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Field application of biodegradable microplastics has no significant effect on plant and soil health in the short term

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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 CO_2, 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 ℃ 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

101 classified as a Haplic Kastanozem (IUSS Working Group WRB, 2015) with initial
102 properties as follows: soil organic carbon, 7.5 g kg^{-1}; total nitrogen, 0.97 g kg^{-1}; mineral
103 nitrogen, 2.0 mg kg^{-1}; available phosphorus, 5.0 mg kg^{-1}; and pH (H_2O), 8.0.
104
105 *2.2. Experimental design and plant and soil sampling*
106 In mid-May 2021, a completely randomized design was established with four
107 replicates (n = 4) for each treatment. Each experimental plot (2.0 × 2.0 m) was then
108 treated with commercial PLA microplastic powder or fiber (Zhonglian Plastics
109 Technology Co., Ltd., Fujian Province, China), at a rate of 0.2% (w/w) by thorough
110 manual mixing with the top 20 cm of soil. That is, there were three treatments in this
111 study: 1) PLA microplastic powder, 2) PLA microplastic fiber, and 3) Control (without
112 microplastic addition). Subsequently, oat (*Avena sativa* L., cv. Bayou 14) and soybean
113 (*Glycine max* (L.) Merr., cv. Jizhangdou 2) were planted. The agricultural practices
114 applied in the experimental plots were based on local recommendations and followed
115 standard farmer practice. Fertilization and artificial irrigation were not arranged during
116 the growing season.
117 After crop harvest in mid-September 2021, soils were sampled from each plot at 0-
118 20 cm. Two sub-samples were pooled to form a mixed soil sample in each of the four
119 field replicates. The samples were passed through a 2-mm sieve after removing the
120 roots, litter, debris, and stones. Each soil sample was then stored at 4 °C for soil enzyme
121 activities and chemical properties analysis which were performed within 5 days.
122 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 (NH_4^+-N) and nitrate (NO_3^--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):

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

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

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

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

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

189 improvement (i.e., SWC, TN, NH_4^+-N, NO_3^--N, Olsen-P, and enzyme activities),
190 otherwise a *less is better* scoring curve (Eq. (7)) was used (i.e., BD, pH, EC).
191 Similarly, the plant growth index (PGI), a single comprehensive index reflecting
192 plant growth, was determined in a similar way to the SQI calculation:
193 $$\text{PGI-area} = 0.5 \cdot \sum_i^n stPP_i^2 \cdot \sin\left(\frac{2 \cdot \pi}{n}\right) \quad (8)$$
194 where n was the number of plant parameters used for the PGI, and $stPP_i$ was the
195 standardized plant parameter i varying from 0 to 1 using the following equation:
196 $$stPP_i = \frac{PP}{PP_{max}} \quad (9)$$
197 where PP and PP_{max} were the measured and maximum mean values of the plant
198 parameter i, respectively. A *more is better* scoring curve (Eq. (9)) was used for all plant
199 parameters.
200
201 *2.6. Quantification of ecosystem multifunctionality*
202 We followed the ecosystem function multifunctionality method (Lozano et al.,
203 2021a) to calculate ecosystem multifunctionality (EMF). Briefly, we identified 19
204 ecosystem functions, which included the majority of soil functions (except for BD,
205 SWC, pH, and EC) and all plant parameters measured in this study (Garland et al.,
206 2021). This cluster analysis allowed us to give more even weights to the ecosystem
207 functions as they are interrelated and shared drivers. We determined the number of
208 clusters by the Elbow method (Kassambara and Mundt, 2020), and weighted each of
209 them equally, irrespective of the number of functions within each cluster. Then, we
210 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

277 mineralization of PLA is slow relative to the PHB (15.5% vs. 84.3%) within the first
278 230 days after application (Schopfer et al., 2022), as well as the distinctly different
279 microbial C partitioning dynamics in the field rater than laboratory (Oburger and Jones,
280 2009). Therefore, PLA may be likely to become bioavailable as a viable C source over
281 a long period (years to decades) (Chamas et al., 2020), and, as such, did not cause major
282 shifts in enzyme activities in the timescale measured here (months). On the other hand,
283 the effect of bio-MPs on enzyme activity may also be concentration-dependent. For
284 example, one study reported by Bandopadhyay et al. (2018) documented that with high
285 concentrations of bio-MPs (i.e., 2.0%, 2.5%, w/w), promoted the growth of
286 microorganisms through labile C addition. In short, the type and loading concentration
287 of bio-MPs incorporated into the soil will likely dictate the biological and ecological
288 effects exhibited.
289
290 *4.2. Effect of bio-MPs on soil quality and plant health*
291 Soil is a fundamental part of the ecosystem and contributes essentially to the cycles
292 of all elements that are critical to crop growth and food production (Bunemann et al.,
293 2018; Kuzyakov et al., 2020). We found bio-MPs had no significant effect on SQI based
294 on many soil indicators (as shown in Fig. 2), which indicated that key soil properties
295 were not fundamentally affected by PLA addition. Specifically, contrary to the common
296 expectation that the degradation of PLA will decrease soil pH due to the generation of
297 lactic acid (Karamanlioglu and Robson, 2013), we observed that the soil exposed to 0.2%
298 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 Elsawy, 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.

https://doi.org/10.1111/1365-2745.13511.

Griffin-LaHue, D., Ghimire, S., Yu, Y.X., Scheenstra, E.J., Miles, C.A., Flury, M., 2022. In-field degradation of soil-biodegradable plastic mulch films in a Mediterranean climate. Sci. Total Environ. 806, 150238. https://doi.org/10.1016/j.scitotenv.2021.150238.

Huang, H.Y., Zhou, L., Chen, J., Wei, T.Y., 2020a. ggcor: Extended tools for correlation analysis and visualization. R package version 0.9.8.

Huang, Y., Liu, Q., Jia, W.Q., Yan, C.R., Wang, J., 2020b. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environ. Pollut. 260, 114096. https://doi.org/10.1016/j.envpol.2020.114096.

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

Jabborova, D., Annapurna, K., Paul, S., Kumar, S., Saad, H.A., Desouky, S., Ibrahim, M.F.M., Elkelish, A., 2021. Beneficial features of biochar and arbuscular mycorrhiza for improving spinach plant growth, root morphological traits, physiological properties, and soil enzymatic activities. J Fungi 7, 571. https://doi.org/10.3390/jof7070571.

Karamanlioglu, M., Robson, G.D., 2013. The influence of biotic and abiotic factors on the rate of degradation of poly(lactic) acid (PLA) coupons buried in compost and soil. Polym. Degrad. Stab. 98, 2063-2071. https://doi.org/10.1016/j.polymdegradstab.2013.07.004.

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

multivariate data analyses. R package version 1.0.7, https://CRAN.R-project.org/package=factoextra.

Khosrozadeh, S., Guber, A., Kravchenko, A., Ghaderi, N., Blagodatskaya, E., 2022. Soil oxidoreductase zymography: Visualizing spatial distributions of peroxidase and phenol oxidase activities at the root-soil interface. Soil Biol. Biochem. 167, 108610. https://doi.org/10.1016/j.soilbio.2022.108610.

Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. Environ. Int. 132, 105078. https://doi.org/10.1016/j.envint.2019.105078.

Kuzyakov, Y., Gunina, A., Zamanian, K., Tian, J., Luo, Y., Xu, X.L., Yudina, A., Aponte, H., Alharbi, H., Ovsepyan, L., 2020. New approaches for evaluation of soil health, sensitivity and resistance to degradation. Front. Agr. Sci. Eng. 7, 282-288. https://doi.org/10.15302/J-FASE-2020338.

Lasota, J., Babiak, T., Blonska, E., 2022. C:N:P stoichiometry associated with biochar in forest soils at historical charcoal production sites in Poland. Geoderma Reg. 28, e00482. https://doi.org/10.1016/j.geodrs.2022.e00482.

Lee, T.-Y., Kim, L., Kim, D., An, S., An, Y.-J., 2022. Microplastics from shoe sole fragments cause oxidative stress in a plant (*Vigna radiata*) and impair soil environment. J. Hazard. Mater. 429, 128306. https://doi.org/10.1016/j.jhazmat.2022.128306.

Li, Z.X., Li, Q.F., Li, R.J., Zhao, Y.F., Geng, J.H., Wang, G.Y., 2020. Physiological responses of lettuce (*Lactuca sativa* L.) to microplastic pollution. Environ. Sci.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

524 1430-1431. https://doi.org/10.1126/science.abb5979.
525 Satti, S.M., Shah, A.A., Marsh, T.L., Auras, R., 2018. Biodegradation of poly(lactic
526 acid) in soil microcosms at ambient temperature: Evaluation of natural attenuation,
527 bio-augmentation and bio-stimulation. J. Polym. Environ. 26, 3848-3857.
528 https://doi.org/10.1007/s10924-018-1264-x.
529 Schopfer, L., Schnepf, U., Marhan, S., Brummer, F., Kandeler, E., Pagel, H., 2022.
530 Hydrolyzable microplastics in soil-low biodegradation but formation of a specific
531 microbial habitat? Biol. Fertil. Soils 58, 471-486. https://doi.org/10.1007/s00374-
532 022-01638-9.
533 Sheteiwy, M.S., Ali, D.F.I., Xiong, Y.C., Brestic, M., Skalicky, M., Hamoud, Y.A.,
534 Ulhassan, Z., Shaghaleh, H., AbdElgawad, H., Farooq, M., Sharma, A., El-Sawah,
535 A.M., 2021. Physiological and biochemical responses of soybean plants
536 inoculated with Arbuscular mycorrhizal fungi and Bradyrhizobium under drought
537 stress. BMC Plant Biol. 21, 1-21. https://doi.org/10.1186/s12870-021-02949-z.
538 Shruti, V.C., Kutralam-Muniasamy, G., 2019. Bioplastics: Missing link in the era of
539 Microplastics. Sci. Total Environ. 697, 134139.
540 https://doi.org/10.1016/j.scitotenv.2019.134139.
541 Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R.,
542 Moorhead, D.L., Shah, J.J.F., 2016. Stoichiometry of microbial carbon use
543 efficiency in soils. Ecol. Monogr. 86, 172-189. https://doi.org/10.1890/15-2110.1.
544 Song, C., Sarpong, C.K., He, J.S., Shen, F., Zhang, J., Yang, G., Long, L.L., Tian, D.,
545 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.

https://doi.org/10.1016/j.hazadv.2021.100024.

Zang, H.D., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y., Jones, D.L., 2020. Microplastics in the agroecosystem: Are they an emerging threat to the plant-soil system? Soil Biol. Biochem. 148, 10. https://doi.org/10.1016/j.soilbio.2020.107926.

Zeb, A.R., Liu, W.T., Meng, L.Z., Lian, J.P., Wang, Q., Lian, Y.H., Chen, C.H., Wu, J.N., 2022. Effects of polyester microfibers (PMFs) and cadmium on lettuce (*Lactuca sativa*) and the rhizospheric microbial communities: A study involving physio-biochemical properties and metabolomic profiles. J. Hazard. Mater. 424, 127405. https://doi.org/10.1016/j.jhazmat.2021.127405.

Zhang, D., Ng, E.L., Hu, W.L., Wang, H.Y., Galaviz, P., Yang, H.D., Sun, W.T., Li, C.X., Ma, X.W., Fu, B., Zhao, P.Y., Zhang, F.L., Jin, S.Q., Zhou, M.D., Du, L.F., Peng, C., Zhang, X.J., Xu, Z.Y., Xi, B., Liu, X.X., Sun, S.Y., Cheng, Z.H., Jiang, L.H., Wang, Y.F., Gong, L., Kou, C.L., Li, Y., Ma, Y.H., Huang, D.F., Zhu, J., Yao, J.W., Lin, C.W., Qin, S., Zhou, L.Q., He, B.H., Chen, D.L., Li, H.C., Zhai, L.M., Lei, Q.L., Wu, S.X., Zhang, Y.T., Pan, J.T., Gu, B.J., Liu, H.B., 2020. Plastic pollution in croplands threatens long-term food security. Glob. Change Biol. 26, 3356-3367. https://doi.org/10.1111/gcb.15043.

Zhao, S.L., Zhang, Z.Q., Chen, L., Cui, Q.L., Cui, Y.X., Song, D.X., Fang, L.C., 2022. Review on migration, transformation and ecological impacts of microplastics in soil. Appl. Soil Ecol. 176, 104486. https://doi.org/10.1016/j.apsoil.2022.104486.

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.
600

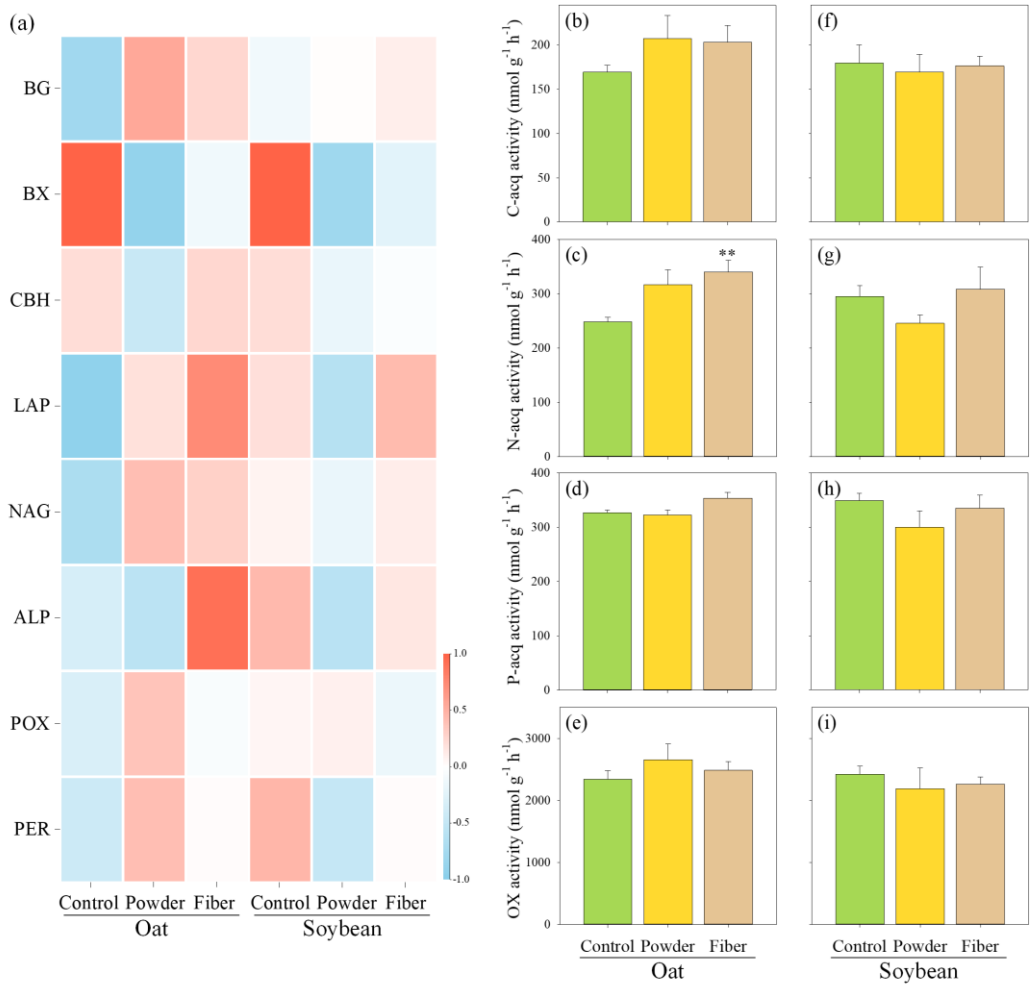
Figure captions

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 ± 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 ± standard errors (n = 4). No statistically significant differences were observed between the polylactic acid microplastics treatments and the Control ($P >$ 0.05).

623 Fig. 3. Plant growth index (PGI) area (b, d) and radar chart of relative response of plant
624 parameters (a, c) in oat (a, b) and soybean (c, d) cropping system. Values are averages
625 ± standard errors ($n = 4$). No statistically significant differences are present between the
626 polylactic acid microplastics treatments and the Control ($P > 0.05$).
627
628 Fig. 4. Ecosystem mutifunctionality (EMF) in oat (a) and soybean (b) cropping system
629 and its correlation with plant and soil parameters (c). Pairwise comparisons of
630 environmental factors are shown in the upper right corner, with a color gradient
631 denoting Pearson's correlation coefficients. Ecosystem multifunctionality was related
632 to each soil environmental factor by Mantel test. Edge width corresponds to the
633 Mantel's r statistic for the corresponding distance correlations, and edge color denotes
634 the statistical significance.
635

636 Fig. 1



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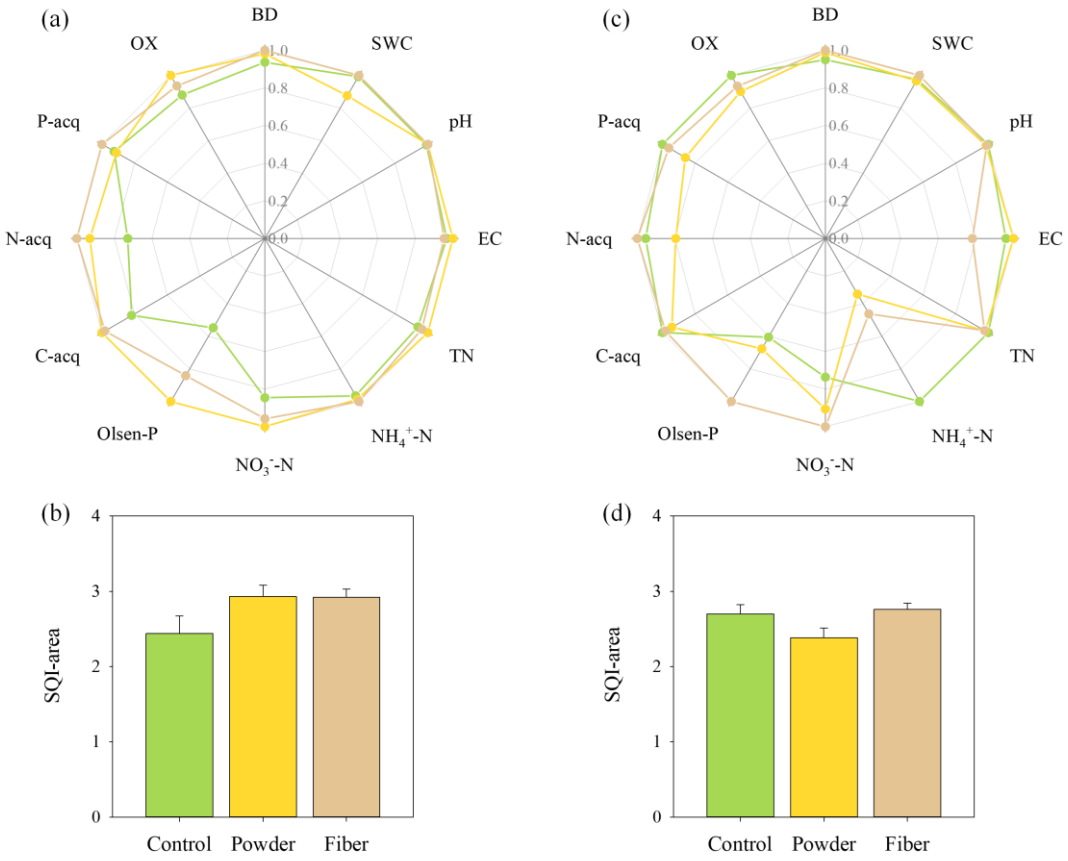
639 Fig. 2



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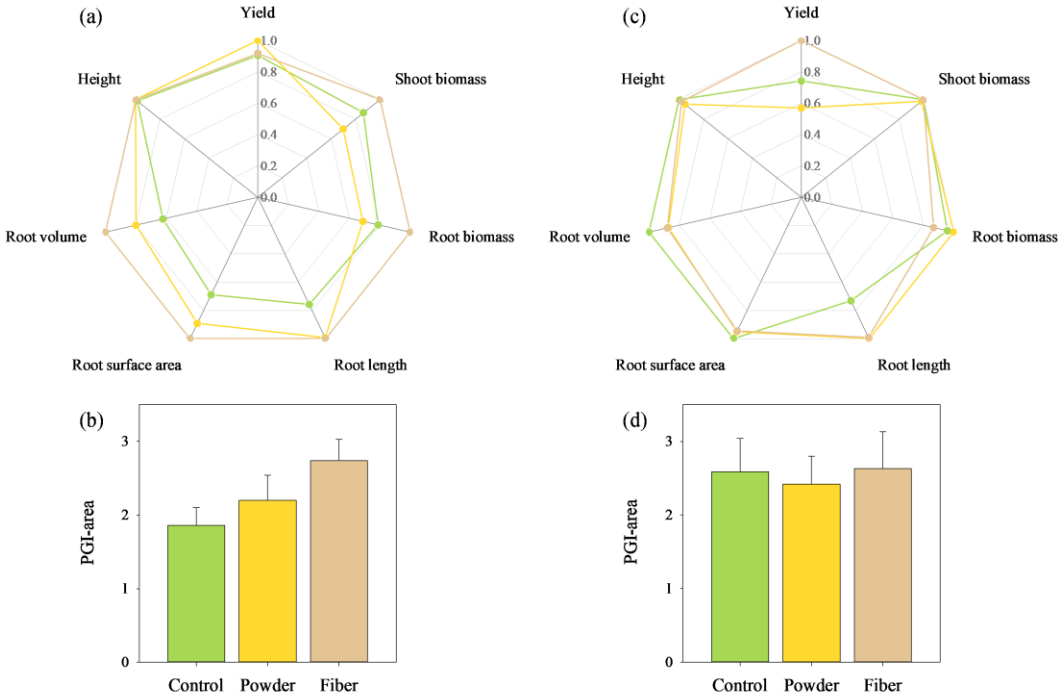
642 Fig. 3



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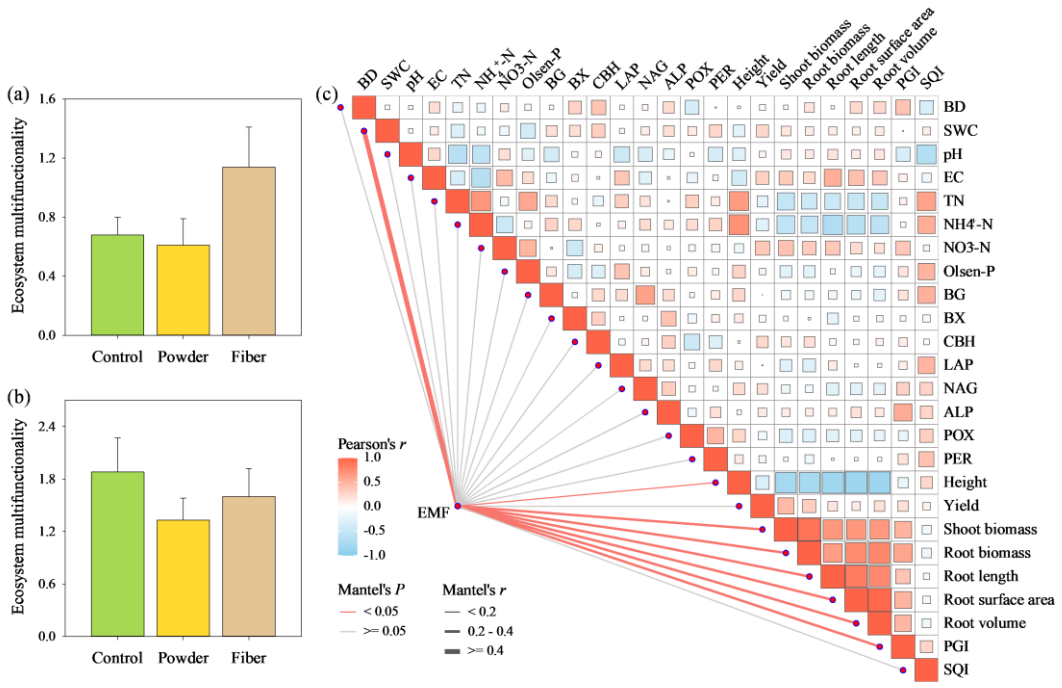
645 Fig. 4



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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