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Size-dependent effects of oxo-degradable plastic contamination on soil quality and the growth of *Zea mays*

Do Thi Kim THANH[1], Robert W. BROWN[1], Martine GRAF[1], Michaela K. REAY[2], Charlotte E. M. LLOYD[3], Gupeng LI[1], David R. CHADWICK[1], Davey L. JONES (✉)[1]

1 School of Environmental and Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK.
2 Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK.
3 School of Geographical Sciences, University of Bristol, University Road, Bristol BS8 1SS, UK.

KEYWORDS

Microplastic pollution, plastic mulch films, oxo-biodegradable plastics, pro-oxidant additives, soil health, soil–plant interactions

HIGHLIGHTS

- First study of micro- vs macro-sized oxo-degradable plastics (ODPs) on soil and plants.
- High dose ODP (> 1%) altered soil quality but typical field loadings had minimal impact.
- Microplastic contamination had stronger effects on soil properties than macroplastics.
- Plant height and chlorophyll decreased at high ODP loadings, but biomass was unaffected.
- ODPs underwent limited degradation over 6 weeks in soil.

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Correspondence: d.jones@bangor.ac.uk

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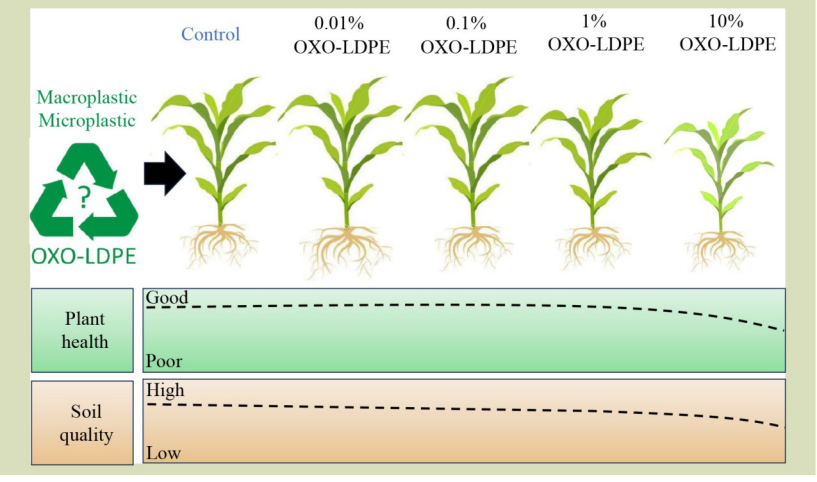
GRAPHICAL ABSTRACT



ABSTRACT

Agricultural plastic mulch film represents a significant source of microplastic contamination in soils, raising concerns about soil health and food security. Oxo-degradable plastics (ODPs) have emerged as a potentially more sustainable alternative to current plastic mulch films, however, uncertainty remains around the degradation rate of ODPs in soil and their impacts on soil quality and crop health. The study evaluated the dynamics and impact of different concentrations of micro- and macroplastics derived from a commercial d_2w ODP (0.01%, 0.1%, 1% and 10% w/w) on the growth of *Zea mays* in an agricultural soil over a 6-week period. Chemical analysis revealed the ODP contained about 0.29% additives by weight, primarily comprising antioxidants (tris(2,4-di-tert-butylphenyl) phosphite and its oxidized form) and lubricants, with minimal heavy metal content. ODP degradation was mainly limited to chain scission and had only partial formation of oxygenated groups, without an increase in carbonyl groups. The rate of ODP degradation was

found to be inversely related to the ODP concentration in soil. Overall, typical field levels of plastic contamination (0.01% w/w) had negligible effect on soil quality or plant performance. However, higher levels of ODP contamination resulted in significant changes in soil pH, EC, NO_3^- and bulk density. At extreme plastic loading rates (10% w/w), both micro- and macro-sized ODPs caused significant reductions in plant growth, with microplastic treatments having consistently greater effects than the macroplastic treatments. Changes in bacterial 16S rRNA community composition were driven by Acidobacteriota and Gemmatimonadota. Macroplastics significantly altered these bacterial communities, while microplastics had minimal effect. These findings indicate that at realistic field concentrations, ODPs are likely to have little effect on agroecosystem functioning in the short-term but might persist in soil for long periods of time, leading to their progressive accumulation in agricultural soils if used over repeated cropping cycles.

1 Introduction

Oxo-degradable plastics (ODPs) were introduced in the 1980s as an innovative solution to address growing concerns about persistent plastic pollution in the environment[1]. ODPs are normal petroleum-based plastics modified with pro-oxidant additives, first commercialized in the 1990s, and have since gained a significant market presence[2]. ODPs undergo a distinctive two-stage degradation process: (1) an initial abiotic phase triggered by exposure to heat and UV, which initiates polymer chain scission, producing oxygen-containing functional groups and increasing hydrophilicity, with the resulting shorter polymer chains leading to fragmentation; and (2) a slower biotic phase where microorganisms mineralize the oxidation products to form CO_2, H_2O and biomass[3,4]. The initial degradation is catalyzed by transition metal salts, such as iron, manganese and cobalt, which promote oxidation and subsequent fragmentation into microplastics[5,6].

While ODPs share similar physical properties with currently-used plastics (e.g., LDPE) in terms of cost, weight and initial durability, their degradation to microplastic is thought to be much more rapid once exposed to environmental conditions[3,7]. This characteristic has made them particularly attractive for agricultural applications, especially in the production of mulch film and food packaging, where they have been used for over four decades[8]. However, their environmental benefits compared to currently used plastics remain controversial, as the efficacy of the secondary biodegradation phase remains largely unproven, and could exacerbate the contamination of soils with nano- and micro-plastics[9,10]. This has led to a banning of their use in some jurisdictions, including the European Union[11,12]. Also, ODPs can be unsuitable for standard waste management approaches, as they degrade slowly in anaerobic landfill conditions and can produce methane, potentially increasing their carbon footprint[13,14]. Also, concerns have been raised about their metal additives, which could pose environmental risks when released during the degradation process[15,16]. Despite these regulatory restrictions, ODPs continue to be widely used in many regions, including parts of the West and East Asia, and North America, where they are used for agricultural applications, packaging materials and single-use carrier bags due to their cost-effectiveness and claimed environmental benefits. Understanding their environmental impacts therefore remains critically important for informing evidence-based policy decisions in these regions, particularly for agricultural applications where significant quantities of plastic enter soil systems annually.

The accumulation of plastic residues in agricultural soils has become a major environmental concern and is now ranked as one of the world's top ten environmental threats due to the impact on soil health, water quality and biodiversity[17,18]. Both non-ODP microplastics ($\leqslant$ 5 mm) and macroplastics (> 5 mm), and have been shown to negatively impact soil systems[19,20]. Macroplastics primarily affect surface-soil properties, reducing aeration, damaging soil structure, limiting

water infiltration and potentially decreasing plant growth[21–23]. In comparison, microplastics pose additional risks as they can move through the soil profile to groundwater and smaller particles can be taken up by plants where they can enter the food chain[24], as well as affecting soil fauna such as earthworms and disrupting nutrient cycling processes[25,26]. While much research has been conducted on the dynamics of non- and biodegradable plastics in soil, aquatic and marine ecosystems, relatively little research has focused on the end-state and impact of ODPs in the environment[12].

Despite the use of ODPs in agriculture and their potential environmental impacts, most research to date has focused on their potential benefits[27–29], while studies examining their effects on soil quality and plant health remains limited[30,31]. This study therefore aimed to address these knowledge gaps by investigating, for the first time, how micro- and macro-sized ODPs affect soil biochemical properties and plant health, using *Zea mays* as a model crop. A further aim was to characterize the chemical composition and additive content of the ODP, providing context for interpreting potential mechanisms of toxicity and the environmental end-state of ODP. We tested three hypotheses: (1) increasing concentrations of ODP will adversely affect soil biochemical properties and microbial communities; (2) ODPs will constrain plant growth by inhibiting root growth, consequently reducing nutrient uptake; and (3) ODP-derived microplastics will have a greater impact on both soil quality and plant health compared to macroplastics. This dose–response study aims to improve fundamental understanding of how ODPs affect soil–plant functioning, while also generating critical limits for ODP contamination in soil, helping to inform future policy decisions on ODP use in agriculture.

2 Materials and methods

2.1 Soil sampling and plastic preparation

The soil was collected from a recently plowed field previously under cereal production at the Henfaes Research Centre, Abergwyngregyn, North Wales, UK (53°14′29″ N, 4°01′15″ W). Soil samples were collected to a depth of 10 cm representing the topsoil Ahp horizon. The soil is classified as a freely draining, sandy clay loam textured Eutric Cambisol with a crumb structure[32]. The climate of the site is characterized by a temperate oceanic climate regime with an annual temperature of 10.8 °C and annual rainfall of 1066 mm·yr^{-1}. The field had no history of plastic mulching or macroplastic contamination. After collection, the soil was prepared by sieving to pass 8 mm before use. Basic properties of the soil are presented in Table 1.

The study used a commercial, black-colored d_2w oxo-biodegradable plastic (thickness 31.3 μm; Symphony Environmental Ltd., Borehamwood, UK) conforming to the British Standard 8472 on biodegradation of plastics[33]. Macroplastic was prepared by cutting the plastic into 1 cm^2 pieces. Microplastic particles were produced through milling in a water-cooled mill (IKA A10, IKA Ltd., Oxford, UK) and then sieved to achieve a size less than 2 mm size.

2.2 Experimental treatments

Previous research examining plastic contamination in soils has often used unrealistic plastic loading rates that far exceed those encountered in agricultural settings[34–36]. This methodological limitation potentially alters our understanding of the actual risks posed by micro- and macroplastics in agricultural systems[37,38]. In this study we used a range of ODP concentrations (0.01%–10% w/w) to evaluate their impact on

Table 1 General properties of the Eutric Cambisol soil at the beginning of the experiment. Values are expressed on a dry weight basis and represent means ± SE (n = 3)

Soil property	Mean ± SE
pH (H_2O)	5.89 ± 0.13
EC (μS·cm^{-1})	58.0 ± 6.00
Soil organic matter (%)	4.15 ± 0.07
Total C (g·kg^{-1})	24.3 ± 1.70
Total N (g·kg^{-1})	2.0 ± 0.20
Available P (Olsen P; mg·kg^{-1})	51.2 ± 2.70
Basal respiration (mg·kg^{-1}·h^{-1} CO_2)	12.9 ± 2.20
Bulk density (g·cm^{-3})	1.15 ± 0.05
Moisture content (g·kg^{-1})	245 ± 44
Available K (mmol·kg^{-1})	1.4 ± 0.10
Available Ca (mmol·kg^{-1})	40 ± 2.0
Available Na (mmol·kg^{-1})	0.15 ± 0.01
Sand (%)	49.0 ± 2.0
Silt (%)	31.3 ± 0.90
Clay (%)	19.7 ± 1.20

plant and soil health. Assuming an ODP density of 0.92 g·cm^{-3}, this equates to an ODP soil contamination level in the topsoil (0–30 cm) of 0.01%, 0.1%, 0.25% and 0.5% (w/w) after 1, 10, 25 and 50 years of repeated ODP use in a field (assuming no degradation and removal). Concentrations therefore ranged from actual field-relevant levels (0.01% w/w) to extreme concentrations (10% w/w) which might represent spent plastic dumped en-masse at field margins. Thus, we consider our lowest ODP doses (0.01% and 0.1%) to be more representative of the likely field contamination levels.

Macro- or micro-sized oxo-degradable plastics were added to independent batches of soil (1 kg) in pots at five rates: 0% (control), 0.01%, 0.1%, 1% and 10% (w/w). A concentration of 0.01% represents typical field contamination levels of microplastics observed in agricultural fields around the world, while higher levels might simulate those seen in successively mulched fields, urban soils or in waste piles at the margins of agricultural fields[39]. Based on the C content of ODP (85.6% C by weight), the addition of plastic at rates of 0.01%, 0.1%, 1% and 10% (w/w) equated to C amendments of about 0.086, 0.86, 8.56 and 85.6 g·kg^{-1} C, respectively (relative to a native soil C content in the control of 24.3 g·kg^{-1} C).

There were five replicates of each plastic treatment and 10 replicates for the control. A higher number of control replicates (n = 10) compared to treatment replicates (n = 5) was used as the control group serves as the comparative baseline for all treatment groups. After setting up the pots, two pre-germinated maize (cv. Debalto; KWS Seeds Inc., Bloomington, MN, USA) seedlings (roots 2 cm long) were placed in each pot and the pots placed in a climate-controlled greenhouse with temperature and moisture conditions similar to the field growing season (about 20 °C daytime and 14 °C nighttime) and daylight hours consistent with UK summer hours (about 15 h). All pots were weighed at the beginning of the experiment and then reweighed three times a week, with water lost by evaporation and transpiration replenished with deionized water[40]. The plants were grown for 6 weeks with fertilizer added to all the pots (NH_4NO_3, KH_2PO_4 and KCl) at a rate of 100 kg·ha^{-1} N, 20 kg·ha^{-1} P and 50 kg·ha^{-1} K, respectively to support growth.

2.3 Plant and soil analysis

Leaf chlorophyll content was measured biweekly using a SPAD chlorophyll meter (Konica Minolta SPAD-502 PLUS). Plant height was measured biweekly using a standard measuring tape by measuring from the soil surface to top of the plant when fully extended. After 6 weeks, the pots were destructively harvested and plant shoot and root biomass measured after drying (80 °C, 72 h). Plant elemental composition was determined on finely ground biomass samples using a S2 Picofox total reflection X-ray fluorescence spectrometer (Bruker Ltd., Coventry, UK).

Soil was recovered from the pots at harvest, the roots removed and key soil quality indicators measured namely; pH, electrical conductivity (EC), moisture content, organic matter, bulk density and available nutrients (NO_3^-, NH_4^+ and P). Standard electrodes were used to measure soil pH and EC after shaking at 200 rev·min^{-1} for 10 min with distilled water (1:2.5 w/v). Soil N availability was evaluated by performing a 1:5 (w/v) soil-to-0.5 mol·L^{-1} K_2SO_4 extract (200 rev·min^{-1}, 1 h), and centrifuging the extracts (18,000 g, 5 min). NH_4^+ and NO_3^- in the extracts was determined colorimetrically[41,42]. Soil organic matterwasmeasuredbyloss-on-ignitioninamufflefurnace(450°C, 16 h)[43]. Soil moisture was determined gravimetrically by oven drying (105 °C, 24 h). P availability was measured in a 1:5 (w/v) 0.5 mol·L^{-1} acetic acid extract and analyzed colorimetrically using molybdate blue[44].

2.4 Effect of soil burial on ODP degradation

At plant harvest, individual plastic pieces (n = 5 per pot) were recovered from the soil of the macroplastic 0.1%, 1% and 10% treatments, their surface was gently cleaned with distilled water, air-dried and analyzed at room temperature on an attenuated total reflectance-Fourier transform infrared (ATR-FTIR). Recovery of a plastic sample from the 0.01% treatment was not possible, and particles from the microplastic treatments (all concentrations) could not be successfully separated from the soil, therefore these treatments were not included in that analysis. The ATR-FTIR analysis was performed using a Cary 630 FTIR bench spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) with a diamond ATR crystal attachment in conjunction with a KBr source with a spectral range of 650–4000 cm^{-1} and a resolution of 8 cm^{-1}. Spectra were recorded in reflectance mode (32 scans per sampling point and background) using the MicroLab Pharma Software (Agilent Technologies Inc., Santa Clara, USA). The crystal was routinely cleaned using acetone and background measurements were taken every 2 min. A total of 25 sampling

points were recorded for the plastic treatments, and 20 sample points for the control (unexposed film). Spectra manipulation and transformation were done using the open-source online application Open Specy[45]. Spectra were transformed to absorbance according to the Kubelka-Munk equation, baseline correction was made with the setting 'Polynomial 8', and the CO_2 region between 2200 and 2400 cm^{-1} was flattened. Peak intensity change in comparison to the control was determined for wavenumbers 1032, 1461, 2844 and 2915 cm^{-1}, representing C=O and CH functional groups. Peak intensity at those wavenumbers corresponds to the visualized peak height in the absorbance spectra. Peak intensity change was calculated by dividing the average peak intensity (n = 25 for plastic treatments and n = 20 for control) at a certain wavenumber of the plastic treatments by the average peak intensity of the control (unexposed) film.

2.5 Soil microbial analysis

At plant harvest, soil (~30 g) was removed from each pot, immediately frozen (−80 °C) to quench microbial activity and preserve the community, and subsequently lyophilized using a Modulyo Freeze Dryer (ThermoFisher Corp, Waltham, MA, USA) equipped with an RV vacuum pump (Edwards Ltd., Crawley, UK). Samples were subsequently shipped on dry ice to Novogene Corp. (Beijing, China) for extraction and analysis. Soil genetic material was extracted using a soil and stool kit (Tiangen, Beijing, China) according to the manufacturer's protocol. Samples were stored at −80 °C before sequencing. Samples were assessed for quantitation and integrity by agarose gel electrophoresis on a fragment analyzer system (Agilent 5400, Agilent, Santa Clara, CA, USA). The bacterial 16S rRNA gene fragments were amplified using primer sets targeting the V3-V4 variable region (primers: 341F-806R). All PCR reactions were performed with 15 μL of Phusion High-Fidelity PCR Master Mix; 0.2 μmol·L^{-1} of forward and reverse primers, and ~10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s and 72 °C for 5 min. Sequencing libraries were generated and indexes added and pooled libraries were sequenced on a paired-end MiSeq platform (Illumina Inc., San Diego, CA, USA).

Raw sequences obtained were cleaned using DADA2, and assigned to amplicon sequence variants (ASVs) were obtained representing the corresponding species information and the abundance distribution based on the species[46,47]. Qiime2 was the used for species annotation based on the SILVA database. The absolute abundance of ASVs was normalized using a standard of sequence number corresponding to the sample with the least sequences. Sequencing read files analyzed in this study can be accessed from the National Center for Biotechnology Information (Sequence Read Archive: SUB14849151).

2.6 Extraction and analysis of plastic additives and heavy metal content

The methods for the extraction and analysis of additives from PE film were as previously reported[48,49]. Briefly, the ODP film (0.1 g) were microwave extracted at 70 °C using dichloromethane-methanol (15 mL of 2:1 v/v), concentrated then functional groups (e.g., alcohols and carboxylic acids) were derivatized using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (30 μL, 70 °C, 1 h). Additives were subsequently quantified by gas chromatography (GC), relative to an internal standard (benzyl benzoate) and identified using GC-mass spectrometry, confirmed using an in-house standard library. For quantification, relative response to the internal standard was used to account for differing responses of compounds with different chemical functionality, determined using external standards.

For the assessment of heavy metal content in plastic samples (~100 mg) were cut into 5 cm × 5 cm pieces, weighed and transferred to a 50-mL glass sample vial before ashing in a muffle furnace (450 °C, 16 h). After cooling, the mass of ash remaining was determined and subsequently dissolved in 2 mL of 1 mol·L^{-1} HCl at high temperature, and after cooling, the sample placed on a shaker (100 rev·min^{-1}, 2 h) to ensure complete dissolution of the ash. The sample was subsequently diluted to 20 mL by adding 10 mL of 1 mol·L^{-1} HCl and 8 mL of ultrapure water, and the mixture shaken (100 rev·min^{-1}, 1 h) to ensure thorough mixing. The solution was then filtered through a Whatman No. 540 filter paper into a 50-mL polypropylene centrifuge tube. The samples were analyzed using an Avio 500 ICP-OES (Perkin Elmer, Waltham, MA, USA) and the concentration of the following metals determined: As, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb and Zn.

2.7 Data analysis

Statistical and graphical analysis was performed using R

version 4.2.1[50]. Two-way analysis of variance (ANOVA) was conducted using the "stats" package to test the effects of plastic size (micro vs macro) and concentration (0%, 0.01%, 0.1%, 1% and 10%) on soil parameters. Post hoc comparisons were performed using Tukey's HSD test using the "agricolae" package[51] to determine significant differences between treatments. Data were tested for normality using Shapiro-Wilk test and homogeneity of variance using Levene's test. Where necessary, data were log-transformed to meet ANOVA assumptions, if parametric assumptions were not met, Kruskal–Wallis tests were performed followed by Dunns post hoc testing. ATR-FTIR spectra graphs were created using the "ggplot2"[52] package in R version 4.3.3[50]. Metagenomic analysis was conducted in the "vegan" package[53] with figures created in "ggplot2." Bacterial ASV richness was calculated using the Chao 1, Shannon and Simpson indices and tested for significant differences between treatments using Kruskal–Wallis analysis. Non-metric multidimensional scaling (NMDS), followed by an analysis of similarities, was used to test for significant differences in community composition among treatment groups (beta diversity), using Bray-Curtis dissimilarity matrices. The statistical cutoff for all analysis was $p < 0.05$.

3 Results

3.1 Effect of oxo-degradable plastics on plant performance

The results provided clear evidence that both ODP size and concentration affected shoot growth rate (both $p < 0.001$) and to a lesser extent foliar chlorophyll content at plant harvest (both $p < 0.001$) (Figs. S1 and S2). The reduction in plant growth rate was most apparent in the first week (data not presented). Overall, plant height was more greatly affected by the presence of macroplastics, although a similar amount of inhibition was seen at the highest microplastic loading rate (Fig. S1). The presence of microplastics increased leaf chlorophyll content, while no effect was seen in the macroplastic treatments. Overall, ODP size and soil loading rate had a weak effect on shoot biomass with a negative effect only evident at the highest ODP loading rate ($p = 0.022$; Fig. 1). The presence of microplastics increased root biomass compared to the macroplastic treatments ($p < 0.001$), particularly at lower ODP concentrations (0.01%). While there was a weak concentration effect ($p = 0.048$), the impact of concentration was less clear than the size effect.

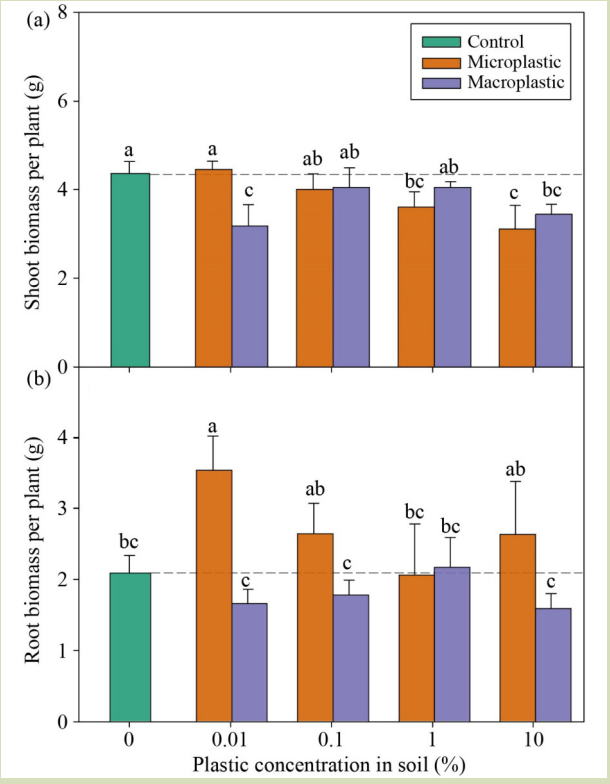


Fig. 1 Effect of increasing amount of oxo-degradable macroplastic and microplastic (ODP) contamination on maize (a) shoot biomass and (b) root biomass. Values are presented as mean ± SE ($n = 5$ for the plastic treatments, $n = 10$ for the 0% control). Different letters represent significant differences between treatments at the $p < 0.05$ level. Analysis based on two-way ANOVA followed by Tukey's HSD test (ODP size $p < 0.001$, ODP concentration, $p = 0.048$; treatment × concentration, $p = 0.126$ for shoots, and ODP size $p = 0.026$, ODP concentration, $p = 0.022$; treatment × concentration, $p = 0.267$ for roots). The dashed line represents the average control value for comparison to the other treatment.

Plant analysis at harvest revealed significant effects of plastic particle size on foliar P, K, Ca, Fe, Cu and Mn concentrations, with macroplastic treatments generally showing higher concentrations than microplastic treatments, particularly for Fe, where macroplastic treatments had up to 4-fold higher concentrations at the highest ODP soil loading rate (Tables S1 and S2). The effect of soil ODP concentration was significant for most nutrients (S, Cl, K, Ca, Mn, Fe and Zn), with higher plastic concentrations generally leading to altered nutrient profiles compared to controls, though the pattern varied by nutrient. Significant interaction effects between size and

concentration were observed for several nutrients (Ca, Mn, Cl and Fe), indicating that the impact of ODP concentration on nutrient uptake depends on particle size. Both micro and macroplastic treatments had distinct concentration-dependent responses, with the most pronounced effects observed at higher concentrations (1% and 10%), particularly for Fe, Mn and Ca, while some nutrients (e.g., Cu and Zn) had relatively minor changes in concentrations across treatments.

3.2 Effect of oxo-degradable plastics on soil physicochemical properties

Overall, ODP presence significantly affected soil quality indicators, with most following a dose-dependent pattern. For soil pH, the presence of ODP microplastics resulted in an increase in pH at all concentrations, although this was most prevalent in the 10% soil ODP loading rate with a pH shift of 0.98 units (Fig. 2(a)). In contrast, the presence of ODP macroplastics had no major effect on soil pH. A similar pattern was observed for soil EC where the presence of ODP microplastics caused a dose-dependent increase with ODP concentration rate, reaching a level double that seen in the unamended control. Only minor differences in EC were seen in the presence of ODP macroplastics, and no consistent dose-dependent patterns were evident. As expected, the percentage of organic matter increased with plastic loading rate due to the samples containing ODP-derived carbon which was not removed before analysis (data not presented).

Both micro and macroplastic treatments significantly reduced soil bulk density compared to the control, with the effect becoming more pronounced as plastic concentration increased, reaching their lowest values at the 10% dose for both ODP sizes (0.59 g·cm^{-3} across all ODP treatments vs 0.95 g·cm^{-3} in the control; Fig. 3(a)). The reduction in bulk density had a clear concentration-dependent pattern, with microplastics generally causing a slightly greater reduction than macroplastics at the same concentration.

Overall, the presence of the ODPs had a minor effect on the amount of NH_4^+ and available P in the soil (Fig. S3), even at the highest plastic loading rates. However, there were significant differences in soil NO_3^- concentration which mirrored the observed changes in EC (Fig. 3(b)). A concentration-dependent change was observed for the microplastic treatments, while no major effect was seen in the presence of the macroplastics ODPs.

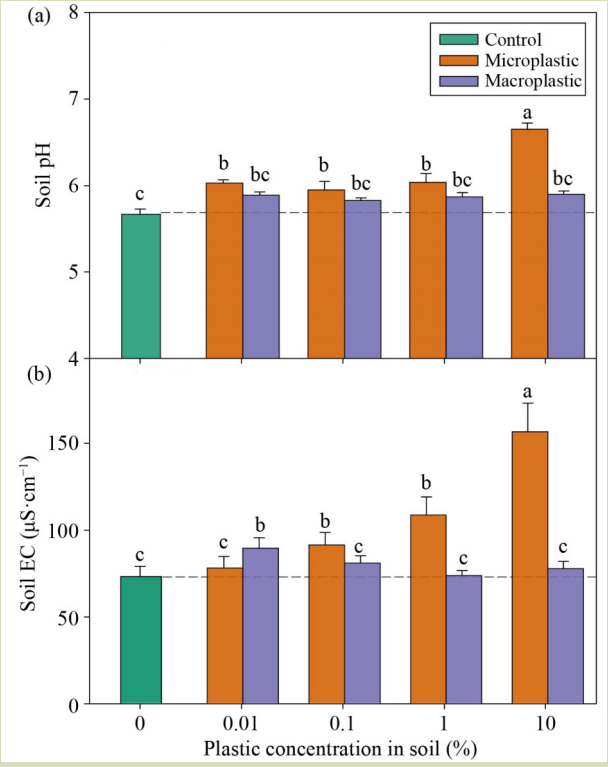


Fig. 2 Effect of increasing amount of oxo-degradable macroplastic and microplastic (ODP) contamination on (a) soil pH and (b) soil electrical conductivity (EC). Values are presented as mean ± SE (n = 5 for the plastic treatments, n = 10 for the 0% control). Different letters represent significant differences between treatments at the $p < 0.05$ level. Analysis based on 2-way ANOVA followed by Tukey's HSD test (ODP size p = 0.378, ODP concentration, $p < 0.001$; treatment × concentration, p = 0.048 for soil pH, and ODP size p = 0.004, ODP concentration, $p < 0.001$; treatment × concentration, p = 0.014 for soil EC). The dashed line represents the average control value for comparison to the other treatments.

3.3 Effect of oxo-degradable plastics on soil microbial communities

In total 22,955 bacteria and archaea were identified across all 16S rRNA gene reads. While community composition varied across the treatments, Proteobacteria, Acidobacteriota, Actinobacteriota and Gemmatimonadota were the most abundant phyla (Fig. 4). Across the 10 most abundant phyla, there was no significant difference between ASV abundance for Actinobacteriota, Bacteroidota, Chloroflexi, Latescibacterota, Proteobacteria and Verrucomicrobiota. However, there were significant differences between Acidobacteriota ($H_{(8)}$ = 20.0,

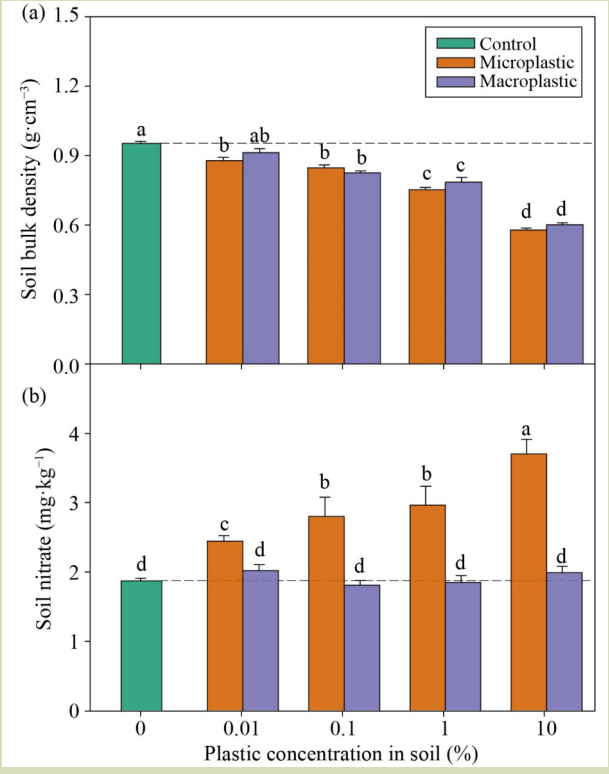


Fig. 3 Effect of increasing amount of oxo-degradable macroplastic and microplastic (ODP) contamination on (a) soil bulk density and (b) soil nitrate content. Values are presented as mean ± SE (n = 5 for the plastic treatments, n = 10 for the 0% control). Different letters represent significant differences between treatments at the $p < 0.05$ level. Analysis based on 2-way ANOVA followed by Tukey's HSD test (ODP size $p < 0.001$, ODP concentration, $p < 0.001$; treatment × concentration, $p = 0.476$ for soil bulk density, and ODP size $p < 0.001$, ODP concentration, $p < 0.001$; treatment × concentration, $p < 0.001$ for soil nitrate). The dashed line represents the average control value for comparison to the other treatments.

$p = 0.01$), Crenarchaeota ($H_{(8)} = 18.4$, $p = 0.02$; however, post hoc testing revealed no significant difference in pairwise comparisons;), Gemmatimonadota ($H_{(8)} = 22.7$, $p = 0.004$) and other phyla (a group of phyla not in the 10 most abundant; $H_{(8)} = 25.1$, $p = 0.002$). Post hoc testing revealed that while no treatments caused significant differences relative to the control (with the exception of other phyla group) there were some weak numerical trends worth considering. In particular, Acidobacteriota has a weak, dose-dependent trend with microplastic addition rate, while the opposite effect was seen in Gemmatimonadota and Actinobacteriota, but further research would be needed to test if these response represent real trends. The group of other phyla had significant decreases relative to the control in the 0.01%, 0.1% and 1% microplastic loading rates. Alpha diversity (measured by Chao1, Shannon and Simpson indices) revealed significant differences between plastic types and loading rates (Fig. 5; $H_{(8)} = 30.4$, $p < 0.001$, $H_{(8)} = 32.5$, $p < 0.001$, $H_{(8)} = 22.8$, $p = 0.004$, respectively). There were similar trends in Chao1 and Shannon diversity with macroplastic 0.1%, 1% and 10% loadings leading to higher values than the control group, whereas alpha diversity in the microplastic groups did not differ significantly from the control treatment. In terms of beta diversity, the NMDS ordination revealed some variation in soil bacterial communities, as confirmed by BetaDisper ($p = 0.001$), however, only the microplastic 10% soil dose gave a significant difference from the control (Fig. S4).

3.4 Changes in oxo-degradable plastics chemistry after burial in soil

After 6 weeks in the soil, spectra of all treatments (0.1%, 1% and 10% macroplastic) had changes in peak intensity relative to the control (Fig. 6(a)). Peak intensity decreased at 1032 cm^{-1} (C–O stretching) and increased at 1461 cm^{-1} (CH scissoring vibration), 2844 cm^{-1} (CH_2 symmetric stretching), and 2915 cm^{-1} (CH_2 asymmetric stretching) (Fig. 6(a,b)). Peak intensity reductions and increases were largest in the 0.1% treatment and decreased with increasing plastic concentration, compared to the control in a relatively linear fashion (Fig. 6(b)). R^2 values for the trend lines were 0.81, 0.99, 0.99 and 0.99 for wave numbers 1032, 1461, 2844 and 1915 cm^{-1}, respectively. There was no observable change to functional groups in the wavenumber intervals of 1700–1800 cm^{-1} (C=O region) and 3200–3600 cm^{-1} (O–H region) (Fig. 6(a)).

3.5 Plastic additives

The total additive content of the ODP was 2.90 $mg \cdot g^{-1}$, with the most abundant class of additives being antioxidants. Tris(2,4-di-tert-butylphenyl) phosphite, a secondary antioxidant largely, was the most abundant additive (1.51 $mg \cdot g^{-1}$). It was also present in its oxidized form (tris(2,4-di-tert-butylphenyl) phosphate; 415 $\mu g \cdot g^{-1}$), which can be produced during plastic film production. A second antioxidant, octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate was also present (211 $\mu g \cdot g^{-1}$) which is a primary antioxidant. There was also evidence of ANOX 20 degradation products

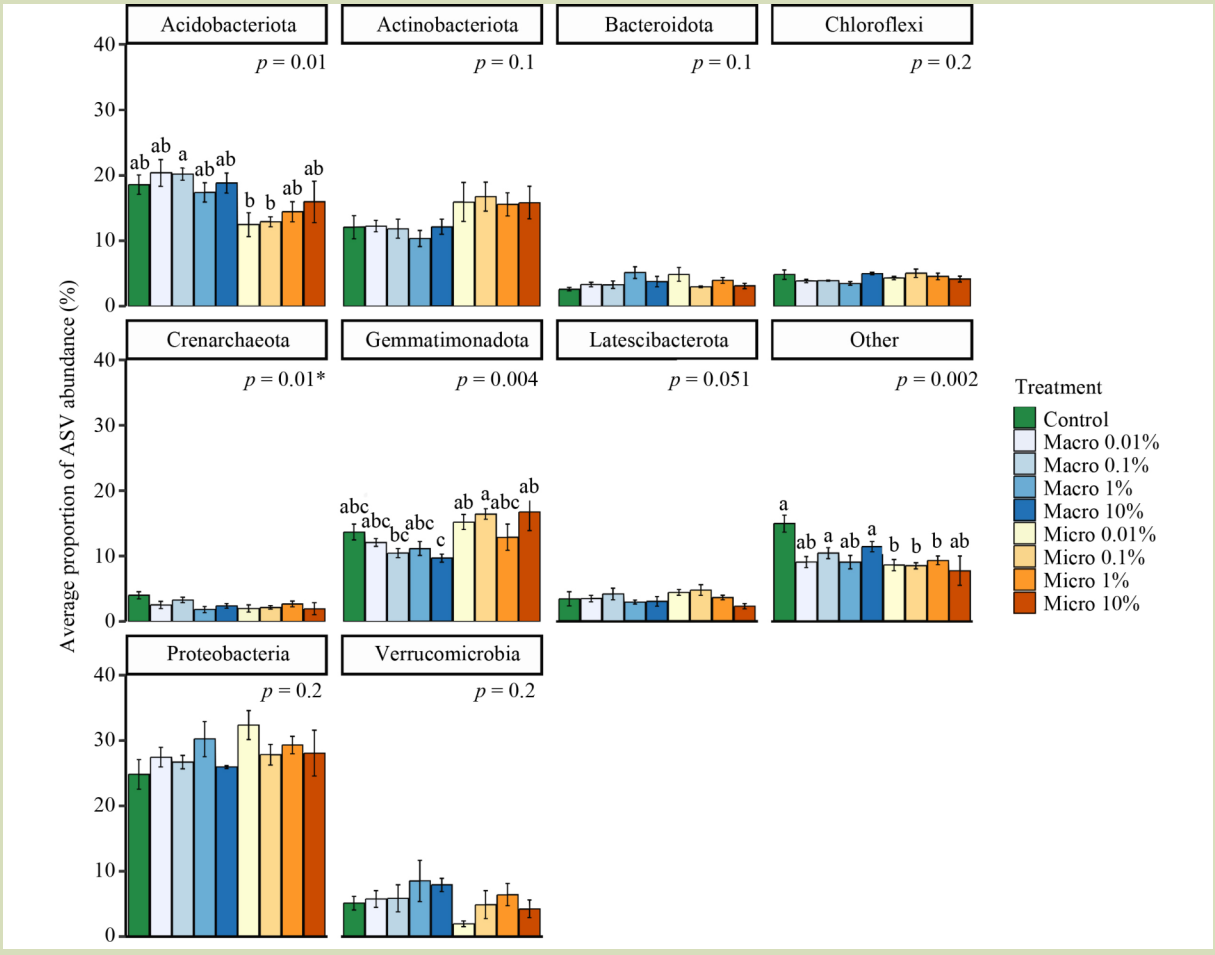


Fig. 4 16S rRNA metabarcoding bacterial community in response to different plastic types (micro and macro) and doses (0%, 0.01%, 0.1%, 1% or 10% w/w) (n = 5). Proportionate abundances of major phyla within each treatment. Statistical differences across treatment groups (size and concentration) were assessed using a Kruskal–Wallis test and significance letters assigned using a Dunn post hoc test with Holm p-value adjustment.

(70.0 μg·g^{-1}), indicating that the high molecular weight hindered phenolic primary antioxidant Anox 20 can also be present, although the parent compound for this degradation product is beyond the analytical window of the GC analytical technique employed here. The only other class of additives present were lubricants. External lubricants were the most abundant (hexadecanoic acid (435 μg·g^{-1}) and octadecanoic acid (54.5 μg·g^{-1}), which aid the production process. Docosenamide (266 μg·g^{-1}) and oleanitrile (15.0 μg·g^{-1}) were present as internal lubricants, probably to improve the slip properties of ODPs.

Alongside intentionally included additives, there were antioxidant degradation products, which can be produced during blown film extrusion to produce the plastic films. These included 2,4-di-tert-butyl-phenol (211 μg·g^{-1}), which is derived from tris(2,4-di-tert-butylphenyl) phosphite, and 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (12.8 μg·g^{-1}), derived from octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate. Linear and branched alkanes and alkenes were observed for even chain lengths between C_{20} to C_{34}, which are likely derived from incomplete polymerization of plastic monomers. The total concentration was 2.22 mg·g^{-1}, and the most abundant chain length was C_{22} for both alkanes and alkenes. Overall the ODP contained few heavy metals as given in Table S3.

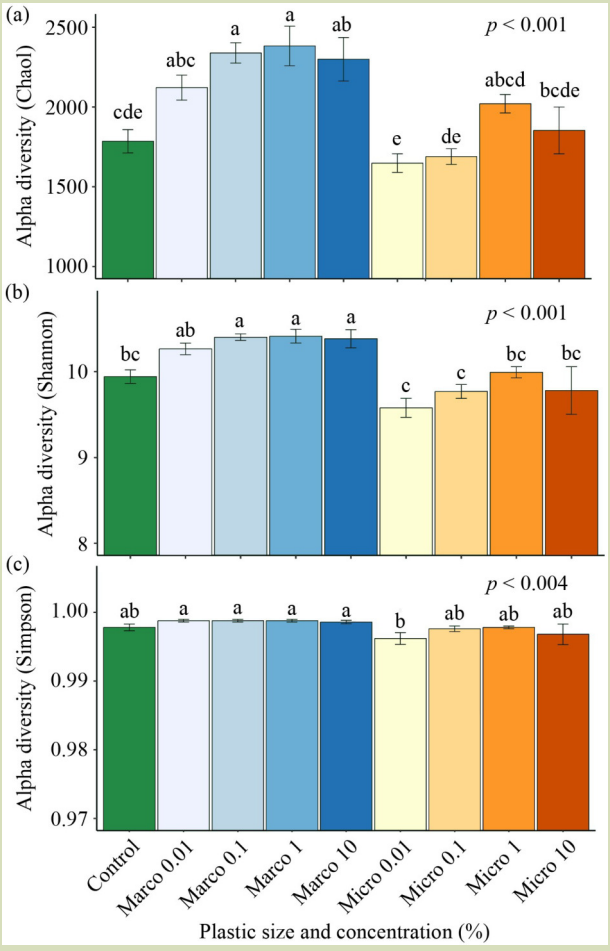


Fig. 5 16S rRNA metabarcoding bacterial community richness ((a) Chao1 index, (b) Shannon, and (c) Simpson) in response to different plastic types (micro and macro) and doses (0%, 0.01%, 0.1%, 1% or 10% w/w) (n = 5). Statistical differences were assessed using a Kruskal–Wallis test and significance letters assigned using a Dunn post hoc test with Holm p-value adjustment.

4 Discussion

4.1 Impact of oxo-degradable plastics on soil physical properties

ODPs contain pro-oxidant additives designed to trigger fragmentation when exposed to UV radiation, oxygen and heat, however, they frequently enter soil systems before these degradation processes have been adequately established. Our experimental design, while potentially underestimating the degradability of ODPs exposed on the soil surface, was designed to determine their ecological impacts where UV exposure can be limited (e.g., under the plant canopy) or absent (i.e., buried in the soil).

Overall, our results showed that low levels of ODP contamination significantly altered soil physical properties, with effects being both size and concentration-dependent. The most pronounced changes were observed in soil bulk density, which decreased by up to 40% at the highest ODP concentrations. This reduction in bulk density partly results from the lower particle density of the ODP plastics (0.92 g·cm^{-3}) relative to mineral soil particles (2.65 g·cm^{-3}), but mostly from their ability to bridge aggregates and generate larger pore spaces. Similar effects have been reported for common microplastics in agricultural soils[21,23,54]. While reduced bulk density might initially appear beneficial for root growth[55], excessive reductions could compromise soil stability and water retention properties, however, this is only likely to occur at extreme ODP contamination levels. The greater impact of microplastics compared to macroplastics on bulk density likely reflects their more uniform distribution throughout the soil matrix and their greater potential to interfere with particle–particle interactions[56,57]. The reduction in bulk density and disruption of soil structure by plastic particles, particularly microplastics, can alter hydraulic conductivity and water retention characteristics[58]. The concentration-dependent effects we observed indicate a threshold response, with minimal impacts at field-relevant concentrations, but substantial physical alterations at higher loading rates, similar to previous observations[59].

4.2 Effects on soil chemical properties and nutrient availability

The differential response of soil chemical properties to micro- and macro-sized ODPs reveals distinct pathways through which these particles influence soil functioning. The elevated soil pH observed in microplastic treatments, particularly at higher concentrations, can be attributed to the release of additives such as $CaCO_3$ fillers, which can make up 7.14% ± 0.20% of the plastic weight (Table S3), rather than alterations in soil microbial processes. This size-dependent effect likely stems from the greater surface area-to-volume ratio and enhanced soil contact of microplastic particles compared to their macro-sized counterparts. Although detectable, these pH shifts

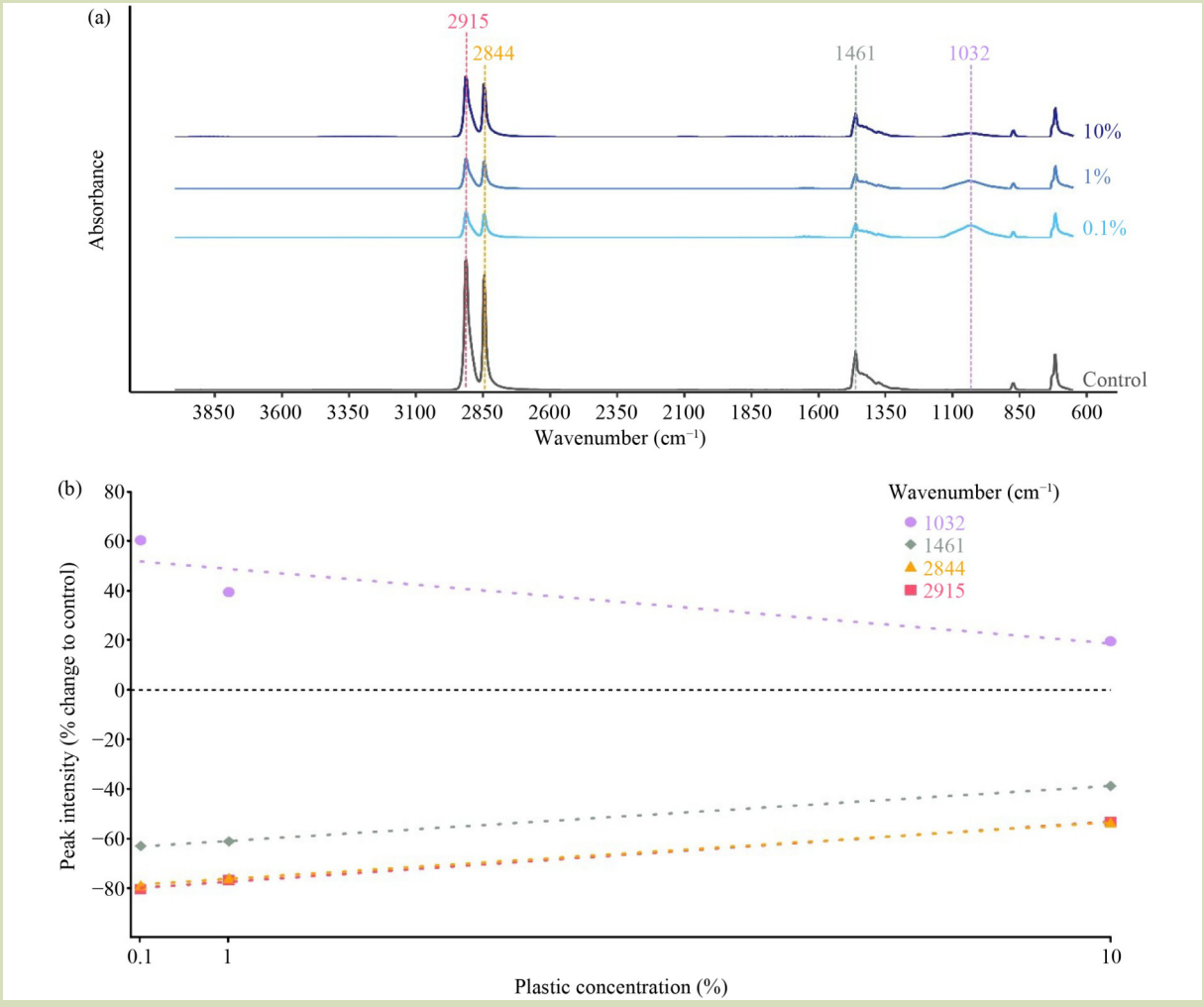


Fig. 6 (a) ATR-FTIR spectra for control and 0.1%, 1%, and 10% plastic treatments after 6 weeks in the soil. Values expressed as mean (n = 20 for control; n = 25 for plastic treatments). Dotted lines represent peaks for selected wavenumbers. (b) Peak intensity of selected wavenumbers expressed as percentage change to the control for 0.1%, 1% and 10% plastic treatments. Values expressed as mean (n = 20 for control; n = 25 for plastic treatments). Dotted lines represent trend lines with R^2 values of 0.81, 0.99, 0.99 and 0.99 for wavenumbers 1032, 1461, 2844, and 1915 cm^{-1}, respectively.

remained within the optimal range for both maize growth and soil microbial activity[60].

The concurrent increases in EC and NO_3^- in the microplastic-amended soils is indicative of a disruption in the soil N cycle[61]. We attribute these changes to two complementary mechanisms: (1) the observed pH increase toward the optimal range for nitrifying bacteria (pH 7.0–8.0) promoting conversion of NH_4^+ to NO_3^-[62], and (2) the reduced bulk density and increased soil porosity created by microplastic particles which improved soil aeration, thereby limiting denitrification losses[63,64]. However, the lack of an ODP effect on soil NH_4^+ and available P concentrations indicates that some nutrient cycling processes do remain unaltered. Based on our measurements of foliar and soil chemistry, our results indicate that at representative field contamination levels, ODP plastics do not greatly affect soil biogeochemical cycling in the short-term. This aligns with recent field studies demonstrating minimal impacts of microplastics on soil N cycling under realistic microplastic loading rates[37,65].

4.3 Plant response to oxo-degradable plastic contamination

The differential plant responses to ODPs reveal the complex nature of plant-plastic interactions in soil systems. Overall, our results indicate that shoot growth was only adversely affected at extreme levels of ODP soil contamination (10% w/w). Conversely, the presence of low amounts of microplastics appeared to promote greater root growth, possibly due to the increase in soil NO_3^- concentrations and pH. Additives present in the film can also produce toxic effects on the roots. For example, the leachate of PVC plastic containing AO168, AO168ox and AO1076 has been shown to reduce wheat root growth and elongation[66]. AO168 and its oxidation product, AO168ox, have both previously been shown to leach from commonly-used plastic mulch films into a soil solution, although AO1076 demonstrated comparatively lower leaching potential[49]. Other abundant additives in the film, including hexadecenoic acid and docosenamide, can also leach into the soil, although such compounds can also be naturally present in soil. However, there were minimal effects on the soil microbial community (see section 4.5), including for high plastic concentrations and the microplastic treatments, where leaching would be highest. In addition, previous studies have not indicated high degradation rates of additives present in this film (e.g., AO1076[49]) which could minimize effects on the soil microbial community. Therefore, further work on the toxicological effects of individual additives is required to determine if the observed effects on plant growth at high ODP concentration in this soil arises from the plastic itself, from the mixture of additives which could leach into the soil, or specific additives leached from the film.

In contrast to previous studies, we did not find any evidence for mechanical impedance of root growth in the macroplastic treatments[67,68]. The elevated chlorophyll content observed in the microplastic treatments indicates greater N acquisition[69]. This contrasts with many previous studies showing that microplastics reduce soil N availability and leaf chlorophyll content[61,68]. Although we observed some differences in foliar chemistry, there was no consistent pattern across the nutrients with either ODP size or dose. Further, in this agricultural soil with no history of metal contamination, there was no evidence for foliar increases in micronutrients or other potentially toxic metals (e.g., Cu, Zn and Pb) in the presence of ODPs. This contrasts with previous studies showing that currently-used plastics can alter the speciation and availability of metals in soil over longer periods[68]. Although Co-stearate and Co- or Mn-carboxylates are sometimes used as pro-oxidant additives (0.1%–1% w/w) in ODPs[70,71], we found no evidence for enhanced foliar Co (which was below the limit of detection; 1–2 $\mu g \cdot kg^{-1}$) or Mn concentrations. We ascribe this to the low amounts of Co and Mn in the ODP film itself, the slow rate of ODP breakdown and thus metal release into the soil and plant uptake (Table S3). We note that low levels of transition metals have been reported in the other types of d_2w branded ODPs[6]. It is also possible that Ca-based pro-oxidants were present (based on the high levels of Ca present), or that an organic pro-oxidant was added[71,72].

4.4 Effect of oxo-degradable plastics on soil microbial community structure/diversity

Differences in community structure were driven by the phyla Acidobacteriota (Gram-negative), Gemmatimonadota (Gram-negative), and the group of other less-abundant phyla. Acidobacteriota and Gemmatimonadota have previously been seen as active plastic-colonizing bacteria[72] and are known for their role in organic matter decomposition and nutrient cycling[73] and rhizosphere processing and N cycling[74,75], respectively. Alpha diversity, which strongly influences microbial community resilience and resistance to change, had increased Chao1 and Shannon index values in macroplastic treatments at concentrations above 0.01%, but remained unaffected in the microplastic treatments. This is unexpected as microplastic pollution is generally considered to decrease the richness of the soil bacterial community[65]. This could indicate that within the short time frame (6 weeks) of this experiment, the ODPs had not broken down sufficiently. Despite pH shifts typically being a major driver of microbial community dynamics[76], the modest increase in soil pH (from 5.7 to 6.7) observed at the highest plastic loading rates did not appear to substantially influence microbial composition changes in this study. Instead, the substantial C addition from plastic amendments (up to 8.56% C at the highest loading rate) is likely to have exerted stronger selective pressure than the modest pH shift. Soil texture is also likely to have been modified with plastic addition (differing particle size and loading rates), this is often suggested as a driver of microbial community change[77]. Additionally, the creation of novel microhabitats on plastic surfaces and potential effects of additives or oxidation products from the ODPs could have made more significant contributions to shaping the microbial community composition than the observed pH changes. Also,

at high plastic loadings, and particularly for microplastic, where the larger surface area will facilitate leaching, any ecotoxicological effects of additives leached from plastics are likely to be most evident. However, there were minimal effects on individual phyla and overall community richness, relative to the control for these treatments to indicate a negative effect of additives on the soil microbial community. Based on the results of the present study, we suggest that ODPs do not perform analogously with either standard plastics (which are generally recalcitrant to microbial breakdown in the short-term) or biodegradable plastics (which are more accessible as a C source to microbial community) in influencing the microbial community.

4.5 Oxo-degradable plastics degradation is inversely related to plastic concentration in soil

The lack of peak formation in the C=O and O–H regions of the spectra after 6 weeks in the soil indicate that oxidative degradation of the polymer had not progressed sufficiently to form ketones, aldehydes or carboxylic acids. This is likely attributed to the lack of prior UV exposure, as UV irradiance is key to the oxidation process of ODPs, and its absence or limited exposure would significantly reduce the rate of oxidation. Variation in soil moisture or competing chemical interactions in the soil could also be compounding factors for limited oxidation.

The peak intensity increase at 1032 cm^{-1} (C–O stretching vibrations) indicates that partial oxidation and formation of oxygenated groups had occurred, although this was insufficient to affect the C=O stretch. However, this increase could have also been affected by the potential adsorption of soil-derived organic compounds to the plastic surface. The decreases in peak intensity at 1461, 2844, and 2915 cm^{-1} (CH bending and stretching vibrations), indicate the process of chain scission and subsequent breakdown of the aliphatic hydrocarbon backbone of the polymer. Also, oxidation of the film will be reduced by the presence of antioxidants (0.2% w/w), quenching free radicals produced in the first steps of chain scission. The ODP we used is therefore likely to require extended periods of UV exposure (potentially weeks to months in direct sunlight) to initiate meaningful degradation (i.e., the antioxidant protection system would need to be depleted first before photooxidation could effectively progress). The observed changes to the ODP FTIR spectra are indicative of surface-level chemical changes at the beginning of the degradation process driven by exposure to soil rather than oxidative chemical reactions, resulting in limited oxidation of the polymer. This is supported by the fact that peak changes occurred inversely to increasing ODP concentration, indicating that direct exposure to soil accelerates the degradation process compared to treatments with high dose-rates where particles have reduced direct surface-soil contact. We ascribe this to a lack of microbial access to ODP surfaces or a saturation in the activity of exoenzymes acting on the ODP additives or lack of natural ODP oxidants when UV is absent[31].

This is consistent with previous studies in fresh water, marine water, sediments and soils that have shown limited breakdown of the polymer[7,78]. This limited degradation aligns with recent studies questioning the effectiveness of pro-oxidant additives to accelerate ODP breakdown under real-world conditions where UV irradiation is limited[11,79,80]. The changes in peak intensity represent the initial stage of degradation, but the overall persistence of original polymer structures and lack of oxidation indicates that degradation remains superficial[81]. These findings indicate that ODPs can persist in agricultural soils for much longer than anticipated, potentially accumulating over multiple growing seasons and leading to progressive changes in soil properties[12]. Clearly, longer-term studies are needed to fully understand these effects, including how UV exposure of plastic film before burial affects degradation.

5 Conclusions

Our results support a precautionary approach to ODP use in agriculture. The combination of incomplete degradation and potential accumulation aligns with recent policy decisions to restrict ODP use in some countries[11]. The lack of acute impacts at field-relevant concentrations on plant and soil health should not reduce concerns about long-term accumulation. This is particularly relevant given the evidence about the potential long-term persistence of ODPs and pro-oxidant additives in soil[5]. Taken together, the evidence indicates that ODPs might not offer significant advantages over standard plastics in terms of environmental safety, supporting recent bans in some countries including Saudi Arabia and parts of Europe[12,82]. While ODPs were introduced as an innovative solution to plastic pollution[1], our results indicate they could instead contribute to the growing challenge of agricultural microplastic contamination. Further research is clearly needed to (1) evaluate the range of ways in which ODPs can enter soil (e.g., via composts and biosolids), (2) better understand their

rate of breakdown and persistence over much longer time periods in the field, (3) their potential to form nanoplastics and release additives, and (4) their potential to transfer to watercourses and the atmosphere.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2025623 contains supplementary materials (Figs. S1–S4; Tables S1–S3).

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Compliance with ethics guidelines

Do Thi Kim Thanh, Robert W. Brown, Martine Graf, Michaela K. Reay, Charlotte E. M. Lloyd, Gupeng Li, David R. Chadwick, and Davey L. Jones declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

Data availability

The data are publicly available in Zenodo open access data repository (doi: 10.5281/zenodo.14003212).

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