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1 **Field application of biodegradable microplastics has no**
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Abstract

Bioplastics (biodegradable plastics) potentially offer an encouraging alternative to conventional (petroleum-based) plastics. However, biodegradable plastics, in practice, inevitably generate a large number of bio-microplastics (bio-MPs, diameter < 5 mm) during the degradation progress. However, the impact of bio-MPs on plant and soil health within the agroecosystem remains incomplete. Here, we investigated the effect of pure polylactic acid (PLA) bio-MPs with 0.2% (w/w) loading in two shapes (fiber and powder) on oat (*Avena sativa* L.) and soybean (*Glycine max* L.) growth, and soil health in a field-based study. Our results showed that PLA application had no significant effect on soil enzyme activities, soil physicochemical properties (soil water content, pH, etc.), root characteristics, plant biomass, and crop yield. Thus, we conclude that soil quality, plant health, and ecosystem multifunctionality were not affected by PLA over one growing season (5 months) in the presence of either bio-MPs shapes (fiber and powder) or crop species (oat and soybean). Overall, we conclude that PLA based bio-MPs may not pose a significant threat to agroecosystem functions in the short term (days to months) and may provide a viable environmentally benign solution to replace traditional non-biodegradable plastics in agroecosystems.

Keywords: Biodegradable microplastics; Soil enzyme activities; Soil quality; Plastic mulch film; Ecosystem multifunctionality

1. Introduction

Plastic mulch films provide multiple benefits for crop production (controlling weeds, reducing evaporation and soil erosion, increasing the soil and air temperature), and are thus widely used in agroecosystems all over the world (Gao et al., 2021; Griffin-LaHue et al., 2022). However, improper disposal of agricultural plastic mulch eventually leads to the dispersal of microplastics (MPs, diameter < 5 mm) into agricultural soils and the wider environment (Astner et al., 2019; Rillig and Lehmann, 2020). This dispersal poses a considerable threat to food and ecological security (Huang et al., 2020b; Zhang et al., 2020). Biodegradable plastic mulch is being used as an alternative to reduce plastic pollution in agricultural soils (Flury and Narayan, 2021), as bioplastics can be readily converted into CO₂, water, nutrient ions and the formation of microbial biomass (Yu et al., 2021). Since bioplastics are more susceptible to rapid degradation, more biodegradable microplastics (bio-MPs) might be generated, in the short term, than conventional plastics within the same time frame, probably leading to more severe bio-MPs pollution and associated effects (Liao and Chen, 2021; Shruti and Kutralam-Muniasamy, 2019). To date, the potential risks of non-biodegradable MPs to the environment and human health have been widely discussed and lots of evidence has shown their detrimental effects on plant and soil health (Li et al., 2020; Xiao et al., 2022; Yu et al., 2022). By contrast, research on the ecological effects of bio-MPs is still in its infancy (Wang et al., 2022; Zhou et al., 2021a). Consequently, fundamental and in-depth studies regarding the effects of bio-MPs on agricultural ecosystems are needed.

Given that soils provide the most basic and diverse services to ecosystems,

maintaining soil health is key to agricultural sustainability (Brown et al., 2022; Kopittke et al., 2019). The potential threat of MPs to the soil ecosystem function and stability has attracted more and more attention (Rillig, 2012; Zhao et al., 2022). Some studies have reported that bio-MPs increased soil aggregate, pH, and nutrient retention (Lozano et al., 2021a; Lozano et al., 2021b); while others have observed no impact on soil biochemical properties (e.g. pH, soil carbon and nitrogen, as well as enzyme activities) (Mazzon et al., 2022; Qi et al., 2020a). The paucity and inconsistency of the available results highlight the need for a comprehensive assessment of how bio-MPs affect soil quality indices (SQI) (Kuzyakov et al., 2020). Bio-MPs can also provide available C to increase microbial biomass (depending on the native soil microbial communities' carbon use efficiency; Sinsabaugh et al., 2016) and intensify soil N immobilization and thus plant N limitation (Yu et al., 2021; Zhou et al., 2021a). This may potentially aggravate the nutrient competition between plants and microorganisms and consequently inhibit plant growth (Zang et al., 2020).

In addition, plants can also be directly affected by MPs (Chen et al., 2022), mainly associated with their physical interaction with roots altering characteristics and consequently plant growth (Lee et al., 2022; Yang and Gao, 2022). The net effect of all these individual functions can alter the overall ecosystem function, however, there is a lack of studies holistically addressing the bio-MPs effect on ecosystem multifunctionality (EMF, the ability of an ecosystem to deliver multiple functions simultaneously) (Manning et al., 2018). Moreover, most studies are limited to laboratory-based experiments (Baho et al., 2021; Zang et al., 2020; Zhou et al., 2022),

so it is imperative that studies on the ecological effects of bio-MPs on agroecosystems are undertaken at the field scale.

Here, as two typical grain crops in the semi-arid region of China (an area with a long history of plastic mulching) (Huang et al., 2020b), oat and soybean have been selected to evaluate the effects of bio-MPs on plant-soil health. Polylactic acid (PLA) is one of the most well-known bioplastics, and it has been proved to be an effective substitute for petroleum-based counterparts (Ainali et al., 2022). Equally, plastic fibers and powders are two of the most ubiquitous forms of MPs in soils, with contrasting impacts on soil functions (Lozano et al., 2021b). Herein, we used two types of PLA (i.e., powder and fiber) with a normal level of agricultural soil pollution (0.2%, w/w) (de Souza Machado et al., 2018; Huang et al., 2020b) to explore the effect on soil biochemical properties and plant growth. We hypothesize that bio-MPs would profoundly change soil quality and plant growth, thereby the *in situ* EMF would be altered by bio-MPs regardless of microplastic shapes and crop species.

2. Materials and methods

2.1. Experimental site

The experiment was carried out at Ertai Town, Zhangbei County (41°21'N, 114°54'E), located northwest of Hebei Province, with a temperate continental monsoon climate. The mean temperature is 16.6 °C and the mean rainfall is 373.8 mm (mainly concentrated in July and August) during the growth period (from May to September) of the past five years. The site has no previous history of plastic application. The soil is

classified as a Haplic Kastanozem (IUSS Working Group WRB, 2015) with initial properties as follows: soil organic carbon, 7.5 g kg⁻¹; total nitrogen, 0.97 g kg⁻¹; mineral nitrogen, 2.0 mg kg⁻¹; available phosphorus, 5.0 mg kg⁻¹; and pH (H₂O), 8.0.

2.2. Experimental design and plant and soil sampling

In mid-May 2021, a completely randomized design was established with four replicates ($n = 4$) for each treatment. Each experimental plot (2.0 × 2.0 m) was then treated with commercial PLA microplastic powder or fiber (Zhonglian Plastics Technology Co., Ltd., Fujian Province, China), at a rate of 0.2% (w/w) by thorough manual mixing with the top 20 cm of soil. That is, there were three treatments in this study: 1) PLA microplastic powder, 2) PLA microplastic fiber, and 3) Control (without microplastic addition). Subsequently, oat (*Avena sativa* L., cv. Bayou 14) and soybean (*Glycine max* (L.) Merr., cv. Jizhangdou 2) were planted. The agricultural practices applied in the experimental plots were based on local recommendations and followed standard farmer practice. Fertilization and artificial irrigation were not arranged during the growing season.

After crop harvest in mid-September 2021, soils were sampled from each plot at 0–20 cm. Two sub-samples were pooled to form a mixed soil sample in each of the four field replicates. The samples were passed through a 2-mm sieve after removing the roots, litter, debris, and stones. Each soil sample was then stored at 4 °C for soil enzyme activities and chemical properties analysis which were performed within 5 days.

Oat and soybean were destructively sampled from each plot, and plants per plot

were cut at the base and divided into aboveground (shoot) and belowground (root) components. With the position of the stem as the centre of the core, we manually excavated the root system from surrounding soils in the 0-20 cm soil layer (more than 50% of the root biomass is located in this soil layer) with a hand trowel. The plant samples were subsequently used for the determination of dry biomass and root characteristic parameters.

2.3. Soil quality assessment

Soil bulk density (BD) was determined on an oven-dry basis by the cutting ring method. Soil water content (SWC) was analyzed by drying at 105 °C until the weight remained stable. Soil pH and electrical conductivity (EC) were measured using a pH meter and conductivity meter (DDS-307, Rex Electric Chemical, China), respectively, in a soil suspension with a soil-water ratio of 1:2.5 (w/v). Total nitrogen (TN) content was analyzed by the semi-micro Kjeldahl method (Bao, 2000). Ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) were both determined using a spectrophotometer (1510, ThermoFisher, USA) after extraction of 5.0 g fresh soil with 20 mL 0.05 M K_2SO_4 . Available phosphorus (Olsen-P) was analyzed by the Olsen method (Olsen et al., 1982) via extracting soil samples with 0.5 M NaHCO_3 .

The activities of six hydrolases enzymes: C-related (β -glucosidase, BG; β -xylosidase, BX; β -cellobiosidase, CBH), N-related (leucine aminopeptidase, LAP; β -1,4-N-acetyl-glucosaminidase, NAG), and P-related (alkaline phosphatase, ALP) cycling were fluorogenically measured using labeled substrates (Zang et al., 2020).

Briefly, 50 mL sterile water was added to 1.0 g of fresh soil and suspended by shaking for 30 min at a speed of 200 rev min⁻¹. An equal amount of 50 µL soil suspension was pipetted into 96-well microplates, and then 50 µL buffer and 100 µL of the substrate at a concentration of 400 µM were added (the enzyme substrates are shown in Table S1). At 60 and 120 min after substrate addition, the microplates were fluorometrically determined at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Phenoloxidase (POX) and peroxidase (PER) activities were spectrophotometrically assayed with 96-well microplates and the substrate of L-DOPA (DeForest, 2009). The enzyme activities are expressed as nmol g⁻¹ dry soil h⁻¹.

Here, we further combined enzyme activities as indicators of specific substrates or nutrient acquisition, which divided into four parts: C acquisition (C-acq), N acquisition (N-acq), P acquisition (P-acq), and oxidative decomposition (OX). They were calculated as the average value of multiple enzyme activities as follows (Luo et al., 2018):

$$\text{C-acq} = (\text{BG} + \text{BX} + \text{CBH}) / 3 \quad (1)$$

$$\text{N-acq} = (\text{LAP} + \text{NAG}) / 2 \quad (2)$$

$$\text{P-acq} = \text{ALP} / 1 \quad (3)$$

$$\text{OX} = (\text{POX} + \text{PER}) / 2 \quad (4)$$

2.4. Plant quality assessment

At the physiological maturity stage, grain yield for each plot was determined by collecting the plants from a full length of a middle row, dried, and corrected to 13%

grain moisture content to calculate grain yield (t ha^{-1}). The root samples were washed with running water to obtain the complete root systems in each plant. WinRHIZO software (Regent Instruments Inc., Canada) was used to analyze scanned images of the roots to determine the root length, root surface area, and root volume. The scanned roots were then collected and dried to constant weight before recording their weights (root biomass). Also, aboveground plant samples were dried at 80 °C for 72 h to determine shoot biomass after the height recorded.

2.5. Calculation of soil quality index and plant growth index

Soil quality index (SQI) was calculated using an SQI-area approach by comparing the area on a radar graph comprising all soil parameters that equal to the sum of individual triangles comprising the whole figure (Kuzyakov et al., 2020):

$$\text{SQI-area} = 0.5 \cdot \sum_i^n stSP_i^2 \cdot \sin\left(\frac{2 \cdot \pi}{n}\right) \quad (5)$$

where n was the number of soil parameters used for the SQI, and $stSP_i$ was the standardized soil parameter i varying from 0 to 1 using the following equation (Eq. (6) or (7)):

$$stSP_i = \frac{SP}{SP_{max}} \quad (6)$$

$$stSP_i = \frac{SP_{min}}{SP} \quad (7)$$

where SP , SP_{max} , and SP_{min} were the measured, maximum, and minimum mean values of the soil parameter i , respectively. According to the response direction of soil quality, soil parameters were divided into two categories. A *more is better* scoring curve (Eq. (6)) was used when the level of the parameter increased with soil quality

improvement (i.e., SWC, TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, Olsen-P, and enzyme activities), otherwise a *less is better* scoring curve (Eq. (7)) was used (i.e., BD, pH, EC).

Similarly, the plant growth index (PGI), a single comprehensive index reflecting plant growth, was determined in a similar way to the SQI calculation:

$$\text{PGI-area} = 0.5 \cdot \sum_i^n stPP_i^2 \cdot \sin\left(\frac{2\pi}{n}\right) \quad (8)$$

where n was the number of plant parameters used for the PGI, and $stPP_i$ was the standardized plant parameter i varying from 0 to 1 using the following equation:

$$stPP_i = \frac{PP}{PP_{max}} \quad (9)$$

where PP and PP_{max} were the measured and maximum mean values of the plant parameter i , respectively. A *more is better* scoring curve (Eq. (9)) was used for all plant parameters.

2.6. Quantification of ecosystem multifunctionality

We followed the ecosystem function multifunctionality method (Lozano et al., 2021a) to calculate ecosystem multifunctionality (EMF). Briefly, we identified 19 ecosystem functions, which included the majority of soil functions (except for BD, SWC, pH, and EC) and all plant parameters measured in this study (Garland et al., 2021). This cluster analysis allowed us to give more even weights to the ecosystem functions as they are interrelated and shared drivers. We determined the number of clusters by the Elbow method (Kassambara and Mundt, 2020), and weighted each of them equally, irrespective of the number of functions within each cluster. Then, we calculated the standardized maximum for each function and placed the function data on

a standardized scale. Thus, we calculated EMF using the threshold approach, in which each ecosystem function that exceeds 50% of the maximum contributed to the ecosystem multifunctionality score with its respective weighted value obtained after clustering. The medium threshold (~50%) was a conservative choice with high responsiveness (Manning et al., 2018).

2.7. Statistical analysis

The Shapiro-Wilk test was conducted to determine the normality of data distribution within each variable group. Levene's test was used to determine the homogeneity of square differences between the two groups of variables with normal distribution. An independent sample t-test was performed once the square differences between the two groups were equal; otherwise, an adjusted t-test (i.e., Welch's t-test) was performed. Mann-Whitney U test was used for comparison between groups with non-normal distribution. All data were analyzed using IBM SPSS Statistics 26 (IBM, USA). The histograms were drawn by SigmaPlot 14.0 (Systat Software Inc., USA), and the radar graphs, as well as the heatmap, were drawn by Origin 2021 (OriginLab Corp., USA). A combination graph of correlation heat map of soil and plant parameters and mantel test line was drawn using the R package ("ggcor") with the R 4.1.2 (Huang et al., 2020a; R Core Team, 2021).

3. Results

3.1. Soil enzyme activity

PLA fiber significantly improved the soil N-acq (the sum of NAG and LAP) activity by 37% compared to Control with oat ($P < 0.01$; Fig. 1c), whilst it did not affect that with soybean ($P = 0.77$; Fig. 1g). Moreover, bio-MPs shapes (i.e., powder and fiber) did not impact the C-acq, P-acq, and OX activities in soil planted with oat and soybean compared with the Control treatment ($P = 0.06-0.89$; Fig. 1).

3.2. Soil quality and plant growth

In soil planted with oat, similar SQI scores were observed between bio-MPs powder and fiber ($P = 0.96$; Fig. 2b), although they marginally increased SQI compared with Control ($P = 0.11$). In soil planted with soybean, bio-MPs powder slightly decreased the SQI compared with Control ($P = 0.12$), while there was no difference in SQI between bio-MPs fiber and Control ($P = 0.70$; Fig. 2d).

Bio-MPs powder and fiber marginally increased the PGI score of oat by 18% ($P = 0.44$) and 47% ($P = 0.06$; Fig. 3b) compared with the Control. Also, the PGI of soybean did not show a difference in response to bio-MPs, regardless of fiber and powder ($P = 0.87$; Fig. 3d).

3.3. Ecosystem multifunctionality

EMF was marginally increased by bio-MPs fiber in soil planted with oat by 69% ($P = 0.17$) and 89% ($P = 0.15$) compared with Control and bio-MPs powder, whilst there was no significant difference in EMF under oat between bio-MPs powder and Control ($P = 0.74$; Fig. 4a). By contrast, both bio-MPs shapes slightly decreased EMF

under soybean by 14-29% compared with Control ($P = 0.29-0.61$; Fig. 4b). The EMF score was mainly influenced by SWC ($r > 0.4$) and plant growth parameters (also known as PGI) ($r > 0.2$, $P < 0.05$; Fig. 4c).

4. Discussion

4.1. Soil enzyme activity response to bio-MPs addition

Soil enzyme activities are vital to a range of soil functions and are considered one of the most sensitive indicators of soil quality (Jabborova et al., 2021; Sheteiwy et al., 2021). The frequent determinations of four combined functional enzyme activities (C-, N-, P-acq, and OX activities) are usually related to soil microbial nutrient limitation and biochemical processes (Khosrozadeh et al., 2022; Lasota et al., 2022). It is generally the case that bio-MPs are C-rich but nutrient-poor (Zhou et al., 2021a), which triggers soil microorganisms to respond to a lack of nutrients (e.g. N, P; Zang et al., 2020). However, legume N-fixation could alleviate soil N deficiency caused by the microbial immobilization of N under bio-MPs addition (as a source of relatively labile C; Song et al., 2020), thus bio-MPs did not increase the N-acq enzyme activity for legume soils (Fig. 1). Additionally, here we observed no significant differences between C-acq enzyme activities for either plastic shape. These findings are contradictory to the observed significant positive effects on soil C-acq enzyme activities previously shown under 10% poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (Zhou et al., 2021a), 1% Mater-Bi (Mazzon et al., 2022), and 2% PLA (Chen et al., 2020) addition. Equally, the discrepancy could be due to the different types of bio-MPs used given that the

mineralization of PLA is slow relative to the PHB (15.5% vs. 84.3%) within the first 230 days after application (Schopfer et al., 2022), as well as the distinctly different microbial C partitioning dynamics in the field rather than laboratory (Oburger and Jones, 2009). Therefore, PLA may be likely to become bioavailable as a viable C source over a long period (years to decades) (Chamas et al., 2020), and, as such, did not cause major shifts in enzyme activities in the timescale measured here (months). On the other hand, the effect of bio-MPs on enzyme activity may also be concentration-dependent. For example, one study reported by Bandopadhyay et al. (2018) documented that with high concentrations of bio-MPs (i.e., 2.0%, 2.5%, w/w), promoted the growth of microorganisms through labile C addition. In short, the type and loading concentration of bio-MPs incorporated into the soil will likely dictate the biological and ecological effects exhibited.

4.2. Effect of bio-MPs on soil quality and plant health

Soil is a fundamental part of the ecosystem and contributes essentially to the cycles of all elements that are critical to crop growth and food production (Bunemann et al., 2018; Kuzyakov et al., 2020). We found bio-MPs had no significant effect on SQI based on many soil indicators (as shown in Fig. 2), which indicated that key soil properties were not fundamentally affected by PLA addition. Specifically, contrary to the common expectation that the degradation of PLA will decrease soil pH due to the generation of lactic acid (Karamanlioglu and Robson, 2013), we observed that the soil exposed to 0.2% PLA did not affect pH in the field (Fig. 2). This could be ascribed to that the natural

environment in the field having a stronger buffering capacity and a higher tolerance for bio-MPs addition (Qi et al., 2020a), which is not reflected in the limited space and controlled temperature and moisture conditions under laboratory conditions. Realistically, bioplastic may accumulate in the soil, particularly in colder and dryer climates (Satti et al., 2018), as application rates may exceed biotic and abiotic degradation rates (Nandakumar et al., 2021). As such, the effect of bio-MPs on soil properties may be concentration-dependent and temporally variable, potentially increasing over time. In the short term, the lower dose (0.2%) of PLA bio-MPs are unlikely to cause a significant shift in soil C/N ratio and N deficiency (Fig. 2), whereas higher doses (or longer term accumulation) of bio-MPs may affect the C/N ratio of the soil (Qi et al., 2020b) and are likely to have a larger impact on soil stoichiometry and associated soil microbial function (Aanderud et al., 2018). Analogously, we found that both bio-MPs shapes had no significant effect on root characteristics and the productivity of either oat or soybean (Fig. 3). This was contrary to a previous greenhouse study, which resulted from the constant soil moisture and other factors in controlled conditions (i.e., temperature, light, nutrient availability) (Yang et al., 2021; Zeb et al., 2022; Zhou et al., 2021a). A previous review also confirmed that the effects of bio-MPs on plant growth are highly dependent on types and concentrations (Zhou et al., 2021b). However, we note that longer term monitoring is required to understand the full extent of the impact of bioplastics and subsequent bio-MPs on the agroecosystem.

4.3. Ecosystem multifunctionality as affected by bio-MPs

Ecosystems have the ability to simultaneously provide multiple functions (Manning et al., 2018), as the biotic and abiotic processes that occur and contribute to ecosystem services either directly or indirectly (Garland et al., 2021), thereby uniformly called EMF. Our results found that bio-MPs incorporation did not affect EMF for both oat and soybean cropping systems (Fig. 4a, b). The non-significant differences in plant growth and soil moisture largely determined the absence of EMF alteration by bio-MPs, confirmed by the significant correlation between PGI, SWC, and EMF (Fig. 4c). Moisture increases the likelihood of hydrolytic breakdown of the ester linkages in PLA polymer (Elsawy et al., 2017), consequently, PLA can be hydrolyzed and form water-soluble low molecular weight oligomers, which may act as additional C sources for microbial assimilation and subsequently affect SQI, PGI, and EMF. However, soil moisture was relatively low in our field site due to limited precipitation (374 mm) in the semi-arid region, which may have hindered the decomposition of bio-MPs and its subsequent effect on EMF. This is supported by Lozano et al. (2021a) who found that bio-MPs fibers reduced soil functions only under well-watered conditions.

4.4. Implications and future research direction

Although the effects of bio-MPs on soil and plant were studied over the course of a one-season field trial, their impact on agricultural ecosystems is still not fully understood. Clearly, the multi-site experimental data and further microbial analysis are needed in the future, in order to better understand the long-term ecological impact of supposedly harmless bio-MPs on the soil environment (Fan et al., 2022). Equally, the

degradation rates of bioplastics and bio-MPs in the field should be monitored over longer time periods (e.g. 10 years) to understand the impact of climate and soil type on the accumulation rates over multiple cropping cycles and the subsequent effect on soil and plant health and EMF. Further, depending on how they were produced, bio-MPs can be divided into natural polymers and synthetic polymers (Pellis et al., 2021). However, research has focused on a limited selection of bio-MPs (i.e., PLA and PHB) that have mainly been used for determination in the greenhouse or field (Liao and Chen, 2021), calling for an expansion of the types and concentrations of bio-MPs.

5. Conclusions

This study showed that the field application of PLA had no significant effect on soil biochemical properties, root characteristics, plant biomass, and ecosystem multifunctionality over one growing season (5 months), regardless of bio-MPs shapes (fiber and powder) and crop species (oat and soybean). Although bio-MPs themselves may not be beneficial to plant-soil health, they do not appear to pose a significant threat to agroecosystem functioning. Our evidence therefore suggests that biodegradable plastics may provide a viable alternative to replace conventional non-biodegradable plastics. Further work should be conducted focusing on the effects of bio-MPs types and concentrations on ecosystem multifunctionality in the multi-site field trials over longer time scales (years to decades).

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Reference

- Aanderud, Z.T., Saurey, S., Ball, B.A., Wall, D.H., Barrett, J.E., Muscarella, M.E., Griffin, N.A., Virginia, R.A., Adams, B.J., 2018. Stoichiometric shifts in soil C:N:P promote bacterial taxa dominance, maintain biodiversity, and deconstruct community assemblages. *Front. Microbiol.* 9, 1401. <https://doi.org/10.3389/fmicb.2018.01401>.
- Ainali, N.M., Kalaronis, D., Evgenidou, E., Kyzas, G.Z., Bobori, D.C., Kaloyianni, M., Yang, X., Bikiaris, D.N., Lambropoulou, D.A., 2022. Do poly(lactic acid) microplastics instigate a threat? A perception for their dynamic towards environmental pollution and toxicity. *Sci. Total Environ.* 832, 155014. <https://doi.org/10.1016/j.scitotenv.2022.155014>.
- Astner, A.F., Hayes, D.G., O'Neill, H., Evans, B.R., Pingali, S.V., Urban, V.S., Young, T.M., 2019. Mechanical formation of micro- and nano-plastic materials for environmental studies in agricultural ecosystems. *Sci. Total Environ.* 685, 1097-1106. <https://doi.org/10.1016/j.scitotenv.2019.06.241>.
- Baho, D.L., Bundschuh, M., Futter, M.N., 2021. Microplastics in terrestrial ecosystems: Moving beyond the state of the art to minimize the risk of ecological surprise. *Glob. Change Biol.* 27, 3969-3986. <https://doi.org/10.1111/gcb.15724>.
- Bandopadhyay, S., Martin-Closas, L., Pelacho, A.M., DeBruyn, J.M., 2018. Biodegradable plastic mulch films: impacts on soil microbial communities and ecosystem functions. *Front. Microbiol.* 9, 819. <https://doi.org/10.3389/fmicb.2018.00819>.

392 Bao, S.D., 2000. Soil agrochemical analysis, China Agricultural Press, Beijing.

393 Brown, R.W., Chadwick, D.R., Thornton, H., Marshall, M.R., Bei, S.K., Distaso, M.A.,
394 Bargiela, R., Marsden, K.A., Clode, P.L., Murphy, D.V., Pagella, S., Jones, D.L.,
395 2022. Field application of pure polyethylene microplastic has no significant short-
396 term effect on soil biological quality and function. *Soil Biol. Biochem.* 165,
397 108496. <https://doi.org/10.1016/j.soilbio.2021.108496>.

398 Bunemann, E.K., Bongiorno, G., Bai, Z.G., Creamer, R.E., De Deyn, G., de Goede, R.,
399 Fleskens, L., Geissen, V., Kuyper, T.W., Mader, P., Pulleman, M., Sukkel, W., van
400 Groenigen, J.W., Brussaard, L., 2018. Soil quality - A critical review. *Soil Biol.*
401 *Biochem.* 120, 105-125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.

402 Chamas, A., Moon, H., Zheng, J.J., Qiu, Y., Tabassum, T., Jang, J.H., Abu-Omar, M.,
403 Scott, S.L., Suh, S., 2020. Degradation rates of plastics in the environment. *ACS*
404 *Sustain. Chem. Eng.* 8, 3494-3511.
405 <https://doi.org/10.1021/acssuschemeng.9b06635>.

406 Chen, G.L., Li, Y.Z., Liu, S.L., Junaid, M., Wang, J., 2022. Effects of
407 micro(nano)plastics on higher plants and the rhizosphere environment. *Sci. Total*
408 *Environ.* 807, 150841. <https://doi.org/10.1016/j.scitotenv.2021.150841>.

409 Chen, H.P., Wang, Y.H., Sun, X., Peng, Y.K., Xiao, L., 2020. Mixing effect of polylactic
410 acid microplastic and straw residue on soil property and ecological function.
411 *Chemosphere* 243. <https://doi.org/10.1016/j.chemosphere.2019.125271>.

412 de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig,
413 M.C., 2018. Impacts of microplastics on the soil biophysical environment. *Environ.*

414 Sci. Technol. 52, 9656-9665. <https://doi.org/10.1021/acs.est.8b02212>.

415 DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on
 416 potential soil enzyme activity in acidic forest soils using MUB-linked substrates
 417 and L-DOPA. Soil Biol. Biochem. 41, 1180-1186.
 418 <https://doi.org/10.1016/j.soilbio.2009.02.029>.

419 Elsayy, M.A., Kim, K.H., Park, J.W., Deep, A., 2017. Hydrolytic degradation of
 420 polylactic acid (PLA) and its composites. Renew. Sust. Energ. Rev. 79, 1346-1352.
 421 <https://doi.org/10.1016/j.rser.2017.05.143>.

422 Fan, P., Yu, H., Xi, B.D., Tan, W.B., 2022. A review on the occurrence and influence of
 423 biodegradable microplastics in soil ecosystems: Are biodegradable plastics
 424 substitute or threat? Environ. Int. 163, 107244.
 425 <https://doi.org/10.1016/j.envint.2022.107244>.

426 Flury, M., Narayan, R., 2021. Biodegradable plastic as an integral part of the solution
 427 to plastic waste pollution of the environment. Curr. Opin. Green Sustain. Chem.
 428 30, 100490. <https://doi.org/10.1016/j.cogsc.2021.100490>.

429 Gao, X.H., Xie, D., Yang, C., 2021. Effects of a PLA/PBAT biodegradable film mulch
 430 as a replacement of polyethylene film and their residues on crop and soil
 431 environment. Agric. Water Manag. 255, 107053.
 432 <https://doi.org/10.1016/j.agwat.2021.107053>.

433 Garland, G., Banerjee, S., Edlinger, A., Oliveira, E.M., Herzog, C., Wittwer, R.,
 434 Philippot, L., Maestre, F.T., van der Heijden, M.G.A., 2021. A closer look at the
 435 functions behind ecosystem multifunctionality: A review. J. Ecol. 109, 600-613.

436 <https://doi.org/10.1111/1365-2745.13511>.

437 Griffin-LaHue, D., Ghimire, S., Yu, Y.X., Scheenstra, E.J., Miles, C.A., Flury, M., 2022.

438 In-field degradation of soil-biodegradable plastic mulch films in a Mediterranean

439 climate. *Sci. Total Environ.* 806, 150238.

440 <https://doi.org/10.1016/j.scitotenv.2021.150238>.

441 Huang, H.Y., Zhou, L., Chen, J., Wei, T.Y., 2020a. ggcor: Extended tools for correlation

442 analysis and visualization. R package version 0.9.8.

443 Huang, Y., Liu, Q., Jia, W.Q., Yan, C.R., Wang, J., 2020b. Agricultural plastic mulching

444 as a source of microplastics in the terrestrial environment. *Environ. Pollut.* 260,

445 114096. <https://doi.org/10.1016/j.envpol.2020.114096>.

446 IUSS Working Group WRB, 2015. World reference base for soil resources 2014, FAO,

447 Rome, Italy.

448 Jabborova, D., Annapurna, K., Paul, S., Kumar, S., Saad, H.A., Desouky, S., Ibrahim,

449 M.F.M., Elkelish, A., 2021. Beneficial features of biochar and arbuscular

450 mycorrhiza for improving spinach plant growth, root morphological traits,

451 physiological properties, and soil enzymatic activities. *J Fungi* 7, 571.

452 <https://doi.org/10.3390/jof7070571>.

453 Karamanlioglu, M., Robson, G.D., 2013. The influence of biotic and abiotic factors on

454 the rate of degradation of poly(lactic) acid (PLA) coupons buried in compost and

455 soil. *Polym. Degrad. Stab.* 98, 2063-2071.

456 <https://doi.org/10.1016/j.polymdegradstab.2013.07.004>.

457 Kassambara, A., Mundt, F., 2020. factoextra: extract and visualize the results of

458 multivariate data analyses. R package version 1.0.7, [https://CRAN.R-](https://CRAN.R-project.org/package=factoextra)
 459 [project.org/package=factoextra](https://CRAN.R-project.org/package=factoextra).
 460 Khosrozadeh, S., Guber, A., Kravchenko, A., Ghaderi, N., Blagodatskaya, E., 2022.
 461 Soil oxidoreductase zymography: Visualizing spatial distributions of peroxidase
 462 and phenol oxidase activities at the root-soil interface. *Soil Biol. Biochem.* 167,
 463 108610. <https://doi.org/10.1016/j.soilbio.2022.108610>.
 464 Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and
 465 the intensification of agriculture for global food security. *Environ. Int.* 132, 105078.
 466 <https://doi.org/10.1016/j.envint.2019.105078>.
 467 Kuzyakov, Y., Gunina, A., Zamanian, K., Tian, J., Luo, Y., Xu, X.L., Yudina, A., Aponte,
 468 H., Alharbi, H., Ovsepyan, L., 2020. New approaches for evaluation of soil health,
 469 sensitivity and resistance to degradation. *Front. Agr. Sci. Eng.* 7, 282-288.
 470 <https://doi.org/10.15302/J-FASE-2020338>.
 471 Lasota, J., Babiak, T., Blonska, E., 2022. C:N:P stoichiometry associated with biochar
 472 in forest soils at historical charcoal production sites in Poland. *Geoderma Reg.* 28,
 473 e00482. <https://doi.org/10.1016/j.geodrs.2022.e00482>.
 474 Lee, T.-Y., Kim, L., Kim, D., An, S., An, Y.-J., 2022. Microplastics from shoe sole
 475 fragments cause oxidative stress in a plant (*Vigna radiata*) and impair soil
 476 environment. *J. Hazard. Mater.* 429, 128306.
 477 <https://doi.org/10.1016/j.jhazmat.2022.128306>.
 478 Li, Z.X., Li, Q.F., Li, R.J., Zhao, Y.F., Geng, J.H., Wang, G.Y., 2020. Physiological
 479 responses of lettuce (*Lactuca sativa* L.) to microplastic pollution. *Environ. Sci.*

480 Pollut. Res. 27, 30306-30314. <https://doi.org/10.1007/s11356-020-09349-0>.

481 Liao, J., Chen, Q.Q., 2021. Biodegradable plastics in the air and soil environment: Low
 482 degradation rate and high microplastics formation. J. Hazard. Mater. 418, 126329.
 483 <https://doi.org/10.1016/j.jhazmat.2021.126329>.

484 Lozano, Y.M., Aguilar-Trigueros, C.A., Onandia, G., Maass, S., Zhao, T.T., Rillig, M.C.,
 485 2021a. Effects of microplastics and drought on soil ecosystem functions and
 486 multifunctionality. J. Appl. Ecol. 58, 988-996. [https://doi.org/10.1111/1365-
 487 2664.13839](https://doi.org/10.1111/1365-2664.13839).

488 Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A., Rillig, M.C., 2021b. Microplastic
 489 shape, polymer type, and concentration affect soil properties and plant biomass.
 490 Front. Plant Sci. 12, 169. <https://doi.org/10.3389/fpls.2021.616645>.

491 Luo, G.W., Li, L., Friman, V.P., Guo, J.J., Guo, S.W., Shen, Q.R., Ling, N., 2018.
 492 Organic amendments increase crop yields by improving microbe-mediated soil
 493 functioning of agroecosystems: A meta-analysis. Soil Biol. Biochem. 124, 105-
 494 115. <https://doi.org/10.1016/j.soilbio.2018.06.002>.

495 Manning, P., van der Plas, F., Soliveres, S., Allan, E., Maestre, F.T., Mace, G.,
 496 Whittingham, M.J., Fischer, M., 2018. Redefining ecosystem multifunctionality.
 497 Nat. Ecol. Evol. 2, 427-436. <https://doi.org/10.1038/s41559-017-0461-7>.

498 Mazzon, M., Gioacchini, P., Montecchio, D., Rapisarda, S., Ciavatta, C., Marzadori, C.,
 499 2022. Biodegradable plastics: Effects on functionality and fertility of two different
 500 soils. Appl. Soil Ecol. 169, 104216. <https://doi.org/10.1016/j.apsoil.2021.104216>.

501 Nandakumar, A., Chuah, J.A., Sudesh, K., 2021. Bioplastics: A boon or bane? Renew.

502 Sust. Energ. Rev. 147, 111237. <https://doi.org/10.1016/j.rser.2021.111237>.

503 Oburger, E., Jones, D.L., 2009. Substrate mineralization studies in the laboratory show
504 different microbial C partitioning dynamics than in the field. Soil Biol. Biochem.
505 41, 1951-1956. <https://doi.org/10.1016/j.soilbio.2009.06.020>.

506 Olsen, S.R., Sommers, L.E., Page, A.L., Miller, R., Keeney, D., 1982. Chemical and
507 microbiological properties. Methods of soil analysis, part 2, 416-418.

508 Pellis, A., Malinconico, M., Guarneri, A., Gardossi, L., 2021. Renewable polymers and
509 plastics: Performance beyond the green. New Biotechnol. 60, 146-158.
510 <https://doi.org/10.1016/j.nbt.2020.10.003>.

511 Qi, Y.L., Beriot, N., Gort, G., Lwanga, E.H., Gooren, H., Yang, X.M., Geissen, V.,
512 2020a. Impact of plastic mulch film debris on soil physicochemical and
513 hydrological properties. Environ. Pollut. 266, 115097.
514 <https://doi.org/10.1016/j.envpol.2020.115097>.

515 Qi, Y.L., Ossowicki, A., Yang, X.M., Lwanga, E.H., Dini-Andreote, F., Geissen, V.,
516 Garbeva, P., 2020b. Effects of plastic mulch film residues on wheat rhizosphere
517 and soil properties. J. Hazard. Mater. 387, 121711.
518 <https://doi.org/10.1016/j.jhazmat.2019.121711>.

519 R Core Team, 2021. R: A language and environment for statistical computing. R
520 Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org/>.

521 Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci.
522 Technol. 46, 6453-6454. <https://doi.org/10.1021/es302011r>.

523 Rillig, M.C., Lehmann, A., 2020. Microplastic in terrestrial ecosystems. Science 368,

1430-1431. <https://doi.org/10.1126/science.abb5979>.

Satti, S.M., Shah, A.A., Marsh, T.L., Auras, R., 2018. Biodegradation of poly(lactic acid) in soil microcosms at ambient temperature: Evaluation of natural attenuation, bio-augmentation and bio-stimulation. *J. Polym. Environ.* 26, 3848-3857. <https://doi.org/10.1007/s10924-018-1264-x>.

Schopfer, L., Schnepf, U., Marhan, S., Brummer, F., Kandeler, E., Pagel, H., 2022. Hydrolyzable microplastics in soil-low biodegradation but formation of a specific microbial habitat? *Biol. Fertil. Soils* 58, 471-486. <https://doi.org/10.1007/s00374-022-01638-9>.

Sheteiwy, M.S., Ali, D.F.I., Xiong, Y.C., Brestic, M., Skalicky, M., Hamoud, Y.A., Ulhassan, Z., Shaghaleh, H., Abdelgawad, H., Farooq, M., Sharma, A., El-Sawah, A.M., 2021. Physiological and biochemical responses of soybean plants inoculated with Arbuscular mycorrhizal fungi and Bradyrhizobium under drought stress. *BMC Plant Biol.* 21, 1-21. <https://doi.org/10.1186/s12870-021-02949-z>.

Shruti, V.C., Kuttralam-Muniasamy, G., 2019. Bioplastics: Missing link in the era of Microplastics. *Sci. Total Environ.* 697, 134139. <https://doi.org/10.1016/j.scitotenv.2019.134139>.

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Shah, J.J.F., 2016. Stoichiometry of microbial carbon use efficiency in soils. *Ecol. Monogr.* 86, 172-189. <https://doi.org/10.1890/15-2110.1>.

Song, C., Sarpong, C.K., He, J.S., Shen, F., Zhang, J., Yang, G., Long, L.L., Tian, D., Zhu, Y., Deng, S.H., 2020. Accelerating phytoremediation of degraded agricultural

546 soils utilizing rhizobacteria and endophytes: a review. Environ. Rev. 28, 115-127.
547 <https://doi.org/10.1139/er-2019-0020>.

548 Wang, F.Y., Wang, Q.L., Adams, C.A., Sun, Y.H., Zhang, S.W., 2022. Effects of
549 microplastics on soil properties: Current knowledge and future perspectives. J.
550 Hazard. Mater. 424, 127531. <https://doi.org/10.1016/j.jhazmat.2021.127531>.

551 Xiao, M.L., Ding, J.N., Luo, Y., Zhang, H.Q., Yu, Y.X., Yao, H.Y., Zhu, Z.K., Chadwick,
552 D.R., Jones, D., Chen, J.P., Ge, T.D., 2022. Microplastics shape microbial
553 communities affecting soil organic matter decomposition in paddy soil. J. Hazard.
554 Mater. 431, 128589. <https://doi.org/10.1016/j.jhazmat.2022.128589>.

555 Yang, C., Gao, X.H., 2022. Impact of microplastics from polyethylene and
556 biodegradable mulch films on rice (*Oryza sativa* L.). Sci. Total Environ. 828,
557 154579. <https://doi.org/10.1016/j.scitotenv.2022.154579>.

558 Yang, W.W., Cheng, P., Adams, C.A., Zhang, S.W., Sun, Y.H., Yu, H.W., Wang, F.Y.,
559 2021. Effects of microplastics on plant growth and arbuscular mycorrhizal fungal
560 communities in a soil spiked with ZnO nanoparticles. Soil Biol. Biochem. 155,
561 108179. <https://doi.org/10.1016/j.soilbio.2021.108179>.

562 Yu, Y.F., Li, J., Song, Y., Zhang, Z.Y., Yu, S.G., Xu, M.L., Zhao, Y.Y., 2022. Stimulation
563 versus inhibition: The effect of microplastics on pak choi growth. Appl. Soil Ecol.
564 177, 104505. <https://doi.org/10.1016/j.apsoil.2022.104505>.

565 Yu, Y.X., Griffin-LaHue, D.E., Miles, C.A., Hayes, D.G., Flury, M., 2021. Are micro-
566 and nanoplastics from soil-biodegradable plastic mulches an environmental
567 concern? J. Hazard. Mater. Adv. 4, 100024.

568 <https://doi.org/10.1016/j.hazadv.2021.100024>.

569 Zang, H.D., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y., Jones, D.L., 2020.

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

571 system? Soil Biol. Biochem. 148, 10.

572 <https://doi.org/10.1016/j.soilbio.2020.107926>.

573 Zeb, A.R., Liu, W.T., Meng, L.Z., Lian, J.P., Wang, Q., Lian, Y.H., Chen, C.H., Wu,

574 J.N., 2022. Effects of polyester microfibers (PMFs) and cadmium on lettuce

575 (*Lactuca sativa*) and the rhizospheric microbial communities: A study involving

576 physio-biochemical properties and metabolomic profiles. J. Hazard. Mater. 424,

577 127405. <https://doi.org/10.1016/j.jhazmat.2021.127405>.

578 Zhang, D., Ng, E.L., Hu, W.L., Wang, H.Y., Galaviz, P., Yang, H.D., Sun, W.T., Li, C.X.,

579 Ma, X.W., Fu, B., Zhao, P.Y., Zhang, F.L., Jin, S.Q., Zhou, M.D., Du, L.F., Peng,

580 C., Zhang, X.J., Xu, Z.Y., Xi, B., Liu, X.X., Sun, S.Y., Cheng, Z.H., Jiang, L.H.,

581 Wang, Y.F., Gong, L., Kou, C.L., Li, Y., Ma, Y.H., Huang, D.F., Zhu, J., Yao, J.W.,

582 Lin, C.W., Qin, S., Zhou, L.Q., He, B.H., Chen, D.L., Li, H.C., Zhai, L.M., Lei,

583 Q.L., Wu, S.X., Zhang, Y.T., Pan, J.T., Gu, B.J., Liu, H.B., 2020. Plastic pollution

584 in croplands threatens long-term food security. Glob. Change Biol. 26, 3356-3367.

585 <https://doi.org/10.1111/gcb.15043>.

586 Zhao, S.L., Zhang, Z.Q., Chen, L., Cui, Q.L., Cui, Y.X., Song, D.X., Fang, L.C., 2022.

587 Review on migration, transformation and ecological impacts of microplastics in

588 soil. Appl. Soil Ecol. 176, 104486. <https://doi.org/10.1016/j.apsoil.2022.104486>.

589 Zhou, J., Gui, H., Banfield, C.C., Wen, Y., Zang, H.D., Dippold, M.A., Charlton, A.,

590 Jones, D.L., 2021a. The microplastisphere: Biodegradable microplastics addition
 591 alters soil microbial community structure and function. *Soil Biol. Biochem.* 156,
 592 108211. <https://doi.org/10.1016/j.soilbio.2021.108211>.

593 Zhou, J., Wen, Y., Cheng, H.G., Zang, H.D., Jones, D.L., 2022. Simazine degradation
 594 in agroecosystems: Will it be affected by the type and amount of microplastic
 595 pollution? *Land Degrad. Dev.* 33, 1128-1136. <https://doi.org/10.1002/ldr.4243>.

596 Zhou, J., Wen, Y., Marshall, M.R., Zhao, J., Gui, H., Yang, Y.D., Zeng, Z.H., Jones,
 597 D.L., Zang, H.D., 2021b. Microplastics as an emerging threat to plant and soil
 598 health in agroecosystems. *Sci. Total Environ.* 787, 147444.
 599 <https://doi.org/10.1016/j.scitotenv.2021.147444>.

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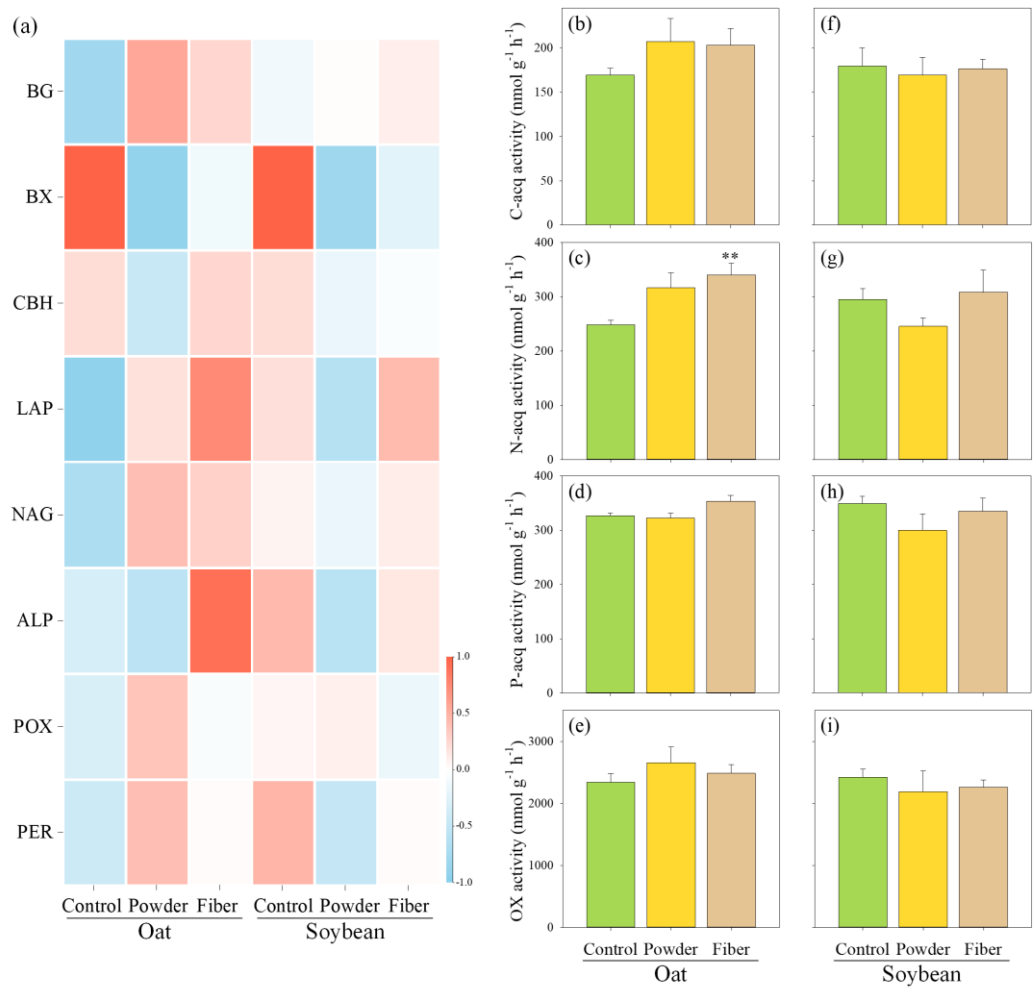
Figure captions

Fig. 1. Soil enzyme activities in oat and soybean cropping system. Panel (a) represent Z-Score standard enzyme activities with a color scale, an increase, and a decrease in the activity being indicated by the intensity of red and blue color. Panels (b-i) show four grouped enzyme activities. C-acquiring enzymes include β -glucosidase, BG; β -xylosidase, BX; and β -cellobiosidase, CBH. N-acquiring enzymes contain leucine aminopeptidase, LAP; and β -1,4-N-Acetyl-glucosaminidase, NAG. P-acquiring enzyme refers to alkaline phosphatase, ALP. Oxidative decomposition enzymes involve phenol oxidase, POX; and peroxidase, PER. Values are averages \pm standard errors ($n = 4$). Asterisk indicates a statistically significant difference from the Control treatment (**, $P < 0.01$).

Fig. 2. Soil quality index (SQI) area (b, d) and radar chart of the relative response of soil parameters (a, c) in oat (a, b) and soybean (c, d) cropping system. BD, bulk density; SWC, soil water content; EC, electrical conductivity; TN, total nitrogen; NH_4^+ -N, ammonium nitrogen; NO_3^- -N, nitrate nitrogen; Olsen-P, available phosphorus; C-acq, carbon acquisition enzyme activity; N-acq, nitrogen acquisition enzyme activity; P-acq, phosphorus acquisition enzyme activity; OX, oxidative decomposition enzyme activity. Values are averages \pm standard errors ($n = 4$). No statistically significant differences were observed between the polylactic acid microplastics treatments and the Control ($P > 0.05$).

Fig. 3. Plant growth index (PGI) area (b, d) and radar chart of relative response of plant parameters (a, c) in oat (a, b) and soybean (c, d) cropping system. Values are averages \pm standard errors ($n = 4$). No statistically significant differences are present between the polylactic acid microplastics treatments and the Control ($P > 0.05$).

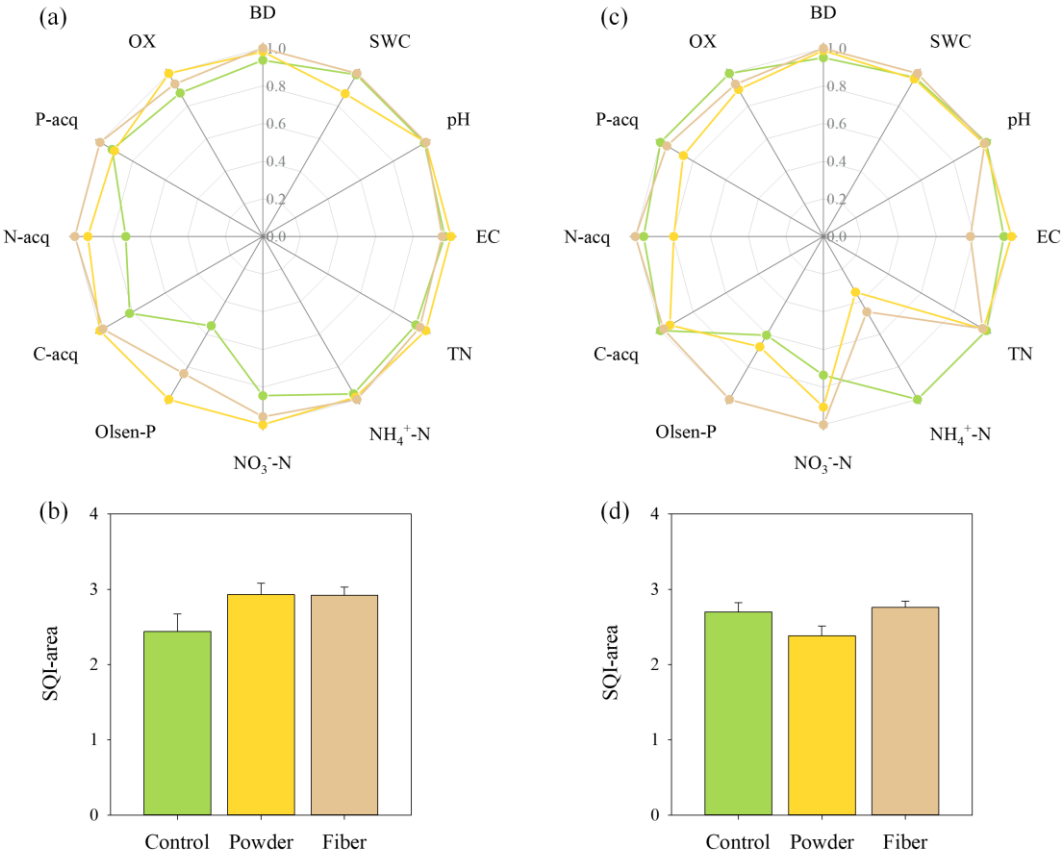
Fig. 4. Ecosystem multifunctionality (EMF) in oat (a) and soybean (b) cropping system and its correlation with plant and soil parameters (c). Pairwise comparisons of environmental factors are shown in the upper right corner, with a color gradient denoting Pearson's correlation coefficients. Ecosystem multifunctionality was related to each soil environmental factor by Mantel test. Edge width corresponds to the Mantel's r statistic for the corresponding distance correlations, and edge color denotes the statistical significance.



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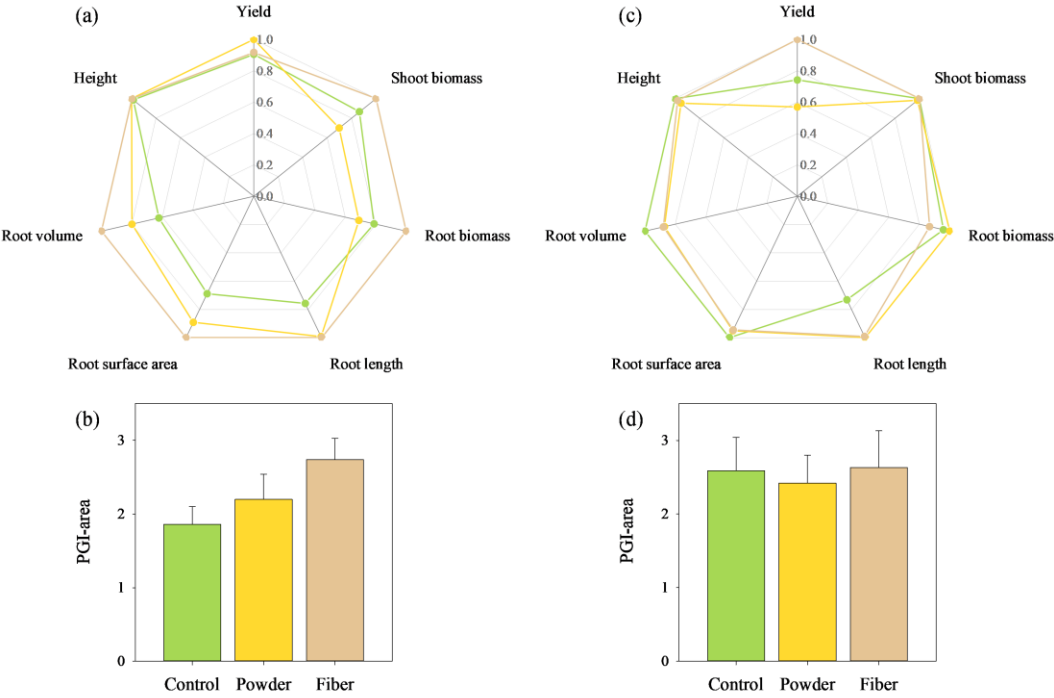
639 Fig. 2



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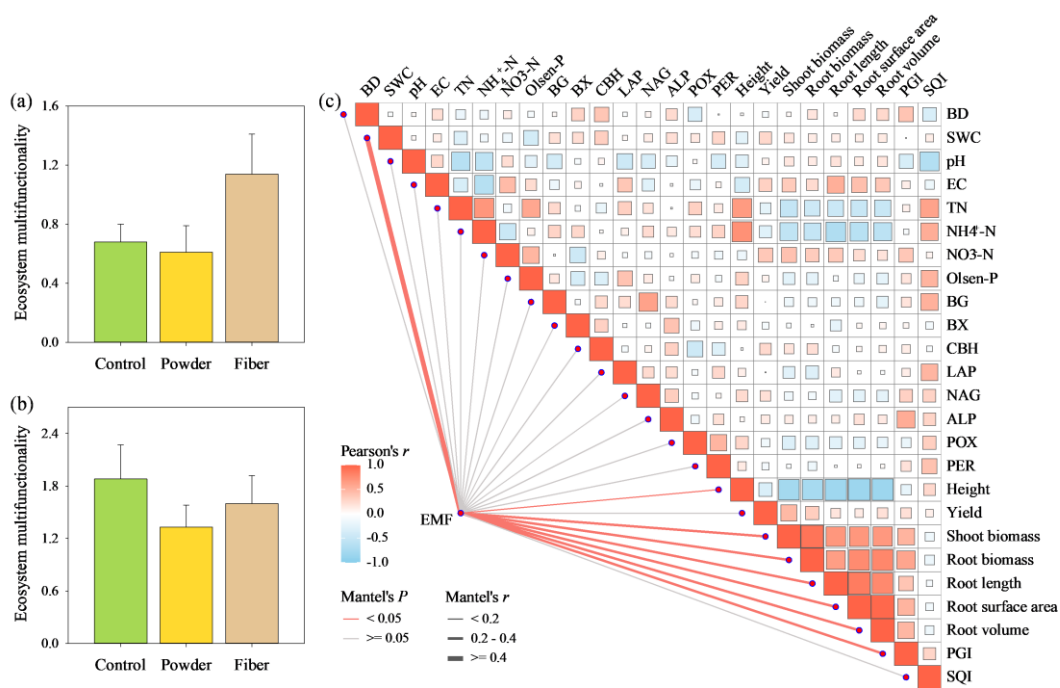
642 Fig. 3



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645 Fig. 4



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Supplementary

Table S1. Enzymes assayed in soil samples, with corresponding enzyme commission (EC) numbers, substrates, functions and abbreviations. 4-MUB, 4-methylumbelliferyl; L-DOPA, L-3,4-dihydroxyphenylalanine.

Enzyme	Substrate	EC	Abbreviation
β -Glucosidase	4-MUB- β -D-glucoside	3.2.1.21	BG
β -Xylosidase	4-MUB- β -D-xyloside	3.2.1.37	BX
β -Cellobiosidase	4-MUB- β -D-cellobioside	3.2.1.91	CBH
L-Leucine aminopeptidase	L-Leucine-7-amino-4-methylcoumarin	3.4.11.1	LAP
β -1,4-N-Acetyl-glucosaminidase	4-MUB-N-acetyl- β -D-glucosaminide	3.2.1.30	NAG
Alkaline phosphatase	4-MUB-phosphate	3.1.3.1	ALP
Phenol oxidase	L-DOPA	1.10.3.2	POX
Peroxidase	L-DOPA	1.11.1.7	PER