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Increasing concentration of pure micro- and macro-LDPE and PP plastic negatively affect crop biomass, nutrient cycling, and microbial biomass

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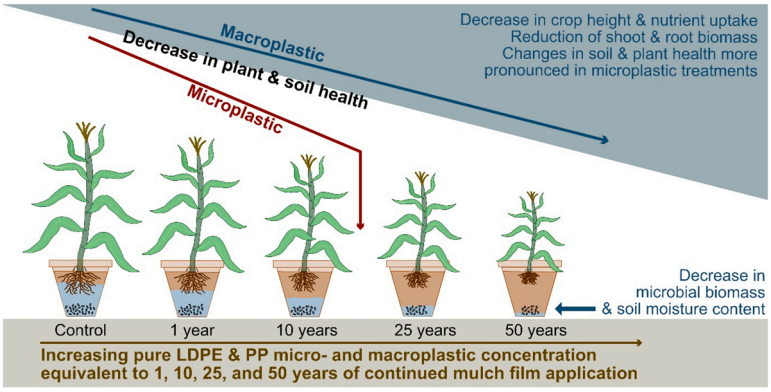
HIGHLIGHTS

- Crop height and nutrient content decreased with increasing plastic concentration.
- Soil moisture content severely reduced with increasing plastic concentration.
- Changes in soil & plant properties were more pronounced in microplastic treatments.
- Effects of plastic addition are negligible in the short term for low concentrations.
- Microbial biomass decreased with increasing plastic concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Over the last 50 years, the intense use of agricultural plastic in the form of mulch films has led to an accumulation of plastic in soil, creating a legacy of plastic in agricultural fields. Plastic often contains additives, however it is still largely unknown how these compounds affect soil properties, potentially influencing or masking effects of the plastic itself. Therefore, the aim of this study was to investigate the effects of pure plastics of varying sizes and concentrations, to improve our understanding of plastic-only interactions within soil-plant mesocosms. Maize (*Zea mays* L.) was grown over eight weeks following the addition of micro and macro low-density polyethylene and polypropylene at increasing concentrations (equivalent to 1, 10, 25, and 50 years mulch film use) and the effects of plastic on key soil and plant properties were measured. We found the effect of both macro and microplastic on soil and plant health is negligible in the short-term (1 to <10 years). However, ≥ 10 years of plastic application for both plastic types and sizes resulted in a clear negative effect on plant growth and

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microbial biomass. This study provides vital insight into the effect of both macro and microplastics on soil and plant properties.

1. Introduction

The use of plastic in agriculture has been increasing over the last 50 years, and it is currently estimated that globally 8–12.5 million tonnes are used annually, representing ca. 2.4–3.5% of global plastic use ([8,17,54]). Most of this is dedicated to the production of agricultural plastic films [17]. One common application of these films is in the form of thin plastic mulch films (< 50 μm thick) that are laid over the soil surface to grow cereal crops, vegetables, and fruit. In 2017, global plastic film usage reached 6.7 million tonnes covering an estimated 25 million hectares [53,64]. The deployment of plastic film confers several advantages including: i) reduced weed growth, ii) increased soil moisture retention, iii) increased soil temperature, iv) enhanced pest control, and v) reduced potential for soil erosion [10]. However, conventional plastic mulch films have an average lifespan of only 6 months [17] and are difficult to recover from the soil after harvest. Plastic mulch films that have been recovered from fields are costly to recycle due to contamination of the plastic with soil particles. This often results in the plastic films being ploughed back into the soil or discarded along field margins [17], creating a legacy of plastic pollution in agricultural fields. Over time, smaller macroplastic (> 5 mm) and microplastic (1 μm to 5 mm diameter; [20]) particles are produced through mechanical abrasion and UV irradiation of the films [46], with different sizes of plastic being generated over time.

The majority of studies to date investigating the impact of plastic on agricultural soils have heavily focused on microplastics [9]. Microplastics have been shown to affect soil biological properties by altering microbial community composition and abundance, and generally negatively affecting macroinvertebrate populations [42,5,7]. Other studies have found soil plastic to negatively impact key soil chemical and physical properties intrinsic to maintaining soil health e.g., water content, aggregate stability, and nutrient content [40,55]. These changes in soil properties subsequently limit plant growth and reduce yields [12,62]. However, there are also field studies that have shown no significant effects of plastic on soil properties (pH, EC, available inorganic nitrogen) and crop yield after polyethylene microplastic application with concentrations of 0.01% [26] and 0.1–10% [6] or biodegradable microplastic application of 0.02% [11]. However, the single focus on microplastic in most of these studies underestimates the proportion of plastic that enters agricultural soils in the form of macroplastic. The rates of plastic degradation in soil under natural conditions are still poorly understood [78] and a large proportion of plastic originating from mulch films likely remains as macroplastic in the soil before breaking down into microplastic over time [30,41,71]. Thus, it is of vital importance to directly compare the effects of macro and microplastic on soil and plant functioning.

Another aspect that is often overlooked, is that plastic mulch films typically consist of a main polymer chain (e.g., polyethylene, polypropylene) but also contain a range of plasticizers and other additives to improve their properties like UV resistance, flexibility, and durability [74]. It is still largely unknown how the different components in the mulch film behave in soil and how these compounds interact once broken down. Most studies use industrially sourced plastics (namely microplastics) which can contain a mixture of unknown quantities of additives from a pool of more than 10,000 substances [74]. Research on plasticizers and additives is concentrated around phthalates, which are known carcinogens and mutagens [15]. Where studies on pure plastics do exist, they have focused on the effect on soil macrofauna, and soil microbial communities and functioning [6,33].

This study therefore aims to provide much needed information on the comparative effects of macroplastic and microplastic, and plastic-only plant-soil interactions on a mesocosms level. Here, we focus on pure plastics of macro and micro size with no or only very low levels of leachable additives to determine a baseline of the effect of polymers on soil and plant health from which to build a comprehensive understanding of how plastic pollution affects soil and crops in agricultural settings. Lozano et al. [45] previously compared 8 polymer types with 4 different shapes and 4 increasing concentrations and showed that all three factors have an effect on soil properties and plant biomass. Here, we investigate the effects of polymer type, concentration, and size, rather than shape. The primary aim of this threshold experiment is to determine the critical plastic loading rate to the system, simulating short- and long-term application of plastic mulch film to the soil. The response of the soil and crops after addition of different polymer types and sizes along a concentration gradient provides insight into realistic conditions and potential future scenarios if the input of agricultural plastic to the soil continues at its current rate. Here, we determined the effect of two commonly used, pure plastic polymers in the forms of macro and microplastic on key soil quality indicators (water content, microbial diversity (16S rRNA), microbial biomass (PLFAs), soil nutrient content, and enzyme activity) and plant properties (shoot and root biomass, chlorophyll content, and nutrient content). We added increasing concentrations of plastic (equivalent to 1, 10, 25, and 50 years accumulation of plastic mulch film in the soil) to determine at which critical threshold crop growth is affected and changes in soil properties are observable. Based on results of previous studies, we hypothesised that i) negative effects on soil and plant health will become more pronounced with increasing plastic concentration, and that ii) microplastic will have a greater effect on soil properties and thus crop growth than macroplastic.

2. Materials and methods

2.1. Soil and plant preparation

Soil was collected from the top 10 cm of a *Lolium perenne* L. dominated grassland located in Abergwyngregyn, North Wales (53°14'19.5''N, 4°00'53.1''W). The site is a flat lowland field with no notable surface runoff, and has no prior history of external microplastic input, e.g. via plastic mulch film use, biosolid applications, irrigation, and use of coated fertilisers. The site is subject to prevailing oceanic winds and has an average intrinsic plastic loading rate of 0.37 microplastic particles per 10 mg soil at a depth of 0 – 10 cm. The microplastic contamination of the soil is mainly attributed to wet and dry atmospheric deposition, and abrasion of equipment used on a regular working farm, with the most prevalent polymer types found being polyamide, polypropylene, and rubber. The field was ploughed two weeks prior to soil sampling. The soil is classified as a Eutric Cambisol [32] or Typic Hapludalf (US Soil Taxonomy) with a sandy clay loam texture and crumb structure. Prior to the experiment, the soil was passed through a

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

Soil property	Unit	Value
pH (H_2O)		6.1 ± 0.1
EC	μS cm^{-1}	119.6
Ammonium	mg N kg^{-1}	2.5 ± 0.5
Nitrate	mg N kg^{-1}	12.4 ± 2.7
Available phosphate	mg P kg^{-1}	41.5 ± 0.6
Potassium	mg K kg^{-1}	165.3 ± 4.3

EC, electrical conductivity

9 mm sieve to remove stones and roots. General soil properties are presented in Table 1.

Two types of conventional petroleum-based plastic were used: low density polyethylene (LDPE) and polypropylene (PP). The plastics used are pure polymers, which is defined within the framework of this study as follows: a main polymer with no or low levels of leachable additives, such as lubricants (e.g., fatty acids, amides) and antioxidants (e.g., Igranox168) that are essential for the production process of films. To our knowledge, there were no co-contaminants present, except for an unknown, likely inorganic, grinding agent used during the PP microplastic manufacturing process (5% *w/w*). For detailed information on leachable additives in each plastic, see supplementary material (Table S1, S2). Pure LDPE and PP plastic film for the macroplastic treatments (50 μm thick with a flat square shape; GoodFellow Cambridge Ltd., Huntingdon, UK. LDPE product code: ET31-FM-000151; PP product code: PP30-FM-000250) were cut into squares of ca. 1 cm × 1 cm. The film thickness was selected to portray the maximum thickness of conventional agricultural mulch films, simulating maximum input into the soil. For the microplastic treatments, LDPE and PP powder waxes were used (4–9 μm particle size with a round shape, Parchvale Ltd., Banbury, UK. LDPE product code: Glissawax PSF-7; CAS number: 9002–88–4. PP product code: Glissawax 14205; CAS number: 9003–07–0). The plastic sizes were selected as representation of the macroplastic (> 500 μm) and microplastic (<500 μm; >1 μm) size ranges. Plastic concentrations added to the soil were determined as an equivalent of years of plastic mulch film usage per area, based on one cropping season per annum. The soil surface area of the pots was calculated and multiplied by 1, 10, 25, or 50 to generate the equivalent volume of mulch film that would have been used on an area this size over the selected number of years. The weight per area (g cm^{-2}) was calculated for both films and the total weight (g) required for each loading rate was determined. Whilst we recognise that microplastics are not directly added to the soil in the same way as macroplastic mulch film, the microplastic treatments were weighed out as an equivalent to the total macroplastic weight for each concentration. The average weight difference between plastic types for all concentrations was ± 1 g. The equivalent weight of macro or microplastic required was then mixed with soil *w/w* (combined total weight of 1.5 kg) in a metal bowl for 2 min, using a metal trowel until homogenised and added to 2 l terracotta clay pots. As a percentage of soil weight these concentrations are 0.06%, 0.6%, 1.6% and 3.2% corresponding to 1, 10, 25 and 50 years of mulch film use, respectively. The concentrations represent the state of current pollution in UK soils, a hotspot of plastic accumulation in the soil, a future potential pollution level, and extreme pollution, respectively. As a reference treatment and to account for dilution of the soil by the plastic, we substituted the weight of plastic in the 50-year treatment with pure acid-washed quartz sand (125–300 μm particle size, Sigma-Aldrich, Poole, UK). This was accompanied by a control treatment where the soil received no plastic or quartz sand. In total, there were 16 plastic treatments and 2 control treatments, each with 5 replicates. The sample number for all analyses described below is $n = 5$ unless otherwise stated. The study was designed in line with previous experimental setups, comparing different plastic types, sizes and concentrations on a mesocosm scale (e.g., [5,59]).

The soil in all pots was pre-fertilised at a rate of 50 kg N ha^{-1} (as NH_4NO_3 as 1 M solution) prior to sowing the seeds; no phosphorus (P) and potassium (K) were initially applied as the soil was freshly collected from the field with sufficient P and K concentration. Pots were randomly placed in trays and rotated weekly. The trays were lined with an absorbent fabric base and water was added every 2 – 3 days, or more frequently when necessary. This watering regime kept the fabric lining saturated with water, allowing water to be taken up into the pots from the bottom. Water was not applied from the top to avoid density separation of microplastic particles in the soil. The pots were filled with the soil-plastic mixture and placed on the trays one week prior to sowing, to allow adequate time for water uptake. Maize (*Zea mays* L. cv. Humboldt) seeds were germinated on a damp paper towel for 4 days before planting two germinated seeds into each pot. Plants were grown in a greenhouse for 8 weeks with a 16:8 light-dark cycle and an average light intensity of 207 W m^{-2} before being destructively harvested. The average temperature during this growing period was 24.5 °C and the relative humidity was 34.6%. A second fertiliser application was added during week 5 of plant growth in response to the appearance of foliar anthocyanin production. Fertiliser was applied as a solution (5 ml per pot) in the following (equivalent) quantities: 50 kg N ha^{-1} as NH_4NO_3, 55 kg P ha^{-1} as $Na_2H_2PO_4$ and 150 kg K ha^{-1} as KCl.

2.2. Plastic additive extraction

An extraction for readily extractable polymer additives was carried out. All solvents were HPLC grade (Rathburn Chemicals Ltd., UK), and all glassware was furnaced prior to use at 450 °C for 2 h to remove any contamination. Polymer (1 g of microplastic or macroplastic cut into 0.5 × 0.5 cm) was extracted using 15 ml 2:1 dichloromethane (DCM)-methanol (MeOH) (*v/v*) via solvent-assisted microwave extraction at 70 °C for 10 min. 5α-Androstane (10 μl of 1.0 mg ml^{-1}) was added as an internal standard for quantification. Following extraction, the total additive extract was transferred, and polymer residues washed with 3 × 3 ml of 2:1 DCM-MeOH. The total additive extract was dried (40 °C, 0.7 ml min^{-1}) then derivatized with MSTFA (*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide, 30 μl) at 70 °C for 1 h. Excess MSTFA was removed at room temperature with a gentle nitrogen flow, and then redissolved in ethyl acetate for analysis. Quantification was achieved using gas chromatography (GC) via comparison to the internal standard. The GC (Thermo 1300, Thermo Fisher Scientific, MA, USA), with a programmable temperate vaporizer inlet (PTV) operated in spitless mode, was fitted with a HP−1 column (polysiloxane, 50 m x 0.32 mm, 0.17 μm film thickness), with a carrier gas of helium at constant flow of 2.0 ml min^{-1}. The temperature programme was as follows: 50 °C (1 min) to 300 °C (15 min) at a rate of 5 °C min^{-1}. Identification was achieved using gas chromatography-mass spectrometry (GC-MS; Thermo 1300-ISQ, Thermo Fisher Scientific, MA, USA), using the same column and temperature program as for GC. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, in a range of 50–700 *m/z*, and at a scan rate of 0.2 s^{-1}. Identification was achieved via comparison to the NIST library alongside analysis of in-house standards. Readily extractable polymer additives for each plastic can be found in the supplementary material (Table S1, S2).

2.3. Plant and soil properties

Plant height and leaf chlorophyll content were measured weekly, the latter using a Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502 PLUS, Konica Minolta, Tokyo, Japan). SPAD measurements were made on the most recently emerged but fully extended leaf. At the end of the experiment (week 8), the shoots were harvested at ca. 5 mm above the soil surface and roots were recovered from the soil by washing under a stream of water. Root and shoot biomass were then determined by oven drying (80 °C, minimum 24 h until constant mass).

Dried shoot and root samples were ground using a stainless-steel ball mill (Retsch GmbH, Haan, Germany) to a size < 63 μm. Total carbon and nitrogen content in the samples was analysed using a TruSpec Leco C/N analyser (Leco Corp., St. Joseph, USA). Phosphorus and potassium content was determined using total reflection X-ray fluorescent spectrometry (S2 Picofox, Bruker, Billerica, USA).

Soil pore water chemistry was determined weekly during the first 4 weeks of the experiment before the second fertiliser application using Rhizon samplers with a pore size of 0.15 μm (Rhizosphere Research Products, Wageningen, Netherlands). The Rhizons were inserted into the soil in a 45° angle in the middle of each pot and a 20 ml vacutainer was attached weekly to collect 1.5–20 ml of water directly around the rhizosphere. Properties measured were pH, electrical conductivity (EC), dissolved organic carbon (DOC) (mg C l^{-1}), ammonium (mg NH_4-N l^{-1}),

nitrate (mg NO_3-N l^{-1}), and phosphate (mg PO_4-P l^{-1}). The Rhizon samplers for the following treatments did not collect enough water due to limited soil moisture content and have hence been excluded for analyses: 50-year treatments for both plastic types and sizes; 25-year micro treatments for both plastic types.

At the end of the experiment, soil gravimetric water content was determined by oven drying (105 °C, minimum 24 h until constant mass). Bulk density of the soil in the pots was determined using 9.8 cm^3 plastic cylinder rings as described in Rowell [63]. Soil hydrophobicity was determined according to Doerr [14]. Briefly, soil was collected from the pots at the end of the trial and air-dried at room temperature. Approximately 10 g of soil were spread evenly across a glass Petri dish. Three droplets (50 µl) of each ethanol concentration were added to the soil surface, starting from the lowest concentration of ethanol (0, 3, 5, 8.5, 13, 24, 36, 50, 100% *v/v*), until droplets penetrated the soil within 3 s.

At the end of the experiment, soil pH and EC were measured in a 1:2 (*w/v*) soil:distilled H_2O suspension using standard electrodes (distilled H_2O pH = 5.59). To measure nutrient availability, the soils were extracted with 0.5 M K_2SO_4 (1:5 *w/v* soil:K_2SO_4 extract). Ammonium (mg NH_4-N kg^{-1}), and nitrate (mg NO_3-N kg^{-1}) concentrations were determined colorimetrically according to the salicylic acid procedure of Mulvaney [51] and VCl_3 procedure of Miranda et al. [50], respectively. Available phosphate (mg PO_4-P kg^{-1}) was measured using the molybdate blue method of Vaz et al. [66], after extracting the soil samples with 0.5 M acetic acid (1:5 *w/v* soil:acetic acid extract). DOC was measured on a Multi-N/C Series TOC/TN analyser (Analytik-Jena, Jena, Germany). All soil properties are expressed on a dry weight basis unless otherwise stated.

Enzyme activities were determined for a sub-set of treatments according to the fluorometric procedure of Marx et al. [47] with minor modifications. The selected treatments were control, macro 10, 25, and 50 years, and micro 10 years only. We did not include any 1-year treatments for either plastic type and size based on the similarities between control and 1-year treatments for key soil properties (Table S4). The high concentration micro treatments (25 and 50 years) were not selected because these treatments resulted in no plant establishment in the mesocosms. We tested 6 different enzymes involved in C, N, and P cycling to determine how plastic type, size and concentration affect soil nutrient cycling (for the list of enzymes and substrates see Table S3). Briefly, soil was collected from the root zone of each treatment using a small metal spatula ($n = 3$). A soil slurry was created by shaking 0.4 g of fresh soil with 40 ml sterile deionised water (250 rev min^{-1}, 30 min). For the assay, 50 µl of soil suspension, 100 µl of 200 µM substrate and 50 µl of buffer (Trizma for AMC and MES for MUF substrates) were added to a 96-well microplate. Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, and a slit width of 20 nm, with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Corp., Santa Clara, CA, USA). Enzyme activities were measured 0 min, 1 h and 2 h after adding the soil solution, buffer, and substrate solution. Microplates were incubated at 20 °C between measurements. The difference between activities at 2 h and 1 h was used to determine AMC/MUF release in nmol g^{-1} dry soil h^{-1}.

2.4. 16S rRNA gene amplicon sequencing

16S rRNA gene amplicon sequencing was conducted for each treatment ($n = 3$–5). At the end of the experiment, soil samples (ca. 15 g) were collected from the root zone of each treatment and stored at −80 °C before freeze-drying. Bacterial DNA was extracted from each sample using the Zymo Research Quick DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA, USA) according to manufacturer's instructions. DNA extractions were carried out with a high-speed bead beating device for each sample. Quality and concentration of extracted DNA were assessed by gel electrophoresis and by Qubit 4.0 Fluorometer dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA). Libraries of 16S rRNA gene amplicons were prepared by duplicate PCR with double-indexed fusion primers as described previously [16]. The hypervariable V4 16S rRNA gene fragment was amplified using modified forward primer F515 (5′-GTGBCAGCMGCCGCGGTAA-3′) and reverse R806 prokaryotic primer (5′-GGACTACHVGGGTWTCTAAT-3′), which amplify an approximately 290 bp region. Primers were designed to contain: the Illumina adapters and sequencing primers, a 12 bp barcode sequence, a heterogeneity spacer to mitigate the low sequence diversity amplicon issue, and 16S rRNA gene universal primers [16]. PCRs were performed using OneTaq DNA Polymerase (New England Biolabs, Ipswich, MA, USA). All reactions were run with no-template negative controls. Thermocycling conditions were: initial denaturation at 95 °C for 2 min, followed by 30 cycles at 95 °C for 30 s, 50 °C for 50 s, and 68 °C for 90 s with a final elongation at 68 °C for 5 min. Amplicons were visualised in a 1.2% tris-acetate agarose gels using a GelDoc System (Bio-Rad, Hercules, CA, USA). DNA bands of approximately 440 bp were gel-purified using the QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany). The purified amplicons were then quantified using Qubit 4.0 Fluorometer, pooled in equimolar amounts and the final pool was run on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using 500-cycle v2 chemistry (2 × 250 bp paired-end reads) at the Centre for Environmental Biotechnology, Bangor, United Kingdom.

Raw sequencing reads were processed according to previously described protocols [16,38]. Briefly, the data was pre-processed to extract the barcodes from sequences, and then cleaned of primer sequences using tagcleaner. The barcodes and the sequences were re-matched again using in-house Python scripts. The resulting filtered reads were analysed using QIIME v2021.2 [2]. First, the libraries were demultiplexed based on the different barcodes. Then, the sequences were classified on operational taxonomic units (OTUs) combining *de novo* and reference-based methods (open-reference I generation algorithm) using the SILVA version 132 reference database. The NCBI BioProject accession number is PRJNA891142.

Analyses of most abundant taxonomic groups were performed using in-house R-based scripts, selecting those groups with a relative abundance of at least 2% in any of the samples. Selection started at genus level and groups are added to the immediate upper taxonomic level when none of the samples of that group reach the 2% threshold.

2.5. PLFA analysis

Phospholipid fatty acid (PLFA) extractions were conducted on freeze-dried soil (2 g), extracted after homogenisation using a modified Bligh Dyer solution (15 ml of 2:1:0.8 (*v/v/v*) methanol, dichloromethane (DCM), and phosphate buffer). This was then sonicated (15 min) and centrifuged (3000 rpm, 12 min). The supernatant was transferred, and the extraction was repeated 3 times. The organic phase was split by addition of phosphate buffer (8 ml) and DCM (8 ml). The DCM layer was transferred, and extraction repeated 3 times, to obtain the total lipid extract (TLE), which was dried at 40 °C under nitrogen. The TLE was redissolved in 5 ml chloroform for lipid fractionation. The activated silica column was conditioned with chloroform before the TLE was added. Neutral lipids (NLFAs) were eluted using 5 ml chloroform, glycolipids were eluted using 10 ml acetone and the phospholipid fraction (PLFAs) were eluted with 5 ml methanol. Only the PLFA fraction was carried forward for analysis. At this stage, 10 µl of 0.1 mg ml^{-1} nonadecane internal standard was added to the PLFA fraction for quantification and dried under a gentle stream of nitrogen. Acid catalysed methylation was carried out to obtain fatty acid methyl esters (FAMEs) using 5% (*v/v*) HCl in methanol (5 ml, 50 °C for 2 h). Saturated sodium chloride solution (5 ml) was added with 3 ml of hexane to exert phase separation, and the hexane fraction collected. This was repeated three times. The derivatised PLFA fraction was dried and redissolved in hexane (50 µl) for GC-MS analysis. The GC was fitted with a VF23-ms column (60 m, 0.32 µm i.d., 0.15 µm film thickness), and the temperature programme was: 50 °C (1 min) to 100 °C (10 °C min^{-1}) to 250 °C (4 °C min^{-1}, 15 min hold), with a helium carrier gas flow of 2.0 ml min^{-1}.

Data was acquired and analysed using Xcalibur (version 4.1). Assignment of PLFAs was based on previous studies [21,35]. The sum of all PLFAs between C14 and C20 were used to represent total PLFAs. The sum of firmicutes-derived PLFAs (i14:0, i15:0a, i16:0a, i17:0, i18, a15:0, a16:0, a17:0, a18:0, a19:0) and actinobacteria-derived PLFAs (10Me16:0, 10Me17:0, 10Me18:0) were used to represent Gram-positive bacteria. The sum of cy17:0, cy19:0, 16:1ω7, 16:1ω9, 17:1ω8, 18:1ω7 was used to represent Gram-negative bacteria. The sum of 16:1ω5c, 18:1ω9c, 18:2ω6c, and 18:3ω6,9,12 was used to represent fungi. 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 20:4ω6,9,12,15 were classed as unspecified.

2.6. Statistical analysis

All graphs and analyses were performed in R v4.1.1. unless otherwise stated [58]. Normality of the data was determined by Shapiro-Wilk test ($p < 0.05$) then visually checked using qqnorm plots. Homogeneity of variance of the data was visually checked using residuals vs. fitted plots. Three-way ANOVAs were used to determine if there was a significant difference between key soil and plant properties with plastic type, size, and concentration ($p < 0.05$). The NMDS plot, alpha- and Shannon diversity graphs (Fig. S1) were generated using SHAMAN [67]. The sand control data is not presented in the main manuscript as there were no significant differences observed for the majority of key soil and plant properties compared to the control (no sand) treatment (Table S4).

3. Results

3.1. Plant health properties

Maize seedlings in the 25- and 50-year concentrations of micro-PP and micro-LDPE treatments failed to establish, hence no plant height and SPAD data could be collected on those treatments. Overall, for microplastic treatments, increasing plastic concentration reduced plant height although there was little difference between the controls and the 1-year plastic treatment (Fig. 1). Although both plastic types showed a reduction in plant growth for the 10-year treatments, PP had a larger effect than LDPE. Plant heights for the macroplastic treatments showed a similar growth trend to the controls for all concentrations. After fertilisation in week 5 the plant height for the 1-year plastic treatment remained comparable to the control, while the higher plastic concentrations failed to increase at the same rate. Similar trends were observed for the SPAD measurements of leaf chlorophyll content (Fig. 2). The chlorophyll content decreased in all treatments after 3 weeks and increased again after the second fertiliser application in week 5 for the control treatments and low concentrations apart from PP micro 10-year treatments. The high (25- and 50-year) concentration macroplastic LDPE treatments did not recover and decreased further over time. The PP 25-year macro treatment showed a slight increase while the 50-year treatment showed no response after fertiliser application. Overall, root and shoot biomass were highest in the control and 1-year plastic treatments irrespective of plastic type and particle size (Fig. 3a, Table 2). The biomass decreased in both root and shoot with increasing concentration for macro and microplastic.

The largest differences in C:N ratio were observed in the macro-LDPE treatments, with C:N ratio increasing with concentration in both shoot and root (Fig. 3b). A similar trend could be observed in the PP treatments. Overall, C:N ratio increased with increasing concentration, with a more profound trend in the macroplastic treatments. C:N ratio was mainly affected by particle size and concentration (Table 2). There were no significant differences between shoot P content across all treatments (Fig. 3c, Table 2). Root P content in the PP treatments increased with increasing concentration, with root P double that of the control in the 50-year macro-PP treatment. Root P content for LDPE treatments were comparable to the control (Fig. 3c, Table 2). Shoot and root K content showed similar trends to the shoot and root P contents (Fig. 3d, Table 2). The N, P, and K use efficiency showed an overall decrease with increasing concentration across both sizes and plastic types (Fig. S2).

3.2. Soil health properties

Bulk density was mainly affected by plastic size and concentration (Table 2), with the highest density present in the 25- and 50-year micro treatments of both plastic types (Fig. 4a). Overall, the macro treatments showed a decrease of bulk density compared to the control with increasing concentrations whilst the micro treatments showed the opposite trend, increasing at higher concentration. The moisture content was similar between the control and 1-year plastic treatments and for

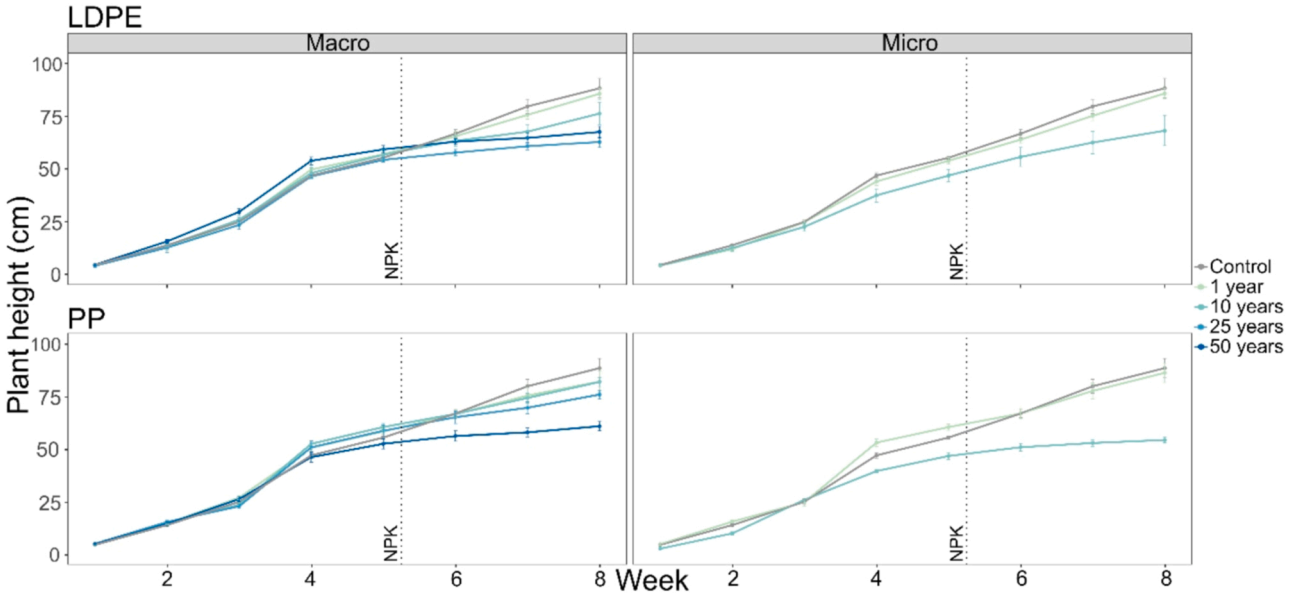

Fig. 1. Height of maize plants over an 8-week period in response to exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP). The concentrations of plastic added to soil reflect 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. Values represent mean ± S.E. ($n = 5$). Dotted lines represent second fertiliser application of N (50 kg ha^{-1}), P (55 kg ha^{-1}), and K (150 kg ha^{-1}). The 25 years and 50 years microplastic treatments for both plastic types are not included, as these treatments failed to establish, hence no plant height data could be collected.

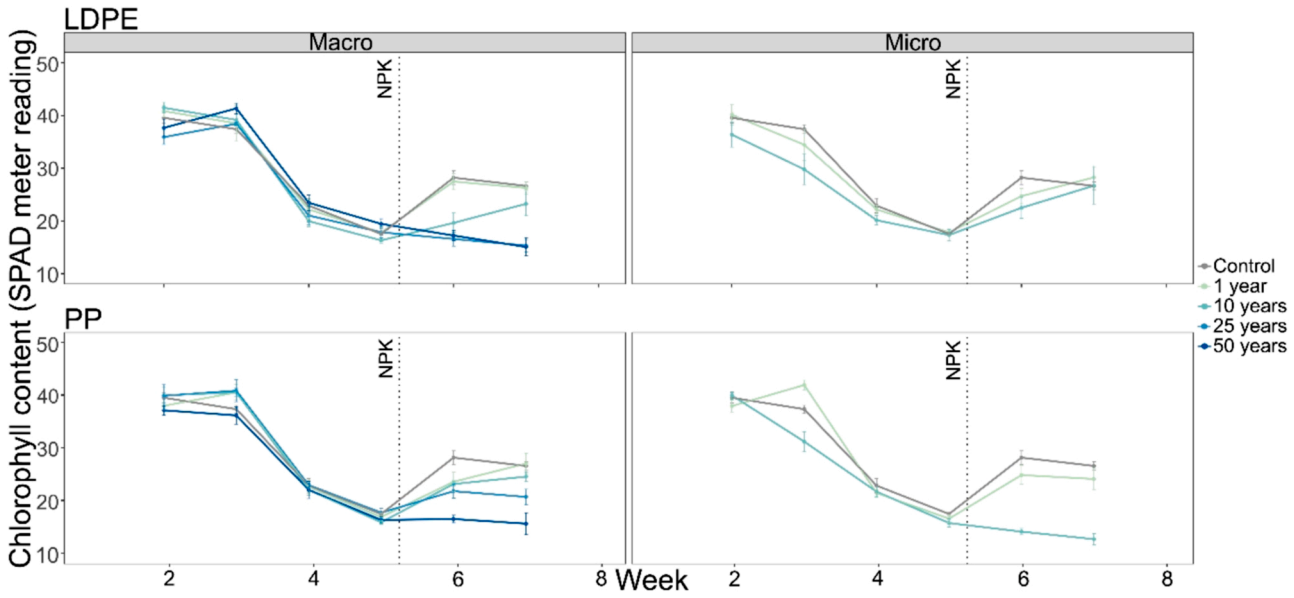

Fig. 2. Chlorophyll content (SPAD) of maize leaves over an 8-week period in response to exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP). The concentrations of plastic added to soil reflect 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. Values represent mean ± S.E. ($n = 5$). Dotted lines represent second fertiliser application of N (50 kg ha^{-1}), P (55 kg ha^{-1}), and K (150 kg ha^{-1}). The 25 years and 50 years microplastic treatments for both plastic types are not included, as these treatments failed to establish, hence no SPAD data could be collected.

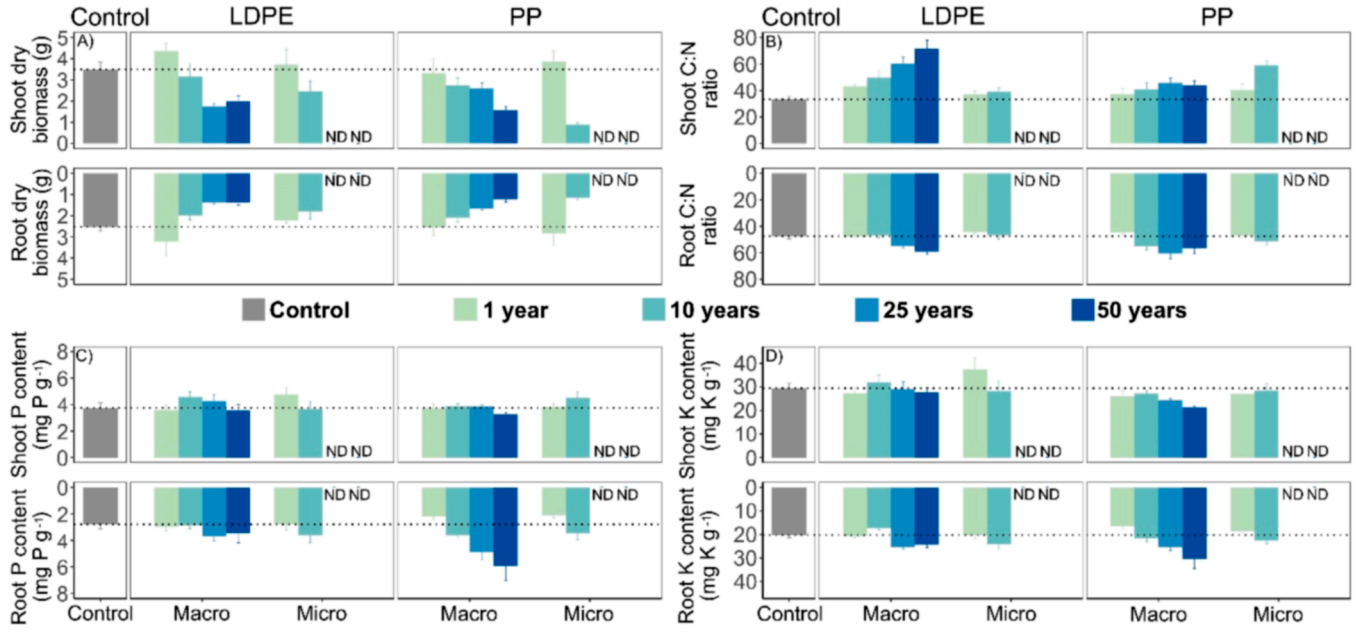

Fig. 3. Plant biomass and nutrient content of shoot and root in response to exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP) for 8 weeks. The concentrations of plastic added to soil reflect 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. A) shoot and root dry biomass (g), B) shoot and root C:N ratio, C) shoot and root P content (mg P kg^{-1}) and D) shoot and root K content (mg K kg^{-1}). Values represent mean ± S.E. ($n = 5$). ND indicates no data due to no germination in those treatments. The dotted lines represent the average control values.

each plastic type and size, but reduced with increasing concentration (Fig. 4b, Table 2).

pH of the soil pore water was consistently highest in the control treatment for the first 4 weeks and reduced with increasing concentration in all treatments (Fig. S8). The same trend was observed at the end of the experiment (Fig. 4c) and pH was significantly influenced by plastic type (Table 2). Initial measurements showed EC was consistently higher in the control up to week 3, while there was little difference between plastic treatments (Fig. S7). However, after 4 weeks the control values decreased to match the remaining treatments. Interestingly, the end measurements show that EC was highest in the 25- and 50-year treatments except for LDPE microplastic and was only significantly affected by difference in particle size (Fig. 4d, Table 2).

Ammonium and nitrate concentrations in the soil pore water both decreased within the first 4 weeks after fertiliser application in all treatments (Fig. S3, S4). The end measurements for both extractable ammonium and nitrate were both highly influenced by plastic type and concentration, while size still played a significant role for ammonium, it was a negligible factor for nitrate (Table 2). The extractable N concentrations were similar in the control treatments and 1-year plastic

Table 2
Summary table of three-way ANOVA outputs of soil and plant health properties at the end of the experiment in response to exposure to plastic type (low-density polyethylene (LDPE) or polypropylene (PP), size (macro- or microplastics) and concentrations of plastic added (equivalent weight of 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil). NS = not significant, * = $p < 0.05$, * * = $p < 0.01$, * ** = $p < 0.001$.

Variable		Plastic type	Size	Concentration
C:N ratio	Shoot	NS	* **	* **
	Root	NS	* **	*
P content	Shoot	NS	* **	* **
	Root	NS	* **	NS
K content	Shoot	* *	* **	* **
	Root	NS	* **	* *
Biomass	Shoot	* **	* **	* **
	Root	* **	* **	* **
Bulk density		NS	* **	*
Moisture content		* *	* *	* **
Ammonium		* **	* *	* **
Nitrate		* **	NS	* **
Phosphate		NS	* **	* **
DOC		NS	*	NS
pH		* **	* *	*
EC		NS	* **	NS
LAP		NS	NS	*
PHOS		*	NS	NS
XYL		NS	NS	NS
NAG		* *	NS	NS
BDG		NS	NS	NS
BG		NS	NS	NS

LAP, leucine aminopeptidase; PHOS, phosphatase; XYL, β-xylosidase; BDG, β-D-glucuronidase; BG, β-glucosidase; NAG, N-acetyl-β-glucosaminidase

treatments, whilst overall inorganic N increased with plastic concentration in the macro treatments of both plastic types, and decreased with plastic concentration in the micro treatments (Fig. 4e-f, Table 2).

The dissolved organic carbon (DOC) in the extractions at the end of the experiment increased significantly in the low concentration macroplastic PP treatments and showed a decrease in both sizes with higher concentrations, whereas it remained similar to the control in the LDPE treatments. (Fig. 4g). The DOC concentrations in soil pore water showed little difference between treatments within the first 4 weeks of the experiment, with the majority of treatments slightly decreasing (Fig. S6).

Plant available extractable phosphate was highly dependent on size and concentration (Table 2) and was lowest in the micro 25- and 50-year plastic treatments. It was elevated for the macroplastic LDPE treatments, whereas the macroplastic PP treatments showed only slight elevation and remained similar to the control (Fig. 4h). There were no significant differences observed for available P in the soil pore water measurements during the first half of the experiment (Fig. S5).

Overall, C cycling enzyme activities were not significantly different between plastic type, size, or concentration (Fig. 5, Table 2). However, N-P cycling enzyme activity did show significant differences. Leucine aminopeptidase (LAP) activity decreased with increasing plastic concentration for LDPE and decreased overall for all concentrations for PP. Phosphatase (PHOS) and *N*-acetyl-β-glucosamindase (NAG) activity showed differences between LDPE and PP treatments, with LDPE macro treatments showing significantly reduced activity compared to PP macro (Fig. 5, Table 2). B-glucosidase (BG) showed a decrease in activity with increasing concentration in LDPE macro treatments, whilst there was no observable difference in PP macro between concentrations. B-xylosidase (XYL) and β-D-glucuronidase (BDG) showed no significant difference in activity between plastic treatments and the control (Fig. 5). C:N enzyme ratios increased with higher concentrations of LDPE macroplastic, yet declined for PP macroplastic, and were elevated relative to the control for both plastic types of the 10-year microplastic treatments (Fig. S9a). There was little effect on C:P enzyme ratios for LDPE macroplastic and PP macroplastic 25- and 50-years, however, it was elevated for the 10-year treatments (Fig. S9b). The N:P enzyme ratio was significantly impacted by concentration and was elevated for both plastic sizes for LDPE 10-years, with no effect on PP plastic treatments (Fig. S9c).

In total, 2092 bacterial ASVs were identified across all 16S rRNA gene reads. Most abundant group analysis identified plastic type as the top influencing factor for most taxa, followed by size and concentration (Fig. 6, Table 3). Most differences in relative abundance were caused by either a single factor or a combination of two; all three factors affecting a

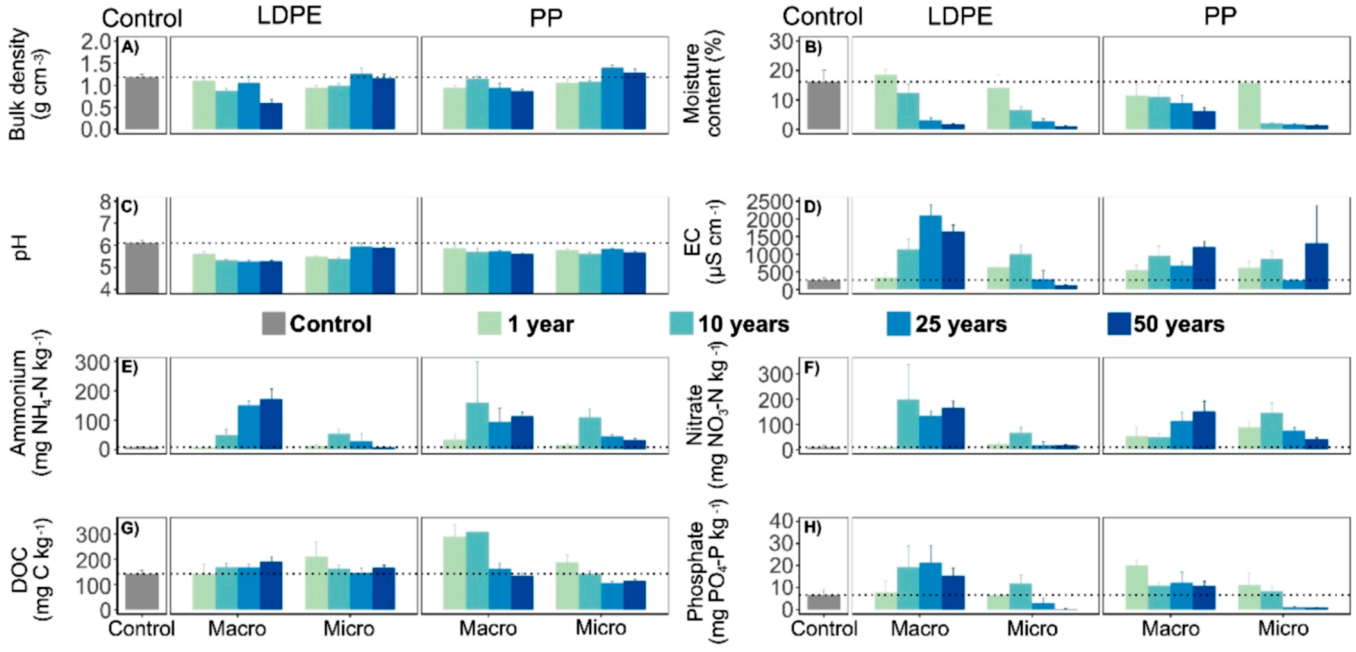

Fig. 4. Soil properties in response to exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP) for 8 weeks. The concentrations of plastic added to soil reflect 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. A) bulk density (g cm^{-3}), B) moisture content (%), C) pH, D) electrical conductivity (EC) (µS cm^{-1}), E) ammonium content (mg NH_4-N kg^{-1}), F) nitrate content (mg NO_3-N kg^{-1}), G) dissolved organic carbon (DOC) (mg C kg^{-1}), and H) phosphate content (mg PO_4-P kg^{-1}). Values represent mean ± S.E. ($n = 5$). The dotted lines represent the average control values.

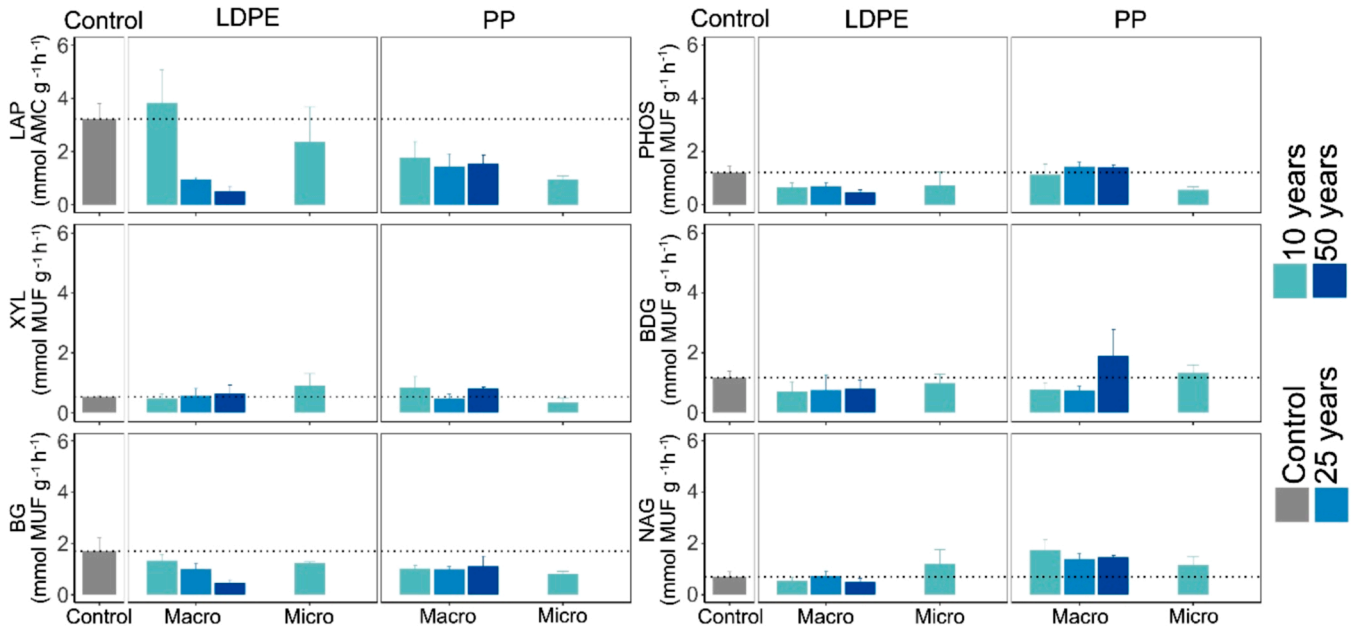

Fig. 5. Soil enzyme activity (nmol AMC/MUF g^{-1} h^{-1}) for leucine aminopeptidase (LAP), phosphatase (PHOS), β-xylosidase (XYL), β-D-glucuronidase (BDG), β-glucosidase (BG), *N*-acetyl-β-glucosaminidase (NAG) in response to exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP) for 8 weeks. The concentrations of plastic added to soil reflect 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. Values represent mean ± S.E ($n = 3$). The dotted lines represent the average control values.

change in abundance was rare (Table 3). Taxa with the most significant differences between treatments were *Rhodococcus, Corynebacteriales, Virgisporangium, Aeromicrobium, Nocardioides, Solirubrobacterales, Conexibacter, Solirubrobacter, Aquabacterium, Massilia, Dyella, Cyanobacteria and Gemmatimonadota* (Table 3). NMDS analysis showed significant clustering within treatments (Fig. S1b, permanova test: $p = 0.001$). Alpha and Shannon diversities did show the lowest diversity with PP microplastic treatment irrespective of the amount of plastic added, with particle size being the only relevant influencing variable (Fig. S1c, Table S5).

The total microbial biomass (Fig. 7) was lower for all plastic treatments than the control. There was no significant effect of size, concentration, and plastic type individually or as a combination of all three. However, there were contrasting effects between plastics of size and concentration. For total biomass, and individual PLFA groups (Fig. S10), PLFA concentration declined at microplastic concentrations above 1 year, except for the 50-year treatment in the unspecified group. This decline was particularly notable for fungal PLFAs. The PP macroplastic treatment for 10-years was higher than the 1-, 25- and 50-year treatments, except for the Gram-positive group. This trend was also observed for the LDPE macroplastic, where the 10-year treatment was higher than the 1-year, followed by the 25- and 50-year treatments in all pools, with a marked decrease above 25-years for fungi. While PLFA concentration for LDPE microplastic 1-, 25- and 50-years decreased gradually with a similar trend for bacterial and unspecified pools, the fungal group had a sharp decrease, similar to PP microplastic. The ratio of Gram-positive to Gram-negative biomass (Fig. S11), indicated by specific PLFAs showed little change for LDPE plastic treatments, except for macroplastic at 25-years loading rate. However, this increased for PP macroplastic at 50-years, and above 10-years for microplastic. The ratio of fungal to bacterial biomass (Fig. S11) decreased relative to the control above 1-year LDPE macroplastic and microplastic. A similar trend was observed for the PP microplastic treatments, while it increased for the 10- and 25-year PP macroplastic treatments.

4. Discussion

4.1. Effect of plastic on plant health

We observed a significant decrease in root and shoot biomass for both plastic types and sizes with increasing concentration, and showed that plastic type, size, and concentration are all major influencing factors for plant development (Fig. 3a, Table 2). Overall, both particle sizes of LDPE and PP had an observable effect on plant health at 10 years of plastic addition. It is likely that the maize seedlings in the 25- and 50-year treatments of micro-PP and LDPE did not establish due to the low water content (Fig. 4b) and high soil hydrophobicity in these treatments (Table S6). A study comparing low and high concentrations of microplastic PE on maize growth showed similar results to our study; low concentrations of PE (0.0125 mg l^{-1}) had negligible effects on plant height, but high concentrations (100 mg l^{-1}) reduced maize growth and plant height [65]. Another study comparing the effects of biodegradable PHB and conventional PET microplastics found that higher concentrations of both microplastic types caused a decrease in water content and water binding strength of organic soil, which was attributed to the disruption in soil structure and the water molecular bonds within [19]. Other studies have found plant growth and biomass not to be affected by microplastic addition rates of 0.1%, 0.4% and 2% *w/w* (e.g., [3,12,45]). Differences could be due to the varying sizes, shapes, polymer types and concentrations used between studies. Shoot growth was reduced in the higher macroplastic concentration treatments after 5 weeks of growth compared to the microplastic addition which were hindered from the start. The delay in response to the second fertiliser application and the overall reduction in plant growth and chlorophyll content with increasing concentrations (Fig. 1, Fig. 2) suggest that the reduced soil moisture content, general hydrophobicity, and reduced root biomass caused by the plastic made the nutrients unavailable to the plants, delaying nutrient uptake or blocking it entirely. This is supported by the remaining high availability of inorganic N and P in the soil after harvest (Fig. 4e, f, h) and the increased plant C:N ratio with increased plastic concentration (Fig. 3b). N, P and K becoming less accessible to the plants is also shown by the lower nutrient use efficiencies in the higher plastic concentrations (Fig. S2).

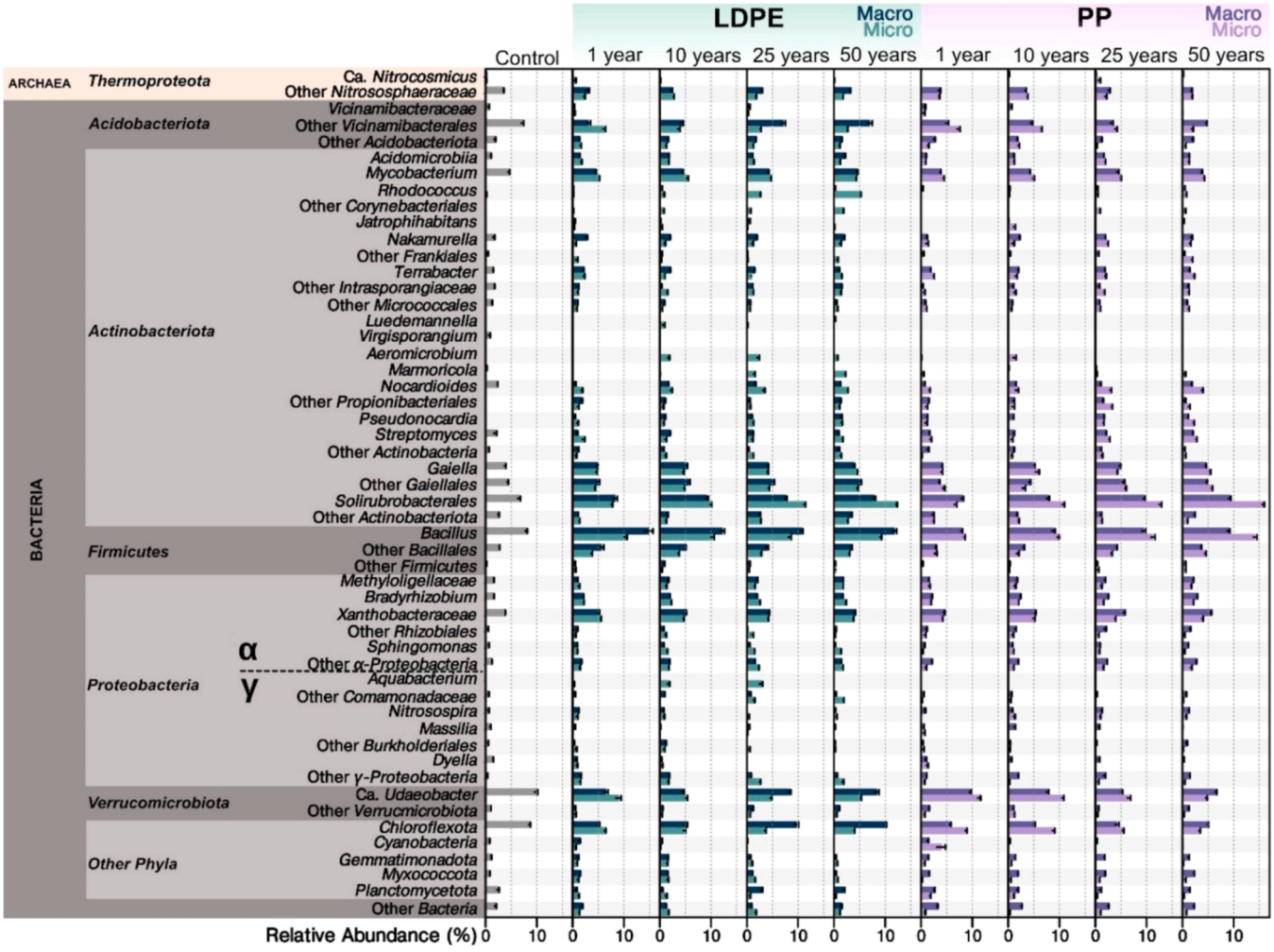

Fig. 6. Analysis of relative abundances of prokaryotic taxa with at least 2% of total SSU rRNA amplicon reads in all samples. Prokaryotic diversity was sampled after exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP) for 8 weeks. The concentrations of plastic added to soil reflect 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. Three-way ANOVA was used to determine significant ($p < 0.05$) differences between plastic addition treatment for each taxon ($n = 3$–5).

Plant nutrient content (N, P and K) is an important indicator of plant health and directly related to the availability of these nutrients in the soil. De Souza Machado et al. [12] found no difference between C:N ratio of onion biomass under no plastic addition and high-density PE or PP (2% *w/w* addition, 650–750 μm), whereas Urbina et al. [65] found a large reduction in plant N content from maize grown with high concentrations of microplastic PE. In our study, we found an increase in root P and K content with increasing concentration for both plastic types. Interestingly, this trend could not be observed for the shoot P and K content of the same treatments (Fig. 3), with all macro treatments showing a decrease in both nutrients with increasing concentration. This, combined with the reduction in nutrient cycling with increasing plastic concentration in the shoot (Fig. S2) and the increased inorganic N pools in the soil under higher concentrations (Fig. 4e-f), confirms that nutrient uptake into the shoot is inhibited in high concentration treatments. Possible mechanisms could be i) the high C content of plastic resulting in microbial immobilisation (less likely for persistent plastic polymers); ii) adsorption of certain nutrients to plastic surfaces making them less available; iii) changes in microbial activity leading to changes in nutrient availabilities; and iv) changes in moisture and aeration of the soil altering nutrient transformations [62]. However, the exact mechanisms for how plastic addition influences nutrient uptake in plants are still unclear and require further research.

4.2. Effect of plastic on soil health

LDPE plastic had a stronger effect on soil health properties with more pronounced differences at concentrations equivalent to 10 years, and above, of plastic addition. Increasing microplastic addition decreased soil moisture content (Fig. 4b) and increased hydrophobicity (Table S6), which resulted in seedlings in the 25- and 50-year microplastic treatments not establishing. Both LDPE and PP are hydrocarbon polymers and are hydrophobic due to their non-polarity and relatively low surface free energy. The PP is likely to be even more hydrophobic compared to the low-density PE used in this study, which explains the increased hydrophobicity in the high concentration microplastic PP treatments compared to the LDPE (Table S6). The increased hydrophobicity in microplastic compared to macroplastic treatments is likely due to the increased surface area and homogeneity in soil of the microplastic particles compared to macroplastic particles with the same total weight. However, no consensus is apparent in studies that have explored the effect of plastic on soil water dynamics with effects varying depending on shape, size, and type of plastic [68]. Qi et al. [57] found 1% LDPE macro and microplastic addition did not affect shoot or root biomass compared to the control. However, it is clear in this study that there is a critical concentration of 25 years equivalent accumulation of microplastic addition where soil water is affected and in turn prevents plant growth. The effect of macroplastic is less clear despite soil moisture

Table 3
Summary table of three-way ANOVA outputs of taxa with most abundant SSU rRNA amplicon reads in response to exposure to plastic type (low-density polyethylene (LDPE) or polypropylene (PP), size (macro- or microplastics) and concentrations of plastic added (equivalent weight of 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil). NS = not significant, * = $p < 0.05$, * * = $p < 0.01$, * ** = $p < 0.001$.

Most abundant group				Plastic type	Size	Concentration
Archaea	*Thermoproteota*		Ca. *Nitrocomicus*[a]	NS	NS	NS
			Other *Nitrososphaeraceae*	NS	NS	*
Bacteria	*Acidobacteriota*		*Vicinamibacteraceae*	NS	NS	NS
			Other *Vicinamibacterales*	NS	NS	NS
			Other *Acidobacteriota*	NS	* *	NS
	Actinobacteriota		*Acidomicrobiia*	*	NS	NS
			Mycobacterium	NS	* *	NS
			Rhodococcus	*	* *	* **
			Other *Corynebacteriales*	*	* **	* **
			Jatrophihabitans[a]	NS	NS	NS
			Nakamurella	NS	* *	NS
			Other *Frankiales*[a]	NS	NS	NS
			Terrabacter	NS	NS	* **
			Other *Intrasporangiaceae*	NS	NS	NS
			Other *Micrococcales*	* *	NS	NS
			Luedemannella[a]	NS	NS	NS
			Virgisporangium	* **	* **	* **
			Aeromicrobium[a]	* *	* **	NS
			Marmoricola[a]	NS	* *	NS
			Nocardioides	NS	* **	* **
			Other *Propionibacteriales*	NS	NS	NS
			Pseudonocardia	*	NS	*
			Streptomyces	NS	NS	NS
			Other *Actinobacteria*	NS	NS	NS
			Gaiella	NS	NS	NS
			Other *Gaiellales*	NS	NS	NS
			Solirubrobacterales	*	* *	*
			Other *Actinobacteriota*	NS	NS	NS
	Proteobacteria	α	*Bacillus*	NS	NS	NS
			Other *Bacillales*[a]	NS	NS	NS
			Other *Firmicutes*	NS	NS	NS
			Methyloligellaceae	NS	NS	NS
			Bradyrhizobium	NS	NS	NS
			Xanthobacteraceae	NS	* *	*
			Other *Rhizobiales*	NS	NS	NS
			Sphingomonas	NS	NS	NS
			Other *α-Proteobacteria*	NS	* *	NS
		γ	*Aquabacterium*[a]	* *	* *	NS
			Other *Comamonadaceae*	NS	NS	NS
			Nitrosospira[a]	NS	NS	NS
			Massilia	*	* **	NS
			Other *Burkholderiales*[a]	NS	NS	NS
			Dyella	* *	NS	* **
			Other *γ-Proteobacteria*	*	NS	NS
	Verrucomicrobiota		Ca. *Udaeobacter*	*	NS	* **
			Other *Verrucmicrobiota*	NS	* *	NS
	Other phyla		*Chloroflexota*	NS	NS	NS
			Cyanobacteria[a]	NS	*	* **
			Gemmatimonadota	NS	* *	*
			Myxococcota	NS	* **	NS
			Planctomycetota	NS	* *	NS
			Other *Bacteria*	NS	* **	NS

[a] Data could not be transformed to normal distribution, Kruskal-Wallis test performed for each factor.

content reducing by around 10 times in the 25-year treatment. The decrease in bulk density in the high macroplastic treatments suggests that the bigger particles caused an increase in porosity of the soil by creating air-filled pockets (Fig. 4a). That in turn could have allowed more water to infiltrate the soil despite the hydrophobicity of the plastic itself, compared to the microplastic treatments. Also, the macroplastic treatments were less homogenised due to the larger particle size, leaving more soil particles plastic-free and allowing for enough water to infiltrate the pore space to facilitate establishment of seedlings.

Soil available C, N and P are paramount to soil fertility and crop growth. Our study showed overall macroplastic increases extractable inorganic N content (Fig. 4e-f) and available phosphate in the soil compared to the controls (Fig. 4h). This trend was particularly marked for the LDPE macroplastic treatments. The increase in available P and N in the soil, and subsequent decrease of P and N use efficiency in the plants (Fig. S2), could have several reasons, one of them being the build-up of biofilm on the plastic surface, accumulating nutrients from the surrounding soil and making them unavailable to the plants. It has been shown that marine plastic debris can act as a nutrient accumulator [49], making them more accessible for microbial communities, but at the same time reducing the nutrient level in the environment, limiting nutrient uptake for other organisms, i.e. plants. If biofilms had formed on the macroplastic surfaces it is likely that this would adsorb nutrients [23], which could be a possible explanation for our study as soil ammonium and nitrate concentrations were highest in the highest plastic addition treatments at the end of the experiment. The microplastic treatments generally showed an increase in inorganic N for low concentrations and a slight decrease with increasing concentration, however most treatments still showed more, or similar levels of available N compared to the control, especially for PP.

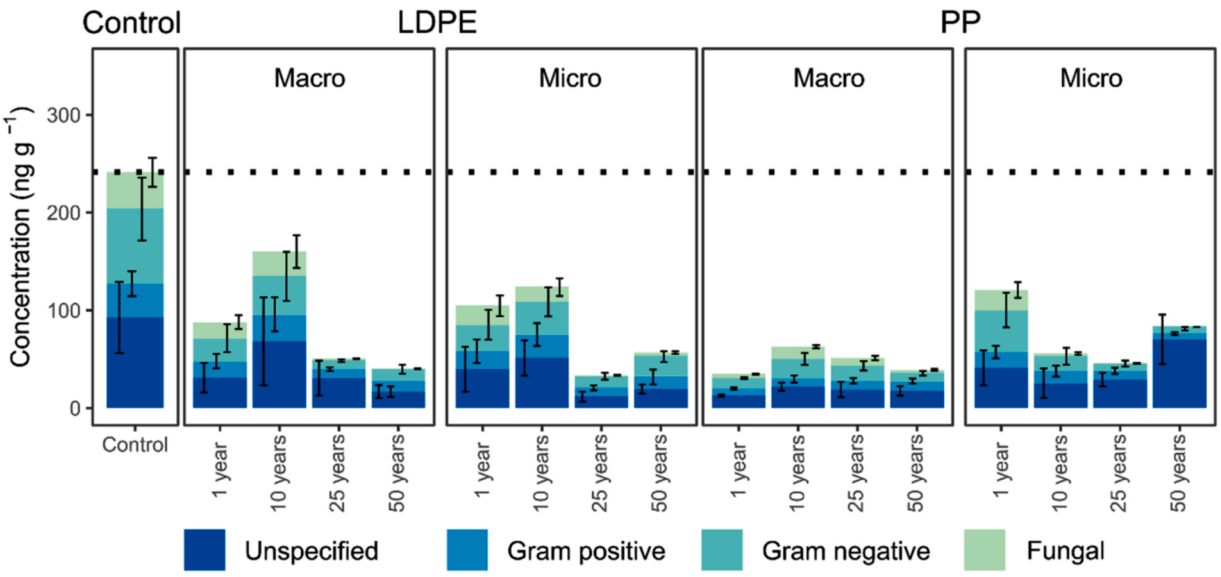

Fig. 7. Microbial PLFAs concentration in response to exposure to either macro- or microplastics of low-density polyethylene (LDPE) or polypropylene (PP) for 8 weeks. The concentrations of plastic added to soil reflect 1, 10, 25 or 50 years of continual mulch film use and ploughing the residues into soil. The stacked bars indicate contribution from Gram positive, Gram negative, fungal and unspecified PLFAs. Values represent mean ± S.E. ($n = 5$) for each contributing pool.

On the other hand, we observed a decrease in available phosphate from 10 to 50 years at the end of the experiment. The decrease in available P and N with increasing microplastic concentration is contradictory to the trend we observed for the macroplastic treatments. The increasing hydrophobicity of the soil could be an influencing factor for nutrient loss within the system, as the fertiliser was applied in liquid form, limiting infiltration into the soil. In a soil-only mesocosm study, high LDPE microplastic concentrations (2.5% *w/w*) caused no difference in nitrate, ammonium or DOC concentrations compared to the control after 46 and 105 days of incubation [48]. In contrast to that, Liu et al. [43] observed an increase in DOC in soil in treatments with high PP microplastic concentrations (7% and 28%) compared to the control. Interestingly, we found that LDPE affected the DOC levels less than PP (Fig. 4g) and that the main influencing factor for varying DOC levels in our study was particle size (Table 2). It is also noteworthy, that we grew plants in our mesocosms, whereas other studies have focused on soil only. It has been shown that roots can release inorganic and organic C into the soil system, thereby altering soil physical, chemical, and biological properties [37]. Jones et al. [36] highlighted a vast number of plant biotic factors influencing rhizodeposition alongside abiotic factors and soil properties, thereby affecting C flow in the rhizosphere. Further, other studies have shown that root exudates can play a crucial role in the regulation of DOC and nutrient cycling in the soil by altering the microbial community structure [27,72], suggesting that plants play a crucial role in soil nutrient dynamics under microplastic addition.

We found that soil pH was affected by plastic type, size, and concentration and was lower in all treatments compared to the control (Fig. 4c, Fig. S8). A similar trend was observed in a study investigating the effects of PE microplastic and cadmium in increasing concentrations on maize and soil properties [69]. They found a decrease in pH with increasing PE concentration in treatments where no cadmium was added. In contrast to this, Zhao et al. [85] found an increase in soil pH over time at a plastic concentration of 0.4% for both PE and PP in different shapes and sizes. An increase in pH and decrease in EC has also been observed by Qi et al. [56] for micro and macro-LDPE mulch film addition to soil. Interestingly, we observed a reduced EC in plastic treatments compared to the control in the first few weeks of the experiment (Fig. S7) but saw an increase with higher concentrations in EC for most treatments at the end of the experiment, except for micro-LDPE which decreased in 25- and 50-year treatments. The elevated EC compared to controls was most apparent in the macro-LDPE samples. Differing results between studies could be due to many factors, for example soil type, plastic type, size, concentration, and surface charge properties but also a huge variety of additives/co-contaminants that are often added to the main polymer [44,57,82]. More research is needed to understand the interaction between all these factors and their influence on soil properties better. Overall, plastic size and concentration were the two main factors influencing soil properties in our study, while the polymer type also had a significant impact on moisture content and inorganic N of the soil.

Soil enzyme activity provides an insight to microbial activity in the soil as well as turnover of key nutrients and their availability. Most studies have focused on microplastic addition and/or assayed fluorescein diacetate hydrolase (FDAse) as an indicator of microbial activity along with urease and catalase [12,13,18,29,43,81]. Here, we explored the effect micro and macroplastics on the activity of a greater number of enzymes by measuring LAP, PHOS, XYL, NAG, BG, and BDG activity. Overall, we found that neither plastic type, nor size or concentration influenced activity of C cycling enzymes (XYL, BDG, BG) (Fig. 5). However, we did see a decrease in N cycling activity for LAP with increasing concentration and a lower activity for NAG in LDPE compared to PP (Fig. 5). This observed decrease in activity of N cycling enzymes matches the observation of available inorganic N in those treatments (Fig. 4e, f). Further, changes in C:N enzyme ratios reflected relative change in DOC and inorganic N. Allison et al. [1] suggested that organisms reduce production of specific enzymes when the key nutrient is abundant in the environment, hence allocating resources to different enzymes instead to synthesise nutrients that are deficient in the soil. We observed a similar trend for the P cycling enzyme PHOS, where activity reduced significantly in the LDPE macro treatments compared to the PP macro treatments, whilst showing no difference in the microplastic treatments (Fig. 5). When we compare this to the available P in the soil at the end of the experiment, we see an increased P in the macro-LDPE treatments, and a decrease in the overall micro treatments (Fig. 4h). Other studies have seen varied effects of microplastic on soil enzyme activity, for example Liu et al. [43] showed that PP addition (28% *w/w*) increased FDAse activity by 2.5 times whilst De Souza Machado et al. [13] found no difference with 2% (*w/w*) PE addition. Lehmann et al. [40] found FDAse variability was determined by plastic type with LDPE and PP leading to a marked decrease in soil FDAse activity. Differing

findings between studies are likely due to the great variation in influencing factors as mentioned above, i.e. polymer types, particle size, and concentration. Thus, it is still unclear whether plastic addition increases or decreases enzyme activity and how the magnitude of change is affected by differing plastic properties. However, our results indicate changes in relative investment in C, N and P enzymes and resultant potential activity, are an effect of changing nutrient availability due to plastic loading.

4.3. Diversity of prokaryotic taxa

Generally, in all treatments and controls, no significant changes in alpha diversity and Shannon diversity indices were observed between different concentrations, sizes, and types of plastics (Table S5). Most-abundant SSU rRNA amplicons derived from typical soil-borne taxa, all retained their high numbers in treatments with both polymer types. Representatives of these taxa are *Bacillus* spp. and *Chloroflexota,* verrucomicrobia of *Ca*. "Udaeobacter", apparently very ubiquitous globally, with low complexity, streamlined genomes [4,75], and members of the order *Vicinamibacterales* (phylum *Acidobacterota*) that have only recently been cultured [31]. Similarly, representatives of the most abundant archaeal ammonium-oxidising family *Nitrososphaeraceae,* were constantly present at the level 2.5–4% of the total numbers of amplicon reads with no significant variations across treatments. However, some minor taxa exhibited dramatic changes in their numbers. For example, *Aquabacterium* spp. increased from 0% to 2.7% total reads on LDPE microplastics with the maximum increase in samples corresponding to the 25-year loading rate. *Aeromicrobium* spp. and members of *Solirubrobacterales* family, all representing actinobacteria, were present in the control soil variants in lower read numbers, but increased significantly: *Aeromicrobium* from 0% to 2% in the micro-LDPE treatment (25-year equivalent), *Solirubrobacterales* from 6% in control to 15.7% (i.e. almost 3-fold) in the micro-PP (50-year equivalent) treatments and doubled in micro-LDPE (50-year equivalent) making up to 12.8% of total reads. In contrast to the above taxa, *Virgisporangium* spp. showed a reduction in numbers of reads to zero, with all plastic types and concentrations, from 0.7% reads in control. Another member of *Actinobacterota*, *Rhodococcus* spp. showed an LDPE microplastics-induced rise in read numbers from 0% to 5.2% of total reads (50-year equivalent). The latter genus harbours a plethora of large-genome, metabolically versatile bacteria, well-known for their ability to degrade a range of recalcitrant organic compounds including chlorinated and non-chlorinated aromatic and aliphatic hydrocarbons [25,39] and potentially, some constituents/leachates of PE [24]. To conclude, no marked changes in most-abundant taxa were observed, probably due to the low solubility and availability of added polymers and the presence of readily utilisable components of soil organic matter, however some minor microbial groups, including biodegrading Gram-positive bacteria with high G+C content in their genomes, showed a very significant rise in their relative population densities.

4.4. Changes in microbial biomass

PLFA analyses indicated consistent decreases in microbial biomass in all plastic treatments relative to the control, with lowest biomass observed at the higher macro and microplastic loadings (Fig. 7). Previous studies quantifying microbial biomass have shown no impact of LDPE microplastic applied at 15 g m^{-2} [42], while others have found an increase in biomass with LDPE or PP microplastics [73]. To our knowledge, no comparable study using PLFAs has been conducted with macroplastics, although microbial biomass has been shown to decrease under plastic mulch film [52]. The contrasting results between studies may reflect the pure polymer, different size and shape, or differing pre-existing microbial community (or resilience) to plastic incorporation. It was assumed there was minimal degradation and minimal C released from the added micro and macroplastics. Hence, the effects of plastic addition on soil properties were likely responsible for the observed decrease in microbial biomass. Decreased soil moisture and increased hydrophobicity will have reduced nutrient mobility (e.g. reduced NH_4^+ in first 4 weeks; Fig S3), and potential supply to the microbial community. Furthermore, there was a decrease in ammonium concentration for the 25- and 50-year treatments, where the effect of plastic was largest on the plant growth. Given the intrinsic link between plant and microbial communities [28] and supply of C, the lower plant biomass (or none) at higher plastic loadings may have reduced microbial biomass. Within the microbial pool, there was little change in the relative abundance of Gram-positive and Gram-negative bacteria, except in higher loadings of PP plastic. Increases in this ratio, alongside decreases in the fungal:bacterial ratio, and very low fungal biomass in 25- and 50-year treatments, were consistent with decreases in nutrient availability at higher plastic loading observed in the first 4 weeks [22,42,79]. Increases in Gram-positive biomass relative to Gram-negative was also consistent with observed increases in actinobacteria reads relative to the control. Preferential colonisation by actinobacteria of macro and microplastics has been observed previously [22,84], alongside degradation of hydrocarbons [39], which may be responsible for the increase in Gram-positive relative to Gram-negative biomass in the bulk soil. The combination of PLFA and 16S rRNA analyses revealed effects of both LDPE and PP microplastics and macroplastics on the microbial community, and this will likely result in changes in microbial community function (e.g. enzyme activity), particularly above 10-years equivalent loading rate of plastic.

4.5. Implications and future outlook

A caveat to this study is that mesocosm studies do not represent real-world conditions. In this environmentally controlled greenhouse study, we have complete homogenisation of the plastic within the soil, however in agroecosystems, sources of plastics mainly originate from the surface, e.g. plastic mulch film application [80], sludge application [83], use of coated fertilisers [34], and atmospheric deposition [76]. Surface pollution will likely create hotspots that will decrease over time with further transport within the soil profile facilitated by regular cultivation practices. However, little research has been conducted on mechanisms, key drivers, and timescales of plastic movement [60]. Thus, our estimate of >10 years of plastic application as the tipping point leading to negative effects on soil and crop health is likely longer at the field scale. It is imperative to transcribe these mesocosm studies to the field scale to determine plastic tipping points under real world agronomic conditions.

It is also worth noting, that we used what has been defined here as 'pure plastic' in this study, that has not been exposed to UV or any other process facilitating degradation before addition to the soil. The low levels of readily extractable polymer additives that were present in the macroplastic, as well as the grinding agent present in the PP microplastic, were necessary additions in the manufacturing process, however, the level and complexity of additives present in the macroplastic used in this study is minimal compared to conventional mulch films. Furthermore, the results of this study strongly suggest that the soil abiotic factors were the main drivers of change, indicating that the low levels of additives or co-contaminants were minor to negligible in the context of our experimental design. The conventional plastic used in agricultural circumstances will almost always have additives (e.g., UV-accelerants, phthalates, heavy metals) to provide the desired properties. These additives can potentially be more harmful than the plastic itself as they are more mobile and create potentially toxic by-products that leach into the soil and could be taken up by plants [55,61,70]. Comparing this study's findings to other literature has been challenging as most studies provide little to no information on the plastic characteristics and potential additives. We therefore strongly suggest being critical when comparing between different studies, and to be mindful of additives as a potential driver for change. We hope that this study can serve as a reference baseline for plastic-only soil-plant interactions, and

that future research can investigate effects of specific additives in more detail using similar experimental designs.

Another point worth noting is that we used concentrations of plastic on an increasing scale to test the critical threshold of plastic in soil, therefore the equivalent weight of microplastic to macroplastic, based on mulch film application to an area per year, was used. However, under normal agricultural circumstances not all macroplastic applied to the soil will turn into microplastic and soil will contain a variety of particle sizes and polymer types. For all these reasons, the selected concentration gradient expressed in 'years of continued mulch film use' should therefore not be seen as a realistic portrayal of plastic pollution levels in agricultural field, but rather a reasoning behind the loading rate selection. This study purposefully did not account for varying degradation rates, plastic movement in the soil and plastic loss in the system to minimise the variables and keep the focus of the experiment on the comparison of plastic type, size, and concentration. Investigating the effect of particle shape in conjunction with polymer type, size and concentration was outside the scope of this study and further research is needed as shape has been shown to be an important factor influencing soil properties [40,45,85].

All points outlined above need to be considered in future experiments to simulate more realistic conditions in mesocosm experiments, including mixtures of micro and macroplastics. Comparing this study's macroplastic findings to other literature was difficult due to a lack of studies investigating macroplastic effects on soil and crop health as well as the quantity of macroplastic in soil [77]. Study bias has been towards the effect of microplastics on indicators of soil health, yet for many agricultural sources, macroplastic is likely to be far more abundant being the first stage in mechanical plastic breakdown. Our study shows macroplastic to have a similar observable effect to microplastic on soil and plant health particularly >10 years equivalent accumulation, though to a lesser extent.

5. Conclusion

Our study investigated the impact of realistic, present-day rates of soil plastic contamination alongside those which may occur in the future. Based on the results presented here, we conclude that the effect of both pure macro and microplastic on soil and plant health is negligible in the short-term (i.e., 1 to <10 years of plastic application). However, equal or more than 10 years equivalent of plastic application has a clear negative effect on plant growth, and microbial biomass, for both LDPE and PP in both macro and micro sizes, and that this is likely to severely affect crop yields and soil function. Future studies must translate these findings to long term field trials to determine the effect of plastic legacy on soil and plant health. In addition, while many studies have looked at macro and micro sized plastics in isolation, these typically occur in soil at the same time. More information is therefore needed on the relative proportion of macro versus microplastic in the soil to inform experiments investigating the effect on the interaction between macro and microplastics in the soil.

Environmental impact statement

Plastic pollution is threatening to undermine many of the UN Sustainable Development Goals and has been identified as a major threat to aquatic life. Continuous agricultural plastic use over the last 50 years has led to an accumulation of legacy plastic in soil, with detrimental implications on soil and crop health, micro and macrofauna. This study investigates the effects of pure plastics on soil and crop health, aiming to determine the relative effects of micro and macroplastic with increasing concentration. Loading rates simulate agricultural plastic use over 50 years, showcasing a critical tipping point when negative effects become observable.

CRediT authorship contribution statement

Martine Graf: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft. **Lucy M. Greenfield:** Conceptualization, Methodology, Investigation, Writing – review & editing, Visualization. **Michaela K. Reay:** Formal analysis, Investigation, Data curation, Writing – review & editing, Visualisation. **Rafael Bargiela:** Software, Visualization. **Gwion B. Williams:** Investigation. **Charles Onyije:** Investigation. **Charlotte E.M. Lloyd:** Supervision, Funding acquisition. **Ian D. Bull:** Supervision. **Richard P. Evershed:** Supervision, Funding acquisition. **Peter N. Golyshin:** Writing – review & editing. **David R. Chadwick:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Davey L. Jones:** Conceptualization, Methodology, Writing – review & editing, Supervision, 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131932.

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