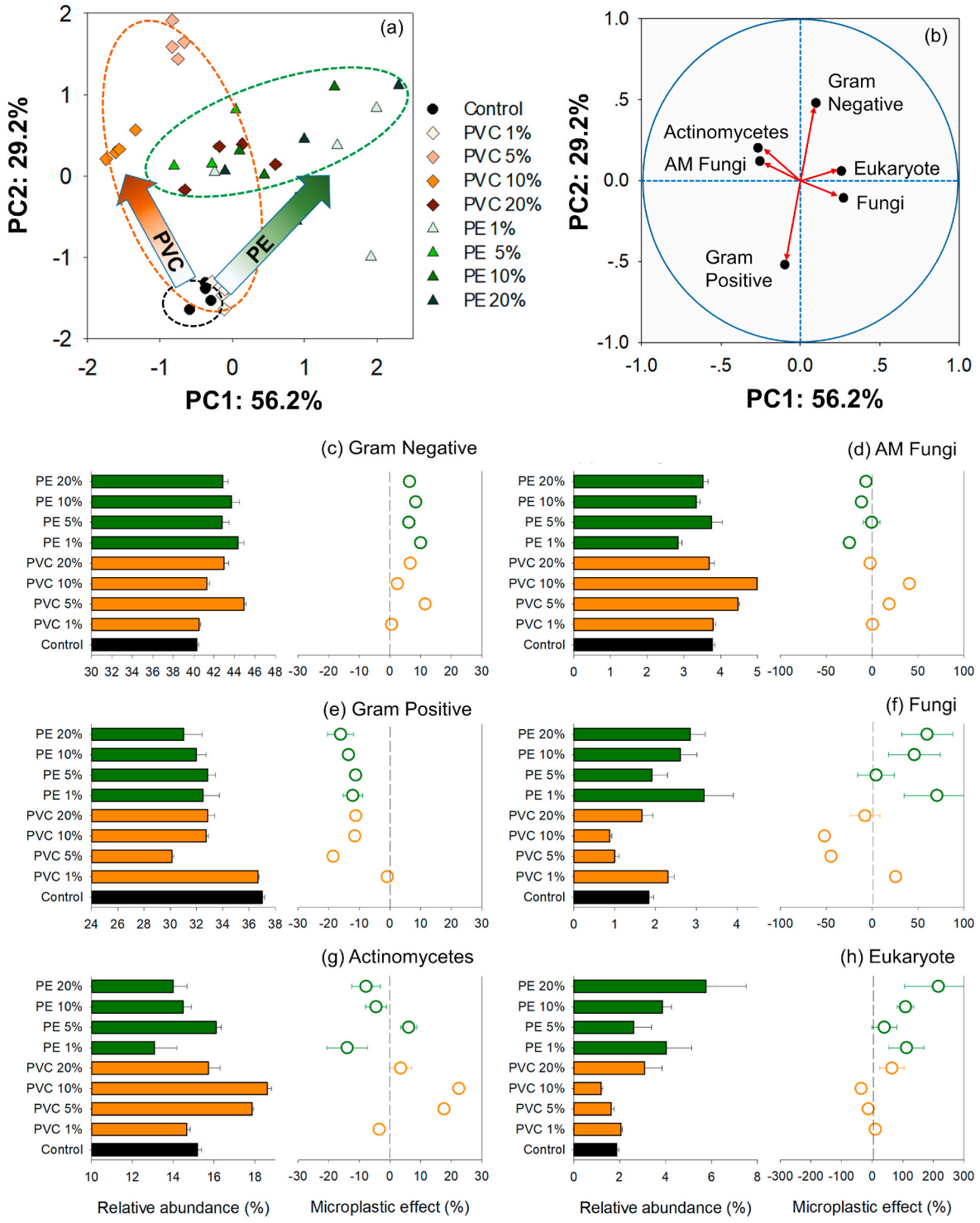

Fig. 4. Principal component analysis of the soil microbial PLFA fingerprints: Gram-negative bacteria, Gram-positive bacteria, fungi, putative AM fungi, eukaryote, and actinomycetes (a). Correlation circle describing the correlation between microbial community and the two PCs (b). Different colours represent the three treatments: control (circles and black), polyvinyl chloride (PVC; rhombus and orange) and polyethylene (PE; triangles and green) addition. The darker colours represent higher microplastics concentration treatments. Ellipses show the within-group variance. PC1 and 2 explained 56.2% and 29.2% of the inertia, respectively. The arrows illustrate the effects of microplastics (corresponding to both PC1 and 2, i.e. x and y-axis). Effect of microplastics type and amount on the relative abundance of different microbial taxonomic groups (PLFAs) in soil (c, d, e, f, g, and h). The microplastics used here were polyvinyl chloride (PVC) and polyethylene (PE) with an increasing concentration of 1%, 5%, 10%, and 20% by soil dry weight. The effect of microplastics on each microbial group was estimated as the relative changes to the control. Values are means ± standard errors (n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

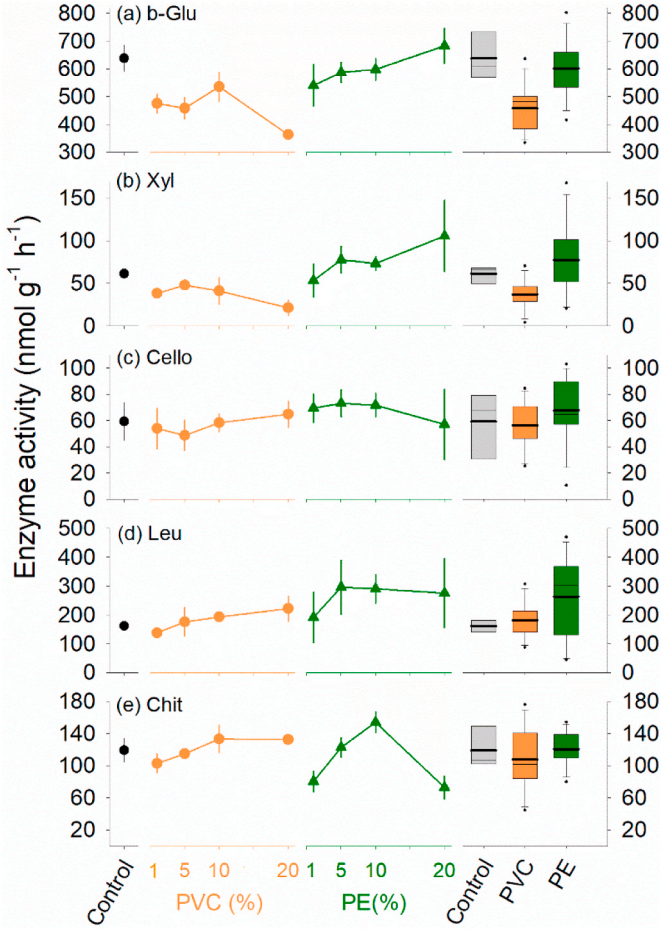

Fig. 5. Effects of microplastics type and amount on soil enzyme activities: b-glucosidase (a), xylosidase (b), cellobiohydrolase (c), leucine aminopeptidases (d), and chitinase (e). The microplastics used here were polyvinyl chloride (PVC) and polyethylene (PE) with an increasing concentration of 1%, 5%, 10%, and 20% by soil dry weight. Left parts: Impact of microplastics on soil enzyme activities over the range of amounts added. Values are means ± standard errors ($n = 4$). Right parts: Summary of the effect of microplastic types on soil enzyme activities combining the amounts added. The black points are outliers beyond the 10th and 90th percentiles; the boxes are 25th and 75th percentiles; central thin horizontal line represents median, and central bold horizontal line represents the mean. The black, yellow, and blue represent the microplastic types as control, PVC, and PE, respectively. Note the different scales on the y-axis in the figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

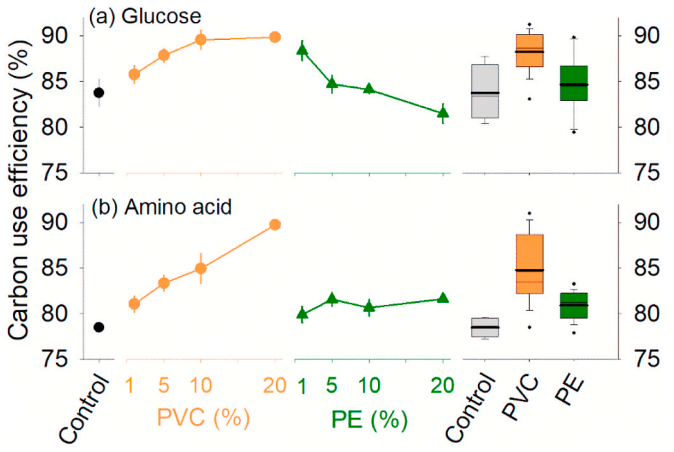

Fig. 6. Effects of the type and amount of microplastics on microbial carbon use efficiency. The microplastics used here were polyvinyl chloride (PVC) and polyethylene (PE) at soil addition rates of 1%, 5%, 10%, and 20% by soil dry weight. Left parts: Impact of microplastics on PLFAs over the range of concentration added. Values are means ± standard errors (n = 4). Right parts: Summary of the effect of microplastic types on PLFAs combining the amounts added. The black points are outliers beyond the 10th and 90th percentiles; the boxes are 25th and 75th percentiles; the central thin horizontal line represents the median, and the central bold horizontal line represents the mean. The black, orange, and green represent the microplastic types as control, PVC, and PE, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

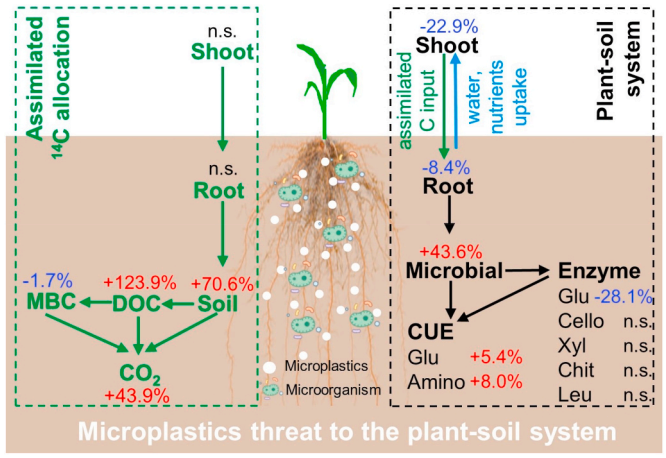

Fig. 7. Schematic overview of microplastics threat to the plant-soil system. The green box in the left shows the assimilated ^{14}C allocation to shoot, root, soil, CO_2 emission, microbial biomass, and dissolved organic C as affected by microplastics. The black box in the right shows the changes in the plant-soil system as affected by microplastics. The red and blue numbers represent the positive and negative effects of microplastics, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

relatively large in comparison to the size of the pores in the root cell wall (4 nm; Carpita et al., 1979; Carpita, 1982) and based on studies with metal nanoparticles are unlikely to be taken up into root cells (Sanaullah et al., 2012). Further work using microscopy, however, is needed to differentiate between MPs sorbed to the root surface versus those present within the root itself.

In contrast to most xenobiotic studies, we found that plant biomass was enhanced under high MPs addition rates. We assume that the changes in soil properties caused by MPs contribute strongly to the increased plant growth. For example, MPs have been suggested to lower soil bulk density (Machado et al., 2018b), which could directly reduce penetration resistance for plant roots, and enhanced soil aeration, and thus increase root growth (Rillig et al., 2019). Furthermore, MPs input also shows a positive relationship with soil water holding capacity but a negative relationship with water stable aggregates (Machado et al., 2018b). Changes in soil bulk density, water holding capacity, and soil aggregates can alter root growth and subsequently plant biomass (Wen et al., 2020). We also found that the effect of MPs was greater on root rather than shoot growth, as proved by the enhanced root-to-shoot ratio with higher MPs addition treatments. However, the mechanistic understanding of MPs addition on the performance of plant and soil still needs further exploration.

4.2. Microplastics-induced changes in ^{14}C allocation in the plant-soil system

To our knowledge, this is the first time the effect of MPs pollution on

the allocation of photosynthetic C in the plant-soil system has been investigated. Generally, MPs seem to increase C allocation to the shoot, which was more pronounced at low rates of PVC addition, and is in agreement with the slightly reduced root-to-shoot ratio (Fig. 7). A greater proportion of C was allocated to the shoots rather than roots under low PVC concentration additions possibly due to the phytotoxic effect of nanoplastics seen in overall root growth (especially with 1% of MPs addition) (Navarro et al., 2008). At increasing MPs concentrations, the physical effect (e.g. soil aeration and bulk density) may counteract the phytotoxic effect (Rillig et al., 2019). Similarly, the increased aeration may stimulate greater native soil organic matter turnover and relieve any nutrient limitations on growth. Therefore, compared to low PVC input, the reduced soil bulk density and increased water holding capacity with the high PVC additions might benefit higher C allocation to roots rather than to shoots (Sanaullah et al., 2012; Machado et al., 2018b; Wen et al., 2020). However, the PE input has no significant effect on C allocation to shoot and root regardless of the amount added. This highlights the complexity of photosynthetic C allocation as affected by different types of MPs. In future studies, a wider range of MPs types and amounts should be applied to evaluate their effect on C, N and P cycling in the plant-soil system.

The proportion of ^{14}C allocation to the soil was increased with low PVC concentration but no effect was seen at high concentrations. We ascribe this to increased root stress and rhizodeposition at low PVC addition rates. PVC inputs reduced the root-to-shoot ratio of incorporated ^{14}C, which means lower ^{14}C remaining in soil per unit of root compared with the control. This is possibly attributed to increased microbial activity and root exudate turnover at higher concentrations of PVC contamination. Similarly, $^{14}CO_2$ emissions increased at high PVC concentrations, which may be linked to enhanced root respiration (higher root biomass than control) and microbial respiration (higher turnover of root exudates) (Kuzyakov and Domanski, 2002; Zang et al., 2017). This is also supported by the higher CO_2 emission under high PVC concentration compared to the unamended control during wheat growth. MPs could also increase dissolved organic matter and nutrient contents in soil (Liu et al., 2017), and thus increase CO_2 release and plant growth. The increased $^{14}CO_2$ release with PVC additions could also be attributed to enhanced soil aeration and/or lower soil bulk density and decreased water stable aggregates (Dorodnikov et al., 2009; Machado et al., 2018b). The increased ^{14}C-DOC under a low rate of PVC addition is indicative of a higher root exudation rate or suppressed root-associated microorganisms (Sanaullah et al., 2012; Zang et al., 2017). However, our results showing unchanged or even increased total PLFAs along with higher ^{14}C incorporation into the microbial biomass with low PVC inputs does not support this. Therefore, the lower root C allocation with PVC input induced a redistribution of C to aboveground biomass and root exudation, potentially indicating higher C sequestration with small amounts of PVC pollution. Remarkably, assimilated C allocation in the plant-soil system was not affected by PE addition, indicating that the type of MPs is important in determining the impact on C cycling in agroecosystem. The different effect of PVC and PE on C allocation was mainly observed belowground (soil, respiration, microbial biomass, and DOC) rather than aboveground (shoot). This we attribute to the different effect of PVC and PE on our observed changes in the size and activity of the soil microbial biomass and its composition (as supported by PLFAs, enzyme activities and CO_2 evolution). Our results highlight that MPs could affect soil ecosystem services through changes in plant and soil function in addition to their direct toxicity.

4.3. Microplastics alterations in the size and structure of the soil microbial community

Generally, both PVC and PE input increased soil microbial biomass, as proved by the higher total PLFAs associated with both MPs types (Figs. 3 and 7). These results are consistent with the increased microbial activity in a loess soil with up to 28% by weight of polypropylene contamination (Liu et al., 2017). The PCA illustrated the similarities between the low PVC input and the control soil (Fig. 4). Both PVC and PE additions resulted in a shift from a Gram-positive to a more Gram-negative dominated microbial community structure suggesting that MPs addition stimulated soil C cycling. However, it should be noted that the PLFA method cannot thoroughly separate Gram-negative and Gram-positive microbial community due to the limitation in biomarkers (Frostegård et al., 2011). Compared to PE, the effect of PVC input on soil microbial community shows a dose effect (i.e. microbial community responds differently at different doses). MPs addition favours microbial activity as indicated by the higher ^{14}C incorporation into microbial biomass and DOC, thus increasing C-substrates which can facilitate microbial growth (Liu et al., 2017). Wang et al. (2016) demonstrated how soil microbial C and N decreased by more than 30% with the addition of plastic residues, however, these results were observed with the addition of macro-rather than micro-plastics. Given that MPs have an impact on both soil physical and chemical parameters (depending on particle size and amount) (Machado et al., 2018b), MPs pollution can be expected to differentially affect different components of the microbial community. Additionally, MPs can also act as an abundant and distinct microbial habitat (i.e. new biofilm surfaces; McCormick et al., 2014), which could potentially stimulate particular microbial groups. Moreover, changes in soil structure (e.g. soil aggregation and bulk density) by MPs may result in a shift in soil microbial community composition, and further affect plant growth (Rillig et al., 2019), although there is little knowledge about the direction of changes and functional consequences.

4.4. Microplastic effects on C substrate turnover in soil

Overall, the presence of MPs did not increase the mineralization of low molecular weight C in soil (Fig. 6). In marine systems, the surface of MPs has been proven to attract and absorb organic chemical pollutants from the water column, particularly those which are hydrophobic (Mato et al., 2001). However, the substrates used here were hydrophilic and carried no charge and are therefore very unlikely to interact strongly with plastic surfaces. Our results do, however, suggest that PVC, and to a lesser extent PE, reduced their mineralization. We ascribe this not to reduced uptake of substrate by the microbial community but due to differences in C partitioning within the community. This is evidenced by the differences in microbial C use efficiency (CUE). Given that PVC increased microbial biomass (supported by PLFAs) but reduced the LMWOS mineralization, the main driver for C mineralization under MPs pollution was the microbial community shift rather than biomass. Overall, CUE increased at higher MPs concentrations, specifically for PVC. This is attributable to (i) an increased allocation of C to cell maintenance (defence and repair) in response to MPs stress, (ii) the presence of an additional C supply which changes how metabolites are used within the cell, (iii) growth of the microbial community, (iv) a shift in microbial community structure and associated C metabolism (e.g. fungal-to-bacterial ratio). The increase in microbial-PLFA data provides direct evidence to support (iii) and (iv), however, we cannot discount (i) and (ii) which could also be occurring simultaneously.

5. Conclusions

Our results clearly show that MPs (especially PVC) negatively affect plant growth and can cause significant shifts in the size, activity, structure, and functioning of the soil microbial community. MPs generally had a minor effect on assimilated ^{14}C allocation in shoot and root, but increased that allocated to the soil (+70.6%) and CO_2 emission (+43.9%). This clearly indicates that both PVC and PE have the ability to greatly alter C partitioning within the plant-soil system. It is expected that this will also lead to significant downstream impacts on the cycling of other macronutrients (e.g. N and P). Moreover, MPs increased the soil microbial biomass (+43.6%; indicated by PLFAs), and changed the structure and metabolic status of the microbial community (indicated by

PLFAs and LMWOS mineralization). The evidence presented here suggests that MPs can have a significant impact on key pools and fluxes within the terrestrial C cycle with the response being both dose-dependent and MP-specific. As the effects of MPs on soil functioning are likely to be multifactorial, involving changes in the chemical, biological and physical attributes of the soil, further multi-scale study is clearly needed to enable the design of effective mitigation measures. In addition, further work should be undertaken on different types, formulations and sizes of MPs to gain a more holistic evaluation of their impact on the plant-soil system.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107926.

References

An, Y.J., Kim, Y.M., Kwon, T.I., Jeong, S.W., 2004. Combined effect of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. The Science of the Total Environment 326, 85–93.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology, Evolution and Systematics 7, 27–49.

Boddy, E., Hill, P.W., Farrar, J., Jones, D.L., 2007. Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biology and Biochemistry 39, 827–835.

Bokern, M., Harms, H.H., 1997. Toxicity and metabolism of 4-n-nonylphenol in cell suspension cultures of different plant species. Environmental Science and Technology 31, 1849–1854.

Carpita, N., Sabularse, D., Montezinos, D., Delmer, D.P., 1979. Determination of the pore size of cell walls of living plant cells. Science 205, 1144–1147.

Carpita, N.C., 1982. Limiting diameters of pores and the surface structure of plant cell walls. Science 218, 813–814.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Marine Pollution Bulletin 62, 2588–2597.

Dimkpa, C.O., McLean, J.E., Martineau, N., Britt, D.W., Haverkamp, R., Anderson, A.J., 2013. Silver nanoparticles disrupt wheat (*Triticum aestivum* L.) growth in a sand matrix. Environmental Science and Technology 47, 1082–1090.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Global Change Biology 15, 1603–1614.

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environmental Sciences Europe 28, 2.

Ekebafe, L., Ogbeifun, D., Okieimen, F., 2011. Polymer applications in agriculture. Biokemistri 23, 81–89.

Frostegård, Å., Tunlid, A., Bååth, A., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621–1625.

Fuller, S., Gautam, A., 2016. A procedure for measuring microplastics using pressurized fluid extraction. Environmental Science and Technology 50, 5774–5780.

Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E., Jones, D.L., 2016. Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil. Soil Biology and Biochemistry 94, 154–168.

Glanville, H.C., Rousk, J., Golyshin, P., Jones, D.L., 2012. Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. Soil Biology and Biochemistry 48, 88–95.

Gu, S.R., Zheng, H., Xu, Q.Q., Sun, C.Z., Shi, M., Wang, Z.Y., Li, F.M., 2017. Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191, 122–130.

Hill, P.W., Kuzyakov, Y., Jones, D., Farrar, J., 2007. Response of root respiration and root exudation to alterations in root C supply and demand in wheat. Plant and Soil 291, 131–141.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. The Science of the Total Environment 586, 127–141.

Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W., Qin, X., 2019. LDPE microplastic films alter microbial community composition and enzymatic activities in soil. Environment and Pollution 254, 112983.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). Environmental Science and Technology 50, 2685–2691.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.

Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163, 459–480.

Jones, D.L., Olivera-Ardid, S., Klumpp, E., Hill, P.W., Lehndorff, E., Bol, R., 2018. Moisture activation and carbon use efficiency of soil microbial communities along an aridity gradient in the Atacama Desert. Soil Biology and Biochemistry 117, 68–71.

Kasirajan, S., Ngouajio, M., 2012. Polyethylene and biodegradable mulches for agricultural applications: a review. Agronomy for Sustainable Development 32, 501–529.

Kim, S., Zang, H., Mortimer, P., Shi, L., Li, Y., Xu, J., Ostermann, A., 2018. Tree species and recovery time drives soil restoration after mining: a chronosequence study. Land Degradation & Development 29, 1738–1747.

Kuzyakov, Y., Domanski, G., 2002. Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labelling of ryegrass. Plant and Soil 239, 87–102.

Lehmann, A., Zheng, W., Rillig, M., 2017. Soil biota contributions to soil aggregation. Nature Ecology and Evolution 1, 1828–1835.

Liu, E., He, W.Q., Yan, C., 2014. 'White revolution' to 'white pollution' - agricultural plastic film mulch in China. Environmental Research Letters 9, 091001.

Liu, H., Yang, X., Liu, G., Liang, C., Xue, S., Chen, H., Ritsema, C.J., Geissen, V., 2017. Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere 185, 907–917.

Machado, A.A.S., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018a. Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biology 24, 1405–1416.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environmental Science and Technology 35, 318–324.

Machado, A.A.S., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018b. Impacts of microplastics on the soil biophysical environment. Environmental Science and Technology 52, 9656–9665.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L., 2017. Microplastics in sewage sludge: effects of treatment. Environmental Science and Technology 51, 810–818.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environmental Science and Technology 48, 11863–11871.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.J., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17, 372–386.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environmental Science: Processes and Impacts 18, 1050–1059.

Qi, Y.L., Yang, X.M., Pelaez, A.M., Lwanga, E.H., Beriot, N., Gertsen, H., Garbeva, P., Geissen, V., 2018. Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. Science of the Total Environment 645, 1048–1056.

Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020. Behavior of microplastics and plastic film residues in the soil environment: a critical review. Science of the Total Environment 703, 134722.

Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environmental Science and Technology 46, 6453–6454.

Rillig, M.C., Lehmann, A., de Souza Machado, A.A., Yang, G., 2019. Microplastic effects on plants. New Phytologist 223, 1066–1070.

Rillig, M.C., Ziersch, L., Hempel, S., 2017. Microplastic transport in soil by earthworms. Scientific Reports 7, 1362.

Rochman, C.M., 2018. Microplastics research-from sink to source. Science 360, 28–29.

Rochaab, R.J.M., Rodriguesab, A., Camposab, D., Cícerob, L.H., Costaab, A., Silvaa, D., Oliveiraab, M., Soaresab, A., Patrício Silvaab, A.L., 2020. Do microplastics affect the zoanthid *Zoanthus sociatus*? The Science of the Total Environment 713, 136659.

Saarma, K., Tarkka, M.T., Itavaara, M., Fagerstedt, K.V., 2003. Heat shock protein synthesis is induced by diethyl phthalate but not by di(2-ethylhexyl) phthalate in radish (*Raphanus sativus*). Journal of Plant Physiology 160, 1001–1010.

Sanaullah, M., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2012. Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labelling. Soil Biology and Biochemistry 55, 132–139.

Sharma, S., Chatterjee, S., 2017. Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science & Pollution Research 24, 21530–21547.

Song, X., Razavi, B., Ludwig, B., Zamanian, K., Zang, H., Kuzyakov, Y., Dippold, M., Gunina, A., 2020. Combined biochar and nitrogen application stimulates enzyme activity and root plasticity. The Science of the Total Environment 735, 139393.
Syberg, K., Khan, F.R., Selck, H., Palmqvist, A., Banta, G.T., Daley, J., Sano, L., Duhaime, M.B., 2005. Microplastics: addressing ecological risk through lessons learned. Environmental Toxicology & Chemistry 34, 945–953.
Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age. Philosophical Transactions of the Royal Society of London B Biological Sciences 364, 1973–1976.
Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environment and Pollution 193, 65–70.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.
Wang, J., Lv, S., Zhang, M., Chen, G., Zhu, T., Zhang, S., Teng, Y., Christie, P., Luo, Y., 2016. Effects of plastic film residues on occurrence of phthalates and microbial activity in soil. Chemosphere 151, 171–177.
Wang, X., Yadong, Y., Pei, K., Zhou, J., Peixoto, L., Gunina, A., Zeng, Z., Zang, H., Rasmussen, J., Kuzyakov, Y., 2020. Nitrogen rhizodeposition of legumes and its fate in agroecosystems: field study and literature review. Land Degradation & Development. https://doi.org/10.1002/ldr. 3729.
Weithmann, N., Möller, J.N., Löder, M.G., Piehl, S., Laforsch, C., Freitag, R., 2018. Organic fertilizer as a vehicle for the entry of microplastic into the environment. Science Advances 4 eaap. 8060.
Wen, Y., Zang, H., Freeman, B., Musarika, S., Evans, C., Chadwick, D., Jones, D., 2019a. Microbial utilization of low molecular weight organic carbon substrates in cultivated peats in response to warming and soil degradation. Soil Biology and Biochemistry 140, 107629.
Wen, Y., Zang, H., Ma, Q., Evans, C.D., Chadwick, D.R., Jones, D.L., 2019b. Is the 'enzyme latch' or 'iron gate' the key to protecting soil organic carbon in peatlands. Geoderma 349, 107–113.
Wen, Y., Freeman, B., Ma, Q., Chadwick, D.R., Evans, C.D., Zang, H., Jones, D.L., 2020. Raising the groundwater table in the non-growing season can reduce greenhouse gas emissions and maintain crop productivity in cultivated fen peats. Journal of Cleaner Production 262, 121179.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environment and Pollution 178, 483–492.
Wu, J., Joergensen, R.G., Pommering, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation extraction - an automated procedure. Soil Biology and Biochemistry 22, 1167–1169.
Yang, J., Cao, W.D., Rui, Y.K., 2017. Interactions between nanoparticles and plants: phytotoxicity and defense mechanisms. Journal of Plant Interactions 12, 158–169.
Zang, H., Wang, J., Kuzyakov, Y., 2016. N fertilization decreases soil organic matter decomposition in the rhizosphere. Applied Soil Ecology 108, 47–53.
Zang, H., Blagodatskaya, E., Wang, J., Xu, X., Kuzyakov, Y., 2017. Nitrogen fertilization increases rhizodeposit incorporation into microbial biomass and reduces soil organic matter losses. Biology and Fertility of Soils 53, 419–429.
Zang, H., Blagodatskaya, E., Wen, Y., Xu, X., Kuzyakov, Y., 2018. Carbon sequestration and turnover in soil under the energy crop *Miscanthus*: repeated ^{13}C natural abundance approach and literature synthesis. GCB Bioenergy 10, 262–271.
Zang, H., Xiao, M., Wang, Y., Ling, N., Wu, J., Ge, T., Kuzyakov, Y., 2019. Allocation of assimilated carbon in paddies depending on rice age, chase period and N fertilization: experiment with $^{13}CO_2$ labelling and literature synthesis. Plant and Soil 445, 113–123.
Zang, H., Blagodatskaya, E., Wen, Y., Shi, L., Cheng, F., Chen, H., Zhao, B., Zhang, F., Fan, M., Kuzyakov, Y., 2020. Temperature sensitivity of soil organic matter mineralization decreases with long-term N fertilization: evidence from four Q_{10} estimation approaches. Land Degradation & Development 31, 683–693.
Zhang, X., Kuzyakov, Y., Zang, H., Dippold, M., Shi, L., Spielvogel, S., Razavi, B., 2020. Rhizosphere hotspots: root hairs and warming control microbial efficiency, carbon utilization and energy production. Soil Biology and Biochemistry 149, 107872.
Zhou, J., Zang, H., Loeppmann, S., Gube, M., Kuzyakov, Y., Pausch, J., 2020. Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. Soil Biology and Biochemistry 140, 107641.