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Microplastics in agricultural soils: Methods, Sources and Fate

by

Freya Radford

ORCID ID https://orcid.org/0000-0002-9742-279X

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Abstract

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Microplastics are an environmental issue of global concern. Although they have been found in a range of environments worldwide, their contamination in the terrestrial environment is poorly understood, particularly in relation to their source and fate. One source of microplastics in soils of particular concern is biosolids. Biosolids are the solid by-product of the wastewater treatment and are commonly spread on agricultural land as a fertilizer, indicating a potential route for microplastics into terrestrial soils. The aim of this thesis was therefore to broaden the understanding of microplastic contamination in agricultural soils in relation to biosolid application.

The lack of suitable methods for microplastic detection and quantification is a major obstacle for determining their concentrations in soil environments. Therefore, an experiment was carried out to determine the best methods for microplastic extraction based on soil characteristics. The efficiency of organic matter removal methods was measured. Soils with a range of particle size distribution and organic matter content were spiked with a variety of microplastic types and density separation methods were tested. The optimal organic removal method was found to be hydrogen peroxide with organic removal rates up to 93%. The recovery efficiency of microplastics was variable across polymer types. Overall, canola oil was shown to be the best method for density separation, however, efficiency was dependent on the amount of organic matter in the soil. This outcome highlights the importance of including matrix-specific calibration in future studies considering a wide range of microplastic types, to avoid underestimation of microplastic contamination.

To understand the sources and fate of microplastics in agricultural soils, these tailored methods were used to extract microplastics from samples collected from agricultural soils in the River Test catchment area in the UK. Soils were collected from fields which had historical biosolid application and these were compared to a similar set of fields which had never received biosolid application during summer and winter. The mean microplastic concentration was high in both the biosolid treated fields (874 MP/kg) and the untreated fields (664 MP/kg) and a wide variety of polymers were found across sites. There was a lack of significant difference between treated and untreated soils suggesting the influence of other sources and environmental processes.

Additionally, soil samples were collected from five separate fields over the course of a year, before and after biosolid application. Microplastic contamination was ubiquitous across these fields up to a maximum concentration of 7950 MP/kg. Despite previous reports of high concentrations of microplastics in biosolids, their concentrations in soils did not significantly increase after application of biosolids. This suggests that biosolids may not be the key influencing factor in microplastic soil concentrations and transport out of soil systems is likely through horizontal (run off) and lateral (percolation) routes. Agricultural soils may thus be acting as a vector for microplastics to freshwater systems and the wider environment.

Overall, the results of this thesis suggest that biosolids, whilst are likely a contributor, are not the sole source of microplastics in agricultural soils. The importance of additional sources and pathways are explored, and the complexities of the soil environment are considered, suggesting the highly dynamic nature of soil environment may determine the variability in microplastic concentrations. The research presented here significantly increased the understanding of microplastic sources and fate in agricultural soil systems while highlighting directions for future soil microplastic research.

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List of Accompanying Materials

An additional paper to which I contributed to the manuscript writing and editing is also included as accompanying material:

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Research Thesis: Declaration of Authorship

Print name: Freya Radford

Title of thesis: Microplastics in agricultural soils: Methods, Sources and Fate

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University;
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- Where I have consulted the published work of others, this is always clearly attributed;
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- I have acknowledged all main sources of help;
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- I am the author of the work, and editing was conducted by my supervisory team of Dr Hudson, Dr Horton, Dr Shaw and Professor Williams. Parts of this work have been published as:

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Definitions and Abbreviations

.

ABS.....Acrylonitrile butadiene styrene

AC.....Acrylic

ANOVA.....Analysis of variance

APV.....Acrylates, Polyurethanes and Varnish

ASA.....Acrylonitrile styrene acrylate

ATR.....Attenuated total reflection

EVA.....Ethylene-vinyl acetate

FTIR.....Fourier transform infrared

GC-MS.....Gas chromatography mass spectrometry

GLMM.....Generalized linear mixed model

HDPE.....High-density Polyethylene

HQI.....Hit quality index

IQR.....Interquartile Range

LDPE.....Low-density polyethylene

LOD.....Limit of detection

LOI.....Loss on Ignition

LOQ.....Limit of quantification

MilliQ.....Ultrapure water, purified using an ion exchange cartridge

MP.....Microplastics

PA.....Polyamide

PAN.....Polyacrylonitrile

PBT.....Polybutylene terephthalate

PC.....Polycarbonate

PCA.....Principal component analysis

PDAP.....Polydiallylphthalate

PDMS.....Polydimethylsiloxane

PE.....Polyethylene

PET.....Polyethylene terephthalate

PLA.....Polylactic acid

PMMA.....Polymethyl methacrylate

PP.....Polypropylene

PS.....Polystyrene

PTFE.....Polytetrafluoroethylene

PUR.....Polyurethane

PVA.....Polyvinyl acetate

PVC.....Polyvinylchloride

PVCA.....Polyvinyl chloride acetate

PVDF.....Polyvinylidene Fluoride

PVS.....Polyvinyl stearate

pyr.....Pyrolysis

SBR.....Styrene-butadiene

SE ..... Standard Error

SRTM .....Shuttle Radar Topography Mission

XRF.....X-ray Fluorescence

Chapter 1 Introduction

Plastics are an integral part of modern-day life. Their intrinsic properties have led to their extensive use and development in a wide variety of sectors including construction, electronics, transport, food production, medicine, packaging, and energy (Gibb, 2019). The desirable characteristics of plastics include their high durability, lightweight build, insulating properties and low costs (van Emmerik & Schwarz, 2020). These have put it in high demand resulting in its production and use increasing exponentially since it became commercially available in the mid-20th century (Villarrubia-Gómez *et al.,* 2018). Plastic is a term which is used to cover a wide range of synthetic and semi-synthetic organic materials made up of polymers. Their high complexity and variability in physical, chemical, and structural properties make them extremely difficult to define as a group (Brydson, 1999). This inherent versatility means that they can take a multitude of forms including rigid items, flexible films, adhesives, foams, and fibres (Napper & Thompson, 2019). They may be made up of a range of resin types, some of the most common of which are polyethylene terephthalate (PET), high density polyethylene (HDPE), low density polyethylene (LDPE), polyvinyl chloride (PVC), polypropylene (PP), and polystyrene (PS) (Table 1.1).

Despite their many benefits, it is important to note the unsustainable nature of plastic materials. The carbon footprint of plastic manufacture is extremely large (Zheng & Suh, 2019). Approximately 4% of the world's oil and gas supplies are used as feedstock for plastics with a further 3–4% used to provide energy for plastic manufacture (Hopewell *et al*., 2009). Additionally, 6% of global coal electricity is used for plastic production (Cabernard *et al*., 2022). Furthermore, with increasing amounts of plastic consumption and the throw-away culture that persists in modern day life, has come an increasing amount of plastic waste. Due to the limited application of waste management strategies, a high proportion of plastic is discarded. Only 9% of all plastic ever made has been recycled, whereas 60% have been discarded either in landfills or the natural environment (Geyer *et al*., 2017). This has led to plastic contamination in the environment becoming one of the biggest environmental concerns of the 21st Century. Its lightweight and durable nature, resulting in its weather and moisture resistance, promotes its longevity and accumulation in the environment (Barnes *et al*., 2009). Long term studies have shown significant increase in plastic presence in the environment over the last 60 years (Ostle *et al*., 2019). Particularly given that some polymers may take hundreds of years to degrade in environmental conditions, the potential for harm may be long lasting (Pol & Thiyagarajan, 2010).

Once in the environment, these plastics may have extensive ecological impact (Thiel *et al*., 2018). These impacts are thought to be extremely widespread effecting a range of species in a variety of environmental compartments (Gall & Thompson, 2015). For example, large plastic items may cause entanglement or be ingested by wildlife (Avery-Gomm *et al*., 2018; Jepsen & de Bruyn, 2019). However, the potential for impact increases as plastics break down into smaller pieces, named microplastics. Microplastic is a term first used in the early 2000's to describe microscopic plastic fragments (Thompson *et al*., 2004). They are a current focus for both the scientific community and the wider public with research increasing exponentially over the decade (Oliveira and Almeida, 2019). The concern for impact comes from their greater potential for bioavailability as a result of their small size (similar to that of plankton or sediment particles) allowing them to reach a wider range of organisms in the environment (Vroom *et al*., 2017; Wright *et al*., 2013). Recently there has also been concern over the implications of their impact on human health as reports of microplastic presence in blood and lung tissue have emerged (Jenner *et al*., 2022; Leslie *et al*., 2022).

Table 1.1 Common polymer types based on the seven resin codes including brief descriptions and example uses for each resin code (Siddique, Khatib and Kaur, 2008).

Resin code	Common name	Abbreviation	Description	Uses
1	*Polyethylene terephthalate*	*PET*	*Clear and tough*	*Water bottles and textile fibres*
2	*High density polyethylene*	*HDPE*	*Very common, white or coloured*	*Cosmetic bottles, milk bottles, shopping bags*
3	*Polyvinyl chloride*	*PVC*	*Hard, ridged and clear*	*Plumbing pipes, blister packs*
4	*Low density polyethylene*	*LDPE*	*Soft and flexible*	*Rubbish bags and bins, black plastic sheeting*
5	*Polypropylene*	*PP*	*Hard and flexible*	*Crips packets, drinking straws*
6	*Polystyrene*	*PS*	*Ridged and brittle (EPS- foamed and lightweight)*	*Food containers, plastic cutlery (EPS-packaging)*
7	*Other (e.g. nylon/ acrylic)*	*O*	*e.g. fibrous and often colourful*	*e.g. Textiles and electrical equipment*

Despite the vastly expanding research area of microplastics, there is still no clear consensus on the definition of a microplastic (Frias and Nash, 2019). They are a multidimensional contaminant which can be considered in terms of their varying characteristics including shape, size, polymer type and chemical composition (Bucci & Rochman, 2022). Broadly, they can be split into two major categories of primary and secondary microplastics. Primary microplastics are those manufactured to micro size and include pellets used as plastic feedstock, microbeads in personal care products (Napper *et al*., 2015) and biobeads from wastewater treatment systems (Hann *et al*., 2018). Secondary microplastics are derived from the breakdown of larger plastic items such as litter, fibres shed from clothing in the washing process (Napper & Thompson, 2016) and fragments of rubber wear from vehicle tyres (Kole *et al*., 2017).

Further classification of microplastics is a highly debated topic due to their diversity and complexity. Oversimplification of microplastics as a single contaminant makes research challenging as they are in fact a diverse suite of contaminants (Rochman *et al*., 2019). One of the most highly debated characteristics is the size of microplastics, for which there is not yet a universally agreed and applied definition. The upper size limit has most commonly been defined as 5mm which was proposed based on the size most likely to be ingested by organisms (Arthur *et al*., 2008). There are also suggestions that size definitions should follow the International System of Units e.g., nanoplastics (1–1000 nm), microplasticss (1–1000 µm), milliplastics (1–10 mm) and centiplastics (1–10 cm) (Underwood *et al*., 2017), however, this has not been broadly adopted and therefore conflicts with the majority of utilised definitions. A recent ISO standard (ISO/TR 21960, 2020) was released that suggests the separation of microplastics and large microplastics into 1 µm -1mm (microplastics) and 1-5mm (large microplastics). This will allow for consistent comparisons between studies, whilst considering currently utilised definitions. Although, the lower limit for size classification remains complicated as it is often determined by sampling equipment (e.g. mesh aperture; Arthur *et al*., 2008) or analysis capabilities (e.g. FT-IR resolution; Frias & Nash, 2019). For the purposes of this thesis, the working size definition will be based on ISO/TR 21960 (2020) to include microplastics (1 µm -1mm) and large microplastics (1-5mm).

Characterisation of microplastics should also include a wide range of properties such as shape, colour and chemical composition to allow for harmonisation in research and mitigation measures (Hartmann *et al*., 2019). Different shapes of microplastics may have different biological implication (Gray and Weinstein, 2017; Qiao *et al*., 2019), while chemical composition provides information on intrinsic polymer characteristics which may relate to environmental persistence and toxicity (do Sul, 2021; Yuan *et al*., 2022). Currently, there is no consensus on which materials constitute a polymer, as both synthetic and semi synthetic materials may be included. Typically

synthetic polymers are made from petroleum oil whereas semi-synthetic polymers can be made of biological materials (do Sul, 2021) and can form the basis of biopolymers which may also be considered as microplastics (Hartmann *et al*., 2019; McGoran *et al*., 2021).

Despite discrepancy between microplastic studies, one thing that is clear is that there are not yet enough data to fully assess the extent of microplastic risk in the environment (Ogonowski *et al*., 2018; Rubin *et al*., 2021). While studies are beginning to establish this, there a need for more information on their toxicity to a wider range of organisms with different modes of life (Prokić *et al*., 2021) as current reports are limited and conflicting (Adam *et al*., 2021; Jacques and Prosser, 2021; Jovanović *et al*., 2018). With increased predictions of up to 12,000 Mt of plastic waste in landfills or in the natural environment by 2050 (Geyer *et al*., 2017), it is essential that the extent of this risk is determined quickly. To do this, both the exposure and hazard of microplastics must be quantified. The focus of this thesis will therefore be on measuring the environmental concentrations of microplastics in the terrestrial environment which, at the time of this thesis commencement, has been relatively overlooked (Horton *et al*., 2017). Although the majority of plastic manufacture and consumption occurs on land (Horton and Dixon, 2018), terrestrial environments have not been the focus for microplastic research until recently. Hence, the aim here is to address this lack of data in this area and report on microplastic contamination on land, with a particular focus on the soil environment.

Chapter 2 A review of microplastics in the soil environment

The microplastic literature relating to the soil environment at the time of this thesis is rapidly expanding. Since the issue of microplastics in soils was first addressed by (Rillig, 2012) research has increased exponentially revealing microplastic contamination in range of soil environments (Yang *et al*., 2021). This Chapter will review the literature relating to sources and pathways of microplastics in soils, including current measured environmental concentrations, and the potential impacts which they pose. Additionally, it will review the current methods used for sampling, extracting, and identifying microplastics in soil matrices.

2.1 Soils

Soils are complex mixtures of inorganic materials (clay, silt, gravel, and sand), decaying organic matter, water, air and living organisms (Williams, 2001). They have a complex mix of biological and physicochemical properties including (pH, bulk density, nutrients and enzymatic activities). They provide an extremely important ecosystem and are one of the most complex and biologically diverse habitats worldwide. Soil biota represent approximately one quarter of described species (Decaëns, 2010), with reports of up to 1 billion bacteria per cm^{-3} of soil (Bardgett and Van Der Putten, 2014). Soil health is therefore extremely important for the functioning and sustainability of land-based ecosystems (Bender *et al*., 2016). The functions of soil are the basis for life on land. For this reason, soils are linked to many of the UN Sustainable Development Goals (United Nations, 2016) specifically in terms of food security, water scarcity, climate change and biodiversity loss (Keesstra *et al*., 2016).

Soils provide ecological functions such as biomass production and biological habitats, they also provide physical functions such as the provision of raw materials and a basis for technical, industrial, and socio-economic structures (Tóth *et al*., 2008). Arguably, one of the most important functioning of soil is its basis for agricultural activities. Agricultural areas represent more than a third of global land area (Piehl *et al*., 2018a) with important crops such as wheat and rice both providing 19% of dietary energy worldwide (Ray *et al*., 2013).

Despite the vital functions they provide, soils are increasingly under threat from multiple pressures. These pressures include erosion, declining organic content, compaction, sealing, salinization, and climate change (Gregory *et al*., 2015). A reduction in soil quality can have large

scale and widespread impacts on ecosystem functioning. Soil erosion has serious implications for soil nutrient cycling and microbiota (Qiu *et al*., 2021) and soil compaction can have negative impacts on crop growth and yield (Liu *et al*., 2022). Moreover, climate induced salinisation is expected to increase significantly by 2050 (Haj-Amor and Bouri, 2019) with detrimental impacts on growth rate of crops (Qados, 2011).

An additional threat to soils globally is contamination (Sun *et al*., 2018). Some of the common and most well understood soil contaminants include petroleum hydrocarbons, pesticides, polychlorinated biphenyl (PCBs), pharmaceuticals, metals and radioactive compounds (Pepper *et al*., 2011). Contamination is of particular concern when considering persistent pollutants, which have long half-lives in the soil environment (Ashraf, 2017). This results in high and consistent concentrations of persistent organic pollutants such as polychlorinated biphenyls (Cabrerizo *et al*., 2018). This can cause degradation in soil quality with knock-on effects for food supply and security (Kopittke *et al*., 2019). Moreover, remediation of soil pollutants can be costly (Khan *et al*., 2021) and difficult to tackle as contaminants are often heterogeneously distributed (Jia *et al*., 2021) making it extremely important that contamination of soils is identified and limited where possible. A relatively new soil contamination threat is microplastics (Nizzetto *et al*., 2016).

2.2 Microplastics in soils

The presence of microplastics in soils has started to be reported in recent years (Liu *et al*., 2018; Scheurer & Bigalke, 2018). However, the focus of microplastics research to date has largely been on the marine realm with relatively few studies focusing on terrestrial environments (Horton *et al*., 2017). Of late, microplastics have been reported in more remote locations stretching from the very bottom of the ocean to the top of mount Everest (Abel *et al*., 2021; Napper *et al*., 2020). Despite this, much less attention has been paid to the terrestrial environment which has more recently been suggested to be a significant sink for microplastics (Huang *et al*., 2022). It is reported that 80% of oceanic plastics are derived from land-based sources (Hann *et al*., 2018) which, given the proximity to anthropogenic activities, puts soils at particularly high risk of microplastic contamination. As a result there is a major research gap for microplastic quantification with particular focus on contributing sources, fate and transport of particles, and their environmental impacts.

2.3 Sources and pathways of microplastics in soils

Determining the sources of microplastics in the environment is particularly challenging due to their small size and fragmentation making them unrecognisable from their origin material (Ballent *et al*., 2016). There are a large variety of sources to consider as plastics are ubiquitous and come in a wide range of configurations. In soils, a range of potential sources have been initially identified. These include agricultural plastics, plasticulture, atmospheric deposition, littering, run-off and soil amendments (Bläsing and Amelung, 2018).

Plastics are used generally in agricultural practices. This may include silage films, bale twines, wraps and packaging such as agrochemical containers and fertilizer sacks (Scarascia-Mugnozza *et al*., 2011a). Plasticulture specifically refers to the use of plastic material in agricultural practices to boost yields by increasing soil temperatures, reducing water losses, preventing the rotting of seedlings and supressing weeds (Liu *et al*., 2014; Schirmel *et al*., 2018). Plastic mulching is an example of this which has been shown to increase plastic residue in soils (Zhang *et al*., 2016). Whilst farmers use plastic mulching for their short-term benefits there is a lack of available evidence for potential longer term environmental damage to inform these decisions (Steinmetz *et al*., 2016); particularly as at the end of use, these plastics are often buried in the soil and shredded when the soil is tilled (Ingman *et al*., 2015).

Littering is considered a major pathway for plastics into soil, with estimates that plastic litter inputs may be 40 times larger in soils than water environments (Kawecki and Nowack, 2019). Additionally, atmospheric fallout may contribute to soil microplastic contamination, with deposition rates up to 1.5 fibres m^{-3} in outdoor areas (MPs measured in the range of 50- 2500 µm: Dris *et al*., 2017) and even higher in urban environments with reports up to 1008 microplastics m^2 day^{-1} (MPs measured in the range of 500µm to 5mm: Dris *et al*., 2015; MPs measured in the range of 25µm -5mm: Wright *et al*., 2020). Similarly, runoff from roads may represent a diffuse input of microplastics to soils, which may include tyre wear particles (Campanale *et al*., 2022; de Jesus Piñon-Colin *et al*., 2020).

On agricultural land, soil amendments are predicted to be one of the biggest sources of microplastics. They include fertilisers used for plant nutrition such as composts, manures and biosolids. Composts, for example, were shown to contain between 12-46 MP/kg (MPs measured in the range of <1mm: Braun *et al*., 2021). Similarly, manures from pigs and chickens have been shown to contain high numbers of common polymer types (e.g., polypropylene, polyethylene and polyester) implying their contribution to microplastic inputs when applied to soils (Wu *et al*., 2021; Yang *et al*., 2021). This contamination is likely to be derived from animal consumption of

feeds containing plastic (Wu *et al*., 2021) and improper waste disposal in the composting process (Braun *et al*., 2021). Given the heterogenous nature of such materials, the amount of microplastics added through this pathway are likely to be variable. Likewise, biosolids have been reported to have highly variable microplastic concentrations; however, their concentrations are much larger, with some studies estimating millions of microplastics per kilogram, implying that they may be one of the largest sources of microplastics in soils (Cunsolo *et al*., 2021; Harley-Nyang *et al*., 2022; Horton *et al*., 2021). These are explored in more detail in the next section.

2.3.1 Biosolids

Biosolids are the treated solid by-product of the wastewater treatment process (otherwise known as sewage sludge; Collivignarelli *et al*., 2019). Since 1998, when its disposal at sea was banned in the UK, application to land is one of the main routes for use of biosolids (Shaddel *et al*., 2019). In Europe and North America half of the produced biosolids are spread on agricultural fields (Bläsing and Amelung, 2018). This process aims to recycle valuable components such as organic matter, nitrogen and phosphorous and improve soil properties such as bulk density, and water holding capacity (Singh and Agrawal, 2008). However, as well as its valuable components, biosolids contain high concentrations of potentially toxic elements (PTE). This includes pharmaceuticals, steroids, hormones, persistent organic pollutants (e.g. POP's), pathogenic microorganisms (Alvarenga *et al*., 2015) and microplastics (Mahon *et al*., 2016; Zhang *et al*., 2020).

Agricultural use of biosolids is regulated in the UK by the 'Sludge (Use in Agriculture) Regulations' (Public Health England and Wales, 1989). These regulations include guidance on the method and conditions of application, for example, spreading is prohibited if land is frozen or waterlogged to avoid run-off. Biosolids and soils must both be tested for quality standards including PTE which limits the amount of permissible chromium, zinc, copper, nickel, cadmium, lead and mercury. However, these regulations were set out 30 years ago and do not account for many emerging contaminants, including microplastics.

More recently there has been concern surrounding the presence of microplastics in biosolids (Edo *et al*., 2020). Anthropogenic activities release microplastics into sewage systems (Prata, 2018). These microplastics originate from domestic sources such as the breakdown of textiles in washing machines, with an average wash load releasing over 700,000 fibres which are destined for wastewater (Napper and Thompson, 2016a). Industrial processes such as air blasting, drilling and cutting plastics also contribute to the formation of secondary microplastics entering wastewater (Prata, 2018). Once in the wastewater systems, it is estimated that 80 - 99% of microplastics are retained in biosolids (Horton *et al*., 2021; Magnusson and Norén, 2014; Talvitie *et al*., 2017). The

amount of microplastics within sludge has been shown to vary by treatment process and wastewater treatment plant (Horton *et al*., 2021). Early studies such as Zubris & Richards (2005), showed that dewatered and digested sludge has the largest quantity of fibres while composted sludge has the least, however this was limited by the identification methods (polarized light microscopy). Recently, advancements in methodologies have allowed for more detailed and accurate studies. Horton *et al*. (2021), used µFTIR to study eight wastewater treatment plants in that UK. They found that even when treatment processes were the same, significant differences in microplastic concentrations were seen between plants, potentially as a result of differences in input of sources and quantities of microplastics at different locations. Additionally, they found temporal differences within sites suggesting that seasonal differences or weather conditions may impact the amount of microplastics in biosolids. What's more, the type of microplastics in biosolids may be impacted by treatment type. Mahon *et al*. (2016), reported a higher abundance of smaller particles in lime stabilisation treated biosolids, compared to other treatment types as a result of a shearing and melting of microplastics during thermal processing, resulting in smaller particles. This may have larger implications, as when the plastics are melted, they can release potentially toxic substances (Tawfik and Huyghebaert, 1998; Whitt *et al*., 2016).

The abundance of microplastic in biosolids has been rapidly increasing since the 1990's, prior to which there were limited concentrations (Okoffo *et al*., 2021). More recently, extremely high concentrations have been reported, although the reported values are often variable. Some studies report lower values of up to 1,946 MP/kg (MPs measured in the range of 50µm – 5mm: Lusher *et al*., 2017) whereas others are up to 286,500 MP/kg (MPs measured in the range of 50µm – 5mm: Harley-Nyang *et al*., 2022), 1,979,740 MP/kg (MP size range measured: 38 - 100µm; Cunsolo *et al*., 2021), and even 10,380,000 MP/kg (MPs measured in the range of 25- 178µm: Horton *et al*., 2021). Highlighting the importance of further investigating the impacts of spreading biosolids with such concentrations on land. However, it is important to consider the differences in methods used between studies as Lusher *et al*., (2017) reported lower concentrations but used visual identification to select a subsample of 10 % of particles for FT-IR analysis with a lower size limit of 50µm whereas Horton *et al*., (2021), reported much higher concentrations using an automated µFTIR approach which eliminated human error in identification and a lower size limit of 25µm.

Estimates for microplastic addition to land during biosolid application have been made from measured concentrations in biosolids. Current estimated values of microplastics added to land from biosolid application range are highly variable. Ng *et al.* (2018), estimated that in the EU, up to 430,000 tonnes of microplastics are applied to land per year in biosolids, whereas in Australia

the maximum predicted value was 19,000 tonnes. These estimates are based on microplastics per million inhabitants values as reported by Nizzetto *et al.,* (2016) which were crudely calculated worst case scenario estimates. While they are currently the best estimates available for microplastic concentrations across large scale areas from biosolids, they should be taken with caution as more recent studies show there are high variations in microplastic concentrations in biosolids (Horton *et al.,* 2021; Cunsolo *et al.,* 2021) therefore there are likely to be localised difference to be considered. It is a complex equation and is likely to be dependent on multiple factors including the source of biosolids, the wastewater treatment type volume added to land and method of application making these estimate extremely rough, preliminary values. Furthermore, the data are presented in units of mass, whereas the current majority of microplastics studies to date report microplastic numbers suggesting that there are uncertainties when converting the data from number of microplastics to mass. More information is required on national and international scale biosolid use as well as more accurate data on biosolid microplastic composition to make detailed predictions. Additionally, large scale temporal and spatial studies are required to confirm these estimates (de Bhowmick and Sarmah, 2022).

2.4 Fate and transport of microplastics in soils

Understanding the influence of sources is vital to understanding microplastics concentrations in soils, however this must be coupled with information on the environmental fate and transport within the soil system (Kim *et al*., 2021). Thus far, land systems have been considered as transport pathways for microplastics, into rivers and ultimately the marine environment (Wagner *et al*., 2014). However, (Zubris and Richards, 2005) reported the presence of microfibres on land which had been treated with sludge up to 15 years previously, similar in appearance to the microfibers found in biosolids, suggesting that the soil environment may preserve and retain microplastics.

Once in soils, there are various pathways of microplastic transport to consider. They may move in the waters associated with soils (surface runoff or vertical percolation). Vertical migration of microplastics in soils may result in contamination of groundwater sources (Wanner, 2021). Soils contaminated with other PTEs have been shown to increase contamination levels in ground or surface water (Aktar *et al*., 2009). However, studies regarding microplastics transport vertically scarce at present. Most studies of microplastics in soils are limited to the topsoil layer. One study considered a 2m depth core which showed a higher occurrence of microplastics in topsoil (<30cm), however they only considered larger microplastics (Weber *et al*., 2022), therefore there is a need to further expand on the understanding of smaller microplastic movement in soils.

Studies have started to consider the horizontal movement of microplastics in the soil environment. This generally occurs as surface runoff, which happens when excess water cannot infiltrate the soil and therefore flows across the surface. This can bring with it any overlying particles including microplastics, particularly lower density and smaller plastics (Han *et al*., 2022). The extent of which may be based on the volume of rainfall at a given time and in agricultural areas, the management of the land (e.g., tillage and crop cover). Although some studies have shown that this pathway only transports a minimal amount of microplastics at approximately 0.2–0.4% in one year (Schell *et al*., 2022).

The amount of water movement within soils is also determined by the soil properties (Mamedov *et al*., 2001). Therefore, there is a need to research how these properties may influence the movement of microplastics within soils (Rillig *et al*., 2017a). The physicochemical properties of soil have been shown to affect concentration of other contaminants (Kim *et al*., 2003). Soil can vary in pH, texture, structure, density, porosity, consistency, organic matter content, temperature, colour, resistivity all of which may have potential impact on microplastic fate. Additionally, on agricultural land there are variances in farming practices to consider (Moss, 2007). For example, different intensities of ploughing may affect the depth to which surface microplastics have the potential to be incorporated into the soil (Gronle *et al*., 2015). Furthermore, it may be important to consider the temporal variations in microplastic concentrations in soils. Chemical contaminants in agricultural soil have been shown to vary significantly with seasonality, making temporal variation important for determining, monitoring and mitigation streams for these contaminants (Fairbairn *et al*., 2016). This may also be applicable to microplastics, however, studies which consider seasonal variation in environmental microplastic concentrations are generally lacking at present (Underwood *et al*., 2017).

Polymer degradation in soils may be considered when determining fate of plastics. The top layer of soil is likely to present an area that promotes degradation of plastics due to its exposure to UV radiation, increased oxygen availability, and higher temperatures (Peng *et al*., 2017). Furthermore, biodegradation may play an important role. For example, the presence of fungi in soil has been shown to increase degradation of PVC (Ali *et al*., 2014). The breakdown of larger plastic items promotes the increase of micro- and nano-plastics in the soil, which may in turn increase bioavailability and subsequent environmental impacts of the plastics. The importance of bioturbation of soils should be considered as a factor influencing microplastic fate as microplastic particles have been shown to be transported by organisms such as earthworms, which can create biopores that move the plastics deeper into the soil (Rillig *et al*., 2017a). Horizontal bioturbation of microplastics by soil biota has been demonstrated, with micro-arthropods transporting

different sizes microplastics depending on their size and species (Maaß *et al*., 2017). Unravelling these controlling processes will enable impact assessment of microplastics in soils (Hurley and Nizzetto, 2018). Given that the soil environment is extremely dynamic with various mechanisms of transport to the wider environment, it is important to assess how this relates to inputs of microplastics, for example, in the application of biosolids to soils.

2.5 Environmental concentrations

To be able to fully understand the risk of microplastics in soils, empirical field data are required to determine their concentrations. Thus far, field studies have often reported the presence of microplastics in soils but little detail on microplastics types, characteristics, and exact quantities (Yang *et al*., 2021). Increasing numbers of studies investigating microplastics in soils, with varying sampling strategies, methods, and results. Studies have been conducted in North and South America, Asia, Europe, and Australia (Corradini *et al*., 2021; Crossman *et al*., 2020; Fuller and Gautam, 2016; Harms *et al*., 2021a; Huang *et al*., 2020; van den Berg *et al*., 2020). Table 2.1 outlines the details of recent microplastic soil studies. There are a wide range of methods used across these studies. Generally, concentrations are reported in soils based on the number of particles per kg of dry soil weight, however some studies have reported numbers of microplastic per area of soil (Fakour *et al*., 2021) and mass per kg of dry soil (Fuller and Gautam, 2016). The reported values will depend on methods of extraction used which, due to lack of standardisation, are often very different between studies. In some cases, this can be as simple as sieving soil fractions to specific size ranges (Weber *et al*., 2022) whereas others use more complicated multistep methods involving multiple reagents and procedures (Zhang *et al*., 2020). This reduces the extent of comparability and highlights the need for consistency in future studies.

Table 2.1 Summary of current studies of microplastics in soils including quantity of microplastics found, methods used and sampling locations.

Number of microplastics found (MP/kg)	Location	Maximum soil depth sampled (cm)	Number of sites sampled	Microplastic size range considered	Microplastic extraction method	Method of identification	Polymer types found	Time of year sampled	Reference
300-67500[$]	Australia	n/a	1	<1mm	A pressurized fluid extractor	ATR-FTIR	PE, PVC, PS	Not specified	(Fuller and Gautam, 2016)
6.8-658.4	Canada	15	4	50um-5mm	Dg: Fenton's reagent, DS: Sodium iodide	Visual ID then ATR-FTIR (>300µm) or µ-FTIR (<300µm)	PP, PE, PET, AC, PBT, PVCA	Spring to Autumn	(Crossman *et al*., 2020)
0-540	Chile	20	4	0.4um-2mm	Ds: centrifuged in water sodium chloride and zinc chloride	Visual ID then µ-FTIR	APV, PE, EVA, PP, Rubber, PS, PET, PA, PLA	Not specified	(Corradini *et al*., 2021)

320-12560	China	5	5	0.02-5mm	Ds: Zinc chloride	Visual ID then μ-Raman	PA, PP, PS, PE ,PVC	Winter	(Chen *et al.*, 2020)
1360-4960	China	10	3	Not specified	Dg: Hydrogen peroxide, Ds: Calcium chloride and sodium chloride	Visual ID then micro-transformed infrared spectroscope	PS, PE, PP, PVC, PET	Summer	(Ding *et al.*, 2021)
1430-3410	China	10	9	Not specified	Dg: Hydrogen peroxide, Ds: Calcium chloride and sodium chloride	Visual ID then μ-FTIR and SEM	PS, PE, PP, HDPE, PVC, PET	Summer	(Ding *et al.*, 2020)
62-103	China	40	19	7um-5mm	Dg: Hydrogen peroxide, Ds: Sodium iodide	Visual ID then μ-FTIR and SEM	PE	Not specified	(Huang *et al.*, 2020)
0-44	China	20	12	2-5mm	Dg: Hydrogen peroxide, Ds: Sodium chloride and sodium iodide	Visual ID then ATR-FTIR (>2mm) or μ-FTIR (<2mm) and SEM	PET, PP, PE, rayon	Summer	(Yang *et al.*, 2021)
0-218	Germany	30	15	1-5mm	Sieved and disaggregated in water	Visual ID then μ-FTIR	PE, PP, PA, PVDF, PDAP, PMMA, PET, PVF, PVA, PVS, PBA	n/a	(Harms *et al.*, 2021a)
0-1	Germany	5	1	1 to 5 mm	Dg: Hydrogen peroxide	Visual ID then ATR-FTIR	PE, PS, PP PVC, PET, PMMA	Autumn	(Piehl *et al.*, 2018)
0.4-6	Germany	200	4	2-5mm	Sieving	Visual ID then ATR-FTIR	PP, LDPE, PA, PS, POM, PET, HDPE, PMMA, PDMS	Summer	(Weber *et al.*, 2022)
5-545	Germany	25	3	25um- 5mm	Dg: Hydrogen peroxide, Ds: Zinc chloride and sodium chloride	Visual ID then μ-FTIR	PE, PP, PB, EVA, PET	n/a	(Zhang *et al.*, 2020)
10-7630	Korea	5	3	20um-5mm	Dg: Hydrogen peroxide, Ds: Zinc chloride and calcium chloride mixed	Visual ID then ATR-FTIR	PP, PE, PS, PVC, PVA, PUR, PTFE, PET, acrylic, nylon, epoxy resin, rayon, ABS, PC, PAN, ASA, polyisoprene	Spring	(Kim *et al.*, 2021)
2116	Spain	10	6	n/a	Ds: Centrifuged in water	Visual ID and reaction to heat	Not specified	Summer	(Beriot *et al.*, 2021)
0-3060	Spain	30	11	11um-5mm	Ds: Centrifuged in water and sodium iodide	Visual ID then μ-FTIR	PP, PVC	n/a	(van den Berg *et al.*, 2020a)
0-593	Switzerland	5	29	125um-5mm	Dg: Nitric acid, Ds: Sodium chloride	Automated μ-FTIR	PE, PA, latex, PS, PVC, SBR, PP	n/a	(Scheurer and Bigalke, 2018)
*12-117 MP/m^2	Taiwan	20	5	1-5mm	Dg: Hydrogen peroxide, Ds: Sodium chloride	Visual ID then ATR-FTIR	PE, LDPE, PP, PS	n/a	(Fakour *et al.*, 2021)

**Data only available in microplastics per m^2; § Data only available in mg/kg; Dg. = method of digestion; Ds. = density separation method*

The amount of microplastics found is likely to depend on the size range being investigated as most studies report a trend of higher abundance with decreasing microplastic size (Chen *et al*., 2020). Therefore, the smaller the lower limit is set, the increased likelihood of finding microplastics. This is true, not only in soils but also other environmental media (Lu *et al*., 2021a) and should be considered when reporting microplastics concentrations. For example, Piehl *et al*., (2018), consider only larger microplastics (1-5mm) and report lower concentrations of microplastics up to 1.3 MP/kg, whereas van den Berg *et al*. (2020), report concentrations as high as 3060 MP/kg but consider a size range with a lower limit (11µm-5mm). The highest reported concentrations of the studies included in Table 2.1 were in agricultural land used to grow vegetables in central China (Chen *et al*., 2020) and they measured concentrations of microplastics in a wide size range (0.02-5mm) up to 12,560 MP/kg and found five different polymer types using Raman spectroscopy. A range of polymer types were reported across the reviewed studies. The most common polymers were polyethylene and polypropylene which were identified in 15 and 14 out of the 17 reviewed studies, respectively. Microplastic abundance may depend on the type of soil environment investigated. For example, one study reported concentrations in flood plain soils to be between 0-593 MP/kg (MP size range measured: 125µm- 5mm; Scheurer and Bigalke, 2018), whereas the majority of studies examining microplastic concentrations in soils are focused on agricultural land. Within these studies there is a large amount of variation. One study compared areas of plastic mulching, rice cultivation, and greenhouse and found the highest reported abundance in greenhouse soils at 215–3315 MP/kg (MP size range measured: 0.1- 5mm; Kim *et al*., 2021b). However, studies which have measured microplastics in soils treated with biosolids have found much higher concentrations up to 5190 MP/kg (MP size range measured: 50µm-5mm; van den Berg *et al*., 2020). Therefore, it is suggested that biosolids are a prime driver for soil microplastic contamination (Corradini *et al*., 2019).

Other considerations when analysing the results of these studies may include the climatic conditions of the study area and the season in which it was sampled. This information is not regularly included which makes comparison challenging between studies. Only one of the 17 reviewed studies in Table 2.1 took samples from locations on more than one occasion. Crossman *et al*. (2020), took samples from Spring to Autumn and found variations in microplastic concentrations particularly in relations to months with high precipitation, suggesting the importance of accounting for temporal variation within studies.

The evidence for microplastic soil contamination is currently limited and is often it difficult to compare between studies because of methodological variability and lack of consistency with units

of measurement and reporting. More empirical studies are therefore required to determine sources and hotspots, for example, areas of biosolid application.

2.6 Methods for microplastic quantification

One of the main limiting factors for microplastic studies in soils are the methods which are currently available for sampling, extracting, and identifying microplastics. Techniques and methods used to quantify microplastics in the environment, particularly in soils, are not standardised and show significant variability throughout the literature. Compared to larger macroplastics, the quantification of microplastics in the environment is challenging, as it is difficult to separate the small particles from environmental matrices to characterise and assess quantities (Cashman *et al*., 2020). Numerous methods have been used to assess microplastics across a range of different environmental samples leaving much room for inconsistencies between studies. Consistency across methodological approaches is required to maximise comparability and enable for boarder conclusions to be made (Lu *et al*., 2021b).

2.6.1 Sampling

The basis for representative results of microplastic studies lies in the sampling strategy (Stock *et al*., 2019). Despite this, there are often inconsistencies between studies making it harder to compare and contrast results. This has led to much criticism of the way in which microplastic research is conducted. A review by Underwood *et al*., (2017) suggested that much of the current microplastic literature is not yet robust enough to provide a full understanding of how microplastics vary in the environment both spatially and temporally. Additionally, a more recent report called for higher quality microplastic studies with a focus on quality control and quality assurance (Provencher *et al*., 2020).

Several recommendations have been made to ensure the quality and consistency of microplastics research. The importance of reporting information on the sampling design, the sample type (e.g. core or surface sample), depth of sample, and an appropriate sample size (ideally based on dry weight) to ensure maximum quality and comparability in studies has been highlighted (Praveena *et al*., 2022). Where possible, the microplastic size definition should be accommodated for by the sampling method (Underwood *et al*., 2017). This extends to the units in which microplastic concentrations will be reported as these concentrations may be expressed as an amount of microplastics per sample, which may include number of particles (Corradini *et al*., 2019) and/or weight of particles (Fuller and Gautam, 2016). The units of the sample itself are also variable and

may be reported as mass, volume, area, or length. For example, microplastics have been reported as particles per mass of dry sediment (Klein *et al*., 2015) or wet sediment (Strand and Tairova, 2016), particles per area sampled (Klein *et al*., 2015) or volume of sediment (Thompson *et al*., 2004). These inconsistencies in units can make comparisons between studies more complex and sometimes impossible.

Replication should be considered when taking environmental samples to ensure patterns may be reliably determined on a spatial and temporal scale (Underwood *et al*., 2017). For example, one study took samples in beach sediments only in the summer months with limited sampling sites (Laglbauer *et al*., 2014). This was later repeated with more sampling sites and across different seasons to assess differences in the microplastic concentrations found (Korez *et al*., 2018). Sampling in the field for microplastics requires careful planning and must take into account the variability of the environment in which it is sampled. There are numerous examples of pseudo replication in the literature (Underwood *et al*., 2017), where multiple samples are taken within the site but there is a lack of multiple site studies where sites with particular characteristics are grouped and compared with those without that characteristic e.g. areas of farmland treated with biosolids compared with those which are untreated. This pseudo replication does not account for the large environmental variability expected in the samples and is not sufficient to make generalised conclusion about types of environments and their microplastic concentrations. In the context of sedimentary environment (e.g. sandy beaches), depth, sampling location and number of replicates are critical parameters for microplastic studies and should therefore be considered (Besley *et al*., 2017). These general principles also apply to soil-based systems. A sampling strategy should be determined by the goal of the research (Stock *et al*., 2019). Basic principles of consistency and repeatability between studies should be applied to maximise the levels of integrity and comparability between studies.

2.6.2 Microplastic extraction

This level of required integrity also applies to the processing of microplastic samples. A range of methods have been used to extract microplastics from environmental matrices with different levels of success. This variation in success may be to be due to the inherent variability and complexity of the environmental matrices in which the microplastics occur. Soils are particularly complex due to their organic content, mix of minerals and associated chemistry. Field data of microplastics concentrations in soils are lacking, perhaps due to the inherent complexities of the medium and the lack of available methods- there is an inherent need for suitable methods to be developed and applied (Corradini *et al*., 2019).

Soils are generally extremely variable in texture, organic content, nutrient content, and depth- all of which are important to consider as they may influence both the distribution of plastic in the sample and the process(es) required to extract them. Currently methods of extraction rarely consider the detailed characteristics of soils and sediments prior to analysis. To obtain optimal results, standardisation within methods, which accounts for the characteristics of a sample is needed. These methods should also be widely accessible to ensure their use consistency within the research community to allow for inter-study comparisons.

A variety of novel methods have been applied to for microplastic extraction. For example, the SMI (Sediment Microplastic Isolation) unit which uses the density separation principle to extract microplastics from marine sediments at rates of 95.8% (Coppock *et al*., 2017) and the Munich Plastic Sediment Separator (MPSS) which also uses density separation principle and has high extraction rates, especially when considering large volumes of inorganic sediments (Imhof *et al*., 2012). The electrostatic behaviour of microplastics has been utilised in a method of extraction where samples are separated into conductor and non-conductor fractions, minimising the volume of sediment, and easing microplastic identification (Felsing *et al*., 2018). Attempts have also been made to extract plastics magnetically. This utilises the hydrophobic properties of microplastic surfaces by binding iron nanoparticles to the plastics and extracting those plastics using a magnet (Grbic *et al*., 2019). However, the problem with these more novel methods is that they often require specialised equipment or expertise to process and therefore may be less accessible and more expensive than other methods. The most commonly employed methods for extraction generally include filtration, density separation, organic matter digestion, visual inspection and identification (de Souza Machado *et al*., 2018a). The two main steps considered here density separation and organic matter removal, as they generally require limited specialist equipment and may be applied to a variety of sample types.

2.6.2.1 Density separation

Density separation involves sorting of microplastics from sediment by particle density. It has been shown to be almost twice as efficient at extracting microplastics than visual sorting (Horton *et al*., 2017a). In theory this approach works on the basis of sediment particles having a greater density than that of the plastics. Therefore when submerged in a dense liquid, the microplastics, which have a lower density, float to the surface while the sediment, with greater density, remains at the bottom. This method has been shown to work for many substrates and is a quick and easy method with few processing steps. Quinn *et al*. (2017), quantified recovery rates of microplastics using various brine solutions including sodium chloride and zinc bromide showing a trend of

increased recovery with increasing solution density suggesting that to maximise the recovery of all microplastics with density separation, higher density solutions should be used. However, the scientific question and requirements should be considered. For example, if the requirements are to quantify the buoyant microplastic load in sea water, it may be sufficient to use a lower density brine solution equivalent to that of seawater (1.025 g cm^{-3}) (Hurley *et al*., 2018). However, to find out the total microplastic contamination in sediments a higher density brine solution such as zinc chloride (1.7g cm^{-3}) must be used to extract those microplastics with a higher density than seawater as common polymers have densities greater than 1.2g cm^{-3} (e.g. PET and PVC; Han *et al*., 2019). There is thus a trade-off between using the generally more expensive and toxic, higher density solutions, and the less effective but cheaper and safer lower density ones. Multiple density separations have also been attempted to ensure a greater recovery, i.e. samples subjected to two (Claessens *et al*., 2011) or more (Martins and Sobral, 2011) rounds of settlement may increase microplastic recovery rates. Alternatively, oils have been used as a separation medium, which works on typical oleophilic/hydrophobic properties of oil which attracts the plastics to the oil, increasing extraction (Crichton *et al*., 2017). This method has been shown to have very high recovery rates in different media types, including soils (Mani *et al*., 2019).

2.6.2.2 Digestion

Although density separation works well for most highly minerogenic substrates, problems occur when the particles of sediment are also of a density lower than that of the solution in which they are placed. For example, the organic portion of sediments often has particles of low density which float in the density separation step, hindering the practicality and reliability of the microplastic identification stage of analysis (Löder *et al*., 2017a). This is particularly a problem in soils where there are high volumes of organic matter that generally has a density of 1.0-1.4 g cm^{-3}, comparable with many common plastic polymers, therefore developed methods should include validation for soils where organic content is high (Bläsing and Amelung, 2018). To overcome this problem, the organic matter that can obstruct the view of microplastics in samples can be removed from the sample prior to, or after the density separation step by digestion to ease identification of particles.

Historically, strong acids have been used to remove organic matter from soil samples as they have strong oxidising potentials (Claessens *et al*., 2013; Lasee *et al*., 2017). However, this is a problem when it comes to microplastic research as the strong acids can degrade the plastics themselves (Cole *et al*., 2014). Therefore, other methods such as the use of hydrogen peroxide have been implemented. Hydrogen peroxide works as an oxidising agent to break down and remove organic

matter, but it can be a lengthy process, sometimes taking in excess of seven days with digestion rates, as low as 50% for biogenic material (Nuelle *et al*., 2014). Another potential problem with the use of hydrogen peroxide is the need for the introduction of heat to the process. Efficiencies have been seen to increase when temperatures are elevated, but increasing the temperature is unfavourable as it runs the risk of exceeding the continuous operating temperature of some plastics, resulting in degradation of the particles (Sujathan *et al*., 2017).

Alternatives to hydrogen peroxide digestion include sodium hydroxide, potassium hydroxide and Fenton's reagent, although sodium hydroxide and potassium hydroxide tend to have lower digestion rates in soils (Hurley *et al*., 2018). Fenton's reagent uses hydrogen peroxide to digest the organic matter with the aid of an iron catalyst. This is beneficial because of its lower temperature and time requirements, making it quicker and less degrading to the microplastic particles. Several studies have now successfully utilised Fenton's as a digestion reagent, with digestion efficiencies in excess of 86.9% in sludge and soil, resulting in recovery rates of 79-100% for polyethylene and polyethylene terephthalate when combined with density separation methods (Hurley *et al.*, 2018).

Enzymatic digestion has been presented as a method in the process of microplastic extraction (Catarino *et al*., 2017) but is rarely utilised in soils. This may be due to the high specificity of enzymes and complex variability of organic matter in soil samples. Möller *et al*. (2021), utilised enzymatic digestion in soil samples however, this required a multistep approach with multiple reagents over many days per sample. While enzymatic digestion has its benefits- e.g. high digestion rates and low impact on microplastic particles (Cole *et al*., 2014), it is a costly approach compared with alternative chemical digestions and may therefore not be suitable for large scale sediment-based extraction.

2.6.3 Identification and Characterisation

After the microplastics have been extracted from environmental samples, further complications may arise when identifying microplastics. This involves quantifying numbers of microplastics and defining their characteristics, for example, shape, size, colour, and polymer type. Most commonly, studies use various microscopy methods and visual inspection to define these characteristics. Only 2 of the 17 studies reviewed in Table 2.1 used identification methods which didn't rely on visual identification; however it has been shown that particles suspected to be microplastics are commonly undercounted or misidentified (Song *et al*., 2015). This is also dependant on the particle shape as it has been shown that plastic fibres are more easily distinguished from natural material that plastic fragments (Lenz *et al*., 2015a). To aid this some studies use heat (e.g. the hot

needle test) to determine if a particle is plastic by testing its reaction to increased temperature although this cannot determine polymer type (Beriot *et al*., 2021). Scanning electron microscopy has also been used to determine microplastic characteristics, although it generally utilised to assess microplastic surface characteristics and inorganic additives as it cannot be used to determine polymer composition (Fries *et al*., 2013).

Additionally, staining techniques may be used to identify microplastics from natural material. For example, Nile Red adsorbs onto the plastics' surface and can be detected to identify plastic materials (Maes *et al*., 2017). However, it must be used in combination with other methods as it has limited detection capability for less hydrophobic polymers such as PET and PVC and does not define by polymer type (Erni-Cassola *et al*., 2017). There are some materials of natural origin e.g., chitin and some algae, that can adsorb Nile Red and may result in false positive identification.

For accurate and detailed microplastic identification, more advanced analytical methods may be used. This can generally be split into vibrational spectroscopy and GC-MS (Gas chromatography-Mass spectrometry), both of which can confirm and quantify polymer type (Chen *et al*., 2020). The choice of which method should be defined by the research questions being asked as both give different types and units of measurement. Spectroscopic methods give details of individual particle size and number whereas GC-MS gives detail of plastic mass. This will influence the way in which reported values may be interpreted and should therefore be carefully considered.

There are two main spectroscopic methods used for microplastic analysis which are Fourier-Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy. FTIR measures the absorbance or transmittance of infrared light to produce a spectrum whereas Raman spectroscopy measures scattering of monochromatic light. Both have been used in microplastic studies with varying degrees of success (Lenz *et al*., 2015b). FTIR can be further split into Attenuated Total Reflection (ATR) FTIR and µFTIR. ATR can generally be used only on larger microplastics (>500µm) and requires direct contact with the crystal (Chen *et al*., 2020). Whereas µFTIR can be used on smaller particles and if used with a focal plane array (FPA) it can automatically scan a sample, removing human bias and error (Tirkey & Upadhyay, 2021). The theoretical lower size limit of µFTIR analysis of 10µm (Shim *et al*., 2017), it provides the benefit of ease of use and direct detection from the filter (Koenig *et al*., 2001). However, its limitations come in the form of initial high cost of the instrument and its reliability with smaller sized microplastics, as detection below 50 µm is less reliable (Shim *et al.*, 2017). Raman spectroscopy, which measures the scattering of light after applying a laser to a sample. It is generally regarded as a more laborious and time-consuming method, however, with developments in automated Raman, this may improve (Araujo *et al*.,

2018a). This may improve detection of smaller microplastics as it has been shown to be more reliable down to a lower limit of 1 µm (Shim *et al.*, 2017).

Both methods are non-invasive and give a detailed profile of polymer composition but require an expert operator to obtain a reliable spectrum (Rocha-Santos and Duarte, 2015). A combination of both techniques could be used to make use of the advantages each one presents as they are complimentary (Qiu *et al*., 2016). Rapid FTIR imaging could be used to screen for larger microplastics (500-50 µm) and Raman could be used to gain detail for smaller particles (50-1 µm) (Käppler *et al*., 2016a). However, a limitation of both methods is the lack of comprehensiveness of the spectral library used to match the obtained spectrum with a lack of environmentally relevant spectra (Araujo *et al*., 2018b). Such polymer libraries are starting to be developed (Cowger *et al*., 2021) which will reduce the requirements for individual researchers and boost material available to the wider research community.

Alternatively, some researchers are choosing to use GC-MS as a microplastic quantification method. It is a destructive technique which aims to determine mass of microplastics. It is a fast method, allowing for quantification of additives and polymer types simultaneously and has no lower size limit (Gomiero *et al*., 2021). Pyrolysis GC-MS in particular, been utilised in favour of its capabilities of identifying chemical indicators of tyre wear particles which may be missed with other identification methods and are thought to contribute to a large proportion of microplastics in the environment (Kole *et al*., 2017; Parker-Jurd *et al*., 2019). However, it does not provide information on the morphological characteristics (Chen *et al*., 2020b) and is still in development, with further work required to determine how well it works for different plastic types, and how applicable it may be for more complex matrices (Kirstein *et al*., 2021). Ideally a combination of microplastic identification techniques should be used to obtain maximum information of particle number, characteristics, and mass, however this will depend on the research question and aims (Primpke *et al*., 2020), and the available resources.

There are many challenges for all these methods particularly as in environmental samples the microplastic particles may have been in the environment for a number of years. Once in the environment the particles may come into contact with weathering processes and chemicals which may alter their physical and chemical structure. This in turn can inhibit the ability to identify the plastic to its source material (Dong *et al*., 2020). Additionally, there is the added complication that the pre-processing steps which involve the use of various chemicals may alter the plastics and thus impede accurate identification (Munno *et al*., 2018). Often studies struggle to identify particles with poor quality spectra resulting in inconclusive analysis (Korez *et al*., 2019). To fully

understand microplastic contamination and its impacts it is necessary to gather as much characterisation data as possible using best available and most appropriate techniques.

2.6.4 Contamination control

Quality control in microplastic research is essential to reporting accurate results. Given that plastics are ubiquitous, including on clothing (Napper and Thompson, 2016a) and indoor air (Dris *et al*., 2017) there is high probability of sample contamination. This contamination may occur at any stage of analysis, including the laboratory setting, and has the potential to disrupt the accuracy of microplastic quantification (Wesch *et al*., 2017). Recently it has been reported that contamination control measures are lacking in microplastics research with most studies only including a few essential control measures (Prata *et al*., 2021). These measures include use of clothing covers (e.g. cotton lab coats, proper pre-cleaning of equipment, covering samples, working in clean environments (e.g. laminar flow cabinets), filtering reagents, and using negative controls (Prata *et al*., 2021). Procedural blanks are essential to measure contamination (Hidalgo-Ruz *et al*., 2012). This should be rigorously reported and accounted for in the final data wherever possible. The most rigorous approach for this involves assessing the limits of detection (LOD) and limits of quantification (LOQ) as recommended by (Horton *et al*., 2021). This ensures that false positive values are not overestimated and should be included where possible when reporting microplastic concentrations.

2.7 Conclusion and research gaps

Although it is a rapidly expanding research areas it is clear that there are still many knowledge gaps in soil microplastic research. Given the complexities of soils, further method development is required to optimise extraction protocols. This is vital to allow for rapid and harmonised expansion of studies considering microplastic contamination in soils. The sources of microplastics in soils remain undefined and transport processes are not well understood. To work out the complexities of source and sink dynamic in such environments, development of these areas is essential. This thesis will therefore develop and optimise methods for extracting microplastics from soils. These methods will then be utilised to quantify the contribution of biosolids, as a potential major source, to overall soil microplastic concentrations and determine the fate of microplastics within these systems.

2.8 Aims and objectives

The overall aim of this thesis is to expand on the knowledge of microplastics as a contaminant in the terrestrial environment with particular relation to biosolids as an identified source of concern. The theses sets out to meet the following aims and objectives.

Chapter 3 aims to develop and test suitable methods for extraction of microplastics from soils with varying characteristics using the following objectives:

- Quantifying the removal of soil organic matter using selected digestion methods.
- Selecting the most suitable technique for separating microplastics from the inorganic fraction of soils with varying particle size (e.g. sand, silt, and clay).
- Assessing the effectiveness of combined organic removal and inorganic separation techniques for extracting microplastics from samples of varying organic matter content.
- Assessing the impact of extraction methods on the integrity of microplastic particles using Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (ATR FT-IR).

Chapter 4 aims to evaluate the contribution of sewage sludge application to microplastic concentrations in agricultural soils by:

- Quantifying and characterising (polymer type and size) baseline values of microplastics in agricultural soil.
- Comparing and contrasting the quantities and characteristics of microplastics in soils which have had biosolids applied to soils which have never been treated with biosolids.
- Investigating the effect of seasonality on the quantities and characteristics microplastics in agricultural soils by comparing across two seasons.

Chapter 5 aims to investigate the fate of microplastics in soils from sewage sludge application by following these objectives:

- Quantifying and characterising (polymer type and size) microplastics in agricultural soil before and after biosolid application at defined time intervals (1 week, 1 month, 3 months, 1 year).
- Quantifying differences in microplastic concentrations between and within agricultural fields across these timepoints.
- Evaluating the relationship between soil characteristics (organic matter and particle size distribution) and microplastic concentrations.

Finally, **Chapter 6** will expand on the findings of Chapters 3, 4 and 5 to interpret and explain the importance and relevance of the results in a wider context with relation to each other and the wider literature.

Chapter 3 Developing a systematic method for extraction of microplastics in soils

A version of this Chapter was published as (Radford *et al*., 2021), prior to thesis submission.

3.1 Introduction

Microplastics have been found globally in a wide variety of environments (Rochman, 2018). However only 3.8% of studies had, until recently, investigated microplastics in terrestrial soils (He *et al*., 2018). This is despite the close proximity of terrestrial environments to many potential sources, as a large proportion of plastic waste is generated and disposed of on land (Jambeck *et al*., 2015). As stated above (§ 2.5), high concentrations of microplastics have been found in soils (Fuller and Gautam, 2016; van den Berg *et al*., 2020a; Vollertsen and Hansen, 2017). Scheurer & Bigalke (2018), found evidence for microplastics in 90% of tested soils, indicating there is a high likelihood of widespread contamination. Highlighting the need to measure and quantify the amount of microplastics in the terrestrial environment over a wide range of spatial and temporal scales to enable the risk of adverse effects to be determined. However, studies of this sort are currently limited by the lack of suitable methods for quantifying microplastics in soils.

As stated previously, there is an absence of standard operating procedures for quantifying microplastics in the environment and this is especially the case for soils, which can be extremely complex matrices (da Costa *et al*., 2018). The heterogeneous nature soils as a mixture of minerals with a range of particle size distributions and organic matter at varying stages of decomposition (Bläsing and Amelung, 2018) and complexity of those organo-mineral interactions makes the collection of soil microplastic data challenging (Möller *et al*., 2021).

Initial attempts have been made to quantify microplastics in soil. Density separation methods are some of the most commonly utilised techniques to isolate microplastics from environmental matrices. These methods isolate microplastics using high density salt solutions, such as sodium chloride (NaCl, 1.2 g cm^{-3}), sodium bromide (1.4 g cm^{-3}) and zinc chloride (ZnCl2, 1.7g cm^{-3}) (Quinn *et al*., 2017; Scheurer and Bigalke, 2018; Thompson *et al*., 2004). Lower density solutions tend to be cheaper and less hazardous, but higher density solutions are required to extract more dense polymers such as polyvinyl chloride (PVC, 1.16-1.58 g cm^{-3}) and polyethylene terephthalate (PET, 1.37-1.45 g cm^{-3}) (Corradini *et al*., 2019; Liu *et al*., 2019; Liu *et al*., 2018). Alternatively, oil extraction methods have been developed, which use a combination of low-density oil and the

oleophilic property of plastic to accumulate microplastics in a layer of oil above an aqueous solution (Crichton *et al*., 2017; Mani *et al*., 2019). Additionally, techniques such as ultrasonication (Lwanga *et al*., 2017; Zhang *et al*., 2018) and centrifugation (Corradini *et al*., 2019) may be used to enhance these extractions.

While density separation techniques tend to target the inorganic fraction of a sample, organic matter, which has similar density to many types of plastic (Bläsing and Amelung, 2018), can obscure the detection of microplastics and interfere with identification (Vermeiren *et al*., 2020). To overcome this problem, digestion methods to remove the organic matter have been used. Established soil organic matter digestion techniques involve strong acids (Hseu, 2004); however they are not recommended for microplastic studies as they are damaging to some polymers (Karami *et al*., 2017). Instead, potassium hydroxide (KOH), hydrogen peroxide (H_2O_2), and enzymatic treatments may be used with minimal impact on microplastics (Hamm *et al*., 2018). Enzymatic treatments can be costly as they target only specific components of organic matter and require multiple types to fully remove organic material (Hurley *et al*., 2018; Löder *et al*., 2017).

Hydrogen peroxide is particularly effective at removing organic material in soil (Liu *et al*., 2018) and can be used in combination with an iron catalyst (Fenton's reagent) to accelerate the reaction. Fenton's reagent has been shown to be particularly effective in sludge and soil samples (Hurley *et al*., 2018; Vollertsen and Hansen, 2017; Zhang and Liu, 2018). Increasing temperature may enhance organic removal (Prata *et al*., 2019), although this should be limited to 50 °C to remain within the heat deflection limits of most common microplastics (Hamm *et al*., 2018; Qiu *et al*., 2016).

An additional consideration of these methods is the sample matrix characteristics. In soils, this includes chemical and physical properties such as organic matter content, particle size distribution, pH, and bulk density. It is very likely that properties such as these impact the efficiency of extraction methods, similar to the matrix effect seen in analytical chemistry techniques, where the sample matrix characteristics influence quantification and identification of contaminants (Zhou *et al*., 2017). Some studies have started to incorporate matrix characteristics into microplastic extraction method design, for example, testing the difference between sandy and clay soils (Zhang and Liu, 2018). It has been suggested that different methods for extracting microplastics from soil should be applied depending on the proportions of clay and organic matter (He *et al*., 2018). However, most studies lack a matrix characterisation, which is particularly important in soils due to their heterogeneous and variable nature. Method suitability must consider the impacts of reagents on microplastics (Munno *et al*., 2018) and microplastics recovery

efficiency. This is particularly important as some studies report recovery efficiencies of 85-100% (Liu *et al*., 2019) while others are much lower (5 to 75%; Wang *et al*., 2018).

This chapter aims to systematically explore and validate methods for extracting microplastics from soils, taking sample matrix characteristics into consideration. Organic removal efficiency and density separation techniques were tested in soils with a range of organic content and particle size. These techniques were combined to establish the most effective extraction methods for a range of microplastics types. Methods were further validated for use by assessing the physical impact on, and subsequent ease of identification of, microplastics. The outcomes of these trials can inform future studies looking to quantify microplastics in soil, enabling the most suitable method to be chosen based on the sample characteristics.

3.2 Methods

Methods of extraction were tested in four stages by measuring the organic matter removal efficiency of selected digestion methods, the extraction efficiency of spiked microplastics with both organic and inorganic soils using density separation techniques, and validation by assessing the impact of extraction reagents on microplastic identification.

3.2.1 Microplastic spikes

A mixed microplastic standard for spiked recovery was created. Consumer materials were used to create fragments and fibres under 5 mm in size (ISO/TR 21960, 2020) of polymers representing the six main resin codes (ASTM, 2020) (Table 3.1). Fragments were created using a household coffee grinder and separated into small (0.25-0.5mm) and large size fractions (0.5-1 mm), and fibres were cut to size (1-5 mm). Polymer type was identified by material labelling and confirmed using Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (ATR FT-IR) (Frontier, Perkin Elmer) with Spectrum infrared spectroscopy software (Perkin Elmer). Microplastic spikes were chosen with distinct characteristics making identification and separation from contamination sources possible. Each sample tested for recovery efficiency was spiked with five particles of each type of microplastic particle (n=60) and shaken thoroughly prior to treatment to ensure microplastic distribution.

Table 3.1 Microplastic types used in spiking experiments: polymer type, shape, size, colour and original product. Density information relates to literature recording of polymer types (Alvim *et al*., 2020; Han *et al*., 2019)

Resin code	Abbreviation	Shape	Size (mm)	Colour	Original product	Density (g cm^{-3})
1	PET	Fragment	0.5-1 mm	Blue	Drinks bottle	1.37-1.45
		Fibre	1-5 mm	Green	Craft ribbon	
2	HDPE	Fragment	0.25- 0.5 mm	Pink	Cleaning product bottle	0.93-0.97
			0.5-1 mm			
3	PVC	Fragment	0.25- 0.5 mm	Red	Tablecloth	1.16-1.58
			0.5-1 mm			
4	LDPE	Fragment	0.25- 0.5 mm	Purple	Carrier bag	0.91-0.92
			0.5-1 mm			
5	PP	Fragment	0.5-1 mm	White	Storage bottle	0.9-0.91
		Fibre	1-5 mm	Purple	Carpet	
6	PS	Fragment	0.25- 0.5 mm	White	Packaging	0.015-0.03
			0.5-1 mm			

PET, Polyethylene terephthalate; HDPE, High-density polyethylene; PVC, polyvinylchloride; LDPE, Low-density polyethylene; PP polypropylene; PS, polystyrene

3.2.2 Soil materials

Soil materials for testing were specifically created for experimental procedures. The organic fraction of soils was represented by a commercial compost (John Innes Manufacturers Association approved, no.1 compost, sieved to 2 mm to remove large debris) and the inorganic fraction was a fine sand. The two materials were mixed in varying ratios to form representative soils with specific levels of organic matter content. For soils of purely inorganic content the focus was particle size composition. Particle size was classified according to the Wentworth Scale (Wentworth, 1922), where clay particles are <4µm and sand particles are 0.125-2mm. A clay material (Bentonite, Sibelco; see Appendix A Table 7) was mixed with a fine sand (85.6% sand) in varying ratios to form six distinct soil types (see Appendix A Table 1). Organic matter content was measured in all samples using loss-on-ignition (LOI) at 550 °C and particle size distribution was

analysed using the hydrometer method (Sheldrick and Wang, 1993). Unless otherwise stated, soils categorised for organic matter removal experiments as 'high organic' had an organic matter content of 73% (±0.6 SE) and 'low organic' had 12% (±0.9 SE). For each sample (both organic and inorganic), 10g of soil was used.

3.2.3 Organic matter removal

The initial phase aimed to assess the amount of organic matter that could be removed from soil. Digestion treatments were tested on samples of low and high organic matter content to represent the extremes likely to be found in the environment (Huat *et al*., 2009), carried out in glass jars (330 mL capacity) and repeated three times per treatment for each sample type.

Fenton's reagent (H_2O_2, 30% w/v + Fe^{2+} catalyst, Fisher Scientific), H_2O_2 (30% w/v, Fisher Scientific) and KOH (10% w/v, Fisher Scientific) were selected for testing based on their reported organic removal efficiency and low impact on microplastics (Hamm *et al*., 2018; Hurley *et al*., 2018; Tagg *et al*., 2015). For H_2O_2 and KOH treatments, a 50 mL aliquot was added to each sample. Fenton's reagent digestion was carried out using 25 mL of H_2O_2 with 25 mL of iron catalyst ($FeSO_4{\cdot}7H_2O$, 1 gL^{-1}, Fisher Scientific) adjusted to pH 3 with concentrated sulphuric acid (H_2SO_4, 95% v/v, Fisher Scientific), an ice bath was used to control the maximum temperature of the reaction to 50 °C until there was no longer a visible reaction. All samples were then placed in a shaking incubator at 100 rpm at 50 °C for 24 hours or until all liquid had evaporated. The samples were then dried at 105°C overnight, organic content was measured again in triplicate and the quantity of removed organic matter calculated. Each digestion was repeated on separate samples at 40 °C to assess the effect of temperature on digestion efficiency. Digestion treatments were additionally tested in combination with a dispersant, with the aim of dispersing the soil particles prior to digestion maximise organic removal efficiency. In separate samples, 50 mL of sodium hexametaphosphate ($Na_6P_6O_{18}$, 1% w/v, Fisher Scientific) was used to soak samples for 24 hours prior to digestion, and each digestion process was then completed on the samples as stated above at 50 °C.

3.2.4 Density separation

Microplastic recovery experiments were conducted using density separation methods to assess efficiency of microplastic separation from inorganic samples only. Each method was tested on six soils with distinct particle size composition in triplicate. Three different density separation media were tested: $ZnCl_2$ (1.7g cm^{-3}, APC pure), NaCl solution (1.2 g cm^{-3}, food grade), and canola oil (food grade).

For $ZnCl_2$ and NaCl trials, 300 mL was added to the sample. The lid was tightly sealed and shaken vigorously by hand for 30 seconds to ensure full contact between the density separation medium and the sample, before leaving to settle overnight to allow dense particles to settle out. The top layer of the sample was then removed using an overflow method (Horton *et al*., 2017), where excess $ZnCl_2$ or NaCl was gently added to the jar to spill the top layer of the sample into a surrounding glass crystallising dish and used to rinse the sides and inside of the lid of the jar.

Canola oil separations were conducted based on the method developed by Crichton *et al*. (2017). 100 mL of distilled water and 5 mL of canola oil was added to each sample and again, shaken for 30 seconds. An additional 200 mL of distilled water was added into the jar to create a further separation between the top layer of canola oil and the bottom of the jar. This solution was then placed in an orbital shaker for 2 hours at room temperature, 100 rpm. The solution was then left to settle overnight, after which the canola oil layer was extracted with the same overflow method using distilled water. The overflowed layer of each treatment, containing microplastics, was then vacuum filtered onto a glass fibre filter (Whatman GF/A, 1.6 µm). The overflow process was completed twice per sample, from initial shaking to vacuum filtering, to achieve maximum extraction efficiency within a reasonable timeframe (see Appendix A Figure 2). All filters were inspected under a low power microscope (Nikon SMZ1000, x40) and recovered microplastics were counted, distinguishable from contamination by their chosen colours (Table 3.1)

Each protocol with the density separation media was separately run and tested with ultrasound to break up the soils. After each time a sample was shaken, it was subjected to 5 minutes of ultrasound in an ultrasonic bath (37 Hz, Fisher Scientific: FB15055). Samples were then overflowed, filtered, and analysed as above.

3.2.5 Method combinations

To combine extraction methods, organic removal was included as an additional step prior to density separation in samples with organic matter. Informed by the results of the organic removal efficiencies (§3.3.1), H_2O_2 at 50 °C was chosen as the optimum digestion method and was used in combination with each of the density separation methods. Each combined method was tested on samples with a range of organic matter content, ranging from 0.2- 72% (n= 18 per method).

For the digestion, 50 mL of H_2O_2 was added to each sample and additional 50 mL once not visible reaction occurred to ensure maximum digestion. The samples were then heated and shaken (50°C, 100 rpm) until all remaining liquid had evaporated. Using the same methods as stated

previously (§3.2.4), $ZnCl_2$, NaCl and canola oil extractions were carried out on samples. Recovery efficiencies were calculated for each sample.

3.2.6 Method reagent impact on the physical and spectroscopic properties of microplastics

To complete the validation, the impact of each method on the plastics was tested. Each type of microplastic particle used as a spike was exposed separately to each treatment involved in the methods in the absence of soil. Spikes subjected to H_2O_2, KOH and Fenton's reagent were tested at 40 and 50 °C to evaluate the effects of the digestion methods. Each of the microplastic types were added to glass vials containing 1 mL of each of the reagents, then heated to 40°C or 50 °C for 24 hours and removed for analysis. Similarly, the digestion methods were evaluated by exposing each of the microplastic types to 1 mL of $ZnCl_2$, canola oil, and NaCl in glass vials. The effects of ultrasound were separately measured by adding 1 mL of distilled water to vials containing the microplastics and exposing them to the ultrasound treatment.

Virgin and exposed microplastics were analysed using ATR FT-IR with a wavenumber range of 4000–600 cm^{-1} with spectral resolution of 4 cm^{-1}. A library of virgin microplastics, which were not exposed to any reagents, was created including each of the 12 microplastic types used in the spiking experiments. The spectrum of microplastics exposed to each treatment (n=3) was compared with the virgin microplastic library and assigned a hit quality index number (HQI, on a scale of 0-1) (Renner *et al*., 2019) to determine the effect of chosen reagents.

3.2.7 Statistical analysis

Organic matter removal rates were measured by calculating the amount of organic matter removed from a sample after digestion (OM_a) as a percentage of the initial organic matter content (OM_i).

$$\text{Organic matter removed } (\%) = \frac{OM_a\ (g)}{OMi\ (g)} \times 100$$

All statistical analyses were performed in RStudio (1.2.1335) software. Normal distribution of data was checked using Shapiro-Wilk tests and homogeneity of variance was checked using Levene's test. Parametric tests were applied where assumptions of normality and equal variance had been met.

Statistical analysis in the form of Kruskal–Wallis tests (non-parametric), one and two-way analysis of variance (ANOVA, parametric) were used to compare differences between groupings, and

pairwise comparisons were made using the post hoc analysis of Dunn's test for non-parametric and Tukey's tests for parametric data. This analysis applied to the amount of organic matter removed from samples with different digestion methods, recovery efficiencies in inorganic and organic samples using the different extraction methods, and differences in identification hit scores for microplastics treated with the different extraction reagents.

Differences between means were tested using a T-test and Wilcoxon rank sum test for the amount of organic matter removed at 40°C and 50 °C and with initially high and low organic content, recovery efficiencies in inorganic samples with and without the use of ultrasound and H_2O_2. Correlations were tested using Spearman's Rank to assess relationships between particle size distribution and recovery of plastics in inorganic samples and the relationship between percentage organic matter content and microplastic recovery across all treatments.

3.3 Results

3.3.1 Organic matter removal

The three organic removal reagents worked at significantly different efficiencies across samples with low and high organic content ($p < 0.05$, Kruskal Wallis test). For samples with low organic content, removal of organic matter was similar for Fenton's and H_2O_2 and both these treatments were more effective than KOH ($p < 0.05$ for both, Dunn's test.). Samples with high organic content did not show a significant difference between the amount of organic matter removed by Fenton's and KOH (Figure 3.1), but H_2O_2removed more organic matter than both other treatments ($p < 0.05$ for both, Dunn's test). H_2O_2at 50 °C removed 93% organic matter. This was significantly more than Fenton's at 40 and 50 °C, which removed 51% and 56% organic matter, respectively ($p < 0.05$ for both, Dunn's test). H_2O_2 at both 40 and 50°C removed more organic matter than KOH at 40°C which removed only 20% in samples with high initial organic content ($p < 0.01$, Dunn's test).

Temperature did not affect the efficiency of organic matter removal in samples with initial low or high organic content ($p > 0.05$ for both, Wilcoxon rank sum). The amount of organic matter removed by $H_2O_2$was significantly reduced by the addition of dispersant ($p < 0.001$, one-way ANOVA) from 93 to -1.9%. There was no difference between the amounts of organic matter removed by KOH or Fenton's reagent with or without dispersant.

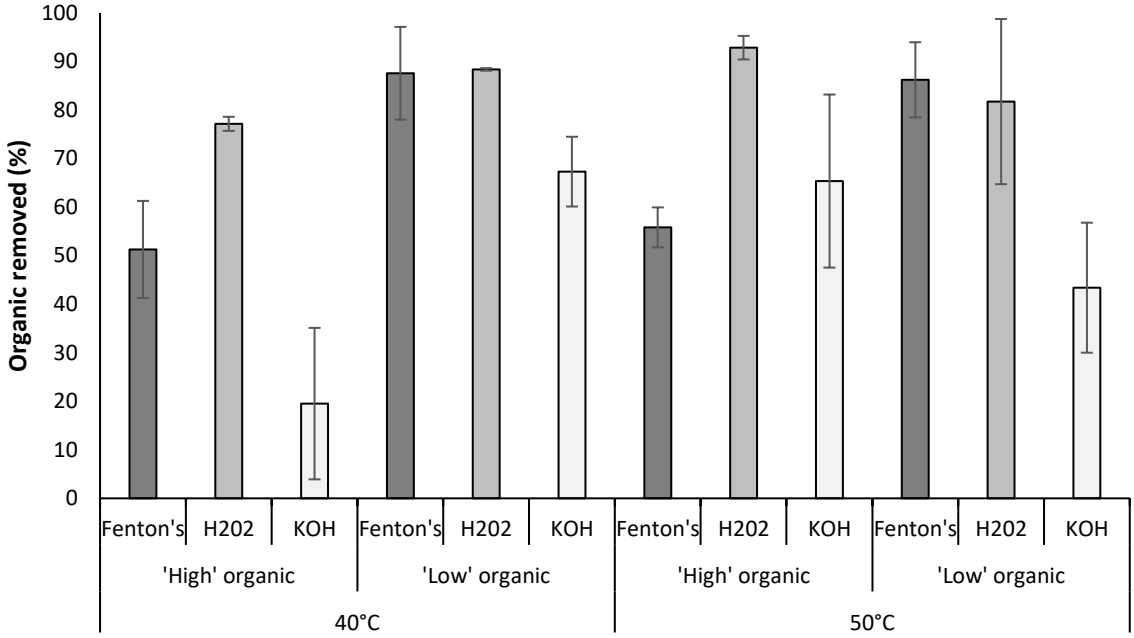


Figure 3.1 Organic removed by tested chemicals (Fenton's reagent, potassium hydroxide (KOH) and hydrogen peroxide (H_2O_2) at 40 and 50°C in samples of initial high (73 ± 0.6 % SE) and low (12± 0.9% SE) organic matter content. Percent organic removed is reported as a mean (n=3) with 95% CI error bars.

3.3.2 Density separation

There was no significant correlation between soil particle size composition (i.e. amounts of clay in a sample) and total microplastics recovery efficiency across all methods (r_s=0.04, p >0.05, Spearman's Rank, (Figure 3.2). There was no significant difference in microplastic recovery efficiency when combining ultrasound with any of the three methods: $ZnCl_2$, NaCl or canola oil (p >0.05 for all, t-test). Ultrasound samples were therefore not considered further. Total microplastic recovery efficiencies from inorganic samples were different between the extraction methods (p <0.01, one-way ANOVA). Mean recovery efficiency was 59% (±1.8 SE) for NaCl, 80% (±1.7 SE) for $ZnCl_2$ and 84% (±2.0 SE) for canola oil extractions (Table 3.2). Canola oil and $ZnCl_2$ recovered significantly more microplastics than NaCl (p <0.01 for both, Tukey's test) but there was no difference in total microplastic recovery between canola oil and $ZnCl_2$ extractions.

The methods recovered fragments and fibres with different efficiencies (p <0.05, one-way ANOVA; p<0.05, Kruskal Wallis); Figure 3.3). $ZnCl_2$ and canola oil recovered more fragments and fibres than NaCl (p <0.01 for all), but there were no significant differences in the recovery of fragments or fibres between $ZnCl_2$ and canola oil methods. There were differences in recovery of large (0.5-1 mm) and small (0.25-0.5 mm) microplastics with the tested methods (p <0.05, Kruskal

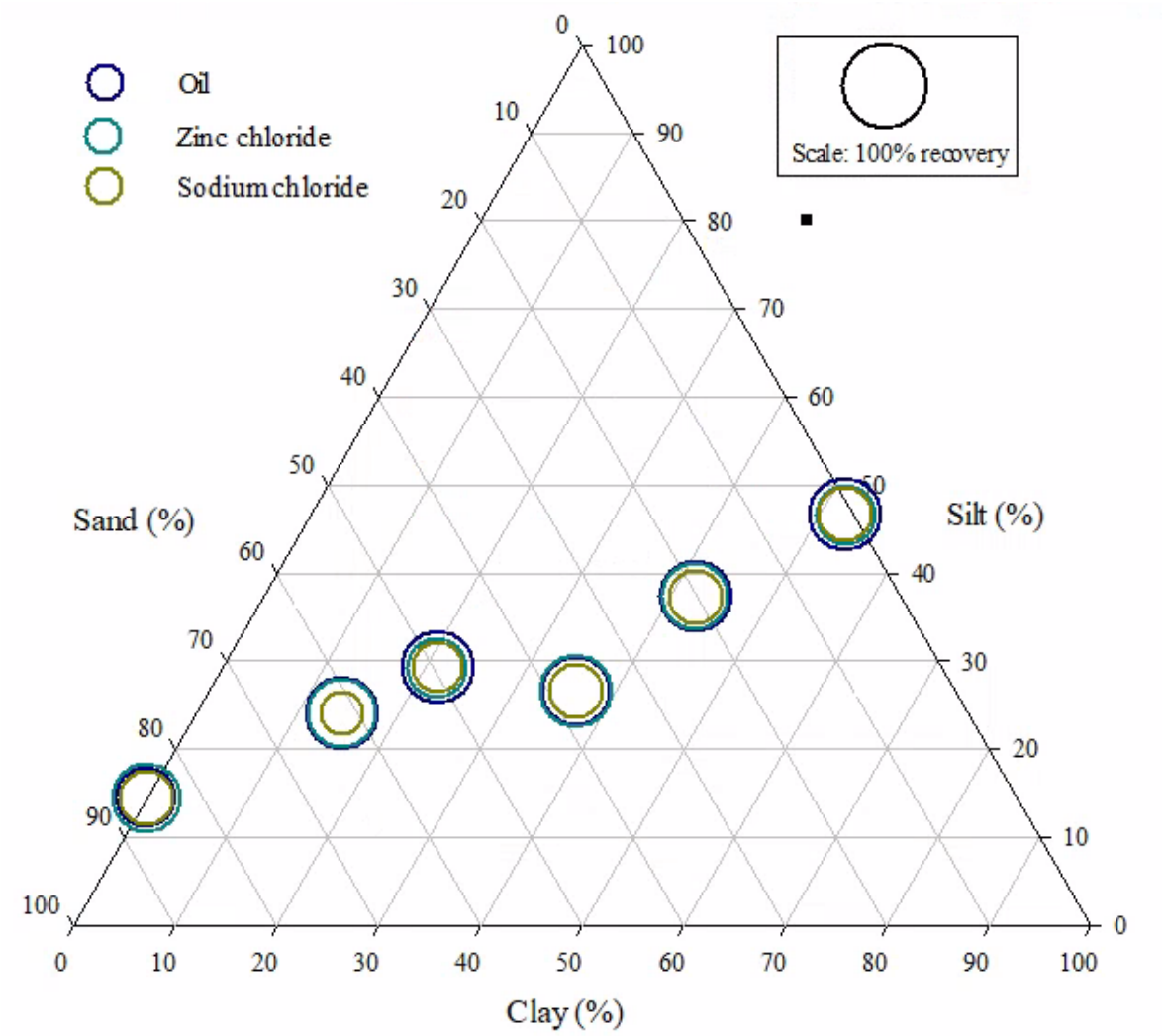


Figure 3.2 Total microplastic recovery efficiencies from inorganic sediments with measuring particle size distributions using canola oil, sodium chloride and zinc chloride. Circle size represents microplastic recovery efficiency.

Wallis test; $p < 0.05$ one way ANOVA). Small microplastics were better recovered with canola oil than NaCl ($p < 0.01$, Tukey's test) but showed no difference between canola oil and $ZnCl_2$ or $ZnCl_2$ and NaCl. Large microplastics had higher recovery efficiencies with canola oil and $ZnCl_2$ than NaCl ($p < 0.01$ for both, Dunn's test) but there was no difference between canola oil and $ZnCl_2$ methods.

Different types of microplastics had different recovery efficiencies with each of the methods of extraction. PET fibres had the lowest recovery efficiency in both NaCl and oil extractions with a mean of 10% (± 4.0 SE) and 68% (± 7.5 SE), respectively. PP fibres had the lowest recovery efficiency using $ZnCl_2$ with a mean of 51% (± 11 SE). The highest recovery efficiency in both $ZnCl_2$ and NaCl extractions was with small LDPE which had mean recoveries of 108% (± 16 SE) and 97% (± 14 SE). Recovery efficiencies were highest for small PVC in oil extractions (99% ± 1.6 SE).

Recovery efficiencies of the densest polymers (PET fragments and fibres, and large and small PVC) were higher with $ZnCl_2$ and canola oil than NaCl ($p < 0.05$, Dunn's test). Large and small HDPE and PS, large LDPE or PP fragments showed similar recovery efficiencies for all three extraction methods.

3.3.3 Method combinations

There was a difference in the total recovery efficiency of microplastics in organic samples when using the three density separation methods ($p < 0.01$, one-way ANOVA; Figure 3.4); 95% confidence intervals for each treatment ranged from 8.6 to 9.0%. In combination with H_2O_2 as a digestion method, $ZnCl_2$ and canola oil showed similar microplastic recovery efficiencies from organic samples and both recovered more microplastics than NaCl ($p < 0.05$ for both, Tukey's test). There was a significant correlation between percent organic content and total microplastic recovery efficiency using the canola oil method ($r=-0.50$, $p < 0.05$, Spearman's Rank); the higher the organic content, the lower the microplastics recovery. However, this was not the case for NaCl or $ZnCl_2$, which both showed no correlation between percent organic content and total microplastic recovery.

Microplastic fragment recovery efficiencies were different across treatments ($p < 0.05$, one-way ANOVA). Higher recovery efficiencies were seen for fragments using $ZnCl_2$ compared with NaCl ($p < 0.01$, Tukey's test). Similarly for large microplastics, the recovery efficiencies were significantly different across treatments ($p < 0.01$, one-way ANOVA). $ZnCl_2$ recovered more large microplastics

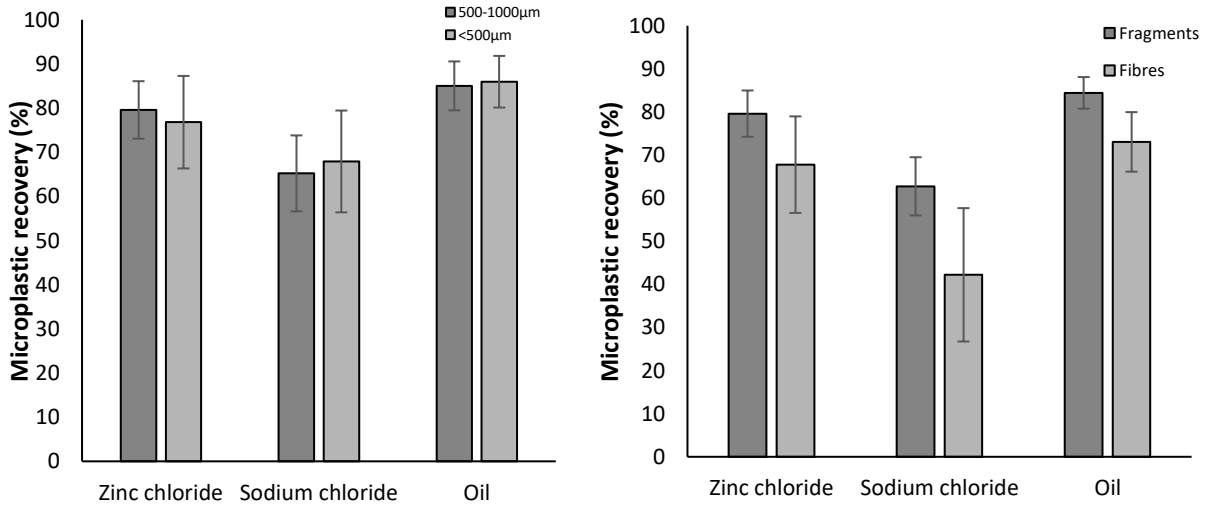


Figure 3.3 Microplastic recovery for large (0.5-1 mm) and small (0.25- 0.5mm) microplastics (left) and fragments and fibres (right) across the three different density separation methods (zinc chloride, sodium chloride and canola oil). Percent recoveries are reported as a mean (fragments: n=3, fibres: n=12, large: n=24, small: n=24) with 95% CI error bars.

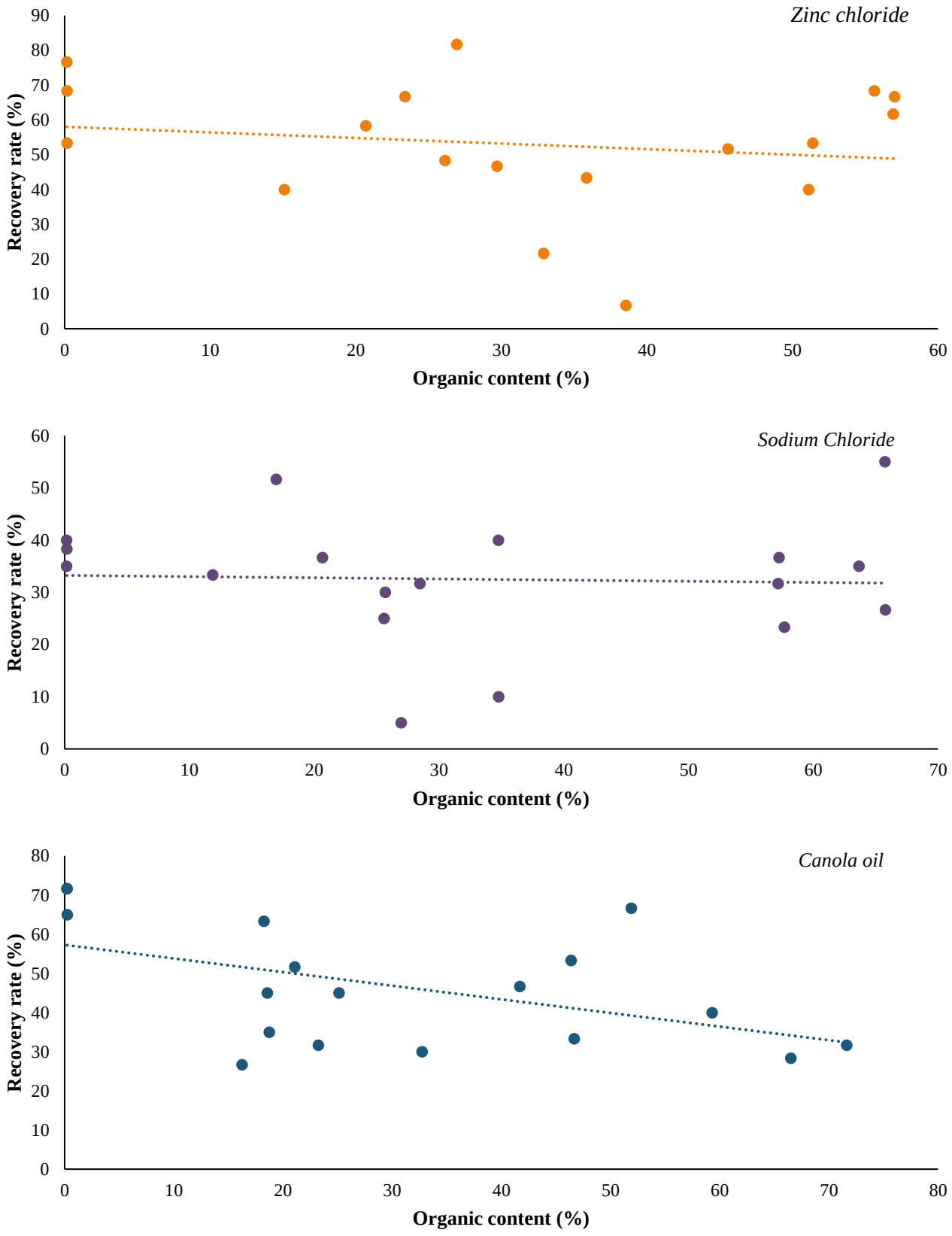


Figure 3.4 Recovery efficiency of total microplastics compared to organic content of samples using hydrogen peroxide to digest and three different density separation techniques: zinc chloride(top), sodium chloride(middle) and canola oil(bottom).

than NaCl ($p < 0.05$, Tukey's test) but there was no difference between these treatments and canola oil. Recovery efficiencies for both fibres and small microplastics did not vary between methods across all these samples ($p > 0.05$ for both, Kruskal Wallis). There was no correlation between fibre recovery efficiency and organic content using any of the treatments or for small microplastics using $ZnCl_2$ or NaCl but there was a significant but weak negative correlation for small microplastics using canola oil ($r=-0.48$, $p < 0.05$, Spearman's Rank).

Recovery efficiencies varied for each microplastic type with the different methods of extraction (Table 3.2.) PET fragments had the lowest recovery efficiency in NaCl with a mean of 10% (± 4.0). Small HDPE had the lowest recovery efficiencies in $ZnCl_2$ (32%, ± 5.4 SE) and large PVC has the lowest recoveries in canola oil (20% ± 6.7 SE). Small LDPE showed the highest recovery efficiencies with NaCl (57%, ± 7.6 SE), large PVC showed the highest recovered microplastic in $ZnCl_2$ (74%, ± 6.6) and PP fibres were the highest in canola oil (66%, ± 8.4). There was a strong negative correlation between recovery efficiencies of PET and small LDPE fragments and organic content using the canola oil method; the higher the organic content, the fewer fragments were recovered ($r_s=-0.69$, $p < 0.01$; $r_s=-0.55$, $p < 0.05$, Spearman's Rank). There were no correlations between recovery efficiencies of any of the other types of microplastics with organic content or density separation technique. In combination with H_2O_2 digestion, multiple density separations significantly increased the number of microplastics recovered using canola oil ($p<0.05$, two-way ANOVA), but not with $ZnCl_2$ or NaCl.

Table 3.2 Mean recovery efficiency (±SE) for individual microplastic types with the tested extraction methods. Inorganic only soils were treated with density separation alone and organic soils were treated with a hydrogen peroxide (H_2O_2) digestion prior to density separation.

Microplastic type	Mean recovery efficiency (%) Inorganic soils			Mean recovery efficiency (%) Organic soils		
	Sodium chloride	Zinc chloride	Canola oil	Sodium chloride	Zinc chloride	Canola oil
PET fibre	10 (±4.0)	62 (±12)	68 (±7.5)	27 (±5.1)	59 (±11)	51 (±9.5)
PET fragment	27 (±11)	93 (±6.4)	77 (±12)	10 (±4.0)	57 (±7.5)	21 (±7.5)
Large HDPE	81 (±8.9)	89 (±7.6)	86 (±11)	42 (±6.8)	46 (±7.6)	59 (±6.8)

Small HDPE	78 (±9.0)	90 (±10)	96 (±3.1)	34 (±6.4)	32 (±5.4)	28 (±6.1)
Large PVC	38 (±12)	83 (±7.6)	96 (±2.0)	11 (±4.6)	74 (±6.6)	20 (±6.7)
Small PVC	34 (±13)	88 (±11)	99 (±1.6)	12 (±4.6)	72 (±8.9)	54 (±8.1)
Large LDPE	77 (±5.3)	80 (±14)	77 (±6.3)	34 (±7.4)	50 (±7.6)	40 (±10)
Small LDPE	97 (±15)	108 (±16)	74 (±6.2)	57 (±7.6)	54 (±8.5)	39 (±5.9)
PP fibre	56 (±9.0)	51 (±11)	78 (±5.8)	47 (±6.0)	57 (±9.5)	66 (±8.4)
PP fragment	71 (±9.6)	76 (±14)	87 (±5.5)	44 (±8.9)	51 (±6.9)	60 (±6.9)
Large PS	72 (±10)	76 (±8.0)	84 (±8.0)	49 (±5.9)	50 (±8.1)	64 (±8.3)
Small PS	73 (±12)	62 (±12)	84 (±11)	22 (±3.2)	33 (±5.6)	56 (±8.2)
Mean recovery	**59 (±1.8)**	**80 (±1.7)**	**84 (±2.0)**	**33 (±2.9)**	**53 (±4.4)**	**47 (±3.7)**

PET, Polyethylene terephthalate; HDPE, High-density polyethylene; PVC, polyvinylchloride; LDPE, Low-density polyethylene; PP polypropylene; PS, polystyrene

3.3.4 Method reagent impact on the spectroscopic properties of microplastics

Organic removal treatments had minimal effects on the ease of identification with FT-IR. All microplastics subjected to organic removal treatments had good mean HQI (>0.7, as defined by Renner *et al*., 2019). The lowest scores were for large PVC treated with KOH at 40°C and 50°C, which had HQIs of 0.71, 0.77, respectively. However, there was no overall difference in HQIs for polymers with different chemical treatments or temperatures. It was not possible to measure the HQIs for PET fibres treated with H_2O_2 at 50^{o}C or KOH at 40^{o}C.

There were some differences in microplastic identification with the different density separation treatments ($p < 0.01$, Kruskal Wallis). NaCl and ultrasound treatments both had significantly better identification HQI scores than canola oil ($p < 0.01$, for both, Dunn's test) and $ZnCl_2$ ($p < 0.01$, for both, Dunn's test) for overall identification. There were significant differences between the different types of microplastic and the identification across density separation treatments ($p < 0.01$, Kruskal Wallis). Despite differences between treatments, all polymers subjected to density separation treatments good mean HQI (>0.7, Renner *et al*., 2019) with the exception of large PVC treated with canola oil, which had a HQI score of 0.49.

Table 3.3 Overall recommendations of method suitability for extracting microplastics from soils of low (<30%) and high (>30%) organic content.

	Reagent	Suitable for use on low organic soils? (<30% organic matter)	Suitable for use on high organic soils? (>30% organic matter)
Organic removal	Hydrogen peroxide	Yes	Yes
	Fenton's reagent	Yes[$]	Yes[$]
	KOH	n/a	No
Density separation	Oil	Yes	Yes[#]*
	Zinc chloride	Yes	Yes*
	Sodium chloride	No	No

*Rinsing with ethanol is required to minimise impacts on identification of microplastics
[#] To be used only on soils of lower organic content
[$] To be used with caution to avoid exceeding polymer heat deflection limits

3.4 Discussion

Standardised methods for quantifying microplastics in the environment, and in particular effective methods for soils, are urgently required. Here methods have been tested and validated several methods and found differences in the efficiency based on the reagents used and soil characteristics. The first step of testing organic matter digestions indicated that KOH is an unsuitable method for removing organic matter from soils, despite its reported success when used on biological samples (Thiele *et al*., 2019). Pre-treatment with the dispersant sodium hexametaphosphate was deemed unsuitable as it decreased the efficiency of H_2O_2 and had no impact on the efficiency of KOH or Fenton's reagent; therefore, it is an unnecessary additional step.

H_2O_2 and Fenton's reagent both resulted in considerable digestion of organic matter (>70%) indicating their suitability for removing organic matter from soils (Figure 3.1). This is in line with previous studies (Vermeiren *et al*., 2020) which also showed that there was minimal difference between organic removal with both treatments in intertidal sediments, although this was also dependant on original organic content. It is recommended here that H_2O_2 is the preferred method as, although both reagents removed similar amounts of organic matter overall, H_2O_2 removed

more from soils with initially high organic content than did Fenton's reagent. Additionally, it is a simpler method to perform, requiring fewer reagents and reduced costs. The reaction can be more easily controlled, as the exothermic reaction of Fenton's reagent requires extra monitoring and control which, if not properly regulated, may result in temperatures >90°C (Qiu *et al*., 2016) leading to the likelihood of polymer damage. As there was no difference in the efficiency of reagents at the two temperatures tested (40 °C and 50 °C), it is suggested that processing samples at 50 °C may be optimal to speed up processing times, allowing for larger number of samples to be processed without altering the chemical structure of polymers by remaining within the heat deflection limits of most common polymers (Qiu *et al*., 2016). Additionally, prolonged exposure to 30% H_2O_2 may cause degradation to some polymer types and therefore should be shortened where possible (Nuelle *et al*., 2014).

In general, density separation has been developed for aquatic sediments and different methods have been tested with good recovery rates (Claessens *et al*., 2013; Coppock *et al*., 2017; Imhof *et al*., 2012). Here it was found that density separation methods had differences in extraction efficiencies in both organic and inorganic soils (Table 3.2). The composition of inorganic soils had no impact on recovery rate for any of the methods tested, similar to previous studies that have shown no difference in recovery efficiency between fine, medium and coarse sediments (Crichton *et al*., 2017). This suggests that particle size does not need to be adjusted for when applying a density separation method when within these ranges, however it may affect to the time required to effectively process a sample, as it relies on particles settling out in a solution, which according to Stoke's Law denotes that the smaller the particle size, the longer it will take to settle (Wang *et al*., 2018). This should be considered when calculating density separation processing time as soils with higher clay content may take longer to separate fully (Filgueira *et al*., 2006). It is suggested that ultrasound is not required in the density separation step of extraction. Despite its use in previous studies (Liu *et al*., 2018a; Lwanga *et al*., 2017a; Zhang *et al*., 2018), it did not increase microplastic recovery efficiency in the present study and can therefore be excluded to simplify methods.

Of the tested density separation methods, NaCl had the lowest extraction rates from both organic and inorganic soils. The recoveries of the higher density polymers PET and PVC, which together make up over 17% of the global plastic demand (Plastics Europe, 2019), were particularly low. Despite this clear bias towards low-density polymers, it is a method that has been used extensively since it was first tested in 2004, predominantly due to its low cost and limited potential for harm (Coppock *et al*., 2017; Pagter *et al*., 2018; Thompson *et al*., 2004). In soils with purely inorganic content, both oil and $ZnCl_2$ had much higher recovery rates than NaCl and which

extended across the different polymer types. Extraction with canola oil recovered the most small microplastics so is preferential for extractions from soils with high inorganic content, as environmental samples tend to be dominated by smaller microplastics (Chen *et al*., 2020; Haave *et al*., 2019; Wang *et al*., 2018). Additionally, $ZnCl_2$ can be more expensive, more hazardous to work with, and more toxic to aquatic biota, whereas oil offers a cheap and relatively safe method (Crichton *et al*., 2017).

In soils containing organic matter, extraction efficiencies were generally much lower (Table 3.2). The organic fraction of soils increases the difficulty of microplastic extraction, even with the addition of a digestion step (Bläsing and Amelung, 2018). In this case, canola oil and $ZnCl_2$ showed similar overall recoveries, however the canola oil method was more obviously impacted by the presence of organic matter as extraction efficiency decreased as organic content increased, particularly for PET fragments and small LDPE. This highlights the importance of including a digestion step to reduce this effect, particularly in environmental samples where microplastics are likely to be coated or aggregated with biological material, which may further reduce efficiencies (Mani *et al*., 2019). Additionally, it is important to note the variability of microplastic recovery within treatments which is higher for zinc chloride than canola oil (Table 3.2), suggesting that canola oil may be a more reliable extraction method for repeatability between samples.

The results how that canola oil can should be recommended to be used for soils with low organic content, but $ZnCl_2$ is required to obtain sufficient extraction efficiencies in soils with higher organic matter content (>30%; Huang *et al*., 2009). It is anticipated that the canola oil method will be suitable for the large majority of soil types as organic matter rarely exceeds 30% (Pulleman *et al*., 2000). Only soils with high organic content, for example peats (Rezanezhad *et al*., 2016), will exceed this and require the use of $ZnCl_2$. When $ZnCl_2$ is used it must be carefully considered in terms of hazards to operators and environmental concern, and precautions must be taken to reduce its impact (Quinn *et al*., 2017; Rodrigues *et al*., 2020). Additionally, oil extraction method may be further optimised by using alternative types of oil, e.g. castor oil, which may be more efficient at extracting microplastics due to their higher viscosity (Mani *et al*., 2019), although this may further reduce suitability for soils with high organic matter content.

Little impact was seen on the identification of microplastics treated with the method reagents, with the majority of HQIs above 0.7. This was expected as reagents were chosen for their previously reported low impact on plastic particles (Hurley *et al*., 2018; Munno *et al*., 2018; Tagg *et al*., 2015). PVC proved to be most susceptible to the tested reagents as it returned the lowest hit scores. This may be due to the characteristically broad C-Cl peak of PVC seen at 690 cm^{-1} which is at the edge of the spectral range measured (4000-650 cm^{-1}) (Käppler *et al*., 2016). This suggests

that consideration of visually matching to reference spectra when identifying PVC with FT-IR is required, and that a more conservative assessment of hit scores may be required to avoid false identification. Additionally, a decrease in HQI scores was seen for some polymers treated with canola oil and $ZnCl_2$. This may be due to the high viscosity of both liquids and hydrophobicity of canola oil, which results in residues remaining on the particles that reduced ease of identification (Renner *et al*., 2019). It is therefore important that a cleaning step (e.g. alcohol rinse) to remove these residues should be further considered (Crichton *et al*., 2017).

This study is the first to compare systematically different methods for extraction of microplastics from soils and highlights the importance of considering sample characteristics when selecting a method for extracting microplastics. Sample-dependent efficiencies should be considered and applied when quantifying microplastics in environmental samples (Table 3.3), similar to the principle of matrix-matched calibrations used in other areas of analytical chemistry (Cuadros-Rodríguez *et al*., 2007). Microplastic recovery efficiency is dependent on the polymer type, shape and size, therefore study-specific calibrations are suggested using a range of different polymers with different shapes and sizes similar to those used here are employed to account for this variation. It should also be considered that microplastics smaller than the size ranges used in this study may have additional complications and are highly likely to be found in the environment, therefore should be considered in future studies, particularly as their large surface area to volume ratio may increase susceptibility to chemical degradation. Additionally the type of recovery microplastics should be tailored to the type of microplastics considered within a study, for example, if a study aims to consider smaller plastics, it should use spiking plastics within that size range. Environmental samples tend to show large compositional differences in types of polymers found (Scheurer and Bigalke, 2018), including different shapes and sizes (Corradini *et al*., 2019b; van den Berg *et al*., 2020) making it especially important to establish methods that will account for this and avoid an underestimation of environmental microplastic concentrations.

3.5 Conclusions

For the majority of common soils, which are likely to have low organic matter content, the preferred method for extracting microplastics from soils involves a digestion step using H_2O_2 at 50°C to remove organic matter followed by a canola oil density separation. These methods proposed do not require specialized equipment, are relatively cheap and have reduced complexity to extract microplastics from soils, while minimising environmental impact and hazard to operators. This approach meets the need of the microplastics research community to allow for method harmonisation, however it is clear that method efficiency must be accounted for to

prevent underestimation of microplastic concentrations and study-specific calibrations must be employed to enable high accuracy within studies. This will allow for the expansion of future research and a greater understanding of microplastic concentrations in soils.

Chapter 4 Agricultural soils and microplastics: are biosolids the problem?

4.1 Introduction

It is now known that microplastics are ubiquitous contaminants globally. As plastic demand continues to rise, with production rates already exceeding 360 Mt (PlasticsEurope, 2021), it is inevitable that plastic pollution in the environment will also increase. This is especially true within the terrestrial environment, as reports estimate much of the plastic litter in the ocean originated on land (Andrady, 2011). There is growing evidence to suggest that terrestrial soils receive microplastics from varying sources with indications that they cause negative impacts on soil ecosystems. In soils, microplastics have the potential to alter physical properties such as bulk density and water holding capacity, with consequences for plant growth (Machado *et al*., 2019).

Of particular concern are agricultural soils. As mentioned previously, they are considered to have high microplastic inputs from plasticulture, fertilisers, atmospheric deposition, irrigation, littering, and surface runoff (Zhu *et al*., 2019; Feng *et al*., 2021; Ramos *et al*., 2015). Managed land has been shown to have elevated numbers of microplastics (Corradini *et al*., 2021), likely due to primary anthropogenic activities including agricultural practices. Of high concern amongst these practices is the use of organic fertiliser, including the application of composts (Watteau *et al*., 2018), animal manures (Yang *et al*., 2021), and biosolids (Crossman *et al*., 2020). Biosolids are applied to land as a fertilizer to improve agricultural yields by increasing essential elements such and nitrogen and phosphorous. Some countries (e.g., Switzerland) have placed restrictions on the use of biosolid application to land, based on their contamination with persistent pollutants and the resulting environmental impacts (Collivignarelli *et al*., 2019; Racek *et al*., 2020). However, in the UK, almost all biosolids produced are spread on agricultural land (Liu *et al*., 2021).
Wastewater treatment systems are designed to remove organic matter and contaminants from wastewater; this includes, for example, natural organic material, pharmaceuticals, metals, and microorganisms, many of which are removed in the form of biosolids (Mohajerani and Karabatak, 2020a). With respect to microplastics, while wastewater treatment systems are reported to remove up to 99.8% of microplastics from final effluents, they instead end up in the solid fraction resulting in their incorporation into biosolids (Horton *et al*., 2021). Numbers of microplastics have been steadily increasing in biosolids since the 1950s (Okoffo *et al*., 2021) with current estimates of millions of microplastic particles per kilogram of biosolid (Cunsolo *et al*., 2021; Horton *et al*., 2021;

Salmi *et al*., 2021). Various sources have been identified as contributing to microplastic loads in wastewater including washing of synthetic textiles (Napper and Thompson, 2016b), personal care products, industrial plastic particles and road runoff (including tyre wear particles) (Ngo *et al*., 2019).

Given these estimates, alongside high rates of biosolid application to land which were reported in 2010 as 1,118,159 tonnes in the UK (Ofwat, 2015), microplastic quantities in soils where biosolids are applied are expected to be high. To date, varying soil microplastic concentrations have been reported. While some studies have found biosolid treated soils to have high microplastic concentrations, up to 10,400 MP/kg (MP size range measured: 8µm – 2mm; Corradini *et al*., 2019), others have found lower numbers, up to 288 MP/kg, (MP size range measured: 55µm – 5mm; Schell *et al*., 2022). Higher concentrations, > 2000 MP/kg have also been observed in soils without previous biosolid treatment (MP size range measured: 50 µm – 1mm; van den Berg *et al*., 2020). In addition, theoretical calculations of soil microplastic concentrations have been made in relation biosolid application (Mohajerani and Karabatak, 2020b; Nizzetto *et al*., 2016). In the UK alone, it is suggested that 2.7×10^{15} microplastics are applied to agricultural soils annually (Horton *et al*., 2021). However, there are limited real-world data addressing this. To date, only 9.2% of microplastic studies looking at soil and biosolid matrices focused specifically on biosolid amended soils (Ziajahromi and Leusch, 2022).

In addition to comparing quantities of microplastics, characterising their properties is key to identifying potential sources. Defining sizes, shapes, and polymer types may enable particles to be traced back to their origins. Some studies have linked specific polymer types to suspected sources. For example, Tagg *et al.,* (2022) reported similar microplastic profiles in biosolid treated soils and biosolids. However, accurate source identification is difficult due to the small size and fragmented nature of microplastics (Ballent *et al*., 2016). Further complications arise as there have been limited studies assessing how microplastic contamination varies temporally. Often studies consider only one sampling timepoint which creates a snapshot in time and may not capture the dynamic variation within the environment (Underwood *et al.,* 2017). Initial studies have reported significant variation in soil microplastic concentrations over time (Crossman *et al.,* 2020) suggesting the importance of multiple sampling timepoints to enable a comprehensive view of soil microplastic contamination.

Sources must be defined and quantified, to be able to target and mitigate microplastic contamination in agricultural soils. The aim of this Chapter was therefore to measure and compare microplastic contamination in agricultural soils with and without biosolid amendment within a defined geographical region (River Test catchment, southern UK). Given the previous

reports of microplastic contamination of biosolids, it is hypothesised that there will be a greater abundance of microplastics in soils treated with biosolids. Quantifying these differences will aid in identifying recommendations for programmes of measures and priorities further research.

4.2 Methods

4.2.1 Study area

Study sites were chosen in the catchment area of the River Test, Hampshire, UK (Figure 4.1). The focus was on one catchment as this location comprises a well-defined landscape unit which allow for assessment of microplastics at an integrating scale (Windsor *et al*., 2019). Characteristics of the catchment area (e.g., slopes and length of river, land use, and soil properties) may be used to inform predictive models inform future mitigation measures for microplastic contamination (Nizzetto *et al*., 2016). The River Test is a designated SSSI (Site of Special Scientific Interest) chalk-bed river (Homewood *et al*., 2005). It is 139 km in length, drains an area of 1269 km^2 (Gallagher *et al*., 2016; Moore *et al*., 1998) and feeds into Southampton water through the Solent estuary. Its catchment area is predominantly rural with high agricultural land use (National Rivers Authority, 1991) and comprises of two main soil types, the Andover and Carstens associations (Cranfield University, 2020). The Andover series is a chalky soil which tends to be shallow and silty with high calcareous content; while the Carstens series is majorly clay based, often with a high flint content. Both have a low carbon content, loamy texture, are freely draining, and commonly utilised for grassland and arable agriculture (Cranfield University, 2020).

4.2.2 Sample collection

Ten arable fields were selected, five of which had historically been treated with biosolids and five that had never received biosolids. Sites were selected to minimise environmental variation between sites. All farms were part of the same cluster group which covers 4550 hectares in the Test Valley and has a total of 20 independent farm members. All field receiving biosolids were from one of two local suppliers. Samples were taken from each field on two occasions during summer (August 2019) and winter (February 2020) seasons. The average rainfall in the month of the summer sampling occasion was 51mm in the South East of England, and 122mm in the month of the winter sampling occasion (Environment Agency, 2020).

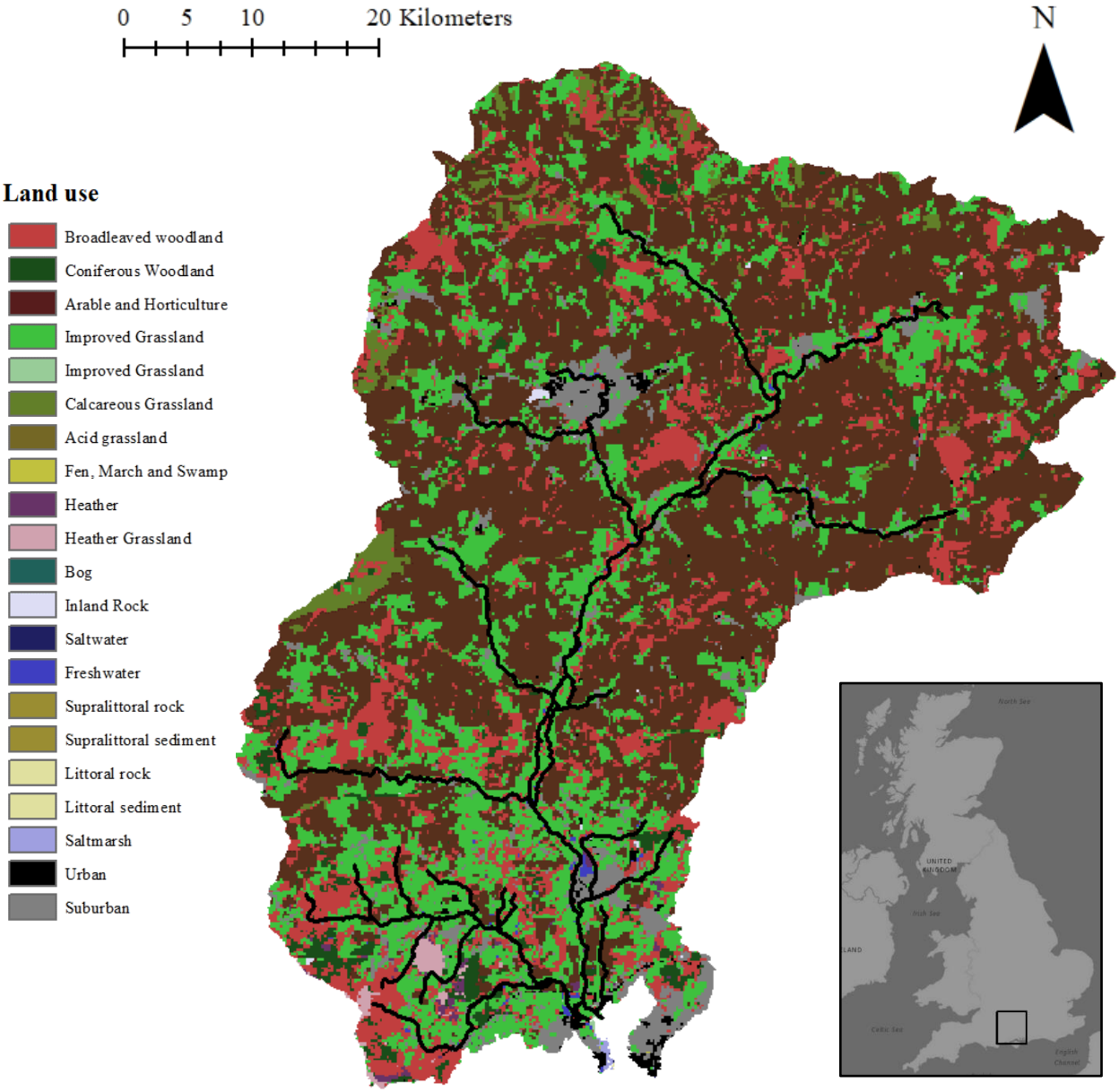


Figure 4.1 The River Test catchment area in Hampshire, UK showing land cover (Rowland *et al*., 2017). Soil samples were collected from 10 fields across the catchment (exact locations of farms are concealed for anonymity).

Environmental factors were accounted for as much as possible when considering the samples to reduce the influence of confounding factors on observations and interpretation thereof. All selected fields were for arable use and had similar agricultural regimes with a typical crop rotation of winter and spring cereal crops and break crop (typically winter wheat, barley, or oilseed rape). Cultivation in all fields was done by using a minimum tillage method, meaning that soil is rotated only in the top layer (typically <10cm). In the treated fields, biosolids were all from the same supplier and had been applied once within the last three years using a spraying method. No

significant plasticulture was used on any of the farms (i.e. plastic mulching or the use of plastic film and tunnel covers).

In anticipation of the likely variability within fields (Harms *et al*., 2021), four replicate samples were taken per field, per sampling occasion, to increase the precision of microplastic measurements. Samples were taken using a stainless-steel pot corer (3 cm diameter, 10 cm height). Each field was split into four quarters, excluding a 4 m buffer zone around the edge to exclude external influences (Piehl *et al*., 2018), and a composite sample was taken for each quarter. Each composite sample was composed of 25 cores taken at random points in accordance with the Sludge (Use in Agriculture) Regulations for soil testing (Public Health England, 1989), and homogenised by thoroughly mixed in a stainless-steel bucket with a metal trowel. A subsample of approximately 300 mL was then removed and stored in a glass jar with a metal lid. Samples were subsequently stored in darkness at ambient temperature.

4.2.3 Site characterisation

Environmental factors that could not be externally controlled were measured and accounted for in the analysis and interpretation. Soil and field characteristics, including organic matter content and particle size distribution, and site characteristics, including slope and distance from roads, were measured. For each field, a further composite sample was taken for these measurements. For each sampling occasion (n=20) equal proportions of the original four replicates were combined for analysis. Soil organic matter content was measured using loss-on-ignition (LOI) at 550 °C. Particle size distribution was measured by sieving for the coarse fraction above 1 mm, and a Malvern Mastersizer 3000 granulometer for the <1 mm fraction. Additionally, these samples were characterised using X-ray fluorescence (XRF) for elemental composition. Metals that are commonly regulated for in biosolids (zinc, copper, nickel; Public Health England and Wales, 1989) were measured using a Niton XL3t GOLDD+ Portable XRF analyser.

Distance to roads and slope of fields were determined using ArcMap (version 10.8.0.12790). The slope of fields was determined using SRTM (Shuttle Radar Topography Mission, USGS) Digital Elevation data at 30 m resolution converted to slope using the Slope (Spatial Analyst) tool. The mean slope was determined within the field boundaries of each site using the Zonal statistics (Spatial Analyst) tool. Distance to roads was determined using the Generate Near Table (Analysis) tool to measure the minimum distance of a field to a major road based on the edge of field boundaries and open roads data (Ordnance Survey open roads, November 2021).

4.2.4 Sample processing

The microplastic extraction method was selected based on the soil characteristics according to Radford *et al*., (2021). Samples were oven dried at 50°C for 7 days. As the soil samples contained a relatively small percentage of organic matter, oil extraction was selected as a density separation technique to remove the inorganic fraction of the soils. A sub-sample of homogenised dry sediment was taken and weighed for extraction (ranging from 14.4 to 35.1g D.W.). Sub-samples were placed into 250mL glass beakers, 50mL of Mili-Q water was added and left to stand for 1 hour to aid soil dispersion. An aliquot of 10mL of canola oil was added to each sample and mixed with a stainless-steel spoon for 30 seconds to break up any agglomerates. Residues on the spoon were rinsed back into the sample with additional Mili-Q water. Each beaker was then filled up with Mili-Q water, leaving a 1 cm gap to the top of the beaker, covered with aluminium foil and left to settle overnight. The top layer of oil was poured into a smaller 150 mL glass beaker, which was covered in aluminium foil and set aside. A second round of oil extraction was performed by adding another 10 mL of canola oil and mixed again with a stainless-steel spoon for 30 seconds. Beakers were then filled up with Mili-Q water and left overnight as before. Again, the top layer of oil was poured into the same 150 mL beaker as previously to combine the two rounds of oil extraction. The entire contents of the 150 mL beaker were filtered using a vacuum pump over a 25 µm stainless steel filter. The filter was placed into a 100 mL glass beaker and any residues from the filtering apparatus were rinsed in with Mili-Q water.

In a fume cupboard, 30 mL of hydrogen peroxide (30% v/v) was then added to the samples, which were covered in aluminium foil and placed in a shaking incubator at 50°C, 100rpm overnight. The same 25 µm stainless steel filters were then rinsed off into the beaker using Mili-Q water and used to filter the contents of the beaker again using a vacuum pump to remove all remaining hydrogen peroxide. This time, the residues of the filter were rinsed, using minimal water, back into the beaker and the filter discarded. Each sample was then topped up with 30 mL of Decon90, chosen to remove any remaining oil residues that would impede later identification methods (Stead *et al*., 2020). After 48 hours, the Decon90 was filtered out using a vacuum pump over a 25 µm stainless steel filter and rinsed with Mili-Q water until no bubble formation occurred. The residues were separated out by size using 1 mm stainless steel mesh and rinsed with ethanol (50% v/v) into a 20 mL glass vial for storage, one containing >1 mm particles and one with <1 mm. Only small microplastics (<1 mm) were analysed and will hereafter be referred to as microplastics.

4.2.5 Polymer identification

Polymers were identified using automated µFourier-transform infrared spectroscopy (µFTIR) (PerkinElmer Spotlight 400). Sub-samples of processed soils were taken to control quality of filters and ensure overloading didn't reduce spectrum quality. Filter areas were limited using a silicone washer (8 mm diameter), placed on to a silver filter on a vacuum filter set up. The <1mm vial for each sample was well mixed by pipetting up and down in the vial using a glass 10 mL pipette, before pipetting a subsample onto the silver filter (3 µm pore size, Sterlitech, Washington USA). The amount of subsample was determined based on the amount of particles present in the sample and quantified by weighing the vial before and after to determine the weight (and thus volume) of the subsample as per (Horton *et al*., 2021).

Filters were left to dry in a glass petri dish at room temperature for at least 24 hours prior to scanning. An area of 8.5 x 8.5 mm was scanned for each sample to cover the filter area. The filter was scanned with 2 × 8 linear arrays in reflectance mode with 2 scans per pixel at a pixel resolution of 25 µm and spectral resolution of 8 cm^{-1} in the range of 4000–700 cm^{-1}. A background spectrum was collected on a clean space of the silver filter with the same settings at 90 scans per pixel prior to each analysis.

Spectral maps were processed and analysed using siMPle software (Primpke *et al*., 2020; available at www.siMPle-plastics.eu). The Aalborg University pipeline using raw and first derivatives was used, with a minimum particle size of one pixel (25 µm). Particles were identified using the siMPle automated IR database (version 1.0.1) and classified by size and mass (as an estimate based on particle volume, polymer density, and an assumed ellipsoid 3-dimensional shape). It should be noted here that while cellulose itself is not considered a plastic it is included here as artificially modified cellulose as it forms the basis of many artificial polymers and is therefore often counted in studies to account for semi-synthetic materials such as viscose and rayon of which cellulose is the base material (Remy *et al*., 2015; Scott *et al*., 2019).

4.2.6 Quality control

Stringent quality control measures were taken throughout the experiment. In the field, only metal and wooden sampling equipment was used, and clothing was limited to natural fibres where possible during sample collection, processing, treatment and analysis. In the laboratory, extractions were carried out in an ISO-5 clean laboratory and in a laminar flow cabinet (Felcon), where non-shreddable Tyvex suits (Dupont, IsoClean) were worn at all times, with the exception of digestions which were carried out in a separate laboratory in a fume hood wearing a cotton lab

coat, for the purpose of health and safety. All processing equipment was glass or metal. Metal equipment (i.e. stainless-steel spoons and aluminium foil) was furnaced at 500°C for 9 hours and all glassware was acid washed and rinsed thoroughly with Milli-Q water prior to use. All reagents were filtered prior to use over a GF/C glass-fibre filters (1.2 µm, Whatman GF-C) and all water used was Milli-Q. PTFE wash bottles were used to dispense Milli-Q and ethanol where required. All stainless steel and GF/C filters were furnaced at 500°C for 9 hours prior to use to remove any particulate contaminants. Sample analysis using FTIR was conducted in a separate laboratory where cotton lab coats were worn. The FTIR microscope was encased with a Spotlight atmospheric enclosure made of Plexiglas to limit atmospheric contamination. Ten procedural blanks were conducted alongside the extractions and used to correct all data for procedural contamination using limit of detection (LOD) values. The LOD value for each was calculated as 3.3 times the standard deviation and accounted for based on individual polymers (Horton *et al*., 2021).

Additionally, spiked samples were processed as positive controls to determine recovery efficiency of the microplastic extraction method. A stock solution was created using known concentrations of four types of microplastic fragments: PET (66 MP/mL, size: 34- 149 µm), PE (8 MP/mL, size: 30-96 µm), PP (711 MP/mL, size: 66-140 µm), PVC (73 MP/mL, size: 99- 333 µm), dispersed in MiliQ water. Six replicates of one soil sample were spiked with this stock solution and were processed as per the soil extraction and identification method. Recovery rate was then calculated as a percentage of the concentrations of each microplastic type added to the sample. This was used as a reference and not directly accounted for as is currently standard in microplastic research as recovery rate is specific to microplastic characteristics (shape, size, polymer type etc.) and therefore a blanket correction is not deemed appropriate given the diversity of environmental microplastics and limited representation in the spikes used.

4.2.7 Statistical analysis

All statistical analyses were conducted in R Studio (1.4.1106). Microplastic count and mass data were blank corrected using LOD values as per Horton *et al*. (2021), whereby only data greater than the average + 3.3 SD of the blank samples were reported. Where required, data were checked for normality using Shapiro Wilk tests. Mixed models were used to analyse differences in microplastics counts, weights and average size across biosolid treatments and seasons. Data were converted to integers and a GLMM (Generalized Linear Mixed Model) was fitted to a Poisson distribution and log link. Field replicates were nested in each sampled field as a random effect to account for repeated measurements of the same field. All models included the two-way

interaction between biosolid application and season, and Tukey's post hoc tests were used to determine differences across treatments. Data were transformed when required to ensure model fit– mass data were cubed, and average size was square rooted. Residual distributions were checked to assess model fit.

For individual polymers, data were transformed to binary (presence or absence of polymer types) and a GLMM was fitted with a binary distribution. Again, field replicates were nested in field ID as a random and a two-way interaction between biosolid application and season was included. Shannon Diversity index was calculated for each field on each sampling occasion to determine polymer diversity (Sun *et al.*, 2021). An average across the four replicates was taken per field and differences across treatments and sampling occasions were calculated using Kruskal Wallis tests as the data did not meet the requirements for parametric assessments. Additionally, a principal component analysis (PCA) was applied to determine which polymers best accounted for the variability between treatments and seasons.

Co-variates were accounted for separately to rule out the influence of soil characteristics, including particle size distribution (as % clay particles), organic matter and metal content which were analysed using a 2-way analysis of variance (ANOVA) or Kruskal-Wallis Rank Sum tests, depending on data distribution, to determine differences between seasons and treatments. Distance to roads and slope of field were analysed using a Mann Whitney-U test and T-test, respectively, to determine differences between biosolid treatment groups. Factors that were not significant between treatments were excluded from the models to improve model accuracy.

4.3 Results

4.3.1 Quality control

There was minimal contamination within the blanks with a mean of 2.5 microplastics per sample. Five polymer types were found across the blank samples which were 'Acrylates, Polyurethanes, and varnishes' (APV), artificial modified cellulose, 'Ethylene-Vinyl-Acetate' (EVA), polyester, and polypropylene. Polyester was the most prevalent with a mean of 1.5 microplastics per sample whereas artificial modified cellulose was only found in one blank sample. The LOD (3.3 x the SD of the blank samples) for individual polymers therefore ranged from 1 to 10.11 microplastics per sample meaning that, for microplastics to be detected, quantities within one sample vial needed to exceed these values for individual polymers. The average recovery of microplastics across the four types of spiked microplastics was 42%. Note, subsequent microplastic concentrations

reported were not corrected for recovery (in line with current microplastic research studies e.g., Horton *et al.,* 2021) due to the high variability between recovery rates of microplastics types (size, shapes, and polymers).

4.3.2 Covariates

Covariate measurements are shown in Figure 3.1. There was no difference in the amount of organic matter in the soils between the biosolid treatments or seasons ($F(1, 16) = 0.47$, $p = 0.505$), the minimum organic matter content was 3.6% and the maximum was 8.9%. The particle size distribution (% clay particles, <0.4µm) did not vary between seasons or biosolid treatments ($W=74$, $p=0.075$; $W=54$, $p=0.796$, Mann Whitney-U tests) and ranged from 1.07 to 4.99%. Additionally, there were no differences in soil metal contents between biosolid treatments or seasons (zinc: seasons $W=56.5$, $p=0.646$, biosolid treatments $W=29.5$, $p=0.126$; copper: seasons $W=66.5$, $p=0.197$, biosolid treatments $W=57.0$, $p=0.600$) and nickel was below the LOD (11ppm; Shand and Wendler, 2014) for all samples. Zinc concentrations ranged from 60 to 170 ppm across

Table 4.1 Mean covariate measurements for soil characteristics and geographical features. Values for particle size, organic matter and metals are given as means across the biosolid treated and untreated groups in summer and winter (n=20). Distance to roads and slope are given as mean per field sampled (n=10).

		Biosolid treated		**Biosolid untreated**	
		Summer	Winter	Summer	Winter
Particle size distribution (%)	**Clay (<4µm)**	*2.36*	*1.99*	*3.25*	*1.92*
	Silt (4-63µm)	*13.47*	*11.95*	*13.33*	*12.15*
	Sand (63-1000µm)	*4.5*	*4.34*	*4.3*	*3.73*
	> Sand (>1000µm)	*79.67*	*81.72*	*79.11*	*82.2*
Organic matter (%)		*6.62*	*5.4*	*6.94*	*6.42*
Zinc (ppm)*		*80*	*90*	*106*	*102*
Copper (ppm)*		*30*	*20*	*24*	*16*
Nickel (ppm)*		*<LOD*	*<LOD*	*<LOD*	*<LOD*
Distance to roads (m)		*60.52*		*7.04*	
Slope (°)		*2.46*		*2.5*	

*LOD for zinc= 2.9 ppm, copper= 11 ppm, nickel= 11 ppm (Shand and Wendler, 2014)

all samples while copper ranged from 0 to 40 ppm. These metal concentrations are in line with background concentrations across England and Wales. Soils collected for the National Soil

Inventory report mean concentrations of zinc, copper and nickel to be 97.1, 10.6 and 24.5 ppm, respectively (McGrath and Zhao, 2006). While the mean concentrations across all fields were 94.5 and 22.5 ppm for zinc and copper and <LOD for nickel. The mean distance of sampling sites to roads was 33.78m there was no difference between biosolid treatments (W=16, p=0.548, Mann Whitney-U test). The mean slope of sampling fields was 2.48° and there were no differences across groups (t (7.83) = -0.0837, p-0.935, T-test).

4.3.3 Microplastic quantities

Microplastics were found in all ten of the fields sampled on at least one occasion. There were only two instances where no plastic was found in fields, both on the summer sampling occasion. The highest number of microplastics was found in one of the biosolid treated fields with a mean of 1486(±1064 SE) MP/kg across the two sampling occasions. While another biosolid treated field had the lowest number of microplastics across the two sampling occasions with a mean of 202(±87 SE) MP/kg.

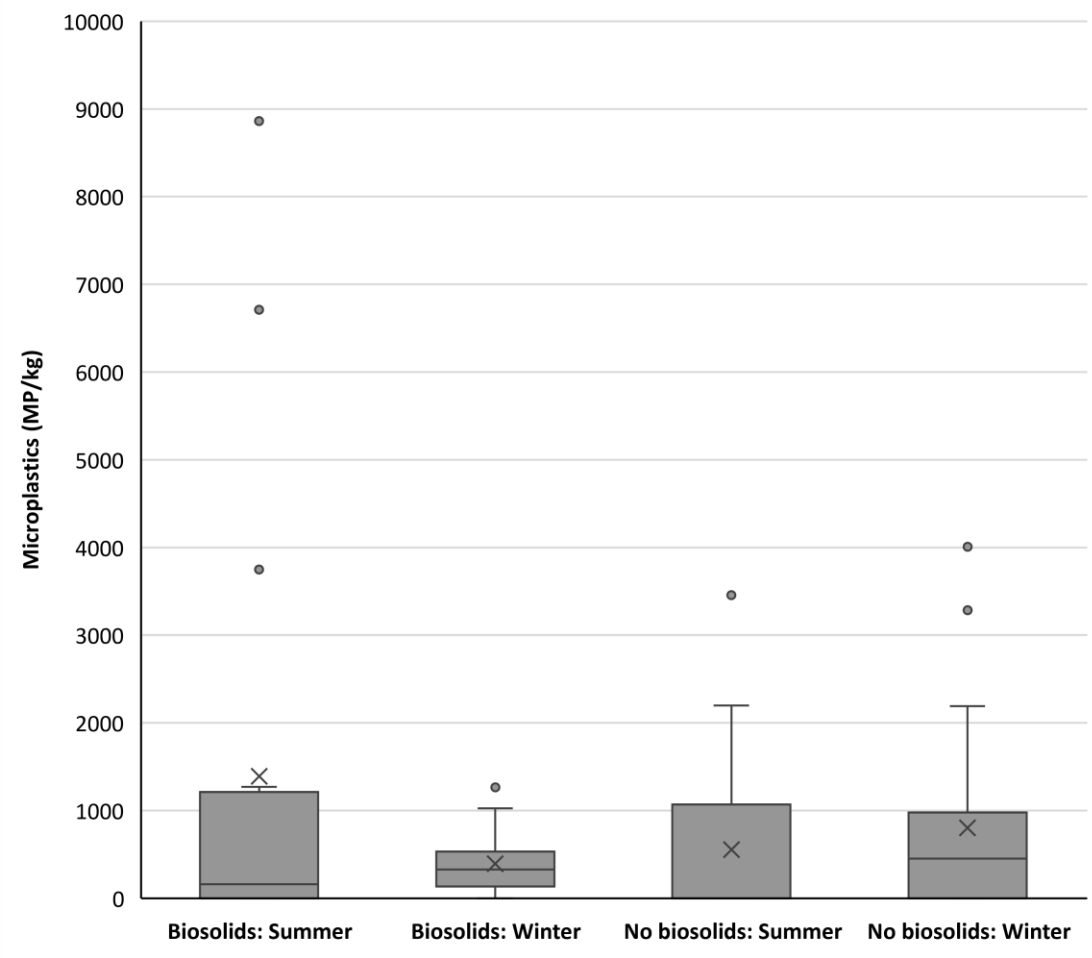


Figure 4.2 Microplastic concentrations in soils with and without biosolid application during summer (August 2019) and winter (February 2020) months.

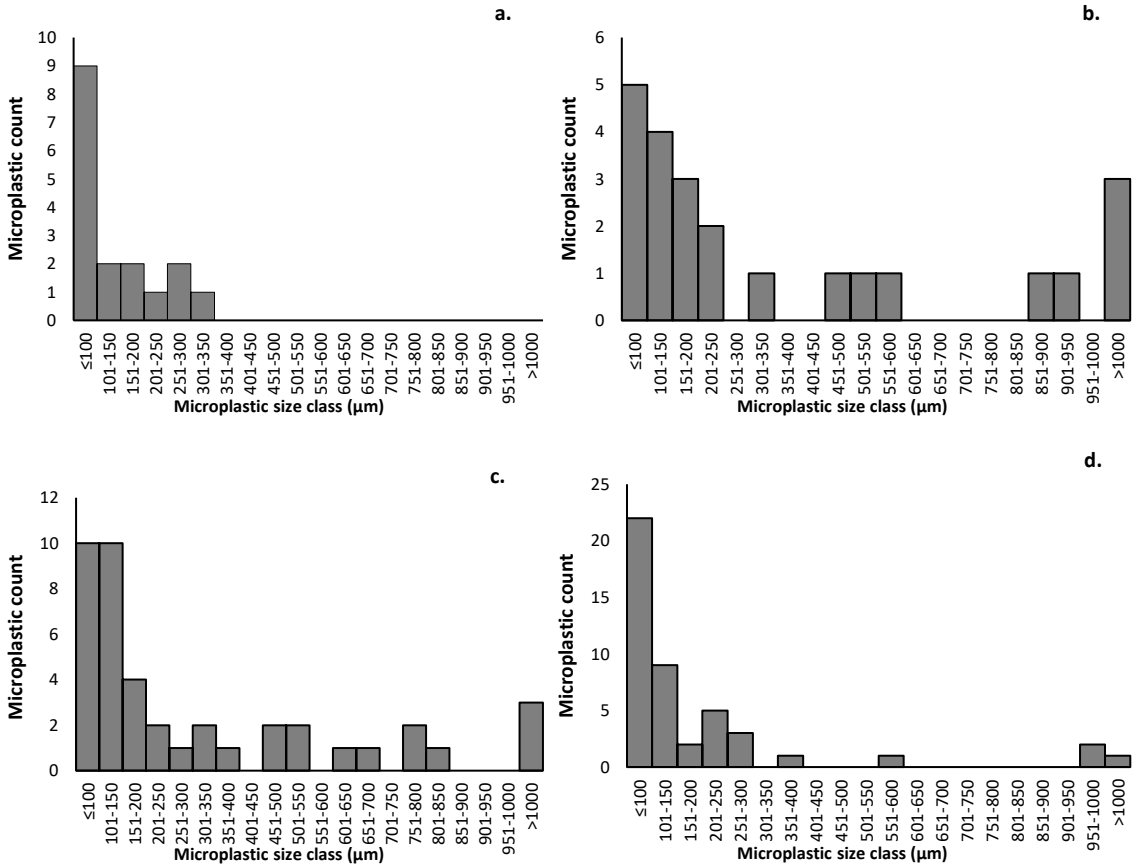


Figure 4.3 Size categories for microplastics found in soils with and without biosolid treatment in summer and winter (a. No biosolids: summer; b. Biosolids: summer; c. No biosolids: winter; d. Biosolids: winter). Microplastic count represents the raw data and have not been blank corrected.

There were no differences in the overall number of microplastics in biosolid treated and untreated soils with medians of 314(809 IQR) and 75(967 IQR) MP/kg, respectively ($\chi2$ (1) = 0.68, p = 0.411, Figure 4.2). The same was true for the median size of microplastics, which was 152 µm (197 IQR) in the untreated soils and 125 µm (134 IQR) the treated soils ($\chi2$ (1) = 0.597, p = 0.441), and the mass of microplastics with medians of 204(262 IQR) and 0(307 IQR) µg/kg, in the treated and untreated soils respectively ($\chi2$ (1) = 0.013, p = 0.971).

Significant differences were evident in microplastic quantities and characteristics between summer and winter. There were more microplastics overall in the samples taken in the summer with a median of 0 (1076 IQR) MP/kg compared with a winter with a median of 402 (797 IQR) MP/kg ($\chi2$ (1) = 2232.04, p < 0.001). The soils treated with biosolids followed this trend and had significantly more microplastics in the summer than the winter, however there was higher variation in the microplastic concentrations in the treated summer soils (median: 161 MP/kg, 1103 IQR) than those in the winter (median: 330 MP/kg, 384 IQR; Tukey Test: z =-98.88, p <0.001).

The soils without biosolid treatment had significantly more plastic in the winter than the summer (Tukey Test: z = 29.87, p <0.001) with medians of 452 (967 IQR) and 0 (889 IQR) MP/kg, respectively. The size of microplastics in the winter was larger than the summer ($\chi 2$ (1) = 441.32, p < 0.001), despite high variation and medians of 115 µm (143 IQR) in the winter compared to summer where the median size was 174 µm (176 IQR). Specifically, within the treated soils the median microplastic size was smaller in the winter at 104 µm (68 IQR), compared to the summer with a median of 177 (337 IQR) µm (Tukey Test: z = 4.24, p <0.001, Figure 4.3).

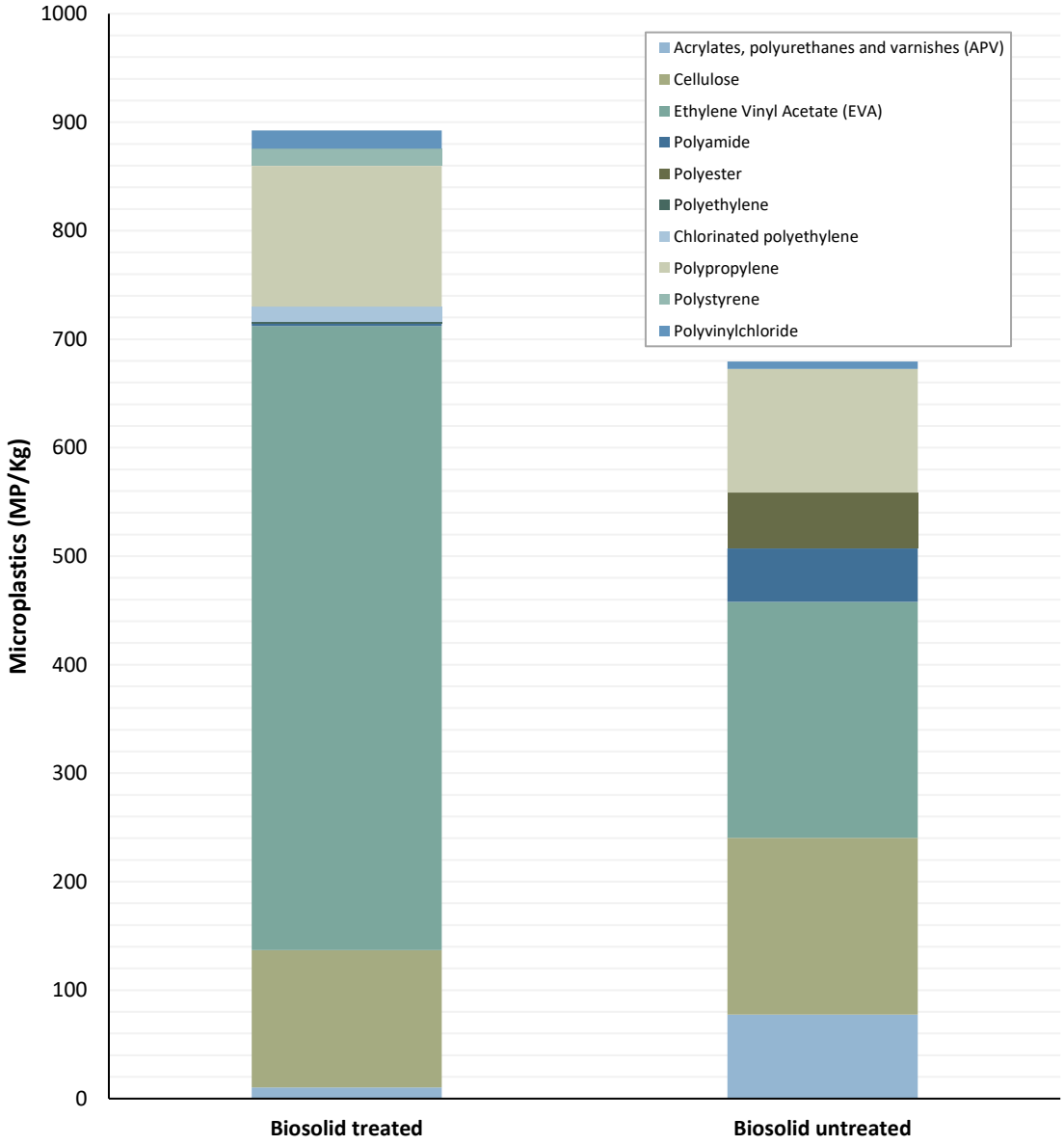


Figure 4.4 Polymer types of microplastics in soils with and without biosolid treatment. Counts are reported as number of microplastics of each polymer type per kilogram of soil and have been blank corrected using calculated LOD's.

Overall, there was a higher mass of microplastics in the summer than the winter sampling occasion ($\chi2$ (1) = 3.95, p=0.045). This overall difference was driven by the significantly higher mass of plastic in the summer month in the treated soils which had a median of 56 (430 IQR) µg/kg compared to the untreated which had a median of 0 (127 IQR) µg/kg (Tukey Test: z = -2.98, p 0.011). When considering the treated vs untreated soils separately there was a higher mass of microplastics in the summer for treated soils (Tukey Test: z = -10.51, p <0.001). However, in line with the differences seen in microplastic numbers, there was more plastic, by mass, in the untreated soils in the winter than the summer (Tukey Test: z = 10.48, p <0.001).

4.3.4 Microplastic composition

Ten different polymer types were identified across the samples (Figure 4.4) The most common polymers were polypropylene and EVA. Polypropylene was found in 24 of the total 80 samples with a mean of 122(±29 SE) MP/kg, whereas EVA was only found in 20 samples but in higher concentrations with a mean of 396(±150 SE) MP/kg. Nine of the polymer types were found in biosolid treated soils, whereas only 7 were found in the untreated soils. Polyethylene, chlorinated polyethylene, and polystyrene were the least common polymers found and were only present in the biosolid treated soils. Polyester was only found in the untreated soils. With the exception of polypropylene and artificial modified cellulose, there were no significant differences in individual polymer types across the treatments and seasons. Polypropylene was found in more frequently in the winter months, occurring in all 10 of the sampling locations, whereas in the summer it was only found in four ($\chi2$ (1) = 12.41, p <0.001). Similarly, artificial modified cellulose was found in eight of the fields in winter but only three in the summer ($\chi2$ (1) = 4.05, p 0.044). Diversity indices for polymers were very low across sites (mean 0.20 ±0.04). Samples had uneven polymer distribution in terms of relative abundance of individual polymers and this diversity did not vary across treatments ($\chi2$ (1) = 0.74, p =0.389, Kruskal Wallis test). However, polymer diversity was higher in the winter months with a mean of 0.29 ($\chi2$ (1) = 6.90, p= 0.009, Kruskal Wallis test).

To prevent distortions polyester, polyethylene, chlorinated polyethylene, and polystyrene were excluded from PCA analysis as they all only occurred once across all samples. The first principal Component (PC1) explained 23.0% of the variance. Polypropylene, artificial modified cellulose and polyamide were all negatively correlated with this axis and made up the majority of the loadings. Principal Component 2 (PC2) explained a further 17.9% of the variation in these data and was largely made up of APV and PVC. APV was negatively correlated with this axis and PVC was positively correlated. The untreated winter soils had the least amount of variation along this axis, indicating low numbers of PVC and APV. Soils from different treatments and seasons cannot be

clearly distinguished from each other based on polymer composition (Figure 4.5). Of the biosolid treated soils, in the summer PC2 looked to increase with PC1 whereas in the winter this was reversed and PC2 decreased with increasing PC1. However, there was an overall lack of differentiation between the composition of microplastics across treatments and seasons suggesting they cannot be clearly distinguished from each other based on polymer composition(Figure 4.5).

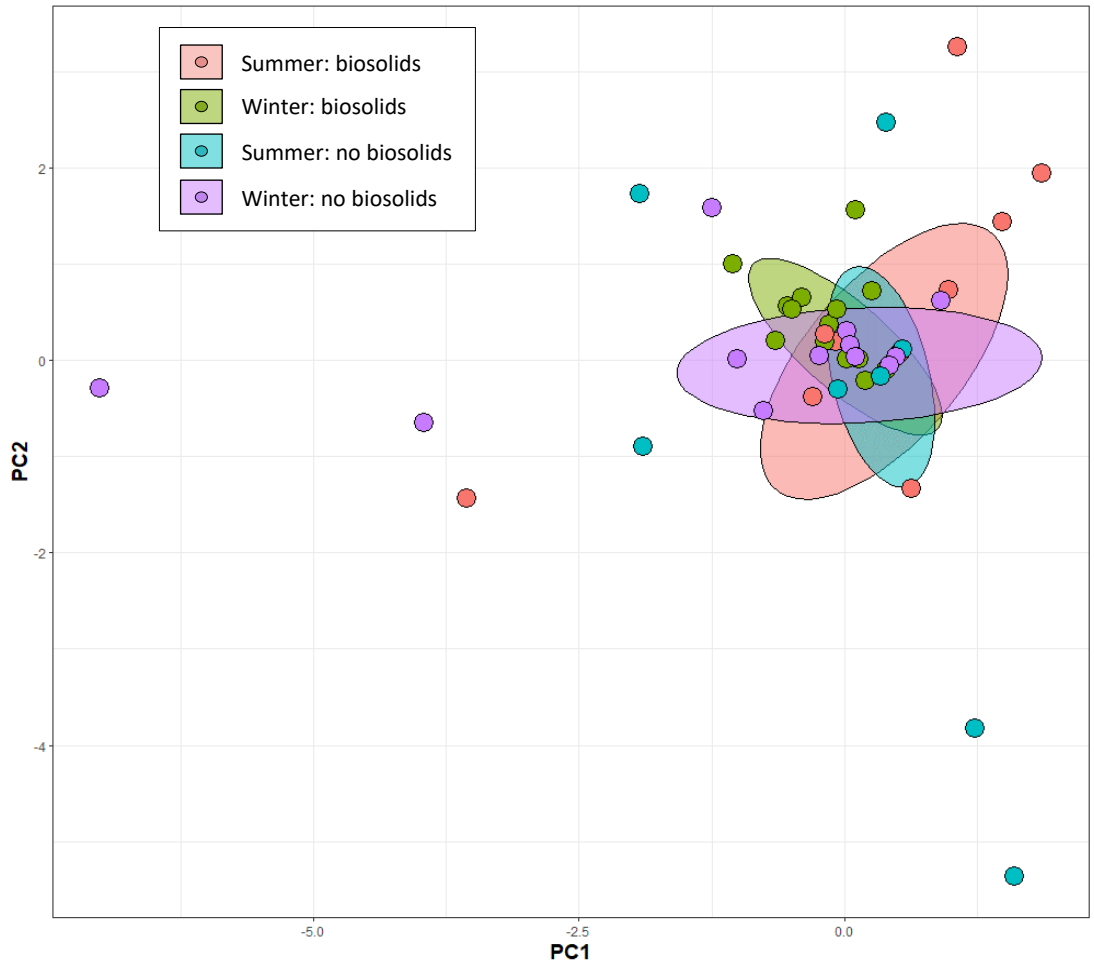


Figure 4.5 Principal component analysis showing the ordination of treatment groups (biosolids or no biosolids) across summer and winter seasons. Polymers with only one occurrence across all samples were excluded from analysis.

4.4 Discussion

The observations of this study suggest that biosolids are not the only source of microplastics in the agricultural soils investigated. While the mean number of microplastics was slightly higher in the biosolid treated fields (874 MP/kg), than the untreated fields (664 MP/kg), there were no significant differences found in the number of microplastics between the two groups. Despite this,

overall concentrations of microplastics were relatively high, with a maximum of 1461 MP/kg, and were found across all the sampled fields on at least one occasion. These results correspond with observations recorded elsewhere. Biosolid amended soils in Chile were found to have microplastic concentrations in the range of 600 to 10,400 MP/kg (MP size range measured: 55µm – 5mm; Corradini *et al*., 2019). In contrast, lower values were found in central Spain where soils recently treated with biosolids had microplastic concentrations ranging from 138 to 288 MP/kg, and 31 to 120 MP/kg in control soils that had not received biosolids (MP size range measured: 50µm – 5mm; Schell *et al*., 2022). Other studies indicate that biosolid application is a main driver of soil microplastic concentrations (Corradini *et al*., 2019), however, the lack of difference between biosolid treated and untreated fields here suggest that they are not solely responsible for microplastic contamination in agricultural soils.

There is no doubt that biosolids contain extremely high quantities of microplastics as this has been reported on multiple occasions. Reports of microplastic concentrations in biosolids vary between studies but have been reported in the millions (per kg D.W.) in several studies with a highest value of 10,380,000 MP/kg reported to date (MP size range measured: 38 - 100µm; Cunsolo *et al*., 2021, MP size range measured: 25- 178µm; Horton *et al*., 2021, MP size range measured: 20– >300µm; Salmi *et al*., 2021). Despite these high numbers, overall indications from the present and previous studies are that numbers of microplastics are generally similar between biosolid-treated and untreated soils, although within both groups there is a great deal of variation within the data (Ziajahromi and Leusch, 2022). For example, Crossman *et al*. (2020), tested soils from three fields and showed that there was an increase in microplastic concentrations following biosolid application for two sites but not the third. There are likely other factors that contribute to the high variation in overall microplastic concentrations in soils.

This lack in difference found in microplastic concentrations between the two groups may have several explanations. Firstly, there may be other sources that input at varying scales, meaning that fields without biosolid treatment may be exposed to high numbers of microplastics. One of the main additional sources to consider are agricultural plastics. Although sites with plastic mulching were avoided, other agricultural items such as plastic films (including silage wrap), nets, irrigation pipe, fertilizers sacks, pesticide cans (Scarascia-Mugnozza *et al*., 2011b) and polymer seed coatings (Clayton *et al*., 2004) may be contributing. The most common polymer types found in this study were polypropylene and EVA. Polypropylene has been frequently found in both biosolids and soils (Horton *et al*., 2021; Liu *et al*., 2018b; Piehl *et al*., 2018); however, it is also often used in agricultural packaging, piping, sheeting, nets, and twines (Scarascia-Mugnozza *et al*., 2011b) and more niche uses such as tree guards which are often found in field margins (Chau *et al*., 2021).

Additionally, EVA is a copolymer frequently used in agricultural films and has previously been reported in soils that have not received biosolid amendments (Corradini *et al*., 2021). The lack of differentiation in polymer diversity between treatments suggests that there is more to consider than the biosolid application. Although there were three polymer types which were only found in the biosolid treated soils (polyethylene, chlorinated polyethylene, and polystyrene), these polymers were rare and only occurred once across all samples making them harder to sample and account for (Cunningham and Lindenmayer, 2005).

Another source that may be considered is atmospheric deposition, which has shown to account for 136.5 to 512.0 MP/m^2/day, even in rural areas (MP size range measured: 50- >300µm; Klein and Fischer, 2019). This deposition will depend on wind direction and speeds (Allen *et al*., 2019) and may be responsible for horizontal migration of microplastics across geographical locations, even with the possibility of the microplastic load in biosolid treated soils to be distributed to non-treated areas (Tagg *et al*., 2021). This deposition is likely to be impacted by proximity to urban areas (Koutnik *et al*., 2021) and distance to main roads (Fakour *et al*., 2021). So, given that all sampled fields were within proximity to roads (<300m) there is high potential for microplastics to be entering these soils through such sources. Additionally, patterns of microplastic patchiness were shown in the present study, evident by the high variation in microplastic concentrations within sampled fields. This is a potential sign of fragmentation of larger plastic debris (Harms *et al*., 2021c). Secondary sources such as the breakdown of larger plastic litter may contribute (Bläsing and Amelung, 2018). The varied polymer composition and diversity found across all sites in the present study suggest a range of sources may be contributing to the overall microplastic contamination. It remains challenging to determine the exact origin of these microplastics, particularly from diffuse sources that are harder to identify (Campanale *et al*., 2022b).

In addition, the variation in the data may be attributable to individual farm management. Whilst all efforts were taken in this study to factor out influencing variables (crop rotations, cultivation methods, use of plasticulture), agricultural practices are complex and often farm-specific. Ploughing frequency and intensity, crop types and the use of fertilizers (inorganic and other organic e.g., animal slurry) may all impact the amount and distribution of microplastics (Harms *et al*., 2021). The rate and frequency of biosolid application may have an impact too. Soils with historic biosolid treatment, as opposed to a recent one-off application, may have higher microplastic concentrations due to cumulative inputs (Corradini *et al*., 2019b; Schell *et al*., 2022). The types of biosolids and associated methods of application may have an impact on the overall microplastic distribution (Yang, Li, Li, *et al*., 2021). For example, with the soils sampled here, spraying of sludge cake was implemented which generally leaves non-uniform distribution of

biosolids and consequently the contained microplastics (see supplementary information, Figure S3). This may explain the patchiness in microplastic concentration and composition within the sampled fields.

Additionally, ploughing and tillage activity may influence microplastic behaviour in soils by further fragmenting plastics and incorporating them into the soil (van den Berg *et al*., 2020). As is common in modern UK farming practices, all farms sampled here used a minimum tillage approach, where only the top layer (<10cm) of soil is turned over. This approach has the potential to reduce the retention of microplastics in soils, particularly at depth, as water infiltration is reduced (Mirzavand and Moradi-Talebbeigi, 2021), increasing the potential for microplastics to be washed off via surface runoff.

The outputs of materials from these systems may influence the number of microplastics found. It is known that large amounts microplastics are being applied in biosolids to these soils, but as this was not reflected in the soil microplastic concentrations, it suggests many of these microplastics are not staying where they are applied. Samples taken are a snapshot in time and outputs due to erosion, runoff and wind transport should be taken into account when considering overall microplastic concentrations, which may not be retained indefinitely. This was indicated by the difference in microplastic numbers between summer and winter samples. During the month of the summer sampling occasion, the total rainfall was 51 mm, and a mean of 967 MP/kg was found across all sites, whereas in the winter month, rainfall was much higher (122 mm) and the mean microplastic concentration was lower at 571 MP/kg. Rainfall and resultant surface runoff may cause microplastics to be removed from the soils (Kim *et al*., 2021). Previous studies have shown similar patterns of lower microplastic concentrations, with losses of 30-45% from soils, following heavy rainfall events (Crossman *et al*., 2020). In addition, the resulting erosion of agricultural land is seasonal, with increased erosion prevalent in winter months (Boardman *et al*., 2020). This will depend on the soil type as more dense soils are more susceptible to runoff and will therefore likely retain less plastic (Crossman *et al*., 2020). The mean microplastic size found here was larger in the winter indicating that smaller microplastics may be more susceptible to transport out of soils subjected to higher rainfall. This transport is also dependent on crop cover, as bare soils become saturated quickly and generate increased runoff. Given that crop cover is generally lower in the winter months after summer crops are harvested, this may be influencing the output of microplastics from these soils. However, in the untreated soils there were more microplastics in the winter and significantly higher, although still relatively low, polymer diversity. This highlights the complexity of these systems and the influence of external factors, for example different types of vegetation cover may influence microplastic concentrations and distributions in different ways

(Ding *et al*., 2021). Moreover, inputs of microplastics from atmospheric deposition have been shown to increase during periods of high rainfall as a result of scavenging of particles into water droplets (Dris *et al*., 2016). This process may be particularly pronounced for light density polymers such as polypropylene, which was found more frequently in the winter month, that are more likely to be transported by wind. In addition, some reports propose that surface runoff only contributes to a small proportion of mobilising microplastics (Schell *et al*., 2022), therefore this may depend on the polymer type, size, and morphology of the plastics, and requires further investigation. Moreover, the influence of biota must be considered, as it has been shown that soil invertebrates such as earthworms are likely to incorporate microplastics into the soil (Lwanga *et al*., 2017a).

Whilst the sampling strategy in this study aimed to gather representative samples across fields, it is possible that soil heterogeneity at field-scale may impact microplastic concentrations. This will depend on individual field and soil characteristics (e.g. slopes, presence of and proximity to hedgerows, soil grain size and density), especially as soil texture and organic carbon have been shown to influence the retention and transport of organic pollutants (Patzold *et al*., 2008). It is important to consider that soils are a complex medium for which the development of microplastic extraction methods are currently in progress (Radford *et al* 2020). Given that the spiked recoveries were generally low (42%), the values reported here may be an underestimate- in line with microplastic extraction methods generally which are estimated to be underestimated by 14% across all environmental media (Way *et al*., 2022a). Additionally, while the use of µFTIR allows for rapid detection of small microplastics, it is limited to the aperture size (10–20 µm) (Song *et al*., 2015b) and is therefore likely to underestimate fibres which generally have a diameter below this limit.

It is now known that there are microplastics in agricultural soils at appreciable levels, but to reduce or prevent the input of these plastics their sources must be elucidated. It is important to understand further the behaviour of microplastics in soils, and their fate once they are transported out of agricultural systems; farm fields may act as a sink and a pathway for microplastic to move through the environment. In particular, this may have implications for surrounding waterbodies that may receive the exported microplastics. In a local context, the River Test estuary downstream of agricultural areas has been shown to have high numbers of microplastics present in the water (Gallagher *et al*., 2016) to which agricultural soils may be contributing. Future studies should focus on how microplastics move through terrestrial systems, ideally at a catchment level (Windsor *et al*., 2019). Simultaneously, we need to know what impact

these microplastics are having in soils, including effects on soil fauna, food security and crop yields (de Souza Machado *et al*., 2020).

4.5 Conclusions

Understanding the sources of microplastic contamination in soils is imperative for future mitigation strategies to be effective. Overall, the results of this study show that biosolids, whilst are likely a contributor, are not the sole source of microplastics in agricultural soils. The variability in results seen here highlights the complexity of determining microplastic concentrations in heterogeneous agricultural soils' and given the variety of microplastic types found here suggests that multiple sources may be contributing. Additionally, the difference in microplastic concentrations between seasons here mean that the dynamic nature of the agricultural soil environment may result in soils being a vector for microplastics into the wider environment, so further research is required to determine source and sink dynamics.

Chapter 5 Temporal variation in soil microplastic abundance related to biosolid application

5.1 Introduction

Microplastic contamination in the terrestrial environment continues to receive increased attention from the scientific community and wider public. Our knowledge of this contamination is based on the existing, limited research in these areas (Dai *et al*., 2022). However, data of this nature is currently scarce and as such our understanding is incomplete. Studies have been restricted on temporal and spatial scales, focusing on a small number of sampling sites or within a short timeframe (Heinze *et al*., 2021). Understanding of microplastic movements in association with environmental factors will allow determination of their distribution in terrestrial and aquatic environments. These systems are interconnected, and as such it has been suggested that soils may act as a vector for microplastics to freshwater systems (Nizzetto *et al*., 2016a). Once in the environment, microplastics have the potential to cause harm to biota and to alter ecosystem functioning. It is therefore imperative to understand their transport and accumulation to determine their potential for harm within environmental domains.

As previously discussed (§4.1), agricultural soils are particularly at risk of microplastic contamination due to the use of biosolids, which have been recognised as a major source of microplastics in terrestrial soils. Agricultural land accounts for 70% of land use in the UK (The World Bank, 2018). In 2021, 801,721 tonnes of biosolids were produced by water companies in the England, of this 94.4% (756,825 tonnes) was used in agriculture covering 150,376 hectares of land (Environment Agency, 2022). Application rates vary between farms and regions, but typically fields are treated with 5- 30 Mg ha^{-1} of biosolids every three years (Brandes *et al*., 2021). This practice is regulated by the Sludge (Use in Agriculture) Regulation (Public Health England and Wales, 1989a), which aims to minimise the impacts of biosolids in the environment. However, it is targeted at only a few potentially toxic elements (e.g., metals) and does not account for emerging contaminants such as microplastics. As biosolids have been shown to contain extremely high numbers of microplastics, ranging up to the millions per gram (Cunsolo *et al*., 2021; Horton *et al*., 2021; Salmi *et al*., 2021), the inputs and subsequent concentrations of microplastics in soils may be extremely high and widespread. However, it is increasingly evident that the situation is more complex. As indicated by the results of Chapter 4 (§4.3), whilst microplastics are universally present in the terrestrial environment, their concentrations are not necessarily elevated in soils

treated with biosolids. Studies have shown high numbers of microplastics across various soil environments (Xi *et al*., 2022). However, predicted hotspots (e.g., areas of biosolid application) do not necessarily have elevated microplastic concentrations (Crossman *et al*., 2020). In addition to their introduction to soils through sources such as biosolids, microplastics are transported out of soil environments through water and wind erosion (Hurley and Nizzetto, 2018; Rezaei *et al*., 2019). It has been suggested that microplastics may subsequently be entering watercourses from soils through vertical (groundwater) or lateral (surface runoff) pathways (Brandes *et al*., 2021).

With increasing plastic production rates (Okoffo *et al*., 2021) and the knowledge of their long-term residence in the environment, it is suspected that over time there will be an increase in microplastic accumulation in the environment (Borrelle *et al.,* 2020). The terrestrial environment is of particular concern as the amount of plastic released annually is estimated to be 4–23 times more than to the marine environment (Horton *et al*., 2017b). Especially in agricultural soils, where microplastic contamination is likely to be high, it is imperative that we understand the drivers of these transport mechanisms and their influence on the fate of microplastics. Initial models have begun to identify transport drivers and pathways (Brandes *et al*., 2021), however current information is lacking, and more empirical data are required for model accuracy to be sufficient.

It is important to understand the fate of microplastics in the soil environment to build a holistic understanding of long-term source and sink dynamics. Information on the fate of these microplastics in relation to environmental variables, such as soil type and climatic conditions, will enable this. For example, precipitation directly impacts the hydraulic characteristics of soils and has been shown to be positively correlated with microplastic concentrations in riparian soils (Zhou *et al*., 2021). What's more, the fate of microplastics in soil systems will likely depend on differences in environmental matrices (Li *et al*., 2021). Microplastics can alter inherent soil characteristics (de Souza Machado *et al*., 2018b) and in reverse, the fate of microplastics may be influenced by soil properties. Characteristics such as organic matter content and particle size distribution should be considered. Soils with high organic matter could reduce microplastic retention as humic acid present in the organic matter may cause repulsion between microplastics and soil particles, increasing their dispersion in water and potential for transport in runoff (Gui *et al*., 2022). Moreover, soil particle size distribution is one of the most important characteristics of the soil environment (Kerry *et al*., 2009). It is likely to affect both horizontal and vertical transport of microplastics as larger grain size causes higher porosity and increased potential for vertical migration of microplastic particles (Castan *et al*., 2021; Waldschläger and Schüttrumpf, 2020). Given that microplastics themselves are heterogenous mix of sizes, shapes, and polymer types (Guo *et al*., 2020), the level of effect may vary depending on their composition. For example, the

size of microplastics may relate to soil grain size as smaller particles will translocate through smaller pore sizes (Yu *et al*., 2021a). Additionally, the inherent characteristics (e.g. specific density) of different polymer types may determine their transport pathways and mechanisms (O'Connor *et al*., 2019).

Seldom do studies in soils consider changes in microplastics over time. Some findings suggest that microplastics applied to soil in biosolids are retained for more than 15 years (Zubris and Richards, 2005) pointing to a potential build-up of concentrations with repeated exposures to new biosolids applications. Alternatively, a high number of microplastics in soils are thought to be exported to rivers through surface runoff, particularly during periods of heavy rainfall (Husrin *et al*., 2022). This is likely to depend on seasonality and, when considering agricultural soils in particular, the anthropogenic processes taking place. In the agricultural environment this seasonality may be linked to the types of farming (e.g. arable or pastoral) and specific methods used. Furthermore, this will depend on the treatments soils receive (e.g. biosolids and composts) and the intra-annual processes (e.g. tillage, cultivation and harvesting of crops).

This Chapter aims to develop our understanding of biosolids as a source of microplastics in soils and their environmental fate. Given previous reports of high concentrations of microplastics in biosolids, it is hypothesised that microplastic concentrations in soils will increase after application. However, a decline in these concentrations over time is anticipated as microplastics are transported out of these systems. The spatial and temporal viability in microplastic concentrations will be measured in relation to soil characteristics (e.g. particle size distribution and organic matter content) while field characteristics will be accounted for (e.g. slope). A year-long sampling campaign in the River Test catchment in the UK was conducted across five independently managed agricultural fields selected on the basis of their treatment with biosolids. This study aimed to determine microplastic concentrations and compositions, in terms of polymer types, across fields and consider these observations in relation to environmental factors which may influence microplastic concentrations and composition enabling a holistic investigation of microplastic abundance in relation to biosolid application.

5.2 Methods

5.2.1 Study area

The United Kingdom has some of the highest rates of biosolid disposal to land (Collivignarelli *et al*., 2019). This study was conducted in arable farmland areas typical to the region of the

southeast of England, UK in an area of high agricultural land use (National Rivers Authority, 1991). To investigate the fate of microplastics in biosolid applications, five representative fields from independent farms were selected as case study sites. All sites were within the catchment area of the River Test (§4.2.1, Figure 4.1) and had similar environmental and physical characteristics; these were selected to minimise the influence of external variables. All sites have historically used crop rotations typical to this region. This included wheat, barley and oilseed rape which are generally drilled in autumn and harvested in the following summer. Fields ranged in size from 7-21 ha and cultivation was done by minimum tillage which rotates the top 10cm of soil. No significant plasticulture was used on any of the farms (i.e. plastic mulching or the use of plastic film and tunnel covers). Three of the fields had received no previous biosolid application while the other two last received biosolids in 2017. All biosolids came from one of two local suppliers.

Each of the five fields was characterised in terms of environmental features. The slope of each field was measured in ArcMap (version 10.8.0.12790) using SRTM (Shuttle Radar Topography Mission, USGS) Digital Elevation data at 30 m resolution converted to slope using the Slope and Zonal statistics (Spatial Analyst) tools. The minimum distance to roads was measured with the Generate Near Table tool (Analysis) using open roads data (Ordnance Survey open roads, November 2021). The average rainfall data for the Southeast of England in the week prior to sampling was acquired for each occasion (Environment Agency, 2020).

5.2.2 Sample collection

Biosolids were applied to fields between August and October 2019 and sampling regimes were designed to match up with this timing. Sites were sampled repeatedly over the course of a year from August 2019 to October 2020. Exact timing of sampling was dependent on the biosolid application date for specific sites. Sites were sampled in the week prior to biosolid application and then at intervals of one week, one month, three months and one year after biosolid application. Fields were tilled before biosolid application and crops were drilled in the winter (between the one month and 3-month sampling points), although exact timing was dependant on the crop type and individual farm.

Fields were split into four quarters, and each treated separately as a replicate for that field. A 4 m buffer zone around the edge of fields was excluded to minimise the influence of external inputs (Piehl *et al*., 2018). Based on observed heterogeneity across the soil surface, composite samples were taken. A stainless-steel pot corer (3 cm diameter, 10 cm depth) was used to take 25 cores randomly spread within each replicate quarter. These cores were then combined to make a homogenous composite sample in a stainless-steel bucket using a metal trowel. A subsample of

approximately 300 mL was then extracted to a glass jar with a metal lid for storage (see section 2.6. for washing and contamination control measures). Replicates therefore consisted of four composite samples per field. All samples were stored in darkness at ambient temperature until processing.

5.2.3 Soil properties

Soil properties were measured for each replicate across all sampling sites and times. Organic matter was measured using the Loss on Ignition (LOI) method at 550°C and reported as percent organic content. Particle size distribution was measured by sieving for the coarse fraction above 1 mm, and a Malvern Mastersizer 3000 granulometer for the <1 mm fraction. Size distributions were then categorised into sand(63µm-1mm), silt(4-63µm) and clay (<4µm) based on the Wentworth scale (Wentworth, 1922).

5.2.4 Microplastic extraction

Microplastic extractions were conducted as outlined in Chapter 4(§4.2.4). In brief, samples were oven dried at 50°C for 7 days and a sub-sample was taken and weighed for extraction (ranging from 8.5 to 22.8g dry weight). This was then placed into a 250 mL glass beaker, and an oil extraction was set up using 10 mL of canola oil and Milli-Q water which was covered with aluminium foil and left to settle overnight. The top layer of oil was poured off into a smaller 150 mL glass beaker and a second round of oil extraction was performed by adding another 10 mL of canola oil and again, leaving overnight. The top layer of oil was poured off and combined with the initial oil extraction. This was filtered using a vacuum pump over a 25 µm stainless steel filter. The filter and all residues were then transferred to a 100 mL glass beaker. A digestion using 30 mL of hydrogen peroxide (30% v/v) was performed in a shaking incubator at 50°C, 100 rpm overnight.

Table 5.1 Study site characteristics including biosolid application dates, suppliers, and history.

Field number	Biosolids applied	Biosolids supplier	Last biosolid application	Size of field (ha)	Distance to road (m)	Slope (%)	Organic matter (%)	Particle size (% clay)	Main crop 2019-2020
1	19.09.2019	1	2017	21	8.7	1.3	8.3	6.1	Winter wheat
2	24.08.2019	1	Never	15	385.0	2.6	5.5	6.7	Oilseed rape
3	17.09.2019	2	Never	7	11.1	1.1	6.6	2.9	Spring barley
4	14.08.2019	2	Never	13	0	3.3	10.7	4.4	Winter wheat

5	23.10.2019	2	2017	17	10.0	1.1	7.0	4.2	Winter wheat

The sample was then filtered back over the same 25 µm stainless steel filter and the residues fromthe filter were washed back into the beaker with MilliQ water, which was topped up with 30 mL of Decon90 to remove any remaining oil residues. After 48 hours, the Decon90 was filtered out using a vacuum pump over a 25 µm stainless steel filter. Subsequently, the residues were separated as size fractions using 1 mm stainless steel mesh and rinsed with ethanol (50% v/v) into 20 mL glass vials for storage. Only small microplastics (<1 mm) were analysed and will hereafter be referred to as microplastics.

5.2.5 Polymer ID

Polymer identification was carried out as per Chapter 4(§4.2.5). Samples were analysed using automated µFourier-transform infrared spectroscopy (FTIR) (PerkinElmer Spotlight 400) to identify polymer types. Sub-samples of processed soils in ethanol were taken to control quality of filters and minimise overloading. Samples were pipetted onto a silver filter (3 µm pore size, Sterlitech, Washington USA) on a vacuum filter set-up through a silicone washer (8 mm diameter). Vials were well mixed by pipetting up and down using a glass 10 mL pipette, before pipetting a subsample onto the filter. Vials were weighed before and after subsampling to determine the weight, and thus volume, of subsamples. Filters were left to dry in a covered glass petri dish within a laminar flow hood at room temperature for at least 24 hours prior to scanning. An area of 8.5 x 8.5 mm was scanned for each sample. Samples were scanned with 2 × 8 linear arrays in reflectance mode with 2 scans per pixel at a pixel resolution of 25 µm and spectral resolution of 8 cm^{-1} in the range of 4000–700 cm^{-1}. Spectral maps were processed and analysed using siMPle software (Primpke *et al*., 2020; available at www.siMPle-plastics.eu) and identified using the siMPle automated IR database (version 1.0.1).

5.2.6 Contamination and quality control

The same stringent quality control measures were used here as in Chapter 4. In the field, only metal and wooden sampling equipment was used and the samplers' clothing was limited to natural fibres where possible. In the laboratory, extractions were carried out in an ISO 14644-1-Class 5 clean laboratory and in a laminar flow cabinet (Felcon), where non-shreddable Tyvex suits (Dupont, IsoClean) were always worn. Digestions were carried out in a separate laboratory in a fume hood where a cotton lab coat was worn at all times. All reagents were filtered prior to use over a glass-fibre filter (1.2 µm, Whatman GF-C) and all water used was Milli-Q. Glass fibre filters

and metal equipment were furnaced at 500°C for 9 hours and all glassware was acid washed and rinsed thoroughly with water prior to use. Milli-Q and ethanol were dispensed using PTFE wash bottles. FTIR analysis was conducted in a separate laboratory. During analysis, cotton laboratory coats were worn and the FTIR microscope was encased with a Spotlight atmospheric enclosure made of Plexiglas to reduce atmospheric contamination. Ten procedural blanks were conducted alongside the extractions to account for laboratory contamination using the entire same process, materials and reagents, in the absence of a sample. These were used to correct all data using limit of detection (LOD) values which were calculated as 3.3 times the standard deviation and accounted for based on individual polymers (Horton *et al*., 2021).

5.2.7 Statistical analysis

All statistical analyses were conducted in R Studio (1.4.1106). Where required, data were checked for normality using Shapiro Wilk tests. Microplastic count and mass data were blank corrected using average blank values and then compared to LOD values as per Horton *et al*. (2021), whereby only data higher than the average + 3.3 SD of the blank samples were reported. Generalized Linear Mixed Models (GLMM) were used to analyse differences in microplastic numbers, mass and size across times and farms. Mass data was square root transformed to ensure model fit. Data were converted to integers and models were fitted to a Poisson distribution with log link. A two-way interaction of sampling time and farm identity was fitted and field replicates were nested in each farm identification as a random effect to account for repeated measurements of the same farm. Tukey's post hoc tests were used to determine individual differences across farms and times. The coefficient of variance was calculated for the microplastics numbers for the four replicates at each site.

For individual polymers that occurred across all samples three or more times, data were transformed to binary (presence or absence of polymer types) and a GLMM was fitted with a binary distribution. The same two-way interaction of sampling time and farm identity was fitted again with field replicates nested in each farm identification as a random effect. The Shannon Diversity Index was calculated for all samples and a Kruskal Wallis test was used to establish differences between time and farms. Additionally, a principal component analysis was applied to determine which polymers best accounted for the variability between treatments and seasons. To prevent distortions polymers that occurred less than three times across all samples were excluded.

Particle size (as percent clay) and organic matter were analysed using a GLMM to determine differences across time and farms. Data were fitted with Gaussian distribution and the same two-

way interaction of sampling time and farm identity was fitted with field replicates nested in farm identification. Tukey's post hoc tests again were used to determine individual differences. Additionally, Spearman's Rank was used to determine correlations between number of microplastics with organic matter and particle size (as % clay) per replicate and with rainfall per farm on each sampling occasion.

5.3 Results

5.3.1 Quality control

Minimal contamination of microplastics was found in the control blanks, with a mean of 7.6 microplastics per sample. Nine polymer types were found across the blank samples, and the most prominent was polypropylene which had a mean of 4.3 microplastics per sample. The LOD values for individual polymers ranged from 1.0 to 24.6 microplastics.

5.3.2 Microplastics across time

The number, mass, and size of microplastics found in the soils varied significantly across the sampled timepoints (Figure 5.1; Number: χ^2 (1) = 10487.57, p <0.001; Mass: χ^2 (1) = 3877.65, p <0.001; Size: χ^2 (4) = 478.43, p <0.001). Before biosolid application, the number and mass of microplastics was highest with a mean of 1070(±391 SE) MP/kg and 1113(±459 SE) µg/kg. This was significantly higher than all other timepoints (p<0.001 for all, Tukey's Tests). Microplastics identified before biosolid application were the largest with a mean of 241.3(±44.9 SE) µm. This was significantly larger than the microplastics found at 1 week and 1 year after biosolid application (p<0.001 for both, Tukey's Tests). However, there were no differences in the size of plastics found before biosolid application and at the 1- and 3-month time points (p>0.05 for both, Tukey's Test). The number of microplastics and rainfall showed no significant correlation (r= -0.20, p=0.334, Spearman's rank). However, the amount of rainfall was lowest before biosolids application, with a mean of 14.24 mm across sites in the week leading up to sampling.

Directly after biosolid application at the 1-week timepoint, the number of microplastics decreased to a mean of 436(±271 SE) MP/kg and the size of microplastics decreased, with a mean of 171.8(±56.1 SE) µm. The number of microplastics then reduced further to 307(±140 SE) MP/kg at the 1-month timepoint, which was the lowest mean microplastic number recorded and was significantly lower than the 1-week and 1-year timepoints (p<0.05 and p<0.001, Tukey's Tests). The smallest microplastics were found at this timepoint with a mean of 110.4(±17.0 SE) µm. Three

months after biosolid application, the number of microplastics increased again to 987(±443 SE) MP/kg. This was significantly higher than the 1 week, 1 month and 1 year time points ($p<0.001$ for all, Tukey's Test). The mean size of plastics found at 3 months was 168.6(±26.9 SE) µm which was

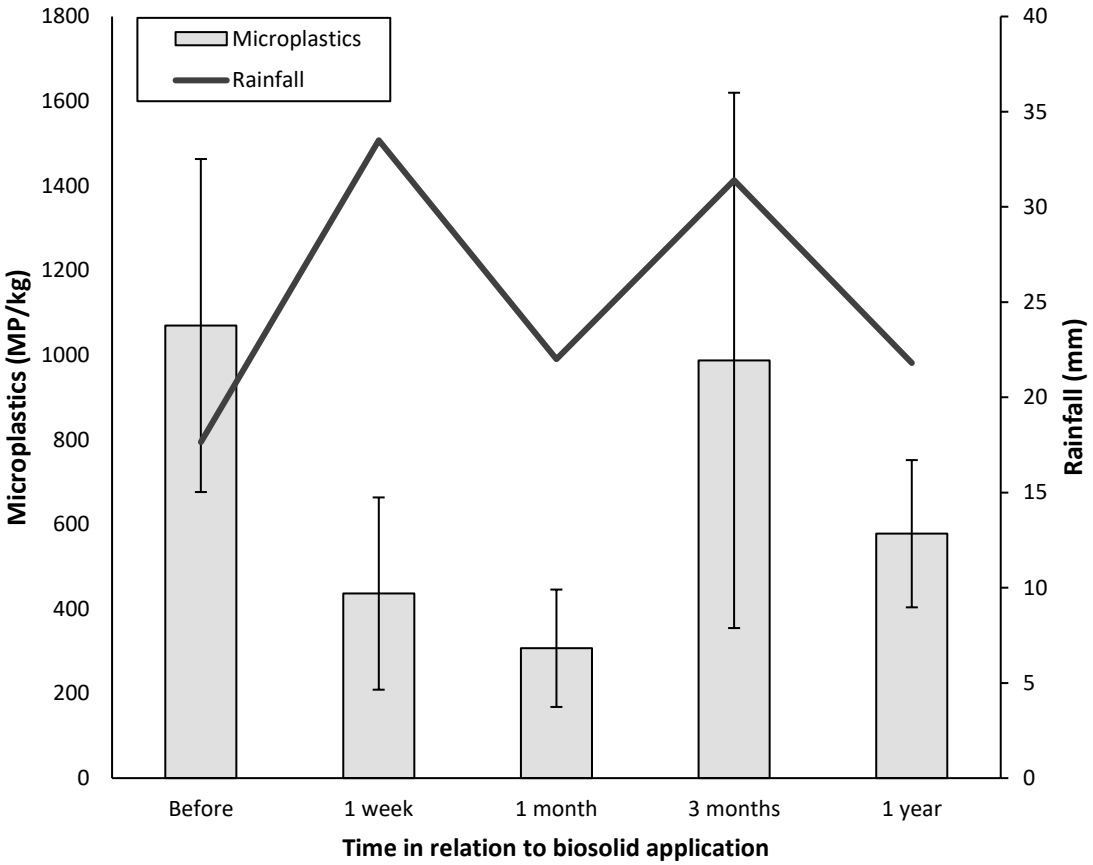


Figure 5.1 Microplastic concentrations (n=20 per timepoint) and rainfall (taken as the value for the week in which sampling falls) across sampled times in relation to biosolid application. Vertical bars show standard error.

significantly larger than the microplastics found at 1 month and 1 year, but smaller than the microplastics found at 1 week after biosolid application ($p<0.001$ for all, Tukey's Test). At the final sampling point (1 year after biosolid application) the mean number of microplastic had reduced again to 578(±193 SE) MP/kg with a mass of 386(±245 SE) µg/kg. At this time the mean size of microplastics decreased to 122.7(±18.0 SE) µm.

Despite differences in numbers, the composition of microplastics across the timepoints did not show great variation (Figure 5.2). The diversity of polymers was similar across all sampled timepoints (χ^2 (4) = 3.590, p=0.464). Additionally, the occurrence of individual polymers was similar across time (χ^2 (4) = <3, p >0.05, for all). The similarity in polymer composition was further emphasised by the PCA, highlighted by the overlapping ellipses. Together, PC1 and PC2 only

explained a total of 38% of the variation in samples. PC1 was dominated by polypropylene and PC2 was dominated by polyethylene and EVA.

In addition to microplastic concentrations, the organic matter and particle size distribution (as % clay) varied over time (

Figure **5.3** Organic matter and particle size distribution (as % clay) across sampled times in relation to biosolid application.

). Organic content was significantly different between timepoints (χ^2 (4) = 10.34, p <0.05) with the lowest organic content at 3 months with a mean of 7.25% compared to a mean of 7.83% which was the highest organic content, measured in the soils before biosolid application. The particle size distribution of the soils varied significantly over time (χ^2 (4) = 943.14, p <0.001). The lowest clay content was before biosolid application with a mean of 2.53% clay; this then increased at the 1 week, 1 month and 3-month sampling occasions to a maximum of 8.25% and decreased again at the 1-year mark to 2.62%.

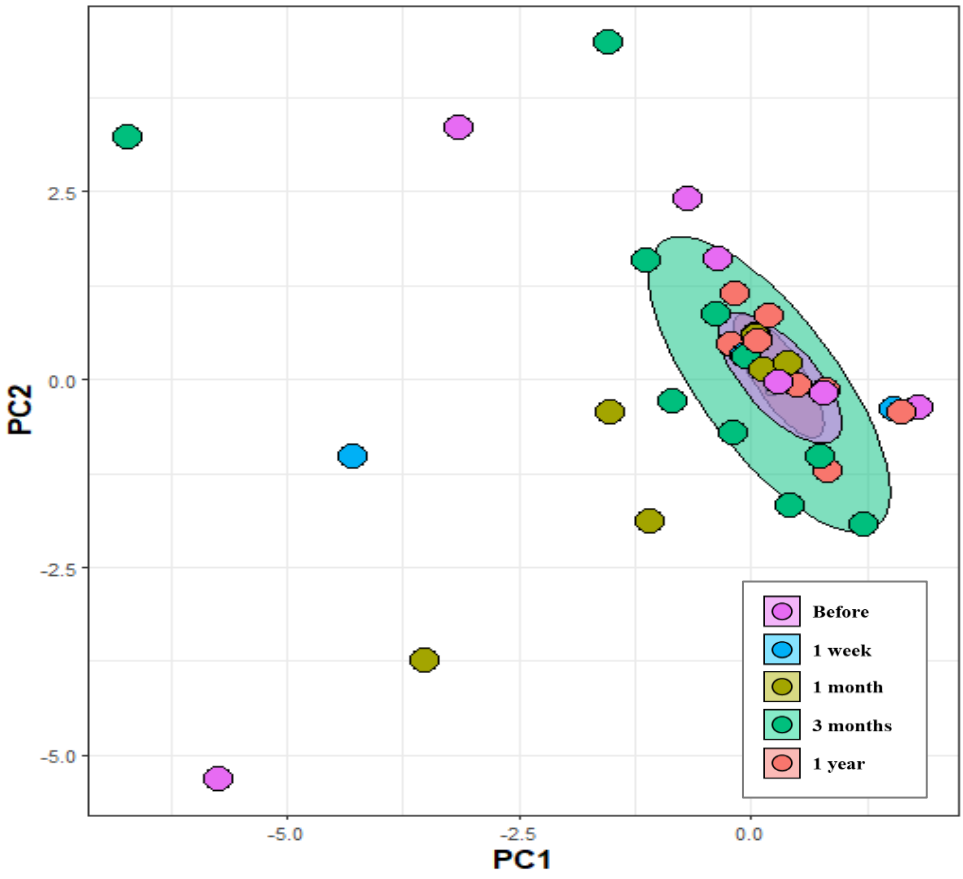


Figure 5.2 Principal component analysis showing the ordination of polymer concentrations between time in relation to biosolid application. Polymers with less than three occurrences across all samples were excluded from analysis.

5.3.3 Microplastics between fields

Microplastics were found in the soils of each sampled field with no significant difference between them (χ^2 4) = 6.82, p =0.145). The same could be said for the mass of plastic and the average size of plastics found (χ^2 (4) =4.490, p =0.344; (χ^2 (4) = 1.92, p =0.751). However, there was high variation in microplastic numbers within fields when considering the four individual replicates. The coefficient of variance between field replicates across sampling occasions was high for all fields ranging from 57.2 to 94.9%.

Field 4 had the highest number of microplastics with a mean value of 1402(±495 SE) MP/kg and the largest microplastics at 194.4(±49.1 SE) µm. It also had the highest slope (3.3%) and the highest proportion of organic matter at 10.7% of the sampled fields. Moreover it was the earliest in the year (August 2019) of the sampled fields to receive biosolid treatment although had never received biosolid treatment prior to the sampling year.

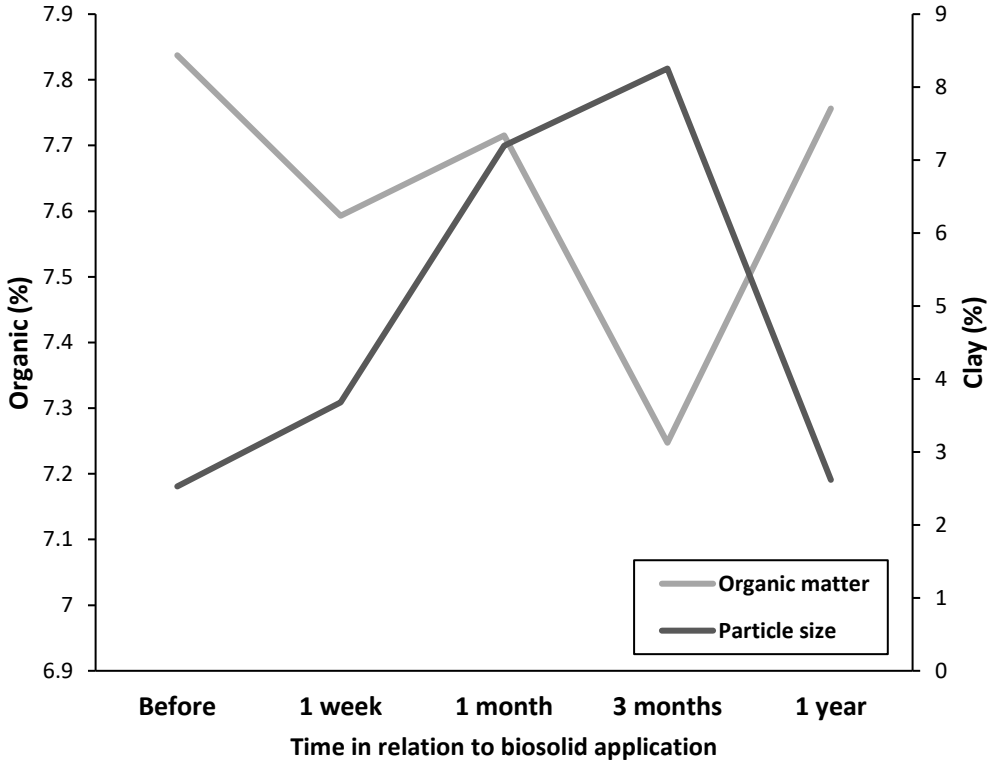


Figure 5.3 Organic matter and particle size distribution (as % clay) across sampled times in relation to biosolid application.

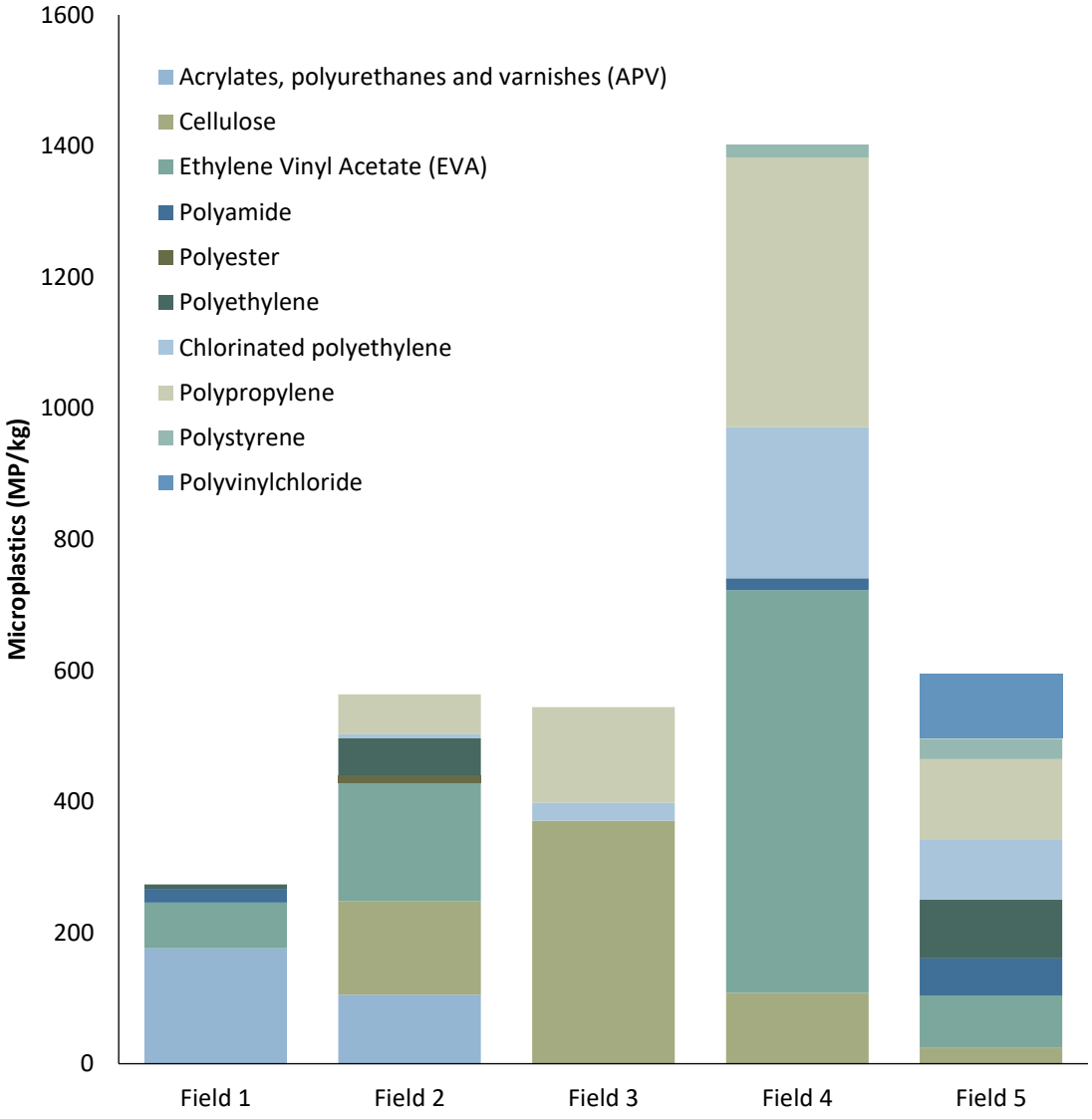


Figure 5.4 Polymer types of microplastics in soils of the sampled fields as a mean across all sampling points (n=20 per field). Counts are reported as number of microplastics of each polymer type per kilogram of soil and have been blank corrected using calculated LOD's.

Field 1 had the lowest number of microplastics with a mean of 274(±139 SE) MP/kg. Field 1 had historic biosolid treatment with the last application prior to the sampling year being in spring 2017. It was the largest of the sampling fields at 21ha. Fields 2, 3 and 5 had similar microplastic concentrations ranging from 544 to 595 MP/kg. Field 3 was the smallest field at 7ha and had the smallest soil particle size distribution with a clay content of 2.9%. It had the lowest polymer diversity with only 3 polymer types found across all samples. Both fields 3 and 5 had the lowest slope with a mean of 1.1%. Field 5 was spread the latest in the year (October 2019) of the sampled fields and had the smallest mean microplastic size at 139.2(±20.3 SE) µm. It had the

greatest polymer diversity found across all samples, with 8 different types. Field 2 had the soil with the lowest organic content (5.5%) and the largest particle size distribution with a clay content of 6.7%.

There was no correlation between the amount of organic matter in soils and number of microplastics present (r= 0.04, p=0.72, Spearman's Rank). However, organic content varied significantly between farms (χ^2 (4) = 14.17, p <0.005). Specifically, Farm 4 had the highest organic content at 10.74%, which was significantly higher than Farm 2 which had the lowest organic content at 5.51%. Additionally, there was no correlation between particle size distribution (as percentage of clay particles, <0.4 µm) and number of microplastics (r= 0.10, p=0.328, Spearman's Rank) and no difference in particle size of soils between the different fields when combining all timepoints (χ^2 (4) 7.60, p =0.107). Similarly, there were no differences in the polymer composition of microplastics between fields (Figure 5.4). Shannon diversity was low with means ranging from ranging from 0 to 0.35 per field and the occurrence of individual polymers was similar across fields (χ^2 (4) = <3, p >0.05, for all).

5.4 Discussion

The concentrations of microplastics in the soils studied here were comparable with those previously reported with the maximum recorded value being 8250 MP/kg (Crossman *et al*., 2020; Lang *et al*., 2022; Scheurer and Bigalke, 2018). However, there were significant differences in the amount of microplastics across the sampled timepoints. Contrary to hypotheses, the highest microplastic abundance was recorded before biosolid application with a mean of 1070 MP/kg across sites (Figure 5.1). This suggests that biosolids are not the main cause of microplastic contamination in these agricultural soils. As reported in Chapter 4, it is likely that other sources are contributing microplastics to these fields. The results indicate that external environmental conditions may be influencing the results seen here; particularly as there was a lack of significant difference in composition of microplastic types over time, which indicates that these changes are a resulting from the fate of microplastics rather than additional sources. The high numbers of microplastics observed before biosolid application coincide with the lowest reported rainfall across the time periods. Although no significant correlations were made between microplastics and rainfall, microplastic abundance was much lower when rainfall was highest at 1 week after biosolid application where the mean number of microplastics was 436 MP/kg. Given that microplastic concentrations were expected to be elevated at this point due to the microplastics contained in the biosolids, there is potential for export of microplastics from these systems with elevated rainfall scenarios. This is contrary to previous studies that showed runoff of microplastics

from biosolid application to be negligible, however this was in a semi-arid region of Spain compared to the temperate climate of southern UK (Schell *et al*., 2022), suggesting that the extent of microplastic runoff from agricultural soils may be climate specific. The extent of runoff from the fields studies here was likely also due to farming management techniques. Measures such as contour ploughing can be utilised to reduce the scale of runoff from soils, however this is often dictated at a farm or field level and therefore will vary between sites (Posthumus *et al*., 2015).

Previous studies have reported that microplastic particles are more readily transported than natural soil particles due to their typically lighter density (Bullard *et al*., 2021). Erosion rates typical for natural soil material will therefore be accelerated for the microplastic fraction of these soils, hence an increase in microplastic losses and lower overall microplastic abundance with heightened rainfall may be likely (Rehm *et al*., 2021a). It has been shown that up to 96% of low density microplastics (e.g. polyethylene) may be removed in surface water movements (Zhang *et al*., 2020). In addition to this, the longer microplastics reside in these environments, the more likely they are to bind with the soil mineral fraction, forming aggregates and increasing stability (Rehm *et al*., 2021a). Therefore recently applied microplastics from biosolids are less likely to be bound to soil minerals and may be more easily transported out of these systems in periods of higher rainfall - as seen at the 1-week sampling point here. This has the potential to impact both vertical and lateral transport of microplastics in these systems (Xu *et al*., 2020). Lateral movement may increase microplastic transport to aquatic systems though surface runoff, while vertical movement may result in groundwater contamination (Ren *et al*., 2021). To put this in a wider context, it is particularly important when considering future climate scenarios, as predicted significantly long dry periods followed by high intensity rain may cause higher levels of runoff and likely export of microplastics from these systems (Vryzas, 2018).

The export of microplastics from these systems may depend on discrete microplastic characteristics. For example, microplastic abundance in this study was lowest at the 1-month timepoint, when the mean number of microplastics was 307 MP/kg. This timepoint also has the smallest average microplastic size (110.4 µm). This suggests preferential transport of larger microplastics out of these systems as before biosolid application the largest mean microplastic size was recorded at 241.3 µm. This outcome may be due to individual soil characteristics. Soil texture, in particular, may determine the movement of different sized plastic. In other words, smaller sized microplastics may translocate through smaller grain sized soils due to smaller pore spaces (Yu *et al*., 2021). However, in the soils examined here, which had a clay content up to 22.1% (

Figure **5.3** Organic matter and particle size distribution (as % clay) across sampled times in relation to biosolid application.

), no significant correlation between soil particle size and number of microplastics was observed. Likewise similar studies found no difference in microplastic abundance across soil texture classes (Scheurer & Bigalke, 2018; Weber *et al*., 2022), so while grain size is an important factor, it is likely not the primary determinant of microplastic concentrations at these sites.

Conversely to this study, some studies have shown that in the presence of a larger particle size distribution, and hence larger pore size, microplastics are more likely to migrate into the deeper layers of the soil (Xing *et al*., 2021a). However this was tested in soils with larger particle size distribution (up to 3.87% clay) that those in this study, alluding to a potential threshold effect level. Particle size of the soils tested here varied over time, the coarsest soils were prior to biosolid application with a mean of 2.53% clay and finest at the 3-month sampling occasions (8.25% clay). Conversely, organic matter was the lowest at the 3-month timepoint, suggesting that there may have been a loss of larger sized organic particles and a higher proportion of remaining inorganic ones. Organic matter may be more likely to be transported in runoff given its lighter densities which are typically range between 1.0 - 1.4 g cm^{-3} (Bläsing and Amelung, 2018). This is important when considering comparison of microplastic transport in runoff as traditional models for natural inorganic particle transport may not be applicable to microplastics as a result of their differing characteristics i.e. density (Waldschläger and Schüttrumpf, 2019).

Additionally, the influence of soil biota on microplastic transport should be considered. Several studies have shown transport of microplastics by soil invertebrates e.g. collembola (Maaß *et al*., 2017b), mites (Zhu *et al*., 2018) and earthworms (Rillig *et al*., 2017). The extent of this transport will depend on the abundance of such biota which will be linked to soil conditions (Martay and Pearce-Higgins, 2018). Moreover, it may be influenced by the presence of biosolids in soils, as it has been shown that biosolid application may increase the abundance of some soil biota (Viketoft *et al*., 2021).

Microplastics may alter the properties of the soil environment itself (de Souza Machado *et al*., 2018b). When considering microplastic abundance by weight, in the soils studied here a maximum of 0.00082% microplastics were found. Initial studies show that at microplastic concentrations in the range of 0.1-7%, there may be changes in bulk density, water holding capacity, and microbial activity, depending on polymer types (de Souza Machado *et al*., 2018b; Lozano *et al*., 2021; Xing *et al*., 2021b). These concentrations are much larger than those reported in this study which are significantly below the impact threshold. However, these impacts are

highly variable and will depend on individual soil and microplastic properties (Mbachu *et al*., 2021). For example, fibres are shown to have increased impact on water holding capacity compared to fragments and films (Lozano *et al*., 2021). Machado *et al*. (2019), demonstrate the links between plant and soil changes with microplastic contamination, indicating that even small changes to individual parts of the model may impact the whole soil environment. A species sensitivity distribution (SSD) conducted by Jacques & Prosser (2021), suggest that based on current studies, 95% of organisms that reside in soil have a LOEC value above 162 MP/kg. All timepoints within the present study had mean values exceeding this therefore at current concentrations, effects on soil organisms may be observed. However, as microplastic concentrations were so variable within sites, this is likely to be highly localised. More information on fate and effect of microplastics is needed to reduce uncertainty and increase accuracy in these risk assessments (Jacques & Prosser, 2021).

Repeated application of biosolids has been suggested to increase microplastic concentrations. Studies showed that the number of microplastics increased significantly consecutive biosolid application, although the relationship is not necessarily linear (Corradini *et al*., 2019). However, the results presented here showed similar microplastic concentrations across all fields, regardless of previous biosolid treatment (some of which had none). This increase may therefore be field specific and will depend on the mass balance of microplastics in the system. That is, if the input from biosolids and other sources overcomes the output e.g., wind and water transport.

There were no differences in the abundance of microplastics between the five sampled fields in this study (Figure 5.4). This lack of difference alludes to the complexity of microplastic contamination in agricultural fields. It suggests that there is not one main driving factor, rather an assortment of contributing ones driven by the balance of microplastic inputs against soil environment dynamics. All the sampled fields were selected for based on their farm management strategies (e.g., tillage and crop rotations), however the complex nature of agriculture means that not all farm-specific characteristics can be accounted for and therefore may go some way to explaining the results seen here. Each of the sampled fields had high levels of intra-field variation with coefficient of variance ranging from 57.2 to 94.9%. These results suggest that the distribution of microplastics within fields is uneven. This localised heterogenous distribution of microplastics may be a result of differences in surface morphology, vegetation, and anthropogenic activities (Weber *et al*., 2022).

Different types of crops have been shown to impact the vertical movement of microplastics in agricultural soils either through direct contact and movement or migration through the fissures created by the roots (Li *et al*., 2021). The extent of this is determined by the type of crop which is

often linked to the specific farming techniques associated with said crop type. For example, Liu *et al*., 2022) showed that there was higher abundance of microplastics in soils surrounding tall crops (e.g., sunflower and maize) than short crops (e.g., potato), which was linked to the different management methods including the use and removal of plastic mulch associated with these different crop types.

In the present study, while the exact crops for the sampling year were different across fields, the crop rotations were the same (i.e., alternate years of wheat, barley, and oilseed rape). This meant that in all fields the crops were typically drilled in the winter (between the 1-month and 3-month sampling occasions) and harvested in the late summer (prior to the 1-year sampling occasion). As a result, crop cover was generally low during all sampling occasions. Areas with little to no crop cover are often associated with higher erosion rates, particularly in winter when rainfall events are more frequent (Boardman *et al*., 2020) which may result in microplastic losses. Moreover, the differences in crop types during sampling occasions may have influenced the amount of microplastics lost through runoff as there can be significant differences between runoff with different types of crops. For example, winter oilseed rape crops prevent runoff to a greater extent than wheat crops (Chowaniak *et al*., 2020). The interactions between crops and fate of microplastics is not currently well understood and should be considered further in future studies to enable a fuller understanding of their role in transport and retention of microplastics.

In addition to crop type, it is likely that the physical characteristics of the fields influenced microplastic concentrations. The highly complex nature of agricultural systems give rise to many potentially influencing factors. For example, Field 4, which had the highest microplastic numbers, had the greatest slope at 3.3%, which is likely to increase microplastic runoff (Campanale *et al*., 2022c). Additionally, Field 4 was spread the earliest in the year, in the late summer, when rainfall was less frequent, meaning there was more time for microplastics to bind to soil particles before rainfall could wash them away (Rehm *et al*., 2021b). It also had the highest recorded organic matter content with a mean of 10.7%, which may indicate a link between organic matter concentration and microplastic concentrations. However, the organic content was high in this field prior to biosolid application, suggesting that this is not the driver as there was no overall correlation between microplastics and organic content. Alternatively, Field 5 showed the greatest variety in terms of polymer composition with a total of eight different polymers throughout the study, but it was spread the latest in the year.

The complex nature of these observations and the variation seen therein indicate that even within individual fields there is high variation in microplastic distribution. Visual observations of the sampled fields show that the distribution of biosolids across fields was uneven (See Appendix C

Figure 1). This may explain the uneven microplastic concentration coupled with the lack of increase in organic matter content in soils after biosolid application. This is a perhaps unexpected result considering that biosolids are added to soils to increase soil organic matter and typically contain 40–70% organic matter (Haynes *et al*., 2009). Again, there was high variation within the data and therefore the patchy distribution of biosolids in this case may explain why an increase in organics was not observed.

Future studies may consider these intra-field differences by coupling the use of tools such as precision farming where fine scale differences in soil properties are mapped to better understand field characteristics (Iticha and Takele, 2019) with the detection of microplastics as this may further unpick the underlying mechanisms driving microplastic distribution in agricultural soils. Recommendations for future studies are to consider even larger sample numbers within fields, and where possible, larger sample volumes, to account for this, as is recommended for such heterogenous environments (Weber *et al*., 2022; Y. Yu & Flury, 2021).

5.5 Conclusions

Overall, the results presented here show that microplastics are widely distributed in the agricultural soils sampled here. Despite expectations, microplastic concentrations were highest in soils prior to biosolid application indicating that external environmental factors strongly influence these concentrations and that sources are not limited to biosolids application. The differences seen in microplastic concentrations between fields suggests that farm management may exert a strong influence on their fate. Additionally, geographical and environmental factors are likely to have bearing on the abundance and distribution of microplastics in soils. The high variation seen in the study fields here suggests that the distribution of microplastics in soils is highly localised and therefore future studies will need to consider this particularly in relation to the understanding of biosolids as a source of microplastics in these environments.

Chapter 6 Discussion

6.1 Summary of findings

The purpose of this thesis was to expand and enhance our understanding of microplastics in agricultural soils. Although microplastic research has vastly expanded in the last decade, studies in the terrestrial environment have been lacking and our knowledge of their presence, fate, and impacts have been limited. In this thesis, Chapters 1 and 2 outlined the state of the knowledge and highlighted the gaps in the literature. These related to insufficiency of suitable methods for quantifying microplastics in soils and a lack of knowledge of the environmental concentrations of microplastics in soils, particularly in reference to their sources (including biosolids) and their fate. These gaps relating to the methods of extraction, biosolids as a microplastic source in soils and the fate of microplastics in soils treated with biosolids, were therefore addressed in Chapters 3, 4 and 5.

Chapter 3 addressed the need for robustness and consistency regarding methods for extracting microplastics from environmental matrices. It focused on soils, which have been identified as one of the most challenging media to extract microplastics from due to their complex mixtures of minerals, wide range of particle size distributions, and organic matter content at varying stages of decomposition (Thomas *et al*., 2020). Organic removal methods were tested, and hydrogen peroxide was found to be the optimal method given its high removal rates and simplicity of use (Figure 3.1). Methods of density separation were then tested on the inorganic fraction of soils with varying particle size distributions, for which, canola oil was the most efficient (Figure 3.2). However, when tested on soils with a range of organic matter content, the oil method reduced in efficiency as organic matter content increased (Figure 3.4). The recommendations are therefore that canola oil should be used in soils with lower organic content, given its efficiency, low cost, and lesser environmental impact. However, despite concern over environmental and health risk (Way *et al*., 2022b), when testing soils with high organic matter it is recommended that zinc chloride should be used to ensure maximum recovery rates. All methods were assessed for impacts on polymer integrity and were deemed suitable for use with ATR FT-IR- as few impacts were evident. This work showed the importance of matrix-specific calibration for microplastic extraction methods. It presented a method which is widely applicable, cost-effective and minimises environmental impact and hazard to operators. Therefore it may be utilised by researchers universally, improving the opportunity for harmonisation across studies.

Chapter 4 quantified and characterised microplastics in a set of agricultural soils which were either treated or untreated with biosolids; the methods developed in Chapter 3 were applied in this work. High numbers of microplastics were found across both treatments and a wide variety of polymer types was found comparable with previous studies (Corradini *et al*., 2021; Crossman *et al*., 2020; van den Berg *et al*., 2020). However, there were no significant differences in the number of microplastics found between fields with and without biosolid treatments (Figure 4.2). Therefore, whilst biosolids are a contributor to soil microplastics concentrations, they are not the sole source to agricultural soils. Recently the importance of additional source such as manure and composts have been suggested (Vithanage *et al*., 2021; Wu *et al*., 2021; Yang *et al*., 2021). There were, however, differences across seasons, with higher microplastic abundance in the summer. While most studies currently only examine one sampling timepoint (Beriot *et al*., 2021; Ding *et al*., 2020; Piehl *et al*., 2018b), these results highlight the importance of considering temporal variations in soil microplastic concentrations. Furthermore, environmental and meteorological conditions, and wider anthropogenic activities (e.g. rainfall, farming practices and littering) may be influencing the presence and retention of microplastics in these soils.

Chapter 5 expanded on the work presented in Chapter 4 by examining microplastic concentrations in soils before and after biosolid application at defined intervals. This aimed to determine the temporal variation in microplastic concentrations in relation to biosolid application. The results of this work further suggest that microplastic concentrations are not solely elevated by biosolid application and are variable over time. The highest microplastic abundance was seen before biosolid application, which coincided with the period of lowest rainfall across the sampled timepoints (Figure 5.1), further indicating that climatic conditions and subsequent runoff may impact overall microplastic concentrations. It has now been reported in the literature that environmental conditions such as rainfall and subsequent runoff may be having an impact on overall concentrations (Schell *et al*., 2022). However, microplastic concentrations were not associated with key soil properties (organic matter content or particle size distribution). Across all fields sampled in Chapters 4 and 5 there was high variation in microplastic concentrations within fields indicating that microplastic concentrations may be patchily distributed to a large extent.

6.2 Methods of microplastic extraction in soils

At the time of commencement of this thesis there was a considerable lack in established methods for microplastic quantification in the environment. Accurately quantifying and characterising microplastics from the environment is vital in determining their sources, pathways, and fate. The

work of Chapter 3 built upon previous methods whilst considering a wide variety of polymers in a range of soil types. It suggests that, while standardisation is highly sought after, with current methods available it is not possible when considering the impact of soil characteristics on recovery, and therefore harmonisation of methods which takes into account these properties is a more suitable approach going forward. This is shown by the differences seen in extraction efficiencies of canola oil (mean 47%) and zinc chloride (mean 53%) in soils with varying organic matter content. In soils with lower organic matter, canola oil is a more appropriate method, whereas in soils with higher organic matter, zinc chloride may be required to ensure optimised extraction efficiency. While some of the methods used in Chapter 3 were clearly not suitable (i.e. NaCl flotation and KOH digestion), it is vitally important to consider and include such information as often studies do not report on challenging or unsuccessful methods resulting in duplication of efforts within the scientific community.

Since Chapter 3 was published (Radford *et al*., 2021), more advancements have been made in the field of methods for microplastic extraction from soils. The use of the oil extraction method, initially developed by (Crichton *et al*., 2017) has increased and more studies are now taking this approach. Oil extraction has been combined with other methods such as the Sediment-Microplastic Isolation (SMI) unit developed by (Coppock *et al*., 2017) particularly with fine sediments (Lechthaler *et al*., 2021). Oil has also been used in studies of sludges, and biota (Courtene-Jones *et al*., 2020; Lekše *et al*., 2021; Song *et al*., 2022). Many studies are still using hydrogen peroxide for organic digestion in soils (Helcoski *et al*., 2020), however, some have incorporated the use of enzymes. Möller *et al*., (2021), utilised enzymatic digestion to increase sample purification, thereby enabling the processing of larger sample volumes (up to 250g). Nevertheless, this required a multistep approach with multiple reagents over many days and while sample size may be increased, the number of samples processed may be limited by time constraints and associated costs. Additionally, the methods used by Möller *et al*. (2021), were shown to impact the integrity of PLA meaning that it may be excluded from analysis using these methods. More novel methods are also in development. For example, a magnetic extraction method utilising hydrophobic properties of plastics to bind with iron particles which can be recovered using a magnet (Grbic *et al*., 2019). However, this method is still under development with tests so far only being with limited conditions and types of microplastics (10-20 µm polyethylene and polystyrene in seawater).

Method development for microplastic quantification in soils is an ongoing process. While improvements and advancements have been made recently, they are yet to be fully optimised and are not yet universally applicable to the wide range of microplastics present in the

environment (He *et al*., 2021). A recent review assessing microplastic analysis methods in soil across 35 studies showed that none of the studies acquired the maximum score when considering quality criteria (Praveena *et al*., 2022); studies are currently limited by equipment, timing, or the available methods. Moreover, while extraction procedures are a vital component, they are just one part of the whole picture (Praveena *et al*., 2022) which includes suitable approaches in sampling, storage, contamination mitigation and polymer identification which should be optimised in combination with the methods developed in Chapter 3.

Going forward, the environmental impact of microplastic research methods should be further considered, as the majority of current methods used cannot be considered as 'green' (Picó and Barceló, 2021). This is essential to ensure that the work of microplastic researchers is not tainted by using potentially more harmful chemicals and techniques. Additionally, it is imperative that the methods used to extract microplastics are applicable to a wide variety of polymer types to understand the full extent of microplastic pollution in the environment. However, given the complexities of microplastics as a contaminant, in some cases it may be appropriate to focus on selected polymers for which microplastic extraction and identification is more reliable or tailor it to those which are more prevalent in environmental situations. There is therefore a trade-off between quantity of microplastic types which can be reliably detected and the quality of those methods (i.e. recovery rate efficiency). Some studies have chosen to focus on a few select polymers. For example, Pabortsava & Lampitt (2020) focused on three of the most common polymer types (polyethylene, polypropylene, and polystyrene) based on their widespread prevalence as the most-littered plastics in the marine environment, although this will depend on the research objectives. As new studies are conducted it is becoming evident that the size distributions of plastics found in the environment are skewed towards smaller sizes. This includes nanoplastics, which may pose even greater risk to the environment as their smaller size makes them increasingly bioavailable, although they present further methodological challenges (Mitrano *et al*., 2021). Therefore it is necessary that we further develop and tailor methods to include these smaller size fractions (Junhao *et al*., 2021; Monikh *et al*., 2021)

The ideal methods for microplastic extraction and analysis would be independent of soil properties, have low environmental impact and cost, and high applicability across all types of microplastics: we are yet to achieve this ambition. Currently methods are limited by the inherent heterogeneity of environmental samples and the accessibility of equipment and reagents. At present, harmonisation is, arguably, the best approach where possible. As outlined in Chapter 3, studies should ideally aim to include full reporting of methods used to account for the differences between studies and allow for comparability between studies.

6.3 Microplastics in soils

The results of Chapters 4 and 5 in combination with recent literature (Crossman *et al*., 2020; Lang *et al*., 2022; Scheurer and Bigalke, 2018) suggested that microplastics are ubiquitous in agricultural soils, as has been evidenced across wider environments. Regardless of treatment, all sites sampled in this study contained some microplastics on at least one occasion. The highest value recorded across the sites in Chapter 4 was 8862 MP/kg and 7590 MP/kg in Chapter 5. Both sites had temporal variations in microplastic concentrations, indicating that microplastic concentrations in agricultural soils are highly variable, corresponding with the dynamic soil environment. In recent years, the call for more empirical data on the contamination of microplastics in the terrestrial environment has become more prominent, but studies meeting this need remain scarce (Yang *et al*., 2021). During the time over which this PhD research was undertaken, a study assessing the concentrations of microplastics in fields treated with biosolids was published (Crossman *et al*., 2020). This study defined small microplastics as 50-300µm and larger microplastics as >300µm, which were measured across three fields in Canada. They found lower concentrations than those sampled in this study (see Chapters 4 and 5). This difference may well be due to differences in the characteristics of the locations in terms of geographical influence or farming practice. Additionally, the study used a larger lower size limit (50µm) than in this thesis(25µm) and applied a method of visual analysis to pick microplastics for identification with the potential for observer bias and underestimation of microplastic concentrations (Song *et al*., 2015). However, Crossman *et al*. (2020), also found that microplastic concentrations were not necessarily elevated with the application of biosolids, further strengthening the conclusions of this thesis that biosolids are not the sole driver of microplastic concentrations in agricultural soils.

6.3.1 Sources of microplastics in soils

In this thesis, the same ten polymers were detected across the sample of agricultural fields in separately managed farms. These were polyethylene, artificially modified cellulose, chlorinated polyethylene, polystyrene, polypropylene, polyvinylchloride, polyamide, polyester, ‘acrylates, polyurethanes and varnishes’ (APV) and ethylene vinyl acetate (EVA). The three most common polymers across both studies (Chapters 4 and 5) were polypropylene, artificial modified cellulose, and EVA. This diversity in polymer types indicates a variety of sources for these microplastics. It is difficult to determine the exact origins of microplastics as they are small in size, often fragmented and the range of potential sources can be extremely large (Ballent *et al*., 2016). This is particularly the case with sources such as biosolids which contain a mixture of microplastic types. Biosolids

have been shown to contain a wide variety of polymer types with the most abundant polymers often being polypropylene and polyethylene (Horton *et al*., 2021; Salmi *et al*., 2021). This makes identifying sources challenging as polypropylene and polyethylene are two of the most abundant polymer types in use worldwide (Plastics Europe, 2020). This is similarly the case for other soil amendment sources such as composts and manures, which contain a mixture of common polymer types generally originating from the breakdown of larger macroplastics (Schwinghammer *et al*., 2021; Watteau *et al*., 2018; Yang *et al*., 2021b). Additionally, inputs of microplastics as a result of environmental processes (e.g. runoff and airborne microplastics) contain multiple types of polymers (Han *et al*., 2006; Kim *et al*., 2006; Prata, 2018).

It may be easier to trace soil microplastics where single polymer sources are concerned. For example, agricultural plastics are often made up of a single polymer type. In soils where plastic mulch sheets are used, microplastics in the soil may be traced back to the mulch itself through polymer identification e.g. polyethylene (Steinmetz *et al*., 2022; Yang *et al*., 2022). Plastic mulch is broken down through photooxidation and mechanical abrasion in the soils to release microplastics directly into soils (Huang *et al*., 2020). However, even in areas where mulching is used there are often other contributing sources to consider (Zhou *et al*., 2020), meaning it is not always straightforward to associate sources with materials observed *in situ*. It may therefore be important to target the less common polymers, for example polystyrene, which is rarely used in agriculture (Astner *et al*., 2019). However this is currently limited by the methods available as it would require more samples and greater volumes to accurately quantify scarce polymers (Mintenig *et al*., 2020).

Although the exact origins of microplastics observed in agricultural fields remain unclear, the lack of difference in abundance between sites presented in this thesis indicates that other sources must also be contributing to soil microplastic concentrations, particularly in areas where biosolids have never been applied. This is contrary to other studies which have suggested that biosolids are the main source of microplastics in such environments (Nizzetto *et al*., 2016; Rolsky *et al*., 2020; van den Berg *et al*., 2020). There have been calls to halt the use of biosolids on land over concern of their safety in use (Ekane *et al*., 2021; Mohajerani and Karabatak, 2020), and further concern stems from reports of high concentrations of microplastics (Cunsolo *et al*., 2021; Horton *et al*., 2021) in addition to other contaminants including polycyclic aromatic hydrocarbons, perfluoroalkyl compounds and phthalates (Rigby *et al*., 2021). Some countries (e.g. Switzerland) operate with a precautionary approach and have banned the application of biosolids to land (Kawecki *et al*., 2021). The risks to the soil environment associated with the compounds must be balanced with the benefits of biosolid application (e.g. increased organic matter and nutrients,

Singh and Agrawal, 2008) to determine if continued use is justified given the associated risks. There is a need to determine if biosolid to land application may be managed prudently while taking advantage of them as a resource for sustainable use (Poornima *et al*., 2021) while considering soils as a vector for contaminants and, in this case, as a vector for microplastics to the wider environment.

6.3.2 Fate and transport of microplastics in soils

Knowledge of microplastic fate in the soil environment is currently limited. A mass balance approach may aid in determining the scale of input from commonly targeted sources (Fahrenfeld *et al*., 2019). In the case of this thesis, this would involve comparing the amount of microplastics measured in these soils with the estimated load of microplastics in biosolids and expected outputs. However, this approach relies on accurate estimates of microplastic concentrations in biosolids, which have been shown to be variable over time and depend on their originating wastewater treatment plant (Horton *et al*., 2021). We are only beginning to understand the processes which may impact microplastic movement and transport in soils. The results presented in this thesis highlight there may be many influencing factors to consider (e.g. soil characteristics, biosolid application history and additional sources of plastic). Figure 6.1 outlines the main sources, pathways, and potential sinks for microplastics surrounding the soil environment. All considered, a meaningful mass balance remains elusive at present. However, the lack of differences between field sites with and without biosolids, and before and after biosolid application, suggests the export of microplastics out of the agricultural soils system, which has implications for the surrounding environments.

Lateral transport may result in contamination of nearby environments. Lateral runoff of microplastics may depend on the shape and size of particles (Zhang *et al*., 2022). One study demonstrated lateral transport of microplastics between fields, spreading from soils which had received biosolids into adjacent soils which had not (Tagg *et al*., 2022). This observation is thought to be a result of wind transport of microplastics across a landscape; and could apply to some of the results seen in Chapter 4 where the numbers of microplastics were similar between fields with and without biosolid application. Additionally, lateral transport of microplastics from soils may result in contamination of freshwater environments. This may be as a result of surface runoff which is likely a significant pathway for microplastics to enter surface waters (Akdogan and Guven, 2019). Such transport is likely to depend on a number of factors such as climate, time of year and surface morphology. While Schell *et al*., (2022), showed that minimal quantities of microplastics (0.2-0.4%) are mobilised in surface runoff, their study was conducted in a semi-arid

region of Spain where rainfall was relatively low and concentrated in the spring and autumn. This may contrast to areas such as those studied in this thesis where rainfall was higher and therefore

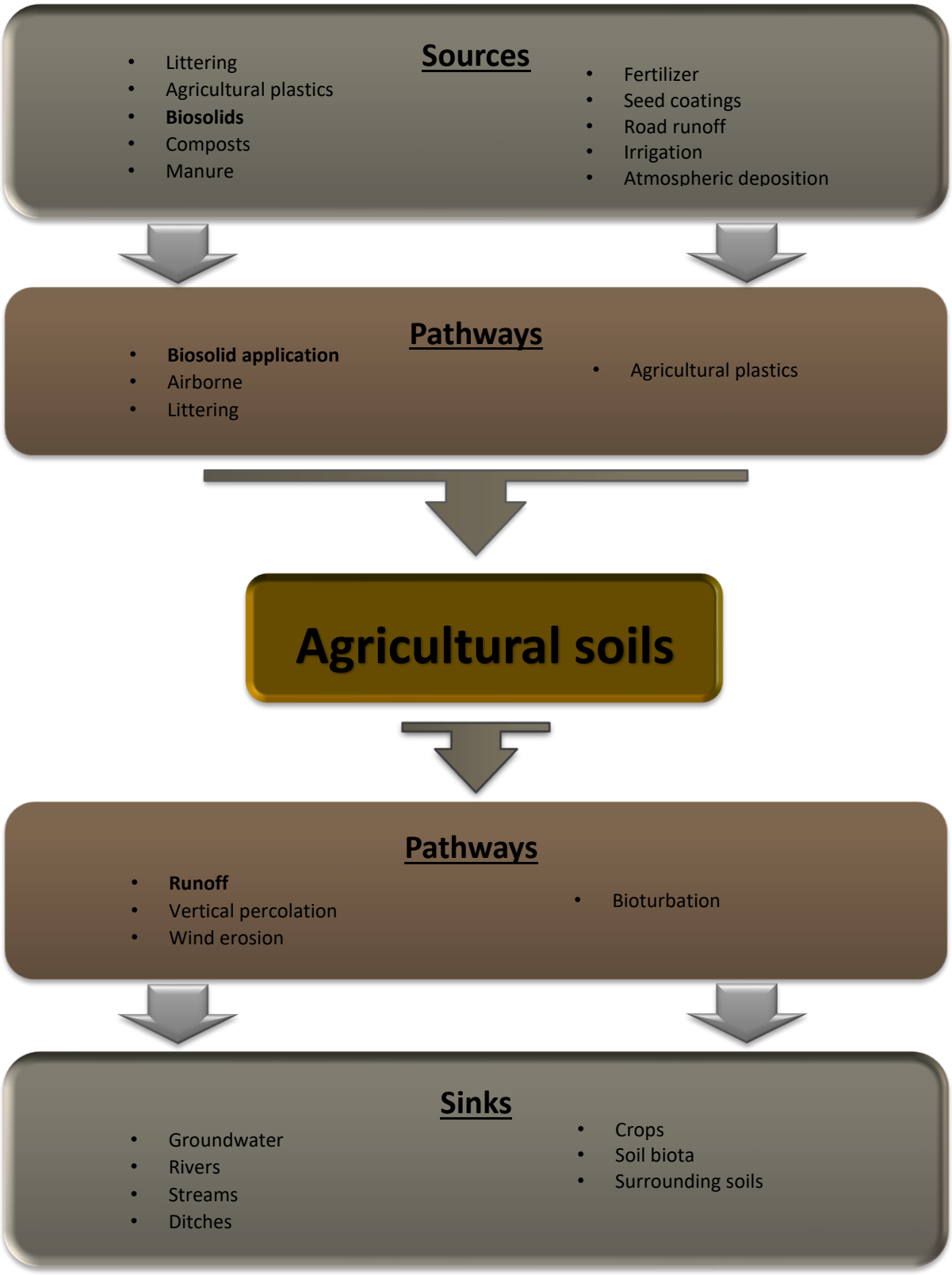


Figure 6.1 Sources and pathways of microplastics entering the soil environment and pathways and potential sinks as a result of microplastics transfer out of soils. The sources and pathways considered directly in this thesis are highlighted in bold.

runoff is more likely. The amount of runoff is likely to be influenced by soil characteristics (Zemke *et al*., 2019). For example, soils with a higher proportion of fine particles will have lower permeability, which may result in higher runoff potential (Castan *et al*., 2021b).

Runoff has implications for surrounding environments, particularly freshwater systems. Rivers have been shown to have extremely high microplastic concentrations (Hurley *et al*., 2018; Vera S Koutnik *et al*., 2021) and high residence times (Drummond *et al*., 2022). All sites in the present study were within the catchment area of the River Test; this river is therefore a likely receiver of microplastics from the sampled sites through runoff. The River Test drains an area of 1269 km^2 (Gallagher *et al*., 2016; Moore *et al*., 1998) predominantly draining rural areas with high agricultural land use (National Rivers Authority, 1991). Downstream the River Test drains into Southampton Water which has previously been shown to contain high numbers of microplastics (Gallagher *et al*., 2016). Given the high likelihood of runoff from the sites sampled in this thesis, it is likely that this downstream contamination may in part be a result of runoff from agricultural fields.

Microplastics have been found to penetrate the soils at depth through vertical translocation. At depth this may reach agricultural drainage systems (60–90 cm), although this may only be up to 1.6% of the surface load of microplastics (Tagg *et al*., 2022). Again, penetration will depend on the soil characteristics and environmental conditions. In times of less rainfall and lower soil water saturation there may be preferential transport of microplastics vertically through the soil profile (Crossman *et al*., 2020). Vertical transport may also occur as a result of bioturbation. For example, the earthworm *Lumbricus terrestris* has been shown to incorporate microplastics in their burrows to over 18 cm deep (Lwanga *et al*., 2017b). In contrast, crop roots tend to move microplastics upward in the soil profile, specifically in the top 12 cm (H. Li *et al*., 2021b). Vertical transport of microplastics from agricultural soils is likely to result in groundwater contamination (Viaroli *et al*., 2022) which is of fundamental concern given the regionally importance of the River Test as an input to an aquifer with potable and agricultural use (Stuart and Smedley, 2009), and globally given that that groundwater is a primary water source worldwide.

6.3.3 Impacts of microplastics on soil physicochemical properties and ecosystems

The results of this thesis have wider implications for the agricultural soil environment. The potential impacts come in two main interlinked compartments: soil properties and soil biota. The presence of microplastics in soils has been shown to impact the physical properties of soils including structure, porosity, bulk density and water content (Wang *et al*., 2022).These effects have been shown to be heavily dependent on soil type.(Ingraffia *et al*., 2021), showed that in soils with a finer particle size (i.e. clay), bulk density was decreased in the presence of polyester microfibres, whereas no effect was seen in soils with lower clay content. However in coarser soils, there was a reduction in runoff compared with finer texture soils with the presence of microplastics. Moreover, these impacts are likely dependent on the type of microplastic. Some polymer types (e.g. polyester) have a more definite impact on soil parameters such as bulk density, water holding capacity and soil structure (de Souza Machado *et al*., 2018b). Similarly, the chemical properties of soils may be impacted by the presence of microplastics. For example Boots *et al*. (2019), showed that in the presence of HDPE microplastics soil pH is lowered.

Changes in soil physicochemical conditions may have effects on soil biota (Khalid *et al*., 2020). Machado *et al*., (2019), outlines a causal model which outlines the interactions between soil parameters such as water holding capacity, evapotranspiration, and duration of water saturation, with secondary impacts on soil biota. For example, changes in soil properties such as a reduction in water retention as a result of microfibres may result in a secondary impact on plants in the form of drought stress (Zhang & Liu, 2018). Though the exact risk of microplastics to biota remains unclear, studies have shown that the presence of microplastics has implications for soil fauna and flora (Ding *et al*., 2022; Wang *et al*., 2021). The reported impacts for terrestrial plants, in particular, are mixed. One study which reports on the impacts of biosolids containing microplastics shows that they may initially increase the growth of tomato plants, but the production of fruits may be delayed or diminished (Hernández-Arenas *et al*., 2021). Additionally, broad bean plants (*Vicia faba*) were exposed to polystyrene microplastics and nanoplastics which showed impacts on enzymatic activity and growth (Jiang *et al*., 2019). This was tested at two microplastic size categories (5 µm and 100 nm), the smaller of which showed increased impact and translocation of the microplastics into the plant roots, likely blocking nutrient uptake and causing oxidative damage. Therefore the impacts on biota may be dependent on the size of plastic particles.

These impacts are likely to be species specific (Lozano and Rillig, 2020). Therefore, it is important to consider individual and community effects as microplastic contamination may lead to

unevenness in natural plant communities resulting in reduced ecosystem functionality (Lozano and Rillig, 2020). In the agricultural environment this may cause concern for food production. Commercially grown fruits and vegetable crops (e.g. apples, pears, cabbages, and carrots) have in some cases been shown to contain over 100,000 MP/g (Conti *et al*., 2020). Initial studies have shown that at high concentrations of microplastics (8% PE), above ground biomass was reduced in commercially important wheat crops (Liu *et al*., 2021). This indicates a reduction in crop yield at high microplastic concentrations. While the concentrations measured in this thesis did not reach such levels, with predicted increase in plastic production worldwide, this may be applicable in future scenarios.

Microplastics have been shown to impact soil invertebrates and microbial communities. Microorganisms are important for the functioning of soils and influence almost all soil properties (Gao *et al*., 2021). Microplastics may have selective effects on microbial communities and impact ecological function such as organic matter decomposition and nutrient cycling (Zhang *et al*., 2021). Additionally, microplastic presence in soils may impact on soil invertebrates. With high concentrations of HDPE in soil (5%), plant-herbivore interactions were significantly impacted as there was a reduced attraction of the black fungus gnat (*Bradysia difformis*) to lentil plants (*Lens culinaris*). Direct impacts on soil invertebrate health have been observed, with reduced reproduction in the annelid worm *Enchytraeus crypticus* reported in the presence of nylon microplastics, particularly in the smaller size range (Lahive *et al*., 2019).

It is important to consider that microplastics do not exist in isolation in the environment. Microplastics are comprised of different polymer types, shapes, and sizes. Furthermore, microplastics are likely to be present in the environment alongside other potential contaminants as they have been shown to co-transport with heavy metals, organic contaminants, and engineered nanoparticles (Ren *et al*., 2021). This is of particular importance in the soils studied in this thesis as microplastics in biosolids are likely to associate with a wide range and variety of contaminants (e.g. pharmaceuticals, hormones, personal care products, pesticides, metals, and herbicides) present in the biosolids matrix (Mohajerani and Karabatak, 2020). The surfaces of microplastics may be laden with metals and metalloids which may have adsorbed to the plastics wastewater treatment processes (Frost *et al*., 2022) Moreover, the environmental conditions in which microplastics are present will influence the overall impacts measured, e.g. in combination with the impacts of climate change (Knox *et al*., 2010; Richter and Semenov, 2005).

The findings presented in this thesis have expanded our understanding of microplastics in the terrestrial environment. This in combination with recent developments of microplastics research

in other environments (e.g. freshwater and marine) has increased public awareness and implications for policy to regulate plastic release to the environment. In the UK, regulators introduced a ban on wash-off cosmetics products containing microbeads in 2017 (DEFRA, 2016) and introduced charges on the use of single use carrier bags (HM Government, 2015). On a global level, at a recent UN Environment Assembly 175 nations signed up to a Global Plastics Treaty which will aim to address the plastic lifecycle from production to disposal. Such steps provide promise for future mitigation strategies which may reduce the release of microplastics into the environment. Considering this in relation to the use of biosolids, there may be a requirement to adapt the Sludge (use in agriculture) Regulations which currently control the use of biosolids in agricultural environments (Public Health England and Wales, 1989). While the results of this thesis showed that biosolids did not directly increase soil microplastic concentrations, regulators may consider the wider implications for this as there may be mobilisation of microplastics applied in biosolids to the wider environment. The current UK regulations were formed over 30 years ago, before people were widely aware of microplastics, and only contain limits for a few contaminants such as metals and biological contaminants. Therefore microplastics as a contaminant may be factored into future adaptations of biosolid regulations which could require a collaboration between water companies and farmers to minimise potential impacts.

6.4 Limitations and challenges

There were a number of limitations and challenges associated with the work presented in this thesis. Methodological limitation can be broadly categorised into aspects of laboratory and the field. There is currently a lack of standardised methods for sampling microplastics in the soil environment, therefore the sampling strategy developed here was done to best consider variation within fields whilst taking a representative sample of all areas. The composite method used here is relatively common for soil microplastic studies (Perez *et al*., 2022) and aims to capture a sample containing representative microplastic concentrations. However, as one composite sample was taken per field quarter, this means the resolution across fields was fairly coarse. This approach was informed by the literature that indicated that large differences in microplastic concentrations between fields with and without biosolid application were likely to be observed (Nizzetto *et al*., 2016); appreciable differences should have been measurable with the sampling strategy as adopted. However, given the results presented here, it is evident that these differences may not be as distinct as anticipated. Some studies consider differences between fields using a limited number of replicates per field (van den Berg *et al*., 2020) and some studies measure finer scale differences within a limited number of fields (Zhang *et al*., 2020). The sampling design used in this

thesis was therefore a trade-off to ensure sufficient replication with treatment groups whilst maintaining sampling integrity within individual fields by taking pseudo-replicates to enhance statistical power.

The laboratory methods used in this thesis presented challenges for microplastic quantification. One of the main limitations was the relatively small sample sizes utilised during the final identification of microplastics using FTIR. This is a result of the methodical constraints involved when using automated µFTIR, which requires considered sampling loading to ensure effective imaging. While comparable sample sizes to previous studies were used from which to extract microplastics (Crossman *et al*., 2020), the overall sample size analysed was relatively small once a subsample was taken for FTIR analysis. This approach has been previously validated (Horton *et al*., 2021) and was taken to avoid reliance on visual identification of particles, which is a less reliable method of microplastic identification which doesn't' provide information on polymer type and often results in a large amount of the microplastic fraction (particularly smaller particles) being missed due to the small size of microplastics and difficulty in discriminating natural materials from microplastics (Wen *et al*., 2021).Therefore automated µFTIR was used for more precise detection without operator bias. However, small subsamples were required to reduce overloading which would impede polymer detection on the FTIR. The advances in microplastic extraction from soils in Chapter 3 made it possible to utilise automated FTIR and ensure precision in microplastic identification, however further work is required to further clean up samples to allow for larger volumes of soil to be measured and improve detection accuracy.

Given that microplastic research is a rapidly developing field of research, there are a number of quality control constraints which apply not just to this thesis but to the wider microplastics literature. One of these relates to the units in which microplastics are reported. This is generally determined by the microplastic identification technique used which is generally one of three methods: FTIR, Raman or GC-MS. Of these, FTIR and Raman indicate the numbers of particles of different polymer types, including information on size and shape of microplastics, whereas GC-MS gives information on the mass of plastics and polymer mix (Chen *et al*., 2020). Therefore the reported units will depend on the methods used in individual studies. While in this thesis the FTIR approach was taken, best efforts were made to make these data comparable by also presenting data on calculated mass of plastics. This was reported as an output of the siMPle software used which is an automated method for analysing FTIR output for microplastic quantification (Primpke *et al*., 2020). This approach aimed to make results more comparable but was limited as these mass values were calculated based on the size of particles and assigned densities in relation to polymer types. This included the assumption that all particles are an elliptical shape and that the

densities of the generic polymer types found in the libraries used are assignable to the specific microplastics found in the soils studied here. This should be considered when interpreting these data and mass values should be considered indicative. Future studies may consider both approaches as a combined effort to report universally microplastic concentrations to obtain information on particle sizes and mass.

Another consideration are the positive control results. Again, this is not currently standard practise in microplastics research as the methods are yet to be fully developed. While it is recommended to correct final data for recovery rate where possible, it was not possible to correct the data of Chapters 4 and 5 for the recovery rates measured here as it was not possible to obtain suitable spiked plastics to cover a sufficiently wide spectrum of polymer types, sizes, and shapes. Therefore the positive controls reported here a guide based on the available plastics. Given that recovery rates measured here were approximately 40%, microplastic concentrations are likely to be underreported by approximately 60%. Therefore the maximum value for samples from fields (Chapters 4 and 5) may be ca. 22,000 MP/kg and ca. 19,000 MP/kg, respectively which is much higher than previously reported numbers (Corradini *et al*., 2021; Crossman *et al*., 2020; van den Berg *et al*., 2020). Until methods are further improved, this should be considered when assessing the risk of such microplastics as with increased concentrations comes increase potential for impact on soil properties and biota. While recovery rates could be improved on in this study it is also an issue within wider microplastics research as the majority of soil analysis methods are lacking in positive controls and contamination mitigation (Praveena *et al*., 2022).

In addition to the methodological constrains, the data in Chapters 4 and 5 were subject to interpretation based on a number of confounding factors. Given that both studies were based in the field, there were a number of variables which could not be controlled for such as additional sources of plastic and the environmental conditions that each site was subjected to. Assumption must therefore be made about the influence of these variable when considering the overall microplastic concentrations in relation to biosolid application.

6.5 Recommendations for future work

Microplastics research has developed rapidly in the last two decades, but there are still major gaps, particularly in relation to the terrestrial environment. Some of these fundamental gaps were addressed during this thesis, while new ones were identified.

In terms of methodological approaches, the work of this thesis highlighted the need for considering matrix characteristics when extracting microplastics from soils. However, there is an

apparent need for further refining of such methods to ensure further separation of microplastics from the matrix in which they reside. This will allow for larger sample sizes to be analysed and for more precise microplastic detection. Additionally, it will allow for sufficient replication within environments for accurate quantification of microplastics. Going forward, methods should consider further the inclusion of enhanced quality control measures including contamination controls and recovery rate estimation.

The results of studies reported in Chapters 4 and 5 suggest that biosolids are likely not the main source of microplastics in the soil environment in this instance. Therefore, it would be appropriate for future studies to consider additional sources of microplastics in agricultural soils. These may include other soil amendments (e.g. composts, manures, and synthetic fertilizers), general littering and agricultural plastics. Determining the input from these sources will allow for a targeted approach to mitigating microplastic contamination of soils. Further investigation of biosolid application may be undertaken. This may include determining if factors such as time of year spread, type of biosolid (e.g. slurry/cake/pellets) or origin of biosolids impact the amount of microplastics transferred to soils and their subsequent retention within the soil matrix. There is also a need to understand as the role of wind and runoff on the delivery of microplastics to soils. This is likely to depend on the surrounding geography of a site and the anthropogenic activities within close proximity.

Consideration of how these sources interact with farm management strategies and practices should be considered. For example, tillage timing, depth and frequency may impact the extent to which microplastics are incorporated into soils and their subsequent retention. The fate of microplastics in soils has not often been studied, however, given the results of this study (Chapter 5) and the potential loss of microplastics from the sampled sites, agriculutral soils may be a vector for microplastic transport in the environment, particularly in relation to freshwater systems.

The above should also be considered in relation to the environmental characteristics. The geomorphology of sites may be studied in relation to the movement of microplastics in the environment. This may be at a landscape scale across differencing topographic morphologies or a smaller scale within field units where there may be more fine scale differences including slope, aspect and evenness. Given the high variation within fields seen (Chapters 4 and 5), these fine scale differences should be investigated to determine how microplastics are distributed across soils. Likewise, differences on a temporal scale should be considered. The work presented in Chapter 4 shows the differences between two seasons, highlighting the importance of considering seasonal variation within future studies.

The work presented in Chapter 5 suggests that microplastics applied through biosolid application may not reside indefinitely in the soil environment; there is considerable opportunity and need to study further the fate of microplastics. This may depend on the land type, proximity to anthropogenic activities and climatic interactions. Additionally, it will be dependent on the soil characteristics. Future studies may consider which soil types are likely to result in increased runoff or vertical percolation to determine sensitive environments. This will be important for determining hotspots of pollution and identifying potential interventions between environmental transfers. It is important to consider where the eventual sinks of microplastics from the soil environment. This may include freshwaters and, as more recently identified, groundwaters.

There is a particular research gap in the impacts of microplastics in the soil environment in relation to biota. This is of importance when considering the crops grown on agricultural land as it is important to establish how microplastics may impact their yields and how this may have consequences for food production. Similarly there is space to expand on our knowledge of the impacts of microplastics on soil fauna. It is important to determine individual and community effects of microplastics in soils. Particularly sensitive species should be identified and both acute and chronic impacts measured. Furthermore, the impact of microplastics on soil biota should be considered in combination with other contaminants, such as those present in biosolids, and in relation to variable environmental conditions such as water content, soil organic matter, particle size distribution, pH and temperature. Going forward, the coordination of research into such impacts is essential to further understand such a complex pollutant in a dynamic environment.

6.6 Conclusions

The results of this thesis have broadly expanded our understanding of methods, sources, and fate of microplastics in the terrestrial environment. The work presented in Chapter 3 improves and advances on the methods used for microplastic detection in the terrestrial environment, providing a widely applicable set of extraction methods to allow for harmonisation across microplastic studies in soils. Furthermore, the work presented in Chapters 4 and 5 utilised these methods to advance our knowledge of environmental concentrations and composition of microplastics in agricultural soils, considering both the sources of microplastics (biosolids in particular) and their fate once in this environment. The work in this thesis has deepened our understanding of microplastics in the terrestrial environment by increasing our understanding of biosolids as a source of microplastics and their associated fate and transport in the environment. In addition, further questions arose from the work in this thesis which were refined by the

research outcomes presented. Therefore, recommendations of future work were suggested to further expand this area of research and our understand of microplastics in terrestrial soils.

Appendix A Developing a systematic method for extraction of microplastics in soils

Appendix A Table 1 Particle size distribution (proportion of clay, silt and sand) for inorganic soils used in the experiment measured using the hydrometer method (Sheldrick and Wang, 1993).

	Clay (%)	Silt (%)	Sand (%)
Soil 1	0.0	14.4	85.6
Soil 2	14.4	24.0	61.6
Soil 3	21.2	29.2	49.6
Soil 4	36.2	26.5	37.3
Soil 5	42.6	37.3	20.2
Soil 6	52.7	46.5	0.8

Appendix A Table 2 Statistical analysis results for Kruskal Wallis tests to measure differences in: the amount of organic removed from samples of initial high and low organic content by different chemicals and by those chemicals at different temperatures; the recovery efficiency of microplastics from inorganic soils using density separation methods; the recovery efficiency of microplastics from organic soils using a combination of digestion and density separation methods; and the ease of identification of microplastics subjected to different treatments, split into temperatures and chemicals.

		Kruskal Wallis			
		X_2	df	p	
Organic matter removed	Low organic	11.41	2	<0.01	Reagent only (H_2O_2, Fenton's and KOH)
	High organic	9.58	2	<0.01	
	Low organic	10.43	5	>0.05	Reagent and temperature (H_2O_2, Fenton's and KOH at 40 and 50^{o}C)
	High organic	15.69	5	<0.01	
Recovery efficiency (inorganic soil)	fibres	17.26	2	<0.01	By treatments (NaCl, $ZnCl_2$ and canola oil)
	big	12.29	2	<0.01	
	PET fragments	48.78	2	<0.01	
	PET fibres	59.25	2	<0.01	
	HDPE (0.5-1 mm)	8.16	2	<0.05	
	HDPE (0.25- 0.5 mm)	15.6	2	<0.01	
	Big PVC	44.31	2	<0.01	
	PVC (0.25- 0.5 mm)	46.33	2	<0.01	
	Big PS	8.32	2	<0.05	
	PS (0.25- 0.5 mm)	9.66	2	<0.01	
	PP fragments	11.99	2	<0.01	
Recovery efficiency (organic soil)	Fibres	9.14	8	>0.05	By treatments (H_2O_2 followed by NaCl, $ZnCl_2$ and canola oil)
	Fragments (0.25- 0.5 mm)	14.84	8	>0.05	
	PET fragments	18.89	2	<0.01	
	PET fibres	6.49	2	<0.05	
	PVC big	25.98	2	<0.01	
	PVC (0.25- 0.5 mm)	23.06	2	<0.01	
	PS (0.25- 0.5 mm)	20.64	2	<0.01	
HQI	Treatments	5.69	5	>0.05	At 40 and 50^{o}C: H_2O_2, Fenton's and KOH. NaCl, $ZnCl_2$ and canola oil, ultrasound.
	Chemicals	2.96	2	>0.05	
	Temperatures	0.02	1	>0.05	

Appendix A Table 3 Statistical analysis results for Wilcox tests to measure differences in: the amount of organic matter removed from samples of high and low initial organic at 40 and 50^{o}C; the amount of organic removed across all treatments in low and high organic; and the recovery efficiency in inorganic soils using canola oil with and without the use of ultrasound.

		Wilcox Test		
		W	p	
Organic matter removed	Low organic	34	>0.05	Between temperatures (40 and 50^{o}C)
	High organic	60	>0.05	
	All treatments	104	>0.05	Between low and high organic
Recovery efficiency (inorganic soil)	With and without ultrasound	-1.805	>0.05	Canola oil only

Appendix A Table 4 Statistical analysis results for one-way ANOVA's to measure differences in: the amount of organic removed across reagents with and without the inclusion of dispersant; recovery efficiency of total microplastics, fragments and small microplastics from inorganic soils using different density separation treatments; and recovery efficiency of total microplastics, fragments and big microplastics from organic soils using different treatments combining digestion and density separation.

		ANOVA			
		F	df	p	
Organic matter removed	Dispersant vs. no dispersant	20.61	2,12	<0.01	Between reagents (H_2O_2, Fenton's and KOH)
Recovery efficiency (inorganic soil)	Total microplastics	20.77	2,51	<0.01	Difference between treatments (NaCl, $ZnCl_2$ and canola oil)
	Fragments	7.34	2,51	<0.01	
	small	4.11	2,51	<0.05	
Recovery efficiency (organic soil)	Total microplastics	7.95	2,51	<0.01	Difference between treatments
	fragment	8.08	2,45	<0.05	
	big	5.83	2,45	<0.01	

Appendix A Table 5 Statistical analysis results for t-tests to measure differences in the recovery efficiency in inorganic soils using ZnCl2 and NaCl with and without the use of ultrasound.

		T Test		
		t	df	p
Ultra sound vs. no ultrasound	$ZnCl_2$	1.14	32	>0.05
	NaCl	1.18	34	>0.05

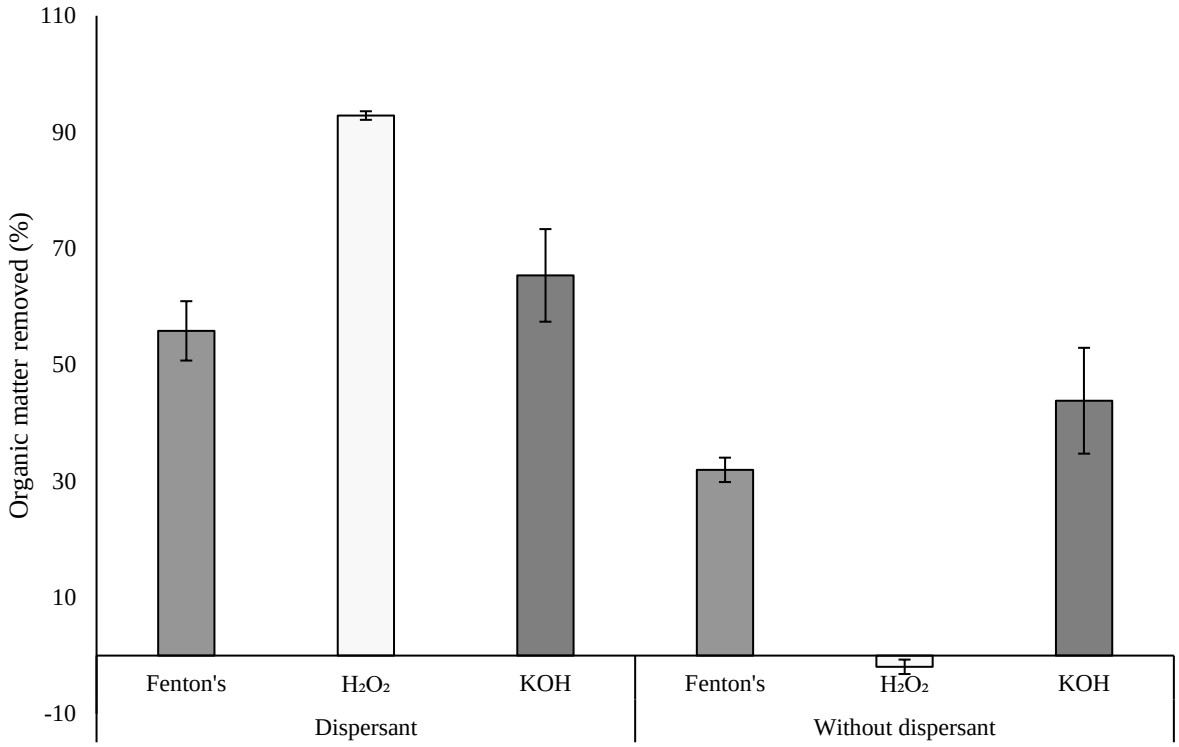
Appendix A Table 6 Statistical analysis results for Spearman's Rank correlations to determine relationships between; the amount of clay in inorganic soils and the recovery efficiency; the organic content in a sample and the recovery of total microplastics, fragments, small microplastics, PET fragments and small LDPE.

		Spearman's rank	
		r_s	p
	Amount of clay in sample vs. recovery of total microplastics	0.04	>0.05
Organic matter content vs. recovery with canola oil	Total microplastics	0.5	<0.05
	Fragment	0.57	<0.05
	small	0.48	<0.05
	PET fragments	0.69	<0.01
	Small LDPE	0.55	<0.05

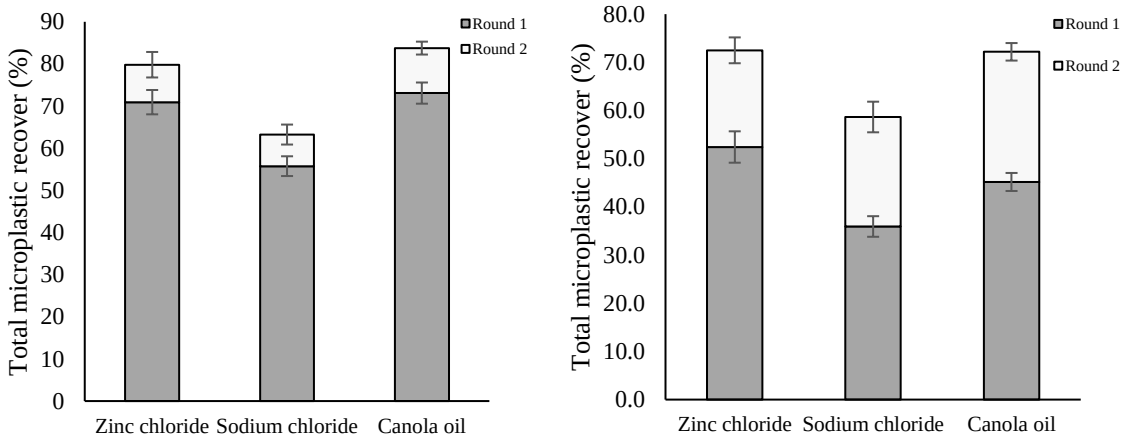
Appendix A Table 7 Particle size distribution of clay used to create artificial soils in Chapter 3. Material is Kaolin sourced from Sibelco, UK.

Fraction	%
Clay (<2um)	25.63
Silt (2 - 50um)	51.74
Very fine sand (50-100um)	1.96
Fine sand (100-250um)	4.14
Medium sand (250-500um)	12.06
Coarse sand (500-1000um)	4.64
Very coarse sand (1000-2000um)	0

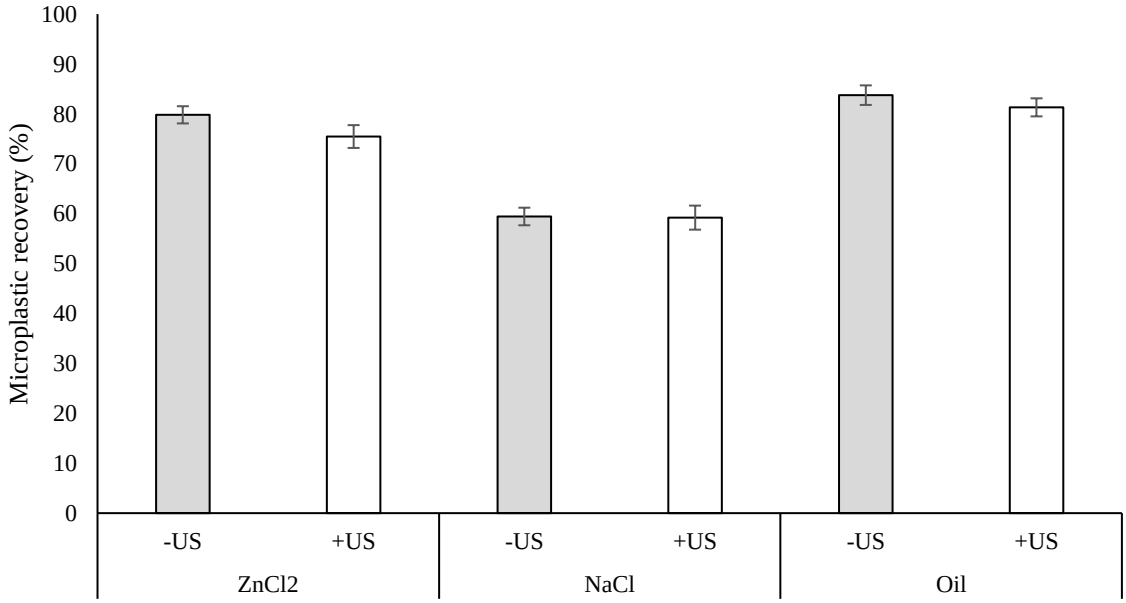
Appendix A Figure 1 Percentage organic matter removed from soils with initially high organic content (~70%) using three digestion methods (Fenton's reagent, hydrogen peroxide and potassium hydroxide) with and without the use of a dispersant (sodium hexametaphosphate)



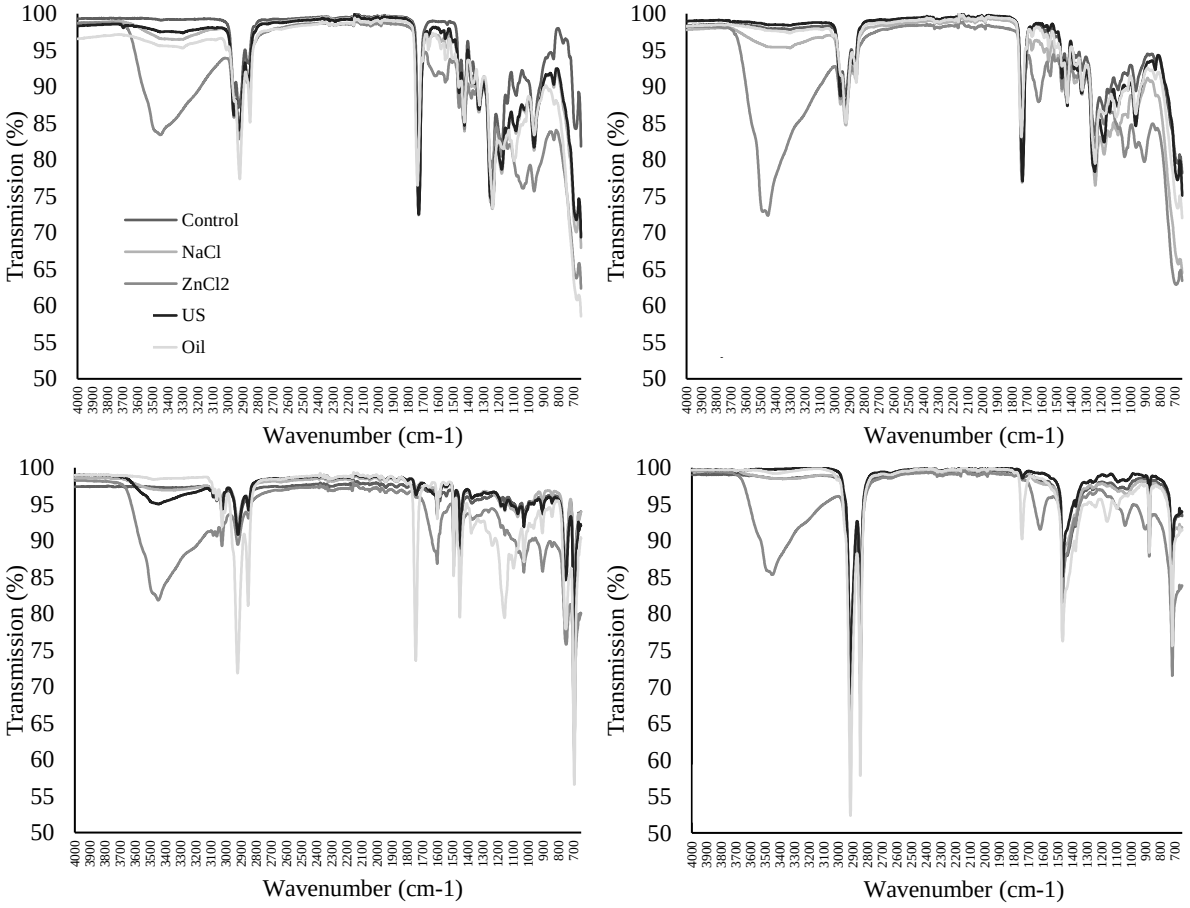
Appendix A Figure 2 Total microplastic recovery from low organic (left) and organic (right) soils using the three density separation methods ($ZnCl_2$, NaCl and canola oil) in the first and second round of extraction.



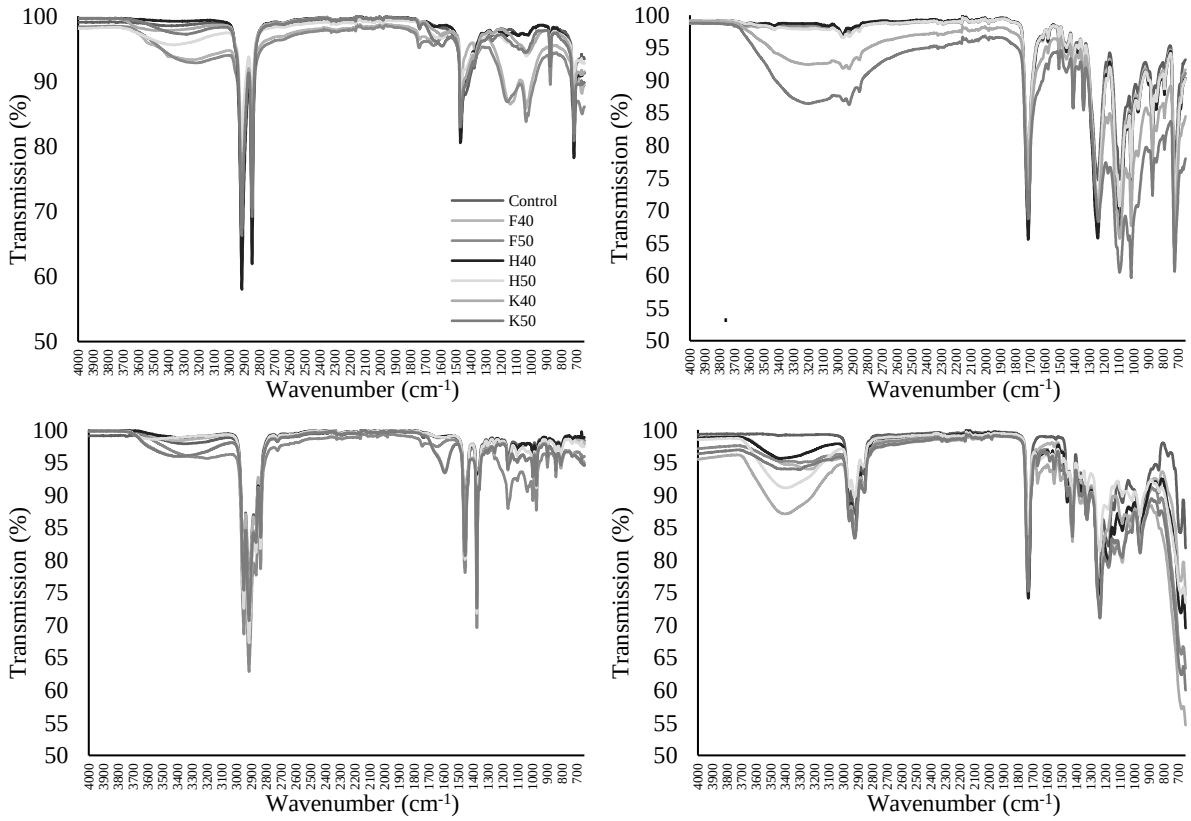
Appendix A Figure 3 Total microplastic recovery from inorganic soils using the three density separation methods ($ZnCl_2$, NaCl and canola oil) with (+US) and without (-US) the inclusion of an ultrasound step



Appendix A Figure 4 FT-IR spectra for a sample of microplastic spikes using the experiment when treated with density treatments involving NaCl, $ZnCl_2$, oil and ultrasound (US) (a. big PVC b. small PVC c. small PS d. small LDPE).



Appendix A Figure 5 FT-IR spectra for a sample of microplastic spikes using the experiment when treated with digestion treatments involving Fenton's reagent, potassium hydroxide and hydrogen peroxide at 40 and 50^{o}C. (a. small LDPE b. PET fragments c. PP fragments d. large PVC)



Appendix B Agricultural soils and microplastics: are biosolids the problem?

Appendix B Table 1 Blank contamination values in samples. Values are reported for whole blank sample (per sample vial). Average blank is the mean of all blanks (n=10) and the Limit of Detection (LOD) is 3.3 x the standard deviation.

	Total microplastics	AVP	Cellulose	EVA	Polyester	Polypropylene
Blank 1	*0*	*0*	*0*	*0*	*0*	*0*
Blank 2	*4*	*1*	*0*	*1*	*0*	*2*
Blank 3	*1*	*0*	*0*	*0*	*1*	*0*
Blank 4	*0*	*0*	*0*	*0*	*0*	*0*
Blank 5	*6*	*0*	*0*	*0*	*5*	*1*
Blank 6	*9*	*0*	*0*	*0*	*9*	*0*
Blank 7	*0*	*0*	*0*	*0*	*0*	*0*
Blank 8	*0*	*0*	*0*	*0*	*0*	*0*
Blank 9	*0*	*0*	*0*	*0*	*0*	*0*
Blank 10	*5*	*2*	*1*	*2*	*0*	*0*
Average blank	***2.5***	***0.3***	***0.1***	***0.3***	***1.5***	***0.3***
LOD	***8.25***	***0.99***	***0.33***	***0.99***	***4.95***	***0.99***

Appendix B Table 2 Field and soil characteristics for individual sampling sites. Clay (<0.4µm), Silt (0.4-63µm), Sand (63-1000µm), Sand (>1000µm).

	Field	Particle size distribution (%)				Organic matter (%)	Zinc (ppm)	Copper (ppm)	Nickel (ppm)	Distance from road (m)	Gradient (°)
		Clay	Silt	Sand	> Sand						
Biosolid treated	*1*	1.7	11.4	4.9	81.9	7.2	0.0	0.0	0.0	259.6	2.9
	2	2.1	13.7	3.4	80.8	5.7	0.0	0.0	0.0	2.1	1.8
	3	3.5	11.4	4.1	81.1	6.4	0.0	0.0	0.0	20.6	3.4
	4	1.8	14.4	4.0	79.9	6.0	0.0	0.0	0.0	0.0	1.8
	5	1.8	12.6	5.8	79.8	4.9	0.0	0.0	0.0	20.3	2.4
Untreated	*6*	3.1	11.7	3.3	81.9	5.3	0.0	0.0	0.0	7.0	2.3
	7	1.4	8.1	2.4	88.2	7.0	0.0	0.0	0.0	0.3	1.9
	8	3.5	11.1	3.9	81.5	6.6	0.0	0.0	0.0	16.5	3.8
	9	2.8	14.1	3.9	79.2	8.5	0.0	0.0	0.0	4.9	2.7
	10	2.2	18.7	6.5	72.5	6.1	0.0	0.0	0.0	6.5	1.8

Appendix B Table 3 Rainfall per week in in Southeast England during the two sampling months (August 2019 and February 2020; Environment Agency, 2020)

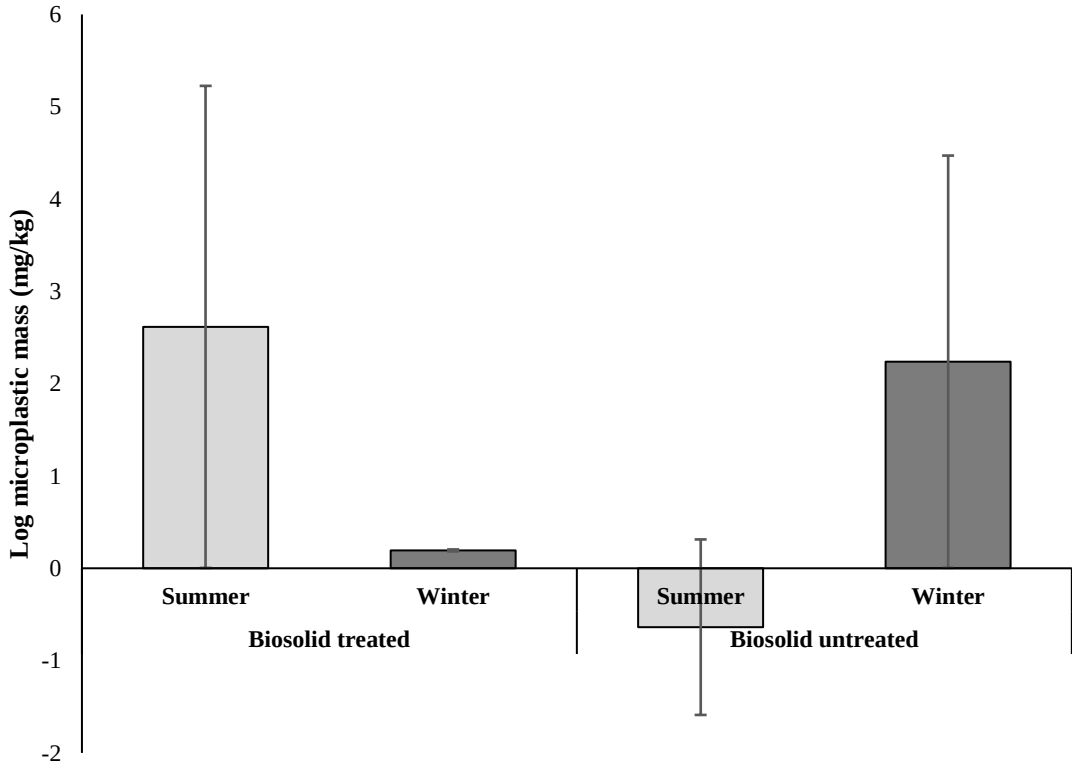
	Week	Total rainfall (mm)
August	*31.07.19-06.08.19*	*2*
	07.08.19-13.08.19	*24*
	14.08.19-20.08.19	*23*
	21.08.19-27.08.19	*0.6*
	28.08.19-03.09.19	*7*
	Total	***51***
February	*29.01.20-04.02.20*	*13*
	05.02.20-11.02.20	*22*
	12.02.20-18.02.20	*57*
	19.02.20-25.02.20	*18*
	26.02.20-03.03.20	*31*
	Total	***122***

Appendix B Table 4 Polymers included in siMPle automated IR database (version 1.0.1) used for polymer identification

Polymer match
Acrylates/Polyurethanes/Varnish (APV)
Cellulose artificial modified
Ethylene-Vinyl-Acetate (EVA)
Nitrile rubber
Polyamide
Polybutadiene
Polycaprolactone
Polycarbonate
Polychloroprene
Polyester
Polyetheretherketone
Polyethylene
Polyethylene chlorinated
Polyethylene oxidized
Polyimide
Polyisoprene-chlorinated
Polylactic acid
Polyoxymethlyene
Polypropylene
Polystyrene
Polysulfone
Polyvinylchloride
Rubber type 1
Rubber type 2
Rubber type 3
Acrylonitrile-butadiene

Appendix B Table 5 Spiked recovery of positive control samples (n=6)

Spike	Recovery (%)			
	PET	**PE**	**PP**	**PVC**
Spike 1	0.0	101.4	30.8	11.2
Spike 2	160.9	110.6	102.1	24.4
Spike 3	56.4	0.0	38.4	51.3
Spike 4	29.0	0.0	29.6	0.0
Spike 5	0.0	0.0	29.0	0.0
Spike 6	0.0	135.2	95.9	0.0
Mean	49.3	69.5	59.4	17.4



Appendix B Figure 1 Mean microplastic concentrations as mass in soils with and without sludge application during summer (August 2019) and winter (February 2020) months. Values are logged for visualisation. Error bars are standard error (n=20).

Appendix B Figure 2 Photographs of example plastic exposure routes in sampled soils which may result in patchy distribution. A+B) Darker brown patches are 'sludge cake' after soil mixing. C) Macroplastic from suspected agricultural activity.

Appendix C Temporal variation in soil microplastic abundance related to biosolid application

Appendix C Table 1 Blank contamination values in samples. Values are reported for whole blank sample (per sample vial). Average blank is the mean of all blanks (n=10) and the Limit of Detection (LOD) is 3.3 x the standard deviation.

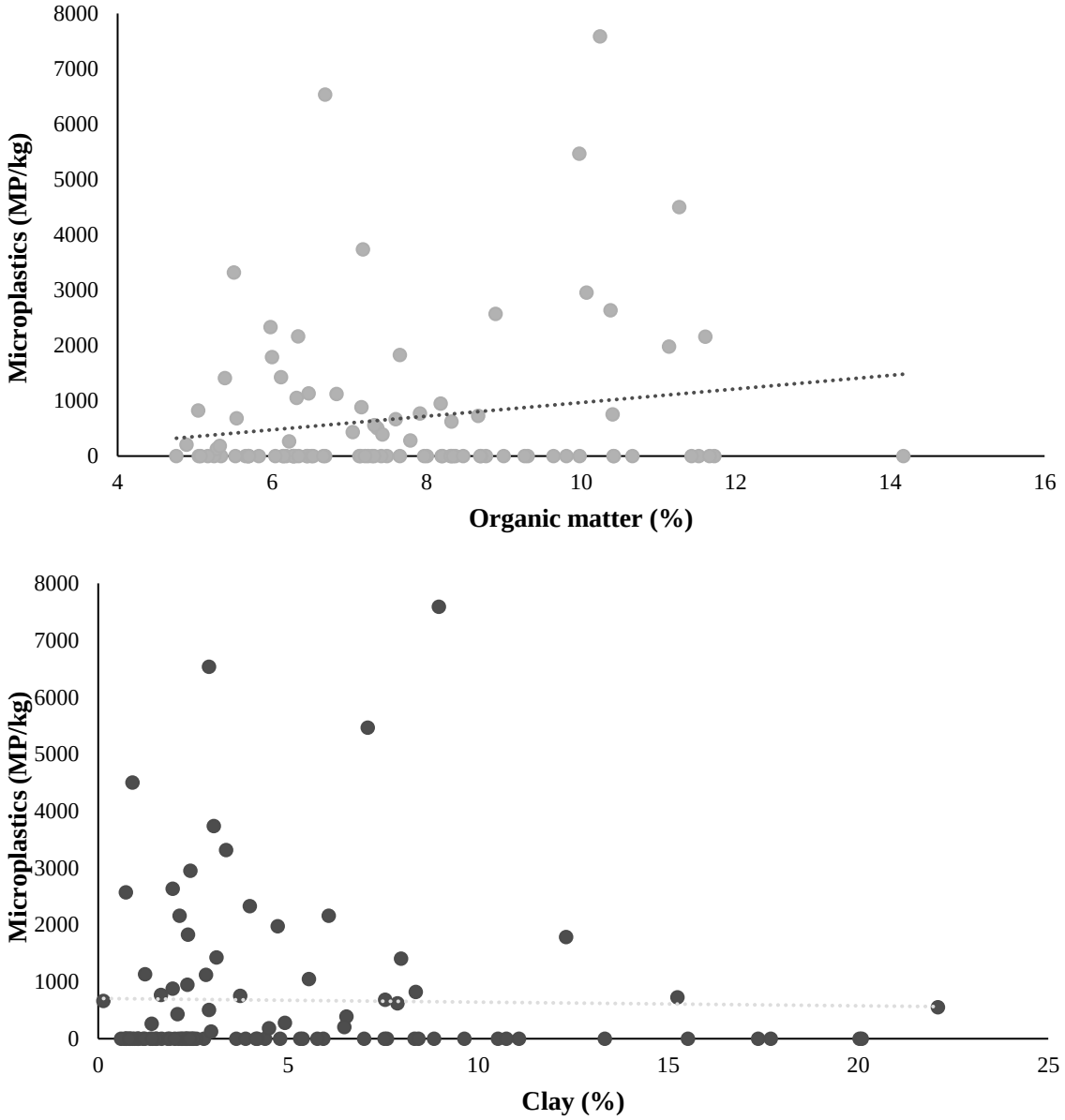
	Total	APV	Cellulose	EVA	Polyamide	Polyester	Polyethylene	PP	PS	PVC
Blank 1	1	0	0	0	0	0	0	0	1	0
Blank 2	1	0	0	0	0	0	1	0	0	0
Blank 3	5	2	0	3	0	0	0	0	0	0
Blank 4	3	0	0	2	0	1	0	0	0	0
Blank 5	14	0	1	2	0	0	0	11	0	0
Blank 6	33	0	0	1	7	0	1	23	0	1
Blank 7	14	0	0	7	1	0	0	5	0	1
Blank 8	4	0	1	0	0	0	0	3	0	0
Blank 9	0	0	0	0	0	0	0	0	0	0
Blank 10	1	0	0	0	0	0	0	1	0	0
Mean	**7.6**	**0.2**	**0.2**	**1.5**	**0.8**	**0.1**	**0.2**	**4.3**	**0.1**	**0.2**
LOD	**34.0**	**2.1**	**1.4**	**7.3**	**7.3**	**1.0**	**1.4**	**24.6**	**1.0**	**1.4**

*APV= 'acrylates, polyurethanes and varnish', EVA= ethylene vinyl acetate, PVC= polyvinylchloride, PS= polystyrene, PP= polypropylene,

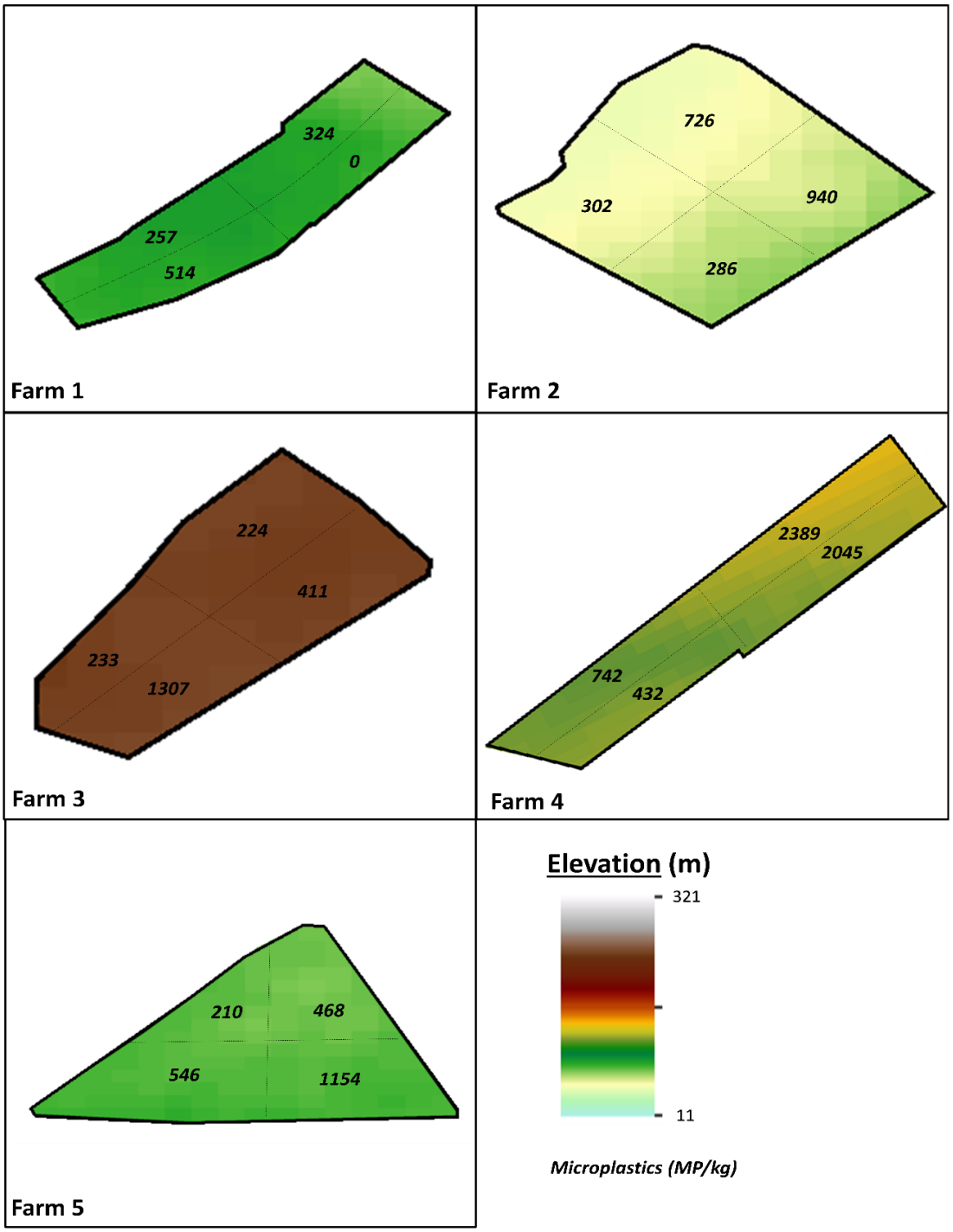
Appendix C Table 2 Microplastic numbers, mass, and size for individual fields across sampling occasions. Rainfall is reported as the total mm in the week of sampling. Organic is reported based on LOI and particle size distribution is reported as percent clay.

Field	Sampling time	Total microplastics (MP/kg)	Mass (ug/kg)	Average size (um)	Rainfall (mm)	Organic (%)	Clay (%)
1	Before	237.5	96.9	37.5	4	8.7	2.1
	1 week	0.0	0.0	20.2	44	8.1	3.6
	1 month	181.7	151.9	51.1	22	8.4	12.3
	3 months	139.3	127.5	106.3	45	8.0	11.8
	1 year	810.1	786.5	93.7	16	8.1	0.7
2	Before	0.0	135.8	87.6	0.6	5.5	3.2
	1 week	829.6	187.9	49.4	7	5.4	6.1
	1 month	779.1	211.2	97.8	41	5.4	7.7
	3 months	98.3	215.9	161.5	27	5.4	12.2
	1 year	1111.2	1096.2	113.1	16	5.8	4.3
3	Before	1633.8	475.5	32.0	0.6	6.7	2.1
	1 week	70.5	43.2	145.1	41	6.7	1.8
	1 month	447.2	0.0	40.5	19	6.5	6.9
	3 months	347.4	239.3	202.9	45	6.9	2.2
	1 year	220.6	4.5	12.5	27	6.2	1.4
4	Before	1892.4	3086.5	211.2	26	10.5	2.8
	1 week	1125.5	2062.4	147.8	23	10.4	1.5
	1 month	0.0	0.0	20.2	1	11.5	6.3
	3 months	3452.4	801.4	157.3	24	9.9	9.3
	1 year	540.2	20.8	95.3	16	11.4	2.3
5	Before	1584.9	1769.0	174.5	40	7.7	2.4
	1 week	155.7	15.7	23.9	26	7.4	5.5
	1 month	126.9	84.7	38.9	27	6.7	2.8
	3 months	899.0	191.9	130.9	16	6.1	5.8
	1 year	206.2	19.6	84.2	34	7.2	4.4

Appendix C Figure 1 Photographs of example biosolid patches on sampled fields suggesting ununiform microplastic dispersal. Patches of biosolids are circled in red.



Appendix C Figure 2 Top: Correlation between soil organic matter and microplastic concentrations (r= 0.04, p=0.72, Spearman's Rank). Bottom: Correlation between particle size distribution of soil as % clay content and microplastic concentrations (r= 0.10, p=0.328, Spearman's Rank).



Appendix C Figure 3 Microplastic concentrations across the sampling fields (1-5) and sampling quarters. Microplastic concentrations are denoted in italics in each of the sampled quarters which are separated by dashed lines and slope is indicated as elevation in metres.

List of References

Abel, S.M., Primpke, S., Int-Veen, I., Brandt, A. and Gerdts, G. (2021) “Systematic identification of microplastics in abyssal and hadal sediments of the Kuril Kamchatka trench,” *Environmental Pollution*, 269, p. 116095.

Adam, V., von Wyl, A. and Nowack, B. (2021) “Probabilistic environmental risk assessment of microplastics in marine habitats,” *Aquatic Toxicology*, 230, p. 105689.

Akdogan, Z. and Guven, B. (2019) “Microplastics in the environment: A critical review of current understanding and identification of future research needs,” *Environmental Pollution*, 254, p. 113011.

Aktar, W., Sengupta, D. and Chowdhury, A. (2009) “Impact of pesticides use in agriculture: their benefits and hazards,” *Interdisciplinary toxicology*, 2(1), pp. 1–12.

Ali, M.I., Ahmed, S., Robson, G., Javed, I., Ali, N., Atiq, N. and Hameed, A. (2014) “Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates,” *Journal of basic microbiology*, 54(1), pp. 18–27.

Allen, S., Allen, D., Phoenix, V.R., le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S. and Galop, D. (2019) “Atmospheric transport and deposition of microplastics in a remote mountain catchment,” *Nature Geoscience*, 12(5), pp. 339–344.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C. and Cunha-Queda, C. (2015) “Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors,” *Waste management*, 40, pp. 44–52.

Alvim, C.B., Mendoza-Roca, J.A. and Bes-Piá, A. (2020) “Wastewater treatment plant as microplastics release source–Quantification and identification techniques,” *Journal of Environmental Management*, 255, p. 109739.

Andrady, A.L. (2011) “Microplastics in the marine environment,” *Marine pollution bulletin*, 62(8), pp. 1596–1605.

Araujo, C.F., Nolasco, M.M., Ribeiro, A.M.P. and Ribeiro-Claro, P.J.A. (2018) “Identification of microplastics using Raman spectroscopy: Latest developments and future prospects,” *Water research*, 142, pp. 426–440.

Arthur, C., Baker, J. and Bamford, H. (2008) “International research workshop on the occurrence, effects, and fate of microplastic marine debris,” in *Conference Proceedings. Sept*, pp. 9–11.

Ashraf, M.A. (2017) “Persistent organic pollutants (POPs): a global issue, a global challenge.” Springer.

ASTM (2020) *D7611 / D7611M-20, Standard Practice for Coding Plastic Manufactured Articles for Resin Identification*. West Conshohocken, PA.

Astner, A.F., Hayes, D.G., O’Neill, H., Evans, B.R., Pingali, S. v, Urban, V.S. and Young, T.M. (2019) “Mechanical formation of micro-and nano-plastic materials for environmental studies in agricultural ecosystems,” *Science of The Total Environment*, 685, pp. 1097–1106.

Avery-Gomm, S., Borrelle, S.B. and Provencher, J.F. (2018) “Linking plastic ingestion research with marine wildlife conservation,” *Science of the Total Environment*, 637, pp. 1492–1495.

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A. and Longstaffe, F.J. (2016) “Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments,” *Marine pollution bulletin*, 110(1), pp. 383–395.

Bardgett, R.D. and Van Der Putten, W.H. (2014) “Belowground biodiversity and ecosystem functioning,” *Nature*, 515(7528), p. 505.

Barnes, D.K.A., Galgani, F., Thompson, R.C. and Barlaz, M. (2009) “Accumulation and fragmentation of plastic debris in global environments,” *Philosophical transactions of the royal society B: biological sciences*, 364(1526), pp. 1985–1998.

Bender, S.F., Wagg, C. and van der Heijden, M.G.A. (2016) “An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability,” *Trends in ecology & evolution*, 31(6), pp. 440–452.

van den Berg, P., Huerta-Lwanga, E., Corradini, F. and Geissen, V. (2020) “Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils,” *Environmental Pollution*, p.261, 114198.

Beriot, N., Peek, J., Zornoza, R., Geissen, V. and Lwanga, E.H. (2021) “Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain,” *Science of the Total Environment*, 755, p. 142653.

Besley, A., Vijver, M.G., Behrens, P. and Bosker, T. (2017) "A standardized method for sampling and extraction methods for quantifying microplastics in beach sand," *Marine pollution bulletin*, 114(1), pp. 77–83.

de Bhowmick, G. and Sarmah, A.K. (2022) "Microplastics contamination associated with land-application of biosolids–A Perspective," *Current Opinion in Environmental Science & Health*, p. 100342.

Bläsing, M. and Amelung, W. (2018) "Plastics in soil: Analytical methods and possible sources," *Science of the Total Environment*, 612, pp. 422–435.

Boardman, J., Burt, T. and Foster, I. (2020) "Monitoring soil erosion on agricultural land: results and implications for the Rother valley, West Sussex, UK," *Earth Surface Processes and Landforms*, 45(15), pp. 3931–3942.

Boots, B., Russell, C.W. and Green, D.S. (2019) "Effects of microplastics in soil ecosystems: above and below ground," *Environmental science & technology*, 53(19), pp. 11496–11506.

Borrelle, S.B., Ringma, J., Law, K.L., Monnahan, C.C., Lebreton, L., McGivern, A., Murphy, E., Jambeck, J., Leonard, G.H., Hilleary, M.A. and Eriksen, M. (2020) "Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution." *Science*, 369(6510), pp.1515-1518.

Brandes, E., Henseler, M. and Kreins, P. (2021) "Identifying hot-spots for microplastic contamination in agricultural soils - A spatial modelling approach for Germany," *Environmental Research Letters*, 16(10), p.104041.

Braun, M., Mail, M., Heyse, R. and Amelung, W. (2021) "Plastic in compost: Prevalence and potential input into agricultural and horticultural soils," *Science of The Total Environment*, 760, p. 143335.

Brydson, J.A. (1999) *Plastics materials*. Elsevier, UK, 7th edition.

Bucci, K. and Rochman, C.M. (2022) "Microplastics: a multidimensional contaminant requires a multidimensional framework for assessing risk," *Microplastics and Nanoplastics*, 2(1), pp. 1–9.

Bullard, J.E., Ockelford, A., O'Brien, P. and Neuman, C.M. (2021) "Preferential transport of microplastics by wind," *Atmospheric Environment*, 245, p. 118038.

Cabernard, L., Pfister, S., Oberschelp, C. and Hellweg, S. (2022) "Growing environmental footprint of plastics driven by coal combustion," *Nature Sustainability*, 5(2), pp. 139–148.

Cabrerizo, A., Muir, D.C.G., De Silva, A.O., Wang, X., Lamoureux, S.F. and Lafrenière, M.J. (2018) "Legacy and Emerging Persistent Organic Pollutants (POPs) in Terrestrial Compartments in the High Arctic: Sorption and Secondary Sources," *Environmental science & technology*, 52(24), pp. 14187–14197.

Campanale, C., Galafassi, S., Savino, I., Massarelli, C., Ancona, V., Volta, P. and Uricchio, V.F. (2022) "Microplastics pollution in the terrestrial environments: Poorly known diffuse sources and implications for plants," *Science of The Total Environment*, 805, p. 150431.

Cashman, M.A., Ho, K.T., Boving, T.B., Russo, S., Robinson, S. and Burgess, R.M. (2020) "Comparison of microplastic isolation and extraction procedures from marine sediments," *Marine pollution bulletin*, 159, p. 111507.

Castan, S., Henkel, C., Hüffer, T. and Hofmann, T. (2021) "Microplastics and nanoplastics barely enhance contaminant mobility in agricultural soils," *Communications Earth & Environment*, 2(1), pp. 1–9.

Catarino, A.I., Thompson, R., Sanderson, W. and Henry, T.B. (2017) "Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues," *Environmental toxicology and chemistry*, 36(4), pp. 947–951.

Chau, C., Paulillo, A., Lu, N., Miodownik, M. and Lettieri, P. (2021) "The environmental performance of protecting seedlings with plastic tree shelters for afforestation in temperate oceanic regions: A UK case study," *Science of the Total Environment*, 791, p. 148239.

Chen, G., Fu, Z., Yang, H. and Wang, J. (2020) "An overview of analytical methods for detecting microplastics in the atmosphere," *TrAC Trends in Analytical Chemistry*, 130, p. 115981.

Chen, Y., Leng, Y., Liu, X. and Wang, J. (2020) "Microplastic pollution in vegetable farmlands of suburb Wuhan, central China," *Environmental Pollution*, 257, p. 113449.

Chowaniak, M., Głąb, T., Klima, K., Niemiec, M., Zaleski, T. and Zuzek, D. (2020) "Effect of tillage and crop management on runoff, soil erosion and organic carbon loss," *Soil Use and Management*, 36(4), pp. 581–593.

Claessens, M., van Cauwenberghe, L., Vandegehuchte, M.B. and Janssen, C.R. (2013) "New techniques for the detection of microplastics in sediments and field collected organisms," *Marine pollution bulletin*, 70(1–2), pp. 227–233.

Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K. and Janssen, C.R. (2011) "Occurrence and distribution of microplastics in marine sediments along the Belgian coast," *Marine pollution bulletin*, 62(10), pp. 2199–2204.

Clayton, G.W., Harker, K.N., O'Donovan, J.T., Blackshaw, R.E., Dosdall, L., Stevenson, F.C., Johnson, E.N. and Ferguson, T. (2004) "Polymer seed coating of early-and late-fall-seeded herbicide-tolerant canola (Brassica napus L.) cultivars," *Canadian journal of plant science*, 84(4), pp. 971–979.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C. and Galloway, T.S. (2014) "Isolation of microplastics in biota-rich seawater samples and marine organisms," *Scientific reports*, 4, p. 4528.

Collivignarelli, M.C., Abbà, A., Frattarola, A., Miino, M.C., Padovani, S., Katsoyiannis, I. and Torretta, V. (2019) "Legislation for the reuse of biosolids on agricultural land in Europe: Overview," *Sustainability (Switzerland)*. MDPI.

Conti, G.O., Ferrante, M., Banni, M., Favara, C., Nicolosi, I., Cristaldi, A., Fiore, M. and Zuccarello, P. (2020) "Micro-and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population," *Environmental Research*, 187, p. 109677.

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M. and Galloway, T.S. (2017) "A small-scale, portable method for extracting microplastics from marine sediments," *Environmental Pollution*, 230, pp. 829–837.

Corradini, F., Bartholomeus, H., Lwanga, E.H., Gertsen, H. and Geissen, V. (2019) "Predicting soil microplastic concentration using vis-NIR spectroscopy," *Science of the Total Environment*, 650, pp. 922–932.

Corradini, F., Casado, F., Leiva, V., Huerta-Lwanga, E. and Geissen, V. (2021) "Microplastics occurrence and frequency in soils under different land uses on a regional scale," *Science of the Total Environment*, 752, p. 141917.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E. and Geissen, V. (2019) "Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal," *Science of the Total Environment*, 671, pp. 411–420.

da Costa, J.P., Paço, A., Santos, P.S.M., Duarte, A.C. and Rocha-Santos, T. (2018) "Microplastics in soils: assessment, analytics and risks," *Environmental Chemistry*, 16(1), pp. 18–30.

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F. and Narayanaswamy, B.E. (2020) "Microplastic accumulation in deep-sea sediments from the Rockall Trough," *Marine Pollution Bulletin*, 154, p. 111092.

Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., de Frond, H., Rochman, C. and Herodotou, O. (2021) "Microplastic spectral classification needs an open source community: open specy to the rescue!," *Analytical Chemistry*, 93(21), pp. 7543–7548.

Cranfield University (2020) *The Soils Guide*, *2020*, [Accessed on 21 April 2022] https://www.cranfield.ac.uk/themes/environment-and-agrifood/landis/soils-guide.

Crichton, E.M., Noël, M., Gies, E.A. and Ross, P.S. (2017) "A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments," *Analytical Methods*, 9(9), pp. 1419–1428.

Crossman, J., Hurley, R.R., Futter, M. and Nizzetto, L. (2020) "Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment," *Science of the Total Environment*, 724.

Cuadros-Rodríguez, L., Bagur-González, M.G., Sánchez-Vinas, M., González-Casado, A. and Gómez-Sáez, A.M. (2007) "Principles of analytical calibration/quantification for the separation sciences," *Journal of Chromatography A*, 1158(1–2), pp. 33–46.

Cunningham, R.B. and Lindenmayer, D.B. (2005) "Modeling count data of rare species: some statistical issues," *Ecology*, 86(5), pp. 1135–1142.

Cunsolo, S., Williams, J., Hale, M., Read, D.S. and Couceiro, F. (2021) "Optimising sample preparation for FTIR-based microplastic analysis in wastewater and sludge samples: multiple digestions," *Analytical and Bioanalytical Chemistry*, 413, pp. 3789–3799.

Dai, Y., Shi, J., Zhang, N., Pan, Z., Xing, C. and Chen, X. (2022) "Current research trends on microplastics pollution and impacts on agro-ecosystems: a short review," *Separation Science and Technology*, 57(4), pp. 656–669.

Decaëns, T. (2010) "Macroecological patterns in soil communities," *Global Ecology and Biogeography*, 19(3), pp. 287–302.

DEFRA (2016) *Proposals to ban the use of plastic microbeads in cosmetics and personal care products in the UK and call for evidence on other sources of microplastics entering the*

marine environment. UK, [Accessed on 21 April 2022], www.gov.uk/government/publications.

Ding, L., Huang, D., Ouyang, Z. and Guo, X. (2022) "The effects of microplastics on soil ecosystem: A review," *Current Opinion in Environmental Science & Health*, p. 100344.

Ding, L., Wang, Xinglei, Ouyang, Z., Chen, Y., Wang, Xuexia, Liu, D., Liu, S., Yang, X., Jia, H. and Guo, X. (2021) "The occurrence of microplastic in Mu Us Sand Land soils in northwest China: Different soil types, vegetation cover and restoration years," *Journal of Hazardous Materials*, 403, p. 123982.

Ding, L., Zhang, S., Wang, X., Yang, X., Zhang, C., Qi, Y. and Guo, X. (2020) "The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-western China," *Science of the Total Environment*, 720, p. 137525.

Dong, M., Zhang, Q., Xing, X., Chen, W., She, Z. and Luo, Z. (2020) "Raman spectra and surface changes of microplastics weathered under natural environments," *Science of The Total Environment*, 739, p. 139990.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V. and Tassin, B. (2017) "A first overview of textile fibers, including microplastics, in indoor and outdoor environments," *Environmental Pollution*, 221, pp. 453–458.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N. and Tassin, B. (2015) "Microplastic contamination in an urban area: a case study in Greater Paris," *Environmental Chemistry*, 12(5), pp. 592–599.

Dris, R., Gasperi, J., Saad, M., Mirande, C. and Tassin, B. (2016) "Synthetic fibers in atmospheric fallout: A source of microplastics in the environment?" *Marine Pollution Bulletin*, 104(1–2), pp. 290–293.

Drummond, J.D., Schneidewind, U., Li, A., Hoellein, T.J., Krause, S. and Packman, A.I. (2022) "Microplastic accumulation in riverbed sediment via hyporheic exchange from headwaters to mainstems," *Science advances*, 8(2), p. eabi9305.

Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F. and Rosal, R. (2020) "Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge," *Environmental Pollution*, 259, p. 113837.

Ekane, N., Barquet, K. and Rosemarin, A. (2021) “Resources and risks: Perceptions on the application of sewage sludge on agricultural land in Sweden, a case study,” *Frontiers in Sustainable Food Systems*, 5, p. 94.

van Emmerik, T. and Schwarz, A. (2020) “Plastic debris in rivers,” *Wiley Interdisciplinary Reviews: Water*, 7(1), p. e1398.

Environment Agency (2020) *The National Archives*, *Weekly rainfall and river flow reports for England*, [Accessed on 21 April 2022] https://www.gov.uk/government/publications/weekly-rainfall-and-river-flow-reports-for-england.

Environment agency (2022) *Water and sewerage companies in England: environmental performance report 2021*, [Accessed on 12 August 2022]. https://www.gov.uk/government/publications/water-and-sewerage-companies-in-england-environmental-performance-report-2021/water-and-sewerage-companies-in-england-environmental-performance-report-2021#conclusions.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C. and Christie-Oleza, J.A. (2017) “Lost, but found with Nile red: a novel method for detecting and quantifying small microplastics (1 mm to 20 µm) in environmental samples,” *Environmental science & technology*, 51(23), pp. 13641–13648.

Fahrenfeld, N.L., Arbuckle-Keil, G., Beni, N.N. and Bartelt-Hunt, S.L. (2019) “Source tracking microplastics in the freshwater environment,” *TrAC Trends in Analytical Chemistry*, 112, pp. 248–254.

Fairbairn, D.J., Karpuzcu, M.E., Arnold, W.A., Barber, B.L., Kaufenberg, E.F., Koskinen, W.C., Novak, P.J., Rice, P.J. and Swackhamer, D.L. (2016) “Sources and transport of contaminants of emerging concern: A two-year study of occurrence and spatiotemporal variation in a mixed land use watershed,” *Science of The Total Environment*, 551–552, pp. 605–613.

Fakour, H., Lo, S.L., Yoashi, N.T., Massao, A.M., Lema, N.N., Mkhontfo, F.B., Jomalema, P.C., Jumanne, N.S., Mbuya, B.H., Mtweve, J.T. and Imani, M. (2021) “Quantification and analysis of microplastics in farmland soils: Characterization, sources, and pathways,” *Agriculture (Switzerland)*, 11(4), p.330.

Felsing, S., Kochleus, C., Buchinger, S., Brennholt, N., Stock, F. and Reifferscheid, G. (2018) “A new approach in separating microplastics from environmental samples based on their electrostatic behavior,” *Environmental Pollution*, 234, pp. 20–28.

Filgueira, R.R., Fournier, L.L., Cerisola, C.I., Gelati, P. and García, M.G. (2006) "Particle-size distribution in soils: A critical study of the fractal model validation," *Geoderma*, 134(3–4), pp. 327–334.

Frias, J. and Nash, R. (2019) "Microplastics: finding a consensus on the definition," *Marine pollution bulletin*, 138, pp. 145–147.

Fries, E., Dekiff, J.H., Willmeyer, J., Nuelle, M.-T., Ebert, M. and Remy, D. (2013) "Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy," *Environmental science: processes & impacts*, 15(10), pp. 1949–1956.

Fuller, S. and Gautam, A. (2016) "A procedure for measuring microplastics using pressurized fluid extraction," *Environmental science & technology*, 50(11), pp. 5774–5780.

Gall, S.C. and Thompson, R.C. (2015) "The impact of debris on marine life," *Marine pollution bulletin*, 92(1–2), pp. 170–179.

Gallagher, A., Rees, A., Rowe, R., Stevens, J. and Wright, P. (2016) "Microplastics in the Solent estuarine complex, UK: An initial assessment," *Marine Pollution Bulletin*, 102(2), pp. 243–249.

Gao, B., Yao, H., Li, Y. and Zhu, Y. (2021) "Microplastic Addition Alters the Microbial Community Structure and Stimulates Soil Carbon Dioxide Emissions in Vegetable-Growing Soil," *Environmental Toxicology and Chemistry*, 40(2), pp. 352–365.

Geyer, R., Jambeck, J.R. and Law, K.L. (2017) "Production, use, and fate of all plastics ever made," *Science advances*, 3(7), p. e1700782.

Gibb, B.C. (2019) "Plastics are forever." *Nature Chemistry*, 11, p. 394–395.

Gomiero, A., Øysæd, K.B., Palmas, L. and Skogerbø, G. (2021) "Application of GCMS-pyrolysis to estimate the levels of microplastics in a drinking water supply system," *Journal of Hazardous Materials*, 416, p. 125708.

Gray, A.D. and Weinstein, J.E. (2017) "Size-and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio)," *Environmental toxicology and chemistry*, 36(11), pp. 3074–3080.

Grbic, J., Nguyen, B., Guo, E., You, J.B., Sinton, D. and Rochman, C.M. (2019) "Magnetic extraction of microplastics from environmental samples," *Environmental Science & Technology Letters*, 6(2), pp. 68–72.

Gregory, A.S., Ritz, K., McGrath, S.P., Quinton, J.N., Goulding, K.W.T., Jones, R.J.A., Harris, J.A., Bol, R., Wallace, P. and Pilgrim, E.S. (2015) "A review of the impacts of degradation threats on soil properties in the UK," *Soil use and management*, 31, pp. 1–15.

Gronle, A., Lux, G., Böhm, H., Schmidtke, K., Wild, M., Demmel, M., Brandhuber, R., Wilbois, K.-P. and Heß, J. (2015) "Effect of ploughing depth and mechanical soil loading on soil physical properties, weed infestation, yield performance and grain quality in sole and intercrops of pea and oat in organic farming," *Soil and Tillage Research*, 148, pp. 59–73.

Gui, X., Ren, Z., Xu, X., Chen, X., Chen, M., Wei, Y., Zhao, L., Qiu, H., Gao, B. and Cao, X. (2022) "Dispersion and transport of microplastics in three water-saturated coastal soils," *Journal of Hazardous Materials*, 424, p. 127614.

Guo, J.J., Huang, X.P., Xiang, L., Wang, Y.Z., Li, Y.W., Li, H., Cai, Q.Y., Mo, C.H. and Wong, M.H. (2020) "Source, migration and toxicology of microplastics in soil," *Environment International*. Elsevier Ltd.

Haave, M., Lorenz, C., Primpke, S. and Gerdts, G. (2019) "Different stories told by small and large microplastics in sediment-first report of microplastic concentrations in an urban recipient in Norway," *Marine pollution bulletin*, 141, pp. 501–513.

Haj-Amor, Z. and Bouri, S. (2019) "Use of HYDRUS-1D–GIS tool for evaluating effects of climate changes on soil salinization and irrigation management," *Archives of Agronomy and Soil Science*, 66(2), 193-207.

Hamm, T., Lorenz, C. and Piehl, S. (2018) "Microplastics in Aquatic Systems–Monitoring Methods and Biological Consequences," in *YOUMARES 8–Oceans Across Boundaries: Learning from each other*. Springer, pp. 179–195.

Han, N., Zhao, Q., Ao, H., Hu, H. and Wu, C. (2022) "Horizontal transport of macro-and microplastics on soil surface by rainfall induced surface runoff as affected by vegetations," *Science of The Total Environment*, p. 154989.

Han, X., Lu, X. and Vogt, R.D. (2019) "An optimized density-based approach for extracting microplastics from soil and sediment samples," *Environmental Pollution*, 254, p. 113009.

Han, Y., Lau, S.-L., Kayhanian, M. and Stenstrom, M.K. (2006) "Characteristics of Highway Stormwater Runoff," *Water Environment Research*, 78(12), pp. 2377–2388.

Hann, S., Sherrington, C., Jamieson, O., Hickman, M., Kershaw, P., Bapasola, A. and Cole, G. (2018) "Investigating options for reducing releases in the aquatic environment of

microplastics emitted by (but not intentionally added in) products," *EUNOMIA Report*, Final Report. London/Bristol.

Harley-Nyang, D., Memon, F.A., Jones, N. and Galloway, T. (2022) "Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK," *Science of The Total Environment*, 823, p. 153735.

Harms, I.K., Diekötter, T., Troegel, S. and Lenz, M. (2021) "Amount, distribution and composition of large microplastics in typical agricultural soils in Northern Germany," *Science of the Total Environment*, 758, p. 143615.

Hartmann, N., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T.M., Brennholt, N. and Cole, M. (2019) "Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris," *Environmental science & technology*, 53(3), 1039–1047.

Haynes, R.J., Murtaza, G. and Naidu, R. (2009) "Inorganic and organic constituents and contaminants of biosolids: implications for land application," *Advances in agronomy*, 104, pp. 165–267.

He, D., Luo, Y., Lu, S., Liu, M., Song, Y. and Lei, L. (2018) "Microplastics in soils: analytical methods, pollution characteristics and ecological risks," *TrAC Trends in Analytical Chemistry*, 109, 163-172.

He, D., Zhang, X. and Hu, J. (2021) "Methods for separating microplastics from complex solid matrices: Comparative analysis," *Journal of Hazardous Materials*, 409, p. 124640.

Heinze, W.M., Mitrano, D.M., Lahive, E., Koestel, J. and Cornelis, G. (2021) "Nanoplastic Transport in Soil via Bioturbation by Lumbricus terrestris," *Environmental Science and Technology*, 55(24), pp. 16423–16433.

Helcoski, R., Yonkos, L.T., Sanchez, A. and Baldwin, A.H. (2020) "Wetland soil microplastics are negatively related to vegetation cover and stem density," *Environmental Pollution*, 256, p. 113391.

Hernández-Arenas, R., Beltrán-Sanahuja, A., Navarro-Quirant, P. and Sanz-Lazaro, C. (2021) "The effect of sewage sludge containing microplastics on growth and fruit development of tomato plants," *Environmental Pollution*, 268, p. 115779.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M. (2012) “Microplastics in the marine environment: a review of the methods used for identification and quantification,” *Environmental science & technology*, 46(6), pp. 3060–3075.

HM Government (2015) *The Single Use Carrier Bags Charges (England)*. London, England.

Homewood, J.M., Purdie, D.A. and Shaw, P.J. (2005) “Influence of sewage inputs and fish farm effluents on dissolved nitrogen species in a chalk river,” *Water, Air, & Soil Pollution: Focus*, 4(6), pp. 117–125.

Hopewell, J., Dvorak, R. and Kosior, E. (2009) “Plastics recycling: challenges and opportunities,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1526), pp. 2115–2126.

Horton, A.A., Cross, R.K., Read, D.S., Jürgens, M.D., Ball, H.L., Svendsen, C., Vollertsen, J. and Johnson, A.C. (2021) “Semi-automated analysis of microplastics in complex wastewater samples,” *Environmental Pollution*, 268. 268(A), p. 115841.

Horton, A.A. and Dixon, S.J. (2018) “Microplastics: An introduction to environmental transport processes,” *Wiley Interdisciplinary Reviews: Water*, 5(2), p. e1268.

Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J. and Lahive, E. (2017) “Large microplastic particles in sediments of tributaries of the River Thames, UK–Abundance, sources and methods for effective quantification,” *Marine Pollution Bulletin*, 114(1), pp. 218–226.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. and Svendsen, C. (2017) “Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities,” *Science of The Total Environment*, 586, pp. 127–141.

Hseu, Z.-Y. (2004) “Evaluating heavy metal contents in nine composts using four digestion methods,” *Bioresource technology*, 95(1), pp. 53–59.

Huang, J., Wu, P., Dong, S. and Gao, B. (2022) “Occurrences and impacts of microplastics in soils and groundwater,” in *Emerging Contaminants in Soil and Groundwater Systems*. Elsevier, pp. 253–299.

Huang, P.-T., Patel, M., Santagata, M.C. and Bobet, A. (2009) “Classification of organic soils.” FHWA/IN/JTRP-2008/2, Purdue University, p.195.

Huang, Y., Liu, Q., Jia, W., Yan, C. and Wang, J. (2020) “Agricultural plastic mulching as a source of microplastics in the terrestrial environment,” *Environmental Pollution*, 260, p. 114096.

Huat, B.B.K., Asadi, A. and Kazemian, S. (2009) “Experimental investigation on geomechanical properties of tropical organic soils and peat,” *American Journal of Engineering and Applied Sciences*, 2(1), pp. 184–188.

Hurley, R., Woodward, J. and Rothwell, J.J. (2018) “Microplastic contamination of river beds significantly reduced by catchment-wide flooding,” *Nature Geoscience*, 11(4), p. 251.

Hurley, R.R., Lusher, A.L., Olsen, M. and Nizzetto, L. (2018) “Validation of a method for extracting microplastics from complex, Organic-Rich, Environmental Matrices,” *Environmental science & technology*, 52(13), pp. 7409–7417.

Hurley, R.R. and Nizzetto, L. (2018) “Fate and occurrence of micro (nano) plastics in soils: Knowledge gaps and possible risks,” *Current Opinion in Environmental Science & Health*, 1, pp. 6–11.

Husrin, S., Hidayat, H., Rahmadya, A., Aisyah, S., Hurley, R., Braaten, H.F.V. and Olsen, M. (2022) “Riverine Plastic Monitoring during the Rainy Season in the Citarum Estuary of Muara Gembong,” in *IOP Conference Series: Earth and Environmental Science*. IOP Publishing Ltd.

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P. and Laforsch, C. (2012) “A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments,” *Limnology and oceanography: methods*, 10(7), pp. 524–537.

Ingman, M., Santelmann, M. v and Tilt, B. (2015) “Agricultural water conservation in China: plastic mulch and traditional irrigation,” *Ecosystem Health and Sustainability*, 1(4), pp. 1–11.

Ingraffia, R., Amato, G., Bagarello, V., Carollo, F.G., Giambalvo, D., Iovino, M., Lehmann, A., Rillig, M.C. and Frenda, A.S. (2021) “Polyester Microplastic Fibers affect Soil Physical Properties and Erosion as a Function of Soil Type,” *SOIL Discussions*, pp. 1–22.

ISO/TR 21960 (2020) *ISO/TR 21960:2020(en) Plastics — Environmental aspects — State of knowledge and methodologies Table*. Available at: http://iso.org/obp/ui/#iso:std:iso-iec:17025:ed-2:v1:es.

Iticha, B. and Takele, C. (2019) “Digital soil mapping for site-specific management of soils,” *Geoderma*, 351, pp. 85–91.

Jacques, O. and Prosser, R.S. (2021) “A probabilistic risk assessment of microplastics in soil ecosystems,” *Science of the Total Environment*, 757.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R. and Law, K.L. (2015) “Plastic waste inputs from land into the ocean,” *Science*, 347(6223), pp. 768–771.

Jenner, L.C., Rotchell, J.M., Bennett, R.T., Cowen, M., Tentzeris, V. and Sadofsky, L.R. (2022) “Detection of microplastics in human lung tissue using µFTIR spectroscopy,” *Science of The Total Environment*, p. 154907.

Jepsen, E.M. and de Bruyn, P.J.N. (2019) “Pinniped entanglement in oceanic plastic pollution: A global review,” *Marine pollution bulletin*, 145, pp. 295–305.

de Jesus Piñon-Colin, T., Rodriguez-Jimenez, R., Rogel-Hernandez, E., Alvarez-Andrade, A. and Wakida, F.T. (2020) “Microplastics in stormwater runoff in a semiarid region, Tijuana, Mexico,” *Science of the Total Environment*, 704, p. 135411.

Jia, X., Cao, Y., O’Connor, D., Zhu, J., Tsang, D.C.W., Zou, B. and Hou, D. (2021) “Mapping soil pollution by using drone image recognition and machine learning at an arsenic-contaminated agricultural field,” *Environmental Pollution*, 270, p. 116281.

Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M. and Klobučar, G. (2019) “Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba,” *Environmental Pollution*, 250, pp. 831–838.

Jovanović, B., Gökdağ, K., Güven, O., Emre, Y., Whitley, E.M. and Kideys, A.E. (2018) “Virgin microplastics are not causing imminent harm to fish after dietary exposure,” *Marine pollution bulletin*, 130, pp. 123–131.

Junhao, C., Xining, Z., Xiaodong, G., Li, Z., Qi, H. and Siddique, K.H.M. (2021) “Extraction and identification methods of microplastics and nanoplastics in agricultural soil: A review,” *Journal of Environmental Management*, 294, p. 112997.

Käppler, A., Fischer, D., Oberbeckmann, S., Schernewski, G., Labrenz, M., Eichhorn, K.-J. and Voit, B. (2016) “Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both?,” *Analytical and bioanalytical chemistry*, 408(29), pp. 8377–8391.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y. bin and Salamatinia, B. (2017) “A high-performance protocol for extraction of microplastics in fish,” *Science of the Total Environment*, 578, pp. 485–494.

Kawecki, D., Goldberg, L. and Nowack, B. (2021) “Material flow analysis of plastic in organic waste in Switzerland,” *Soil Use and Management*, 37(2), pp. 277–288.

Kawecki, D. and Nowack, B. (2019) “Polymer-specific modeling of the environmental emissions of seven commodity plastics as macro-and microplastics,” *Environmental science & technology*, 53(16), pp. 9664–9676.

Keesstra, S.D., Bouma, J., Wallinga, J., Tittonell, P., Smith, P., Cerdà, A., Montanarella, L., Quinton, J.N., Pachepsky, Y. and van der Putten, W.H. (2016) “The significance of soils and soil science towards realization of the United Nations Sustainable Development Goals,” *Soil*, 2(2), pp. 111–128.

Kerry, R., Rawlins, B.G., Oliver, M.A. and Lacinska, A.M. (2009) “Problems with determining the particle size distribution of chalk soil and some of their implications,” *Geoderma*, 152(3–4), pp. 324–337.

Khalid, N., Aqeel, M. and Noman, A. (2020) “Microplastics could be a threat to plants in terrestrial systems directly or indirectly,” *Environmental Pollution*, 267, p. 115653.

Khan, S., Naushad, M., Lima, E.C., Zhang, S., Shaheen, S.M. and Rinklebe, J. (2021) “Global soil pollution by toxic elements: Current status and future perspectives on the risk assessment and remediation strategies–A review,” *Journal of Hazardous Materials*, 417, p. 126039.

Kim, D.-S., An, K.-G. and Kim, K.-H. (2003) “Heavy metal pollution in the soils of various land use types based on physicochemical characteristics,” *Journal of Environmental Science and Health, Part A*, 38(5), pp. 839–853.

Kim, L.H., Kang, J., Kayhanian, M., Gil, K.I., Stenstrom, M.K. and Zoh, K.D. (2006) “Characteristics of litter waste in highway storm runoff,” in *Water Science and Technology*, pp. 225–234.

Kim, S.-K., Kim, J.-S., Lee, H. and Lee, H.-J. (2021) “Abundance and characteristics of microplastics in soils with different agricultural practices: Importance of sources with internal origin and environmental fate,” *Journal of Hazardous Materials*, 403, p. 123997.

Kirstein, I. v, Hensel, F., Gomiero, A., Iordachescu, L., Vianello, A., Wittgren, H.B. and Vollertsen, J. (2021) “Drinking plastics?–Quantification and qualification of microplastics in drinking water distribution systems by µFTIR and Py-GCMS,” *Water research*, 188, p. 116519.

Klein, M. and Fischer, E.K. (2019) “Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany,” *Science of the Total Environment*, 685, pp. 96–103.

Klein, S., Worch, E. and Knepper, T.P. (2015) “Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany,” *Environmental science & technology*, 49(10), pp. 6070–6076.

Knox, J., Morris, J. and Hess, T. (2010) “Identifying future risks to UK agricultural crop production: Putting climate change in context,” *Outlook on AGRICULTURE*, 39(4), pp. 249–256.

Koenig, J.L., Wang, S.-Q. and Bhargava, R. (2001) “Peer Reviewed: FTIR Images,” *Analytical Chemistry*, 73(13), pp. 360 A-369 A.

Kole, P.J., Löhr, A.J., Van Belleghem, F. and Ragas, A. (2017) “Wear and tear of tyres: a stealthy source of microplastics in the environment,” *International journal of environmental research and public health*, 14(10), p. 1265.

Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A. and Lombi, E. (2019) “Soil and the intensification of agriculture for global food security,” *Environment international*, 132, p. 105078.

Korez, Š., Gutow, L. and Saborowski, R. (2019) “Microplastics at the strandlines of Slovenian beaches,” *Marine pollution bulletin*, 145, pp. 334–342.

Korez, S., Reuter, K., Gutow, L. and Saborowski, R. (2018) “Microplastic at Slovenian beaches: study repeated,” conference paper, Micro2018 - Fate and Impacts of Microplastics: Knowledge, actions and Solutions, Lanzarote, Spain.

Koutnik, Vera S, Leonard, J., Alkidim, S., DePrima, F.J., Ravi, S., Hoek, E.M. v and Mohanty, S.K. (2021) “Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modeling,” *Environmental Pollution*, 274, p. 116552.

Laglbauer, B.J.L., Franco-Santos, R.M., Andreu-Cazenave, M., Brunelli, L., Papadatou, M., Palatinus, A., Grego, M. and Deprez, T. (2014) “Macrodebris and microplastics from beaches in Slovenia,” *Marine pollution bulletin*, 89(1–2), pp. 356–366.

Lahive, E., Walton, A., Horton, A.A., Spurgeon, D.J. and Svendsen, C. (2019) “Microplastic particles reduce reproduction in the terrestrial worm Enchytraeus crypticus in a soil exposure,” *Environmental Pollution*, 255, p. 113174.

Lang, M., Wang, G., Yang, Y., Zhu, W., Zhang, Y., Ouyang, Z. and Guo, X. (2022) "The occurrence and effect of altitude on microplastics distribution in agricultural soils of Qinghai Province, northwest China," *Science of the Total Environment*, 810(1), p. 152174.

Lasee, S., Mauricio, J., Thompson, W.A., Karnjanapiboonwong, A., Kasumba, J., Subbiah, S., Morse, A.N. and Anderson, T.A. (2017) "Microplastics in a freshwater environment receiving treated wastewater effluent," *Integrated Environmental Assessment and Management*, 13(3), pp. 528–532.

Lechthaler, S., Esser, V., Schüttrumpf, H. and Stauch, G. (2021) "Why analysing microplastics in floodplains matters: Application in a sedimentary context," *Environmental Science: Processes & Impacts*, 23(1), pp. 117–131.

Lekše, N., Griesseler Bulc, T. and Žgajnar Gotvajn, A. (2021) "Oil extraction of microplastics from communal dewatered suldge," *Proceedings of Socratic Lectures*, 6, pp. 49–55.

Lenz, R., Enders, K., Stedmon, C.A., Mackenzie, D.M.A. and Nielsen, T.G. (2015a) "A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement," *Marine Pollution Bulletin*, 100(1), pp. 82–91.

Lenz, R., Enders, K., Stedmon, C.A., Mackenzie, D.M.A. and Nielsen, T.G. (2015b) "A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement," *Marine pollution bulletin*, 100(1), pp. 82–91.

Leslie, H.A., van Velzen, M.J., Brandsma, S.H., Vethaak, D., Garcia-Vallejo, J.J. and Lamoree, M.H. (2022) "Discovery and quantification of plastic particle pollution in human blood," *Environment International*, p. 107199.

Li, H., Lu, X., Wang, S., Zheng, B. and Xu, Y. (2021) "Vertical migration of microplastics along soil profile under different crop root systems," *Environmental Pollution*, 278, p. 116833.

Li, M., Zhang, X., Yi, K., He, L., Han, P. and Tong, M. (2021) "Transport and deposition of microplastic particles in saturated porous media: Co-effects of clay particles and natural organic matter," *Environmental Pollution*, 287, p. 117585.

Liu, E.K., He, W.Q. and Yan, C.R. (2014) "'White revolution'to 'white pollution'—agricultural plastic film mulch in China," *Environmental Research Letters*, 9(9), p. 91001.

Liu, Hui, Colombi, T., Jäck, O., Keller, T. and Weih, M. (2022) "Effects of soil compaction on grain yield of wheat depend on weather conditions," *Science of the Total Environment*, 807, p. 150763.

Liu, Hao, Wang, X., Shi, Q., Liu, Y., Lei, H. and Chen, Y. (2022) “Microplastics in arid soils: Impact of different cropping systems (Altay, Xinjiang),” *Environmental Pollution*, p. 119162.

Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X. and He, D. (2018) “Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China,” *Environmental Pollution*, 242, pp. 855–862.

Liu, M., Song, Y., Lu, S., Qiu, R., Hu, J., Li, X., Bigalke, M., Shi, H. and He, D. (2019) “A method for extracting soil microplastics through circulation of sodium bromide solutions,” *Science of the total environment*, 691, pp. 341–347.

Liu, S., Wang, Jiawei, Zhu, J., Wang, Jia, Wang, H. and Zhan, X. (2021) “The joint toxicity of polyethylene microplastic and phenanthrene to wheat seedlings,” *Chemosphere*, 282, p. 130967.

Löder, M.G.J., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I. and Laforsch, C. (2017) “Enzymatic purification of microplastics in environmental samples,” *Environmental science & technology*, 51(24), pp. 14283–14292.

Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A. and Rillig, M.C. (2021) “Microplastic shape, polymer type, and concentration affect soil properties and plant biomass,” *Frontiers in plant science*, 12, p. 169.

Lozano, Y.M. and Rillig, M.C. (2020) “Effects of microplastic fibers and drought on plant communities,” *Environmental science & technology*, 54(10), pp. 6166–6173.

Lu, H.-C., Ziajahromi, S., Neale, P.A. and Leusch, F.D.L. (2021) “A systematic review of freshwater microplastics in water and sediments: recommendations for harmonisation to enhance future study comparisons,” *Science of the Total Environment*, 781, p. 146693.

Lusher, A.L., Hurley, R., Vogelsang, C., Nizzetto, L. and Olsen, M. (2017) “Mapping microplastics in sludge.” Technical report, Norwegian Institute for Water Research.

Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A. and Geissen, V. (2017) “Incorporation of microplastics from litter into burrows of Lumbricus terrestris,” *Environmental Pollution*, 220, pp. 523–531.

Maaß, S., Daphi, D., Lehmann, A. and Rillig, M.C. (2017) “Transport of microplastics by two collembolan species,” *Environmental Pollution*, 225, pp. 456–459.

Maes, T., Jessop, R., Wellner, N., Haupt, K. and Mayes, A.G. (2017) "A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red," *Scientific reports*, 7(1), pp. 1–10.

Magnusson, K. and Norén, F. (2014) "Screening of microplastic particles in and down-stream a wastewater treatment plant." Technical report, IVL Swedish Environmental Research Institute, Stockholm.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R. and Morrison, L. (2016) "Microplastics in sewage sludge: effects of treatment," *Environmental Science & Technology*, 51(2), pp. 810–818.

Mamedov, A.I., Levy, G.J., Shainberg, I. and Letey, J. (2001) "Wetting rate, sodicity, and soil texture effects on infiltration rate and runoff," *Soil Research*, 39(6), pp. 1293–1305.

Mani, T., Frehland, S., Kalberer, A. and Burkhardt-Holm, P. (2019) "Using castor oil to separate microplastics from four different environmental matrices," *Analytical methods*, 11(13), pp. 1788–1794.

Martay, B. and Pearce-Higgins, J.W. (2018) "Using data from schools to model variation in soil invertebrates across the UK: The importance of weather, climate, season and habitat," *Pedobiologia*, 67, pp. 1–9.

Martins, J. and Sobral, P. (2011) "Plastic marine debris on the Portuguese coastline: a matter of size?," *Marine pollution bulletin*, 62(12), pp. 2649–2653.

Mbachu, O., Jenkins, G., Kaparaju, P. and Pratt, C. (2021) "The rise of artificial soil carbon inputs: Reviewing microplastic pollution effects in the soil environment," *Science of The Total Environment*, 780, p. 146569.

McGoran, A., Maclaine, J., Clark, P. and Morritt, D. (2021) "Synthetic and semi-synthetic fibre ingestion by mesopelagic fishes from Tristan da Cuhna and St Helena, South Atlantic." Frontiers in Marine Science, 8, pp. 2296-7745.

McGrath, S.P. and Zhao, F.J., 2006. Ambient background metal concentrations for soils in England and Wales. Science Report SC050054. Environmental Agency, *31p*.

Mintenig, S.M., Kooi, M., Erich, M.W., Primpke, S., Redondo- Hasselerharm, P.E., Dekker, S.C., Koelmans, A.A. and van Wezel, A.P. (2020) "A systems approach to understand microplastic occurrence and variability in Dutch riverine surface waters," *Water Research*, 176, p. 115723.

Mirzavand, J. and Moradi-Talebbeigi, R. (2021) “Relationships between field management, soil compaction, and crop productivity,” *Archives of Agronomy and Soil Science*, 67(5), pp. 675–686.

Mitrano, D.M., Wick, P. and Nowack, B. (2021) “Placing nanoplastics in the context of global plastic pollution,” *Nature nanotechnology*, 16(5), pp. 491–500.

Mohajerani, A. and Karabatak, B. (2020a “Microplastics and pollutants in biosolids have contaminated agricultural soils: An analytical study and a proposal to cease the use of biosolids in farmlands and utilise them in sustainable bricks,” *Waste Management*. Elsevier Ltd, pp. 252–265.

Möller, J.N., Heisel, I., Satzger, A., Vizsolyi, E.C., Oster, S.D.J., Agarwal, S., Laforsch, C. and Löder, M.G.J. (2021) “Tackling the challenge of extracting microplastics from soils: a protocol to purify soil samples for spectroscopic analysis,” *Environmental Toxicology and Chemistry*, 41(4), p. 844-857.

Monikh, F.A., Doornhein, N., Romeijn, S., Vijver, M.G. and Peijnenburg, W.J.G.M. (2021) “Method for extraction of nanoscale plastic debris from soil,” *Analytical Methods*, 13(13), pp. 1576–1583.

Moore, A., Ives, S., Mead, T.A. and Talks, L. (1998) “The migratory behaviour of wild Atlantic salmon (Salmo salar L.) smolts in the River Test and Southampton Water, southern England,” in *Advances in Invertebrates and Fish Telemetry*. Springer, pp. 295–304.

Moss, B. (2007) “Water pollution by agriculture,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), pp. 659–666.

Munno, K., Helm, P.A., Jackson, D.A., Rochman, C. and Sims, A. (2018) “Impacts of temperature and selected chemical digestion methods on microplastic particles,” *Environmental toxicology and chemistry*, 37(1), pp. 91–98.

Napper, I.E., Bakir, A., Rowland, S.J. and Thompson, R.C. (2015) “Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics,” *Marine Pollution Bulletin*, 99(1–2), pp. 178–185.

Napper, I.E., Davies, B.F.R., Clifford, H., Elvin, S., Koldewey, H.J., Mayewski, P.A., Miner, K.R., Potocki, M., Elmore, A.C. and Gajurel, A.P. (2020) “Reaching new heights in plastic pollution—preliminary findings of microplastics on Mount Everest,” *One Earth*, 3(5), pp. 621–630.

Napper, I.E. and Thompson, R.C. (2016) “Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions,” *Marine pollution bulletin*, 112(1–2), pp. 39–45.

Napper, I.E. and Thompson, R.C. (2019) “Marine plastic pollution: Other than microplastic,” in *Waste*. Elsevier, pp. 425–442.

National Rivers Authority (1991) “River Test catchment management plan: phase 1.” Technical report, NRA, Bristol.

Ng, E.L., Huerta Lwanga, E., Eldridge, S.M., Johnston, P., Hu, H.W., Geissen, V. and Chen, D. (2018) “An overview of microplastic and nanoplastic pollution in agroecosystems,” *Science of the Total Environment* 627, pp. 1377-1388.

Ngo, P.L., Pramanik, B.K., Shah, K. and Roychand, R. (2019) “Pathway, classification and removal efficiency of microplastics in wastewater treatment plants,” *Environmental Pollution*, 255, p. 113326.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D. and Whitehead, P.G. (2016) “A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments,” *Environmental Science: Processes & Impacts*, 18(8), pp. 1050–1059.

Nizzetto, L., Futter, M. and Langaas, S. (2016) “"Are agricultural soils dumps for microplastics of urban origin?." (2016) *Environmental Science and Technology,* 50(20), pp. 10777–10779.

Nuelle, M.-T., Dekiff, J.H., Remy, D. and Fries, E. (2014) “A new analytical approach for monitoring microplastics in marine sediments,” *Environmental Pollution*, 184, pp. 161–169.

O’Connor, D., Pan, S., Shen, Z., Song, Y., Jin, Y., Wu, W.-M. and Hou, D. (2019) “Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles,” *Environmental Pollution*, 249, pp. 527–534.

Ofwat (2015) *Water 2020: Regulatory framework for wholesale markets and the 2019 price review*. [Accessed on 21 April 2022] https://www.ofwat.gov.uk/consultation/water-2020-consultation/.

Ogonowski, M., Gerdes, Z. and Gorokhova, E. (2018) “What we know and what we think we know about microplastic effects–A critical perspective,” *Current Opinion in Environmental Science & Health*, 1, pp. 41–46.

Okoffo, Elvis D., Donner, E., McGrath, S.P., Tscharke, B.J., O’Brien, J.W., O’Brien, S., Ribeiro, F., Burrows, S.D., Toapanta, T., Rauert, C., Samanipour, S., Mueller, J.F. and Thomas, K. v.

(2021) “Plastics in biosolids from 1950 to 2016: A function of global plastic production and consumption,” *Water Research*, 201, p. 117367.

Oliveira, M. and Almeida, M. (2019) “The why and how of micro (nano) plastic research,” *TrAC Trends in analytical chemistry*, 114, pp. 196–201.

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M. and Johns, D.G. (2019) “The rise in ocean plastics evidenced from a 60-year time series,” *Nature communications*, 10(1), pp. 1–6.

Pabortsava, K. and Lampitt, R.S. (2020) “High concentrations of plastic hidden beneath the surface of the Atlantic Ocean,” *Nature communications*, 11(1), pp. 1–11.

Pagter, E., Frias, J. and Nash, R. (2018) “Microplastics in Galway Bay: A comparison of sampling and separation methods,” *Marine pollution bulletin*, 135, pp. 932–940.

Parker-Jurd, F.N.F., Napper, I.E., Abbott, G.D., Hann, S., Wright, S.L. and Thompson, R.C. (2019) “Investigating the sources and pathways of synthetic fibre and vehicle tyre wear contamination into the marine environment” Report prepared for the Department for Environment Food and Rural Affairs, [Accessed on 21 April 2022], https://eprints.ncl.ac.uk/267269.

Patzold, S., Mertens, F.M., Bornemann, L., Koleczek, B., Franke, J., Feilhauer, H. and Welp, G. (2008) “Soil heterogeneity at the field scale: A challenge for precision crop protection,” in *Precision Agriculture*, pp. 367–390.

Peng, J., Wang, J. and Cai, L. (2017) “Current understanding of microplastics in the environment: occurrence, fate, risks, and what we should do,” *Integrated environmental assessment and management*, 13(3), pp. 476–482.

Pepper, I.L., Gerba, C.P. and Brusseau, M.L. (2011) *Environmental and pollution science*. Elsevier, 3rd Edition, USA.

Perez, C.N., Carré, F., Hoarau-Belkhiri, A., Joris, A., Leonards, P.E.G. and Lamoree, M.H. (2022) “Innovations in analytical methods to assess the occurrence of microplastics in soil,” *Journal of Environmental Chemical Engineering*, p. 107421.

Picó, Y. and Barceló, D. (2021) “Analysis of microplastics and nanoplastics: How green are the methodologies used?,” *Current Opinion in Green and Sustainable Chemistry*, 31, p. 100503.

Piehl, S., Leibner, A., Löder, Martin G.J., Dris, R., Bogner, C. and Laforsch, C. (2018) "Identification and quantification of macro- and microplastics on an agricultural farmland," *Scientific Reports*, 8(1).

Plastics Europe (2019) *Plastics- the facts 2019*. [Accessed on 21 April 2022], https://plasticseurope.org/knowledge-hub/plastics-the-facts-2019/.

Plastics Europe (2020) *Plastics the Facts 2020*. [Accessed on 21 April 2022], https://plasticseurope.org/knowledge-hub/plastics-the-facts-2020/.

Pol, V.G. and Thiyagarajan, P. (2010) "Remediating plastic waste into carbon nanotubes," *Journal of environmental monitoring*, 12(2), pp. 455–459.

Poornima, R., Suganya, K. and Sebastian, S.P. (2021) "Biosolids towards Back–To–Earth alternative concept (BEA) for environmental sustainability: a review," *Environmental Science and Pollution Research*, pp. 1–42.

Posthumus, H., Deeks, L.K., Rickson, R.J. and Quinton, J.N. (2015) "Costs and benefits of erosion control measures in the UK," *Soil use and management*, 31, pp. 16–33.

Prata, J.C. (2018a) "Airborne microplastics: consequences to human health?," *Environmental pollution*, 234, pp. 115–126.

Prata, J.C. (2018b) "Microplastics in wastewater: state of the knowledge on sources, fate and solutions," *Marine pollution bulletin*, 129(1), pp. 262–265.

Prata, J.C., da Costa, J.P., Duarte, A.C. and Rocha-Santos, T. (2019) "Methods for sampling and detection of microplastics in water and sediment: a critical review," *TrAC Trends in Analytical Chemistry*, 110, pp. 150–159.

Prata, J.C., Reis, V., da Costa, J.P., Mouneyrac, C., Duarte, A.C. and Rocha-Santos, T. (2021) "Contamination issues as a challenge in quality control and quality assurance in microplastics analytics," *Journal of Hazardous Materials*, 403, p. 123660.

Praveena, S.M., Aris, A.Z. and Singh, V. (2022) "Quality Assessment For Methodological Aspects Of Microplastics Analysis In Soil," *Trends in Environmental Analytical Chemistry*, p. e00159.

Primpke, S., Cross, R.K., Mintenig, S.M., Simon, M., Vianello, A., Gerdts, G. and Vollertsen, J. (2020) "Toward the Systematic Identification of Microplastics in the Environment: Evaluation of a New Independent Software Tool (siMPle) for Spectroscopic Analysis," *Applied Spectroscopy*, 74(9), pp. 1127–1138.

Primpke, S., Fischer, M., Lorenz, C., Gerdts, G. and Scholz-Böttcher, B.M. (2020) "Comparison of pyrolysis gas chromatography/mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics," *Analytical and bioanalytical chemistry*, 412(30), pp. 8283–8298.

Prokić, M.D., Gavrilović, B.R., Radovanović, T.B., Gavrić, J.P., Petrović, T.G., Despotović, S.G. and Faggio, C. (2021) "Studying microplastics: Lessons from evaluated literature on animal model organisms and experimental approaches," *Journal of Hazardous Materials*, 414, p. 125476.

Provencher, J.F., Covernton, G.A., Moore, R.C., Horn, D.A., Conkle, J.L. and Lusher, A.L. (2020) "Proceed with caution: The need to raise the publication bar for microplastics research," *Science of The Total Environment*, p. 141426.

Public Health England and Wales (1989a) *The Sludge (Use in Agriculture) Regulations 1989*. Available at: http://www.legislation.gov.uk/uksi/1989/1263/made.

Public Health England and Wales (1989b) *The Sludge (Use in Agriculture) Regulations 1989*. PUBLIC HEALTH, ENGLAND AND WALES. Available at: http://www.legislation.gov.uk/uksi/1989/1263/made.

Pulleman, M.M., Bouma, J., van Essen, E.A. and Meijles, E.W. (2000) "Soil organic matter content as a function of different land use history," *Soil Science Society of America Journal*, 64(2), pp. 689–693.

Qados, A.M.S.A. (2011) "Effect of salt stress on plant growth and metabolism of bean plant Vicia faba (L.)," *Journal of the Saudi Society of Agricultural Sciences*, 10(1), pp. 7–15.

Qiao, R., Deng, Y., Zhang, S., Wolosker, M.B., Zhu, Q., Ren, H. and Zhang, Y. (2019) "Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish," *Chemosphere*, 236, p. 124334.

Qiu, L., Zhang, Q., Zhu, H., Reich, P.B., Banerjee, S., van der Heijden, M.G.A., Sadowsky, M.J., Ishii, S., Jia, X. and Shao, M. (2021) "Erosion reduces soil microbial diversity, network complexity and multifunctionality," *The ISME journal*, 15(8), pp. 2474–2489.

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M. and Zhan, Z. (2016) "Extraction, enumeration and identification methods for monitoring microplastics in the environment," *Estuarine, Coastal and Shelf Science*, 176, pp. 102–109.

Quinn, B., Murphy, F. and Ewins, C. (2017) “Validation of density separation for the rapid recovery of microplastics from sediment,” *Analytical Methods*, 9(9), pp. 1491–1498.

Racek, J., Sevcik, J., Chorazy, T., Kucerik, J. and Hlavinek, P. (2020) “Biochar – Recovery Material from Pyrolysis of Sewage Sludge: A Review,” *Waste and Biomass Valorization*. Springer, pp. 3677–3709.

Radford, F., Zapata-Restrepo, L.M., Horton, A.A., Hudson, M.D., Shaw, P.J. and Williams, I.D. (2021) “Developing a systematic method for extraction of microplastics in soils,” *Analytical Methods*, 13(14), pp. 1695–1705.

Ray, D.K., Mueller, N.D., West, P.C. and Foley, J.A. (2013) “Yield trends are insufficient to double global crop production by 2050,” *PloS one*, 8(6), p. e66428.

Rehm, R., Zeyer, T., Schmidt, A. and Fiener, P. (2021) “Soil erosion as transport pathway of microplastic from agriculture soils to aquatic ecosystems,” *Science of the Total Environment*, 795, p. 148774.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G. and Lepoint, G. (2015) “When microplastic is not plastic: the ingestion of artificial cellulose fibers by macrofauna living in seagrass macrophytodetritus,” *Environmental science & technology*, 49(18), pp. 11158–11166.

Ren, Z., Gui, X., Xu, X., Zhao, L., Qiu, H. and Cao, X. (2021) “Microplastics in the soil-groundwater environment: aging, migration, and co-transport of contaminants–a critical review,” *Journal of Hazardous Materials*, 419, p. 126455.

Renner, G., Nellessen, A., Schwiers, A., Wenzel, M., Schmidt, T.C. and Schram, J. (2019) “Data preprocessing & evaluation used in the microplastics identification process: A critical review & practical guide,” *TrAC Trends in Analytical Chemistry*, 111, pp. 229–238.

Rezaei, M., Riksen, M.J.P.M., Sirjani, E., Sameni, A. and Geissen, V. (2019) “Wind erosion as a driver for transport of light density microplastics,” *Science of the Total Environment*, 669, pp. 273–281.

Rezanezhad, F., Price, J.S., Quinton, W.L., Lennartz, B., Milojevic, T. and van Cappellen, P. (2016) “Structure of peat soils and implications for water storage, flow and solute transport: A review update for geochemists,” *Chemical Geology*, 429, pp. 75–84.

Richter, G.M. and Semenov, M.A. (2005) “Modelling impacts of climate change on wheat yields in England and Wales: assessing drought risks,” *Agricultural Systems*, 84(1), pp. 77–97.

Rigby, H., Dowding, A., Fernandes, A., Humphries, D., Jones, N.R., Lake, I., Petch, R.G., Reynolds, C.K., Rose, M. and Smith, S.R. (2021) “Concentrations of organic contaminants in industrial and municipal bioresources recycled in agriculture in the UK,” *Science of the Total Environment*, 765, p. 142787.

Rillig, M.C. (2012) “Microplastic in terrestrial ecosystems and the soil?,” *Environmental Science and Technology*, 46(12), 6453–6454.

Rillig, M.C., Ingraffia, R. and de Souza Machado, A.A. (2017) “Microplastic incorporation into soil in agroecosystems,” *Frontiers in plant science*, 8, p. 1805.

Rillig, M.C., Ziersch, L. and Hempel, S. (2017) “Microplastic transport in soil by earthworms,” *Scientific reports*, 7(1), pp. 1–6.

Rocha-Santos, T. and Duarte, A.C. (2015) “A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment,” *TrAC Trends in Analytical Chemistry*, 65, pp. 47–53.

Rochman, C.M. (2018) “Microplastics research—from sink to source,” *Science*, 360(6384), pp. 28–29.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H. and Munno, K. (2019) “Rethinking microplastics as a diverse contaminant suite,” *Environmental toxicology and chemistry*, 38(4), pp. 703–711.

Rodrigues, M.O., Gonçalves, A.M.M., Gonçalves, F.J.M. and Abrantes, N. (2020) “Improving cost-efficiency for MPs density separation by zinc chloride reuse,” *MethodsX*, p. 100785.

Rolsky, C., Kelkar, V., Driver, E. and Halden, R.U. (2020) “Municipal sewage sludge as a source of microplastics in the environment,” *Current Opinion in Environmental Science & Health*, 14, pp. 16–22.

Rowland, C.S.; Morton, R.D.; Carrasco, L.; McShane, G.; O'Neil, A.W.; Wood, C.M. (2017) “Land Cover Map 2015 (25m raster, GB),” NERC Environmental Information Data Centre. (Dataset). https://doi.org/10.5285/bb15e200-9349-403c-bda9-b430093807c7

Rubin, A.E., Sarkar, A.K. and Zucker, I. (2021) “Questioning the suitability of available microplastics models for risk assessment–A critical review,” *Science of The Total Environment*, 788, p. 147670.

Salmi, P., Ryymin, K., Karjalainen, A.K., Mikola, A., Uurasjärvi, E. and Talvitie, J. (2021) "Particle balance and return loops for microplastics in a tertiary-level wastewater treatment plant," *Water Science and Technology*, 84(1), pp. 89–100.

Scarascia-Mugnozza, G., Sica, C. and Russo, G. (2011) "Plastic materials in European agriculture: actual use and perspectives," *Journal of Agricultural Engineering*, 42(3), pp. 15–28.

Schell, T., Hurley, R., Buenaventura, N.T., Mauri, P. v., Nizzetto, L., Rico, A. and Vighi, M. (2022) "Fate of microplastics in agricultural soils amended with sewage sludge: Is surface water runoff a relevant environmental pathway?," *Environmental Pollution*, 293, p. 118520.

Scheurer, M. and Bigalke, M. (2018) "Microplastics in Swiss floodplain soils," *Environmental science & technology*, 52(6), pp. 3591–3598.

Schirmel, J., Albert, J., Kurtz, M.P. and Muñoz, K. (2018) "Plasticulture changes soil invertebrate assemblages of strawberry fields and decreases diversity and soil microbial activity," *Applied Soil Ecology*, 124, pp. 379–393.

Schwinghammer, L., Krause, S. and Schaum, C. (2021) "Determination of large microplastics: wet-sieving of dewatered digested sludge, co-substrates, and compost," *Water Science and Technology*, 84(2), pp. 384–392.

Scott, N., Porter, A., Santillo, D., Simpson, H., Lloyd-Williams, S. and Lewis, C. (2019) "Particle characteristics of microplastics contaminating the mussel Mytilus edulis and their surrounding environments," *Marine pollution bulletin*, 146, pp. 125–133.

Shaddel, S., Bakhtiary-Davijany, H., Kabbe, C., Dadgar, F. and Østerhus, S.W. (2019) "Sustainable sewage sludge management: From current practices to emerging nutrient recovery technologies," *Sustainability*, 11(12), p. 3435.

Shand, C.A. and Wendler, R. (2014) "Portable X-ray fluorescence analysis of mineral and organic soils and the influence of organic matter," *Journal of Geochemical Exploration*, 143, pp.31-42.

Sheldrick, B.H. and Wang, C. (1993) "Particle Size Analysis," In: Carter, M.R., Ed., Soil Sampling and Methods of Analysis, Lewis Publishers, Boca Raton, pp. 499-517.

Shim, W.J., Hong, S.H. and Eo, S.E. (2017) "Identification methods in microplastic analysis: a review," *Analytical methods*, 9(9), pp. 1384–1391.

Siddique, R., Khatib, J. and Kaur, I. (2008) “Use of recycled plastic in concrete: A review,” *Waste management*, 28(10), pp. 1835–1852.

Singh, R.P. and Agrawal, M. (2008) “Potential benefits and risks of land application of sewage sludge,” *Waste management*, 28(2), pp. 347–358.

Song, Xiaowei, Wu, X., Song, Xiaoping and Zhang, Z. (2022) “Oil extraction following digestion to separate microplastics from mussels,” *Chemosphere*, 289, p. 133187.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J. and Shim, W.J. (2015) “A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples,” *Marine Pollution Bulletin*, 93(1–2), pp. 202–209.

de Souza Machado, A.A., Horton, A.A., Davis, T. and Maaß, S. (2020) “Microplastics and their effects on soil function as a life-supporting System,” in *Microplastics in Terrestrial Environments*. Springer, pp. 199–222.

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S. and Rillig, M.C. (2018) “Microplastics as an emerging threat to terrestrial ecosystems,” *Global change biology*, 24(4), pp. 1405–1416.

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S. and Rillig, M.C. (2019) “Microplastics can change soil properties and affect plant performance,” *Environmental science & technology*, 53(10), pp. 6044–6052.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R. and Rillig, M.C. (2018) “Impacts of microplastics on the soil biophysical environment,” *Environmental science & technology*, 52(17), pp. 9656–9665.

Stead, J.L., Cundy, A.B., Hudson, M.D., Thompson, C.E.L., Williams, I.D., Russell, A.E. and Pabortsava, K. (2020) “Identification of tidal trapping of microplastics in a temperate salt marsh system using sea surface microlayer sampling,” *Scientific Reports*, 10(1).

Steinmetz, Z., Löffler, P., Eichhöfer, S., David, J., Muñoz, K. and Schaumann, G.E. (2022) “Are agricultural plastic covers a source of plastic debris in soil? A first screening study,” *Soil*, 8(1), pp. 31–47.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O. and Schaumann, G.E. (2016) “Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation?,” *Science of the Total Environment*, 550, pp. 690–705.

Stock, F., Kochleus, C., Bänsch-Baltruschat, B., Brennholt, N. and Reifferscheid, G. (2019) "Sampling techniques and preparation methods for microplastic analyses in the aquatic environment–a review," *TrAC Trends in Analytical Chemistry*, 113, pp.84-92.

Strand, J. and Tairova, Z. (2016) "Microplastic particles in North Sea sediments 2015," Scientific Report from Danish Centre for Environment and Energy, pp.20.

Stuart, M.E. and Smedley, P.L. (2009) "Baseline groundwater chemistry: the Chalk aquifer of Hampshire." British Geological Survey Open Report, OR/09/052, pp. 61.

Sujathan, S., Kniggendorf, A.-K., Kumar, A., Roth, B., Rosenwinkel, K.-H. and Nogueira, R. (2017) "Heat and bleach: a cost-efficient method for extracting microplastics from return activated sludge," *Archives of environmental contamination and toxicology*, 73(4), pp. 641–648.

do Sul, J.A.I. (2021) "Why it is important to analyze the chemical composition of microplastics in environmental samples," *Marine Pollution Bulletin*, 165, p. 112086.

Sun, J., Pan, L., Tsang, D.C.W., Zhan, Y., Zhu, L. and Li, X. (2018) "Organic contamination and remediation in the agricultural soils of China: a critical review," *Science of the Total Environment*, 615, pp. 724–740.

Tagg, A.S., Brandes, E., Fischer, F., Fischer, D., Brandt, J. and Labrenz, M. (2022) "Agricultural application of microplastic-rich sewage sludge leads to further uncontrolled contamination," *Science of the Total Environment*, 806, p. 150611.

Tagg, A.S., Sapp, M., Harrison, J.P. and Ojeda, J.J. (2015) "Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging," *Analytical chemistry*, 87(12), pp. 6032–6040.

Talvitie, J., Mikola, A., Setälä, O., Heinonen, M. and Koistinen, A. (2017) "How well is microlitter purified from wastewater?–A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant," *Water research*, 109, pp. 164–172.

Tawfik, M.S. and Huyghebaert, A. (1998) "Polystyrene cups and containers: styrene migration," *Food additives & contaminants*, 15(5), pp. 592–599.

The World Bank (2018) *Agricultural land (% of land area)*. [Accessed on 21 April 2022] https://data.worldbank.org/indicator/AG.LND.AGRI.ZS.

Thiel, M., Luna-Jorquera, G., Álvarez-Varas, R., Gallardo, C., Hinojosa, I.A., Luna, N., Miranda-Urbina, D., Morales, N., Ory, N. and Pacheco, A.S. (2018) "Impacts of marine plastic pollution from continental coasts to subtropical gyres—fish, seabirds, and other vertebrates in the SE Pacific," *Frontiers in Marine Science*, p. 238.

Thiele, C.J., Hudson, M.D. and Russell, A.E. (2019) "Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size," *Marine pollution bulletin*, 142, pp. 384–393.

Thomas, D., Schütze, B., Heinze, W.M. and Steinmetz, Z. (2020) "Sample preparation techniques for the analysis of microplastics in soil—a review," *Sustainability*, 12(21), p. 9074.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D. and Russell, A.E. (2004) "Lost at sea: where is all the plastic?," *Science*, 304(5672), p. 838.

Tirkey, A. and Upadhyay, L.S.B. (2021) "Microplastics: An overview on separation, identification and characterization of microplastics," *Marine Pollution Bulletin*, 170, p. 112604.

Tóth, G., Montanarella, L. and Rusco, E. (2008) "Threats to soil quality in Europe." Technical report, EUR 23438 EN, Italy.

Underwood, A.J., Chapman, M.G. and Browne, M.A. (2017) "Some problems and practicalities in design and interpretation of samples of microplastic waste," *Analytical Methods*, 9(9), pp. 1332–1345.

United Nations (2016) "Transforming our world: The 2030 agenda for sustainable development." [Accessed on 21 April 2022] https://sdgs.un.org/2030agenda.

Vermeiren, P., Muñoz, C. and Ikejima, K. (2020) "Microplastic identification and quantification from organic rich sediments: A validated laboratory protocol," *Environmental Pollution*, p. 114298.

Viaroli, S., Lancia, M. and Re, V. (2022) "Microplastics contamination of groundwater: Current evidence and future perspectives. A review," *Science of The Total Environment*, p. 153851.

Viketoft, M., Riggi, L.G.A., Bommarco, R., Hallin, S. and Taylor, A.R. (2021) "Type of organic fertilizer rather than organic amendment per se increases abundance of soil biota," *PeerJ*, 9, p. e11204.

Villarrubia-Gómez, P., Cornell, S.E. and Fabres, J. (2018) “Marine plastic pollution as a planetary boundary threat–The drifting piece in the sustainability puzzle,” *Marine Policy*, 96, pp. 213–220.

Vithanage, M., Ramanayaka, S., Hasinthara, S. and Navaratne, A. (2021) “Compost as a carrier for microplastics and plastic-bound toxic metals into agroecosystems,” *Current Opinion in Environmental Science & Health*, 24, p. 100297.

Vollertsen, J. and Hansen, A.A. (2017) “Microplastic in Danish wastewater: Sources, occurrences and fate,” *The Danish Environmental Protection Agency. Environmental Project*, pp.55.

Vroom, R.J.E., Koelmans, A.A., Besseling, E. and Halsband, C. (2017) “Aging of microplastics promotes their ingestion by marine zooplankton,” *Environmental pollution*, 231, pp. 987–996.

Vryzas, Z. (2018) “Pesticide fate in soil-sediment-water environment in relation to contamination preventing actions,” *Current Opinion in Environmental Science & Health*, 4, pp. 5–9.

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J. and Marti, T. (2014) “Microplastics in freshwater ecosystems: what we know and what we need to know,” *Environmental Sciences Europe*, 26(1), pp. 1–9.

Waldschläger, K. and Schüttrumpf, H. (2019) “Erosion behavior of different microplastic particles in comparison to natural sediments,” *Environmental science & technology*, 53(22), pp. 13219–13227.

Waldschläger, K. and Schüttrumpf, H. (2020) “Infiltration behavior of microplastic particles with different densities, sizes, and shapes—from glass spheres to natural sediments,” *Environmental science & technology*, 54(15), pp. 9366–9373.

Wang, F., Wang, Q., Adams, C.A., Sun, Y. and Zhang, S. (2022) “Effects of microplastics on soil properties: Current knowledge and future perspectives,” *Journal of Hazardous Materials*, 424, p. 127531.

Wang, Q., Adams, C.A., Wang, F., Sun, Y. and Zhang, S. (2021) “Interactions between microplastics and soil fauna: A critical review,” *Critical Reviews in Environmental Science and Technology*, pp. 1–33.

Wang, Z., Taylor, S.E., Sharma, P. and Flury, M. (2018) “Poor extraction efficiencies of polystyrene nano-and microplastics from biosolids and soil,” *PloS one*, 13(11), p. e0208009.

Wanner, P. (2021) “Plastic in agricultural soils–a global risk for groundwater systems and drinking water supplies?–a review,” *Chemosphere*, 264, p. 128453.

Watteau, F., Dignac, M.F., Bouchard, A., Revallier, A. and Houot, S. (2018) “Microplastic Detection in Soil Amended With Municipal Solid Waste Composts as Revealed by Transmission Electronic Microscopy and Pyrolysis/GC/MS,” *Frontiers in Sustainable Food Systems*, 2, pp. 2571-581X.

Way, C., Hudson, Malcolm D., Williams, I.D. and Langley, G.J. (2022) “Evidence of underestimation in microplastic research: A meta-analysis of recovery rate studies,” *Science of the Total Environment*, 805, p. 150227.

Way, C., Hudson, Malcolm D, Williams, I.D., Langley, J. and Marsh, R. (2022) “Assessing the effectiveness of microplastic extraction methods on fishmeal with different properties,” *Analytical Methods*, 6(14), pp. 606-619.

Weber, C.J., Opp, C., Prume, J.A., Koch, M., Andersen, T.J. and Chifflard, P. (2022) “Deposition and in-situ translocation of microplastics in floodplain soils,” *Science of The Total Environment*, 819, p. 152039.

Wen, D., Chen, Y., Tong, Y., Wang, H., Zhang, H. and Luo, Y. (2021) “Quantification of Microplastics in Soils Using Accelerated Solvent Extraction: Comparison with a Visual Sorting Method,” *Bulletin of Environmental Contamination and Toxicology*, 107(4), pp. 770–777.

Wentworth, C.K. (1922) “A scale of grade and class terms for clastic sediments,” *The journal of geology*, 30(5), pp. 377–392.

Wesch, C., Elert, A.M., Wörner, M., Braun, U., Klein, R. and Paulus, M. (2017) “Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data,” *Scientific reports*, 7(1), p. 5424.

Whitt, M., Brown, W., Danes, J.E. and Vorst, K.L. (2016) “Migration of heavy metals from recycled polyethylene terephthalate during storage and microwave heating,” *Journal of Plastic Film & Sheeting*, 32(2), pp. 189–207.

Williams, I.D. (2001) *Environmental chemistry: a modular approach*. Wiley, UK, p. 412.

Windsor, F.M., Durance, I., Horton, A.A., Thompson, R.C., Tyler, C.R. and Ormerod, S.J. (2019) “A catchment-scale perspective of plastic pollution,” *Global change biology*, 25(4), pp. 1207-1221.

Wright, S.L., Thompson, R.C. and Galloway, T.S. (2013) “The physical impacts of microplastics on marine organisms: a review,” *Environmental pollution*, 178, pp. 483–492.

Wright, S.L., Ulke, J., Font, A., Chan, K.L.A. and Kelly, F.J. (2020) “Atmospheric microplastic deposition in an urban environment and an evaluation of transport,” *Environment international*, 136, p. 105411.

Wu, R.-T., Cai, Y.-F., Chen, Y.-X., Yang, Y.-W., Xing, S.-C. and Liao, X.-D. (2021) “Occurrence of microplastic in livestock and poultry manure in South China,” *Environmental Pollution*, 277, p. 116790.

Xi, B., Wang, B., Chen, M., Lee, X., Zhang, X., Wang, S., Yu, Z. and Wu, P. (2022) “Environmental behaviors and degradation methods of microplastics in different environmental media.” Chemosphere, 299, p.134354.

Xing, X., Yu, M., Xia, T. and Ma, L. (2021) “Interactions between water flow and microplastics in silt loam and loamy sand,” *Soil Science Society of America Journal*, 85(6), pp. 1956–1962.

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Zhijiang, He, Y., Wang, H., Lu, Zhenmei, Brookes, P.C. and Tang, C. (2020) “Microplastics in the soil environment: occurrence, risks, interactions and fate–a review,” *Critical Reviews in Environmental Science and Technology*, 50(21), pp. 2175–2222.

Yang, J., Li, L., Li, R., Xu, L., Shen, Y., Li, S., Tu, C., Wu, L., Christie, P. and Luo, Y. (2021) “Microplastics in an agricultural soil following repeated application of three types of sewage sludge: A field study,” *Environmental Pollution*, 289, p. 117943.

Yang, J., Li, R., Zhou, Q., Li, L., Li, Y., Tu, C., Zhao, X., Xiong, K., Christie, P. and Luo, Y. (2021) “Abundance and morphology of microplastics in an agricultural soil following long-term repeated application of pig manure,” *Environmental Pollution*, 272, p. 116028.

Yang, L., Zhang, Y., Kang, S., Wang, Z. and Wu, C. (2021) “Microplastics in soil: A review on methods, occurrence, sources, and potential risk,” *Science of the Total Environment*, 780, p. 146546.

Yang, Y., Li, Z., Yan, C., Chadwick, D., Jones, D.L., Liu, E., Liu, Q., Bai, R. and He, W. (2022) "Kinetics of microplastic generation from different types of mulch films in agricultural soil," *Science of The Total Environment*, 814, p. 152572.

Yu, L., Zhang, J., Liu, Y., Chen, L., Tao, S. and Liu, W. (2021) "Distribution characteristics of microplastics in agricultural soils from the largest vegetable production base in China," *Science of the Total Environment*, 756, p. 143860.

Yu, Y. and Flury, M. (2021) "How to take representative samples to quantify microplastic particles in soil?," *Science of the Total Environment*, 784, p.147166.

Yuan, Z., Nag, R. and Cummins, E. (2022) "Ranking of potential hazards from microplastics polymers in the marine environment," *Journal of Hazardous Materials*, 429, p. 128399.

Zemke, J.J., Enderling, M., Klein, A. and Skubski, M. (2019) "The influence of soil compaction on runoff formation. A case study focusing on skid trails at forested andosol sites," *Geosciences*, 9(5), p. 204.

Zhang, D., Liu, H., Hu, W., Qin, X., Yan, C. and Wang, H. (2016) "The status and distribution characteristics of residual mulching film in Xinjiang, China," *Journal of integrative agriculture*, 15(11), pp. 2639–2646.

Zhang, G.S. and Liu, Y.F. (2018) "The distribution of microplastics in soil aggregate fractions in southwestern China," *Science of the Total Environment*, 642, pp. 12–20.

Zhang, L., Xie, Y., Liu, J., Zhong, S., Qian, Y. and Gao, P. (2020) "An overlooked entry pathway of microplastics into agricultural soils from application of sludge-based fertilizers," *Environmental Science & Technology*, 54(7), pp. 4248–4255.

Zhang, S., Liu, X., Hao, X., Wang, J. and Zhang, Y. (2020) "Distribution of low-density microplastics in the mollisol farmlands of northeast China," *Science of the Total Environment*, 708, p. 135091.

Zhang, S., Yang, X., Gertsen, H., Peters, P., Salánki, T. and Geissen, V. (2018) "A simple method for the extraction and identification of light density microplastics from soil," *Science of the Total Environment*, 616, pp. 1056–1065.

Zhang, X., Chen, Y., Li, X., Zhang, Y., Gao, W., Jiang, J., Mo, A. and He, D. (2022) "Size/shape-dependent migration of microplastics in agricultural soil under simulative and natural rainfall," *Science of The Total Environment*, 815, p. 152507.

Zhang, X., Li, Y., Ouyang, D., Lei, J., Tan, Q., Xie, L., Li, Z., Liu, T., Xiao, Y. and Farooq, T.H. (2021) "Systematical review of interactions between microplastics and microorganisms in the soil environment," *Journal of Hazardous Materials*, 418, p. 126288.

Zheng, J. and Suh, S. (2019) "Strategies to reduce the global carbon footprint of plastics," *Nature Climate Change*, 9(5), pp. 374–378.

Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S., Tong, Y., Wen, D., Luo, Y. and Barceló, D. (2020) "Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, east China: Multiple sources other than plastic mulching film," *Journal of Hazardous Materials*, 388, p. 121814.

Zhou, W., Yang, S. and Wang, P.G. (2017) "Matrix effects and application of matrix effect factor." *Future Science*, 9(23), pp. 1839-1844.

Zhou, Y., He, G., Jiang, X., Yao, L., Ouyang, L., Liu, X., Liu, W. and Liu, Y. (2021) "Microplastic contamination is ubiquitous in riparian soils and strongly related to elevation, precipitation and population density," *Journal of Hazardous Materials*, 411, p. 125178.

Zhu, D., Bi, Q.-F., Xiang, Q., Chen, Q.-L., Christie, P., Ke, X., Wu, L.-H. and Zhu, Y.-G. (2018) "Trophic predator-prey relationships promote transport of microplastics compared with the single Hypoaspis aculeifer and Folsomia candida," *Environmental Pollution*, 235, pp. 150–154.

Zhu, F., Zhu, C., Wang, C. and Gu, C. (2019) "Occurrence and Ecological Impacts of Microplastics in Soil Systems: A Review," *Bulletin of Environmental Contamination and Toxicology*. Springer New York LLC, pp. 741–749.

Ziajahromi, S. and Leusch, F.D.L. (2022) "Systematic assessment of data quality and quality assurance/quality control (QA/QC) of current research on microplastics in biosolids and agricultural soils," *Environmental Pollution*, 294, p. 118629.

Zubris, K.A. v and Richards, B.K. (2005) "Synthetic fibers as an indicator of land application of sludge," *Environmental pollution*, 138(2), pp. 201–211.