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LDPE and biodegradable PLA-PBAT plastics differentially affect plant-soil nitrogen partitioning and dynamics in a *Hordeum vulgare* mesocosm

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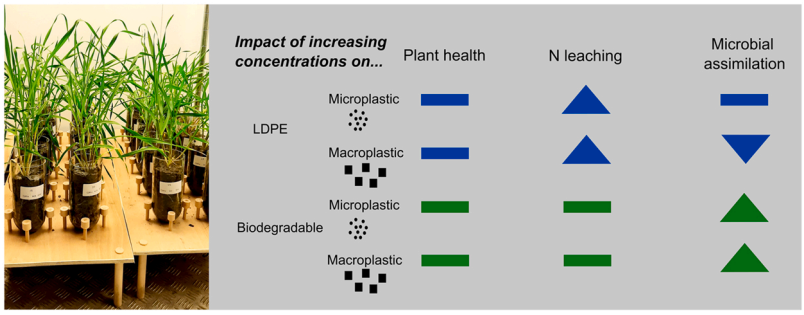
HIGHLIGHTS

- Barley was exposed to biodegradable and LDPE macro and microplastic.
- ^{15}N-tracing indicated LDPE microplastic reduced plant ^{15}N uptake due to N losses.
- LDPE plastics altered partitioning of ^{15}N within soil N pools.
- Biodegradable macro and micro plastic increased microbial N uptake.
- Biodegradable and LDPE plastics had differing impacts on plant-soil N partitioning.

GRAPHICAL ABSTRACT



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ABSTRACT

Micro and macroplastics are emerging contaminants in agricultural settings, yet their impact on nitrogen (N) cycling and partitioning in plant-soil-microbial systems is poorly understood. In this mesocosm-scale study, spring barley (*Hordeum vulgare* L.) was exposed to macro or microplastic produced from low density polyethylene (LDPE) or biodegradable plastic at concentrations equivalent to 1, 10 and 20 years of plastic mulch film use. Partitioning of ^{15}N-labelled fertiliser into plant biomass, soil and leachate yielded a partial mass balance. Soil N partitioning was probed via compound-specific ^{15}N-stable isotope analyses of soil microbial protein. Concentration-dependent decreases in plant ^{15}N uptake occurred with increased leached nitrogen for LDPE microplastic. Assimilation into soil microbial protein was higher for biodegradable plastics, which we associate with early-stage biodegradable plastic degradation. Partitioning of ^{15}N into inorganic soil N pools was affected by LDPE size, with lower assimilation into the microbial protein pool. While microplastics and macroplastics altered soil N cycling, the limited impacts on plant health indicated the threshold for negative effects was not reached at agriculturally relevant concentrations. This study highlights the difference between conventional and biodegradable plastics, and emphasises that the interplay of micro and macroplastics on soil N cycling must be considered in future studies.

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1. Introduction

Over the last > 50 years, plastic addition as macro or microplastic to agricultural cropping systems has been increasing due to the direct use of plastic mulch films and polymer-coated fertilisers, and indirectly via sewage irrigation, sludge/compost application and aerial deposition [1-4]. Inappropriate disposal of plastic mulch, due to prohibitive costs and limited facilities, has resulted in soils likely receiving more plastics than the oceans [5]. Both conventional, and newly emerging biodegradable mulches are subsequently fragmented into macro and microplastics via both abiotic (e.g. tillage, UV) and biotic (microbial and mesofauna) mechanisms [6,7]. While biodegradable macro and microplastics should further degrade to CO_2 and biomass (e.g. 90% within 24 months, EN17033), conventional mulches persist on a decadal timescale [8,9]. It is not clear how these inputs of varying size affect soil nitrogen (N) dynamics prior to further degradation, despite the expected continued use and accumulation of terrestrial plastic [10,11].

Previous studies have indicated effects of plastics on both soil biological properties (i.e. microorganism composition, N assimilation, enzyme activities; [12]), and physical properties (i.e. soil porosity and water content; [13]). Changes in microbial community composition, including species and genes directly linked to N processes, have been found to vary following exposure to low density polyethylene (LDPE) microplastic [14]. Direct measures of N processing are limited, and there is also wide variability in microplastic size, concentration and shape used in previous studies. In the presence of 2% (*w/w*) of high-density polyethylene (HDPE) and polypropylene (PP) microplastic, no observable effect on soil microbial activity was seen using hydrolysis of fluorescein diacetate in the presence of *Allium fistulosum* [15]. However, at extreme PP concentrations (28% *w/w*), stimulation of microbial activity was observed, enhancing soil organic matter decomposition [16]. While lower levels of synthetic microplastics have shown little impact on N cycling, Meng et al. [13] found biodegradable microplastics (10% polylactic acid (PLA), 85% polybutylene adipate terephthalate (PBAT), 5% calcium) had stronger effects on soil N cycling, increasing dissolved organic N. Biodegradable plastics are likely to replenish soil organic carbon pools when the plastic particles decompose, thus, affecting soil C: N ratios and rates of N mineralisation [17]. However, the type and production of decomposition compounds will depend upon the type of bioplastic, environmental conditions and time [18,19].

To date, research has focused on microplastic yet macroplastic is likely to be more abundant in the soil after mechanical breakdown of plastic mulch film. Particularly LDPE mulch is a potential major source of macroplastic, taking decades to show evidence of decomposition [20]. At high synthetic macroplastic concentrations, above the equivalent of 31 years of mulch film use, soil available N was significantly reduced in cotton fields [21]. Biodegradable (starch based) macroplastic in soil (1% *w/w*) has previously been shown to inhibit wheat growth, potentially arising from reduced N availability with increases in microbial immobilisation, primed from the addition of degradable C, comparable to residue mulching [22]. Conversely, when relatively inert LDPE was applied same concentration, there was no impact on wheat growth [23]. Given the potential abundance of macroplastics in soil [24,25], more studies are needed to determine the effect of macroplastic on soil N cycling.

^{15}N stable isotope probing (^{15}N-SIP) is a powerful tool to determine N partitioning and quantify nutritional relationships [12]. ^{15}N-tracing under plastic mulch with maize (*Zea mays* L.) saw increased plant uptake, with losses from ridges under mulch from both lateral and vertical flow [26]. Furthermore, Liu et al. [27] found increased retention of ^{15}N-urea under LDPE mulch, both with and without maize. However, the application of ^{15}N-SIP to agricultural systems impacted by micro and macroplastic contamination rather than intact mulch has so far been limited. Importantly, such studies have not considered how the soil microbial community may be impacted by micro and macroplastics. The application of compound-specific ^{15}N-SIP has previously enabled direct quantification of microbial N processing into the soil protein pool of fertiliser [28]. This approach has elucidated both the extent of microbial N assimilation, and provided mechanistic level detail of the flow of N through microbial portion biosynthetic pathways [29]. Combining a ^{15}N mass balance approach with compound-specific ^{15}N-SIP has the ability to provide both insight into partitioning of fertiliser N and additional mechanistic detail regarding fertiliser N assimilation into the microbial community. This is key to determining the impact of micro and macroplastics on soil health, and potential impacts on the ability of soil microbes to support plant N supply.

The primary aim of this study was to determine a partial N balance (gaseous losses were not experimentally examined) in a *Hordeum vulgare* mesocosm experiment using ^{15}N-labelling under the addition of different plastic types (LDPE and a PLA/PBAT biodegradable blend, termed biodegradable hereon), size (macro and micro) and concentration (1, 10 (both plastic types) and 20 (only LDPE) years equivalent plastic addition). The differing concentrations of plastic aimed to determine a critical threshold for the impact of macro and microplastics on N partitioning, and plant and microbial N uptake. We hypothesise that at high macro and microplastic concentrations, plant uptake will decrease, with increased potential for microbial N assimilation and N leaching due to lower plant N uptake and changes in soil physical properties. Additionally, we hypothesise that increased C availability due to degradation of the biodegradable plastic will promote microbial N assimilation, increasing with higher plastic addition rate.

2. Materials and methods

2.1. Soil and plant preparation

Soil was collected from the top 10 cm of a grassland (*Lolium perenne* L.) located in Abergwyngregyn, Wales (53°13′ N, 4°00′ W) with no prior history of plastic use. The field was ploughed one month before soil sampling. The soil is classified as a Eutric Cambisol (IUSS Working Group WRB, 2015) or Typic Hapludalf (US Soil Taxonomy) with a sandy clay loam texture and crumb structure. Prior to the experiment, the soil was 9 mm sieved to remove stones, and air dried. General soil properties are presented in Table 1.

Two types of plastic were used: conventional (LDPE, 30 μm thickness, GroMax Industries Ltd, Hadleigh, UK) and biodegradable (PLA/PBAT; 15:85 *w/w*; 15 μm thickness, GroMax Industries Ltd). Both plastic films were cut into squares of ca. 1 cm × 1 cm for the macroplastic treatment. For microplastic treatments, pieces of film were blended with an A10 basic batch mill (IKA Ltd, Oxford, UK) and then sieved to obtain microplastic below 63–500 μm. The size range was measured on an 8700 Laser Direct Infrared (LDIR) Imaging System (Agilent Inc., Santa Clara, CA). The biodegradable microplastic size range was 20–498 μm, the median size was 103 μm, and the 95th percentile was 412 μm. The LDPE microplastic size range was 20–431 μm, the median size was 40 μm, and the 95th percentile was 84 μm. The amount of plastic added to the soil was determined as an equivalent of years of plastic mulch film use,

Table 1
General properties of the soil used in the experiments. Values are expressed on a dry weight basis and represent mean ± SE ($n = 3$).

Soil property	Unit	Value
pH(H_2O)		6.5 ± 0.1
EC	μS cm^{-1}	55.1 ± 3.5
Soil C	g kg^{-1}	23.9 ± 0.8
Soil N	g kg^{-1}	2.4 ± 0.1
Sand	%	15.3 ± 0.9
Silt	%	42.3 ± 0.3
Clay	%	42.3 ± 0.9
NH_4^+	mg kg^{-1}	0.8 ± 0.1
NO_3^-	mg kg^{-1}	3.1 ± 0.2
DOC	mg kg^{-1}	8.0 ± 0.6

where we weighed the plastic film that would cover the surface area of the mesocosms and multiplied by the number of years. This assumed one cropping season per year, consistent with UK practices. The plastic was homogenised with soil (combined total of 700 g) and then added to 1 l glass mesocosms ($n = 4$ per treatment; Fig. S1). As a percentage of soil weight these concentrations are 0.02%, 0.2% and 0.4% to represent 1-, 10-, 20-years of plastic mulch film use, respectively. The 20-year treatment was only conducted for LDPE, and was not included for the biodegradable plastic given the expected shorter life span of this plastic type in soil. Two no-plastic controls treatments were included, with and without ^{15}N fertiliser ($n = 4$). Soil was wetted by adding 500 ml of artificial rainwater without N to 70% field water holding capacity (WHC) [30]. Spring barley (*Hordeum vulgare* L., var. Firefoxx Pre-Basic, Elsoms UK) seeds were pre-germinated in a damp paper towel. Spring barley was selected as a relevant crop for the region, and based on previous field studies with pure microplastics [31].

After 3 days, one germinated seed was planted into each container. Soil was fertilised with 40 kg N ha^{-1} (as NH_4NO_3), 45 kg P ha^{-1} (as Na_2HPO_4) and 45 kg K ha^{-1} (as KCl) on the seedbed. The ammonium nitrate (NH_4NO_3) fertiliser was labelled with ^{15}N at 20% atom enrichment. Plants were grown in a growth room for 5 weeks before being sampled. The average temperature was 20 ± 2 °C. The average relative humidity was 69% with a minimum and maximum relative humidity of 54% and 84%, respectively. The average solar radiation was 97.8 μmol m^{-2} s^{-1}, with a 16 h photoperiod. The containers were watered twice weekly to field capacity (100% WHC), with each container receiving between 100 and 150 ml of artificial rainwater without N to obtain leachate. Volume was increased as plants developed, due to increased plant water use. The leachate was collected in a glass jar underneath the container and combined per mesocosm across the 5 week experimental period and stored at 4 °C prior to analysis for total N and total ^{15}N content (see methods below). Cumulative mass of total N and ^{15}N leached from the mesocosms was calculated by multiplying the leachate volume by the total N and ^{15}N content.

2.2. Plant and soil properties

Plant height was measured from the base of plant to the top of the stem. Leaf chlorophyll content was also measured by Soil Plant Analysis Development (SPAD) chlorophyll meter (Konica Minolta SPAD-502 PLUS). Both were measured twice a week for the duration of the experiment ($n = 9$). Root and shoot dry biomass were determined at the end of experiment by drying at 80 °C for 24 h and then weighing.

Soil samples were taken from each pot at the end of the experiment. Gravimetric water content was determined by oven drying soil (105 °C, 24 h). Soil pH and electrical conductivity (EC) were measured in soil solution (1:2 (*w/v*) soil: distilled H_2O) using standard electrodes. The following analyses were measured in soil extracts (1:5 (*w/v*) soil:0.5 M KCl). Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were both determined colorimetrically according to the salicylic acid procedure of Mulvaney [32] and VCl_3 procedure of Miranda et al. [33], respectively. Available phosphorus (P) was measured in soil extracts (1:5 (*w/v*) soil:0.5 M acetic acid) using the method of Vaz et al. [34]. Dissolved organic C (DOC) was measured in soil extracts (1:5 (*w/v*) soil: distilled H_2O) on a Multi-N/C Series NPOC-TN analyser (Analytik Jena, Germany). Total dissolved N (TDN) of the leachate was also measured on a Multi-N/C Series NPOC-TN analyser.

2.3. ^{15}N balance analysis

2.3.1. Sample preparation

Soil, and leachate samples were frozen (−20 °C), freeze dried and homogenised by grinding. Plant biomass was oven dried (see Section 2.1) and ground. Extractable soil NH_4^+ and NO_3^- (see Section 2.1) were collected onto acidified filter discs (50 μl of 2.5 M $KHSO_4$ on Whatman 3 filter) [35]. Briefly, NH_4^+ was volatilised using MgO and collected on an acidified filter over 7 days. Subsequently NO_3^- was converted to NH_4^+ using Devarda's alloy (0.2 g), and collected in the same manner. Filter discs were dried in a desiccator prior to analysis.

2.3.2. Amino acid preparation

The total hydrolysable amino acid (THAA) portion of soil was extracted, isolated and derivatised to their *N*-acetyl, *O*-isopropyl (NAIP) derivatives [28,36]. Briefly, THAAs were extracted from soil (200 mg) using 6 M HCl under an N_2 atmosphere (24 h at 100 °C) and isolated using cation exchange chromatography (Dowex® 50WX8). Subsequently, the isolated THAAs were propylated using isopropanol and acetyl chloride (4:1 *v/v*), then acetylated using triethylamine, acetic anhydride and acetone (2:1:5 *v/v/v*). Norleucine (Nle; 100 μl of 400 μg ml^{-1} in 0.1 M HCl) was added as an internal standard.

2.3.3. Analyses

The elemental C and N content and ^{15}N-enrichment of soil, plant biomass, leachate and filters were determined via an elemental analyser (EA; vario PYRO cube; Elementar Analysensysteme GmbH, Hanau, Germany) coupled to a continuous flow isotope ratio mass spectrometer (Elementar Isoprime Precision; Elementar Analysensysteme GmbH). The EA was calibrated with sulfanilamide (N: 16.26%, C:41.81%, S: 18.62%) and the precision as a relative standard deviation (RSD) was < 5% for both C & N. The IRMS was calibrated against international reference standards (caffeine: USGS61 (−2.87‰ $\delta^{15}N$), USGS62 (20.17‰ $\delta^{15}N$), USGS63 (37.83‰ $\delta^{15}N$), and IAEA 311 (2.05 ± 0.2 atom% ^{15}N)) and the precision as a standard deviation (SD) was < 0.08‰.

Amino acid derivatives were quantified separately using an Agilent Technologies 7890B GC (Agilent Technologies, CA, USA) with a flame ionisation detector (FID). Data was acquired and analysed using Agilent OpenLab CDS Chemstation (Rev C.01.07[27]; Agilent Technologies). The analysis for THAAs was as follows: using a DB-35 coated capillary column (60 m × 0.32 mm i.d., 0.5 μm phase thickness) and He carrier gas at a constant flow of 2.0 ml min^{-1}, the temperature programme was 70 °C (2 min) to 150 °C at 15 °C min^{-1}, to 210 °C at 2 °C min^{-1} and a final temperature of 270 °C (8 °C min^{-1}; 10 min) [37]. An external standard of amino acids was used to monitor instrument performance, identification and calculation of AA-specific response factors for quantification. The external standard for amino acids comprised of 14 amino acids (alanine; Ala, aspartic acid; Asp, glutamic acid; Glu, Glycine; Gly, hydroxyproline; Hyp, leucine; Leu, lysine; Lys, Nle, phenylalanine; Phe, proline; Pro, serine; Ser, threonine; Thr, tyrosine; Tyr and valine; Val) in 0.1 M HCl.

^{15}N enrichment for individual AAs, as their NAIP derivatives, was determined using a continuous flow isotope ratio mass spectrometer (Elementar Isoprime Precision; Elementar Analysensysteme GmbH) coupled to a GC (Agilent 7890 B fitted with an Agilent 7693a autosampler, Agilent Technologies) and a combustion oven, with a combustion reactor head at 1030 °C (Elementar GC5). The carrier gas was He at a flow rate of 1.4 ml min^{-1}. The GC was fitted with a DB-35 column and the temperature programme employed was 40 °C (5 min) to 120 °C at 15 °C min^{-1}, to 180 °C at 3 °C min^{-1}, then 210 °C at 1.5 °C min^{-1} and finally to 270 °C (5 °C min^{-1}). Data was acquired and analysed using IonOS (4.3.7.9012). All analyses were completed in duplicate. Determined $\delta^{15}N$ values were corrected using in-house AA standards of known ^{15}N enrichment, traceable to secondary standards, and values were only accepted when the calibration r^2 greater than 0.95.

2.4. Data analysis

^{15}N enrichments of plant tissues, soil, leachate, and soil N pools (NH_4^+, NO_3^-, THAAs) are reported as atom fractions, and as percentage retained (%R) in each pool as derived from applied ^{15}N-fertiliser at $t = 0$ following:

$$\%R = \frac{mol^{E}(^{15}N)p}{mol(^{15}N)_{g}}100$$

Where mol(^{15}N)$_g$ is the ^{15}N in applied ^{15}N-labelled fertiliser and mol^E(^{15}N)p is the total amount of ^{15}N in excess (above background/ambient concentration) in the analysed pool, calculated from the pool size, N content and atom fraction excess ($x^{E}(^{15}N)$):

$$x^{E}(^{15}N) = x(^{15}N)_{p} - x(^{15}N)_{C}$$

Where $x(^{15}N)_p$ is the atom fraction of ^{15}N in the pool at each sampling timepoint, and $x(^{15}N)_C$ is the atom fraction of ^{15}N in the pool in the control treatment where no fertiliser was applied.

Unless otherwise stated, all graphs and data analysis were carried out in R v4.1.2. (R Studio 2021). Normality was checked visually using qqplot plots and heterogeneity using residual plots. Linear mixed-effects modelling was applied to evaluate the significance of plastic type, concentration, and size in explaining the variation in soil properties, THAA concentrations, and ^{15}N partitioning at the end of the experiment. Plastic type, size and concentration were set as fixed effects, while the mesocosms were a random effect.

3. Results

3.1. Plant growth and properties

Plant height was not influenced by plastic type, size or concentration across the 5-week experimental period (Fig. S2; ($F(1,396) = 0.495$, $p = 0.950$)), and plastic treatments showed a similar growth trend as the control treatment. Additionally, similar trends for all treatments in SPAD measurements of leaf chlorophyll content were also observed (Fig. S3; ($F(1,396) = 0.930$, $p = 0.535$)). Shoot and root biomass at the end of the experimental period are shown in Fig. S4. Mixed effects models revealed plastic type had a significant effect on shoot biomass ($F(1,30) = 4.32$, $p = 0.046$), with indications of decreased biomass at higher macro and microplastic concentrations. There were no significant effects on root biomass for any variable ($F(1,33) = 0.061$, $p = 0.807$). While there was a significant effect on shoot biomass, there was no effect on shoot N offtake (Fig. 1a) ($F(1,33) = 0.132$, $p = 0.719$). Similarly, plastic type, size and concentration had no effect of total N in roots (Fig. 1b) at the end of the experimental period ($F(1,33) = 0.034$, $p = 0.855$).

3.2. Soil physicochemical properties

Soil moisture content, phosphate and pH did not vary from the control treatment (Fig. S5). There was some variation in soil EC with plastic type (Fig. S5d), particularly for the LDPE treatment, although effect of size varied between plastic type, and there was a significant effect of plastic × size ($F(1,33) = 4.732$, $p = 0.037$). For the total N content of soil, there was no difference between the control, LDPE and biodegradable treatments (Fig. 2a; ($F(1,30) = 0.020$, $p = 0.965$)). Similarly, both NH_4^+ and NO_3^- concentrations (Fig. 2b and c, respectively) did not vary with treatment ($F(1,30) = 0.185$, $p = 0.671$ and $F(1,33) = 0.486$, $p = 0.490$, respectively). While inorganic N concentration did not vary, the THAA concentration (Fig. 2d) in soil was significantly influenced by plastic type ($F(1,30) = 6.820$, $p = 0.014$), and the interaction of plastic × concentration ($F(1,30) = 8.917$, $p = 0.006$). LDPE microplastic treatments had lower THAA concentrations at higher rates of plastic loading. For both micro and macro biodegradable treatments, THAA concentration increased with higher plastic loading (Fig. 2d). Soil DOC increased at higher plastic concentrations for both LDPE and biodegradable plastic (Fig. S5c), and concentration had a significant effect ($F(2,30) = 5.981$, $p = 0.007$).

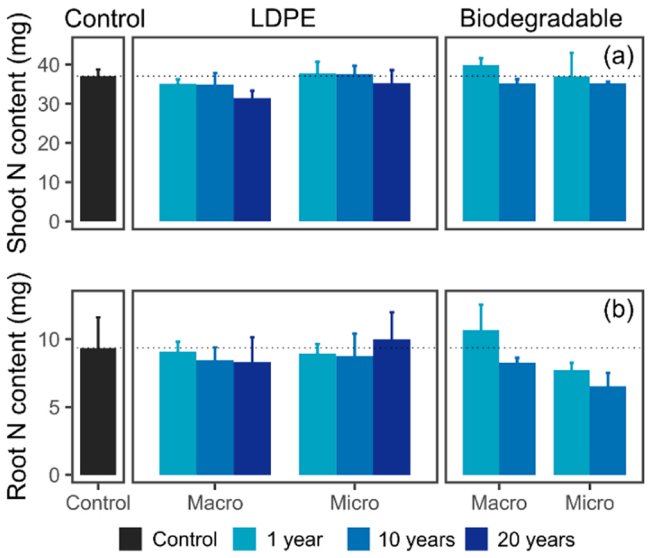

Fig. 1. Barley biomass N per plant present in (a) shoots and (b) roots following exposure to either macro- or micro-plastics of low-density polyethylene (LDPE) or biodegradable (PLA/PBAT) plastic for 5 weeks. The concentrations of plastic added to soil reflect 1, 10 and 20 (LDPE only) years of continual mulch film use and ploughing the residues into soil. Values represent mean ± SE ($n = 4$) and the dotted line indicates the control mean value.

3.3. Leached N

The total volume leached across the 5-week experiment (Table S1) did not vary between treatments. With respect to total N leached across the experimental period (Table 2), there were differing trends between plastic type, size and concentration, as indicated by the significant three-way interaction ($F(1,30) = 5.405$, $p = 0.027$). Leached N increased with concentration of LDPE macro and microplastic. An increase at higher concentration was also observed for the biodegradable macroplastic treatment, although there was no difference for the biodegradable microplastic.

3.4. Partial ^{15}N mass balance

The partitioning of applied ^{15}N-fertiliser is shown in Fig. 3. For LDPE microplastic, and both biodegradable plastic sizes, soil ^{15}N retention decreased at higher concentrations, while the opposite was the case for LDPE macroplastic (Fig. 3a). While there were differences in soil retention, this did not vary significantly from the control for the plastic treatments. N uptake into aboveground barley biomass (Fig. 3c) decreased with increasing LDPE macro and microplastic concentrations, which was also the case for the biodegradable macroplastic. There was a significant effect of plastic × size on aboveground ^{15}N uptake ($F(1,33) = 4.133$, $p = 0.043$), although all treatments were comparable to the control. Root uptake was significantly influenced by plastic concentration ($F(2,33) = 3.899$, $p = 0.030$), with a variable effect of size between plastic treatments (Fig. 3d). Root ^{15}N uptake for the LDPE macroplastic treatment only increased at 20 years, while uptake decreased for increasing LDPE microplastic concentration. Biodegradable macroplastic did not have an effect on root ^{15}N uptake, while biodegradable microplastic showed increased root uptake at 10 years concentration. Finally, leached ^{15}N (Fig. 3b) largely reflected the opposite trends observed for aboveground ^{15}N uptake, and there was a significant effect of both plastic × size ($F(1,30) = 18.83$, $p < 0.001$) and size × concentration ($F(2,30) = 4.564$, $p = 0.019$). It should be noted, while there were differences within treatments, and observable trends, the majority of parameters did not significantly vary from the control treatment. The average unaccounted for ^{15}N was 1.2 ± 2.7% of applied

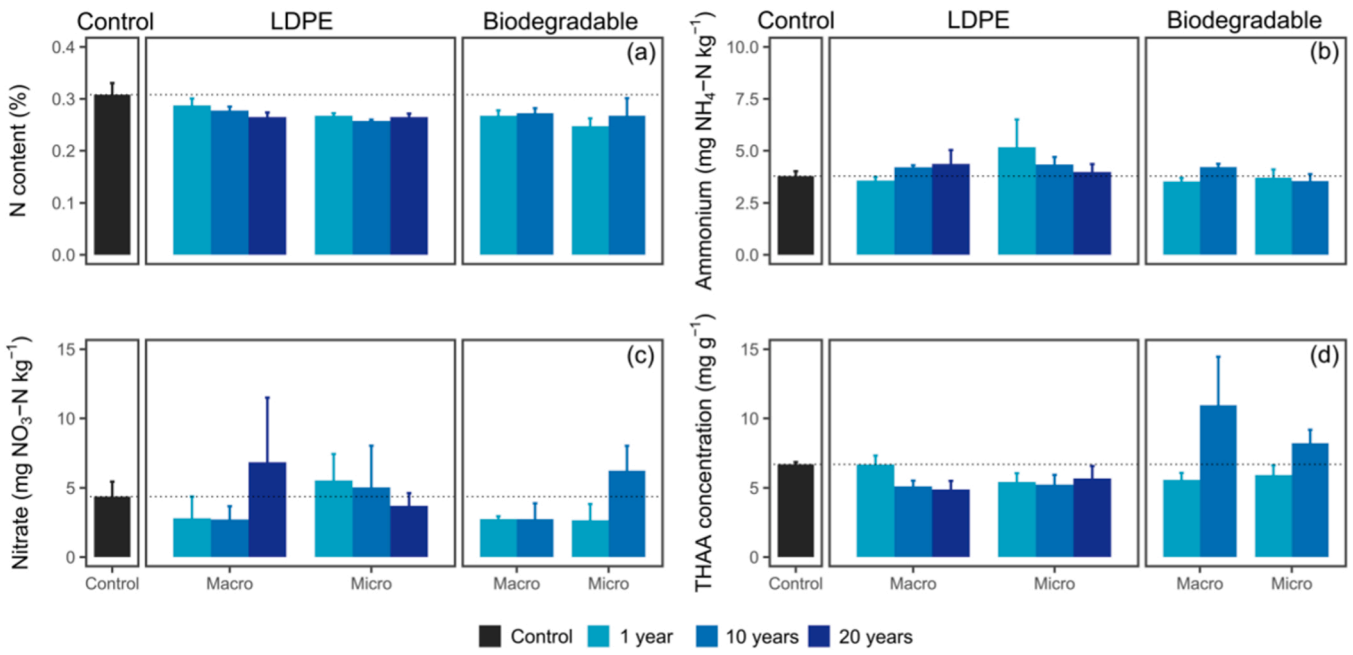

Fig. 2. Soil N properties in response to exposure to either macro- or micro-plastics of low-density polyethylene (LDPE) or biodegradable for 5 weeks. The concentrations of plastic added to soil reflect 1, 10 and 20 (LDPE only) years of continual mulch film use and ploughing the residues into soil (i.e. 1 year = 1 cropping cycle). (a) Soil N content, (b) ammonium content, (c) nitrate content, and (d) total hydrolysable amino acid (THAA) concentration, on a dry weight basis. Values represent mean ± SE ($n = 4$) and the dotted line indicates the control mean value.

Table 2
Cumulative leached N from soil mesocosms across 5 weeks from the barley mesocosm after exposure to either macro or microplastics of LDPE or biodegradable. The concentrations of plastic added to soil reflect 1, 10 and 20 (LDPE only) years of continual mulch film use and ploughing the residues into soil. Values represent mean ± SE ($n = 4$). Values were corrected for leached volume to account for any variation in leaching for N mass balance.

Plastic	Size	Concentration	Leached N (mg mesocosm^{-1})
Control	Control	Control	10.4 ± 2.3
Biodegradable	Macro	1 year	9.0 ± 1.0
		10 years	15.9 ± 1.3
	Micro	1 year	13.1 ± 2.0
		10 years	12.2 ± 1.0
LDPE	Macro	1 year	13.7 ± 1.3
		10 years	13.1 ± 2.6
		20 years	12.5 ± 1.0
	Micro	1 year	8.8 ± 1.4
		10 years	10.3 ± 0.2
		20 years	13.1 ± 1.9

fertiliser and did not vary between treatments. It is suggested unaccounted for N was lost via gaseous emissions, with nitrification likely the dominant process at 70% WHC, with increases in dentification following addition of water for leaching. This was a minor fate in this study and consistent with UK agriculture GHG and ammonia inventories [38,39].

In addition to the partial ^{15}N mass balance, partitioning into inorganic N (NH_4^+ and NO_3^-) and the largest defined ON pool in soil (THAAs) was determined (Fig. 4). For ^{15}N retained in the NH_4^+ pool, LDPE macro and microplastic increased with concentration, except for LDPE microplastic at 20 years equivalent concentration (Fig. 4a). ^{15}N in the NH_4^+ pool did not vary for the biodegradable macroplastic treatments, while it was higher at 10 years vs. 1 year for the biodegradable microplastic treatment. There was a significant size × concentration ($F(2,33) = 3.429$, $p = 0.044$) effect for ^{15}N retained in this pool. There was variability for ^{15}N retained in extractable NO_3^-, (e.g. decreased ^{15}N in NO_3^- with higher concentrations for LDPE microplastic, increased ^{15}N in NO_3^- for biodegradable microplastic, Fig. 4b). However, there was no significant influence of plastic, size or concentration for ^{15}N retained in this pool.

Partitioning into THAAs is shown in Fig. 4c. Generally, assimilation into this pool largely showed the opposite trends as observed for total soil ^{15}N retention. Incorporation into THAAs decreased with increased LDPE macroplastic concentration, although it was variable with concentration for LDPE microplastic. For both biodegradable macro and microplastic, assimilation into THAAs increased in the 10 year treatment compared to 1 year, and there was a significant effect of plastic × concentration ($F(1,30) = 8.737$, $p = 0.006$). Normalising THAA incorporation to account for the ^{15}N retained in the soil pool was used to see if there were differences in allocation to THAAs other than soil available ^{15}N (Fig. S7). Incorporation into the THAA pool of soil ^{15}N was significantly higher than the control treatment for 10-year biodegradable plastic treatments. Plastic type ($F(1,33) = 4.206$, $p = 0.048$), all two way interactions, and the combined effect of plastic type, concentration and size ($F(1,33) = 5.074$, $p = 0.031$) were significant for THAA incorporation normalised to soil-retained ^{15}N. Within the THAA pool, incorporation into individual AAs (Fig. S8) largely reflected trends for the THAA pool for all treatments. Incorporation into these pools were influenced by biosynthetic proximity to incorporation of fertiliser into AAs, via glutamate dehydrogenase or glutamine synthetase [40], as indicated by high incorporation into Glx and Asx pools, compared to Hyp and Tyr. Furthermore, AAs with larger pool size (e.g. Ala and Gly) also accounted for a larger proportion of ^{15}N within the THAA pool. Further probing of partitioning of assimilated ^{15}N within the AA pool (Fig. S9) revealed that assimilation into some hydrophobic (Pro, Leu, Val) and aromatic (Phe) AAs was lower in the biodegradable treatments compared to the control, although there was not a clear trend for LDPE treatments. Assimilation into Lys, the only basic AA quantified, was elevated relative to the control treatments for all plastic types.

4. Discussion

4.1. Plant development and N uptake

There was no significant effect of plastic addition on key plant

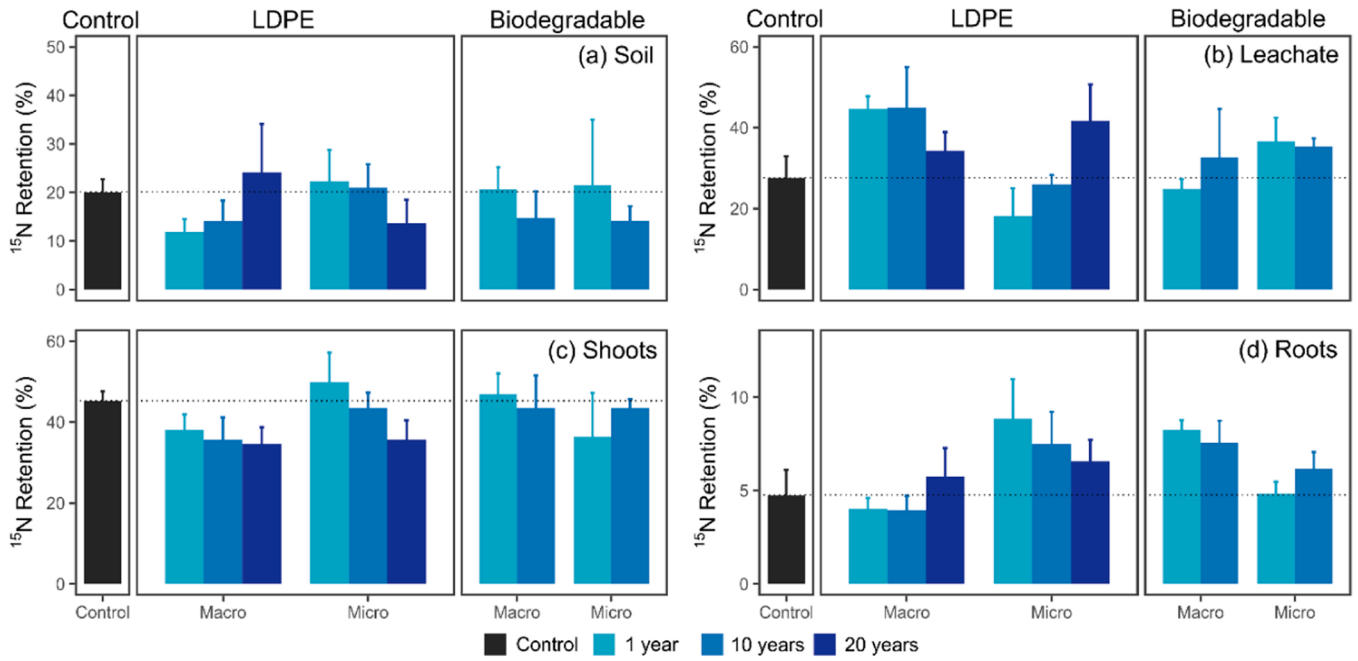

Fig. 3. Partitioning of ^{15}N fertiliser into (a) total soil, (b) leachate, (c) barley shoots and (d) roots after exposure to either macro or microplastics of LDPE or biodegradable for 5 weeks. The concentrations of plastic added to soil reflect 1, 10 and 20 (LDPE only) years of continual mulch film use and ploughing the residues into soil. Values represent mean ± SE ($n = 4$) and the dotted line indicates the control mean value.

productivity indicators, including plant height, chlorophyll content, and plant N offtake. A common effect of PLA microplastic on plants has been identified as reduced chlorophyll content [41], however, there was no effect of the PLA/PBAT blend used in this study. While shoot biomass decreased at higher plastics loadings for LDPE and biodegradable microplastics, any observable effect was not significant. Combined, this indicated that the threshold for effects on barley growth was not reached in this study. This is consistent with previous studies which used low LDPE and biodegradable microplastic concentrations with barley [31, 42] and other crop types [43,44]. Furthermore, no ecotoxicological effects were found for *Allium cepa* for PLA, PBAT and their blends [45]. Some studies have found negative impacts on *Lolium perenne* germination and shoot growth for PLA only at lower concentrations (0.01% *w/w*), suggesting different blends or plant systems may have lower thresholds for significant effects on development [46].

For macroplastics, low concentrations of LDPE (1% *w/w*) showed no impact on wheat growth [23], as was found for barley in this study, with only concentrations equivalent to over 100 years of mulch use significantly impacting cotton plant growth [21]. To our knowledge, there have not been any previous studies on PLA/PBAT macroplastic on plant performance. However, other studies of biodegradable macroplastic (e.g. a pullulan/PET/PBT blend, 1% *w/w*) indicated significant impacts on plant growth [23]; caution is therefore required in extrapolating to other biodegradable plastics. Given that macroplastics will be a major input from biodegradable plastic mulch films as they degrade [6,7,47], this study sheds new light onto effects of barley growth (or lack of) at low, but agriculturally relevant, concentrations for a PLA/PBAT blend.

While there was little evidence of negative effects on overall plant health, as indicated by chlorophyll content, fertiliser N uptake into barley was altered by the presence of macro and microplastics. For the LDPE plastics, aboveground ^{15}N uptake decreased, while soil retention and leached N increased for increasing concentrations of macro and microplastics, respectively. As plastic additions had no effect on plant health, alongside similar trends in soil ^{15}N retention, these changes in ^{15}N partitioning were likely governed by changes in ^{15}N availability and leaching, as a result of abiotic effects of micro and macroplastic, rather than a direct impact on the barley. This was supported by the observation of little effect on ^{15}N uptake for both biodegradable plastic sizes, where leaching did not vary significantly compared to the control. A similar response was observed for polyester fibres in soil (0.5% *w/w*) which decreased N uptake in maize due to increased leaching [48]. Thus, while plant health (e.g., growth, chlorophyll content, N content) was not impacted by the realistic soil concentrations of macro and microplastics used, compared to no plastic, uptake of fertiliser N can be altered in the early stages of barley growth.

4.2. Soil nitrogen partitioning and microbial N biosynthesis

Varying patterns in soil N partitioning between plastic type, size and concentration-dependent effects revealed potential complexities of impacts of N cycling for macro and microplastics. In terms of differences between LDPE and biodegradable plastic, assimilation into the soil microbial protein pool reflected the differences in degradation of the two plastics, with LDPE inert while decomposition of the PLA/PBAT blend will act as a potential source of C. This is supported by the concentration-dependent increase in THAA concentration for the biodegradable plastics, despite a decrease in soil N content. Changes in THAA concentration have not previously been observed with fertiliser application only [28]. Furthermore, assimilation of fertiliser ^{15}N increased at higher loadings of macro and microplastic relative to the control, which is more evident when corrected for soil retained ^{15}N for biodegradable microplastics. There was no increase in soil DOC, consistent with previous studies with PLA/PBAT microplastics at similar loadings, given rapid microbial utilisation of available C [13,49]. Utilisation of biodegradable plastic derived C by the soil microbial community has previously been confirmed by increased microbial growth [50], and directly traced microbial assimilation of ^{13}C-labelled PBAT [51]. There was also an indication of changes in the distribution of assimilated ^{15}N into individual AAs relative to the no plastic control, particularly for hydrophobic AAs. From the findings herein, it is not possible to confirm the mechanism for this. Breakdown products of the biodegradable plastic, such as lactate from PLA, are linked to AA biosynthesis and metabolism via pyruvate metabolism [52]. It is likely a change in production or turnover rates altered ^{15}N partitioning within the AA pool, affecting the equilibrium

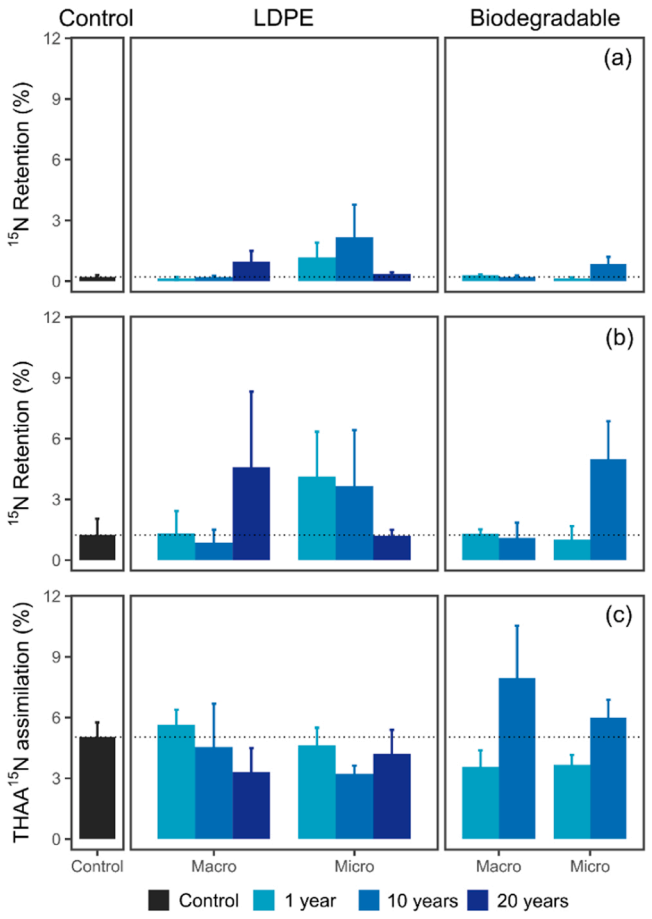

Fig. 4. Partitioning of ^{15}N fertiliser into soil N pools of (a) ammonium, (b) nitrate and (c) THAAS after exposure to either macro or microplastics of LDPE or biodegradable for 5 weeks. The concentrations of plastic added to soil reflect 1, 10 and 20 (LDPE only) years of continual mulch film use and ploughing the residues into soil. Values represent mean ± SE ($n = 4$) and the dotted line indicates the control mean value.

^{15}N assimilation observed after 5 weeks exposure, a hypothesis which needs further testing.

It was hypothesised that ^{15}N assimilation would have been highest for biodegradable microplastic at 10 years, due to C availability from bioplastic degradation, which was not the case. Instead, retention in the soil ammonium and nitrate pools were elevated. As both inorganic N pools exhibited elevated ^{15}N retention in this treatment, it may be that both nitrification and denitrification were suppressed. Previous studies have found variable responses to PLA microplastics for N transformations, including suppression [53,54], no changes [55] and promotion [56,57]. THAA ^{15}N incorporation reflects the equilibrium between microbial assimilation and mineralisation. Increased incorporation into this pool may reflect increases in microbial assimilation, consistent with increased THAA concentration. It may also reflect decreased mineralisation of the THAA pool, similar to that observed by Shi et al. [58] with 0.5% and 1.0% (*w/w*) PLA microplastic, although there were no significant changes in NH_4^+ concentration. With the added insight from the organic N pool, it is apparent that higher PLA/PBAT microplastic concentrations altered soil N cycling, despite no change in plant ^{15}N uptake. Further work is required to disentangle the direction and magnitude these N cycle processes (e.g., ^{15}N isotope tracing [59,60] and will help build on the N partitioning findings herein.

For LDPE microplastics, there was little change in microbial assimilation relative to the control, indicating there was no toxic effect on microbial biosynthesis even at high plastic loadings. There was little difference in the distribution of assimilated ^{15}N within individual AAs, except lysine, the only basic AA quantified by this approach, which was elevated compared to the control. Links have previously been identified between lysine-rich proteins and responses to abiotic stress [61]. It is not possible to confirm the mechanism for this change in ^{15}N partitioning within the THAA pool presented herein, and requires further investigation. At lower microplastic loading, higher ^{15}N retention in inorganic N pools suggested suppression of nitrification and denitrification for fertiliser-derived ^{15}N, similar to PLA [53,62]. Furthermore, it may also reflect increases in mineralisation of assimilated ^{15}N, which is also consistent with the opposite trend in THAA assimilation compared to ^{15}N retention in the NH_4^+ pool. Increased N mineralisation has been found with LDPE microplastic, associated with changes in microbial community structure [63-65]. At 20 year microplastic concentration, there was a decrease in ^{15}N in both inorganic N pools, and the total soil pool. We speculate that this reflects changes in physical pore flow pathways which may have promoted leaching, rather than changes in N cycling processes, given no change in total accounted for ^{15}N, and increased N leaching, alongside concurrent decreased soil and plant ^{15}N retention [48]. Conversely, for LDPE microplastic, a predominantly biotic effect on ^{15}N partitioning is suggested, with no change in leached N, yet suppression of microbial ON biosynthesis and elevated ^{15}N in both inorganic N pools. Thus, changes in N availability, and potential suppression of nitrification and denitrification may be responsible, rather than changes in N loss via leaching.

4.3. Implications and outlooks

While there were differential responses between plastic types, and sizes with plastic concentration, there were few parameters for plant health that were significantly different compared to the control treatment. This indicated while there was some influence of the differing treatments, at the realistic concentrations used in this study, the threshold to significantly alter N cycling, and thus impact plant health was not reached, at least in the short term. Hence, the approach herein offers valuable insights into the effect of micro and macroplastics in real-world scenarios, including using higher concentration (e.g. LDPE 20 years) to reflect more extreme plastic inputs. Further improvements should consider the potential synergistic effects of macro and microplastics together, given potential differences in responses, particularly those observed for LDPE, given they will co-occur in the environment.

This study looked at effects occurring during early stage plant growth, however, it does not reveal potential impacts of plastic loadings later in the cropping season, or subsequent years. Further, biodegradable macro and microplastics will continue to degrade, hence have been termed “dynamic stages” of bioplastic; [9]). Longer-term studies, over a whole growing season, and multiple growing seasons, would elucidate effects of longer term degradation of plastics and C release. For pure LDPE, previous studies at the same site, even with a higher microplastic concentration (10% *w/w*), did not indicate any changes in wheat yield, nor soil function over a growing season [43]. Consideration of the starting soil, and its ability to buffer, or resist, abiotic and biotic effects of plastic inputs on a longer-term scale is needed. This should also include earthworms, given their role in N cycling and microplastic transport [66,67]. Furthermore, this study used macro and microplastic produced from plastic mulch, which will also contain additives prone to leaching into soil [68]. These are both available for plant [69-72] and microbial uptake [73-75], and can have toxic effects. Given the wide variety of additives used in plastic production it is likely the composition of additives varied between the two plastic types, and with previous work [76]. For example, phthalates have been identified as key drivers for changes in soil microbiota and function, while pure PVC did not [77]. Thus, the differing additive composition for plastics in this study, and in future work, and their impact on N cycling must be explored.

5. Conclusions

Our results demonstrate the complexity of concentration-dependent N cycling with differing plastic types and sizes. While significant changes in N partitioning, and microbial protein biosynthesis were observed, the impacts in plant health parameters were limited at the agriculturally relevant concentrations used herein. Differences in plant N uptake were largely governed by losses via leaching, or soil N partitioning between inorganic and organic N pools. There was evidence of suppression of N transformations, via changes in ^{15}N retention in inorganic N pools, although further work is required to determine the mechanisms underlying this impact, and longer term impact on soil N and plant N supply. Microbial protein biosynthesis was promoted for biodegradable plastic treatments, likely due to C released during early stages of plastic degradation. Differences in assimilation into the protein pool with LDPE macro and microplastics were due to changes in N movement and loss via leaching. The combination of ^{15}N mass balance, and compound-specific ^{15}N-SIP revealed these abiotic and biotic effects to differ between the two plastic types. The results provide valuable insight into potential impact of micro and microplastic on N cycling in the soil ecosystem. As microbial N transformation play a key role in supporting plant N supply during turnover, further research is required to determine impacts in a wider range of soil-plant systems, and whether this will impact plant N supply on a longer timescale.

CRediT authorship contribution statement

M. K. Reay: Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **L. M. Greenfield:** Conceptualization, Investigation, Data curation, Visualization, Writing – review & editing. **M. Graf:** Investigation, Data curation, Writing – review & editing. **C. E. M. Lloyd:** Writing – review & editing, Funding acquisition. **R.P. Evershed:** Writing – review & editing, Funding acquisition. **D. R. Chadwick:** Writing – review & editing, Funding acquisition. **D. L. Jones:** Writing – review & editing, Funding acquisition.

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

Data will be made available on request.

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Environmental implications

This manuscript is of high environmental relevance due to increasing inputs of plastics in terrestrial settings from agriculture. Conventional plastic mulch films are used globally to improve agricultural yields, and biodegradable plastics are now being widely adopted as a green alternative. Yet the impact of microplastics and macroplastics on nitrogen cycling in cropping systems is relatively unknown. Our results indicate significant changes in soil nitrogen processes, leaching and plant uptake of fertilizer. As such, increasing concentrations of microplastics and macroplastics are likely to alter nitrogen use efficiency, and agricultural productivity.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.130825.

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