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Review

The long-term uncertainty of biodegradable mulch film residues and associated microplastics pollution on plant-soil health

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HIGHLIGHTS GRAPHICAL ABSTRACT

Micro

N-enzyr

- Bioplastics (Bio-MPs) are unlikely to be important in promoting soil C storage.
- Bio-MPs act as labile C sources to stimulate microbial growth and soil N and P cycling.
- Bio-MPs are much easier to form nanoplastics and cause stronger toxic to plants.
- Uncertainty of bio-MPs pollution remains on plant-soil health.

ABSTRACT

Mineral N

Mineral N

Biodegradable mulch film potentially offers an encouraging alternative to conventional (petroleum-based) plastic films. Since biodegradable films are more susceptible to rapid degradation, more microplastics (MPs) are likely to be generated than conventional films within the same time frame, probably leading to more severe MPs pollution and associated effects. However, the effect of biodegradable mulch film residues and associated MPs pollution on plant-soil health remains uncertainty. Here, we evaluated the potential effect of bio-MPs pollution on soil carbon (C) and nutrient (i.e., N and P) cycling, soil biology (microorganisms and mesofauna), and plant health, as these are crucial to agroecosystem functioning and the delivery of key ecosystem services. Unlike the inert (and therefore recalcitrant) C contained within petroleum-based MPs, at least 80% of the C from bio-MPs is converted to CO₂, with up to 20% immobilized in living microbial biomass (i.e., < 0.05 t C ha⁻¹). Although biodegradable films are unlikely to be important in promoting soil C storage, they may accelerate microbial biomass turnover in the short term, as well as CO₂ production. Compared to conventional MPs, bio-MPs degradation is more pronounced, thereby inducing greater alterations in microbial diversity and community composition. This may further alter N_2O and CH_4 emissions, and ultimately resulting in unpredictable

 $\left(\begin{matrix} 1 & 0 \\ 0 & 0 \end{matrix}\right)$

Necromass

Soil mineral

Necromass

Bacteria ^oFungi

 \circ Bio-MPs

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consequences for global climate warming. The extent to which this may occur, however, has yet to be shown in either laboratory or field studies. In addition, bio-MPs have a large chance of forming nanoplastics, potentially causing a stronger toxic effect on plants relative to conventional MPs. Consequently, this would influence plant health, crop productivity, and food safety, leading to potential health risks. It is unclear, however, if these are direct effects on key plant processes (e.g. signaling, cell expansion) or indirect effects (e.g. nutrient deficiency or acidification). Overall, the question as to whether biodegradable mulch films offer a promising alternative to solve the conventional plastic legacy in soil over the long term remains unclear.

1. Introduction

Plastics provide a vital role within almost all agronomic management regimes, however, their use is receiving increased attention and scrutiny due to their potential to contaminate and pollute land, and migrate to freshwater and marine habitats ([Maraveas, 2020a\)](#page-8-0). Plastic mulch films are one of the most important plastic products used in agriculture and are widely used to suppress weeds and reduce water usage in crop production [\(Kasirajan and Ngouajio, 2012](#page-8-0)). The global mulch films market is currently valued at US\$ 5×10^9 and is expected to have a compound annual growth rate of 5.9% over the next 5 years ([TMR,](#page-9-0) [2022\)](#page-9-0). Although values vary widely, it is currently estimated that 2.5 \times $10⁶$ tonnes (t) of plastic film are used annually within greenhouses and for mulching (ca. 0.5% of global plastic production) and that this covers an area of land ca. 25×10^6 ha (Oadeer et al., 2021; OECD, 2022). These films, however, have a limited lifespan (*<*1 y for mulches and ca. 5 y for greenhouses) due to progressive chemical, physical and biological degradation. Removing the plastic mulch films from soil in the agroecosystem is time-consuming (estimated ~42 h per ha; [Velandia et al.,](#page-9-0) [2019\)](#page-9-0), and expensive (estimated to be $£176.5, £186$ and $£192$ per ha for removal, landfill and recycling, respectively) ([Marí et al., 2019\)](#page-9-0). At the end of their working lifetime, complete removal from fields is often therefore unviable economically, if not impossible, leading to a substantial accumulated legacy of both macro- and microplastics (MPs, diameter *<* 5 mm) in agroecosystems [\(Rillig et al., 2012](#page-9-0)). The detection of MPs in edible vegetables (e.g. carrots, lettuce, broccoli, potatoes) and fruits (e.g. apples and pears) suggests that MPs also transfer into the food chain and may pose a threat to human health ([Li et al., 2020; Conti et al.,](#page-8-0) [2020; Wang et al., 2022a](#page-8-0)). This is supported by recent evidence showing that human exposure to MPs can lead to intestinal inflammation, as well as accumulation in organs (including the kidney, liver, gut and placenta) resulting in metabolic changes [\(Kannan and Vimalkumar, 2021;](#page-8-0) [Schwarzfischer and Rogler, 2022; Shi et al., 2022a](#page-8-0)), although a causal link to more serious medical conditions has yet to be established. It is therefore clear that strategies are needed to minimize the formation, persistence, movement, and toxicity of MPs within agriculture.

One way to lessen plastic pollution in agroecosystems is by the substitution of petroleum-based plastic with biobased plastics (examples include polyhydroxyalkanoates (PHAs), polyhydroxybutyrate (PHB),

polybutylene succinate (PBS) and polylactic acid (PLA)). Biodegradable film are progressively replacing conventional polyethylene (PE)-, polypropylene (PP)- and polyvinyl chloride (PVC)-based films, and their use is predicted to increase from 1.5×10^6 t in 2021 to 5.3×10^6 t by 2026 ([Plastics Europe, 2020\)](#page-9-0). Although biobased films are typically more expensive than their petroleum-derived counterparts [\(Jogi and Bhat,](#page-8-0) [2020\)](#page-8-0), the cost is often offset by the absence of removal and disposal costs ([Malinconico et al., 2008](#page-8-0)). Biodegradable plastic mulch films have also shown equivalent agronomic performance compared with conventional polyethylene mulches in many cases (Fig. 1). A meta-analysis conducted in China by [Liu et al. \(2021\)](#page-8-0) found that the performance of biodegradable mulch film differed little from that of conventional mulch films, increasing the water use efficiency of crops by 19–25% and soil temperature by 0.9 ◦C, consequently leading to an enhancement in crop yields (i.e., maize, wheat, cotton, and potato) by 18–26%. However, it is still too early to promote the use of biodegradable films on a large scale, due to the questions about *in situ* degradation particularly in different (natural) climates and over longer temporal periods, as well as the uncertainties about the long-term impacts on plant-soil health and ecosystem multifunctionality in agroecosystems (Figs. 1, 2). It has been widely accepted that biodegradable plastic mulch films are designed to break down into carbon dioxide $(CO₂)$ and water (usually by hydrolysis) with some carbon (C) incorporated into the soil microbial biomass within less than one year, according to the standard laboratory analysis at temperatures from 20 ◦C to 28 ◦C [\(Liwarska-Bizukojc, 2021\)](#page-8-0). However, it cannot be guaranteed that biodegradable mulch films will indeed degrade in the field within a 24-month time frame as environmental conditions, such as the microbial community present, temperature, and moisture content, vary from soil to soil, and are also dependent on climatic conditions [\(Sintim et al., 2020\)](#page-9-0). Therefore, biodegradation is most likely much slower as average soil temperatures and moisture contents seldom reach those used in laboratory test conditions ([Tabasi and Ajji,](#page-9-0) [2015\)](#page-9-0). For example, [Liao and Chen \(2021\)](#page-8-0) found that the weight loss was only 1.1–8.0% for PLA, and 0.8–6.8% for poly(butylene adipate-co-terephthalate) (PBAT) after a 180 day incubation in soil. Of note, numerous MPs were formed after poly(*p*-dioxanone) degradation, leading to the presence of 2103 plastic items/g in the soil [\(Liao and](#page-8-0) [Chen, 2021](#page-8-0)). Thus, the question as to whether biodegradable mulch films offer a promising alternative to solve the conventional plastic

Fig. 1. Uncertainty of biodegradable plastic films in the agroecosystem.

accumulation/legacy problem in soil over the long term, remains unclear. Therefore, investigation and assessment of the degradation processes, as well as the timescale of biodegradable mulch films degradation in the natural environment are required. This is of particular importance given biodegradable mulch films are designed to be tilled into the soil and not removed after use.

Equally, while the main plastic biopolymer in the film may be classified as environmentally benign [\(Maraveas, 2020a, 2020b\)](#page-8-0), mulch films also contain a multitude of undeclared additives (e.g. metals, volatile organic chemicals) that are used to provide additional functionality (e.g. UV resistance, flexibility, improved tensile strength) [\(Marra et al., 2016;](#page-9-0) [Arza et al., 2018](#page-9-0)). While some of these are added to accelerate degradation, the short- and long-term impact of these on plant and soil health remains virtually unknown (Fig. 2). Since biodegradable mulch films are designed to degrade within a few years, it is highly likely that more MPs will be generated over short periods in comparison to conventional plastics [\(Liao and Chen, 2021\)](#page-8-0). Consequently, this may lead to even more serious bio-MPs pollution in soils with implications for nutrient (i. e., N and P) cycling processes, with consequences for soil quality, ecosystem functions and multifunctionality ([Ma et al., 2022\)](#page-8-0), and therefore on the food security (Beltrán-Sanahuja et al., 2021). In this perspective piece, we therefore discuss the potential effect of bio-MPs from the decomposition of biodegradable mulch films with a focus on: C storage, nutrient (i.e., N and P) cycling, greenhouse gas emission, soil biology (microorganisms and mesofauna), and plant health, as these are crucial to agroecosystem functions and the delivery of key ecosystem services.

2. Soil carbon storage

Biodegradable mulch films are C-rich (typically around 60–80%), and may influence soil organic matter (SOM), and biogeochemical cycling. Assuming an average film thickness of 25 μ m and an average density of 1.2 $g \text{ cm}^{-3}$, we calculate that biodegradable mulch films will typically contribute 0.30 t C ha $^{-1}$ y $^{-1}$. For context, annual inputs from cereal root systems and crop residues (e.g. maize or wheat) can exceed

5 t ha⁻¹ y⁻¹, even excluding C inputs from root exudation (Zhang et al., [2013; Komainda et al., 2018](#page-9-0)), while native SOM levels typically range from 50 to 100 t C ha⁻¹. Unlike the inert (and therefore recalcitrant) C contained within petroleum-based MPs [\(Brown et al., 2022b\)](#page-8-0), at least 80% of the organic C from bio-MPs is converted to $CO₂$, with up to 20% immobilized in living microbial biomass (i.e., <0.05 t C ha⁻¹) (Ding [et al., 2021a, 2021b](#page-8-0)). Therefore, while bio-MPs are unlikely to be important in promoting soil C storage per se, they may still influence microbial growth, based on the active component of the microbial biomass (0.01 t C ha⁻¹; [Blagodatsky et al., 2000\)](#page-8-0). This is supported by studies using 13C-labeled polymers and isotope-specific analytical methods which have demonstrated that the C from each monomer unit of PBAT was metabolized by soil microorganisms, including filamentous fungi, ultimately contributing to microbial biomass [\(Zumstein et al.,](#page-9-0) [2018\)](#page-9-0). However, there is no direct evidence to support whether the C from bio-MPs can further form mineral-associated organic matter or be encapsulated in soil aggregates ([Fig. 3](#page-3-0)), thus contributing to C sequestration over longer periods.

The presence of bio-MPs can also significantly alter soil hydrological processes and damage the soil structure, i.e., aggregate formation ([Fan](#page-8-0) [et al., 2022\)](#page-8-0), which is highly likely to affect plant growth and the allocation of photosynthetic C and rhizodeposition ([Zang et al., 2020](#page-9-0)). Rhizodeposition is one of the primary mechanisms which regulates C flow and organic matter replenishment rates in soil [\(Jones et al., 2009;](#page-8-0) [Zhou et al., 2020](#page-8-0)), however, it is not clear how rhizodeposition might be affected by bio-MPs ([Fig. 3](#page-3-0)). Shifts in rhizodeposition quality and/or quantity would have far-reaching effects on plant-microbial interaction, function and signaling ([Sasse et al., 2018; Zhou et al., 2022a\)](#page-9-0), specifically SOM stabilization, as belowground C input is now thought to be an effective way of C sequestration [\(Sokol and Bradford, 2019; Xu et al.,](#page-9-0) [2022\)](#page-9-0). Another area of uncertainty is whether the high C:N ratio of the biodegradable mulch films and associated bio-MPs may induce accelerated microbial biomass turnover in the short term; either through positive SOM priming (the release of exoenzymes to mine organically bound nutrients (e.g., N, P) from SOM (Chen et al., 2014; [Zhou et al.,](#page-9-0) [2021c](#page-9-0))), or negative priming through breakdown of the comparably

Fig. 2. Hotspots in microplastic research: map of microplastic topics based on The size of each circle represents the frequency of that keyword. All graphs were produced based on the ISI Web of Science (WOS) database for the following combinations of terms within a date range of 1991–2020: "biodegradable microplastic OR bio-MPs", AND "soil OR agroecosystem", AND "plant OR soil property OR enzyme activity OR microbial community OR greenhouse gase".

Fig. 3. Schematic diagram showing the effect of biodegradable microplastics on soil carbon and nitrogen cycling, microbial communities, and plant growth. Red and blue solid line indicate the effect of biodegradable microplastics on C and N cycling, respectively. Grey dotted line indicates the effect of biodegradable microplastics on soil physical properties. '+ ' indicates positive response under biodegradable microplastics, '-' indicates a negative response and '?' indicates uncertainty.

more labile bio-MPs substrate ([Kuzyakov et al., 2000\)](#page-8-0), leading to $CO₂$ production and a relative reduction in soil C stocks. Given that bio-MPs may weaken the ability of soil aggregates to protect organic C this is likely to make C more bioavailable [\(Rillig et al., 2021a\)](#page-9-0), inducing DOC ([Table 1](#page-4-0)). Previous studies have documented that bio-MPs such as PLA and 3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) can increase the soil DOC (Zhou et al., 2021b; Schöpfer [et al., 2022; Shi et al., 2022b](#page-9-0)). Bio-MPs degradation in soil might lead to the release of soluble C [\(Wang](#page-9-0) [et al., 2021](#page-9-0)). For example, both PBS and PLA can be hydrolyzed via the cleavage of ester linkages and form water-soluble low molecular weight oligomers. Their intermediates then can be utilized as the extra available C source by specific heterotrophic microorganisms, which stimulates microbial respiration (Sanz-Lázaro et al., 2021; [Sun et al., 2022c\)](#page-9-0), and consequent $CO₂$ production ([Table 1;](#page-4-0) Fig. 3). Also, bio-MPs can increase soil DOC by increasing enzymes activities (i.e., phenol oxidase) to decompose insoluble high-molecular-weight material [\(Zhou et al.,](#page-9-0) [2022b\)](#page-9-0). This in turn, would be beneficial for microorganisms to produce CO2. High CO2 released from soils could also be due to excessive C consumption by bacteria that derives from the rise in the C:N ratio ([Zhou](#page-9-0) [et al., 2022a](#page-9-0)). Since bio-MPs could increase the proportion of macropores, it would improve soil O_2 supply and stimulate SOM mineralization ([Rillig et al., 2021b\)](#page-9-0). However, we were not able to distinguish the source of this extra $CO₂$ released from the soil after the addition of biodegradable mulch films and the associated bio-MPs. As such, we suggest that polymers labeled with 13 C should be used to provide unambiguous proof of conversion of polymer C to $CO₂$, which will supply a clear distinction between the polymer-derived $CO₂$ and the $CO₂$ formed by SOM mineralization. Taken together, the bio-MPs-derived C mediated alterations in microbial necromass formation, rhizodeposition input, as well as SOM mineralization and $CO₂$ emission, would cause a change in soil C stock (Fig. 3). Thus, it is clear that research on the effects of bio-MPs on soil microbial C use efficiency (i.e., biologically assimilated C vs. respired C), and SOM priming effects are urgently needed.

3. Influence of bio-MPs on nutrient cycling and GHG emissions

Since bioavailable C is the dominant element in bio-MPs, their degradation could supply C for microbial communities, which potentially induces the cycling of other macro and micro nutrients [\(Sun et al.,](#page-9-0) [2022a\)](#page-9-0). N turnover was significantly altered by bio-MPs addition, documented by the changes in NH $^+_4$, and reduction in NO₃ ([Table 1](#page-4-0)). The accumulation of NH $_4^+$ is dependent on its balance between production (e. g. ammonia oxidization and mineralization) and depletion (e.g.

nitrification and microbial immobilization). MPs were reported to influence soil biophysical environment and increase soil porosity [\(de](#page-9-0) [Souza Machado et al., 2018](#page-9-0)), which might enhance the air flow in soil as well as the stimulation of ammonia oxidization for providing sufficient O_2 , as a consequence cause a higher production of NH $_4^+$. The very high C: N ratio of biodegradable mulch film, however, is likely to promote microbial N immobilization [\(Brown et al., 2022a](#page-8-0)). The demand for N may stimulates the microbial N mining from SOM decomposition ([Zhou et al.,](#page-9-0) [2021c](#page-9-0)). Since biodegradable mulch films are a complex substrate that need to be deconstructed through enzyme activities to make its C available to the microorganisms ([Mazzon et al., 2022\)](#page-9-0), the production of enzymes also requires N and can further contribute to N sequestration ([Mooshammer et al., 2014\)](#page-9-0). This, in turn, decreases the content of NH₄ and NO₃ ([Table 1](#page-4-0)), consequently reducing nitrification and denitrification. The sorption of NH₄ cations to bio-MPs (i.e., bio-MPs contained carbonyl (=O) and hydroxyl (-OH) groups) is likely to decrease the accessibility of NH $_4^+$, and suppress the nitrification [\(Chen et al., 2020](#page-8-0)). Theoretically, the mineralization of bio-MPs (i.e., PLA, PHB, and PBS) will also produce organic acids (e.g. lactic acid, 3-hydroxybutyric acid) that may lower soil pH (depending on its inherent buffering capacity and the quantity of bioplastic present) (Fig. 3), due to the broken ester bond and release of H^+ [\(Altaee et al., 2016; Qi et al., 2020](#page-8-0)), which may inhibit nitrification (Jiang et al., 2015). It may thus lead to a transitory reduction in NO₃ leaching, which would be seen as beneficial in most agroecosystems. Another finding is that soil available phosphorus (AP) content was decreased by bio-MPs ([Table 1\)](#page-4-0). Since the availability of inorganic P is controlled by soil pH, the lower soil pH induced by bio-MPs may indirectly explains the lower AP content. On the other hand, the higher soil C:N imbalance induced by bio-MPs as discussed above may facilitates the assimilation of inorganic P by microorganisms and therefore exacerbate P deficiency. In short, the above results suggest that the addition of bio-MPs would decrease the content of NO₃ and AP, and subsequent affect the N and P cycling process in the agroecosystem.

When the bioplastic-derived C is utilized by soil microorganisms, it may also alter N₂O and CH₄ emissions (Fig. 3). For example, bio-MPs can increase the microbially available organic C through stimulated SOM decomposition and then may provide more energy for denitrifiers to produce N2O ([Zou et al., 2022c\)](#page-9-0). This potential C source can further promote N-related enzymes (i.e., chitinase and leucine aminopeptidase), which in turn may increase the content of mineral N and subsequently promote nitrification and denitrification [\(Zhou et al., 2021b](#page-9-0)). Generally, MPs have lower densities than the soil particles and thus tend to lower soil bulk density [\(Zhou et al., 2021a\)](#page-9-0). This can substantially increase soil

Table 1

The impact of biodegradable microplastics (bio-MPs) on dissolved organic carbon (DOC), available nutrient $(NH_4^+, NO_3^-,$ and available phosphorus (AP)), and greenhouse gas (GHG) emission in soil system. $\overline{}$

 $^{\rm a}$ + : positive; $-$: negative; n: neutral. Note, the 'positive' and 'negative' effects mean the significant difference between with and without MPs at p < 0.05 level. $^{\rm b}$ PLA: Polyacrylic; PBS: poly (butylene

polyhydroxyalkanoates; PHB: Polyhydroxybutyrate. $^\mathrm{c}$ /: not mentioned in the study.

aeration and air circulation, which may promote denitrification and stimulate N_2O emissions [\(Smith et al., 2018\)](#page-9-0). Under soil aeration, however, bio-MPs can decrease $NO₃$ and then $N₂O$ production during denitrification ([Rillig et al., 2021b](#page-9-0)). Also, the resultant lower soil N availability due to microbial N immobilization cause a further reduction in nitrification-related N₂O emissions from soils [\(Fig. 2\)](#page-2-0). However, evidence from a field study have shown no significant reduction or increase in N_2O emissions relative to no MP application (Greenfield et al., [2022\)](#page-8-0). Due to limited publications, it is still unclear whether bio-MPs addition positively or negatively influence soil N_2O emissions, which hamper efforts to accurately assess the contribution of agroecosystem to global N2O budgets under unpredictable bio-MPs pollution future especially if plastic use is inevitable in the agroecosystems. Equally, when the diffusion of O_2 into soils is enhanced, the soil oxidation ability would thus be enhanced, which in turn may increase oxidation-reduction potential and subsequently improve the oxidation of CH4 [\(Burgin et al., 2011\)](#page-8-0). Thus far, no study has been conducted to evaluate the impact of bio-MPs on CH4, and understanding of the underlying mechanisms are still scarce.

4. Influence of bio-MPs on the microbial community

The zone of soil surrounding bio-MPs is likely to induce a shift in the microbial community leading to the formation of microbial hotspots (so termed the microplastisphere; [Zhou et al., 2021b\)](#page-9-0). As soil contains numerous spatial niches (i.e., rhizosphere, fertisphere, detritusphere, drillosphere), a key question is whether this shift in soil community has any major influence on soil biodiversity and function. Compared to conventional MPs, bio-MPs disintegration is more rapid and formation of biofilms more pronounced, and consequently can be utilized by microorganisms, thereby suggesting stronger alterations in microbial diversity ([Fan et al., 2022; Wang et al., 2022c](#page-8-0)). Coupled with previous case studies (Table 2), we found that soil bacterial diversity was affected by bio-MPs addition positively [\(Seeley et al., 2020; Li et al., 2022](#page-9-0)), neutrally ([Zhou et al., 2021b](#page-9-0)), or negatively [\(Wang et al., 2022d\)](#page-9-0). The inconsistent results may depends on the types, concentrations, shapes, and sizes of bio-MPs [\(Zhou et al., 2021a; Zang et al., 2022\)](#page-9-0). Generally, bio-MPs at the high concentration could cause more profound alterations in bacterial diversity compared with conventional MPs due to the greater substrate utilization. On the contrary, soils commonly have an intrinsic buffer capacity for additives to counterbalance external

Table 2 The impact of biodegradable microplastics (bio-MPs) on soil fauna and microbial community.

Bio-MPs				Duration	Index ^a	Effectb	Reference
Type ^c	Size ^d (μm)	Shape	Concentration (%, w/w)	(day)			
PLA	\prime		1, 10	100	Bacterial diversity	\overline{a}	Wang et al. (2022d)
PLA			0.1, 1 10	\prime	AMF diversity	$\mathbf n$ $^{+}$	Wang et al. (2020)
PLA	100-154		0.1, 1, 10	30	AMF diversity	$\mathbf n$	Yang et al. (2021)
PLA	39-80	\prime	0.2, 2	120	AMF diversity	$\mathbf n$	Feng et al. (2022)
PBS	$39 - 80$	\prime	0.2, 2	120	AMF diversity	$\mathbf n$	
PHB	$39 - 80$		0.2, 2	120	AMF diversity	$\mathbf n$	
PBS	150-180	\prime	$\mathbf{1}$	60	Actinobacteria	\overline{a}	Sun et al. (2022a)
					Proteobacteria	$^{+}$	
					Bacterial diversity	$^{+}$	
PLA	150-180	\prime	$\mathbf{1}$	60	Actinobacteria	$\overline{}$	
					Proteobacteria	$^{+}$	
					Bacterial diversity	$+$	
PLA	$20 - 50$	Powder	2	70	Bacterial diversity	\overline{a}	Chen et al. (2020)
PBAT	< 1000	Particle	0.02	120	Bacterial diversity		Li et al. (2022)
			0.2, 2, 5		Bacterial diversity	$\mathbf n$	
PLA	$20 - 60$	Particle	0.1	49	Actinobacteria, Chloroflexi	$^{+}$	Lian et al., 2022
					Baceroidota, Firmicutcs	$\overline{}$	
					Bacterial diversity	$\! +$	
PLA	70	Powder	0.3, 1	20	Bacterial diversity	$\mathbf n$	Sun et al. (2022b)
					amoA	$\overline{}$	
					nirS	$\mathbf n$	
					n ir K	$\mathbf n$	
PHBV	\prime	Powder	0.01	210	Bacterial diversity	$\mathbf n$	Greenfield et al. (2022)
PBAT+PLA	2000	Fragment	0.1, 1, 5	90	Bacterial diversity	$\overline{}$	Hu et al., 2022
PLA	\prime	Film	0.5, 1	53	Actinobacteriota, Chloroflexi, Acidobacteriota, Bacteroidota	$^{+}$	Shi et al. (2022b)
					Bacterial diversity	$\mathbf n$	
PHBV	180	Particle	10	25	Acidobacteria, Proteobacteria, Chloroflexi,	$\! +$	Zhou et al. $(2021b)$
					Firmicutes	\overline{a}	
					Bacterial diversity	$\mathbf n$	
Animal							
PLA	$0.6 - 365$	Particle	0.1	30	Earthworm mortality	$\mathbf n$	Boots et al. (2019)
					Earthworm weight	$\overline{}$	
PLA	$\sqrt{2}$	Particle	0.0125, 0.125, 1.25, 12.5, 25, 50	28	Earthworm mortality	$^{+}$	Ding et al. (2021)
PLA	150	Particle	0.5, 1, 2, 5, 7, 14	28	Oxidative stress of earthworm	$\! +$	Yu et al., 2022
PLA	150	Particle	0.5, 1, 1.5	130	Collembola abundance	$=$	Huang et al., 2023
PBS	150	Particle	0.5, 1, 1.5	130	Collembola abundance	$=$	

^a Bacterial diversity was indicated by the Shammon index in the study, AMF: arbuscular mycorrhizal fungi

^b + : positive; - : negative; n: neutral. Note, the 'positive' and 'negative' effects mean the significant diff Polyhydroxybutyrate.

 d /: not mentioned in the study.

disturbances when present at low level, which can explain the weaker effects by bio-MPs with low-level [\(Sun et al., 2022a\)](#page-9-0). Morevoer, previous studies have evidenced that the release of additives from small-sized MPs was facilitated by their larger surface area when compared to that of larger MPs, resulting in greater chemical toxicity in soil microbial diversity ([Fan et al., 2022](#page-8-0)).

Current evidence suggests D-3-hydroxybutyric acid, produced as a secondary product from PHA breakdown, provided optimal microbial growth niches and enhanced enzyme production [\(Zhou et al., 2021b](#page-9-0)). Consequently, the unique environment might have a advantage on microbial survival in soil amended with bio-MPs, potentially influencing the soil ecological functions and subsequent biogeochemical processes ([Fig. 3](#page-3-0)), which may induce a stimulation of soil C and nutrients (e.g. N) cycling as shown in marine sediments (Sanz-Lázaro et al., 2021). This may offset some negative effects of physical disturbance and its indirect impact on the microbiome, thus increasing soil ecosystem multifunctionality (the average response of all measured ecosystem functions) [\(Wagg et al., 2014; Jia et al., 2022](#page-9-0)). As expected, the higher soil DOC with bio-MPs addition may cause a shift in the microbial community to fast-growing copiotrophs (i.e., *r*-strategists), that thrive in environments of high C availability, particularly in the short term. Since their hydrolysis products can serve as the preferred C sources for microorganisms under bio-MPs, the relative abundances of functions related to carbohydrate metabolism, amino acid metabolism, nucleotide metabolism, and energy metabolism were lower ([Sun et al., 2022a\)](#page-9-0). As documented by previous studies, the addition of bio-MPs (i.e., 10% PLA and 10% PHBV) induced a higher abundance of the *Acidobacteria*, *Proteobacteria*, *Chloroflexi,* and a lower abundance of *Firmicutes* [\(Table 2](#page-5-0); [Chen et al., 2020](#page-8-0); [Zhou et al., 2021b](#page-9-0); [Wang et al., 2022b\)](#page-9-0). The different responses within the bacterial community to bio-MPs could be explained as follows. First, alterations in soil biophysical properties (i.e., pH, bulk density, soil aggregate) may affect the bacterial community. For example, the large amount of 3-hydroxybutyric acid released during PHBV degradation has been shown to reduce soil pH, thus supporting the growth of *Acidobacteria* [\(Zhou et al., 2021b\)](#page-9-0). Second, the N deficient environment due to microbial N immobilization caused by the bio-MPs as discussed above could stimulate the proliferation of *Chloroflexi* since they tend to dominate in oligotrophic environments where N availability is low ([Ho et al., 2017](#page-8-0)). Furthermore, the dominance of *Proteobacteria* under bio-MPs polluted soils could be explained by the fact that they are involved in the degradation of complex organic compounds (e.g. hydrolysis of bio-MPs) ([Han et al., 2021\)](#page-8-0). Although once the initial liable C fraction is depleted, the higher nutrient limitation in the microplastisphere may also favor the growth of oligotrophs (i.e., *K*-strategists) as they have a lower N demand [\(Waring et al., 2013](#page-9-0)). Several previous studies have focused on the impacts of bio-MPs on soil bacteria (as shown in [Table 2](#page-5-0)), however, only a few studies evaluated the influence on fungi [\(Wang et al., 2020](#page-9-0)c; [Yang et al., 2021](#page-9-0)). As reported by [Accinelli](#page-8-0) [et al. \(2020\)](#page-8-0), Bio-MPs are rich C resources and can increase the abundance of specific fungal genera such as *Aspergillus*, *Fusarium*, and, *Penicillium* was increased since bio-MPs contained rich C resources. Similarly, PLA also induced some alterations in arbuscular mycorrhizal fungi (AMF) community composition, but had no negative impacts on AMF diversity [\(Table 2](#page-5-0)). PLA (10%, w/w) still showed no significant negative impacts on AMF diversity, implying its low fungitoxicity ([Yang](#page-9-0) [et al., 2021](#page-9-0)). Therefore, the responses of soil bacteria to bio-MPs addition are more stronger than those of soil fungi. This could be explained by the fact that bacteria are more sensitive and respond relatively faster to environmental changes (e.g. increased labile C, changed soil biophysical properties, etc.) compare to fungi (e.g. [Fierer et al., 2003](#page-8-0); [Barnard et al., 2013](#page-8-0)). Another possible explanation is the C utilization efficiency by bacterial and fungal guilds. However, the mechanisms underlying these changes are still unclear and more mechanistic-focused experiments are needed to better understand these changes within the soil system.

microbial functional genes involved in N cycling. For instance, [Seeley](#page-9-0) [et al. \(2020\)](#page-9-0) documented that PLA (0.5%, w/w) increased the abundance of *amoA* gene and *nirS* gene and decreased the abundance of *nirK* gene in sediments, as a consequence promote nitrification and denitrification. Since $amoA$ gene can oxidize $NH₄⁺$ to $NH₂OH$ (Zhang et al., 2015), we assume that NH₄ was oxidized by *amoA* and then used as a substrate for nitrification. Also, [Feng et al. \(2022\)](#page-8-0) found that bio-MPs like PLA, PBS, and PHB (0.2%, w/w) increased the Nitrospirae abundance, which may enhanced nitrification, and consequently decreased soil NH_4^+ content. By contrast, in a paddy soil added with PLA (0.3% and 0.1%, w/w), [Sun et al. \(2022b\)](#page-9-0) found a reduction in *amoA* levels whilst *nirS* and *nirK* remained stable. In short, we concluded that bio-MPs may affected NH4 ⁺ metabolism by changing the abundance of the *amoA* and glutamate dehydrogenase (GDH), as well as affecting NO₃ metabolism by regulating the abundance of the *nirS* and *nirK* genes. However, detailed research is still required to evaluate the impact of bio-MPs with various types on microbial genes and consequent biogeochemical cycles.

5. Influence of bio-MPs on mesofauna

Agroecosystem sustainability is supported by an ecological network comprised of a diversity of organisms. Therefore, understanding the impact of bio-MPs on a range of trophic levels is required to gain a holistic view of soil biology change. Bio-MPs share some common features with MPs, from which some parallels may be drawn. That is, bio-MPs can cause weight loss, reduce growth rates and casting yield, increase mortality, decrease reproduction rate, as well as induce DNA damage and oxidative stress, via a wide range of toxicity mechanisms ([Wang et al., 2022c](#page-9-0)). Theoretically, due to their small size, bio-MPs can be adhered to soil fauna and consequently induce surface damage [\(Yu](#page-9-0) [et al., 2020; Wang et al., 2022c\)](#page-9-0). For instance, earthworms suffered physical damage and lost surface mucous upon moving into the bio-MPs-polluted soil, resulting in burns and lesions on their bodies and thus inducing a adverse effect on earthworms [\(Baeza et al., 2020](#page-8-0)). Second, bio-MPs (like PLA and PPC) can be ingested by some mesofauna (i.e., earthworm) because of their higher degradability and subsequently larger associated biofilm and microbial load [\(Zhang et a, 2018\)](#page-9-0). Once bio-MPs accumulate in the gut of organisms by ingestion (like nematodes and springtails) (Kim et al., 2021), they can elicit an inflammatory response to invasive heterogenic substances by causing physical tearing of organs and tissues [\(Mueller et al., 2020\)](#page-9-0). Third, bio-MPs smaller than 20 µm can pass through (be taken up across) the gut/extracellular barrier and be translocated to other tissues via the circulatory system, then inhibiting the growth and reproduction of earthworms ([Kim and An,](#page-8-0) [2020\)](#page-8-0). Like conventional MPs, bio-MPs can adversely influence soil bulk density, soil aggregates, and water holding capacity, which may induce mesofaunal stress due to less favorable movement conditions and soil ingestion, as well as lead to increases in intracellular reactive oxygen species (ROS) levels, and impairment of membrane integrity ([Wang](#page-9-0) [et al., 2022b; Zhang et al., 2022\)](#page-9-0), which ultimately induces oxidative stress. The higher biofilm and nutritional content of bio-MPs might also cause a reduction in their toxicity. For example, [Ding et al. \(2021a\),](#page-8-0) [\(2021b\)](#page-8-0) found that conventional MPs (i.e., PE) were more toxic at large concentrations (12.5–50%, w/w), while seemingly less harmful than bio-MPs (e.g. PLA and PPC) at lower contamination levels (0–12.5%, w/w). Further, [Boots et al. \(2019\)](#page-8-0) documented that PLA MPs (0.1%, w/w) reduced soil earthworm biomass but did not necessarily induce mortality. Similarly, [Zhang et al. \(2018\)](#page-9-0) reported that there were interactions between bio-MPs fragments and earthworms, but fragments did not cause mortality. It is thus worth mentioning that, the avoidance behavior of soil mesofauna may be relatively lower under bio-MPs compared to conventional MPs. However, there are still few studies on the effect of bio-MPs on earthworms, and the mechanistic basis and factors affecting mesofauna avoidance behavior need to be investigated further.

Although no studies have evaluated the impact of bio-MPs on

nematode communities, we specluate that bio-MPs may indirectly affect nematode performance (e.g., growth, reproduction and abundance) by changing soil water dynamics, since nematode abundance is often positively correlated with soil moisture ([Liao et al., 2021\)](#page-8-0). Furthermore, the influence of bio-MPs on nematode are dependent on its' functional guilds. For example, omnivorous and predatory nematodes with larger body and buccal cavity size may be more sensitive to bio-MPs pollution due to the direct ingestion than small-bodied nematodes [\(Franco et al.,](#page-8-0) [2019; Andriuzzi et al., 2020](#page-8-0)). On the contrary, omnivorous and predatory nematodes are relatively impressible to environmental disturbance ([Shao et al., 2016](#page-9-0)). Although insufficient data exists on nematode responses to bio-MPs, we hypothesize that bio-MPs pollution will have strong impacts on the composition of soil nematode communities through direct and indirect ways.

6. Influence of bio-MPs on plant health

Plants are fundamental to terrestrial ecosystem functioning as well as agroecosystem service provision, and therefore a fuller understanding of MPs-plant interaction is needed. Like conventional MPs, toxic effects of bio-MPs on plants have also been recorded (as reviewed by [Zhou et al.,](#page-9-0) [2021a\)](#page-9-0). It is unclear, however, if these are direct effects on key plant processes (e.g. signaling, cell expansion) or indirect effects (e.g. nutrient deficiency due to microbial immobilization of N and P, or acidification due to film breakdown; [Chen et al., 2020](#page-8-0); [Zang et al., 2022\)](#page-9-0). We assume that bio-MPs may induce an adverse impact by 'dilution effect' reducing the bioavailability of soil available C and N to plants as suggested by [Rillig and Lehmann \(2020\).](#page-9-0) Due to the relatively rapid degradation of bio-MPs, releases of additives, monomers, and possibly harmful intermediates are more pronounced compared to conventional MPs. Since bio-MPs are more vulnerable to weathering than conventional MPs [\(Ribba et al., 2022\)](#page-9-0), bio-MPs may have a large chance of forming nanoplastics, potentially causing a stronger toxic effect on plants relative to conventional MPs. Petroleum-based nano- (*<* 100 nm) and micro-plastics (0.2–2 μ m) have been found to be capable of being taken up and transported in wheat and lettuce plants via water and nutrient flow through transpiration, thus potentially leading to negative impacts on growth and metabolism [\(Li et al., 2020\)](#page-8-0). However, the uptake and fate of bio-MPs in plants remain very unclear with more evidence required to understand its effects on plant health. Bio-MPs with nano size may accumulate near the root hair, adhere to the root surfaces and clog the pores in the seed capsule ([Fan et al., 2022](#page-8-0)). Thus, this physical blocking could inhibit water and nutrients uptake, and respiration of plants, consequently delaying seed germination and above-/under-ground biomass accumulation. It is also likely that bio-MPs could be degraded within root cells, once assimilated, by the diverse array of enzymes present (e.g. esterases, lipases, and cutinases), however, there has been no documentation of this to date. Although current evidence seems to show that bio-MPs are more toxic to plants than conventional MPs, most of these studies have been undertaken on plants and seedlings at early stages of growth, at unnaturally high plastic concentrations (*>* 1%, w/w). Up to now, there is still a scarcity of information on potential risk of bio-MPs on plant growth, metabolism and yield, especially in the field under realistic contamination levels and climate.

7. Current uncertainties about the impact of biodegradable mulch film and associated bio-MPs on agroecosystems

7.1. Uncertainty 1

Although biodegradable mulch films are designed to break down into CO2 and water in the field, a paucity of information exists about their physical disintegration and subsequent degradation under realistic field conditions. If the biodegradation is much slower under cooler, drier conditions, then more bio-MPs (and nanoplastics) may accumulate in the soil in comparison to conventional plastics. Hence, it is still unclear

whether current biodegradable plastic mulches will solve the problem of legacy plastic in the long term. Further, the breakdown products of the plastics and their additives, their phytotoxicity and persistence in soil remain to be established. The concentration of metabolic by-products formed from bioplastic breakdown in soil, their residence time and impact on plant growth and soil function, however, remains very uncertain. The formation and ecotoxicity of nanosized fragments formed from bio-MPs also need to be investigated.

7.2. Uncertainty 2

The input of bioplastics into soil is likely to significantly affect soil biogeochemical cycling which may impact soil C storage and future agronomic fertilizer recommendations. For example, there is still a lack of information about whether bio-MPs-derived contribute to soil C storage directly, affect rates of rhizodeposition or promote SOM priming and consequently influence soil C storage indirectly. As soluble C will be released from bio-MPs, the activity and composition of the soil microbial community, as well as the microbial functional genes (e.g. *AOA* and *AOB*) are likely to be altered which may in turn alter GHG (i.e., CO₂, N2O, CH4) emissions. The extent to which this may occur, however, has yet to be shown either in simulated (i.e., laboratory) or natural (i.e, field) conditions. Also, the majority of studies undertaken to date on microorganism-plastic interactions have been based on laboratory incubations, typically at very high (unrealistic loading rates such as between 1% and 10%, w/w). This has created a disconnect between the scientific evidence base and real-world field scenarios. Thus, there is a need for an understanding of microorganism-plastic interactions in the field at realistic loading rates (*<*0.1%, w/w).

7.3. Uncertainty 3

Commercial bioplastics vary widely in their physicochemical properties, such as their molecular weight and structure, density, hydrophobicity and the presence of functional additives (e.g., UV activators). Each polymer contains different functional groups on its surfaces, and thus possesses different properties, which may strongly determine their impact in the plant-soil system. It is currently too early to suggest that bio-MPs may be a threat to the plant-soil system due to the lack of convincing data; only a handful of studies, using a limited range of bio-MPs, have identified changes in plant-soil functioning in response to bio-MPs addition. Further, if changes in soil properties occur in response to bio-MP addition (e.g., microbial community structure) these should not always be viewed as a negative outcome, particularly as the long-term impacts of change are notoriously difficult to predict. Interpreting the significance of change within the system boundaries also remains difficult, particularly as nearly all bio-MP experiments lack a C-addition control (e.g., ground straw) as a comparator. As such, it is difficult to pinpoint specific threats that bio-MPs might induce for organisms and ecosystem health. Hence, a broader, more holistic picture is needed whereby testing is undertaken in a diverse range of soil types to enable a more complete assessment of the impact of bio-MPs on soil quality, ecosystem multifunctionality, food safety, and human health.

7.4. Uncertainty 4

The effects of biodegradable mulch film and associated bio-MPs on the diversity of organisms in agroecosystems and other natural ecosystem environments is still unexplored. Nevertheless, available results indicate the potential for several biodegradable mulches and associated bio-MPs to alter the growth and reproductive functions of some trophic levels of soil organisms, containing bacteria, and earthworms, whilst limited studies focus on the fungi, nematodes, and protozoa. The sustainability of agricultural soils is strongly dependent on the relationships among the diversity of organisms they host; this should be considered in the evaluation of biodegradable mulch films and MPs

environmental influence.

Environmental Implications

Bioplastics potentially offer an encouraging alternative to conventional (petroleum-based) plastics. Although bioplastics are designed to degrade within a few years, it is highly likely that more MPs will be generated over short periods in comparison to conventional plastics. Consequently, this may lead to even more serious bio-MPs pollution in soils with implications for soil and plant health. However, the short- and long-term impact of bio-MPs on plant-soil health remains virtually unknown. Therefore, it is still too early to promote biodegradable mulch film on a large scale, due to the uncertainties around agronomic performance and questions about in situ degradation.

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.

Data Availability

No data was used for the research described in the article.

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