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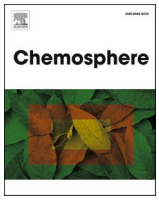

# Microplastics in agricultural soils following sewage sludge applications: Evidence from a 25-year study

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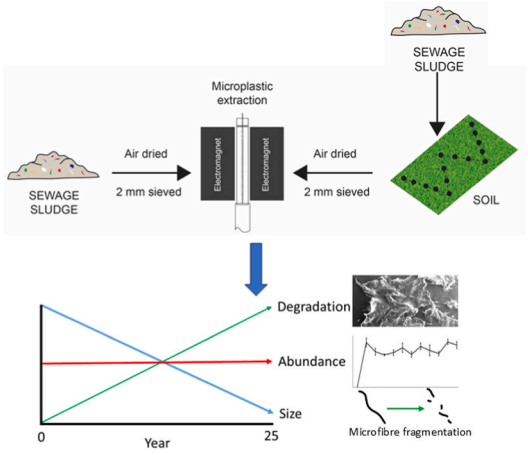
## HIGHLIGHTS

- Application of sewage sludges significantly increased microplastic abundance in soil.
- Microplastic abundance remained relatively unchanged over 22 years.
- Microfibres and microfilms were susceptible to degradation while other morphologies were resistant.
- Textile dyes may have been leached to soil potentially posing further toxic effects.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Sewage sludges applied to agricultural soils are sources of microplastic pollution, however, little is known about the accumulation, persistence, or degradation of these microplastics over time. This is the first study to provide long-term, high temporal resolution quantitative evidence of microplastics in agricultural soils following sewage sludge application. The abundance and degradation of microplastics was assessed in soils sampled biennially from an experimental field over a 25-year period managed under an improved grassland regime following the application of five different sewage sludges. The sludges contained different microplastic compositions reflecting the different sources of the sludges. Microplastic abundance increased by 723–1445% following sewage sludge applications ($p < 0.05$) and remained constant over time (22 years and possibly beyond) ($p > 0.05$). All sludges predominantly added white/transparent microfibres to soil. Microfilms, microfibres, and fragments were most

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susceptible to degradation, potentially creating micro(nano)plastics. Of note was the discoloration of coloured microfibres, which may be environmentally hazardous due to the toxicity of textile dyes in soil ecosystems. We also found that plastic composition could be used to trace its source. This evidence is useful in informing regulation on sewage sludge use and management, and in assessing the fate and impact of microplastics in soil.

## 1. Introduction

Microplastics are typically removed from wastewater at wastewater treatment works (WWTWs) and accumulate in the solid waste (sludge) (Carr et al., 2016; Murphy et al., 2016; Bayo et al., 2020) thus reducing microplastic output to receiving waters. Sewage sludge is commonly applied to agricultural soils worldwide as a fertiliser because it is rich in organic matter and macro- and micronutrients, which benefit soil fertility and function (Elmi et al., 2020), but this also leads to the accumulation of microplastics in the receiving soils. This practice is projected to continue, potentially resulting in further accumulation of microplastics over time (Corradini et al., 2019). High-income countries (HICs) including the United Kingdom, United States of America, Japan, Australia, New Zealand, and the European Union have legislations and regulations on the use and management of sewage sludge applications (Jimenez et al., 2004; Christodoulou and Stamatelatou, 2016). However, microplastics are not currently one of regulated constituents in sludges.

The fate of microplastics in agricultural soils after sewage sludge applications is dependent on soil attributes, land management practices, and environmental and biological factors. Soil texture, porosity, and bulk density can have indirect effects on microplastic abundance, with soils containing higher amounts of powdered-light clay, with low porosity and low bulk density accumulating higher quantities of microplastics (Yang et al., 2023). Microplastics at shallower soil depths will be subjected to photodegradation, however, this is also dependent on soil components, with clay, iron oxides, and manganese (IV) oxide ($MnO_2$) enhancing photodegradation, and organic carbon inhibiting it (Ding et al., 2022; Pan et al., 2024). Tillage causes mechanical fragmentation and degradation (Duan et al., 2021) and can incorporate microplastics to greater depths (Rillig et al., 2017a; Xu et al., 2020; Zhao et al., 2021), preventing photodegradation (Bonyadinejad et al., 2022). Microplastics can also be translocated to deeper layers of soil through bioturbation by earthworms and other soil biota (Huerta Lwanga et al., 2017; Maaβ et al., 2017; Rillig et al., 2017b). Furthermore, smaller microplastics can be transported deeper into soil by vertical drainage of groundwater and rainwater during wet-dry cycles (O'Connor et al., 2019), and the free movement of smaller microplastics into soil pores (Yang et al., 2021a), thereby increasing their persistence.

To date, studies investigating the long-term fate of microplastics in soils have typically only been carried out using contemporary soil samples which had historically been treated with sewage sludges or mulching film. These studies have only been able to confirm the presence of microplastics, characterise them, and comment on their degree of weathering (e.g., van den Berg et al., 2020; Yang et al., 2021b), therefore losing the temporal aspect to microplastic accumulation, persistence, and degradation. Recently, Ji et al. utilised the rate of sedimentary layer formation in a lake to generate a timeline for microplastic pollution and related their findings to historical industrial revolution and societal events (Ji et al., 2024). Although Ji et al.'s study investigated microplastic accumulation over time, the results cannot be translated to soils.

It is critical that high temporal resolution, long-term studies be conducted to elucidate factors affecting the fate of microplastic pollution in soils. This is especially important in terms of the impact microplastics have on soils and soil function. Microplastics affect the bulk density, water holding capacity, and the functional relationship between the microbial activity and water stable aggregates (de Souza Machado et al., 2018). This in turn can affect plant biomass, tissue elemental composition, and root traits, with detrimental consequences to plant performance, e.g., quality and quantity of crop yield (de Souza Machado et al., 2019). They can also damage soil fauna, particularly earthworms and nematodes, affecting critical ecosystem functions such as litter decomposition, nutrient cycling, and energy flow (Wang et al., 2022). In an experiment by Huang et al., the authors found that soil fauna responses to additions of microplastics (both degradable and conventional) was time-dependent and advocated for evaluation of long-term effects of microplastics (Huang et al., 2023). In addition, weathered microplastics release toxic leachates and have increased sorption of pollutants compared to pristine microplastics, exacerbating their toxic effects (Liu et al., 2020).

The aim of this study was to quantify and assess the fate of microplastics in soil over a substantial period of time (25 years) following sewage sludge applications to gain a better understanding of the factors that affect microplastic persistence. To address this, we utilised archived soil samples from a 25-year sewage sludge amendment experiment and characterised the microplastic loads within them. The numbers, size, morphology, colour, composition, and weathering of microplastics were assessed. To our knowledge, this is the first study to provide long-term, high temporal resolution quantitative evidence of microplastics in agricultural soils following sewage sludge application. This provides a better understanding of the consequences of sewage sludge applications and fate of microplastics in soil, which informs the agricultural, food, and environmental sectors on future regulation of sewage sludge application to soil.

## 2. Materials and methods

### 2.1. Experimental set-up and sampling

A field at Hartwood, North Lanarkshire (Scotland, UK) consisting of sandy clay loam soil was included in a UK-wide sludge experiment from 1994 to 2019 investigating long-term impacts of sewage sludge applications (refer to Gibbs et al. (2006) for detailed information regarding the original Hartwood experiment and initial outcomes). The field was previously managed under an improved grassland regime and there is no knowledge that this land was used for anything else in the past. No animals grazed on the land once the sludges were applied to the soils.

Three sludges (Sludge A-C) used in the experiment were from different industrial waste streams, so were contaminated with metals (Table 1). To ensure that there was no difference in organic matter content amongst the industrial sludges, they were supplemented with either digested (Sludge D) or undigested sludge (Sludge E), both of which were from municipal waste streams and therefore did not contain

**Table 1**
Types and sources of sludges applied to experimental plots.

| Treatment Name | Type of Sludge | Source and Information |
|---|---|---|
| Sludge A | Digested | WWTW catchment area included a number of leather processing plants (tanneries). Elevated levels of Zn. |
| Sludge B | Undigested | WWTW catchment area included a number of electronics factories. Elevated levels of Cu. |
| Sludge C | Digested | Composite sludge. Elevated levels of Cd. |
| Sludge D | Digested | Not recorded. |
| Sludge E | Undigested | Not recorded. |

elevated metal concentrations. As such, plots receiving sludges A and C were supplemented with sludge D, and plots receiving sludge B were supplemented with sludge E. Therefore, the sludges from industrial waste streams were not mixed with each other.

Due to the presence of heavy metals in plots treated with sludges originating from industrial waste streams, biological functions were affected (Campbell et al., 2009) which may have influenced the findings of this study.

In the first four years of the experiment, individual sludge cakes were applied annually. A plot that was not treated with any sludge was used as a negative control. The treatments were replicated in three blocks of randomised plots. The plots were 8 × 4.5 m (36 $m^2$) and bordered by 2–3 m of permanent grassland to prevent soil movement during cultivations (Fig. S1). The sludge cakes were evenly spread over the surface of the plot manually then incorporated into the soil using a spading machine to a depth of 20–25 cm, with blade cleaning between treatments (see Supplementary Information). Plots were maintained under an improved grassland regime (see Supplementary Information). Plots were sampled annually when the sludges were being applied, and then biennially thereafter.

Archived soil samples from each treatment that originated from one of the three blocks were used for this study. Original composite topsoil samples (0–25 cm) were collected from each plot using a 2.5 cm diameter auger in a 'W' pattern (15 cores) to produce a total of 2.5 kg soil per plot. Stones and plant material were removed using a sieve with a mesh size of 6.5 cm, before air-drying and sieving to 2 mm. The <2 mm soils were then stored in paper bags and archived within The National Soil Archive of Scotland at the James Hutton Institute, Scotland, UK.

Several kilograms of the sludges were stored in large plastic bags at −20 °C. The archived sludges were partially thawed then subsampled from the centre to avoid the outer layer which had been in contact with the plastic bag, to minimise any potential microplastic contamination. The subsampled sludges were left at room temperature to fully thaw and air-dry before sieving to 2 mm. Large microplastics (2–5 mm in size) were removed by hand before microplastic extraction.

### 2.2. Microplastic extraction by high-gradient magnetic separation

After thorough mixing of the archived soil samples, 8 mL of the dried 2 mm sieved soils and sludges were subsampled using an 8 mL metal scoop in triplicate (average weight recorded) and subjected to high-gradient magnetic separation (HGMS), following the procedure outlined in Ramage et al. (2022) to recover microplastics (see Supplementary Information). Briefly, the soil samples were resuspended in ethanol and passed through an electromagnet and allowed to settle for 3 min, twice, to remove the magnetically susceptible fraction of the sample (Stage 1). The non-magnetic material (containing the microplastics) was then mixed and incubated at room temperature for 10 min with 10 mg of hexadecyltrimethoxysilane-modified iron nanoparticles (25 nm), while frequently stirring with a glass rod to magnetise the microplastics (Stage 2). The sample was then passed through the electromagnet and the magnetic fraction containing microplastics bound to the surface-modified iron nanoparticles was retained (Stage 3). Stages 2 and 3 were repeated a second time. Based on the electromagnetic parameters prescribed to the soil types used in Ramage et al. (2022), the magnetic flux densities for HGMS were 0.25 and 0.34 T (T), respectively.

The HGMS method was tested for use on the sludges (dried) following the same protocol detailed in Ramage et al. (2022) by spiking the sludges with microplastics (approximately 10 of each of the following: 63–75, 106–125, 300–355 and 600–710 μm polyethylene microbeads (Cospheric LLC, USA), 2–4 mm PE fibres, 2.0 × 2.0 × 0.4 mm polyethylene terephthalate flakes, and 650–850 μm polytetrafluoroethylene fragments, which were prepared in the laboratory (7 replicates)), and the performance was compared to that of the density separation extraction method, currently the most common method for extracting microplastics from soil (Zhang et al., 2019). It was confirmed that recovery was significantly better with the HGMS method ($p < 0.05$) (Fig. S2), and the optimal magnetic flux densities prescribed for microplastic extraction from dried sludges were 0.16 and 0.29 T for Stages 1 and 3, respectively.

The performance of each new batch of modified iron nanoparticles was checked using spiked soil samples. The test soil was spiked at 1% (w/w) with 300–355 μm polyethylene microbeads (Cospheric LLC, USA) and extracted as outlined above. The extracted material was vacuum filtered onto Whatman GF/C filter papers and stored in muffled glass petri dishes until further analysis. A blank measurement was taken after every 10 samples (8 in total). The recovery of a spiked soil sample was checked with every new batch in order to ensure that a change in batches of modified iron nanoparticles did not affect microplastic recovery. Filter papers were assessed under an optical microscope and batches were accepted if recoveries were >90%.

Since sewage sludge has a high organic matter content, an additional digestion step prior to HGMS extraction was explored. To test this, Sludge B (an undigested sludge) was spiked with 10 of each of the microplastics previously mentioned (7 replicates). The spiked sludge sample was then digested using Fenton's reagent (hydrogen peroxide with a $Fe^{2+}$ catalyst) following the protocol detailed by Masura et al. (2015). Briefly, a 0.05 M Fe(II) solution was prepared by adding 7.5 g $Fe_2SO_4.7H_2O$ (Sigma-Aldrich) to 500 mL filtered ultrapure water followed by 3 mL of 0.05 M sulphuric acid (Fisher Scientific). Twenty mL of the 0.05 M Fe(II) solution and 30% (v/v) $H_2O_2$ were added to 8 mL spiked sludge samples, mixed thoroughly, and incubated at room temperature for 5 min before heating to 60 °C for 1 h with regular stirring with a glass rod. A further 20 mL of $H_2O_2$ was added once boiling subsided. The spiked sludge was then directly filtered onto filter papers (i.e., no HGMS extraction) and the spiked microplastics enumerated using the optical microscope to assess for potential microplastic loss during this initial digestion step. For comparison, the sludge samples were subjected to microplastic extraction by HGMS without the initial Fenton's reagent digestion step.

### 2.3. Categorisation and enumeration of microplastics

All extracted microplastics were examined using a Nikon SMZ1500 stereomicroscope under 7.5-50x magnification. Microplastics were found by traversing the filter paper systematically and subsequently removed from the filter papers using sterile tweezers and stored on acetate sheets by overlaying with adhesive tape for further analysis (see Supplementary Information).

When a microplastic was located, it was categorised based on its morphology using terminologies derived from Rochman et al. (2019), including fibres, fibre bundles, films, flakes, particles, and fragments (see Supplementary Information). The microplastic abundance (enumerated by each morphology) in soils prior to the addition of the sludges (i.e., the baseline number of microplastics) was subtracted from the results from all years following sewage sludge application. The average number of microplastics measured in HGMS blank controls was also subtracted from all final recorded abundances.

The colour of microplastics were also recorded. Microfibres that were predominantly transparent but with areas of colour were recorded as a separate colour category.

An extremely high number of microplastics (5745 in total) were recovered from the soil samples. To allow a reasonable number of microplastics for size measurements, only 10% of the total number of microfibres were selected because they were the predominant morphology. The total number of all other morphologies were also selected. Size measurements were done at three time points, which were after the last addition of sludge (1997; n = 75), the middle of the time course (2009; n = 75), and the end of the experiment (2019; n = 73) (n is per treatment). Measurements were taken along their longest axes using the Leica Application Suite 4.13 software on a Leica DM5000B microscope.

### 2.4. Spectroscopic analysis using Fourier-transform infrared (FTIR) microscopy

Microplastics that were ≥50 μm in size were subjected to FTIR analysis in reflectance mode using a Nicolet™ iN10 infrared microscope. Microfilms were analysed using attenuated total reflectance (ATR)-FTIR using a Ge tip due to their larger surface area. Microplastics were removed from the tape using xylene to dissolve the adhesive and rinsed with water prior to analysis – the process and an assessment of the influence of xylene on microplastic FTIR spectra are detailed in Supplementary Information. Briefly, FTIR spectra was obtained from polypropylene film at 0 s, 30 s, 1 min, and 2 min of continuous exposure to xylene. A change in spectra was only observed after 1 min exposure and since microplastics in this study were only exposed to xylene for a maximum of 10 s, we are confident that the use of xylene did not significantly impact this work. Data points were recorded over 128 scans using a liquid nitrogen-cooled MCT detector in the range of 4000-650 $cm^{-1}$ with a resolution of 8 $cm^{-1}$. Air blank spectra were recorded after each measurement (using the same number of scans and resolution). The carbonyl indices of polyethylene microfilms recovered from soil samples every four years where possible (1997, 2001, 2005, 2009, and 2019), were also determined to assess microplastic degradation over time (see Supplementary Information).

### 2.5. Scanning electron microscopy (SEM) imaging

Topographic images were taken of the surface of the same microfilms used for carbonyl index measurements (those recovered from soil samples in 1997, 2001, 2005, 2009, and 2019), using a Zeiss EVO LS10 SEM in variable pressure mode. The specimens were sputter coated in gold and imaged using a variable pressure secondary electron detector at a 6.0 mm working distance. The chamber pressure was set to 100 Pa with a beam current of 100 μA, probe current of 100 pA, and an accelerating potential of 25 kV.

### 2.6. Quality control

To minimise potential contamination throughout the extraction and enumeration stages, the use of plastic utensils was avoided by using metal or glass alternatives. All glassware was muffled at 450 °C before use (Dris et al., 2018) and kept covered in aluminium foil prior to use. All reagents were filtered through Whatman GF/C filter papers before use. Sample preparation and filtration was conducted in a clean environment within a HEPA filtered laminar flow hood (0.3 μm pore size) to minimise airborne contamination. To eliminate bias, all samples were anonymised using laboratory barcodes during processing.

### 2.7. Data analysis

Recovered microplastics were recorded as microplastic number $kg^{-1}$, scaled up from the 8 mL soil and sludge subjected to HGMS extraction, whereby the average weight of the 8 mL subsamples was taken for conversion from volume to weight. Statistical *t*-tests were performed to determine the significance of changes in microplastic abundance, microplastic size, and carbonyl indices of polyethylene microfilms over time (Welch, 1938), with a significance level of $p < 0.05$ using R (version 4.3.1). To determine changes in microplastic abundance in the treatment plots, *t*-tests were performed on microplastic concentrations recorded in the treatment plots minus the microplastic concentrations from the control plot at each respective time point. This was to eliminate the possibility of other additional sources of microplastic input during the study time period (e.g., atmospheric deposition).

Primer v7 (PRIMER-e) was used to generate non-metric multidimensional scaling (nMDS) plots to evaluate the similarity in microplastic 'communities' (i.e., the group of microplastics found within a soil sample) between the different treatments and sludges and to determine the factors driving dissimilarly. Data was square root transformed to remove the dominance of the most commonly found microplastics and subjected to a Bray-Curtis dissimilarity test. The similarity matrix was then used to generate the nMDS plots. A 2D stress value of <0.2 was considered to give an accurate representation of the dissimilarities between the microplastic communities. The variation in microplastic community composition was assessed against environmental data using Pearson's correlation. Pearson's correlations, where $r < 0.5$, were overlaid on the resulting nMDS plots. Environmental data included pH, lime and fertiliser quantities (total, N, P, and K values), %CN, total and extractable metals, biomass C, and microbial respiration available from the original Hartwood sludge experiment. The same was repeated with the post-sludge application (1997–2019) soil samples only to evaluate the similarity in microplastic communities between the different treatments and assess factors driving dissimilarly in the microplastic community in these soils. In this instance, Pearson's correlations, where $r < 0.3$, were overlaid on the resulting nMDS plots for morphology, and Pearson's correlations, where $r < 0.5$, were overlaid on the resulting nMDS plots for colour.

## 3. Results and discussion

### 3.1. Analysis of sewage sludge does not require organic matter digestion

During the digestion step with Fenton's reagent, 30–55% of the smaller sized microplastics used for spiking and recovery (polyethylene microbeads (63–75, 106–125, and 300–355 μm) and microfibres) were lost. By comparison, losses from HGMS extraction without the digestion step were only 5%. Therefore, it was deemed unfavourable to add a digestion step prior to or following HGMS microplastic extraction. Crucially, microplastic extraction from sewage sludge using HGMS without the use of organic matter digestion achieved higher recovery rates (>95% for all microplastic types and sizes tested) compared to density separation (average of 79%, excluding PTFE which had a recovery of 0%) (Fig. S2).

### 3.2. Sewage sludges contain high concentrations of microplastics

Microplastic abundance differed between the sludges, with the highest in sludge B (41880 ± 2669 microplastics $kg^{-1}$) and lowest in sludge C (14035 ± 1149 microplastics $kg^{-1}$) (Table S1). These abundances are comparable to sludge microplastic abundances in other HICs (i.e., 7.91 to 495,000 particles $kg^{-1}$ of dry weight sludge) (Mahon et al., 2017; El Hayany et al., 2022; Harley-Nyang et al., 2022). Sludges in low- and middle-income countries (LMICs) generally contain much lower microplastic abundances (830 microplastics $kg^{-1}$), but this probably reflects the fact that most wastewater in LMICs is directly discharged into water bodies, bypassing any treatment (Kamble et al., 2019). Furthermore, clothing may predominantly be made from natural fibres in LMICs and rural areas in these countries may lack automated washing facilities and wastewater infrastructure. HICs have legislations and regulations on the use and management of sewage sludge applications (Jimenez et al., 2004; Christodoulou and Stamatelatou, 2016). Due to financial constraints and poor enforcement of environmental legislation, sludge treatment and management is inadequate, or in some cases, non-existent in LMICs (Jimenez et al., 2004).

Microfibres were the predominant microplastic morphology within all the sewage sludges (>65%) (Fig. S3 and Table S1). On average, microfibre lengths were longest in sludge A (3861 ± 2102 μm) and shortest in sludge B (3121 ± 2237 μm) (Table S1). White, transparent, black, and red were the most abundant microfibre colours (Fig. S3). The dominance of microfibres is consistent with previous studies (Gies et al., 2018; Li et al., 2018; van den Berg et al., 2020; Yang et al., 2021b) as is the dominance of the colours (e.g., Li et al., 2018). Knowledge on characteristics of microplastics is important because variation in microplastic shape, polymer type, and concentration affect soil

processes differently, though this was not explicitly tested in this study. For example, shapes are important modulators of responses in soil aggregation and organic matter decomposition (Lehmann et al., 2021), while films decrease soil bulk density, and foams and fragments increase soil aeration and macroporosity, which promote plant performance (Lozano et al., 2021). Microplastic colour can be used to infer sources (Zhang et al., 2022), while some researchers are advocating for research on the effects of colour on soil fauna responses, knowing that they are attracted to certain colours (Büks et al., 2020). Certainly, terrestrial wildlife, who are visual predators and scavengers (e.g., mammals, birds, and reptiles), will be more affected by colour; studies reporting the prevalence of coloured microplastics in the gastrointestinal tract suggest that coloured microplastics may be mistaken for food, raising concern for the population and health of terrestrial fauna (Teampanpong and Duengkae, 2024).

### 3.3. Microplastics from sewage sludges persist in soils at a constant level

Microplastic abundance in soils prior to sewage sludge applications (1994) averaged 542 ± 292 microplastics $kg^{-1}$ soil. The negative control plot showed an increase in microplastic abundance between 1994 and 1997, but this was not significant ($p > 0.05$). However, the treatment plots did show significant increases ($p < 0.05$). Microplastics could possibly have been introduced to the control plot by cross-contamination in the field from the treatment plots (Fig. S1) through surface water runoff, atmospheric transportation, or from microplastics introduced by the inorganic fertiliser pellets (Cusworth et al., 2024). It is unlikely that there was much microplastic transfer between plots by the spading machine because the negative control plots were tilled before the treatment plots and the blades were cleaned between tillage of each plot. Similarly, a previous study investigating microplastic concentrations in surface run-off from sludge-amended croplands highlighted the significance of microplastic transfer from contaminated sites to unadulterated locations through surface run-off (Naderi Beni et al., 2023). In addition, an increase in societal use of plastics over this time period may have presented a route of entry to the soils. However, it is also worth noting that this slight increase only coincides with the sludge application period (Fig. 1), suggesting that transfer from the treatment plots were most likely. Nevertheless, to eliminate the potential that other sources of microplastic input may have been present, the control plot microplastic concentrations were subtracted from the respective treatment plots at each time point.

Abundance increased Sludge plot E increased by 1107%, 879% in the sludge D plot, 723% in the sludge C plot, 1199% in the sludge B plot, and 1445% in the sludge A plot (Fig. 1 and Table S1), indicating significant microplastic accumulation over the first 4 years when the sludges were

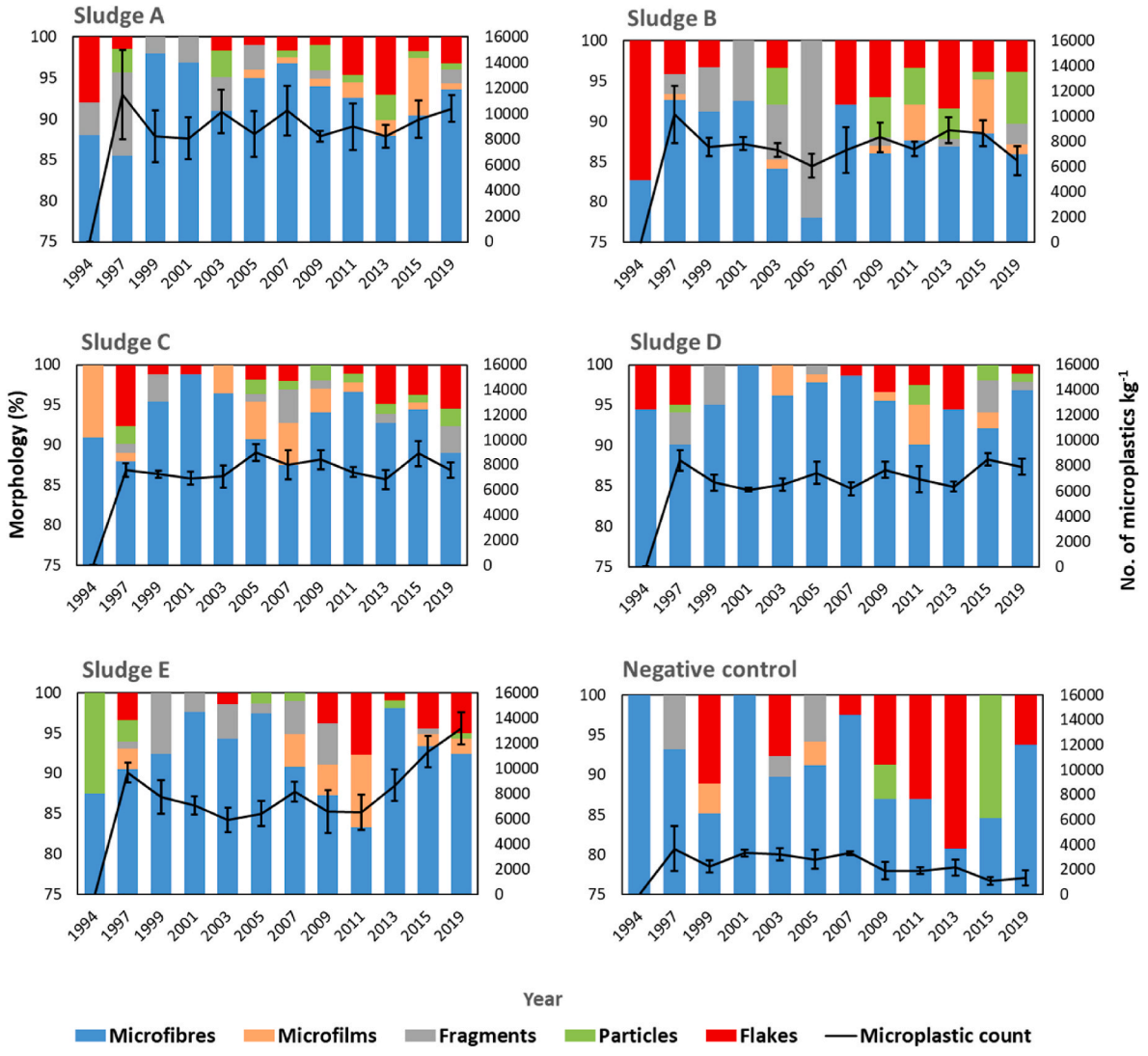


**Fig. 1.** Numbers of microplastic $kg^{-1}$ soil over time (right axis and line graph) and proportions of microplastic morphologies over time (left axis and bar graph). Graphs are labelled with their respective treatments. Error bars depict one standard deviation (n = 3).

applied. The differences in microplastic abundance between treatments are possibly due to the conditions of sludge production at the WWTWs because the servicing area, proportion of industrial waste and treatment processes, including secondary treatments and sludge dewatering, can affect the abundance of microplastics in sewage sludges (Harley-Nyang et al., 2022; Li et al., 2018). It may also be due to differences in sludge application rates (data not available).

Overall, microplastic abundance remained relatively constant after cessation of sludge applications, with no significant change in abundance ($p > 0.05$; Fig. 1 (A-D and negative control)), indicating that they persisted in soil over 25 years. This also indicates that the contribution of microplastics from the inorganic fertiliser pellets was minimal because the pellets were applied to all plots throughout the experiment, but there was no significant increase in microplastic abundance following cessation of the sludge applications in any of the plots. The role of inorganic fertiliser pellets as a source of microplastics to soil was not explicitly tested in our study but should be elucidated in future studies. Since the degradation of microplastics to detectable secondary microplastics (≥50 μm) would increase abundance, and surface run-off and degradation to micro(nano)plastics (<50 μm) would decrease abundance, it is possible that these two processes are occurring at a similar rate such that there were no detectable changes to microplastic abundance overall. The exception was the sludge E plot, which significantly increased in abundance between 1997 and 2019 ($p < 0.05$; Fig. 1), possibly due to the production of detectable secondary microplastics, though why this process would occur more on this plot compared to the others is unclear. Fluctuations in the abundance of the different microplastic morphologies may be due to the heterogenous nature of soil and the constant redistribution of microplastics from annual tillage. The relatively consistent within-treatment standard deviation in the HGMS extraction (Fig. 1) gives confidence in the methodology and that deviations are attributed to heterogeneity within the soil.

Microfibres were the dominant microplastic morphology found in soils from all the treatment and negative control plots across all years, accounting for 81–99% (average 92%) of microplastics found (Fig. 1), consistent with the fact that the sewage sludges used in this study also predominantly contained microfibres. Fibres were the most abundant microplastic morphology found in soils treated with sewage sludge (Liu et al., 2022; Yang et al., 2021b). Fibres come from clothing and textiles during commercial and industrial washing released into WWTWs (Hernandez et al., 2017). High concentrations of synthetic fibres in agricultural soils are indicative of sewage sludge applications (Piehl et al., 2018). Microfilms are commonly derived from mulching film or

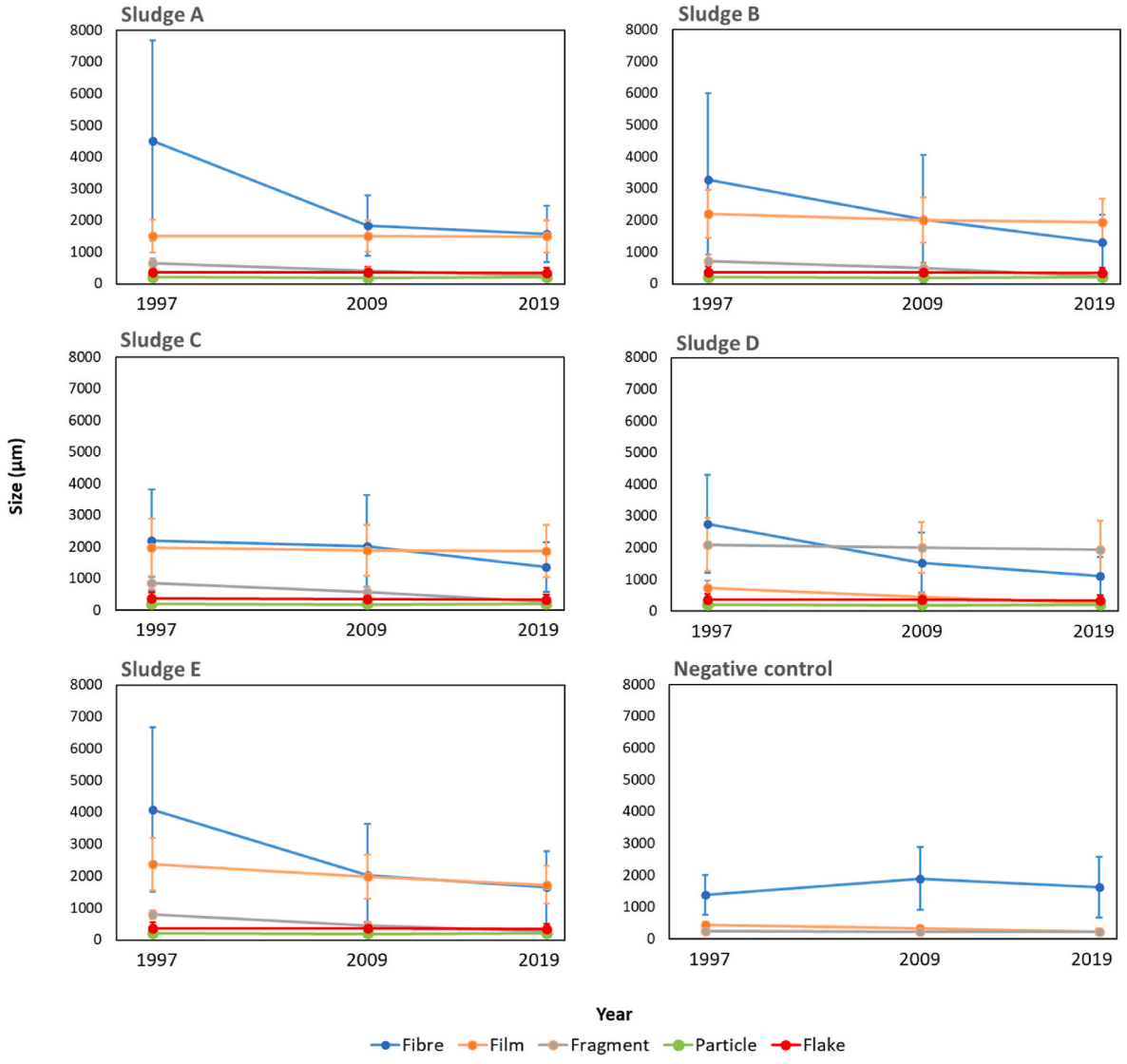


**Fig. 2.** Decline in microplastic sizes over three of the sampling points (n > 70 per treatment). Graphs are labelled with their respective treatments. Error bars depict one standard deviation (n was variable between treatments and years; n = 11–61).

food package, particles from cosmetics, flakes from food and drink packaging, and fragments from a variety of larger plastic items (Surendran et al., 2023).

### 3.4. Size of microfibres and fragments decreased over time

The sizes of microplastics were assessed after the final addition of sludge (1997), halfway through the experiment (2009), and at the end of the experiment (2019) (Fig. 2). Although the sizes of microplastics were only monitored at three time periods, we used a high number of samples (>70 per each treatment) for data analysis for good statistical power. Microfilms were not detected in the 1997 soil samples for sludge A- and sludge D-treated plots, and particles were not detected in the sludge B-treated 1997 soil samples (likely due to soil heterogeneity), therefore, the average sizes of those morphologies recovered from the respective sludges were used as time zero (1997) points instead (Fig. 2). It is appreciated that the sizes of these microplastics may have decreased between the first sludge application (1994) and the final application (1997), however, the size of other microplastics recovered from the soil plots in the 1997 samples were not significantly different to the sizes of microplastics recovered from their respective sewage sludges (*t*-test; $p > 0.05$). Therefore, the use of the sizes for microfilms recovered from sludges A and D and particles in sludge B as a proxy for the corresponding 1997 soil samples is justified. Microfibres displayed a wide range in length (70–9000 μm), which all significantly decreased by the end of the experiment ($p < 0.05$) (Fig. 2 and Table S1). Microfibres in the negative control plot showed no significant change in length ($p > 0.05$), possibly due to their smaller size making them less susceptible to mechanical fragmentation.

All but one of the treatment plots (sludge C) showed a larger decrease in microfibre length between 1997 and 2009 followed by a smaller decrease from 2009 to 2019. This is possibly due to long microfibres (approximately ≥3 mm) being susceptible to mechanical fragmentation caused by tillage, and then as microfibre lengths decreased (approximately ≤1 mm), susceptibility to further mechanical fragmentation decreased. The extremely small diameter of synthetic microfibres (13–25 μm) likely makes them more susceptible to shearing. Microfibres in the sludge C-treated plot and negative control plot did not show this trend, possibly due to the smaller microfibre lengths as noted previously (2202 ± 1604 μm and 1390 ± 625 μm, respectively). It was noted that the time of sludge applications that Sludge C was powdery in texture with large, solid aggregates. While attempts were made to fully incorporate these aggregates, it is possible that some microfibres and other microplastics may have been entrapped within them and thus protected until the aggregates broke down physically, releasing the microfibres. At smaller microfibre lengths, the main degradation process would be through chemical weathering or biodegradation, but these are longer processes and are dependent on soil depth and microbial community composition (Huang et al., 2021).

Fragments were the only other morphological category to significantly decrease in size over time, from 751 ± 74 to 180 ± 43 μm ($p < 0.05$), likely because fragments are brittle.

It is possible for microbial degradation to mediate the reduction in microplastic size since fungi and bacteria can degrade polyethylene (Muhonja et al., 2018). However, microbial biodegradation may have been hindered in our plots treated with sludges containing the heavy metals due to the heavy metals impairing biological functions (Campbell et al., 2009; Lemire et al., 2013), thus contributing to microplastic persistence. Sewage sludges typically contain high metal concentrations (McGrath et al., 1994); therefore, our study reflects the typical scenario of sewage sludge application to soils and subsequent microplastic degradation. To our knowledge, there is no evidence that the heavy metals itself directly affect microplastic degradation (i.e., through sorption). Biodegradation of microplastics is highly dependent on the presence of particular micro-organisms that have the ability to degrade synthetic polymers. Therefore, it does not contribute significantly to microplastic degradation (Sutkar et al., 2023). Given this, mechanical and chemical degradation are more likely to be the major drivers of microplastic degradation. We were unable to explicitly test the relative contributions of biological, chemical, and physical microplastic degradation mechanisms in our study because we used archived samples.

The reduction in microplastic size has implications for soil function. Degradation to smaller sized microplastics increases surface area for pollutants to adsorb to (Liu et al., 2020). They also become more digestible to soil fauna due to a limited size in buccal cavity. Ingestion of microplastics causes shifts in the gut microbiome and adverse effects on motility, growth, metabolism, reproduction, and mortality in various combinations, especially at higher concentrations and small size (Büks et al., 2020). These are effects that could be tested in a future study.

### 3.5. Other microplastic morphologies did not decrease in size over time

Flakes did not significantly decrease in size over time ($p > 0.05$) (Fig. 2 and Table S1), possibly because of their thick (250–890 μm) but flexible morphology and their chemical composition. Particles remained at a constant size throughout (Fig. 2 and Table S1), possibly because of their very small sizes. Microfilms also did not show a significant decrease in overall size ($p > 0.05$), possibly because they are thin (25–150 μm), soft, and pliable, and can easily stretch or form different shapes, allowing them to withstand mechanical shearing. The lack of degradation over the 25-year period suggests that these microplastic morphologies are more resistant to degradation, however, this may be a reflection of the conditions of the experiment. The amount of mechanical, chemical and biological degradation can differ between environments giving different rates of degradation (Cocoran, 2022; Zhang et al., 2021).

### 3.6. Microfilms weather over time

To better understand microplastic degradation over time, the recovered polyethylene microfilms were analysed using SEM (Fig. 3 and S4) and compared to virgin polyethylene mulch film obtained from a local farm (Fig. 3A). Microfilms were chosen due to the ease of characterisation. The microfilms in soil samples after the final application of sludge (1997; $n = 3$) displayed early signs of ageing shown by cracking and folding (Fig. 3B). Over time, the surface of the microfilms began to flake (2001; $n = 1$) (Fig. 3C) before developing cracks and pitting (2005; $n = 3$) (Fig. 3D). These became enlarged, forming holes or tears, while the edges became more ragged (2009 and 2019; $n = 2$ and $n = 2$, respectively) (Fig. 3E–F). This degradation was only on the surface; as mentioned previously, the microfilms did not significantly reduce in size, which is due to their pliability and malleability (Yu et al., 2023). The degree of degradation was visually similar across all treatment plots; therefore, it was independent of the sludge environment they originated from. Films degrade faster at shallower soil depths (Huang et al., 2021), and since topsoils were used in this study, the microfilms within them would have been more susceptible to degradation. Plastic films are regularly used in agriculture as mulch, but if improperly disposed, the resulting microfilms can impede plant root growth and water flow within soils (Hu et al., 2022).

Weathering changes microplastic crystallinity, specific surface area, and oxygen functional groups, which increases sorption of other pollutants (Liu et al., 2020). Therefore, the oxidation of the microfilm surfaces was assessed by calculating the carbonyl indices obtained from their FTIR spectra prior to the SEM imaging process. The carbonyl index for polyethylene microfilms recovered from the 1997 soils was 0.04 ± 0.04 ($n = 3$), 0.15 for 2001 ($n = 1$), 0.40 ± 0.15 for 2005 ($n = 3$), 1.28 ± 0.43 for 2009 ($n = 2$), and 3.13 ± 0.86 for 2019 ($n = 3$). This indicates that oxidation significantly increased over time (*t*-test between 1997 and 2019; $p < 0.05$), which corresponds to the visual degradation observed over time (Fig. 3).

Taken together, microplastic morphology appears to be the greatest

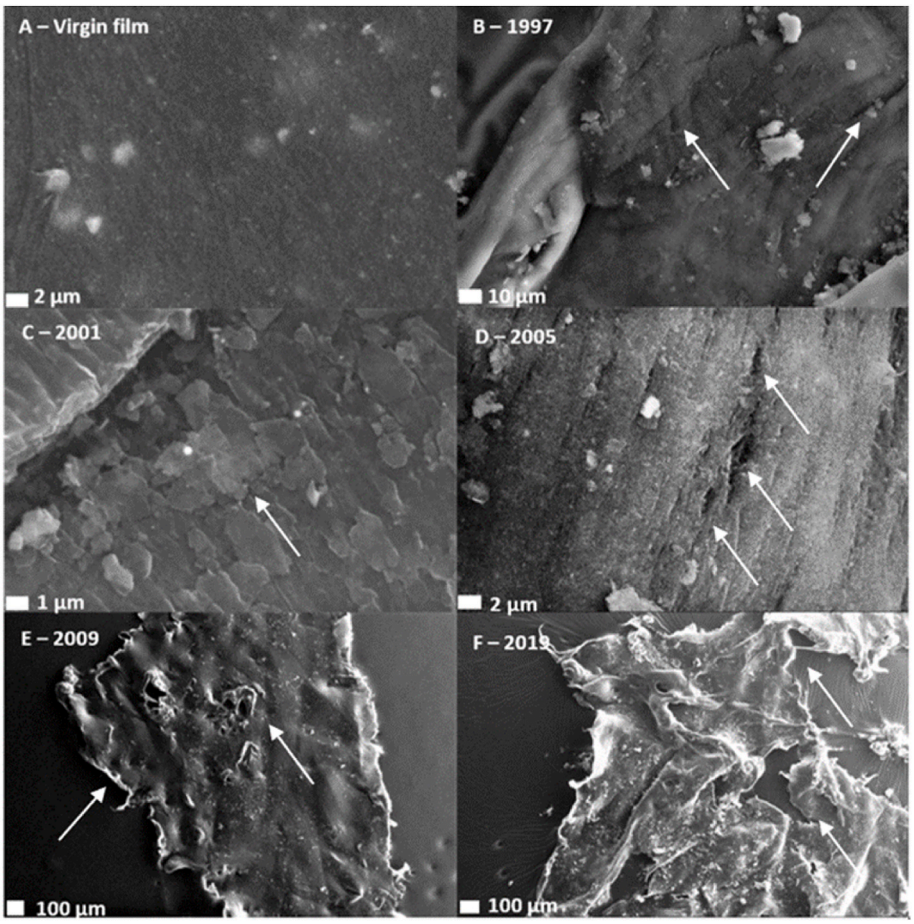


**Fig. 3.** SEM micrographs of films displaying progressive weathered features. The panels show: virgin plastic film with a relatively smooth surface (A); early signs of degradation as shown by cracking and folding (B); surface flaking (C); cracks and pitting (D); holes/tears with ragged edges (E–F). The white arrows indicate each weathering characteristic as described. The panels are labelled with the year that the soil was sampled. The scale bars are shown in the bottom left of each micrograph image. Further examples of similar microfilm weathering are shown in Fig. S4.

intrinsic factor in determining the susceptibility of microplastics to degradation. Microfibres and fragments are susceptible to degradation in size, while microfilms are susceptible to degradation in surface characteristics. However, flakes and microparticles are more resistant to degradation.

### 3.7. Coloured microfibres lose dyes over time

The microfibres were predominantly white or transparent in all treatment plots (>67.8%) and control plot (61.7%) (Fig. 4), which is consistent with previous findings in soils (Yang et al., 2021a). Several microfibres were transparent with patches of blue or pink (Fig. 5A–C). The coloured regions of the microfibres were irregularly spaced along the microfibres' length and were either coloured in blocks (striped) or speckled (dots), and their pigmentations were faded in colour (Fig. 5A–C). By contrast, black, red, green, and purple microfibres had the boldest colouration (i.e., no signs of fading) (Fig. 5D–H).

The appearance of partially coloured microfibres in soil samples only occurred from 2001 onwards and increased over time; they significantly increased in 2003 ($p < 0.05$) and peaked in 2005 at 7.22 ± 0.76% (Fig. 4). At the same time, transparent microfibres in some of the treatment plots (sludge A-, B-, and E-treated soil – Fig. 4) increased in number. This indicates that the coloured microfibres lost their dyes over time, which contributed to the increasing numbers of partially dyed and then transparent microfibres observed in some of the treated soil plots (Fig. 4). Importantly, partially dyed microfibres were not detected in any of the sludge samples, also suggesting that they came from coloured microfibres losing their dyes. It is unlikely that atmospheric deposition could account for the appearance of the partially dyed microfibres in the soil because these microfibres only appeared in the treatment plots after a specific time point; very few appeared in the control plot at two time points (2005 and 2011), and this increase was not significant ($p > 0.05$). Similarly, atmospheric deposition could not account for the increase in transparent microfibres towards the end of the experimental period because this was only observed in sludge A, B, and E-treated plots and not in the control plot. Instead, our observations suggest that this increase is likely attributed to dye loss of coloured microfibres followed by transparent microfibre fragmentation. Future studies should be performed to confirm our observations.

If indeed the fibres are losing their dyes, this has implications for the soil environment. Textile dyes can persist in soils and have negative effects on soil microbial community composition and function (Imran et al., 2015; Rehman et al., 2018; Lellis et al., 2019), and plant germination and growth (Çiçek et al., 2012). To our knowledge, there are no studies on the loss of dyes from microplastics and the effects this has on soil; this warrants further study.

The differences in dye loss could be due to the manufacturing and dyeing process or the molecular stability of the dye. Our results suggest that microfibres were much less likely to lose black, red, and dark blue/navy dyes (Fig. 5). Falk et al. (2000) attributed the loss of colour in natural fibre-thermoplastic composites over time to the susceptibility of the synthetic polymer component to UV exposure, chemical degradation, and environmental stresses such as thermal- and moisture-induced expansion. The rate of colour loss was dependent on the polymer's

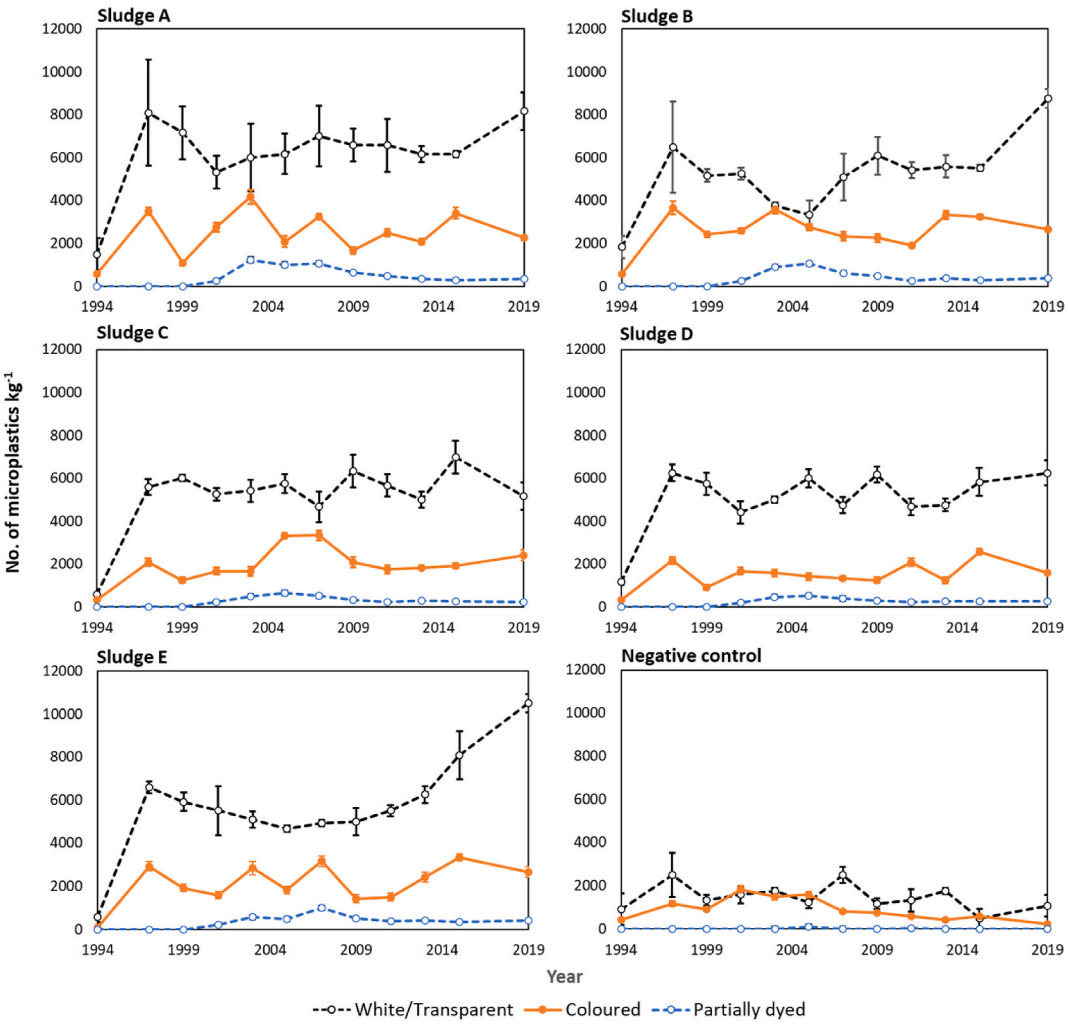


**Fig. 4.** Number of different colours of microfibres over time. Graphs are labelled with their respective treatments. Error bars depict one standard deviation (n = 3).

composition and whether any other additives were present in the polymeric fraction. Microbial degradation may also account for dye loss (Patel et al., 2021), however, as previously mentioned, biological functions in these soils were severely impaired due to high heavy metal concentrations from the sewage sludges (Campbell et al., 2009, Table 1), therefore, significant biodegradation of the dyes was unlikely. It may be possible that UV radiation could have degraded the textile dye of microfibres which were present at or near the surface of the soil, and these microfibres would have been reincorporated into the soil with each annual tillage. While the mechanism through which microfibre dyes were lost could not be determined in our study, the ecological risks associated with the presence of environmentally toxic textile dyes in soils warrants further investigation in future studies.

### 3.8. Microplastic composition as a potential indicator of source

Prior to FTIR analysis, the influence of xylene (used in the collection and preservation of microplastics, see Section 2.3) on microplastic spectra was investigated. This showed there was no spectral changes in the test spectra until after 2 min of continuous exposure to xylene (see Supplementary Information; Fig. S5) indicating that this method did not have any impact on the spectra obtained in this study. Furthermore, the long-established use of this process in forensic science to isolate fibres from adhesive tapes (Schotman and van der Weerd, 2015) provides further confidence that this is an acceptable method to use.

Overall, the compositions of the microplastics recovered from soils corresponded to the compositions of the microplastics recovered from the sewage sludges (Table 2). In both the sludges and the soils, polyester was the most abundant microplastic polymer ($p < 0.05$) corresponding to the most abundant morphology (i.e., microfibres). This is consistent with the findings of other published studies on sewage sludge and sludge-treated soils (Schell et al., 2022; El Hayany et al., 2022; Yang et al., 2021b).

Of the five sludge treatments, rarely observed high-density microplastics (e.g., polymethyl methacrylate, polyurethane) were only identified in sludges A, B, and C, and their corresponding soil plots (Fig. S6). This reflects that these sludges originated from WWTWs servicing industrial wastes (Table 1). Polyester and polyurethane fibres, which are commonly used as co-polymers for base materials in upholstery furnishings (Tables 1 and 2), specifically faux leather, were found in sludge A and its corresponding soil plot. Polymethyl methacrylate and polytetrafluoroethylene fragments, which are commonly used in electrical appliances (Tables 1 and 2), were identified in sludge B and its corresponding soil plot. Sludges A and B are known to originate from WWTWs that serviced wastewater from the tannery and electronics industries, respectively (Tables 1 and 2). It is unknown where sludge C originated from, however, polyurethane was identified in this sludge and its corresponding soil plot, which strongly indicates that it came from a WWTW that served an industrial location (hence its high metal concentration (Table 1)). Sludges D and E, and their corresponding soil plots, did not contain any high-density or rarely observed polymers indicating that the associated WWTWs served household waste rather

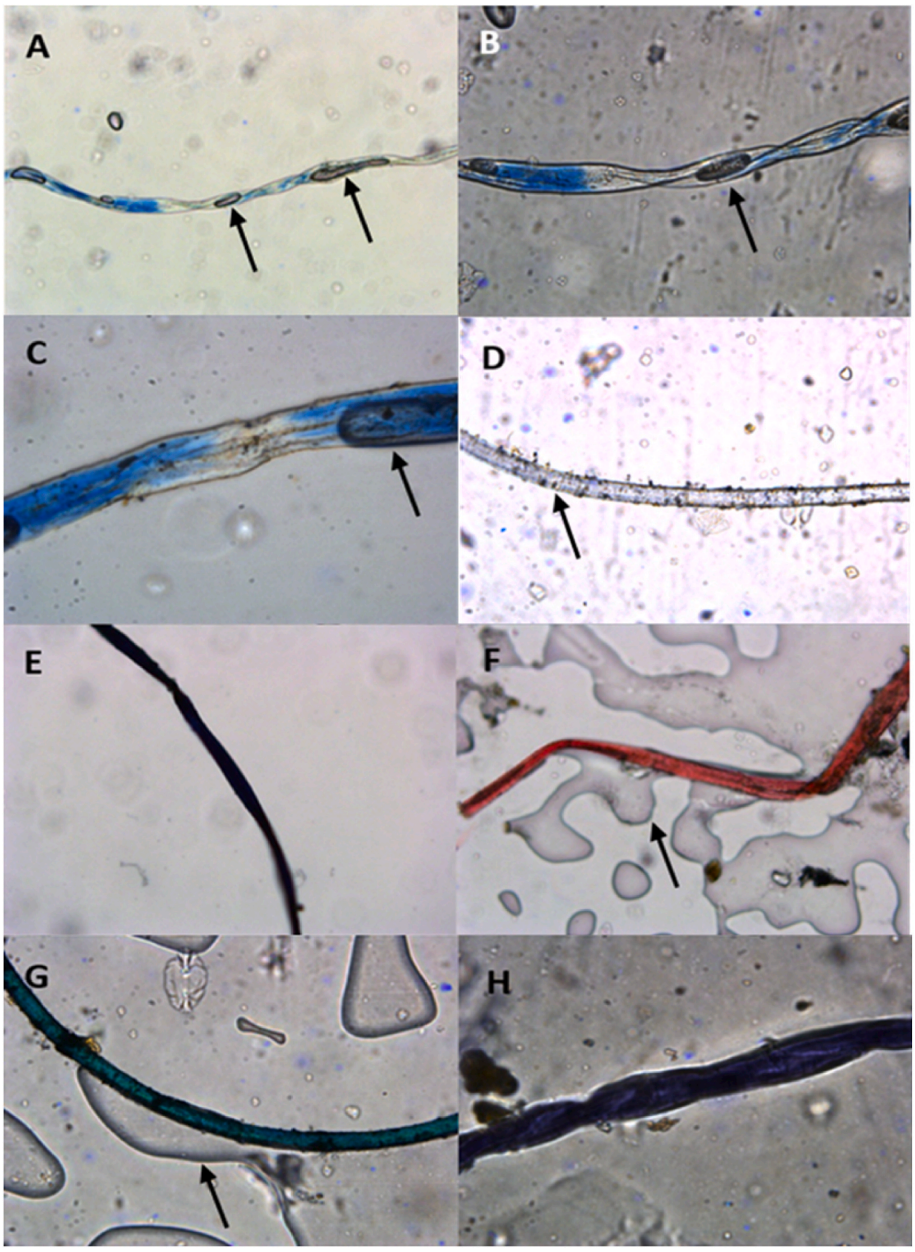


**Fig. 5.** Images of different colours of microfibres. Examples of microfibres that had lost their dyes (A–C), a colourless, transparent fibre (D), and microfibres where their dyes have not been lost – black (E), red (F), green (G), and purple (H). Arrows indicate air bubbles trapped between the tape and the fibres.

than industrial waste (hence, the low metal concentrations (Table 1)). These results demonstrate that determination of the composition of microplastics in the environment is a useful tool for tracing potential sources of microplastic pollution. Since the industrial waste sludges were not mixed with each other, it was possible to assign microplastic source back to the sludge based on microplastic composition. Exemplar FTIR spectra are presented in Fig. S6.

### *3.9. Soil microplastic communities were influenced by sewage sludge microplastic communities*

'Microplastic communities' is a relatively new concept that uses microplastic characteristics (colour, shape, composition, etc.) to distinguish microplastics between locations. It was used to show a significant distance decay relationship within aquatic environments, suggesting sampling sites with close geographical locations had similar pollution sources and could exchange microplastics more easily (Li et al., 2021). We therefore employed similar methods to distinguish differences between our treatments.

Clustering within the nMDS plots indicated that the microplastic communities within samples from the control soil and soils before sludge applications (1994) were similar but distinct from soil samples post-application (1997–2019) and the sludges (Fig. 6), with the latter two groupings forming distinct clusters on their own. The clustering of the control soil and soils before sludge applications gives confidence that the plots were similar (in terms of microplastic communities) prior to treatment, and any differences observed later were as a result of the sludge additions. Moreover, the microplastic communities within the control plots remained relatively unchanged throughout the experiment as microplastics were not introduced through sewage sludge additions. The sludges formed a distinct cluster indicating the microplastic communities in the sludges were different to the soil samples. The post-application (1997–2019) treated soil samples formed its own distinct cluster, between the sludge cluster and the control and pre-treatment

**Table 2**
Compositions of microplastics ≥50 μm in size recovered from sludge and soil samples after the application of sewage sludge.

| Sludge/ Treatment | Microplastic composition | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fibres | | | | | | | | Film | Fragment | Particle | Flake |
| | White and transparent | Black | Red | Pink | Blue | Navy | Green | Purple | Yellow | | | |
| Undigested | Polyester | Acrylic | Acrylic | Polyester | Acrylic | Nylon | PVC | – | Nylon | PE | – | PP, PE | PET, PE |
| Digested (sludge) | Polyester | Acrylic | Acrylic | – | Polyester, Acrylic | Polyester, Acrylic | PVC | – | – | PE, PP | PTFE, PP | PE, PP | PET |
| Digested (soil) | Polyester | Acrylic | Acrylic | – | Polyester | Polyester | PVC | – | – | PE, Ethylene-ethyl acrylate co-polymer | PTFE | PE | PET |
| Zn-digested (sludge) | Polyester, PU | Acrylic | Polyester | Acrylic | Polyester | Polyester | PVC, Acrylic | – | – | PP, PVC | PE | PS, PE, PP | PET |
| Zn-digested (soil) | Polyester, Polyester-PU copolymer | Acrylic | Polyester, Acrylic | Acrylic | Polyester | Polyester | PVC | – | Nylon | PVC | PE | PS, PE | PET, PE |
| Cu-undigested (sludge) | Polyester | Acrylic, Nylon | Acrylic | – | Polyester | Polyester, Acrylic | Polyester, Acrylic | Acrylic | Nylon | PU | PMMA, PTFE | PMMA, PE | PMMA, PET |
| Cu-undigested (soil) | Polyester | Nylon | Acrylic | – | Polyester | Polyester | Polyester, Acrylic | Acrylic | Nylon | Polyether PU | PMMA, PTFE, PET | – | PMMA, PTFE, PET |
| Cd-digested (sludge) | Polyester | Acrylic | Polyester, Acrylic | Acrylic | Polyester | Nylon | Polyester | – | – | – | PU | PS | PET |
| Cd-digested (soil) | Polyester, PP | Acrylic | Polyester | Acrylic | Polyester, Acrylic | Nylon | Polyester, Acrylic | – | Nylon | PP | PU, PE | PS | PET |
| Negative Control | Polyester | – | – | – | PE | – | Polyester | – | – | – | – | – | – |

PU – polyurethane; PVC – polyvinyl chloride; PE – polyethylene; PS – polystyrene; PET – polyethylene terephthalate; PMMA – polymethyl methacrylate; PTFE – polytetrafluoroethylene.

soils cluster, presumably as a result of the mix of microplastic communities from the sludge with the soils. There was little overlap between the sludge cluster and the soils post-application cluster, which might be because once transferred from the sludge to the soil, the microplastics changed, e.g., if the partially coloured fibres resulted from dye loss, then they would have been classed differently to the original fully coloured microfibres. Additionally, microplastic communities may have been altered through the introduction of additional microplastics from atmospheric deposition. Certainly, there was much less overlap between the sludge and soils post-application clusters for colour. There was little change over time in the post-application plots, supporting the finding of the relatively stable microplastic abundances discussed previously. Further investigation of the soils post-application cluster showed that there was no separation by sludge treatments, likely because plots receiving industrial sludges were supplemented with municipal sludges (Fig. S7A).

Total Mn, Cu, and Pb and most probable number (MPN) per g significantly influenced variation in microplastic community by colour (Fig. 6A), while total Mn, Cu, and Pb and %N influenced variation in microplastic community by morphology (Fig. 6B) in the nMDS plots. The metals presented as factors within the nMDS plots reflect the different industrial waste stream sludges (as each sludge contained varying concentrations of heavy metals - Table 1). Therefore, this indicates that it is the source of the sludges that predominantly influenced the microplastic communities in the soil. Industrial and municipal sludges typically contain different concentrations of both nitrogen (King, 1984) and numbers of micro-organisms (Zeng et al., 2022). Since the sludges in our study contained different levels of nitrogen and bacteria, the significance of %N and most probable number per g as factors in the nMDS plots further reflect the influence of sewage sludge source (i.e., different industrial and municipal waste streams) on soil microplastic community compositions. Identifying the potential sources of sludges to industrial waste streams through microplastic community determination could be further investigated in future studies. The concept of microplastic communities in this study was a useful tool in distinguishing microplastic distribution amongst the matrices and indeed shed light on the influence of sewage sludge source on soil microplastics. We therefore advocate for this to be used in future microplastics studies.

### 3.10. Study limitations

In the experimental design of the original long-term sewage sludge experiment at Hartwood, plots treated with sludges from industrial waste streams were supplemented with municipal sludge to minimise differences in organic matter content (see Gibbs et al. (2006) for details on the experimental design and Supplementary Information). Because of this, it was not possible to explore which of the sludges contributed the highest number of microplastics or whether sludge differences affected microplastic fate in the soils. Similarly, establishing a mass balance was not possible, especially because sludge E was not available for analysis (no original sample remained at time of this study, possibly because it was all used up for analysis in previous studies).

The sizes of microplastics were only monitored at three time periods, however, we used a large number of samples (>70 per each treatment) for data analysis to achieve good statistical power. The number of replicates of microfilms that were observed under SEM was small (1–3 replicates) and varied between years and treatments, therefore, further work on the degradation of microfilms is required.

### 3.11. Implications of the findings

The findings from this study showed that microplastics rapidly accumulate in soil through sewage sludge applications and can persist for a prolonged period of time but can slowly degrade depending on their morphologies. The impact of microplastics on soil health and quality in our study were not investigated because we used archived soil

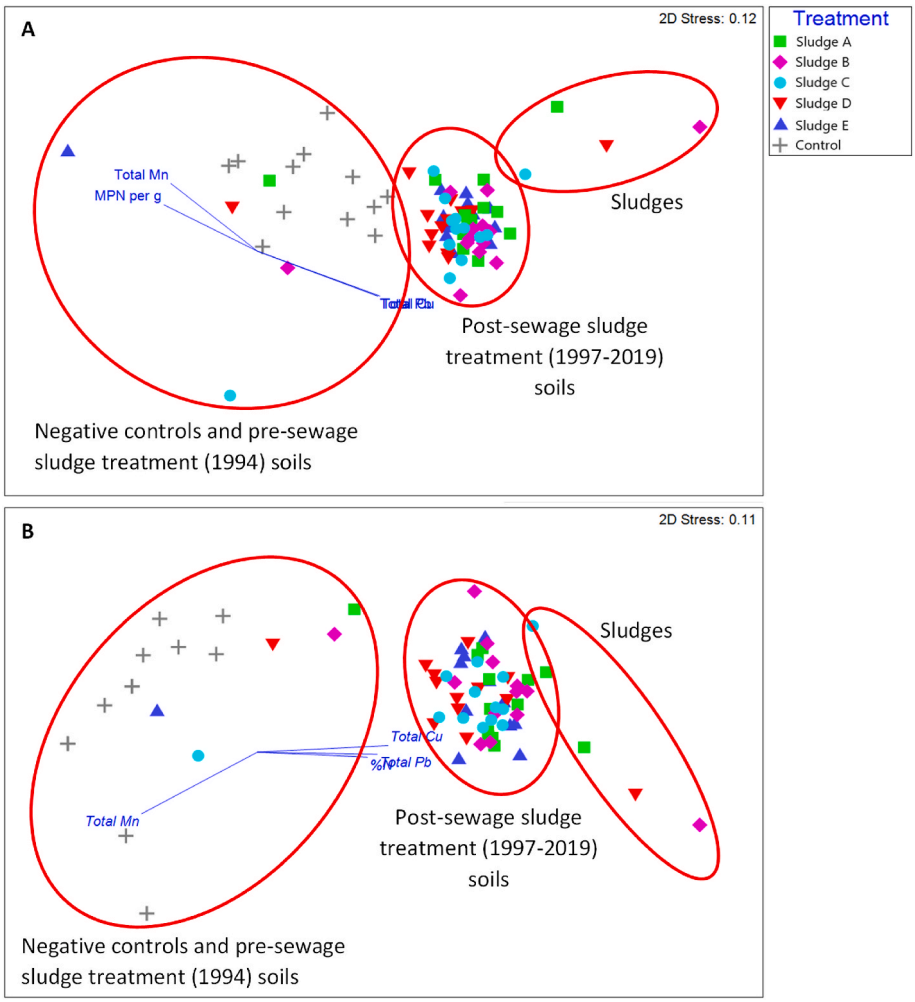


**Fig. 6.** Non-metric multidimensional scaling plot (nMDS) of the microplastic communities within soil and sludge samples. The communities are characterised either by colour (A) or morphology (B). Pearson's correlations with explanatory variables (total Mn, Cu, and Pb, most probable number per g, and %N) are overlaid (where $r < 0.5$). Clusters are encircled in red (added manually) and annotated for illustrative purposes only.

samples. However, as observed by other researchers, the persistence of some microplastic morphologies and degradation of others, as observed in this study, have the potential to negatively impact soil health long-term. This can include the disruption of soil structure, altered physico-chemical properties (e.g., soil aggregation and water holding capacity), reduced nutrient availability, and altered microbial activities and immobilisation. Additionally, some countries (including China and Tunisia (Marzougui et al., 2022; Zhang et al., 2023)) apply sewage sludges to agricultural land used for crop production, and studies have reported that the presence of microplastics can impair the development and growth of crops (Sajjad et al., 2022). Although sewage sludge has positive effects on crop production due to the addition of nutrients, the long-term impacts from the presence of microplastics may outweigh the benefits.

To prevent the potential decline in soil health that could be caused by microplastics introduced to soil through the application of sewage sludge, better legislation could be implemented to impose tolerance limits on microplastic concentrations within sludges (similar to those in place for metals and organic contaminants in some countries) to prevent mass inputs of microplastics to the soil which could persist for substantial periods of time as reported in this study. Additionally, new sludge treatment and management practices could be explored to remove or reduce microplastic contamination before release from WWTWs, for example, through the use of electronic beams (Siwek et al., 2023).

## 4. Conclusions

To our knowledge, this study is the first to provide high temporal resolution long-term data (25 years) on microplastics in agricultural soils following sewage sludge application. Quantitative data showed that sewage sludge is a huge source and significant pathway of microplastics into soils, and that microplastics accumulate rapidly and persist at relatively constant levels across at least 25 years after application, and possibly beyond. Microfibres, fragments, and microfilms were more susceptible to degradation than other microplastic morphologies, releasing further micro- and nanoplastics into soil. Dye loss from microfibres was evident, although, the fate of these dyes is unknown. Given the environmental toxicity of some dyes, this may be of concern and warrants further investigation. Furthermore, determining the composition of microplastics is a useful tool in tracing potential sources of the microplastics.

The findings of this study raise questions on the potential impact of the microplastics, secondary micro(nano)plastics, and microfibre dyes on soil ecosystem function, and whether these contaminants are able to be transported away from the site of contamination, leading to further effects in other locations. Of interest is the potential for these microplastics to act as vectors for other pollutants present in sewage sludge (e.g., organic pollutants, pathogens, and heavy metals), causing further negative effects on soil ecosystem functions, and potentially animal and human health. This study demonstrates that sewage sludge applications

create long-term hot-spots of increased microplastic abundance, which have not yet been included in regulatory standards on sewage sludge use and management.

## CRediT authorship contribution statement

**Stuart J.F.F. Ramage:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Malcolm Coull:** Writing – review & editing, Resources. **Patricia Cooper:** Writing – review & editing, Resources. **Colin D. Campbell:** Writing – review & editing, Resources. **Radhakrishna Prabhu:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Kyari Yates:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Lorna A. Dawson:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Sandhya Devalla:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Eulyn Pagaling:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2025.144277.

## Data availability

Data will be made available on request.

## References

Bayo, J., Olmos, S., López-Castellanos, J., 2020. Microplastics in an urban wastewater treatment plant: the influence of physicochemical parameters and environmental factors. Chemosphere 238, 124593. https://doi.org/10.1016/j.chemosphere.2019.124593.

Bonyadinejad, G., Salehi, M., Herath, A., 2022. Investigating the sustainability of agricultural plastic products, combined influence of polymer characteristics and environmental conditions on microplastics aging. Sci. Total Environ. 15, 156385. https://doi.org/10.1016/j.scitotenv.2022.156385.

Büks, F., Loes van Schaik, N., Kaupenjohann, M., 2020. What do we know about how the terrestrial multicellular soil fauna reacts to microplastic? SOIL 6, 245–267. https://doi.org/10.5194/soil-6-245-2020.

Campbell, C.D., Cooper, P., Crooks, W., Sinclair, A., White, D., 2009. Impact of long term sludge additions to biological function. In: *Scottish Soils*, CR/2008/03. Scottish Government, Edinburgh.

Carr, S.A., Lin, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182. https://doi.org/10.1016/j.watres.2016.01.002.

Christodoulou, A., Stamatelatou, K., 2016. Overview of legislation on sewage sludge management in developed countries worldwide. Water Sci. Technol. 73, 453–462. https://doi.org/10.2166/wst.2015.521.

Çiçek, N., Efeoğlu, B., Tanyolaç, D., Ekmekci, Y., Strasser, R.J., 2012. Growth and photochemical responses of three crop species treated with textile azo dyes. Turk. J. Bot. 36, 529–537. https://doi.org/10.3906/bot-1109-6.

Cocoran, P.L., 2022. Degradation of microplastics in the environment. In: Rocha-Santos, T., Costa, M.F., Mouneyarac (Eds.), Handbook of Microplastics in the Environment. Springer Cham, Gewerbesraße, pp. 531–542.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V., 2019. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Sci. Total Environ. 671, 411–420. https://doi.org/10.1016/j.scitotenv.2019.03.368.

Cusworth, S.J., Davies, W.J., McAinsh, M.R., Gregory, A.S., Storkey, J., Stevens, C.J., 2024. Agricultural fertilisers contribute substantially to microplastic concentrations in UK soils. Communications Earth & Environment 5, 7. https://doi.org/10.1038/s43247-023-01172-y.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Rillig, M.C., 2018. Impacts of microplastics on the soil biophysical environment. Environmental Science & Technology 52, 9656–9665. https://doi.org/10.1021/acs.est.8b02212.

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics can change soil properties and affect plant performance. Environmental Science & Technology 53, 6044–6052. https://doi.org/10.1021/acs.est.9b01339.

Ding, L., Ouyang, Z., Liu, P., Wang, T., Jia, H., Guo, X., 2022. Photodegradation of microplastics mediated by different types of soil: the effect of soil components. Sci. Total Environ. 802, 149840. https://doi.org/10.1016/j.scitotenv.2021.149840.

Dris, R., Gasperi, J., Rocher, V., Tassin, B., 2018. Synthetic and non-synthetic anthropogenic fibers in a river under the impact of Paris megacity: sampling methodological aspects and flux estimations. Sci. Total Environ. 618, 157–164. https://doi.org/10.1016/j.scitotenv.2017.11.009.

Duan, J., Bolan, N., Li, Y., Ding, S., Atugoda, T., Vithanage, M., Sarkar, B., Tsang, D.C.W., Kirkham, M.B., 2021. Weathering of microplastics and interaction with other coexisting constituents in terrestrial and aquatic environments. Water Res. 1996, 117011. https://doi.org/10.1016/j.watres.2021.117011.

El Hayany, B., Rumpel, C., Hafidi, M., El Fels, L., 2022. Occurrence, analysis of microplastics in sewage sludge and their fate during composting: a literature review. J. Environ. Manag. 317, 115364. https://doi.org/10.1016/j.jenvman.2022.115364.

Elmi, A., Al-Khaldy, A., AlOlayan, M., 2020. Sewage sludge land application: balancing act between agronomic benefits and environmental concerns. J. Clean. Prod. 250, 119512. https://doi.org/10.1016/j.jclepro.2019.119512.

Falk, R.H., Felton, C., Lundin, T., 2000. Effects of weathering on color loss of natural fiber thermoplastic composites. Natural Polymers and Composites 14, 382–385.

Gibbs, P.A., Chambers, B.J., Chaudri, A.M., McGrath, S.P., Carlton-Smith, C.H., Bacon, J. R., Campbell, C.D., Aitken, M.N., 2006. Initial results from a long-term, multi-site field study of the effects on soil fertility and microbial activity of sludge cakes containing heavy metals. Soil Use Manag. 22, 11–21. https://doi.org/10.1111/j.1475-2743.2006.00003.x.

Gies, E.A., LeNoble, J.L., Noël, M., Etemadifar, A., Bishay, F., Hall, E.R., Ross, P.S., 2018. Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. Mar. Pollut. Bull. 133, 553–561. https://doi.org/10.1016/j.marpolbul.2018.06.006.

Harley-Nyang, D., Memon, F.A., Jones, N., Galloway, T., 2022. Investigation and analysis of microplastics in sewage sludge and biosolids: a case study from one wastewater treatment works in the UK. Sci. Total Environ. 823, 153735. https://doi.org/10.1016/j.scitotenv.2022.153735.

Hernandez, E., Nowack, B., Mitrano, D.M., 2017. Polyester textiles as a source of microplastics from households: a mechanistic study to understand microfiber release during washing. Environ. Sci. Technol. 51, 7036–7046. https://doi.org/10.1021/acs.est.7b01750.

Hu, J., He, D., Zhang, X., Li, X., Chen, Y., Gao, W., Zhang, Y., Ok, Y.S., Luo, Y., 2022. National-scale distribution of micro(meso)plastics in farmland soils across China: implications for environmental impacts. J. Hazard Mater. 424, 127283. https://doi.org/10.1016/j.jhazmat.2021.127283.

Huang, D., Xu, Y., Lei, F., Yu, X., Ouyang, Z., Chen, Y., Jia, h., Guo, X., 2021. Degradation of polyethylene plastic in soil and effects on microbial community composition. J. Hazard Mater. 416, 126173. https://doi.org/10.1016/j.jhazmat.2021.126173.

Huang, M., Zhu, Y., Chen, Y., Liang, Y., 2023. Microplastics in soil ecosystems: soil fauna responses to field applications of conventional and biodegradable microplastics. J. Hazard Mater. 441, 129943. https://doi.org/10.1016/j.jhazmat.2022.129943.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2017. Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*. Environmental Pollution 220, 523–531. https://doi.org/10.1016/j.envpol.2016.09.096.

Imran, M., Shaharoona, B., Crowley, D.E., Khalid, A., Hussain, S., Arshad, M., 2015. The stability of textile azo dyes in soil and their impact on microbial phospholipid fatty acid profiles. Ecotoxicol. Environ. Saf. 120, 163–168. https://doi.org/10.1016/j.ecoenv.2015.06.004.

Ji, Q., Zhang, Y., Xia, Y., Wang, X., He, M., Yang, Y., Sabel, C.E., Huang, B., Zhu, F., Shao, M., Xie, E., Yan, G., Li, G., Zhou, A., He, H., Zhang, L., Jin, Z., 2024. Centennial records of microplastics in lake cores in huguangyan maar lake, China. Environ. Sci. Technol. 58, 11140–11151. https://doi.org/10.1021/acs.est.4c01970.

Jimenez, B., Barrios, J.A., Mendez, J.M., Diaz, J., 2004. Sustainable sludge management in developing countries. Water Sci. Technol. 49, 251–258. https://doi.org/10.2166/wst.2004.0656.

Kamble, S., Singh, A., Kazmi, A., Starkl, M., 2019. Environmental and economic performance evaluation of municipal wastewater treatment plants in India: a life cycle approach. Water Sci. Technol. 79, 1102–1112. https://doi.org/10.2166/wst.2019.110.

King, L.D., 1984. Availability of nitrogen in municipal, industrial, and animal wastes. J. Environ. Qual. 13, 609–612. https://doi.org/10.2134/jeq1984.00472425001300040020x.

Lehmann, A., Leifheit, E.F., Gerdawischke, M., Rillig, M.C., 2021. Microplastics have shape- and polymer-dependent effects on soil aggregation and organic matter loss – an experimental and meta-analytical approach. Microplastics and Nanoplastics 1, s43591. https://doi.org/10.1186/s43591-021-00007-x, 021-00007-x.

Lellis, B., Fávaro-Polonio, C.Z., Pamphile, J.A., Polonio, J.C., 2019. Effects of textile dyes on health and the environment and bioremediation potential of living organisms. Biotechnology Research and Innovation 3, 275–290. https://doi.org/10.1016/j.biori.2019.09.001.

Lemire, J., Harrison, J., Turner, R., 2013. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat. Rev. Microbiol. 11, 371–384. https://doi.org/10.1038/nrmicro3028.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018. Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res. 142, 75–85. https://doi.org/10.1016/j.watres.2018.05.034.

Li, C., Gan, Y., Zhang, C., He, H., Fang, J., Wang, L., Wang, Y., Liu, J., 2021. "Microplastic communities" in different environments: differences, links, and role of diversity index in source analysis. Water Res. 188, 116574. https://doi.org/10.1016/j.watres.2020.116574.

Liu, P., Zhan, X., Wu, X., Li, J., Wang, H., Gao, S., 2020. Effect of weathering on environmental behavior of microplastics: properties, sorption and potential risks. Chemosphere 242, 125193. https://doi.org/10.1016/j.chemosphere.2019.125193.

Liu, Z., Bai, Y., Ma, T., Liu, X., Wei, H., Meng, H., Fu, Y., Ma, Z., Zhang, L., Zhao, J., 2022. Distribution and possible sources of atmospheric microplastic deposition in a valley basin city (Lanzhou, China). Ecotoxicol. Environ. Saf. 233, 113353. https://doi.org/10.1016/j.ecoenv.2022.113353.

Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A., Rillig, M.C., 2021. Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. Front. Plant Sci. 12, 616645. https://doi.org/10.3389/fpls.2021.616645.

Maaß, S., Daphi, D., Lehmann, A., Rillig, M.C., 2017. Transport of microplastics by two collembolan species. Environmental Pollution 225, 456–459. https://doi.org/10.1016/j.envpol.2017.03.009.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L., 2017. Microplastics in sewage sludge: effects of treatment. Environ. Sci. Technol. 51, 810–818. https://doi.org/10.1021/acs.est.6b04048.

Marzougui, N., Ounalli, N., Sabbahi, S., Fezzani, T., Abidi, F., Jebari, S., Melki, S., Berndtsson, R., Oueslati, W., 2022. How can sewage sludge use in sustainable Tunisian agriculture Be increased? Sustainability 14, 13722. https://doi.org/10.3390/su142113722.

Masura J., Baker J., Foster G., Arthur C., Herring C. 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48.

McGrath, S.P., Chang, A.C., Page, A.L., Witter, E., 1994. Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. Environ. Rev. 2, 108–118. https://doi.org/10.1139/a94-006.

Muhonja, C.N., Makonde, H., Magoma, G., Imbuga, M., 2018. Biodegradability of polyethylene by bacteria and fungi from Dandora dumpsite Nairobi-Kenya. PLoS One 13, e0198446. https://doi.org/10.1371/journal.pone.0198446.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50, 5800–5808. https://doi.org/10.1021/acs.est.5b05416.

Naderi Beni, N., Karimifard, S., Gilley, J., Messer, T., Schmidt, A., Bartelt-Hunt, S., 2023. Higher concentrations of microplastics in runoff from biosolid-amended croplands than manure-amended croplands. Communications Earth & Environment 4, 42. https://doi.org/10.1038/s43247-023-00691-y.

O'Connor, D., Pan, S., Shen, Z., Song, Y., Jin, Y., Wu, W.-M., Hou, D., 2019. Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. Environmental Pollution 249, 527–534. https://doi.org/10.1016/j.envpol.2019.03.092.

Pan, J., Zhang, S., Qiu, X., Ding, L., Liang, X., Guo, X., 2024. Molecular weights of dissolved organic matter significantly affect photoaging of microplastics. Environmental Science & Technology 58, 13973–13985. https://doi.org/10.1021/acs.est.4c04608.

Patel, Y., Chhaya, U., Rudakiya, D.M., Joshi, S., 2021. Biological decolorization and degradation of synthetic dyes: a green step toward sustainable environment. In: Panpatte, D.G., Jhala, Y.K. (Eds.), Microbial Rejuvenation of Polluted Environment, Microorganisms for Sustainability, vol. 26. Springer, Singapore. https://doi.org/10.1007/978-981-15-7455-9_4.

Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C., Laforsch, C., 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. Sci. Rep. 8, 17950. https://doi.org/10.1038/s41598-018-36172-y.

Ramage, S.J.F.F., Pagaling, E., Haghi, R.K., Dawson, L.A., Yates, K., Prabhu, R., Hillier, S., Devalla, S., 2022. Rapid extraction of high- and low-density microplastics from soil using high-gradient magnetic separation. Sci. Total Environ. 831, 154912. https://doi.org/10.1016/j.scitotenv.2022.154912.

Rehman, K., Shahzad, T., Shr, A., Hussain, S., Mahmood, F., Siddique, M.H., Siddique, M. A., Rashid, M.I., 2018. Effect of Reactive Black 5 azo dye on soil processes related to C and N cycling. PeerJ Microbiology 6, e4802. https://doi.org/10.7717/peerj.4802.

Rillig, M., Ingraffia, R., de Souza Machado, A.A., 2017a. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 8, 1805. https://doi.org/10.3389/fpls.2017.01805.

Rillig, M.C., Ziersch, L., Hempel, S., 2017b. Microplastic transport in soil by earthworms. Sci. Rep. 7, 1362. https://doi.org/10.1038/s41598-017-01594-7.

Rochman, C.M., et al., 2019. Rethinking microplastics as a diverse contaminant suite. Environmental Toxicity and Chemistry 38, 703–711. https://doi.org/10.1002/etc.4371.

Sajjad, M., Huang, Q., Khan, S., Khan, M.A., Liu, Y., Wang, J., Lian, F., Wang, Q., Guo, G., 2022. Microplastics in the soil environment: a critical review. Environmental Technology & Innovation. 27, 102408. https://doi.org/10.1016/j.eti.2022.102408.

Schell, T., Hurley, R., Buenaventura, N.T., Mauri, P.V., Nizzetto, L., Rico, A., Vighi, M., 2022. Fate of microplastics in agricultural soils amended with sewage sludge: is surface water runoff a relevant environmental pathway? Environmental Pollution 293, 118520. https://doi.org/10.1016/j.envpol.2021.118520.

Schotman, T.G., van der Weerd, J., 2015. On the recovery of fibres by tape lifts, tape scanning, and manual isolation. Sci. Justice 55, 415–421. https://doi.org/10.1016/j.scijus.2015.05.007.

Siwek, M., Edgecock, T., Chmielewski, A.G., Rafalski, A., Walo, M., Sudlitz, M., Lin, L., Sun, Y., 2023. The potential of electron beams for the removal of microplastics from wastewater and sewage sludge. Environmental Challenges 13, 100760. https://doi.org/10.1016/j.envc.2023.100760.

Surendran, U., Jayakumar, M., Raja, R., Gopinath, G., Chellam, R.V., 2023. Microplastics in terrestrial ecosystem: sources and migration in soil environment. Chemosphere 318, 137946. https://doi.org/10.1016/j.chemosphere.2023.137946.

Sutkar, P.R., Gadewar, R.D., Dhulap, V.P., 2023. Recent trends in degradation of microplastics in the environment: a state-of-the-art review. Journal of Hazardous Materials Advances 11, 100343. https://doi.org/10.1016/j.hazadv.2023.100343.

Teampanpong, J., Duengkae, P., 2024. Terrestrial wildlife as indicators of microplastic pollution in western Thailand. PeerJ 12, e17384. https://doi.org/10.7717/peerj.17384.

van den Berg, P., Huerta-Lwanga, E., Corradini, F., Geissen, V., 2020. Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. Environmental Pollution 261, 114198. https://doi.org/10.1016/j.envpol.2020.114198.

Wang, Q., Adams, C.A., Wang, F., Sun, Y., Zhang, S., 2022. Interactions between microplastics and soil fauna: a critical review. Crit. Rev. Environ. Sci. Technol. 52, 3211–3243. https://doi.org/10.1080/10643389.2021.1915035.

Welch, B.L., 1938. The significance of the difference between two means when the population variances are unequal. Biometrika 29, 350–362. https://doi.org/10.1093/biomet/29.3-4.350.

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Z., He, Y., Wang, H., Lu, Z., Brookes, P.C., Tang, C., Gan, J., Xu, J., 2020. Microplastics in the soil environment: occurrence, risks, interactions and fate – a review. Crit. Rev. Environ. Sci. Technol. 50, 2175–2222. https://doi.org/10.1080/10643389.2019.1694822.

Yang, J., Zhang, S., Kang, S., Wang, Z., Wu, C., 2021a. Microplastics in soil: a review on methods, occurrence, sources, and potential risk. Sci. Total Environ. 780, 146546. https://doi.org/10.1016/j.scitotenv.2021.146546.

Yang, J., Li, L., Li, R., Xu, L., Shen, Y., Li, S., Tu, C., Wu, L., Christie, P., Luo, Y., 2021b. Microplastics in an agricultural soil following repeated application of three types of sewage sludge: a field study. Environmental Pollution 289, 117943. https://doi.org/10.1016/j.envpol.2021.117943.

Yang, X., Zhang, Z., Guo, X., 2023. Impact of soil structure and texture on occurrence of microplastics in agricultural soils of karst areas. Sci. Total Environ. 902, 166189. https://doi.org/10.1016/j.scitotenv.2023.166189.

Yu, Y., Zhang, Z., Zhang, Y., Jia, H., Li, Y., Yao, H., 2023. Abundances of agricultural microplastics and their contribution to the soil organic carbon pool in plastic film mulching fields of Xinjiang, China. Chemosphere 316, 137837. https://doi.org/10.1016/j.chemosphere.2023.137837.

Zeng, T., Wang, L., Zhang, X., Song, X., Li, J., Yang, J., Chen, S., Zhang, J., 2022. Characterization of microbial communities in wastewater treatment plants containing heavy metals located in chemical industrial zones. Int. J. Environ. Res. Publ. Health 27, 6529. https://doi.org/10.3390/ijerph19116529.

Zhang, S., Wang, J., Liu, X., Qu, F., Wang, X., Wang, X., Li, Y., Sun, Y., 2019. Microplastics in the environment: a review of analytical methods, distribution, and biological effects. TrAC, Trends Anal. Chem. 111, 62–72. https://doi.org/10.1016/j.trac.2018.12.002.

Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K.H., Wu, C., Lam, P.K.S., 2021. Understanding plastic degradation and microplastic formation in the environment: a review. Environmental Pollution 274, 116554. https://doi.org/10.1016/j.envpol.2021.116554.

Zhang, Y., Wang, K., Chen, W., Ba, Y., Khan, K., Chen, W., Tu, C., Chen, C., Xu, L., 2022. Effects of land use and landscape on the occurrence and distribution of microplastics in soil, China. Sci. Total Environ. 847, 157598. https://doi.org/10.1016/j.scitotenv.2022.157598.

Zhang, B., Zhou, X., Ren, X., Hu, X., Ji, B., 2023. Recent research on municipal sludge as soil fertilizer in China: a review. Water, Air, Soil Pollut. 8, 119. https://doi.org/10.1007/s11270-023-06142-w.

Zhao, Z.-Y., Wang, P.-Y., Wang, Y.-B., Zhou, R., Koshei, K., Munyasya, A.N., Liu, S.-T., Wang, W., Su, Y.-Z., Xiong, Y.-C., 2021. Fate of plastic film residues in agro-ecosystem and its effects on aggregate-associated soil carbon and nitrogen stocks. J. Hazard Mater. 416, 125954. https://doi.org/10.1016/j.jhazmat.2021.125954.

**Supplemental Information for "Microplastics in agricultural soils following sewage sludge applications: evidence from a 25-year study"**

## Materials and Methods

### Experimental set-up and original sample collection

In the long-term sewage sludge experiment, three replicate blocks of different plots were set up, and treatments assigned to those plots were randomised. This study focussed on plots within a single block (Figure S1).

The sludge cakes were evenly spread over the surface of the plot manually then incorporated into the soil using a spading machine to a depth of 20-25 cm in the following order using the spading machine: negative control → sludge D → sludge E → sludge A → sludge B → sludge C. Mixing of plots of the same treatment were done across all three blocks before the blades of the spading machine and hand tools were cleaned ahead of mixing of the next treatment by running them several times through 'clean' soil at the edge of the field away from the plots to prevent cross-contamination between the treatments. Plots treated with sludges A and C were supplemented with sludge D, while plots treated with sludge B were supplemented with sludge E (see Gibbs et al., 2006).

Soil collected from plots treated with sludge D (plots 2 and 4) were combined at time of sampling to make a composite soil sample. Similarly, the negative control plots (plots 7 and 8) were combined to make a composite sample.

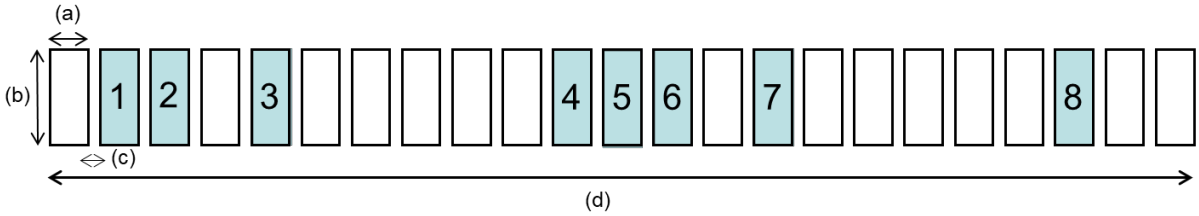



**Figure S1:** Layout and dimension of plots. Highlighted plots indicate those selected for microplastic analysis. Plot 1 – sludge E; Plots 2 and 4 – sludge D; Plot 3 – sludge C; Plot 5 – sludge B; Plot 6 – sludge A; Plots 7 and 8 – Negative Control. Unlabelled plots are other plots in the original experimental set-up that were not used in this study – data not shown. Dimensions of plots are: (a) 4.5 m, (b) 8 m, (c) 2-3 m, and (d) 164.4-204 m.

**Improved grassland management regime**

Ryegrass (*Lolium* spp.) seedlings were sown annually in all the treatment and control plots between July-August, and lime and inorganic fertiliser pellets were applied after sowing to maintain a constant pH of around 5.8 (the recommended pH level for Scottish grassland) to encourage plant growth. The pellets were applied to the plots by hand broadcasting. The plots were subjected to grassland cropping and cut regularly to deplete mineral nitrogen supply to the soil. The soil was tilled before every yearly cycle (June-July).

**Handling of microplastics**

To store microplastics recovered from soil and sludge samples for further analysis, sterile tweezers were used to remove them from the filter papers and attach them to the adhesive side of transparent, colourless tape. The length of exposed tape on the tape dispenser was discarded before each use to minimise potential contamination from any microplastics present. The positions of the microplastics on the tape were immediately marked on the reverse side (the non-adhesive side) with a permanent marker pen to differentiate between microplastics from the laboratory environment that may have been accidentally trapped on the tape. After the filter paper had been completely searched, the tape was secured to an acetate sheet (backing material) so that the collected microplastics were trapped between the tape and the acetate. The collected microplastics were further circled so they could be located more easily, and the acetates stored

prior to FTIR analysis. This is a common method of storing fibres in forensic science (Schotman and van der Weerd, 2015).

Microplastics were later removed from the tape by incising the tape in an L-shape using a scalpel dipped in xylene to dissolve the adhesive, making the tape easily retractable from the acetate using tweezers. While still held in the tweezers, the microplastic was shaken in ultrapure water briefly then air dried before placing on a carbon adhesive pad mounted on a glass slide. To ensure that the use of xylene did not chemically alter the microplastics and affect their spectra, FTIR measurements of a test microplastic (pristine polypropylene mulch film) were taken before exposure to xylene, and 30 s, 1 min, and 2 min after continuous exposure.

**Categorisation of microplastics**

Microplastics were categorised based on morphology (Rochman et al., 2019). Fibres were defined as flexible textile fibres of equal thickness throughout its length, while fibre bundles were defined as tightly wound masses of entangled fibres, which could not be separated. Films were defined as flat, thin fragments of plastic that were malleable, whereas flakes were defined as flat, thin fragments that were not malleable. Particles were defined as spherical in shape with a smooth surface, but also included hemispheres (i.e., fragmented spheres). Finally, fragments were defined as rigid microplastics, which had irregular or angular shapes.

**Spectroscopic analysis of microplastics using FTIR microscopy**

Some of the interpretation of the IR spectra was achieved using searchable in-house and commercial libraries of reference IR spectra. However, due to the level of weathering present in the spectra (i.e., additional bands), library matches often could not always be made, so the majority of spectra were manually interpreted and comparisons with published spectra of weathered microplastics were made to confirm (Fernández-González et al., 2021; Phan et al., 2022; Zvekic et al., 2022).

The carbonyl index (CI) of polyethylene films recovered from soil samples in 1997, 2001, 2005, 2009, and 2019, was calculated using the following equation from Syranidou et al. (2023):

$$CI = \frac{I_{1725}}{I_{1472}}$$

$I_{1725}$ refers to the area of the carbonyl band present at ~1725 $cm^{-1}$ and $I_{1472}$ refers to the area of the methylene band present at ~1472 $cm^{-1}$. The band areas were generated using the peak area function tool in the OMNIC software.

## Results and Discussion

### Effect of xylene on FTIR

FTIR spectra was obtained from polypropylene film at 0 s, 30 s, 1 min, and 2 min of continuous exposure to xylene (Figure S4). No changed occurred in the spectrum after 30 s, however, the band at ~1600 $cm^{-1}$ showed an increase after 1 min of exposure, and a further increase after 2 min. The hydroxyl (OH) band between 3500-3000 $cm^{-1}$ did not show any alteration until 2 min. The microplastics in this study were only exposed to xylene for a maximum of 10 s as part of the tape removal process before rinsing with ultrapure water and air drying, therefore, we are confident that the use of xylene to dissolve the tape adhesive did not affect the subsequent FTIR spectra generated. Furthermore, the long-established use of this process in forensic science (Schotman and van der Weerd, 2015) also provides further confidence that this is an acceptable method to use.

## Supplemental Tables and Figures

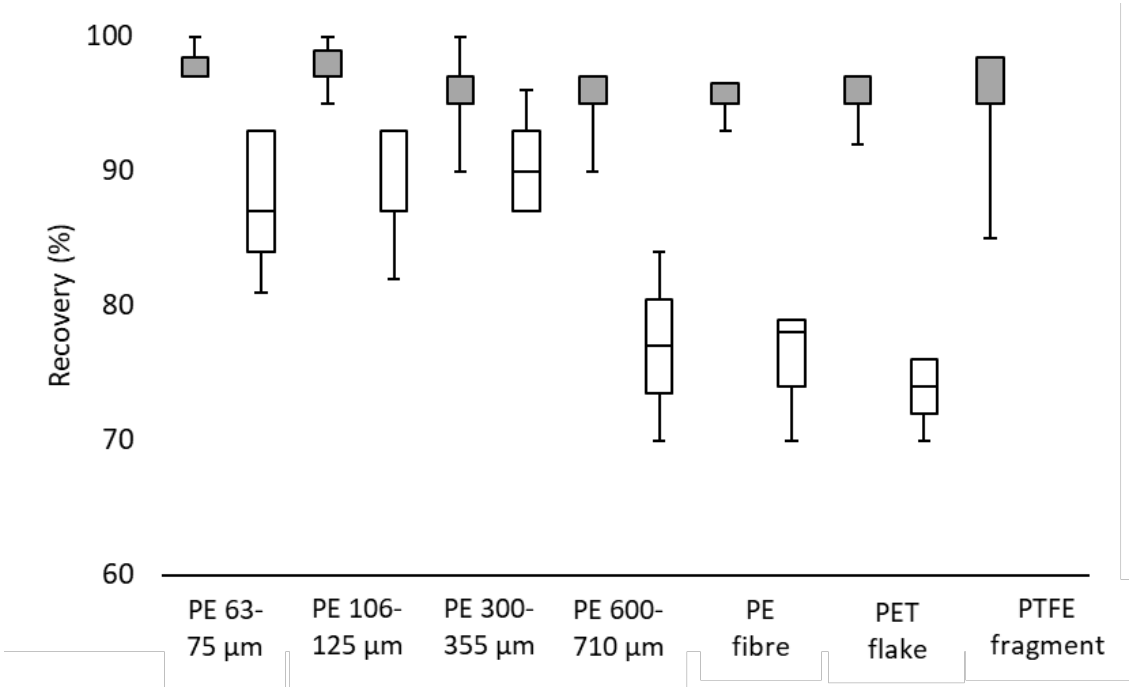



**Figure S2:** Box plots comparing microplastic recovery using HGMS (grey boxes) and density separation (white boxes) in dried sewage sludge (n = 7). N.B.: PTFE fragment was not recovered using density separation.

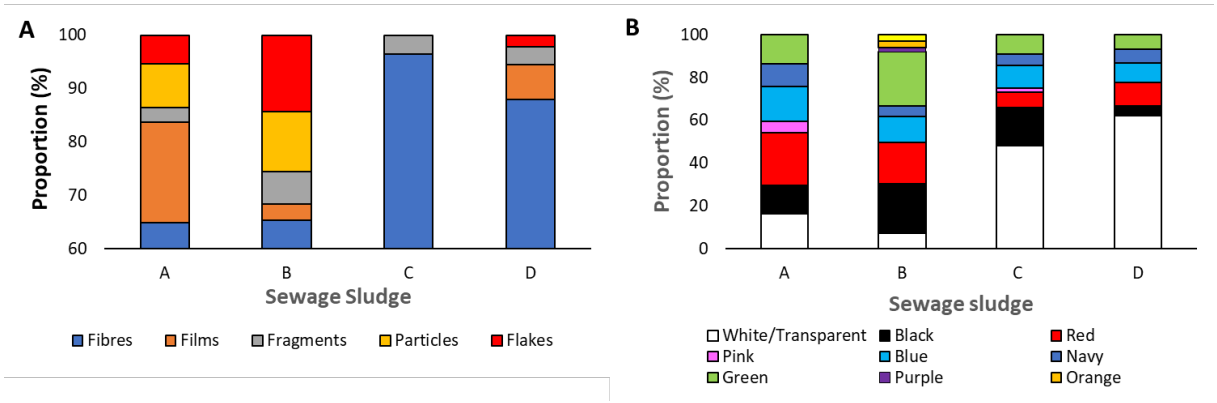
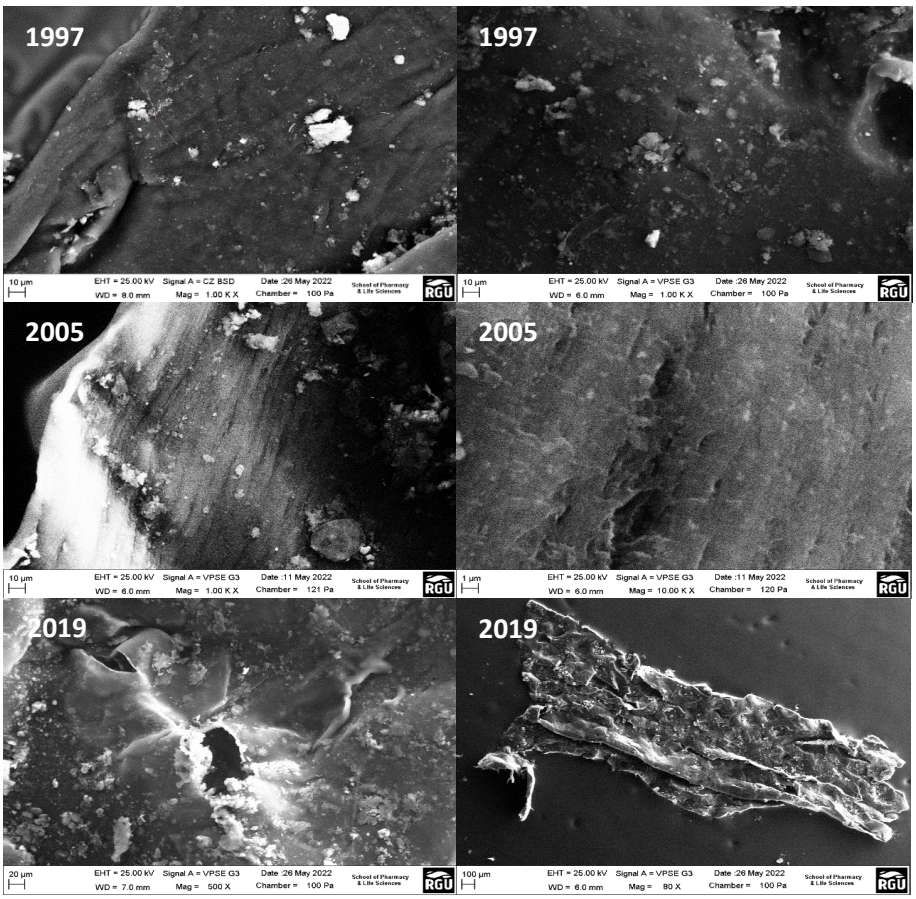


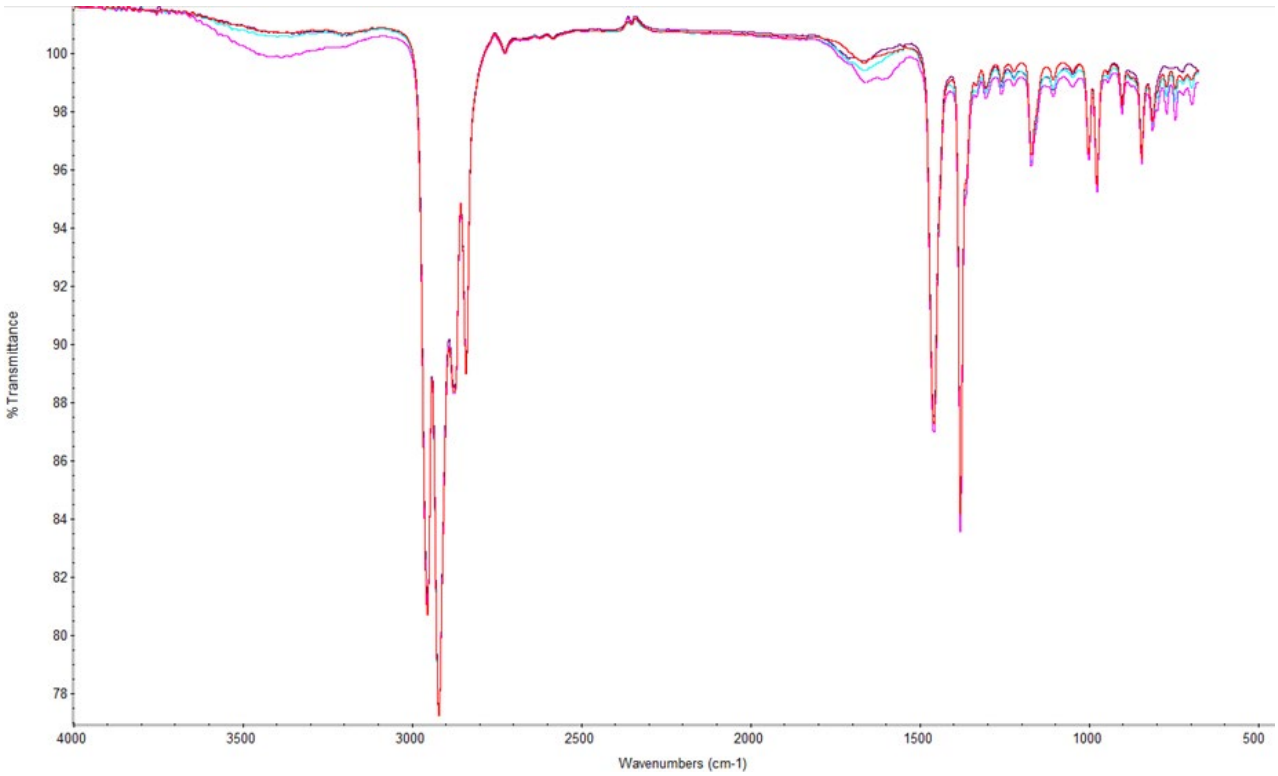

**Figure S3:** Proportions of (A) microplastic morphologies and (B) fibre colours recovered from the sewage sludges. Sludge E was not available for analysis.




101

102 **Figure S4:** Further SEM micrographs displaying progressive weathered features similar to those described in
103 the main paper. The panels are labelled with the year that the soil was sampled. All films were identified as
104 polyethylene through FTIR analysis.

105

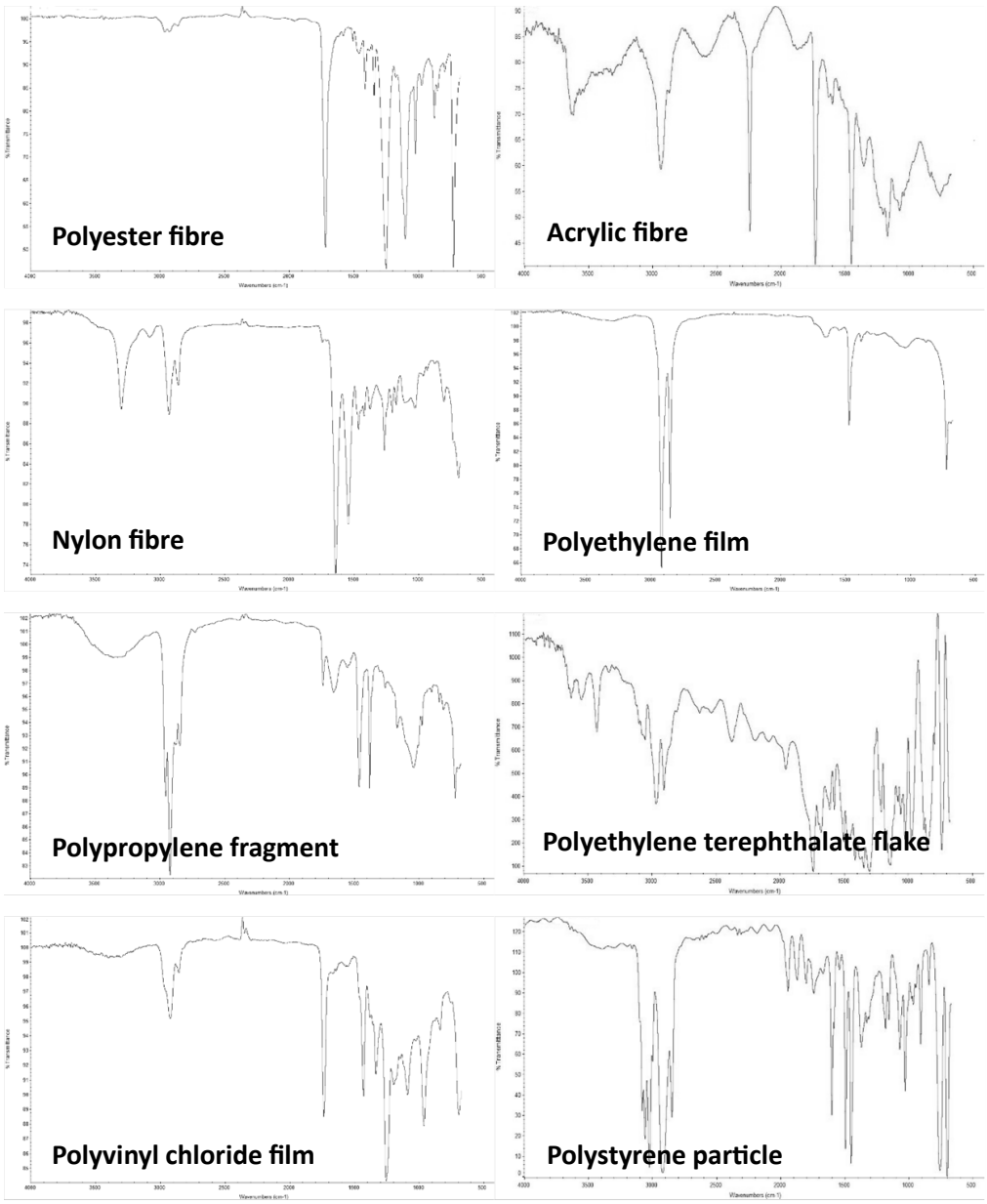



106

107 **Figure S5:** Composite FTIR spectrum of polypropylene exposed to xylene for 0 s (dark blue), 30 s (red), 1 min

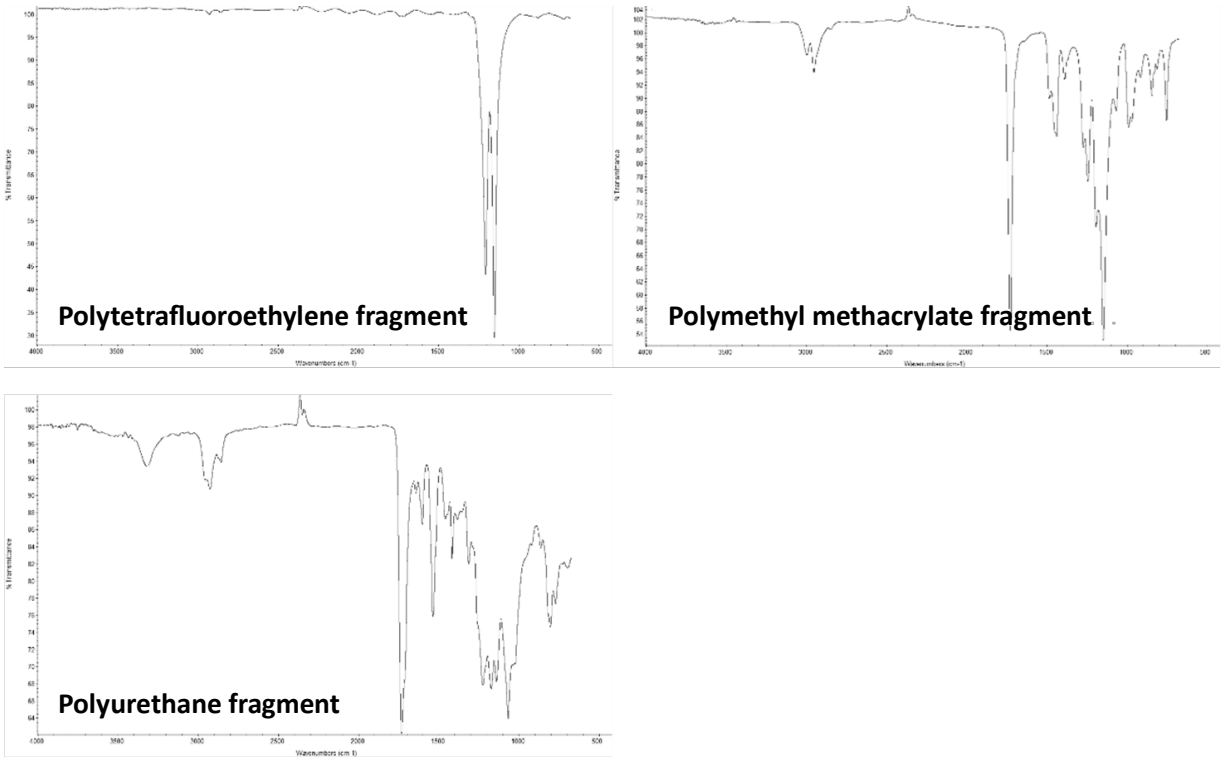
108 (light blue), and 2 min (pink).

109




110

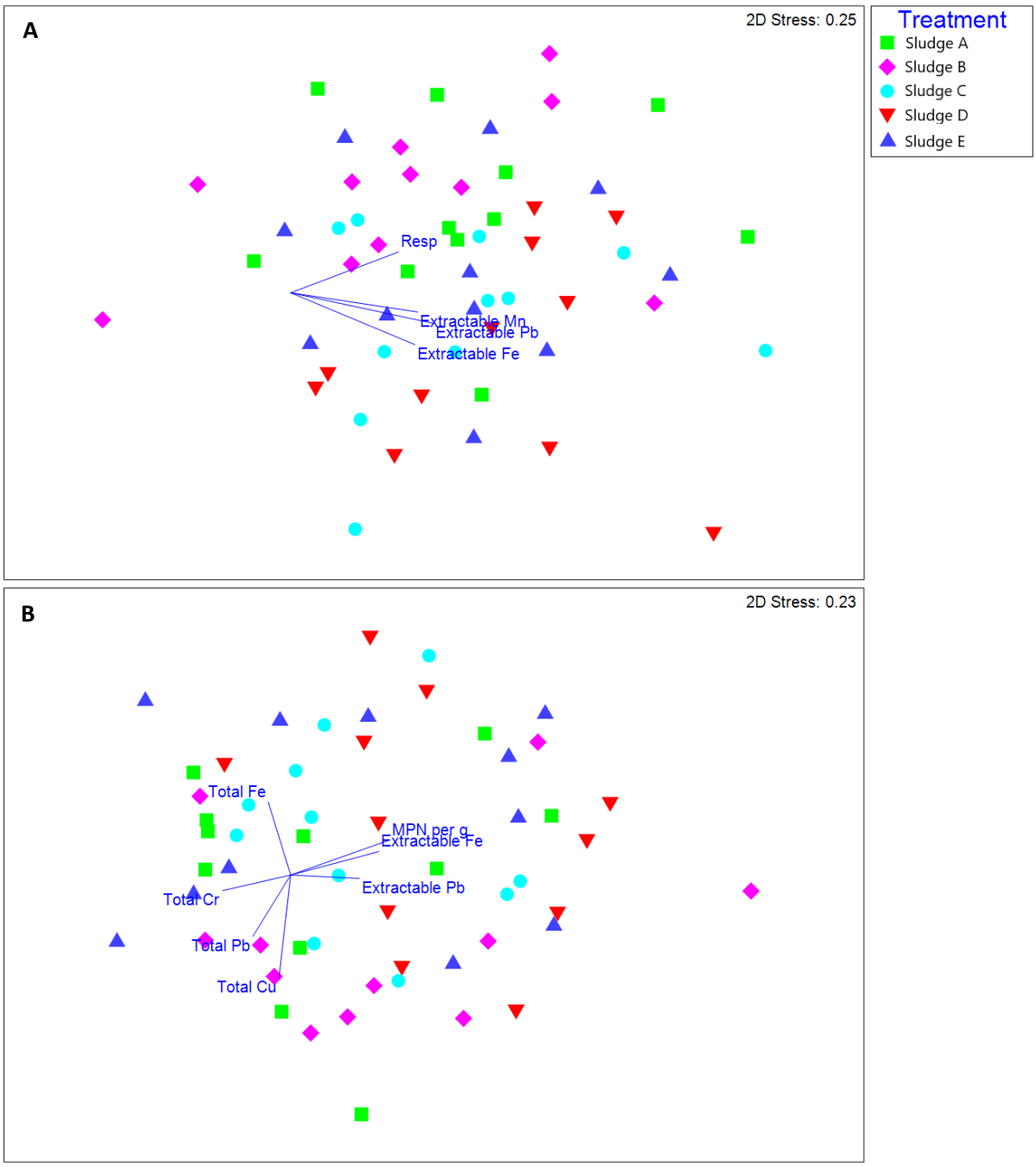
111 **Figure S6:** FTIR spectra of individual microplastics recovered from soil samples.




112

113 **Figure S6 continued:** FTIR spectra of individual microplastics recovered from soil samples.

114




115

116 **Figure S7:** Non-metric multidimensional scaling (nMDS) plot of the microplastic communities within the post-
117 sewage sludge treatment (1997-2019) soil samples. The communities are characterised either by colour (A)
118 or morphology (B). Pearson's correlations with explanatory variables (extractable Fe and Pb, biomass C, and
119 %N) are overlaid (where $r > 0.3$).

120 **Table S1.** Numbers and dimensions of different microplastic morphologies detected in the sewage sludge and the receiving soil plots. The average value across the

121 replicates is shown, while the range of the values are given in brackets, those without did not have a range (i.e., were the same size). *N.D.* = Not detected.

| Sample | | | No. of microplastics $kg^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. $kg^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| A | Sludge | 14510 (12941-16471) | 9412 (8235-11765) | *N.D.* | 2745 (2353-3529) | 392 (0-1177) | 1177 (0-2353) | 784 (0-2353) | 3861 (850-7520) | - | 1506 (995-2100) | 733 (520-840) | 211 (80-260) | 341 (195-460) |
| | Soil (1997) | 11500 (9000-15500) | 9833 (7500-13500) | *N.D.* | *N.D.* | 1167 (750-1750) | 333 (250-500) | 167 (0-250) | 4517 (600-9000) | - | - | 654 (490-780) | 220 (60-295) | 341 (195-460) |
| | Soil (1999) | 8250 (6500-10500) | 7917 (6500-9750) | 167 (0-250) | *N.D.* | 167 (0-500) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2001) | 8083 (6500-9750) | 7833 (5750-9750) | *N.D.* | *N.D.* | 250 (0-750) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2003) | 10167 (8250-11500) | 9000 (7500-10250) | 250 (0-500) | *N.D.* | 417 (250-500) | 333 (250-500) | 167 (0-250) | - | - | - | - | - | - |
| | Soil (2005) | 8417 (7250-10500) | 8000 (7250-9750) | *N.D.* | 83 (0-250) | 250 (0-500) | *N.D.* | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2007) | 10250 (8000-11500) | 9750 (7500-11250) | 167 (100-250) | 83 (0-250) | *N.D.* | 83 (0-250) | 167 (0-250) | - | - | - | - | - | - |

| Sample | | | No. of microplastics $kg^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. $kg^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| A (*continued*) | Soil (2009) | 8250 (7750-8500) | 7667 (7250-8000) | 83 (0-250) | 83 (0-250) | 83 (0-250) | 250 | 83 (0-250) | 1828 (150-3760) | - | 1502 (990-2000) | 413 (300-525) | 200 (100-270) | 358 (160-475) |
| | Soil (2011) | 9000 (7000-10500) | 8083 (6250-9750) | 250 (0-500) | 167 (0-250) | *N.D.* | 83 (0-250) | 417 (0-1000) | - | - | - | - | - | - |
| | Soil (2013) | 8250 (7500-9250) | 7167 (6750-7500) | 83 (0-250) | 167 (0-500) | *N.D.* | 250 | 583 (250-1000) | - | - | - | - | - | - |
| | Soil (2015) | 9583 (8500-11250) | 8583 (8750-9250) | 83 (0-250) | 667 (250-1500) | *N.D.* | 83 (0-250) | 167 (0-250) | - | - | - | - | - | - |
| | Soil (2019) | 10417 (9250-11250) | 9750 (8250-10500) | *N.D.* | 83 (0-250) | 167 (0-250) | 83 (0-250) | 333 (0-750) | 1575 (850-3400) | - | 1502 (995-2010) | 264 (150-360) | 202 (105-275) | 342 (170-445) |
| B | Sludge | 14035 (12782-15308) | 27350 (24359-30769) | *N.D.* | 1282 | 2564 (1282-3864) | 4701 (3846-6410) | 5983 (5128-6410) | 3121 (250-7100) | - | 2001 (1950-3700) | 727 (500-890) | 206 (60-270) | 378 (20-495) |
| | Soil (1997) | 10167 (8500-12750) | 9417 (7500-12500) | *N.D.* | 83 (0-250) | 250 (0-500) | *N.D.* | 417 (0-1250) | 3277 (450-8600) | - | 2201 (1800-3560) | 725 (525-940) | - | 350 (210-500) |
| | Soil (1999) | 7583 (6750-8000) | 6920 (6500-7500) | *N.D.* | *N.D.* | 417 (0-1000) | *N.D.* | 250 (0-750) | - | - | - | - | - | - |

| Sample | | | No. of microplastics kg$^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. kg$^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| B (*continued*) | Soil (2001) | 7833 (7250-8250) | 7167 (6750-7500) | 83 (0-250) | *N.D.* | 583 (250-1000) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2003) | 7333 (6750-7750) | 6167 (5750-6500) | *N.D.* | 83 (0-250) | 500 (250-750) | 333 (0-750) | 250 (0-500) | - | - | - | - | - | - |
| | Soil (2005) | 6333 (5000-7000) | 4750 (3250-6000) | *N.D.* | 83 (0-250) | 1333 (500-1750) | 83 (0-250) | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2007) | 7333 (5250-8750) | 6750 (4750-7750) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 583 (250-1000) | - | - | - | - | - | - |
| | Soil (2009) | 8333 (7000-9000) | 7167 (5500-8750) | *N.D.* | 83 (0-250) | 83 (0-250) | 417 (0-750) | 583 (250-1000) | 2030 (400-6300) | - | 2000 (1800-3500) | 490 (380-530) | 200 (100-230) | 358 (205-500) |
| | Soil (2011) | 7417 (6750-7750) | 6500 | *N.D.* | 333 (0-750) | *N.D.* | 333 (0-500) | 250 (0-250) | - | - | - | - | - | - |
| | Soil (2013) | 8916.67 (8000.00-10000.00) | 7750 (6500-7750) | *N.D.* | *N.D.* | 83 (0-250) | 333 (0-750) | 750 (500-1000) | - | - | - | - | - | - |
| | Soil (2015) | 8667 (7500-9500) | 7583 (7000-8000) | 83 (0-250) | 583 (500-750) | *N.D.* | 83 (0-250) | 333 (0-750) | - | - | - | - | - | - |

| Sample | | | No. of microplastics kg$^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. kg$^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| B (*continued*) | Soil (2019) | 6500 (5250-7500) | 5500 (4750-6250) | 83 (0-250) | 83 (0-250) | 167 (0-250) | 417 (250-500) | 250 (0-500) | 1315 (350-3100) | - | 1943 (1750-3300) | 242 (200-290) | 202 (150-220) | 342 (200-490) |
| C | Sludge | 41880 (39744-44872) | 13534 (12030-15037) | *N.D.* | *N.D.* | 501 (0-752) | *N.D.* | *N.D.* | 3716 (650-14000) | - | - | 900 (600-1000) | - | - |
| | Soil (1997) | 7583 (7250-8250) | 6667 (5750-7750) | *N.D.* | 83 (0-250) | 83 (0-250) | 167 (0-250) | 583 (250-1000) | 2219 (275-7300) | - | 1984 (1000-2850) | 850 (645-960) | 206 (90-375) | 345 (210-495) |
| | Soil (1999) | 7250 (7000-7500) | 6920 (5750-7750) | *N.D.* | *N.D.* | 250 (0-250) | *N.D.* | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2001) | 6917 (6500-7500) | 6833 (6500-7250) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2003) | 7083 (6250-8000) | 6833 (6000-7750) | *N.D.* | 250 | *N.D.* | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2005) | 9000 (8500-9750) | 8000 (7500-9000) | 167 (0-250) | 417 (250-500) | 83 (0-250) | 167 (0-500) | 167 (0-500) | - | - | - | - | - | - |
| | Soil (2007) | 8000 (7000-9250) | 6920 (6750-7000) | 83 (0-250) | 417 (250-7500) | 333 (0-1000) | 83 (0-250) | 167 (0-250) | - | - | - | - | - | - |

| Sample | | | No. of microplastics kg$^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. kg$^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| C (*continued*) | Soil (2009) | 8500 (7750-9500) | 7833 (7000-8750) | 83 (0-250) | 250 (0-250) | 83 (0-250) | 167 (0-250) | 83 (0-250) | 2027 (450-8000) | - | 1898 (1100-2750) | 566 (450-600) | 200 (60-300) | 358 (200-450) |
| | Soil (2011) | 7417 (7000-7750) | 7000 (6250-7500) | 167 (0-250) | 83 (0-250) | *N.D.* | 83 (0-250) | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2013) | 6833 (6000-7500) | 6167 (5250-9250) | 167 (0-250) | *N.D.* | 83 (0-250) | 83 (0-250) | 333 (0-500) | - | - | - | - | - | - |
| | Soil (2015) | 8917 (8000-10000) | 8250 (7500-9250) | 167 (0-250) | 83 (0-250) | *N.D.* | 83 (0-250) | 333 (0-750) | - | - | - | - | - | - |
| | Soil (2019) | 7667 (7000-8500) | 6750 (6000-7250) | *N.D.* | 83 (0-250) | 250 (0-500) | 167 (0-250) | 417 (250-750) | 1370 (450-3000) | - | 1876 (1000-2700) | 268 (150-300) | 202 (80-250) | 342 (210-460) |
| D | Sludge | 35271 (30232-39535) | 30620 (24419-32558) | 129 (0-388) | 2326 | 1163 (0-2326) | *N.D.* | 776 (0-1163) | 3803 (450-7100) | - | 2380 (1750-2975) | 862 (630-970) | - | 421 (240-465) |
| | Soil (1997) | 8417 (7500-9000) | 7583 (7000-8250) | *N.D.* | *N.D.* | 333 (0-1000) | 83 (0-250) | 417 (0-750) | 4087 (1100-8360) | - | - | 792 (590-850) | 201 (105-310) | 369 (240-450) |
| | Soil (1999) | 6667 (6000-7250) | 6333 (6000-7000) | *N.D.* | *N.D.* | 333 (0-1000) | *N.D.* | *N.D.* | - | - | - | - | - | - |

| Sample | | | No. of microplastics kg$^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. kg$^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| D (*continued*) | Soil (2001) | 6083 (6000-6250) | 6083 (6000-6250) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2003) | 6500 (6000-7000) | 6167 (5750-7000) | 83 (0-250) | 250 (0-500) | *N.D.* | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2005) | 7417 (6500-8250) | 6917 (5750-6750) | 333 (0-1000) | 833 (0-250) | 83 (0-250) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2007) | 6167 (5750-6750) | 6000 (5750-6250) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 83 (0.00-250) | - | - | - | - | - | - |
| | Soil (2009) | 7833 (7000-8750) | 7083 (6500-7750) | *N.D.* | 83 (0-250) | 83 (0-250) | 83 (0-250) | 250 | 2027 (760-5450) | - | 1983 (1500-2250) | 451 (400-590) | 200 (125-290) | 358 (100-550) |
| | Soil (2011) | 6917 (5750-7500) | 6000 (4500-7000) | 83 (0-250) | 333 (0-500) | *N.D.* | 167 ( 0-250) | 167 (0-250) | - | - | - | - | - | - |
| | Soil (2013) | 6333 (6000-6750) | 5667 (5500-5750) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 333 (250-500) | - | - | - | - | - | - |
| | Soil (2015) | 8500 (8000-9000) | 7750 (7500-8000) | 83 (0-250) | 167 (0-500) | 333 (250-500) | 167 (0-500) | *N.D.* | - | - | - | - | - | - |

| Sample | | | No. of microplastics kg$^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. kg$^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| D (*continued*) | Soil (2019) | 8000 (7250-8750) | 7500 (7000-7750) | 83 (0-250) | 83 | 83 (0-250) | 83 (0-250) | 83 (0-250) | 1643 (350-4700) | - | 1724 (1300-1950) | 270 (200-360) | 202 (110-300) | 342 (125-475) |
| E | Sludge | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Soil (1997) | 9667 (8750-10750) | 8583 (8000-9000.) | 167 (0-250) | 250 (0-500) | 83 | 250 (0-500) | 333 (0-500) | 2749 (70-8600) | - | 2102 (1750-3950) | 735 (475-925) | 201 (70-215) | 367 (210-480) |
| | Soil (1999) | 7750 (6750-9250) | 6916 (6250-8250) | 250 (0-500) | *N.D.* | 583 (0-1000) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2001) | 7083 (6250-7500) | 6833 (6000-7250) | 83 (0-250) | *N.D.* | 167 (0-250) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2003) | 5917 (5250-7000) | 5583 (4500-6750) | *N.D.* | *N.D.* | 250 (0-500) | *N.D.* | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2005) | 6417 (5500-7500) | 6333 (5250-7500) | *N.D.* | *N.D.* | 83 (0-250) | 83 (0-250) | *N.D.* | - | - | - | - | - | - |
| | Soil (2007) | 6583 (5250-8500) | 5750 (4500-7250) | *N.D.* | 250 (0-500) | 333 (250-500) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2009) | 8250 (7250-9000) | 7333 (6000-8250) | 83 (0-250) | 333 (0-750) | 333 (250-500) | 83 (0-250) | 83 (0-250) | 1527 (350-5600) | - | 2005 (1600-3425) | 436 (350-470) | 200 (95-230) | 358 (200-425) |

| Sample | | | No. of microplastics kg$^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. kg$^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| E (*continued*) | Soil (2011) | 6500 (5250-8000) | 5250 (4500-6750) | 167 (0-500) | 583 (500-750) | *N.D.* | *N.D.* | 500 (250-1000) | - | - | - | - | - | - |
| | Soil (2013) | 8667 (7500-10000) | 8167 (7500-9750) | 333 (250-500) | *N.D.* | *N.D.* | 83.33 (0-250) | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2015) | 11333 (10500-12750) | 10500 (9750-11750) | 83 (0-250) | 167 (0-250) | 83 (0-250) | *N.D.* | 500 (250-750) | - | - | - | - | - | - |
| | Soil (2019) | 13333 (11750-14500) | 12167 (10250-13250) | 83 | 250 (0-500) | 83 (0-250) | 83 | 667 (500-1000) | 1104 (300-3000) | - | 1934 (1550-2950) | 254 (225-375) | 202 (70-230) | 342 (210-420) |
| Negative Control | Soil (1997) | 4000 (2500-6000) | 3417 (2000-5500) | *N.D.* | *N.D.* | 250 (0-500) | *N.D.* | 83 (0-250) | 1390 (550-2100) | - | - | 452 (350-495) | - | 250 (210-300) |
| | Soil (1999) | 2250 (1750-2750) | 1917 (1250-2250) | *N.D.* | 83 (0-250) | *N.D.* | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2001) | 3333 (3000-3500) | 3250 (2750-3500) | 83 (0-250) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2003) | 3250 (2750-3750) | 2917 (2750-3000) | *N.D.* | *N.D.* | 83 (0-250) | *N.D.* | 250 (0-500) | - | - | - | - | - | - |

| Sample | | | No. of microplastics $kg^{-1}$ | | | | | | Dimensions (µm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Type | Total no. $kg^{-1}$ | Microfibres | Fibre bundles | Microfilms | Fragments | Particles | Flakes | Microfibres | Fibre bundles | Microfilms (longest edge) | Fragments | Particles | Flakes |
| Negative Control (*continued*) | Soil (2005) | 2833 (2000-3000) | 2583 (1750-3000) | *N.D.* | 83 (0-250) | 167 (0-250) | *N.D.* | *N.D.* | - | - | - | - | - | - |
| | Soil (2007) | 3333 (3250-3500) | 3250 | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 83 (0-250) | - | - | - | - | - | - |
| | Soil (2009) | 1917 (1500-2750) | 1667 (1250-2250) | *N.D.* | *N.D.* | *N.D.* | 83 (0-250) | 167 (0-250) | 1900 (1100-3500) | - | - | 326 (270-400) | - | 237 (180-290) |
| | Soil (2011) | 1917 (1750-2250) | 1667 (1250-2000) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 250 (0-500) | - | - | - | - | - | - |
| | Soil (2013) | 2167 (1500-2750) | 1750 (1500-2000) | *N.D.* | *N.D.* | *N.D.* | *N.D.* | 417 (0-750) | - | - | - | - | - | - |
| | Soil (2015) | 1083 (750-1250) | 917 (500-1250) | *N.D.* | *N.D.* | *N.D.* | 167 (0-250) | *N.D.* | - | - | - | - | - | - |
| | Soil (2019) | 1417 (750-2250) | 1250 (750-2000) | *N.D.* | *N.D.* | 83 | *N.D.* | 83 (0-250) | 1629 (300-4100) | - | - | 230 (240-290) | - | 235 (180-285) |

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## References


Fernández-González V., Andrade-Garda J.M., López-Mahía P., Muniategui-Lorenzo S. Impact of weathering on the chemical identification of microplastics from usual packaging polymers in the marine environment. *Analytica Chimica Acta*. 2021; 1142, 179-188. DOI: https://doi.org/10.1016/j.aca.2020.11.002

Gibbs P.A., Chambers B.J., Chaudri A.M., McGrath S.P., Carlton-Smith C.H., Bacon J.R., Campbell C.D., Aitken M.N. Initial results from a long-term, multi-site field study of the effects on soil fertility and microbial activity of sludge cakes containing heavy metals. *Soil Use and Management*. 2006; 22, 11-21. DOI: https://doi.org/10.1111/j.1475-2743.2006.00003.x

Phan S., Padilla-Gamiño J.L., Luscombe C.K. The effect of weathering environments on microplastic chemical identification with Raman and IR spectroscopy: Part I. polyethylene and polypropylene. Polymer Testing. 2022; 116, 107752. DOI: https://doi.org/10.1016/j.polymertesting.2022.107752

Rochman C.M. *et al*. Rethinking microplastics as a diverse contaminant suite. *Environmental Toxicity and Chemistry*. 2019; 38, 703-711. DOI: https://doi.org/10.1002/etc.4371

Schotman T.G., van der Weerd J. On the recovery of fibres by tape lifts, tape scanning, and manual isolation. *Science & Justice*. 2015; 55, 415-421. DOI: https://doi.org/10.1016/j.scijus.2015.05.007

Syranidou E., Karkanorachaki K., Barouta D., Papadaki E., Moschovas D., Avgeropoulos A., Kalogerakis N. Relationship between the Carbonyl Index (CI) and Fragmentation of Polyolefin Plastics during Aging. *Environmental Science & Technology*. 2023; 57, 8130-8138. DOI: https://doi.org/10.1021/acs.est.3c01430

Zvekic M., Richards L.C., Tong C.C., Krogh E.T. Characterizing photochemical ageing processes of microplastic materials using multivariate analysis of infrared spectra. *Environmental Science: Processes Impact*. 2022; 24, 52-61. DOI: https://doi.org/10.1039/D1EM00392E