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Enzymes for microplastic-free agricultural soils

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ABSTRACT

Plastic mulch films and biofertilizers (processed sewage sludge, compost or manure) have helped to increase crop yields. However, there is increasing evidence that these practices significantly contribute to microplastic contamination in agricultural soils, affecting biodiversity and soil health. Here, we draw attention to the use of hydrolase enzymes that depolymerize polyester-based plastics as a bioremediation technique for agricultural soils (*in situ*), biofertilizers and irrigation water (*ex situ*), and discuss the need for fully biodegradable plastic mulches. We also highlight the need for ecotoxicological assessment of the proposed approach and its effects on different soil organisms. Enzymes should be optimized to work effectively and efficiently under the conditions found in natural soils (typically, moist solids at an ambient temperature with low salinity). Such optimization is also necessary to ensure that already distressed ecosystems are not disrupted any further.

1. Introduction

Plastic debris in the environment breaks down into microplastics, defined as plastic fragments less than 5 mm in length. Microplastics are accumulating in agricultural soils worldwide, causing negative effects on soil ecosystems while increasing the risk to food safety. Here, we call attention to the use of engineered microbes and plastic-degrading enzymes as a bioremediation technique for agricultural soils and biofertilizers, highlighting the need for fully biodegradable plastic mulches. We also discuss the potential hazards of the proposed approach and the need for ecotoxicity assessment before this promising solution can be used to reduce the level of microplastic contamination in soils.

2. The problem with microplastics in agricultural soils

2.1. Understanding the source of microplastics

Most plastics in agricultural soils originate from farming activities such as the use of mulch films to cover and protect crops, and the application of biofertilizers and irrigation water that is already contaminated with microplastics. A recent study compared 16 agricultural fields in the Netherlands, five using plastic mulches, six using a compost biofertilizer, and five controls that used neither product in at least 5 years (Huerta-Lwanga et al., 2023). The highest microplastic concentration was found in soils exposed to plastic mulch film, followed by those treated with biofertilizer. Lower amounts were detected in the control fields, including microplastics from banned plastic mulch material such as pro-oxidant additive containing (PAC) plastics (oxo-degradable plastics). Plastics that were applied 10–20 years ago therefore persist in the soil as microplastics today.

Plastic mulch films are easily applied and cost-effective, increasing crop yields by limiting water evaporation, keeping soil warm (thus promoting plant growth) and reducing pesticide use. However, it is expensive and time consuming to remove the mulch after the growing season, so at least some is left on the soil, where it disintegrates into microplastics. Processed sewage sludge, compost and manure are often applied as biofertilizers because they contain large amounts of nutrients and organic matter for the crops, increasing soil health and water absorption capacity. However, biofertilizers are also contaminated with microplastics, consequently polluting the soil. Processed sewage sludge is polluted with microplastics (mainly synthetic microfibers), coming from household products, which end up in the domestic grey water for example during laundering. Such grey water is treated in a wastewater treatment plant, where most microplastics sediment in the sludge, although some remain in the effluent water, which is then used for irrigation. Manure is contaminated with microplastics when farm animals are exposed to plastics in their feed, such as plastic nets and films intended to preserve straw. Compost is polluted with microplastics through contamination of the municipal organic waste (usually composed of food or garden waste) with plastic due to improper disposal and insufficient waste separation. Additionally, plastics labelled as "compostable" are collected with such organic fraction, and can remain in the final product depending on the characteristics of the compost treatment.

The repeated use of plastic mulches, biofertilizers and contaminated irrigation water causes the total amount of microplastics to increase each season. Additional sources of plastics in agricultural soil may come from littering (*i.e.* improper disposal of waste, for example on the side of the road) brought to the field by wind, together with airborne microplastics (Huerta-Lwanga et al., 2023).

Abbreviations: EG, ethylene glycol; LCC, leaf-branch compost cutinase; PAC, pro-oxidant additive containing; PHAs, polyhydroxyalkanoates; PLA, polylactic acid; PBAT, polybutyrate adipate co-terephthalate; PET, polyethylene terephthalate; TPA, terephthalic acid.

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2.2. *How do microplastics affect the soil ecosystem?*

A recent meta-analysis summarizes the effects of microplastics on physicochemical soil properties, soil animals, vegetation and microorganisms (Zhang et al., 2022). Based on the summary effect, soil properties are negatively affected, although for some properties, the effect varies depending on polymer type (and their additives), shape, size and abundance. For instance, polyacrylic fibers decreased soil's water holding capacity, whereas polyester microplastic increased it. Independently of shape and type, horizontal and vertical migration of soil water is significantly inhibited by microplastics. Similarly, the total content of carbon, nitrogen and phosphorous in soil were reduced by most types of microplastics. Concerning soil animals, all types of microplastics inhibited animal growth and life span to different degrees. This correlates with the smaller population of soil invertebrates and lower biodiversity found on Dutch farms with a higher amount of microplastics (Huerta-Lwanga et al., 2023). Similarly, many studies have shown a dose-response relationship between microplastic concentration and a decrease in soil microorganism abundance, biomass and diversity. Seed germination and plant growth are also inhibited by microplastics, and crop plants that do grow successfully take up nano and microplastics *via* their roots (Luo et al., 2022) and translocate them to edible tissues such as fruits and storage organs, which are then consumed by humans. This presents a risk to food security and food safety. The accumulation of hazardous materials in crops should be avoided, so we need solutions for the rapid remediation of agricultural soils.

3. Can microplastics biodegrade in soil?

3.1. *Current biodegradable mulch films need enhanced biodegradation options*

Although there are many sources of microplastics, as mentioned above, plastic mulches should be prioritized for action because this is where we purposefully bring plastic in contact with soil. Biodegradable plastics are special polymers whose chemical bonds can be cleaved by microbial enzymes within a reasonable time frame. Specifically, EU norm EN 17033 indicates that biodegradable mulch film has to be 90 % degraded within 2 years in the soil. Most mulch films marketed as fully biodegradable are made from starch and/or polyesters, the latter including polyhydroxyalkanoates (PHAs), polylactic acid (PLA), polybutyrate adipate co-terephthalate (PBAT) and polyethylene terephthalate (PET) and their blends. However, the biodegradation rate varies according to the ambient conditions, so complete breakdown in soil within 2 years is not guaranteed. The rapid degradation of PLA requires temperatures of ~60 °C in industrial compost heaps, and residues therefore remain in the soil for a long time. Accordingly, we require urgent biodegradation options for intended-release products such as plastic mulches (Wei et al., 2020). One potential solution is the use of hydrolytic enzymes, either embedded in the mulch film or applied to the field afterwards to depolymerize remaining fragments.

3.2. *Naturally occurring enzymes are neither efficient nor ubiquitous in the soil ecosystem*

Several organisms in terrestrial ecosystems have been shown to produce plastic-degrading enzymes. One example is leaf-branch compost cutinase (LCC), found in a compost of leaves and branches at 67 °C in Japan, which hydrolyzes ester bonds in PET (Sulaiman et al., 2012). Furthermore, two polyester hydrolases were identified in the bacterium *Ideonella sakaiensis* 201-F6 isolated from a sediment sample outside a bottle-recycling facility in Japan (Yoshida et al., 2016). By comparing the protein sequences with various terrestrial metagenome databases, > 500 genes encoding candidate polyester hydrolases were identified, mainly in *Actinobacteria* and *Proteobacteria* (Danso et al., 2018). Not only microorganisms are candidates for cleaning up the soil. Soil invertebrates, including insect larvae and earthworms, can also ingest plastic and break it down by mechanical grinding, which increases its surface area (Meng et al., 2023). Furthermore, two enzymes in the saliva of waxworms were shown to depolymerize polyethylene (Sanluis-Verdes et al., 2022). However, these organisms are not yet ubiquitous and they break down microplastics very slowly, so neither the organisms nor their enzymes are yet suitable for the remediation of contaminated agricultural soils.

4. Engineered enzymes as bioremediation strategy

To reduce the concentration of microplastics in soil, we recommend the implementation of bioremediation strategies based on biotechnology. This might involve the use of engineered enzymes or microbes, but the latter are currently ruled out by legislation forbidding the deliberate release of genetically modified microorganisms into the environment. We therefore focus solely on enzymatic bioremediation.

4.1. *Enzymes must be optimized for soil bioremediation*

Advances in genetic engineering and enzyme immobilization techniques have facilitated enzyme-based remediation strategies to remove pollutants worldwide using laccases, peroxidases and hydrolases. Hydrolases could be applied as a bioremediation strategy to prevent microplastic pollution (*ex situ* bioremediation of biofertilizers and irrigation water before its addition to the field) and to remove microplastics already present in the soil (*in situ* bioremediation of agricultural soil and mulch residues).

Currently, one of the most active hydrolases against polyester substrates is $\mathrm{LCC}^{\mathrm{ICCG}}$ which surpasses wild type LCC by 160 % (Tournier et al., 2020). This has a temperature optimum of ~70 °C, and large deviations from the optimum significantly reduce its activity. $\mathrm{LCC}^{\mathrm{ICCG}}$ is therefore suitable for the degradation of microplastics in compost (which reaches ~60 °C) but less useful in soil conditions, where the temperature is only ~20 °C. This type of application is logical, given that $\mathrm{LCC}^{\mathrm{ICCG}}$ was originally isolated from a compost sample (Sulaiman et al., 2012). However, it may be possible to adapt hydrolases for the bioremediation of agricultural soil, sewage sludge, manure, and irrigation water by protein engineering, aiming to find structural variants with lower optimal temperatures.

The efficiency of enzymes for bioremediation can also be increased by immobilization because this improves their stability by reducing the risk of denaturation. For example, *Is*PETase was immobilized on supermagnetic iron oxide nanoparticles by adding a poly-histidine tag (Schwaminger et al., 2021). This method is amenable because the same tag is commonly used worldwide for protein purification. Iron oxide nanoparticles can also be captured using magnets to recover the enzyme after the bioremediation treatment.

Finally, there is a need to optimize enzyme activity in the presence of moist solids (rather than diluted aqueous solutions, as most research currently does) because this is a more realistic representation of the soil environment and is in many cases the natural environment of enzymes. Since LCC (Sulaiman et al., 2012) and *Is*PETase (Yoshida et al., 2016) were isolated from a compost and sediment sample respectively, as mentioned above, we do not expect a negative effect of the physical and chemical properties of the soil on the effectiveness of such enzymes or their engineered variants. However, it might be interesting to study interactions that could possibly affect it's activity, particularly since the presence of microplastics in soil have been linked to an stimulation or inhibition on soil enzyme activity (for example of phosphatases, catalases and ureases) depending on whether the microplastics present increased or decreased the soil's water holding capacity (Zhang et al., 2022).

We believe that the combination of protein engineering and immobilization could lead to the development of hydrolases that can effectively treat (1) agricultural soils polluted with microplastics and (2) biofertilizers (processed sewage sludge, compost and manure) or irrigation water before their application to the soil. Additionally, because higher surface area correlates to increased depolymerization (Kawai et al., 2022), we envision a dual approach where soil invertebrates reduce the size of microplastic particles and enzymes then depolymerize the polymer chains.

4.2. *Effect of enzymes on the soil ecosystem: Introducing new risks?*

We also need to ask whether new hazards would be introduced by bringing enzymes into the soil environment. Risk assessments on hydrolases and microplastic degradation products are needed to exclude further negative effects on an already stressed ecosystem. To gain further insight, we carried out ecotoxicity tests evaluating the effect of different enzyme concentrations, diluted in a range of phosphate buffers, on two earthworm species widely used in ecotoxicology: *Lumbricus terrestris* and *Eisenia fetida*. The enzyme caused no significant harm or mortality, suggesting the absence of toxicity (Table 1). These results were anticipated because the polyester hydrolase was originally isolated from compost samples, which are rich in soil invertebrates such as earthworms (Sulaiman et al., 2012). However, we found that 50, 100 and 200 mM phosphate buffers were lethal to both earthworm species, whereas 25, 12.5 and 6.25 mM phosphate buffers were safe. Earthworm mortality has previously been linked to salinity, with $\geq$ 100 mM NaCl causing 100 % mortality in *E. fetida* (Owojori et al., 2008). This is probably because earthworms cannot maintain osmotic balance under highly saline conditions, resulting in plasmolysis. Therefore, hydrolases for *in situ* remediation should be engineered to work optimally at ambient temperature and under low salinity conditions.

It should be mentioned that data on the growth and development of earthworms, which is another parameter for ecotoxicity, is not included in Table 1. At the same time, we did not test the toxicity of PET degradation products for this article, however the monomers of PET – namely terephthalic acid (TPA) and ethylene glycol (EG) – are regarded as environmentally benign. Both are taken up and metabolized by microbes (Yoshida et al., 2016; Wei et al., 2020). However, TPA might temporarily decrease soil pH, as seeing by some compostable plastics upon degradation (Zhang et al., 2022). Other potential degradation products from PET include released plastic additives that cause secondary pollution (Sridharan et al., 2022), and this process should be investigated in more detail. Ecotoxicity tests should be performed in depth and with other soil organisms and plants. The effect of polyester hydrolases on plants, specifically the effect of cutinases (immobilized or not), is interesting because cutinases naturally degrade the cutin in leaves and the suberin in bark (Sulaiman et al., 2012).

5. Concluding remarks

Sustainable Development Goal 15 of the United Nations (Life on Land) defines target 15.5 as “Taking urgent and significant action to reduce degradation of natural habitats and halt the loss of biodiversity”. Given the ubiquity of microplastics in the environment and its effects on soil health, we call for the exploration of biotechnology (enzymes or microorganisms) as a bioremediation strategy to prevent and reduce further degradation and loss of biodiversity in agricultural soils. This could be achieved by including enzymes in plastic mulch, applying enzymes to the soil (for example with irrigation water) or to biofertilizers during their processing, or by spraying microbes that produce the enzyme naturally. In this article, we presented preliminary evidence that the enzymatic hydrolysis of PET at ambient temperatures is not toxic toward the earthworms *L. terrestris* and *E. fetida*. We hope this will encourage further research to 1. optimize hydrolases for soil bioremediation, 2. assess their ecotoxicology in more detail, and 3. study the logistics of their deployment.

Table 1
Impact of phosphate buffer and hydrolase LCC^{ICCG} on the mortality rate of the earthworm species Lumbricus terrestris and Eisenia fetida.

Buffer molarity (mM)	Enzyme concentration (μg/mL)	Earthworm mortality rate (%)
200	0	100
100	0	100
50	0	100
25	0	0
12.5	0	0
6.25	0	0
6.25	5	0
6.25	50	0

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Author contribution

Conceptualization: CPM, EHL; Data curation: KM, LLP, CPM: Formal analysis: KM, CPM; Investigation: LLP, KM; Methodology: CPM, KM, EHL, ESR; Project administration: CPM, KM, EHL; Resources: EHL, CPM, ESR; Supervision: CPM, KM, EHL, LMB; Visualization: CPM; Writing – original draft: CPM; Writing – review & editing: CPM, KM, ESR, EHL, LMB.

Declaration of Competing Interest

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

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