Microplastics in soils and their associated effects on agricultural crop development.

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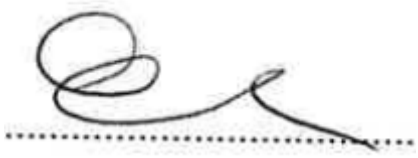
“The more clearly we can focus our attention on the wonders and realities of the universe about us, the less taste we shall have for destruction.”

Rachel Carson.

I certify that the work in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution. All references to ideas and work by other researchers have been specifically acknowledged.



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Abstract

Plastic pollution is a global problem and will increase as plastics continue to be produced, used and discarded. Microplastics (< 5 mm in size) are ubiquitous, being found in every environmental compartment. Plastics are thought to occur in terrestrial environments at rates 4 – 23-fold higher than in aquatic environments and have the potential to pose significant risks to flora and fauna. However, the majority of studies on microplastics have focused on the impacts in aquatic systems, with less research focused on the terrestrial system. Even less research has focused on the agricultural system, and the risks microplastics pose in this environment, despite agricultural soils being proposed as acting as a sink for microplastic pollution.

This thesis presents information from the two aspects of this work, firstly an assessment of the microplastic abundance, morphology and polymer types of microplastics in conventionally managed farmlands in soils from the Midlands, UK. To do this, a methodology was created to enable the extraction of microplastics from soils, which used Fenton's reagent to digest organic matter prior to density separating plastics from the remaining soil using zinc bromide. This research found that Fenton's reagent was the most appropriate methodology to extract microplastics from soil media. Subsequently, the method utilizing Fenton's reagent was used to generate quantitative data on the microplastics present in eight farms throughout the Midlands. A total of 24 fields were sampled, with a range of pasture and arable fields sampled to assess the common polymer types, shapes, and concentrations found in these soils. The assessment of agricultural soils found that microplastics were present in all the fields sampled and that fields that had anthropogenic additions (such as fertilizers, sewage sludge, or composts) contained higher levels of microplastic pollution than fields that did not. There were no differences in the microplastics per kg between arable and grazing fields. In the arable fields, the number of microplastics found in the centrally farmed area, as opposed to the unfarmed margins, was significantly higher but this did not occur in the pasture fields. Fibres were the most common shape of microplastic accounting for 95 % of the total microplastic shapes found, and polyester was the most common type of plastic found accounting for 41 % of the total plastic types.

Based on findings from the agricultural fields, the second aspect of this work assessed the impact of the most commonly found fibre, polyester, in four agricultural crop species (*Brassica napus* (rapeseed), *Sinapis alba* (mustard), *Triticum aestivum* (wheat), and *Hordeum vulgare* (barley)) reviewing any changes to germination, development and reproduction as a result of microplastic polluted soils. This research utilized germination bioassays to review changes to the germination rates, alongside chronic toxicity mesocosm studies to assess any changes to the early development and reproduction of plant species. The research indicated that the addition of polyester fibres resulted in reductions to the germination rates for all species test (at 5 % w/w resulting in reductions between 5 & 17 % reduction), but that this also occurred when a natural fibre was added to *Sinapis alba* (9 % reduction for polyester and 13 % reduction for keratin) , suggesting a physical change in the soil matrix resulted in the changes to germination rates for the four species tested. However, changes were not demonstrated in the shoot and root development when testing with natural fibres but were with polyester fibres. An assessment of chemical effects from plastics was performed using leachate (produced using 5 % w/w polyester fibres), where no effects were demonstrated in germination, however, changes in root development were shown when polyester leachates were used.

For further insight into the impacts of microplastics on plant development, *Sinapis alba* were grown in soils containing two different concentrations of polyester fibres (0.1 % and 1 % w/w) to the fruiting stage. This research indicated that the Fv/Fm measurements (which can demonstrate changes to the PSII system – the first protein complex in light-dependent reactions of photosynthesis) were reduced in the 1 % treatment compared to the control. Additionally, the total flower numbers produced, and the pod-to-seed ratio were reduced when exposed to 0.1 % and 1 % w/w microplastics compared to the control. This suggests that the addition of microplastics to soils could result in reduced yield for agricultural plastics.

The results from this thesis provide strong evidence that microplastics in high concentrations can change the germination, development, and reproduction of crop species. The current concentration of microplastics in UK agricultural soils as of present are not high enough to cause these effects. However, if plastic continues to be added to agricultural soils at the current rate, it is expected that these effects will pose issues for agricultural crop development. Further research is needed into the effects of different polymer types and on a wider range of agricultural crops to ascertain whether the shape or type of polymer has an impact on the development of plants.

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Chapter 1 Introduction

1.1 Background

Plastic pollution is now considered a global threat to different ecosystems, with plastic being found in almost every environmental compartment, from arctic snow to deep-sea sediments (Barnes et al., 2009; Lusher et al., 2018). The term plastic refers to an array of synthetic polymers which have a relatively recent history, being invented just over a century ago (Lusher et al., 2017b). The origin of synthetic polymers can be traced to the early 1900s when Leo Baekeland discovered the first fully synthetic plastic, Bakelite (phenol-formaldehyde) which was used frequently in the automobile and radio industries at the time (Millet et al., 2018). In the years following, there was a rapid expansion in the discovery of plastic polymers, with polyvinyl chloride (PVC) and polyvinyl acetate being discovered just five years later (Chauhan et al., 2019). Plastic was not mass-produced, however, until World War II, during which plastics played a key role in the military supply chain, with polymers such as nylon being used in parachutes, ropes and body armour (Napper & Thompson, 2020). In addition, various new plastics were discovered during the war, many of which are still used today, such as polystyrene, polyester, and polyethylene (Napper & Thompson, 2020). The production of plastic during wartime quadrupled from 213 million pounds produced in 1939 to 818 million pounds produced in 1945 (Freinkel, 2011). After the war ended, plastic products found their way onto the consumer market, offering a low-cost replacement for many items unavailable during wartime (Smithers, 2020). Subsequently, plastic items became incorporated into the home and became a large part of the clothing industry, with polyester, lycra and nylon fabrics widely used (Sajn, 2019). The economic and societal benefits that plastic has produced are undeniable, from extending the shelf life of foods to reducing shipping costs (Thompson et al., 2009). There has since been rapid growth in the production of plastics, with plastics being utilised in numerous industries (Dijkstra et al., 2020), and as such, the age of plastics ensued.

Today there are approximately 30,000 different plastic polymer materials registered by the European Union (EU) (Horton et al., 2017), belonging to seven major plastic groups. Polymers are derived from fossil hydrocarbons and have a unique molecular structure, long chain-like molecules made of repeated chemical structural units (Napper & Thompson, 2020). They can be split into two main groups: thermoplastics and thermosets. Thermosets comprise approximately 16 % of polymers authorised for use in the EU; they cannot be re-melted or reshaped and include materials such as epoxy resins and the aforementioned Bakelite (Horton et al., 2017). Conversely, thermoplastics are characterised by their ability to be remoulded and shaped almost indefinitely and makeup 84 % of the 30,000 polymer materials approved by the EU (Horton et al., 2017). Thermoplastics include commonly used materials such as polyamides (PA), polyesters, and polypropylenes (Nikiema & Asiedu, 2022). Additives can also be included to enhance polymer performance and appearance, such as plasticisers to increase flexibility, flame retardants and antimicrobial agents (Sridharan et al., 2022). The versatility, durability, and longevity of plastics have led to the use of polymers for a broad range of applications. This is reflected in the growth of plastic production, which rose from 1.5 million metric tons in the 1950s to 367 million metric tons in 2020 (see figure 1.1) (Statista, 2021). It is predicted that by 2040, plastic production will increase to 700 million metric tons globally (Lau et al., 2020).

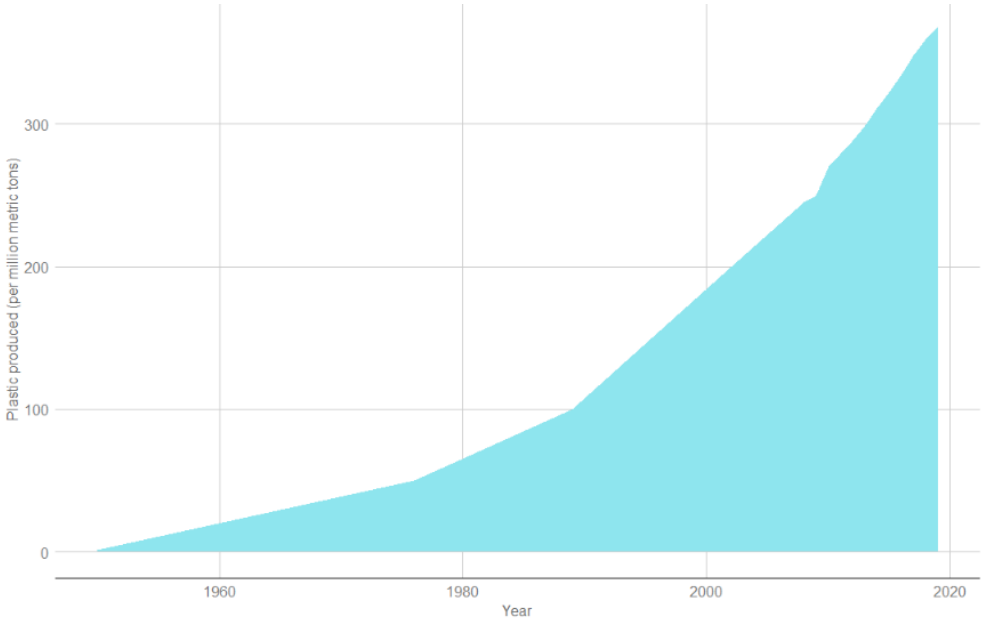


*Figure 1.1: Annual plastic production worldwide between 1950 and 2020, produced using data from PlasticsEurope (2021), which includes thermoplastics, polyurethane, thermosets, adhesives, coatings, sealants, elastomers and polypropylene fibres; * it does not include the following fibres: PA. PET, PP and polyacrylic fibres.*

Plastics have become an irreplaceable part of the modern world, and an increased generation of plastic waste has accompanied the rapid increase in plastic production (Lusher et al., 2017b). The same characteristics that make plastics so beneficial as a material, such as their durability, longevity and resistance to degradation, also lead to difficulties in managing waste (Rigamonti et al., 2014). Additionally, the chemical complexity of plastics makes them challenging to degrade in the natural environment (Chamas et al., 2020). Most of the plastics produced since the 1950s are still around today, and as of 2015, it was estimated that only 9% of plastics ever made have been recycled (Lusher et al., 2017b). The accelerated generation of plastic waste has created a global crisis, leading to negative impacts on many ecosystems (Kumar et al., 2021). For these reasons, plastic pollution has become one of the most topical environmental issues of the 21st century, known as the plastic pollution crisis (Horton, 2022).

1.2 Plastic pollution

Estimates of global emissions of plastic waste to aquatic environments (including rivers, lakes, and the ocean) ranged from 9 to 23 million metric tons in 2016 (Lusher et al., 2017b). Terrestrial environments were suspected to be even higher, with an estimated emission of plastic waste between 13 and 25 million metric tons per year as of 2016 (Borrelle et al., 2020; Lau et al., 2020). Various factors have been noted that influence the proliferation of plastic pollution in the environment, such as a rise in the increase of single-use plastics and the fact that waste management systems do not have the capacity at a global scale to dispose of or recycle waste plastic (Welden, 2020). For example, in 2014, the European demand for plastics was approximately 47.8 million metric tons, but only 25.8 million metric tons of this entered waste stream management (Plastics Europe, 2015). In 2020 the European demand for plastics was 50.3 million metric tons, with 29.5 million metric tons entering waste stream management (Plastics Europe, 2022). It is considered that 80 % of this waste occurs from terrestrial sources such as single-use consumer items (Sheavly & Register, 2007). However, as most plastics are used and disposed of on land, terrestrial environments likely contain higher levels of plastic pollution, with Horton et al. (2017) estimating that pollution in terrestrial systems is 4 – 23-fold higher than in aquatic environments.

Plastic pollution is extremely diverse in the environment due to the wide variety of plastic materials available on the consumer market (Kumar et al., 2021). The sources of plastic pollution are manifold; however, pollution generally originates from anthropogenic activities such as mismanaged waste, landfills (where items can be transported to other habitats by wind and runoff), and agricultural activities (Mai et al., 2018). The first accounts of plastic in the environment were reported from the carcasses of seabirds collected from shorelines in the early 1960s, and many adverse effects of plastics in the environment have been noted (Ryan, 1987). Over 260 species have been reported to have ingested or entangled in plastic debris in the marine environment, resulting in ill health and early mortality (Derraik, 2002; Ozturk & Altinok, 2020). However, most of this work has pertained to the consequences of plastic debris in marine environments, and there is limited understanding of the

impacts on terrestrial and freshwater habitats (Duis & Coors, 2016). Once in the environment, plastic litter can undergo ageing processes which result in disintegration and degradation (Chamas et al., 2020). Plastic degrades due to a range of abiotic and biotic processes, such as mechanical degradation (erosion and abrasion), chemical degradation (photo-oxidation and hydrolysis) and biological degradation (degradation by organisms such as fungi and bacteria) (Julienne et al., 2019). As plastic degrades, it could form smaller fragments, which can become biologically and chemically available to different organisms (Botterell et al., 2019).

1.3 Microplastics

Microplastic (MPs) are defined as particles from 100 nm to 5 mm in size (Thompson et al., 2004; Masura et al., 2015; Zhang et al., 2020d) and are considered an emerging anthropogenic pollutant (Avio et al., 2017). However, there has been controversy regarding the size classifications of microplastics, with Hartmann et al. (2019) suggesting that microplastics should follow logical differentiation along standard international units (5 mm – 1 μm). Hartmann et al. (2019) suggest that the ambiguity in categorising plastic debris results in incomparable data. Despite the ambiguity, evidence suggests that MP pollution is ubiquitous and is estimated to account for 92 % of all global plastic counts (Eriksen et al., 2014). MPs are divided into two main categories, primary and secondary, and have an extensive range of shapes, sizes, and polymer types (Horton et al., 2017). Primary microplastics are industrially manufactured at sizes < 5 mm and include nurdles (the raw material for manufacturing plastic products) and cosmetic products such as microbeads (Rillig, 2012). Secondary microplastics are fragmented particles from larger products which break down via degradation (Lusher et al., 2017b). Microplastics are generally divided into four shape profiles, fibres, fragments, films and spheres (Rosal, 2021). However, as with size, there is debate regarding how fragments should be categorised, Hartmann et al. (2019) suggest six shape profiles, spheres, spheroids (imperfect but approximately spherical), cylindrical pellets (rod-shaped cylindrical objects), fragments, films and fibres. Spheres are uniformly shaped microplastics commonly found in cosmetic products, which are generally primary plastic particles (Rettinger & Huber, 2016). As of 2018,

microplastic spheres in cosmetics have been banned in Europe (Kentin & Kaarto, 2018), but due to the longevity of plastics in the environment, the spheres are likely to be still found in the environment. Fragments, fibres and films are generally considered secondary plastic particles, occurring from the degradation of larger materials (Tanaka & Takada, 2016). Microplastic films are fragmented from thin plastic items such as plastic bags and food wrappers; this group includes low-density polyethylene (LDPE) films, which protect crops (Barnes et al., 2009). Fragments are irregularly shaped and form via the degradation of larger plastic items (Botterell et al., 2019). Fibres are generally released from clothing and fishing gear and are believed to account for the largest proportion of microplastic pollution found in the environment accounting for around 90 % of all plastics found in the marine environment (Gaylarde et al., 2021), and likely similar statistics in the terrestrial system. In 2013, 54.4 million tons of synthetic fibres were produced worldwide, with 81.8 % of these being polyester fibres, which are commonly used in fabrics (European Environment Agency, 2020). Research by Burns & Boxall (2018) found that the most common microplastic types in water samples were fibres (52 %) then fragments (29 %), with a smaller percentage of spheres, films and foams found. In sediment samples, fibres were the most common fibre type found (45 %), but more fragments were found in the aquatic environment (35 %). Corradini et al. (2021) found similar findings in soils, with fibres being the most common microplastic shape, accounting for 68 % of all particles. Films were the second most common shape (23 %), and fragments and pellets were observed less frequently (7 % and 2 %, respectively).

MPs can also be classified by the type of polymer, with some of the most common plastics produced globally being polypropylene, polyethylene, polyvinyl chloride, polystyrene, polyethylene terephthalate and polyurethane (Esterhuizen & Kim, 2022). Research by Erni-Cassola et al. (2018) assessed the diversity of plastic types found in the marine environment, reviewing 39 studies. Their research found that polyethylene (used commonly as a packaging material) was the most abundant type of plastic in aquatic environments, followed by polypropylene (used in fishing gear) and polystyrene (used as a packaging material). Dioses-Salinas et al. (2020) reviewed the diversity of

plastic types found in terrestrial soils, assessing ten studies which extracted MPs from natural, industrial, or agricultural soil environments. Polyethylene was the most common polymer identified within soils and agricultural soils, with polyethylene, polyamide and polypropylene being the three most commonly identified polymers.

Microplastics present a significant challenge when they enter the environment as, unlike larger plastic debris, MPs cannot be cost-effectively detected or collected to be recycled (Cole et al. 2011). Due to the longevity of plastics and the constant input of plastics into the ecosystem, the accumulation of plastics in the environment is inevitable. The lifetimes of plastics in different environments range from years to decades, but this depends on the material's physical and chemical properties (O'Brine & Thompson, 2010). The degradation of polymers can be divided into four main processes: 1) physical degradation occurring by weathering, abrasive forces and mechanical degradation (such as ploughing). 2) Photodegradation occurs when polymers are exposed to UV light; 3) Chemical degradation by oxidation or hydrolysis and 4) biodegradation, which can occur from various organisms such as bacteria, fungi, algae and invertebrates (Yousif & Haddad, 2013). Plastics show high resistance to ageing and minimal biological degradation, with research from Ohtake et al. (1998) finding that additive-free low-density polyethylene may take more than 100 years for the mineralisation of the plastic to occur. Plastic additives include plasticizers, flame retardants and antioxidants which can prolong the material's longevity (Hahladakis et al., 2018); thus, plastics containing additives could take significantly longer to degrade in the environment. The degradation process manifests as changes in the polymer's physical properties, such as surface erosion and discolouration (Chamas et al., 2020). Degraded plastics could also enable polymers to carry chemicals of a smaller molecular size, such as persistent organic pollutants, dichloro-diphenyl-trichloroethane (DDT), and polychlorinated biphenyls and potentially transport these to different organisms (Van et al., 2012; Rodrigues et al., 2019). However, research to date has not found evidence of POPs accumulating in organisms due to microplastics.

1.4 Microplastics in soil environments

Soils are key ecosystem components that provide many ecological services, such as carbon sequestration, promoting biodiversity, and providing plant nutrients (Guo et al., 2020). However, globally, soils are affected by pollution from anthropogenic activities such as mining, improper waste disposal, and agriculture (Jie et al., 2002). Ultimately, this can impact crop productivity, affecting global food security and human health (De-la-Torre, 2020). Therefore, microplastics are considered an emerging persistent pollutant in soils (Kumar et al., 2020b; UNEP, 2022).

Microplastics have been extensively studied in aquatic environments; however, there is a dearth of information about microplastics in the terrestrial system (see figure 1.2). This is surprising, considering that the majority of plastics are used and disposed of in terrestrial ecosystems. Rillig (2012) considered that research was limited in the terrestrial environment because of the comparative difficulty of extracting microplastics from the soil matrix compared with the comparative ease of extracting them from water. Campanale et al, (2022) also discussed this limited understanding of microplastics and stated that monitoring MPs in terrestrial environments is challenging. He et al. (2018) suggested that of all the studies conducted on microplastics between 2004 and 2018, only 3.86 % of these pertained to pollution within soils. As of 2021, the number of papers produced concerning microplastics in soil systems had increased to 7.01 % but still lagged behind aquatic systems, which accounted for 47.02 % of microplastics research (Yang et al., 2021). As terrestrial microplastic studies are a more recent area compared to their marine counterparts, there is still very little known about terrestrial MP sources, release, transport, fate and impacts.

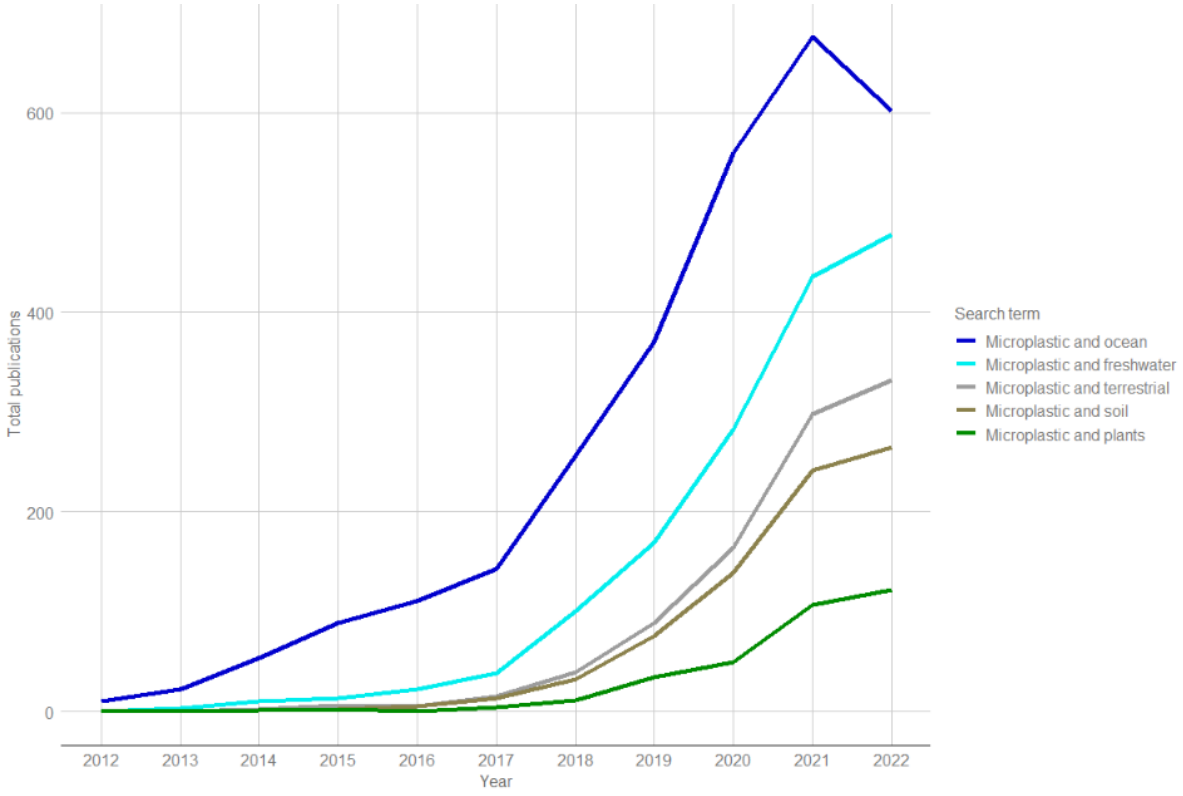


Figure 1.2: The number of publications by year by search term. Produced using data from Web of Science (2022).

There are various inputs of microplastics into terrestrial ecosystems, with urbanised and agricultural environments being the most polluted due to anthropogenic activity (see figure 1.3) (Kallenbach et al., 2022). The sources of microplastics in soils consist of inputs from sewage sludge, compost, street runoff, littering, atmospheric deposition and landfill & industrial waste (Zhang et al., 2020).

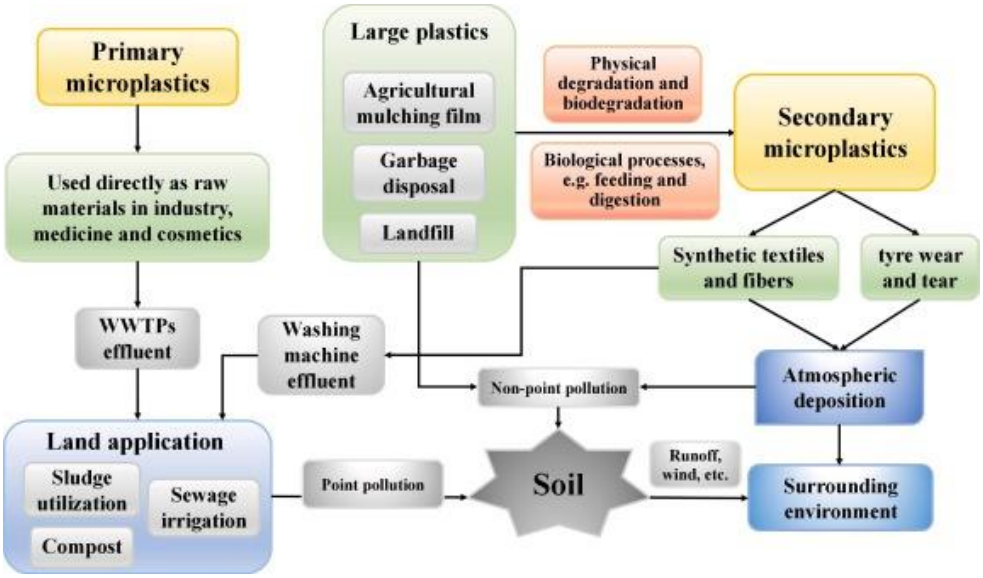


Figure 1.3: The inputs of microplastics into soils, Figure sourced from Guo et al. (2020)

Sewage sludge from wastewater treatment plants is used as a soil amendment on agricultural land and is widely used as a fertilizer (Milojevic & Cydzik-kwiatkowska, 2021). However, research increasingly suggests that the application of sewage sludge contributes to the incorporation of microplastics into soils (van den Berg et al., 2020). Wastewater treatment plants (where sewage sludge is produced) are receptors for microplastics from industry, domestic wastewater and stormwater (Mahon et al., 2017). Sun et al. (2019) found that wastewater treatment plants removed up to 99.9 % of microplastic particles from wastewater, which results in microplastics being concentrated in sewage sludge. Li et al. (2018) investigated microplastics in sewage sludge from 28 wastewater treatment plants (WWTP) in China, finding an average of 22,700 ± 12,100 microplastic particles kg^{-1} of dry sewage sludge. This research highlights considerable amounts of plastic within sewage sludge; however, it should be noted that China is considered one of the most polluted countries in the world when considering plastic pollution (Yang et al., 2015a; OurWorldinData, 2019; WPR, 2023). Research conducted in Ireland also investigated MPs in biosolids, finding an average range between 4,196 to 15,385 particles kg^{-1} of biosolids; however, the authors note this is likely an underestimation of the actual amount due to the efficiency of the method, believing there is up to a 20 % underestimation leaving the total being between 5,033 and 18,462 (Mahon et al., 2017). When considering the aqua sphere, it is apparent that the majority of microplastics are retained in sewage

sludge compared to being released in wastewater effluent. This is demonstrated by research from Murphy et al. (2016), who found untreated wastewater contained 15,000 particles m^{-3}; however, after treatment contained 250 particles m^{-3}. This has particular implications for the agroecosystem; conservative estimates by Nizzetto et al. (2016) suggest that between 125 – 850 tons MP/million inhabitants (equating to 0.2 – 8 mg MP/ha/yr) are added annually to European agricultural soils via the direct application of sewage sludge and processed biosolids. Clearly, sewage sludge is acting as a pathway for microplastics in terrestrial environments.

Compost is considered to be one of the sources of microplastics in agricultural environments (Vithanage et al., 2021; Scopetani et al., 2022). Research by Gui et al. (2021) found that the composting of rural domestic waste was a source of soil microplastics, reporting an average abundance of 2400 ± 358 items/kg (dry weight). Braun et al. (2021) quantified the prevalence of plastic in eight different composts from composting plants and hardware stores. This research found between 0.05 ± 0.08 to 1.36 ± 0.59 g kg^{-1}, which the authors suggest could result in the annual application of 84,000 to 1,610,000 plastic items ha^{-1} into agricultural lands. The aforementioned research suggests that compost application is a major pathway for plastic into agricultural and horticultural soils.

Another significant input of microplastics into the terrestrial environment is tyre and road wear particles (TRWP), which have been estimated to account for half the total MPs produced in Northern Europe (Magnusson, 2016, Sundt, 2014). Furthermore, TRWPs have been estimated to account for 5 – 10 % of plastics found in the oceans (Kole et al. 2017), though due to their high density are mainly deposited in the soils close to roads (Wagner 2018), meaning they are likely found in higher concentrations in soils that in the ocean. TRWPs are a mix of particles, including natural and synthetic rubbers (composed of styrene butadiene or butadiene rubber), paints and various chemical compounds, most of which are still unknown (Wagner 2022). The complex mixture of compounds

contained within TRWPs could also result in complex leachates, which could result in adverse effects on flora and fauna (Baensch-Baltruschat et al., 2020). TRWPs could contribute to increased levels of microplastics within terrestrial environments and, due to the heterogenous nature of the particles, could cause a range of toxicological effects from both the physical contaminant and the chemical contaminants.

Landfills and industrial waste processing centres may also contribute to the input of microplastics into the terrestrial environment (Nizzetto et al., 2016a). This can occur through improper waste management, the accidental loss of particles and the generation of contaminated soils (contaminated with plastic) and aerosols (De Souza Machado et al., 2018). Landfills are a widely applied strategy for waste disposal and have been estimated to store between 21 - 42 % of global plastic waste (Nizzetto et al., 2016). The conditions within landfills, such as high salinity, fluctuating temperatures, gas generation and microbial degradation, can lead to the fragmentation of plastics into microplastics (Chamas et al., 2020). Some of the earliest identified microbes which can break down some polymers, such as *Xanthomonas* sp, *Sphingobacterium* sp and *Bacillus* sp, STR-Y-O, were identified in landfills (Eisaku et al., 2003; Ru et al., 2020). He et al. (2019) assessed microplastic pollution found in active and closed landfills and found between 0.42 - 24.58 particles L^{-1} in leachate derived from landfills in southern China. However, this research did not include fibres to avoid false positives, which suggests the actual number is much higher. Microplastics in leachate can be carried into different environments, which could act as a potential pathway for microplastics to infiltrate aquatic and wider terrestrial environments (Boyle & Örmeci, 2020).

Once microplastics have entered soils, they are subject to less degradation than their ocean counterparts due to low mechanical processes (such as wave action in ocean habitats) and UV degradation for floating plastic particles and biological degradation (Chamas et al., 2020). Soils are porous media comprising mineral particles, organic matter, air and water (Wild, 1993). Soils contain macro and mesopores, enabling the migration of dissolved chemicals and small particles (Guo et al.,

2020). Grayling et al. (2018) demonstrated that small microplastics (0.1 – 6 μm) could move vertically in the soil column, moving through soil pores with the addition of water. Heinze et al, (2021) also demonstrated that bioturbation by *Lumbricus terrestris* (common earthworm) could result in the vertical migration of nanoplastic particles in soils. However, larger microplastic particles will likely be retained in soils, acting as a sink for these particles. Zubris and Richards (2005) found that MP fibres could be found in field site soils 15 years after the initial application of sewage sludge, demonstrating the ability for soils to act as a sink of microplastics. External forces, such as bioturbation, farming activities and soil erosion may contribute to the movement of larger microplastics in soils. Additionally, soil organisms such as the collembola *Folsomia candida* have been demonstrated to disperse PVC particles (80 – 250 μm) in a laboratory setting, although further tests are needed to assess whether this happens in real world environments. Additionally, Huerta Lwanga (2016) and Rillig (2017) observed that microplastics could adhere to earthworms and be ingested and secreted resulting in the movement of microplastics. The transport of microplastics in the soil matrix can also be attributed to plant roots, which may help to facilitate the vertical transport of plastics in soils (Ullah et al., 2021).

Microplastics' movement in soils depends on the properties of the polymer, such as their density, shape and size (Lozano et al., 2021). Smaller particles were most likely to move downward and reach deep soils due to their ability to pass through small soil pores (Campanale et al., 2022). However, most studies to date considering microplastic transport have focused on spheres and small particles, and fibres would likely behave differently (Campanale et al., 2022). Studies investigating the occurrence of microplastics in farmland soils in Northern China found that the abundance of microplastics decreased with increasing soil depth (Zhang et al., 2018). Additionally, they found that the greatest level of microplastic concentration was in the 0 – 10 cm depth of soil.

Currently, there is no agreed definition for the amount of plastic in soil which classifies the soil as polluted soil. Wang et al. (2019) suggested the first definition of plastic-polluted soils, considering that soils containing a concentration of 0.1 % w/w plastic soil should be considered polluted, though

this has not been widely accepted. It is vital to understand the extent of plastic pollution in soils, to accurately conduct toxicological assessments of the effects plastics could pose in the soil environment. Soil health is associated with the soil's biophysical and chemical properties, both of which have been demonstrated to undergo changes as a result of the addition of microplastics; ultimately, these properties determine plant health (Tang, 2020). Changes to the soil biophysical and chemical properties of soil could have subsequent effects on plant development, De Souza Machado et al, (2019) found that changes to the soil structure occurred as a result of microplastic addition. The researchers found subsequent effects on plant root development, with an increase in root biomass which the authors suggested was due to decreased root penetration resistance. De Souza et al, (2019) also found that polyester microplastic particles had the strongest effects on soil structure. However, further considerations are needed regarding how microplastic particles may impact the biophyisical and chemical properties within soils and plant health. Currently, there is limited understanding of the mechanisms that result in germination changes, with further information needed as to whether changes to the soil impact germination or whether this is related to chemical changes due to the addition of microplastics.

1.5 Microplastics in agriculture

Plastic products are widely used in the agricultural industry, which results in the incorporation of microplastics into agricultural soils (Nizzetto et al., 2016b). Mulch films are utilised to keep suitable temperatures and improve yield (Kasirajan & Ngouajio, 2012). Fertilisers are added to improve soil nutrient status, which can contain microplastics and other toxicants such as heavy metals and pesticides (Sajjad et al., 2022; Alengebawy et al., 2021). Seed coatings often contain a polymer product (such as polyvinyl alcohol (Ryu et al., 2006)), which helps to protect seeds (Sajjad et al., 2022). The sources of plastics in the agricultural environment are vast, and farming activities such as tilling may break down larger plastic products into microplastics which are then incorporated into the

soil (Sajjad et al., 2022). In agricultural soils, the primary microplastic pollution sources are sewage sludge, wastewater irrigation, mulching films and composts and atmospheric deposition (see figure 1.4) (Tian et al., 2022).

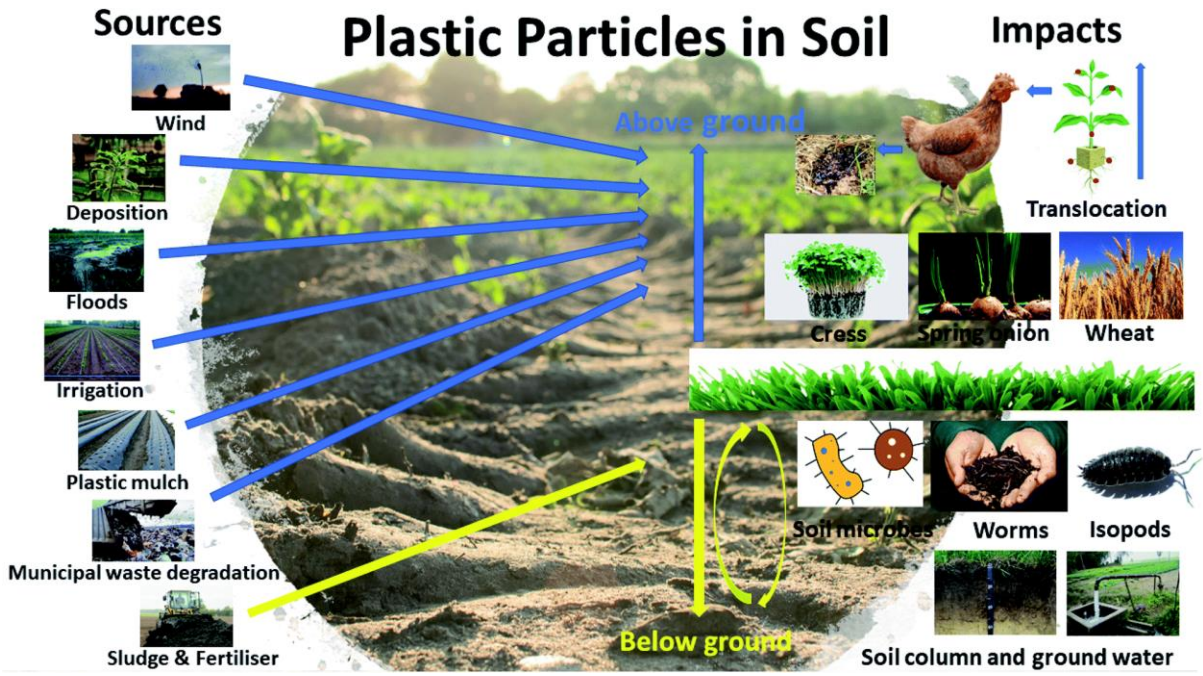


Figure 1.4: The sources of microplastics in the agricultural environment (Okoffo et al., 2021).

Mulch films (often made of LDPE polymers (Espí et al., 2006)) are considered one of the major sources of plastic pollution in agricultural soils due to the extensive use and often improper disposal of waste products (Kasirajan & Ngouajio, 2012). In 2020, the agricultural film market was worth 9.92 billion USD and is expected to grow to 15.37 billion in 2028 (Fortune Business Insights, 2018). Approximately 20 million hectares of farmland worldwide practice mulching, with China accounting for the largest proportion (Steinmetz et al. 2016). Removing plastic mulch is time-consuming, and remnants of plastic films are often left in agricultural soils, degrading over time and becoming brittle, breaking down into micro-sized particles (Huang et al., 2020). Alongside the natural degradation of mulch films, agricultural practices such as ploughing often result in further generation of microplastics (Huang et al., 2020). As plastics are resistant to degradation, the repeated application of

mulch films will result in the accumulation of microplastics in agricultural soils. Huang et al. (2020) investigated agricultural plastic mulching as a source of microplastics in soils, sampling 19 provinces in China. Microplastic residues were found in all soil samples at an average concentration of 83.6 kg ha^{-1}. Microplastics were also found in all fields, with a clear trend of increased MPs the longer mulch films had been applied. In fields with five years of mulching, the average abundance of microplastics was 0.08 ± 0.04 pieces g^{-1}. In fields with 24 years of continuous mulching, the average abundance of microplastics was 1.8 ± 0.3 pieces/g, which was significantly higher than the fields with only five years of mulching ($p < 0.05$). However, as China uses the highest proportion of mulch films worldwide (accounting for 60 % of the plastic film used for agricultural land mulching worldwide (Sun et al., 2020)), it is likely that microplastics from mulch films are lower throughout the rest of the world, however as of current this has not been investigated, as such further research is needed.

Another significant input of microplastics into agricultural land is the application of sewage sludge (Tagg et al., 2022). As discussed in 1.4, sewage sludge is a by-product from wastewater treatment plants, which is noted to contain high levels of microplastics. Berg et al. (2020) investigated the abundance of microplastics in fields in Spain that had repeated applications of sewage sludge. This work found that soils with a history of sewage sludge application, on average, have a 256 % (2070 ± 1310 MPs kg^{-1} in soils without sewage sludge and 5190 ± 2630 MPs kg^{-1} in soils with sewage sludge) higher microplastic content than soils without sewage sludge application. Research from China found that sewage sludge from WWTPs had an average of 22.7 ± 12.1 particles g^{-1}. This varies per country, with Mahon et al. (2016) finding lower levels in Ireland, with sewage sludge containing between 4.2 to 15.4 pieces g^{-1} (N = 21 10 g samples). Nizzetto et al. (2016) estimated that 125 – 850 tons of MP/ million inhabitants are added annually into European agricultural soils by directly applying sewage sludge and processed biosolids.

The European Union's policy on the sustainability and recycling of resources favours the recycling of sewage sludge. EU legislation, such as the renewable energy directive (2009/28/EC) and the landfill

directive (1999/31/EC), have redirected sewage sludge from landfills and incineration into agricultural lands and energy production. Statistics from Eurostat (2022) indicated that in 2012, 42.8 % of all sewage sludge disposed of across 28 European countries was diverted to agricultural lands (see figure 1.5). The amount of sewage sludge redirected to agricultural lands was much higher in the United Kingdom, with 78.3 % applied to agricultural lands (see figure 1.6). Harley-Nyang et al. (2022) found that sewage sludge from a WWTP in Devon contained 17.6 MPs/g w/w of sewage sludge for anaerobically digested material and 14.8 MPs/g w/w in limed sludge cake. This suggests that sewage sludge application will likely increase MPs on agricultural lands.

In the UK, only one study has assessed the amounts of microplastics found in soils in the UK, with the study considering increases from the applications of biosolids. Radford et al, (2023) investigated whether sewage sludge resulted in an increase in microplastics present in agricultural soils in the UK, finding no significant differences between treated soils and untreated soils ($\chi 2$ (1) = 0.597, p = 0.441). Untreated soils had an average of 679 ± 165 MPs kg $^{-1}$, and treated soils had an average of 892 ± 289 MPs kg $^{-1}$. The authors consider that sewage sludge was not the only source of microplastics in UK soils but that further research was needed to determine the sources of microplastics in soils.

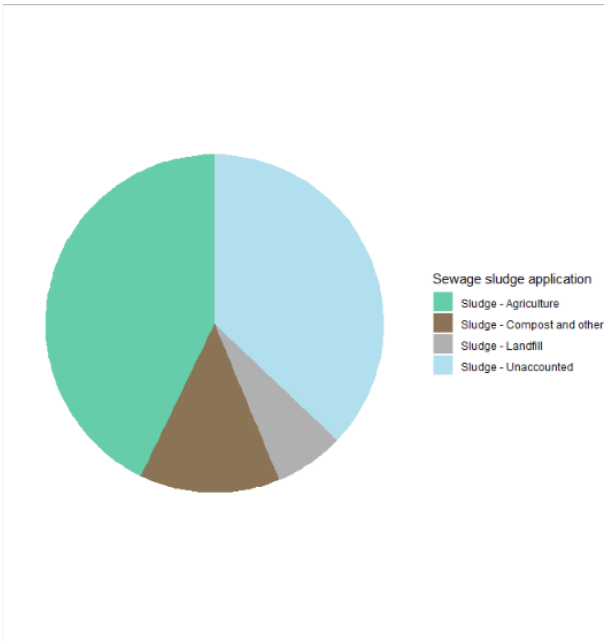


Figure 1.5 EU statistics for 2012 on sewage sludge usage per thousand tonnes across 28 countries, created using R ggplot2 package.

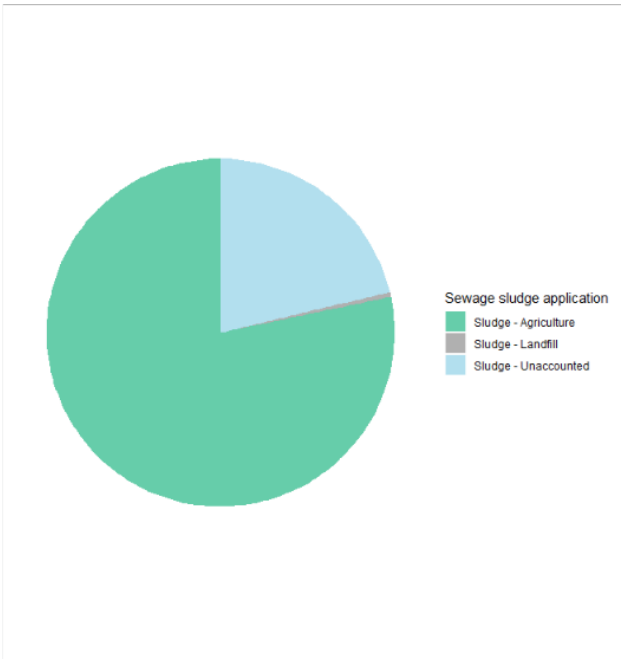


Figure 1.6: EU statistics for 2012 (most recent available date for the data for the UK) for the production and usage of sewage sludge per thousand tonnes in the UK, created using R ggplot2 package.

In addition to sludge, compost is widely applied in agriculture to improve soil fertility which aids with plant development (Visconti et al., 2022). Composts are sourced from various materials, including sewage sludge, green cuttings and waste from agriculture and food processing (Vithanage et al., 2021). However, composts have been recognised as a pathway for microplastics and macroplastics to enter agricultural soils (Zhang et al., 2020). Weithman et al. (2018) identified 24 MPs kg^{-1} in German compost from municipal organic waste and green clippings, despite Germany having one of the strictest regulations on foreign matter within fertiliser (Weithmann et al., 2018). Braun et al. (2021) investigated the prevalence of plastics in composts derived from different source materials, finding that all composts contained plastics. This research suggested that the load of plastics in samples was highly variable and that the microplastics contributed only a small amount to the total weight of the compost, with the calculated weights being between 0.05 ± 0.08 g – 1.36 ± 0.59 g kg^{-1}. However, when this is multiplied to consider an application of 7 – 35 tons of compost per hectare (based on common recommendations for composting loads), this equates to a plastic load of 84,000 to 1,610,000 plastic items ha^{-1} per year or 0.34 – 47.53 kg of plastic ha^{-1}. The authors further state that compost application is a source of microplastics in the environment and that horticultural and agricultural soils are particularly susceptible due to the requirements to apply compost to improve soil health.
Another source of microplastics in the agricultural landscape is atmospheric deposition which subsequently leads to contamination of the terrestrial system (Büks & Kaupenjohann, 2020). For example, the deposition of microplastics in central London was 575 – 1008 particles m^{-2} day^{-1} (Wright et al., 2020). However, urban sites are likely to have a higher deposition rate, and thus countryside areas may be at lower risk (Büks & Kaupenjohann, 2020). Atmospheric conditions play a pivotal role in microplastic deposition, with rainfall and snow influencing microplastic fallout (Allen et al., 2019).

Zubris and Richards conducted the first study pertaining to microplastic accumulation in farmland soils (2005). The authors reported an average count of 1.21 ± 0.25 fibres g^{-1} of soil five years after sludge application. Liu et al. (2018) assessed the microplastic content of farmland soils in Shanghai, China finding an average of 0.078 ± 0.013 pieces g^{-1} in shallow soils and 0.062 ± 0.013 pieces g^{-1} in

deep soils (with the research considering the proliferation of microplastics into soils). The authors considered that sewage sludge application and plastic mulching may have influenced the concentration of microplastics found, suggesting the site with the highest reported abundance of microplastics with 0.275 pieces g^{-1} in the shallow soils was likely influenced by this. This work could suggest that microplastics are primarily contained within shallow soils and not transported into deeper soil depths, being available to more organisms. Work has subsequently assessed the level of microplastics in China when sewage sludge was applied, with Ding et al. (2020) investigating agricultural lands which had received sewage sludge and plastic mulching in north-western China, finding an average concentration of between 2.131 ± 0.371 items g^{-1}. The dominant shape was fibres, and six different plastic types were found, although this study did not report the most dominant plastic type. Although these studies contribute significantly to the research, it is worth noting that, due to China being one of the countries with the most mismanaged plastic waste (Meijer et al., 2021), these numbers are likely significantly higher than in countries with better waste management systems (see figure 1.7).

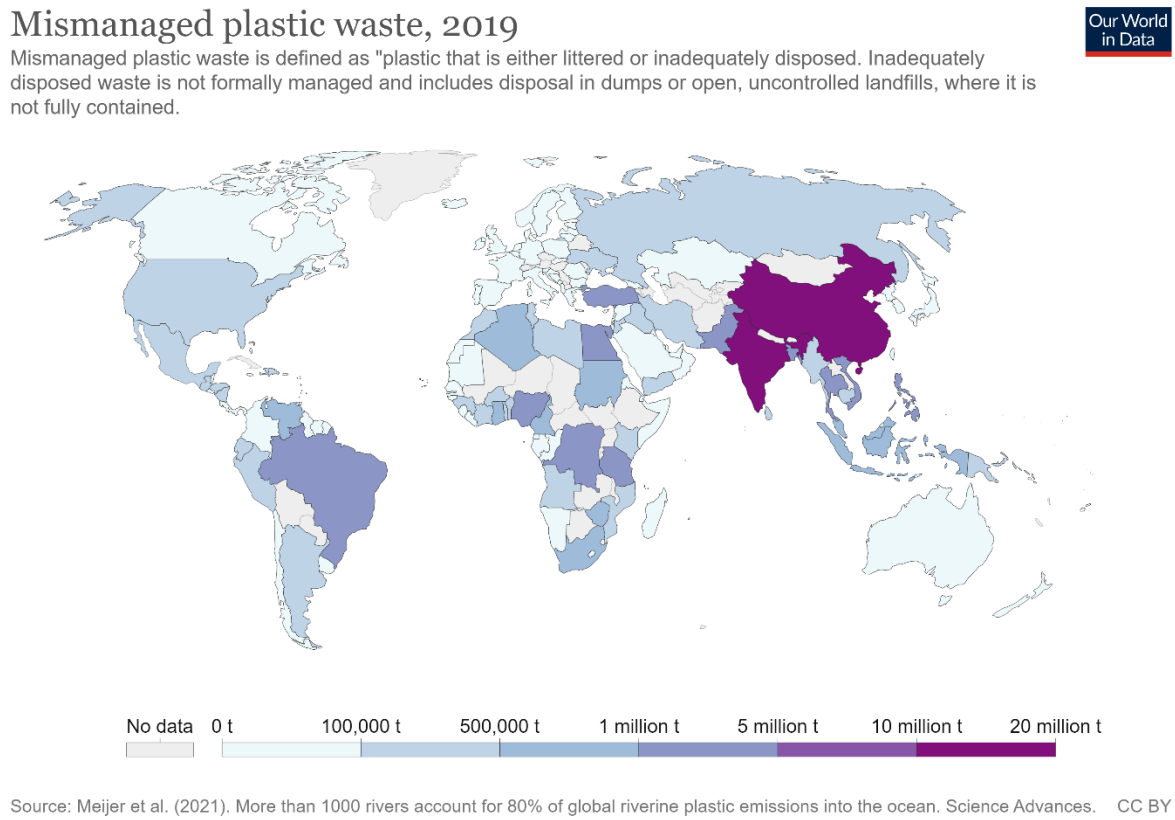


Figure 1.7: The amount of mismanaged plastic waste globally as of 2019 (Meijer et al., 2021).

Corradini et al. (2021) assessed the numbers of microplastics found in agricultural soils in Chile from sewage sludge disposal, finding between 0.6 – 10.4 pieces g^{-1}. In the control site (with no sewage sludge applied), 0.4 pieces g^{-1} were found, whereas the highest counts were found in fields with three sewage sludge applications (0.46 – 3.8 pieces g^{-1}). This work concurred with previous findings by Mahon et al. (2017) that the microplastic counts increased when increased sewage sludge was applied.

Of the European studies, Berg et al. (2020) investigated sewage sludge application as a potential source of pollution in agriculture in Spain. The research sampled agricultural fields with between 0 – 8 sludge applications and the sewage sludge itself. Microplastics were found in 97 % of the analysed soil samples, with the control fields (no sludge application) containing 1.015 pieces g^{-1} ± 0.66 and the soils with sludge application containing an average of 2.595 ± 1.315 pieces g^{-1}.

The vast majority of studies have assessed the levels of MPs in fields with anthropogenic activities, such as sewage sludge and mulching films, which may not be representative of typical farming soils (Harms et al., 2021). Harms et al. (2021) explored the concentrations, distribution, and composition of microplastics in agricultural soils in Northern Germany. The fields were all conventionally managed and primarily used animal-based fertilisers. They found that all 15 sites contained microplastics, with an average number of 3.7 ± 11.9 pieces/kg^{-1}. This is similar to Piehl et al. (2018), who assessed microplastic concentrations in fields in Southern Germany, finding an average of 0.34 ± 0.36 pieces kg^{-1} DW soil. However, it is worth noting that Piehl et al. did not assess plastics smaller than 1 mm due to the labour-intensive analysis of assessing large soil volumes and small fragments of plastic. Limited information exists within the UK; the first study pertaining to UK soils has recently been published by Radford et al, (2023), which found microplastics present in both biosolid treated soils (874 MP/kg) and untreated soils (664 MP/ kg). The research suggested that there was high variability for the amount of microplastics present in the fields and suggested further research is needed to assess microplastic concentrations in soils. Researchers at Lancaster University have recently also begun to investigate the amount of microplastics in agricultural soils in the UK; however, as of current, no research has been published from this study (Cusworth, 2022).

1.6 Sampling of microplastics from soils

As highlighted, few studies consider the extent of microplastic pollution in agricultural soils. This is partly due to the lack of analytical tools to detect microplastics in the soil in situ and the complexity of the soil matrix, making extraction of microplastics from soils difficult (Möller et al., 2020). A requirement for identifying microplastic particles is that they are extracted and isolated from the environmental matrix. This can cause particular challenges in soils, as they are often rich in plant debris and humus, which are difficult to remove (Guo et al., 2020). The simplest method for

microplastic isolation is the sieving and sorting of the soil using stereo microscopy (Möller et al., 2020). However, this method is time-consuming and prone to misidentification bias; thus, underestimation of MPs is likely (Möller et al., 2020).

Manual extraction can be employed to separate microplastics from soils, which is considered one of the simplest methods (Möller et al., 2020). This is considered one of the most common methods for the extraction of microplastics (Huang et al., 2023). This technique requires sieving and manual sorting using a stereomicroscope with which the viewer can exclude mineral or biogenic materials (Möller et al., 2020). Additionally, this method can utilize density separation and the removal of organic matter to aid in the extraction of microplastics (Huang et al., 2023). This technique can be combined with the hot needle test, where the tip of a thin needle is heated, and then the suspected microplastic is touched with the tip of the needle (Cutroneo et al., 2020). If the particle is plastic, it will melt or wrinkle on contact with the needle, providing confirmation of the material but no information regarding the type of polymer (Cutroneo et al., 2020). Other identification methods can be employed alongside stereomicroscopy, such as Fourier transformation infrared spectroscopy (FTIR) and Raman spectroscopy, which can help to aid with identification (Löder & Gerdts, 2015). Manual extraction methods are low-cost but are time and labour extensive, making the method difficult for large sample volumes (Möller et al., 2020). However, manual extraction can be used with a range of other techniques (such as FTIR & Raman), which can help to speed up the processes.

A novel methodology for the extraction of microplastics from soil and sediment is the use of electrostatic separation (Felsing et al., 2018), which is a technique which has been used in the recycling industry to isolate microplastics from sand and sediment samples which remove samples based on the surface charge of the synthetic materials. The method has a high recovery rate, ranging between 90% and 100 %; however, there is a time effort associated with each 150 g sample taking 4 hours to process (Felsing et al., 2018). Additionally, this method cannot be used with moist samples and cannot extract microplastics from soil aggregates (Möller et al., 2020). Electrostatic separation results in a high mass reduction for sand samples (98.9 ± 0.21 %); however, this is lower for sediment samples (69.7 ± 0.1% - 78 ± 6%) (Kurzweg et al., 2022). Kurzweg et al, (2022) noted that factors

such as grain size distribution impacted the recovery rate of MPs, which they suggested could limit the applicability of this method for soil samples, with a density separation step still being required. Additionally, the agglomeration of particulates in soils could further reduce the applicability of this method for soil samples. The methodology still requires further testing to consider recovery rates of MPs from soil media prior to utilizing for field samples. More data is needed regarding the recovery rates of different MP types and soil types before this methodology can be widely used.

Many studies rely on a two-step procedure of removing the mineral fraction and then removing organic matter to isolate the microplastics (Möller et al., 2020). Among these methods, density separation protocols are the most commonly applied in soil sampling, having been pioneered for use in the marine environment. In a study by Thompson et al. (2004), NaCl solution was utilised to separate microplastics from the sand. After mixing and sedimentation of the plastic containing supernatant, the supernatant can be added to a clean flask and filtered. However, NaCl solution can only reach a maximum density of 1.2 g cm^3, meaning that polymers with a higher density, such as PVC and PET, cannot be extracted using this salt solution (Schütze et al., 2022). In addition, acidic and alkaline digestions are frequently reported in the literature to remove the organic fraction of the matrix (Möller et al., 2020). As soils contain a high organic matter content, it is imperative that this step is undertaken to ensure undisturbed analytical analysis (Möller et al., 2020). One of the earliest studies on the extraction of microplastics from soils using density separation and an organic matter removal step was conducted by Hurley et al. (2018). The study conducted a series of tests to remove organic matter from soils and sewage sludge, finding that oxidisation with Fenton's reagent (H_2O_2 + Fe(II)) with a density separation step had the highest recovery rates across eight different common polymer types. This research also suggested that no spectral changes occurred post-treatment using Fourier Transformation Infrared Spectroscopy (FTIR). Hurley et al. (2018) demonstrated that Fenton's reagent could reduce organic matter and provided a cost- and time-efficient methodology. Subsequently, many studies have concurred with these findings suggesting that this method has a high potential for analysing terrestrial samples.

1.7 Analysis of microplastics from soils

After the removal of the microplastics from the background media, it is important to characterise the particle, providing further information on the polymer type and the morphological characteristics (Lusher et al., 2020). Lusher et al. (2020) suggest that individual particles are assessed based on physical and visual characteristics, including particle size, shape, colour and surface characteristics, alongside identifying the microplastic type.

There are three main techniques to identify microplastics after extraction, microscopy (visual), spectroscopy (chemical) and thermal analysis (Woo et al., 2021). Thermal identification enables the identification of polymers based on thermal stability (Woo et al., 2021) and allows the researcher to quantify a large number of samples at once compared to visual and chemical analysis (Zainuddin & Syuhada, 2020). Additionally, samples analysed with thermal analysis can be combined with Gas Chromatography-Mass spectrometry (GC-MS) to provide the total microplastic concentration data by weight, providing the researcher with a quantification of the percentage of microplastics in the sample (Bitter & Lackner, 2021). However, thermal analysis is a destructive technique, meaning that the microplastic particles cannot be characterised unless applied with visual techniques prior to the thermal analysis (Woo et al., 2021). Additionally, information regarding the number, size and shape of the particle cannot be provided with bulk analysis using thermal identification (Woo et al., 2021). Thermal identification methods have proved to have limitations for specific polymers such as PP, PA and PET, with Dümichen et al, (2017) finding that identification of these polymers could not be achieved using this method in water samples from river environments. Further research is needed to consider how any pretreatment steps may affect the identification of microplastics using thermal methods and whether different soil types could affect the identification of polymers (Peñalver et al., 2020).

Chemical analysis can provide information about the specific chemical bonding of the particles and often uses spectroscopic methods such as Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy (Woo et al., 2021). Additionally, combined techniques such as micro-FTIR, which

combines microscopy with FTIR, enable microscopic particle observation prior to spectroscopic confirmation (Morgado et al., 2021). Raman spectroscopy is similar to FTIR in that it combines a non-destructive chemical analysis technique with microscopy (Kniggendorf et al., 2019). However, spectroscopic techniques can be difficult for heavily bio-fouled samples and incur a high initial cost to purchase and maintain the equipment (Woo et al., 2021).

Microscopy is widely used to identify microplastics, with coloured plastics easier to identify with an optical microscope (Dehghani et al., 2017). Light microscopy can be used to identify plastics in the range of several hundred micrometres (Woo et al., 2021). However, as microplastics can be transparent, they can be difficult to identify with light microscopy alone (Löder & Gerdts, 2015). Other microscopy techniques, such as polarized light microscopy (PLM), can be used to successfully identify plastic types for fibres and is a commonly applied technique in forensics (Saadat et al., 2020). Fibres are considered one of the most predominant microplastic types in soils (Lozano et al., 2021). Therefore, microscopy techniques that can help identify this plastic shape are beneficial. Synthetic fibres can be identified by determining the fibres optical properties, and features such as colour, cross-section shape and surface texture can prove useful for further classification (Robertson & Grieve, 1999). The birefringence of a fibre can be used to identify the fibre type; the birefringence of a fibre is estimated from the retardation colours, shown when the polars are crossed, then compared to a Michel-Levy chart (Robertson & Grieve, 1999). The use of PLM to classify synthetic from natural fibres is well established and enables the rapid identification of the polymer type of a fibre (Farah et al., 2015). Thus, using PLM alongside spectroscopic methods to identify films and fragments can provide a high level of information regarding a polymers type and morphological characteristics.

1.8 The effects of microplastics on soil characteristics

1.8.1 The effects on soil physical properties

Various laboratory-based studies have noted changes to soil characteristics due to microplastics in soils (Zhang et al., 2019; Zhao et al., 2021; Gharahi & Zamani-Ahmadmahmoodi, 2022).

Soil physical properties include porosity, soil moisture, and soil structure, which influence microorganisms, soil fauna and terrestrial plants (Bullock, 2005). Research has found that MPs may affect the soils physical, chemical and biological properties and release toxic substances that have been absorbed during the degradation process (De Souza Machado et al., 2018b; Ullah et al., 2021; Qi et al., 2018; Cao et al., 2017; Fu et al., 2021). De Souza et al. (2018) found that polyester fibres significantly decreased water-stable aggregates, which became more pronounced with increasing fibre concentrations. Zhang et al. (2019) found that fibres can entangle soil particles, resulting in clod formation and subsequently impacting the soil structure. Soil structure is related to soil hydraulic properties, soil fertility, thermal properties and soil aeration (Zhang et al., 2021). Soil structure is also a key factor in the migration of soil materials, such as decomposing organic matter and the movement of nutrients (Bronick & Lal, 2005). Lozano et al. (2021) investigated the influence of particle shape on the formation of soil aggregates, finding that fibres, films, foams and fragments all reduce soil aggregation. Fibres were noted to show the greatest effect, which the authors considered could be because fibres are the most different from soil particles in terms of flexibility, size and structure. However, differences have been noted in pot-based experiments and field experiments. In pot-based experiments, there was an increase in macroaggregate formation when polyester fibres were applied; however, this did not occur in the field experiments (Zhang et al., 2019). The authors note that this may be due to differences in the soil textures, which in the pot-based experiment had been sieved at 0.25 mm, allowing fine soil particles to more efficiently contact the polyester fibres. In the field-based experiment, the microfibres adhered to the surface of the macroaggregates instead of becoming entangled within them. The study also found that the contents of water-stable aggregates increase with increasing microfibre concentrations, implying that microfibre contamination can increase aggregation. This is counter to De Souza Machado et al. (2018) study, which found that increasing polyester concentrations decreased water-stable aggregates. This could be due to differences in the soil texture used throughout the studies, with Zhang et al. (2019) using soil with heavy clay content and De Souza Machado et al. (2018) using loamy sand soil. Different observations regarding aggregates are likely due to soil texture, mineralogy, and the capacity of soil particles to interact with microfibres.

Microplastics have also been experimentally demonstrated to affect soil porosity (Meng et al., 2022). Soil pores provide essential habitats for soil fauna and rhizosphere microorganisms (Ramesh et al., 2019). Zhang et al. (2019) found that polyester microfibres in clay soils increase the volume of soil pores greater than 30 μm but decrease the volume of those < 30 μm. The change in porosity will subsequently impact the distribution and movement of soil fauna. Soil porosity also determines soil aeration and water flow, which can alter the abundance of microorganisms and the root uptake of water and nutrients. Lozano et al. (2021) found that foams and fragments increase the microporosity of soils. Differing MP shapes are likely to have varying effects, with spheres potentially occupying pore spare this decreasing pore volume. The addition of organic materials can increase soil granulation and increase the proportion of macropores in soils (Yazdanpanah et al., 2016). Zhang et al, (2019) found that polyester microfibres can entangle particles, increasing clods and soil macropores, potentially acting like natural fibrous material in soils. Thus, it is likely, that microplastics with similar shapes to natural particles could increase the porosity of soils. Further research is required to consider how microplastics influence porosity in different soil types, as the distribution of sand, silt and clay determines soil porosity.

Soil bulk density is an important index of soil fertility and is related to soil quality, plant rooting, and porosity (Negassa et al., 2015). Microplastics generally have lower densities than soil particles; thus, the accumulation of MPs will decrease soil bulk density (De Souza Machado et al., 2018). De Souza Machado et al. (2018) found that polyacrylic, polyamide, polyester and polyethylene all impact soil bulk density resulting in a decrease in the bulk density. It was also found that the polyester fibres could change the water holding capacity, which could have impacts on the soil moisture and evapotranspiration. De Souza Machado et al. (2019) found that soil bulk density was reduced when PE, PET, PP and PS fragments were added to soils, and when polyester fibres were added, but were not changed by polyamide beads. This could be due to beads being more similar to natural soil particles, thus fibrous microplastics would be more likely to affect soil physical properties. Zhang et

al. (2019) found that polyester microfibres caused no changes on bulk density using similar concentrations to De Souza Machado et al. (2019), and the authors considered that the low concentrations used were unlikely to affect the bulk density.

Soil water is another critical factor in soil health, as it determines the availability of nutrients and pollutants (Bünemann et al., 2018). Additionally, soil water content influences the survivability and reproduction of plants and soil organisms (Lowery et al., 2015). Microplastics can change water-holding capacity due to their hydrophobic surfaces and the impacts listed previously on the soil's physical structure (Sajjad et al., 2022). De Souza Machado et al. (2018) found that polyester microfibres increased soil water-holding capacities. This was also noted by Lozano and Rillig (2020), who found that polyester microfibres improved water retention. Changes to water holding capacity can affect the soil moisture content, which changes water availability. De Souza Machado (2019) found that polyester fibres and polyamide beads increase evaporation by 50 % and 35 %, respectively. However, the authors noted that the increases in evaporation were smaller than the increase in water-holding capacity for the polyester fibres, which resulted in higher availability in the soils with plastic fibres. Microfibres may benefit crop productivity by improving water-holding capacities; however, this may be negated by changes to evapotranspiration (De Souza Machado et al., 2019). Changes to soil physical properties are likely to have effects on soil chemistry and subsequent effects to flora and fauna.

1.8.2 The effects of microplastics on soil chemical properties

The chemical properties of soil are critical for soil fertility, plant growth and reproduction (Arévalo-Gardini et al., 2015). The chemical properties of soil include pH, total carbon, nitrogen, and phosphorus and are influenced by organic matter (Ahmadpour et al., 2015). Increased soil nutrient availability can improve plant health and increase yield potential (Morgan & Connolly, 2013). Soil pH is a major abiotic factor which determines the binding capacity and bioavailability of nutrients, the

total soil organic carbon and the binding capacity of minerals (Neina, 2019). Research has confirmed that MPs can increase soil pH, with Yang et al. (2021) finding that High-density polyethylene (HDPE) and polylactic acid (PLA) both increased soil pH. Qi et al. (2020) found that LDPE and biodegradable plastics increased soil pH after two and four months; however, similar changes were also demonstrated in the control treatments. Varied effects are noted across studies; where the four studies mentioned above-found increases in the soil pH, other studies have found that MPs decrease or cause no changes. Boots et al. (2019) compared changes in soil pH after 30 days of exposure to PLA and HDPE MPs and clothing fibres, finding that HDPE significantly reduced pH but that PLA and clothing fibres caused no significant effects. It is considered that the degradation of the MPs may cause the release of chemical compounds contained within the MPs, which subsequently can alter pH. This may also help to explain varied responses, as different soil types, environmental conditions, and the presence of microbial and fungal species may influence the degradation.

Soil nutrients are derived from soil minerals and the decomposition of organic matter in soils (Hoffland et al., 2020). Dong et al. (2021) found that polytetrafluoroethylene (PTFE) and polystyrene MPs reduced soil available nitrogen and phosphorous contents. Lozano et al. (2021) found that microplastics reduced NO_3 – N leaching by 70 %, demonstrating a positive impact of microfibre enabling soils to retain nutrients, likely due to increased soil aggregation. Yan et al. (2021) found that the content of available phosphorus was increased by di(2-ethylhexyl) phthalate (DEHP) plasticised PVC but not increased by unplasticised PVC MPs, suggesting that additives can influence phosphorus solubility and mineralisation. The varied impacts on soil nutrients could be due to the complex chemical composition of polymers, with some additives contained within MPs containing phosphorus, nitrogen (Polyaramide) and chloride (PVC) (Sridharan et al., 2022).

Additionally, as plastics degrade, they have the ability to absorb nutrients, therefore changing the availability of these in soil (Rillig, 2018). The more aged the particle is, the higher its absorption capacity (Mao et al. 2020); therefore, the more nutrients can adhere to it. As the absorption capacity increases, MPs can also absorb metals such as Cu^{2+}, MPs with differently charged surfaces due to

electrostatic interactions may lead to absorbing negatively or positively charged nutrients (Azeem et al., 2021). Additionally, the availability of soil nutrients is largely mediated by microbial communities and mycorrhizal fungi; thus, any changes to these communities related to MPs are likely to result in changes to the soil nutrients (Moreno-Jiménez et al., 2022). Finally, microplastics may affect soil nutrients through the alteration of the physicochemical properties listed above, such as aeration or aggregation (Abel De Souza Machado et al., 2019). Aggregation is likely to help with the retention of soil nutrients, and changes in soil porosity and aeration may enhance oxygen diffusion, facilitating processes such as ammonia oxidation (which helps to control the availability of nitrogen for plants and microbes (Trivedi et al., 2019)) (Mangalassery et al., 2013).

Soil organic matter (SOM) is an important component of soil, playing key roles in plant nutrition and microbial activity (Jacoby et al., 2017). Liu et al. (2021) found that adding polyethylene mulch films influenced soil organic carbon (SOC), but more pronounced effects were demonstrated under alkaline conditions as opposed to acidic conditions. There was a dose-dependent response, with higher reductions in SOC with the increase of MP concentrations. Studies have also investigated the effects of MPs on SOM, focusing on dissolved organic matter (DOM). DOM is important for the transportation of nitrogen and phosphorus; Yu et al. (2020) found that polyethylene decreased the content of dissolved organic carbon (DOC). Liu et al. (2017) found that soils treated with 7 % w/w polypropylene microplastics decreased the decomposition rates of DOM. The authors found that phenol oxidase activities decreased in the microplastic treatment, which slowed the decomposition of humic-like materials when microplastics were added, changing the decomposition rate of DOM. When treated with 28 % w/w MPs, total dissolved nitrogen was increased, along with dissolved organic nitrogen, total dissolved phosphorus and dissolved organic phosphorous. This suggests that MPs may result in the release of soil nutrients and the accumulation of carbon, nitrogen and phosphorus (Liu et al., 2017). Rillig et al. (2021) discussed that MPs entering the soil system could act as organic carbon. Soil bacteria may have the ability to convert microplastics, especially biodegradable plastics, into soil-soluble carbon, which helps to explain why most studies find

increases in dissolved carbon concentrations in soils with a dose-dependent response. Chen et al. (2020) found that adding 2 % PLA biodegradable plastics increased soil DOC concentration. However, Ren et al. (2020) found that 5 % w/w PE MPs did not influence soil DOC. These differences could be more noticeable in biodegradable plastics, as they will likely become bioavailable to microbes in the study periods. Considering the roles of SOM in plant growth, an understanding of the response of plants to MP addition is imperative.

Another key consideration for soil chemistry is the potential of MPs to adsorb and desorb soil pollutants (Sajjad et al., 2022; Iqbal et al., 2023). MPs can affect the bioavailability, bioaccumulation, and toxicity of different organic pollutants (Khan et al., 2021). The complex chemical nature of MPs means that some additives contained within them could be released into the soil during ageing. For example, plastics may contain Polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH), and flame retardants (particularly brominated flame retardants (BRFs)) which may be released into the soil (Sajjad et al., 2022). Due to their small size and surface features, microplastics have an increased sorption capacity for organic and inorganic compounds, facilitating the transport of environmental pollutants (Joo et al., 2021). Studies have confirmed the capacity of MPs to absorb heavy metals such as cadmium, zinc, copper, nickel and lead (Zong et al., 2021; Munier & Bendell, 2018; Liu et al., 2021; Davranche et al., 2019) and act as carriers for heavy metals (Liu et al., 2021). Different polymer types, however, have different carrying capacities, with PE and PVC MPs having a high affinity to lead, chromium and zinc; however, PET showed little absorption capacity for these compounds (Brennecke et al., 2016). Research has indicated that lead has a strong electrostatic interaction with MPs, which leads to stronger absorption (Liu et al., 2021). The ageing of plastics also increased the capacity of MPs to sorb heavy metals (Wang et al., 2021; Lang et al., 2020).

Conversely, research has suggested that PE MPs can transform heavy metals from bioavailable forms to organic bound forms, decreasing their bioavailability through indirect changes to soil properties, such as changes to the pH (Yu et al., 2021a). The soil pH can affect the chemical specification of heavy metals, changing their adsorption-surface stability and adsorption positions (Yu et al., 2021a).

Li et al. (2020) found that biodegradable plastic such as poly(butylene adipate-co-terephthalate) (PBAT) degraded faster and adsorbed more heavy metals compared to PE films, which is likely due to changes to the surface during the MPs degradation. MPs and toxic metals can produce combined toxicity, which influences soil microbiota and plant development (discussed in 1.9).

As well as heavy metals, microplastics may adsorb organic pollutants; MPs have hydrophobic surfaces and therefore have strong adsorption for organic pollutants such as pesticides and antibiotics (Fu et al., 2021). As with heavy metals, the properties of the MP, such as polymer type, structure and surface properties, alongside environmental conditions, influence the absorption of organic contamination (Joo et al., 2021). Wang et al. (2020) investigated the adsorption behaviour of five pesticides (carbendazim, trichlorfon, diflubenzuron, malathion and difenoconazole) on polyethylene films in soils, finding that MPs reached equilibrium within 120 minutes. This research indicates that pesticides could be transported via microplastics, and the authors state that MPs could cause an environmental pollution risk in agricultural fields. Huffer et al. (2019) evaluated the effects of PE MPs on the adsorption of atrazine and 4-(2,4-dichlorophenoxy) butyric acid in the soil, finding that 10 % w/w NP concentrations reduced the overall adsorption within soils. This suggests that MPs' presence may enhance organic pollutants' mobility. Studies by Ding et al. (2020) found that PAHs contained in tyre tread particles can be released into the soil and could ultimately impact *Enchytracus crypticus* (common pot worms). MPs can act as vectors of pollutants within soils, altering their bioavailability.

1.9 Microplastics and their effects on plant development

There is limited information on microplastics' impact on terrestrial plants (De Souza Machado et al., 2018) (see table 1.1 for plant species used in microplastic studies). As microplastics are ubiquitous in terrestrial soils, it is critical to understand the toxicological impacts that MPs could have on terrestrial plants (Yu et al., 2021b). Several mechanisms have been considered to understand microplastics' effects on plant germination and development.

Microplastics can be considered a physical soil contaminant, with research by De Souza Machado et al. (2018) finding that microfibres lowered soil bulk density and increased evapotranspiration. This could result in changes to root development, with increased evapotranspiration resulting in soil drying, which could negatively impact plant performance. In addition, lowered soil bulk density could increase root length due to better soil aeration, and a lower soil bulk density could reduce penetration resistance for plant roots. This is supported by Machado et al. (2018) research, who found that PES and PS triggered increases in root biomass, with longer and finer roots being demonstrated in *Allium fistuolsum* (spring onion). The authors suggested that decreases in soil bulk density resulted in increased root biomass, with PES demonstrating a 40 % increase in root biomass. Boots et al. (2019) found similar results, with the root biomass of *Lolium perenne* (perennial rye grass) being increased in the presence of HDPE.

Table 1-1 Plant species tested with exposure to microplastics – modified from Li et al. (2022). PP *polypropylene,* PS *polystyrene,* PE *polyethylene,* PVC *polyvinyl chloride,* PA *polyamide,* PTFE *polytetrafluoroethylene,* PLA *polylactic acid,* LDPE *low-density polyethylene plastics,* HDPE *high-density polyethylene plastics,* PES *polyether sulfone,* PET *polyethylene terephthalate,* PU and PUR *polyurethane,* PC *polycarbonate,* PMMA *polymethyl methacrylate,* PCF *plastic clad fibre,* PBAT *polybutyleneadipate*-co-*terephthalate*

Microplastic type	Microplastic shape	Plant species	Exposure time	References
PS, PTFE	Fragment	*Oryza sativa*	10 days	Dong et al., 2022
PE, PLA	Fragment	*Zea mays*	30 days	Wang et al., 2020
PE	Sphere		10 days, 15 days	Urbina et al., 2020
PE	Sphere	*Lactuca sativa*	14, 28 days	Gao et al., 2019
PVC	Fragment		21 days	Li et al., 2020a
PS	Sphere		28 days	Gao et al., 2021
PS	Fragment	*Vicia faba*	2 days	Jiang et al., 2019
LDPE, Biodegradable plastic	Fragment	*Triticum aestivum*	139 days	Qi et al., 2018 Lian et al., 2020
PS	Sphere		21 days	Zong et al., 2021
PS	Fragment		8 days	Guo et al., 2022
PE	Fragment		43 days	
PES, PA, PP, LDPE, PET, PU, PS, PC	Fibre, film, foam and fragment	*Daucus carota*	28 days	Lozano et al., 2021

PES	Fibre	*Festuca brevipila, Holcus lanatus, Calamagrostis epigejos, Achillea millefolium, Hieracium pilosella, Plantago lanceolata,* and *Potentilla argentea*	60 days	Zhao et al., 2021a
PS	Sphere	*Allium cepa*	3 days	Maity et al., 2020
PA, PES, HDPE, PP, PS, PET	Fibre & fragment	*Allium fistulosum*	30 days	Abel De Souza Machado et al., 2019
PS **PS**	Sphere Sphere	*Cucumis sativus*	65 days 21 days	Li et al., 2021 Li et al., 2020c
HDPE, PLA	Fragment	*Lolium perenne*	30 days	Boots et al., 2019
PE, PVC, PP	Fragment	*Lepidium sativum*	6 days, 21 days	Pignattelli et al., 2020

PS, PMMA	Sphere	*Hordeum vulgare*	14 days	Li et al., 2021a
PS	Sphere	*Arabidopsis thaliana, Triticum aestivum*	5 days, 12 days	Taylor et al., 2020
PP, PE, PVC, PET	Fragment	*Cucurbita pepo*	28 days	Colzi et al., 2022
PE	Fragment	*Brassica napus*	60 days	Jia et al., 2022
LDPE, PLA, PBAT	Fragment	*Phaseolus vulgaris*	105 days	Meng et al., 2021
PS	Sphere	*Glycine max*	30 days	Xu et al., 2021

Changes to soil structure will also have subsequent effects on the soil microbial community composition, which has functional consequences. This was demonstrated by Qi et al. (2022), who investigated the impacts of plastic mulch films on soil microbial communities. This research found significant changes to the rhizosphere, with Rhizoctonia and Arthrobotrys significantly enriched due to the biodegradable polymers. Rhizoctonia and Athrobotrys are facultative plant pathogens, which can lead to various crop diseases such as Rhizoctonia root rot in commercially important plants (such as Nicotiana tabacum – tobacco) (Gonzalez et al., 2011). Thus, incorporating biodegradable polymers into soils could result in increased habitat for fungal pathogens, causing disease for commercially important crop species. Research by Fei et al. (2020) also considered the impacts of MP exposure on soil microbial communities, reviewing the impacts of PE and PVC MPs in soils. They found that the relative abundance of the nitrogen-fixing bacteria Burkholderiaceae was significantly increased in the presence of PE but not in the presence of PVC, which could benefit plant development for some species due to the increase of nitrogen.

Additionally, there was a significant decline in *Sphingomonadaceae* and *Xanthobacteraceae* in both groups, which may result in an inhibition to degrade xenobiotics in soil, leading to increased toxicological effects. Sun et al. (2022) found no significant difference between the microplastic and control treatments when considering species diversity. The authors did find differences in the bacterial community composition, finding a dose dependant response and that abundances of *Gemmatimonadetes* and *Bacteroidetes* were increased with PE and PP treatments. These species are involved in the cycling of soil elements and the decomposition of cellulose. This could subsequently have adverse effects on the degradation of complex organics. Additionally, Sun et al. (2022) found that proteobacteria were less abundant when microplastic particles were added than when fibres, film or foam were added. This may be due to some species of Proteobacteria's ability to colonise low bulk-density soils. *Firmicutes* were enriched in fibre and foam treatments and are considered important chitinolytic bacteria in soils (Wieczorek 2019). This research suggested that different polymers, shapes and types of microplastics can cause shifts in the bacterial community composition. Changes in microbial community composition are likely to result in changes to plant development, but research

indicates that this is polymer specific and shape-specific, which means that in real-world systems, the impacts are difficult to understand due to the mixture of shapes and polymer types found.

Another mechanism that microplastics have been considered to alter is soil nutrients. Rillig (2018) discusses how plastic particles have a high content of carbon, which will be slowly degraded over time, changing the C: N ratio in soils, leading to microbial immobilisation due to the priming effect (Bernard et al., 2022). Qi et al. (2022) found that LDPE microplastics altered the soil's micronutrient content, with the Cu content increasing compared to the other treatments. This could have adverse effects on the development of plants, with Qi et al. (2018) finding plant performance indicators such as leaf area decreased in the presence of biodegradable plastic residues. Although nutrient content was not measured in this study, the authors suggested that this could be the result of microbial immobilisation, which has subsequent effects on the nutrient content of soils.

Microplastics have been demonstrated to impact plants at various stages of development. Perhaps the most important development phase is germination; it is also when plants are most susceptible to stress (Wolny et al., 2018). Germination begins with the rapid imbibition of water, which activates the seeds metabolic response (Sen & Puthur, 2020). Research has suggested that micro and nanoplastics can reduce the germination rates of plants, though the mechanisms for this are currently unknown. Bosker et al. (2019) reviewed the impacts of nanosized PVC particles on the germination of *Lepidium sativum* (Cress), finding that the seed germination rate was reduced for three different size fractions of plastic (50, 500 and 4800 nm) but that the effect was short-lived, with all eventually reaching 100 % germination. The authors suggested a physical blocking response, where the plastics blocked the seed pores, reduced imbibition, slowing germination, however this is likely only applicable for plastics in the nanoplastic range. Pflugmacher et al. (2020) found that polycarbonate leachates resulted in reduced germination rates finding that germination was inhibited by 20 % in a 1:10 dilution of

leachate in *Lepidium sativum* (Cress). They also found that germination was inhibited in the presence of nanoplastic granules and demonstrated a dose dependent response, with 1 % particle addition resulting in a 27 % reduction and a 10 % concentration resulting in a 55 % reduction in germination compared to the untreated controls. This research suggested that microplastic leachates and particles can result in a reduction in germination success, though it was unclear in this research whether this was due to the physical changes to the soil matrix or due to chemical changes as a result of microplastic addition. Wang et al. (2021) tested the effects of polyethylene microplastics on the germination of *Glycine max* (soybean) and *Vigna radiata* (mung bean), they found no significant differences in germination rate for *Vigna radiata* (mung beans) but found that the mean germination speed for *Glycine max* (soybeans) was longer in those treated with the PE MPs. Varied responses have been noted throughout the literature, with plant species responding in different ways as a result of microplastic addition in soils, though as of current, explanations for this have been limited.

Subsequent effects have been noted on plant biophysical development; however, most works conducted relate to aquatic plants. Colzi et al. (2022) reviewed the effects of PP, PE, PVC and PET on *Cucurbita pepo* (Field Pumpkin); this research found that all MP types impaired root and shoot growth, finding that pigment content, photosynthetic efficiency and leaf size were reduced. Wu et al. (2020) assessed the impacts of polyester microplastics on *Oryza sativa* (rice) using a metabolomic approach. This research found that after 21 days of growth, there was a significant difference in the biomass and shoot lengths of the PS-treated plants and the control plants but no significant differences in the root length and biomass. At a concentration of 0.005 % w/w, shoot lengths were reduced by 12 % and at 0.25 % w/w by 21 % compared to the control. At the highest concentration, 0.5 % shoot length was reduced by 27 %.

Hernandez-Arenas et al. (2021) reviewed the effects of the application of sewage sludge on *Lycopersicon esculentum* (tomato). The research reviewed the levels of microplastics in sewage sludge, assessed the biomass and length of the shoot and root of *Lycopersicon esculentum (*tomato) and then reviewed fruit production. The research found that in low concentrations, plant growth was enhanced; however, at high concentrations, there was a reduction in plant biomass. Wang et al. (2021)

reviewed the effects of MPs on the growth and development of *Glycine max* (soybean) and *Vigna radiata* (mung beans), finding that the *Glycine max* (soybean) seedlings had a longer root length than the control treatment, but no adverse effects were demonstrated in the *Vigna radiata* (mung beans) seedlings. The elongation of the root is likely due to the changes to the soil bulk density, as speculated by Machado et al (2019). Qi et al. (2018) assessed the impacts of biodegradable mulch films and LDPE mulch films on *Triticum aestivum* (wheat) development, finding that the biodegradable mulch films inhibited plant growth, with no apparent effects demonstrated from the LDPE films until the stem extension stage. The LDPE microplastic treatment had the lowest plant height, though this was not significant compared to the control. They found that tillering was delayed in the biodegradable plastic treatments by two weeks; however, the number of fruits produced for either the biodegradable plastic or the LDPE plastic was not significantly different to the control. Liu et al. (2021) also assessed the toxicity of MPs on *Triticum aestivum* (wheat), finding that PE MPs stimulated root elongation but inhibited the shoot weight of the *T. aestivum* (wheat) at high concentrations (8 % w/w). Various effects have been demonstrated across different crop species; however, limited research has focused on seed and fruit yield, which is arguably the most important factor for farmers due to the economic implications of reduced yield. In addition, limited studies have focused on terrestrial plants, and thus there is a need to increase the understanding of the potential implications for MPs on agricultural crop development

1.10 Microplastics and soil organisms

Soil organisms are an integral part of the soil ecosystem, playing an important part in nutrient cycling and energy flow (Jacoby et al., 2017; Bach et al., 2020). MPs have been noted to cause various impacts on soil fauna, from direct toxicity from ingestion or the ability to cause surface damage to fauna (Lin et al., 2020; Tian et al., 2022). Studies have confirmed that MPs can be ingested by soil fauna (Sajjad et al., 2022), including earthworms which are noted as key ecosystem engineers (Le Bayon et al., 2017). Due to the low degradation rates of MPs, bioaccumulation of MPs would likely occur through the ingestion of microplastics. Lei et al. (2018) analysed the distribution of MPs in *Caenorhabditis elegans* (Roundworm) in body tissues after exposure to fluorescent MPs and found

that after 48 hours, MPs could be found in all segments of the nematode. Research has also noted weight loss and decreased growth rates in *Lumbricus terrestris* (Common earthworm) in high concentrations (28 %, 45 % and 60 % w/w) (Huerta Lwanga et al., 2016). Mortality was increased in the 28 and 60 % treatments, but this was not demonstrated in the lower concentrations. Boots et al. (2019) found no mortalities in more environmentally realistic concentrations (0.2 – 1.2 % w/w) on *Aporrectodea rosea* (rose-tipped earthworm) but did note significant differences in the weight of earthworms in the MP treatment. An average increase of 5.7 ± 3.1 % was demonstrated in the control group, compared to a 3.1 ± 1.1 % decrease in biomass in the MP treatments. The authors suggested that ingestion was likely, though this was not measured during the study and further considered that MPs may result in the obstruction and abrasion of the digestive tract, subsequently, limiting the bioavailability and absorption of nutrients.

Aside from the impacts demonstrated on earthworms, research has also found adverse effects on Lobella (springtails) (Kim & An, 2019), which play a vital role in the cycling of nutrients and formation of the soil microstructure. Kim & An (2019) found that MPs moved into soil pores which subsequently immobilised springtails, which the authors considered could lead to high mortality rates. This occurred in extremely low concentrations of MPs (10 mg/L) and thus is likely to occur in actual soil environments. Research by Selonen et al. (2020) assessed the effects of polyester textile fibres at concentrations ranging between 0.02 % to 1.5 % w/w on various soil invertebrates (*Enchytraeus crypticus*; pot worms, *Folsomina candida*; springtails, *Porcellio scaber*; isopods, *Oppia nitens*; Oribatid mites). All invertebrates were exposed to one of two fibre lengths, long fibres (4-24 mm) and short fibres (0.012 mm – 2.87 mm); however, the authors note the effect of polyester fibres on soil invertebrates was slight. Noticeable effects included up to a 30 % decrease in reproduction when enchytraeids were exposed to long polyester fibres; however, no noticeable effects when exposed to short plastics. Isopods and enchytraeids ingested short fibres, with the ingestion rate correlating to the fibres' concentration. The authors note that in the short term, the plastics presented little harm to soil invertebrates; however, long-lasting, multigenerational studies are needed to assess the long-term risk

of plastics in soils. Soil fauna are crucial for healthy soils; thus, adverse effects on the fauna are likely to have subsequent effects on plant development.

Further understanding is needed of the effects and amounts of microplastics in terrestrial ecosystems, specifically, this review has identified knowledge gaps pertaining to the impact of microplastics on terrestrial plants. For example, in the UK, only one study has quantified the concentrations of microplastics in fields with sewage sludge applied, but further research is needed to understand the quantities of microplastics across a range of agricultural field types (grazing and arable, with and without sewage sludge additions). In addition, this research could help with further toxicological studies by determining the types and sizes of microplastics currently found in agricultural landscapes.

There is also limited understanding as to how microplastics are causing an impact on germination rates and the subsequent development of plants. Therefore, research is needed to understand the mechanisms by which these pollutants are causing changes to germination rates and subsequent plant development. Additionally, in the agricultural system, the yield of crops is of key importance; if effects are demonstrated in the early stages of plant development, it is important to understand whether these impacts are subsequently occurring in the reproduction and fruiting of crop species as this is the economically important part of some crop species. As such, this PhD thesis will undertake studies to understand microplastics in agricultural landscapes and the subsequent impacts to crop development.

1.11 Aims and objectives

Microplastics have been recognised for over a decade as rapidly emerging environmental pollutants. However, limited research has been conducted on terrestrial environments, specifically in agricultural soils that are likely to act as sinks for microplastics. Therefore, this study aims to advance research into the understanding of microplastics in UK agricultural soils and assess the risks that MPs may pose to crops, considering germination and further development. This study also aims to advance the understanding of how microplastics impact terrestrial plants, shedding light on the potential mechanisms that result in plant development changes.

Firstly, proposed methods were evaluated to assess their applicability to retrieve MPs from soils (Chapter 2). Secondly, samples were collected from field studies to assess levels of plastics in typical agricultural soils in the Midlands of England; this enabled an understanding of the common polymers and the concentrations found (chapter 3). Then laboratory studies were used to assess the impacts of a common microplastic (as determined in chapter 3) on terrestrial plant development. Next, germination and seedling development were investigated using polyester fibres (as these were determined to be the most common type and shape of polymer after studies conducted in chapter 3) as soil pollutants (chapter 4) and leachate-based studies (chapter 5); this enabled an understanding of physical and chemical effects on plant development. Finally, *Sinapis alba* (mustard) was grown through its entire lifecycle to assess whether any impacts were demonstrated on seed yield.

Thus, this PhD aims to assess the following:

1. A method will be created to enable the extraction of microplastics from soils, which can be used to sample soils from UK farmlands.

2. Assess the types and numbers of microplastics found in sampled UK agricultural soils.

3. Generate data to assess any impacts on the germination phase from the addition of polyester microfibres; this enables an understanding of whether microplastics act as a plant stressor in the early stages of development.

4. Elucidate the potential mechanisms of any impacts on germination to understand further what may cause any changes demonstrated.

5. To review whether impacts demonstrated to plant development are localised at the germination phase or whether impacts are shown in plant reproduction and seed development.

Chapter 2 Method development for the extraction of microplastics from soils.

2.1 Introduction

Soils and sediments represent a challenge for microplastic analysis, as particles must be separated from the soil matrix to allow characterisation and quantification (Möller et al., 2020). Due to this, removing the bulk of the sample matrix is usually necessary to isolate the particles and remove any adhering substances (Junhao et al., 2021). Furthermore, as soils are a heterogenous matrix consisting of minerals, organic matter, gases and liquids, isolation can be more challenging due to removing multiple classes of materials (Peller et al., 2022). Additionally, soils can form relatively stable aggregates that may enclose microplastic particles, making them more challenging to isolate (Dong et al., 2022).

Various methods have been proposed to remove plastics from soils; the most common method relies on a two-step technique where plastics are density separated to remove them from the bulk of the soil and digestion to remove any soil organic matter (SOM) (Nava & Leoni, 2021; Perez et al., 2022). Density separation involves placing materials of different densities into a liquid of an intermediate density. The less-dense materials will float, and the denser materials will sink. Historically NaCl has been utilised as a density separation technique, with a specific gravity of 1.2 g/cm$^{3;}$ however, this methodology has limitations (Catarino et al., 2017). One of the main limitations of density separations using NaCl is that this liquid is often less dense than the polymers to be extracted. Many plastics have a density of >1.2 g/cm^3, such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET), which make up 17 % of the global demand for plastics (Quinn et al., 2017). Thus, utilising any liquid with a density of less than 1.4 g/cm^3 will likely lead to an underestimation of the total MP particles within the sample (Harris, 2020). This is particularly important as denser plastic particles are less likely to be moved around environmental compartments due to natural processes (Pathan et al., 2020), such as atmospheric deposition. Higher-density plastics are more likely to be retained in soils than lower-density plastics, which are likely to be transported through aeolian transport mechanisms (Muthuvairavasamy, 2022).

It is vital to consider plastic densities prior to selecting chemicals that allow the floatation of all plastic types. Of the five most common plastics used (PET, HDPE, PVC, LDPE and PP), the highest density found is that of PVC and PET, being 1.38 g/cm$^{3;}$ therefore, the treatment used to float out these plastics must be greater than 1.38 g/cm^3. Research by Quinn et al. (2017) compared different solutions for the extraction of microplastics, using sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide (NaI) and zinc bromide ($ZnBr_2$). Their research indicated that $ZnBr_2$ and NaI (both of which were diluted to have densities of 1.7 g/cm^3) had significantly higher recovery rates than the other chemicals tested. In addition, $ZnBr_2$, with a density of 1.7 g/cm^3, gave the highest recovery rates for eight of the twelve plastic types tested, providing a 99 % recovery rate in a mixed microplastic sample, which is more representative of an environmental sample.

The second step in microplastic recovery involves the removal of SOM, as it often has similar densities (1 – 1.4 g/cm^3) to that of the MP particles, which may make subsequent recovery more complex. Therefore, it is important to remove SOM to aid in analysis; this is generally achieved using acid digestion; however, this approach has the potential to damage the polymer particles, which can make subsequent analysis more difficult (Lusher et al., 2017a). As such, it is imperative that methods used to digest the SOM are investigated for their potentially deleterious effects on the MPs. Various suggestions have been made in the literature to remove SOM from samples, including; NaOH, Fenton's reagent (H_2O_2 + Fe(II)), H_2SO_4, HCl and H_2O_2, due to either their use in digestions for biota or their use for removal of SOM in other soil testing procedures (Cole et al., 2011; Hurley et al., 2018; Scheurer & Bigalke, 2018; Bläsing & Amelung, 2018).

At the time of this study (2018), although a few studies had been conducted which investigated MP extraction from soils, there was limited information on the effect of these different processes on the MPs and information regarding recovery rates. Therefore, this study assessed the recovery rates of MPs (< 5 mm) (prepared PVC, PP and PET from consumer products) from commercial topsoil using various digestion techniques with the aim of suggesting an appropriate method for both high and consistent recovery rates for field sampling.

When this research was conducted, few studies were available that discussed microplastics' recovery rates from complex media such as soil. As such, this research aimed to review methods suggested in the literature to extract MPs from complex media. Subsequently to the research being conducted, more literature has become available that reviews this topic, concurring with the results of this study. The following paragraphs will detail research that was available prior to this study (conducted between November 2018 and January 2019) and subsequently will detail research that has become available since the completion of the study.

Nuelle et al. (2014) tested H_2O_2 as a digestion solution for the removal of SOM from marine sediment samples, and their research suggested that to remove 92 % of the organic matter from the samples, one week of treatment in 35 % H_2O_2 solution was appropriate. Although this research reports a good reduction in organic matter, it does limit sample numbers due to the long processing time. It has been suggested that Iron II could be used to act as a catalyst to speed up the reaction with H_2O_2 (also known as Fenton's reagent) (Walling, 1975), and as such, this may improve the processing time significantly, suggesting that Fenton's reagent may be an applicable method for the extraction of microplastics from soils.

Hurley et al. (2018) investigated the use of H_2O_2, Fenton's reagent, NaOH and KOH to recover microplastics from complex media. The research concluded that Fenton's reagent was the most suitable treatment for those tested when removing microplastics from complex media, as it caused little degradation to the polymer and removed sufficient amounts of SOM to recover the plastics. Furthermore, this research suggested the efficacy of between 86 % to 100 % depending on the size fraction and shape of the plastic material used with Fenton's reagent, indicating that this technique may be applicable as a method for the extraction of microplastics.

Herrera et al. (2018) tested five different digestion protocols on marine vegetal-rich samples to assess the suitability of these treatments for the extraction of microplastics. This research tested the following protocols: 3 % HCl, 40 % NaOH, 4 % NaOH + SDS, 10 % KOH and a catalytic 30 % H_2O_2 (Fenton's reagent – H_2O_2 with a catalyst of 0.05 M Fe(II)). Each treatment was tested on six different polymers (PP, PE, PVC, PUR, PET and PS). In addition, the researchers assessed whether the particles were damaged or degraded and noted differences in sample weight before and after the treatments. This research noted that the 3 % HCl, 40 % NaOH, and 10 % KOH did not entirely remove the biological material, but 4 % NaOH + SDS and Fenton's reagent partially digested the biological material. Also, it was noted that the NaOH (40 %) damaged the polyester fibres, causing bleaching. It may be that the length of the NaOH treatment (24 hours) could be shortened, and as such, damage to the particles would be limited, though this may impact the efficacy of the method for the removal of organic matter.

Zhang et al. (2018) investigated the recovery rates of low-density microplastics (PP and LDPE) from soils and utilised H_2O to density separate the SOM and plastics. The SOM and plastic mixture were then filtered, dried and subsequently analysed using microscopy to confirm the presence of plastics. The SOM and plastic mixture were heated to 130 °C to remove impurities; however, this also led to changes in the polymers used, changing them from irregular particles to circular transparent particles. These particles could be easily identified against irregular materials such as organic matter and silicates. The authors noted that this method had a 90 % extraction efficacy and concluded that the microplastics were easily distinguishable from the impurities. Although this method is an interesting approach, it is worth considering that the thermal changes to the plastics may pose difficulties for later analysis, especially in field samples where a multitude of different polymer types may be present. Additionally, this method would not enable further characterisation of the plastics present (such as the shape or colour of the polymer), which means it is then challenging to consider the sources of MPs and gives no further information which could be beneficial for the reduction of microplastics into soils.

Li et al. (2019) trialled three methods for the digestion of SOM from samples, these being 30 % H_2O_2, 30 % H_2O_2 + H_2SO_4 (3:1 v/v) and 30 % H_2O_2 + HNO_3 (3:1 v/v) at 70 °C. They noted that methods excluding the 30 % H_2O_2 caused detrimental effects on polyamide fibres. It must be noted here that the samples were field samples, and as such, the plastic quantities were unknown, so this paper does not provide further insight into the efficacy of these extraction methods but does allow some insight into the impacts of H_2SO_4 and HNO_3 on polyamide. The research also noted that various colours of MPs were found, including white, red, blue, black, yellow and green, and found that white fibres were the most prevalent. As HNO_3 has been noted to bleach some fibres (Naidoo et al., 2017), it must be considered that there is potential that white fibres were the most prevalent due to damage from the methodology, as opposed to these being the most prevalent colour. The colour of the fibre can help with the identification of sources of pollution; thus, this method may not provide enough information to consider sources. The research noted that significantly more plastics were found when digestion of SOM occurred instead of no digestion of SOM, indicating that this is a crucial step for microplastic recovery. This research recommended H_2O_2 as a method for removing SOM and noted that as long as temperatures less than 70 °C were used, little damage was observed to the particles by this methodology.

Duan et al. (2020) developed a digestion method for determining microplastics in vegetal-rich clayey mangrove sediments. This research tested concentrated HNO_3, 2 mol/ L NaOH, and H_2O_2 and used 50 g of dried sediment to test the treatments. The researchers spiked the dried sediment samples with 40 pieces of microplastics using six different polymer types (PET, PS, PE, PVC, PA and PP) and found H_2O_2 to be the most appropriate methodology. However, Duan et al. (2020) noted that it took a week for the H_2O_2 to remove the appropriate amount of organic matter to extract the microplastics, which again would cause limitations for larger-scale applications due to the time taken to process the samples.

Radford et al. (2021) investigated the use of potassium hydroxide (KOH), Fenton's reagent and H_2O_2 for the removal of SOM in both high organic matter contents (73%) and low organic matter contents (12%). Both Fenton's reagent and H_2O_2 were more effective than KOH for the low organic matter groups. However, H_2O_2 removed more organic matter than KOH and Fenton's reagent for the high organic matter treatment. Soil organic matter content in UK soils has generally been reducing; however, the soil association aims to increase total organic matter in agricultural farms to 20 % in the next 20 years (Soil Association, 2016). The soil association states that degraded soils in the UK currently have 1-2 % organic matter content, suggesting that the low organic matter treatment is more likely to represent SOM contents within soils than the high organic matter treatment. This suggests that for most soils, Fenton's reagent may be the most appropriate treatment, but soil type and organic matter content should be considered when choosing an appropriate treatment to reduce SOM.

It is considered that the method's efficacy is not the only determining factor for the optimum method, and factors such as sample processing time and cost must also be taken into account as this is a limitation for larger sized studies investigating polymer particles in soils. The optimum method would; cause little to no degradation to the plastic in such a way that analysis could still be completed to analyse the plastic type and ideally allow for full characterisation of the MP, e.g., colour, shape etc. The optimum method would also allow researchers to process a large number of field samples, meaning that quicker processing methods are encouraged, and the method would have an extraction rate above 80 % for all plastic types. As such, this research was conducted to find a method that fits the criteria and was suitable for large scale field studies.

From the research available at the time of the study, it was decided that five methods were promising for the removal of SOM to enable microplastic extraction. The five methods used included HNO_3, HCl, H_2SO_4, H_2O_2 + Iron II (Fenton's reagent) and NaOH; from the literature, these methods appeared to fit the criteria (low cost, quick processing time and the potential to remove SOM) that the researchers deemed necessary for the use in field studies, this work was conducted to enable an

optimum method to be employed in further works. The experiment tested the hypotheses that Fenton's reagent would be the optimum method for the removal of organic matter from soils and provide the highest recovery rates for the three different microplastics in soils.

2.2 Materials and methods

2.2.1 Preparation of soils

The soil was obtained from a commercially available source (Westland's topsoil, Westlands, Evesham, UK) a clay loamy soil with high humus content (Organic matter = 19 %, sand = 62 %, clay = 21 % and silt = 17 %). The soil was sieved to 5 mm to remove any large stones or debris and homogenise the soil. This soil type was selected as a large proportion of UK soils have a clay component (49.1 % of UK soils having some clay component) (Cranfield, 2014), so the soil was chosen to be representative of this. The soil was air-dried at room temperature for two weeks to remove excess moisture and then weighed into 10 g samples. Soils were searched manually for evidence of plastic contamination, and any plastics found were removed. The soil was added to secure polypropylene collection pots (height = 9 cm, width = 2.5 cm with a total volume of 44 cm^3).

2.2.2 Preparation of plastics

The types of plastics chosen for this study were PVC, PET and PP with different shapes (fibres and fragments), which enabled an assessment of different densities and shapes of microplastics. Consumer products were broken down into secondary microplastics by manually cutting the items to sizes less than 5 mm (see figure 2.1). The PVC fragments were obtained from 100 % PVC sleeving; these fragments had a mean length of 4939 µm (maximum = 6700 µm, minimum = 3260 µm, N = 100). PET fragments were sources from 100 % PET braised wire loom sleeving; the fibres had a mean length of 4512 (maximum = 7600 µm, minimum =1600 µm, N = 100) and a mean diameter of 194.7 µm (maximum = 260 µm, minimum = 150 µm, N = 100). PP fibres were obtained from PP blue stranded rope with a mean length of 4999 µm (maximum 7300 µm, minimum 3980 µm, N = 100). Measurements of plastics were taken using Moore & Wright Vernier Callipers (Model number: 110-15DDL) with an accuracy of ± 20 µm.

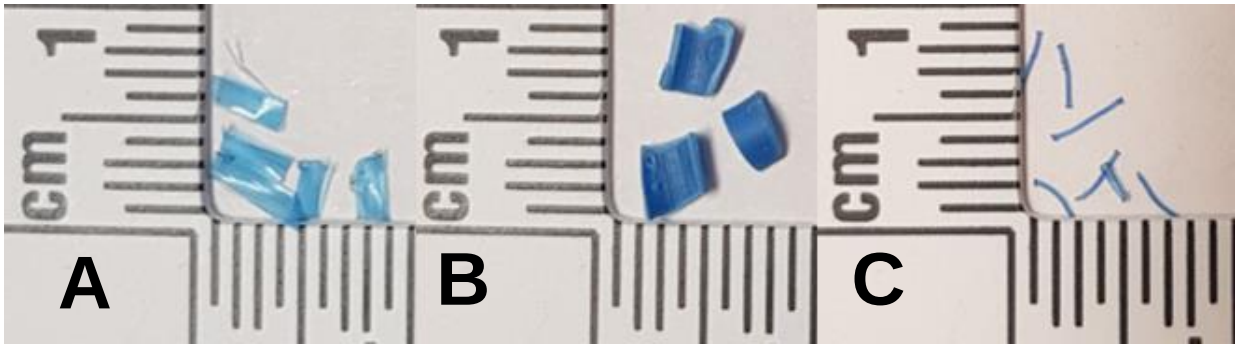


Figure 2.1: Three microplastic types used in the current study before treatment. PP fibres (A), PVC fragments (B) and PET fibres (C).

2.2.3 Microplastic additions to soil

Twenty pieces of one plastic type (either PP, PVC or PET) were added to the secure collection pots (20 pots per treatment, each containing 20 plastics, N of pots = 240) containing 10 g of soil. The pots were stirred using a clean glass rod and manually shaken to ensure the plastics were thoroughly mixed into the substrate. Samples were stored at room temperature for up to four months during the study. During this time, they were kept in a dark environment to ensure plastics could not be further degraded by UV.

2.2.4 Digestion of plastics without soil

Prior to the trials of the efficacy of the treatments to digest soil organic matter (SOM), five treatments were tested to assess the potential for the treatments to damage the plastic. Five treatments were tested due to their noted potential for removing soil organic matter. Table 2.1 (below) summarises the five treatments used, including the time each protocol took. For each protocol, 20 pieces of each type of plastic were added to clean glass test tubes and submerged in 10 ml of each treatment for the appropriate processing time (20 pieces per pot, 20 pots per treatment, N = 300 pots). After processing, the plastics were filtered using vacuum filtration onto Whatman 3 cellulose papers (70 mm). Subsequently, the plastics were examined for any visual changes such as colour changes, increasing brittleness or pitting and sheering. The polymers were qualitatively graded out of 5 points, with a

point for each of the following: original colour still intact, general shape intact (fibre or fragment with no apparent damages to the edges under microscopy), no evidence of brittleness (reduced in size/ powdery appearance), no evidence of general pitting when viewed under a stereomicroscope (Nikon C-Lens, Nikon, Normanton, UK) and finally no reduction in overall weight. Any protocols that showed detrimental damage to all polymer types (which meant that post-processing characterisation could not be completed) were excluded from the second testing phase.

Table 2-1: The five protocols used to test whether they caused damage to the polymer particles.

Protocol number	**Treatment**	**Processing time**
1	HCl - 1 M	24 Hours (20 °C)
2	H_2SO_4 – 15.8 M	4.5 Hours (60 °C)
3	NaOH – 10 M	6 Hours
4	Fenton's Reagent (9.8 M H_2O_2 + $FeSO_4$ (20 g dissolved in 1 L of distilled water – 0.155 moles))	1 Hour
5	HNO_3 – 14 M	4 Hours

2.2.5 Digestion of SOM

After the trials on the plastic particles (HNO_3 caused damage to all polymers tested and thus was removed), four treatments were deemed suitable for use in this study. First, digestion techniques were used to remove the SOM before floating the plastics out of the samples, as SOM is often in the same density range as that of plastic (1- 1.4 g/cm^{-1}) (Mbachu et al., 2021), using the four protocols listed below. The following protocols were either suggested as viable treatments within the literature or used

to digest SOM previously. These treatments were tested to assess which was the most suitable for microplastic extraction from the soil media.

Protocol one: The digestion of SOM using HCl (1 M) (Fisher Scientific, Loughborough, UK) as suggested by Cole et al. (2014), 20 ml of HCl was added to the 10 g of soil and allowed to digest at room temperature (20 °C) for 24 hours. HCl is a method used commonly for the extraction of microplastics in biota (Lusher et al., 2017a). HCl has been suggested as a potential method for the extraction of MPs in soil samples (Pinto da Costa et al., 2018) but has not been tested in soils. It has been shown to be highly efficient in removing organic matter from samples; however, it has also been noted that these acids may have adverse effects on the microplastics, potentially leading to degradation (Stock et al., 2020).

Protocol two: 10 ml of H_2SO_4 (15.8 M) (Fisher Scientific, Loughborough, UK) in a water bath at 60 °C for four and a half hours. This method has been suggested in the literature for the extraction of microplastics from complex media; however, this has not been tested for its applicability for microplastic extraction (Scheurer & Bigalke, 2018). In addition, H_2SO_4 can be used to remove SOM; however, it has been noted to have a negative effect on PET and has also been suggested as a means to recycle PET.

Protocol three: 20 ml NaOH (Fisher Scientific, Loughborough, UK) was processed in the water bath for six hours (Cole et al., 2014) at 60 °C. This technique has been used to extract microplastics from biota using varying concentrations of NaOH. Literature suggests potential adverse effects on microplastic particles (Catarino et al., 2017); however, as this is a standard technique for the extraction of MPs, it is imperative to understand whether this may be applicable for the extraction of MPs from soil matrices.

Protocol four: Fenton's reagent (10 ml of H_2O_2 + 10 ml $FeSO_4$ solution). The solution of $FeSO_4$ was comprised of 20 g of Iron II sulphate heptahydrate (Fisher Scientific, Loughborough, UK) diluted in 1 L of distilled water (Hurley et al., 2018). 10 ml of this solution was added to the beaker and then

combined with 10 ml of 9.8 M of H_2O_2 (Fisher Scientific, Loughborough, UK). This treatment was left for one hour at room temperature. The $FeSO_4$ acts as a catalyst to speed up the reaction time by increasing the generation of OH (Bissey et al., 2006) and may negate the adverse effects that H_2O_2 has on plastic polymers (Tagg et al., 2017). It has been considered in the literature as a promising technique for extracting MPs from complex media.

Protocol five: 10 ml of HNO_3 (Fisher Scientific, Loughborough, UK) was tested on the plastics without soils for four hours at 60 °C. Scheurer and Bigalke (2018) suggested this technique, which found that it removed the most organic matter from soils. Lusher et al, (2017) tested this protocol by digesting fish and invertebrate samples but noted that they did not find any fibres in the samples, likely due to the destructive nature of this acid. As the methodology shows promise for the removal of organic matter, it is important to test any effects this technique may cause to plastics.

2.2.6 Density separation

Following the SOM removal, density separation was used to remove plastics from the remaining soil constituents after digestion. The chemical selected for this was $ZnBr_2$ (Fisher Scientific, Loughborough, UK) for this separation, and a 25% saturated solution was made following the methodology by Quinn et al. (2017). The $ZnBr_2$ solution was made by dissolving 1126 g L^{-1} of $ZnBr_2$ salt into 1 L of water, which gave a density of 1.7 g/cm^3; the density was checked using the equation below. This density allowed all plastic types in this study to be floated out as the highest density plastic used was 1.38 g/cm^3. The density of the solution was calculated using the following equation:

$$\text{Density} = \frac{\text{(Weight of vessel and solution (g) – weight of vessel (g))}}{\text{Volume of vessel (cm}^3\text{)}}$$

The same brine solution was reused throughout the experiment, being filtered three times through Whatman grade 3 filter papers (Fisher scientific, Loughborough, UK) before being reused. A 3:1 ratio of salt solution to soil was used as described by Claessens et al, (2013), with 30 ml added to each 10 g

soil sample. The samples were stirred using a clean glass rod for 5 minutes to aid the separation of the plastics; these were then left to stand for ten minutes to allow heavier particles to sink and the plastics to float in the suspension. The particles which accumulated at the top of the suspension were recovered using vacuum filtration. This top layer of solution was filtered with a ceramic Buchner funnel using Whatman 3 cellulose paper (70 mm), which was then added to a clean glass Petri dish and closed to ensure no airborne contamination.

2.2.7 Searching of soil samples.

Soil samples were visually searched for a maximum of ten minutes per sample. All microplastics found were removed with metal tweezers and placed in a clean petri dish with a clean Whatman grade 3, 70 mm cellulose filter paper. A stereo microscope (Nikon C-LEDS) was used once it was determined that all plastics that could be seen macroscopically had been removed, allowing for a closer inspection of samples to find the remaining plastic. After the 10-minute search time had elapsed, the MP particles recovered were counted (out of N = 20) and recorded for subsequent statistical analysis.

2.2.8 Remaining soil

Once the plastic had been removed, the soil was air-dried at room temperature for between 2-4 weeks (some samples required a longer processing time than others to achieve total dryness). The samples were then weighed to determine the mass (g), which remained post-searching.

2.2.9 Contaminant mitigation

As all plastic samples were blue, the researchers could easily identify environmental contamination. Additionally, the following contamination mitigation was adhered to during all steps of the procedure. Firstly, a clean white cotton lab coat was worn, with no synthetic fibres worn underneath. Next, blank

runs were undertaken in-between samples, post rinsing glassware three times with distilled water to ensure that the glassware was clean. Finally, blank runs involved running the density separation protocols with no soil or MP samples, as this allowed an assessment of whether any previous samples remained adhered to the glassware.

2.2.10 FT-IR analysis

Fourier Transformation Infrared Spectroscopic (FT-IR) analysis was conducted on the plastic particles after the digestion to assess any chemical changes to the particles. MIR-FTIR spectra were measured in the range of 4000 – 650 cm^{-1} by a Perkin Elmer Spectrum 3 spectrometer (Perkin Elmer, Buckinghamshire, UK) using an ATR diamond/ZnSe crystal. Each spectrum was an accumulation of four scans at a resolution of 4 cm^{-1}. After each measurement, the surface of the crystal was cleaned using ethanol.

Match rates were determined against a self-created library of control sample spectra of untreated polymers from the same source. Ten different MP particles (from ten different pots) were spectroscopically analysed for each treatment and polymer type. Match rates were calculated using the Hit Quality Index (HQI) in the Spectrum 10 software (Perkin Elmer, Buckinghamshire, UK); the match rates are calculated based on the correlation coefficient between the test spectrum and the library spectrum.

2.2.11 Statistical analysis

Microplastic recovery data (%) from soils was expressed as the mean and the standard deviation. Sample numbers of N = 20 pots, each containing 20 plastics, a total N of 240 pots were used for the three polymer types (PP, PVC and PET) across all four treatments (Fenton's, HCl, NaOH, H_2SO_4), giving a total number of 240 samples tested across the study. Data normality was tested using the Shapiro-Wilks test with a significance level of 0.05. Normality was demonstrated in all groups tested; thus, a one-way ANOVA was performed (significance level = 0.05) considering the polymer recovery

rate for each treatment, and the differences between groups were appraised using a Tukey's HSD posthoc test.

The remaining SOM was analysed using a one-way ANOVA, 20 remaining SOM samples were weighed for each treatment (N = 80 samples) considering the final weight of the remaining matter and differences between the four treatments were appraised using a Tukey's HSD posthoc test. Statistical testing for this section of the study was conducted using IBM SPSS version 26.

FTIR match rate data were analysed using R v3.6.1 (CoreTeam, 2019). First, data were screened for normality using a Shapiro-Wilks test from the stats package (version 3.6.1, CoreTeam 2019). A Kruskal-Wallis test was subsequently used (as data did not conform to parametric testing) (alpha level = 0.05), and Dunn's post hoc test from the Dunn.test package (Dinno, 2017) was applied to test for differences between the treatments for each polymer type. Finally, graphs were created using R v3.6.1. utilising the ggplot2 package (version 3.4.2) (Wickham, 2016).

2.3 Results

2.3.1 Plastic samples without soils

Samples were processed with each treatment to observe any damage caused by the treatment to the polymers. Each treatment was scored to provide the researchers with a qualitative method of determining the applicability of these treatments for future studies (a table of these results can be viewed below in table 2.3). Of the five treatments tested, HNO_3 damaged all polymer types, causing significant colour changes and brittleness in all samples, with the PET samples becoming powder-like and fragmenting easily. In addition, weight loss occurred in all samples treated with HNO_3, with PET losing the largest average mass of 0.17 g, PVC reducing by an average mass of 0.026 g, and PP losing 0.00016 g. These weight changes indicated that the treatments physically damaged the polymers, which was subsequently confirmed using FT-IR analysis. Due to the detrimental changes across all groups, HNO_3 was excluded from further studies (images of the changes to the samples can be seen in Table 2.2).

HCl caused no visible damage to the plastic, with all colours appearing unchanged and visual inspection did not indicate that the polymers had undergone any structural changes. Furthermore, no weight loss occurred in any samples, indicating no significant damage to the polymers. As such, this method represented good potential for the removal of SOM from samples. NaOH caused no visual changes to the PP or the PVC, and no weight loss was demonstrated in either of these polymers. However, PET was found to have slight colour changes, and a residue formed on the surface of the fibres, causing a slight weight gain. Additionally, the fibres became more brittle, causing them to fragment. Thus, it was decided to trial the treatment in soils, with the note that PET fibres may be damaged. Fenton's reagent caused no visible detrimental damage to either PP or PET; however, a slight colour change was noted in the PVC group. In addition, no weight changes were noted for any of the polymer types tested. As such, this treatment represented a viable option for further testing. H_2SO_4 resulted in slight colour changes to the PP fibres but did not result in complete colour changes such as bleaching; no weight changes occurred for the PP fibres. For the PVC group, minor weight

loss of the samples occurred with an average reduction of 0.0029 g, suggesting some changes to the fragments. PET was significantly changed when treated with the H_2SO_4, resulting in a complete breakdown of the fibre, causing PET to form a liquid substance. The fibres were not weighed, as removing these from the treatment was impossible. The colour of the fibres did not change; however, structural damages meant that this method would have limitations for future studies.

Table 2-2: Representative images of three different plastic types after the five treatments, samples were photographed at x15 magnification using a light microscope. The scale lines represent 1 mm for each segment.

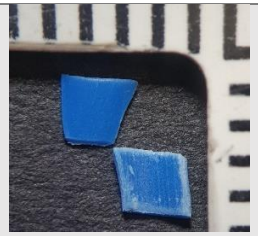
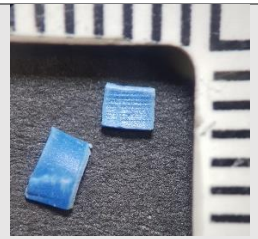
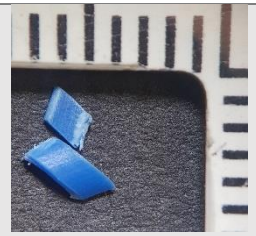
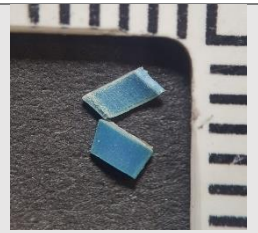
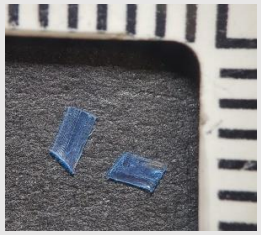
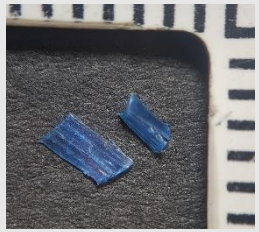
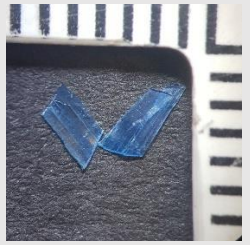
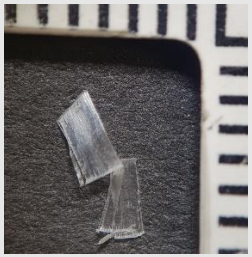
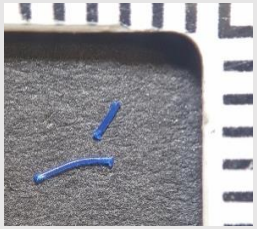
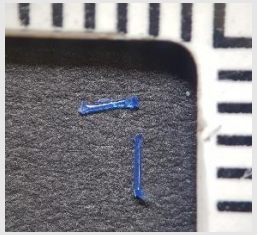
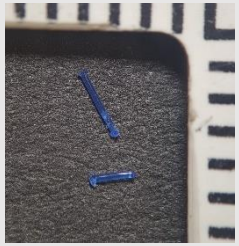
	Control	Fenton's Reagent	HCl	NaOH	H_2SO_4	Nitric acid
PVC						
PP						
PET					Sample too damaged to photograph	

Table 2-3: The quantitative scoring of the plastic particles, a score of 1 means no change was seen and a score of 0 means that changes were observed. The total column is the combined score from the characteristics, with 5 being the highest possible and 0 the lowest.

Plastic-type	Treatment	Colour change	Shape change	Brittleness	Pitting	Weight change	Total
Polypropylene	HCl	1	1	1	1	1	**5**
	NaOH	1	1	1	1	1	**5**
	Fenton's reagent	1	1	1	1	1	**5**
	H_2SO_4	0	1	1	1	1	**4**
	HNO_3	0	1	1	1	0	**3**
Polyvinyl chloride	HCl	1	1	1	1	1	**5**
	NaOH	1	1	1	1	1	**5**
	Fenton's reagent	1	1	0	1	1	**4**
	H_2SO_4	0	1	1	1	1	**4**
	HNO_3	0	1	1	1	0	**3**
Polyethylene terephthalate	HCl	1	1	1	1	1	**5**
	NaOH	0	1	0	0	1	**2**
	Fenton's reagent	1	1	1	1	1	**5**
	H_2SO_4	1	0	0	0	0	**1**
	HNO_3	0	1	0	0	0	**1**

2.3.1 FTIR of polymers

A statistically significant difference was demonstrated in the match rates across the treatment and plastic types, as determined by a Kruskal-Wallis test ($\chi^2(13) = 106.71$, $p < 0.0001$) (see figure 2.3). For the PET treatment, HCl demonstrated the highest match rate of 98.9 ± 0.73 % compared to Fenton's reagent, which had a match rate of 98.67 ± 0.65 %; there were no significant differences ($p = 0.373$). HNO_3 showed an average match rate of 27.45 ± 4.05 %, which was significantly different from both HCl ($p = 0.0004$) and Fenton's reagent ($p = 0.0006$), but no significant differences compared to NaOH ($p = 0.108$). NaOH had the lowest average match rate of 17.94 ± 3.5 %, indicating significant changes from the control. When tested against both HCl and Fenton's reagent, this was determined to be significantly different, with a p-value of < 0.0001 for both treatments.

In the PP group, Fenton's reagent had the highest average match rate of 98.95 ± 1.53 % based on data from FTIR analysis; however, this was not significantly different to the match rates of any other treatments tested. For example, HCl had a match rate of 98.65 ± 1.65 % ($p = 0.32$), H_2SO_4 a match rate of 97.47 ± 1.57 % ($p = 0.039$), NaOH a match rate of 97.92 ± 1.79 % ($p = 0.105$), and HNO_3 a match rate of 98.37 ±1.67 % ($p = 0.21$).

For the PVC groups, NaOH demonstrated the highest match rate of 98.89 ± 4.98 %, and Fenton's had the second-highest match rate of 97.06 ± 0.93%, which was significantly different to the match rate of NaOH ($p = 0.022$). HCl had a match rate of 94.65 ± 1.91%, which was significantly different to the NaOH ($p = 0.0018$), but there were no significant differences between HCl and Fenton's reagent ($p = 0.153$). HNO_3 had a match rate of 27.45 ± 4.99 %, which was significantly different from NaOH ($p < 0.0001$) and Fenton's ($p = 0.0066$) but not significantly different from HCl ($p= 0.0727$). H_2SO_4 showed the lowest match rate for PVC of 16.86 ± 1.55 %, which was significantly different from the NaOH ($p < 0.0001$), HCl ($p = 0.0082$) and Fenton's treatments ($p = 0.0004$).

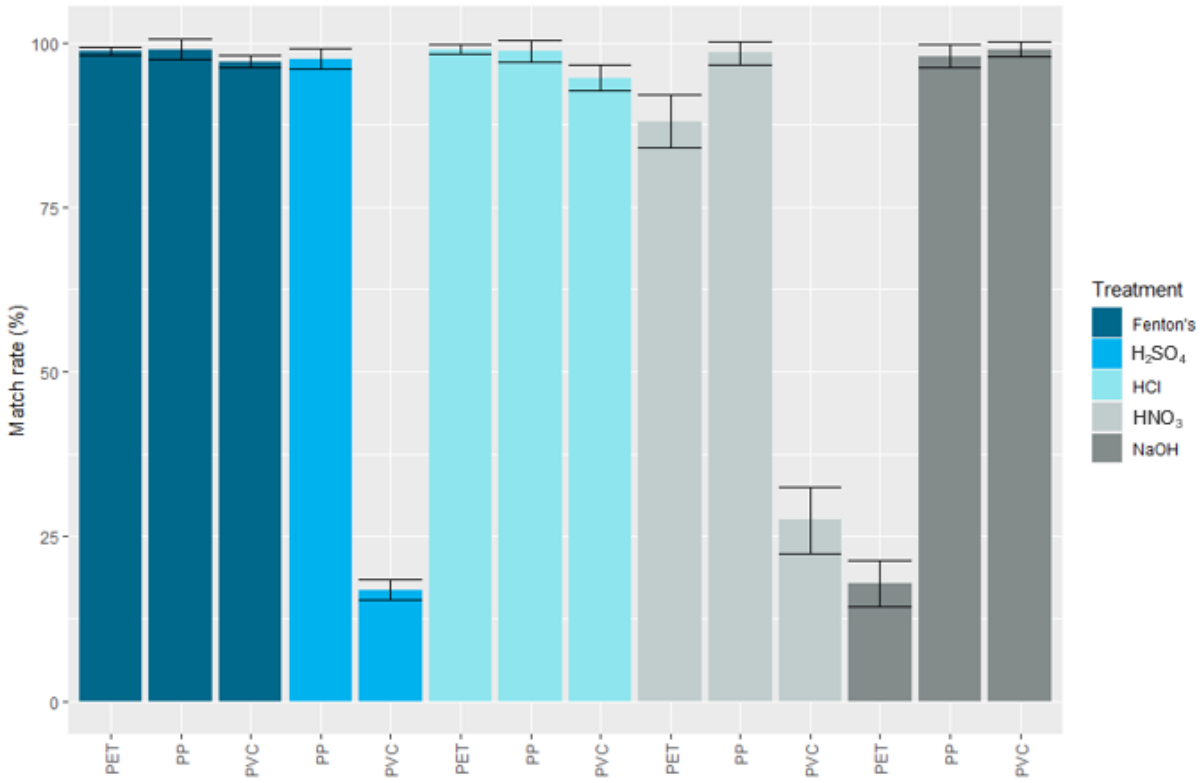


Figure 2.2: The mean match rates of each polymer type (n = 20 per polymer from 20 different pots) after treatment. Data is presented as the average and the error bars indicate the standard deviation from the mean.

2.3.2 Recovery rates of plastics with soils

There was a statistically significant difference between the treatments across all polymer types as determined by a one-way ANOVA ($_F$ (11,228) = 94.0, $p < 0.0001$) (see figure 2.3). NaOH was significantly different from all other treatments investigated across all polymer types ($p < 0.0001$) and demonstrated the lowest recovery rates of all the treatments tested (PVC = 35.75 %, PP = 32 %, PET = 2.25 %). NaOH also had the most considerable standard deviations for PP and PVC recovery, indicating a high level of variability of recovery in the MPs tested.

There was minimal variation between the remaining three protocols (HCl, Fenton's reagent and H_2SO_4) in terms of recovery rates (see figure 8). For two of the three plastic types, Fenton's reagent demonstrated the highest recovery rates (PP = 95.5 % and PVC = 92.75 %); however, this was not statistically significant when compared to the recovery rates of HCl (PP = 93.75, p = 1.000, PVC = 89.25 %, p = 1.000) or H_2SO_4 (PP = 91.25 %, p = 0.995, PVC = 81%, p =0.252). For PET recovery, HCl showed the highest recovery rates (66 %) compared to Fenton's reagent (63.5 %) and H_2SO_4 (53.25 %); however, there was no statistical difference between these results (p = 1.000, p = 0.152, respectively).

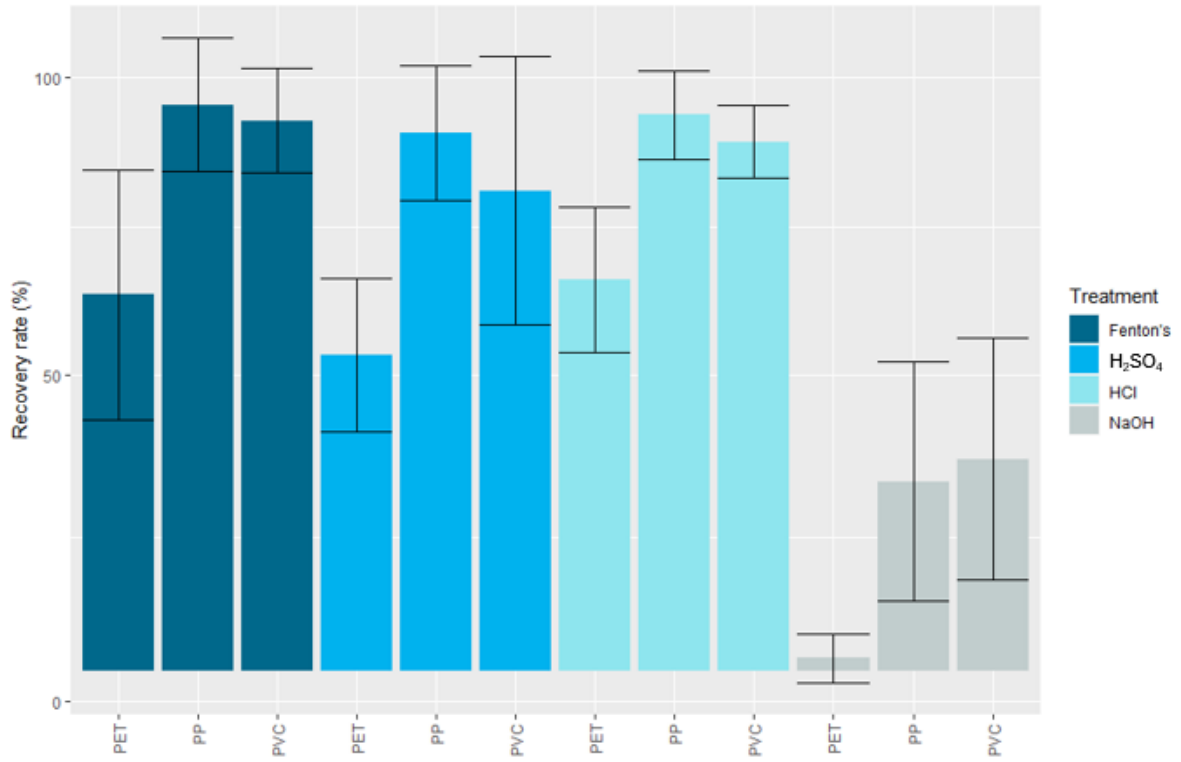


Figure 2.3: The recovery rates of the three polymer types across the four treatments (n = 240 pots, 4,800 plastics). The mean recovery rate is expressed as a percentage and the error bars indicate the standard deviation from the mean.

2.3.3 Efficacy of treatments to remove SOM.

There was a statistically significant difference between all groups as determined by a one-way ANOVA ($_F$ (3, 76) = 212.425, $p < 0.0001$) when comparing the weight of the remaining SOM (see figure 2.4). Post hoc comparisons using Tukey's HSD test indicated that Fenton's reagent (M = 2.265, SD = 1.112) was significantly different from all other treatments ($p < 0.0001$) with the lowest amounts of remaining soil organic matter. Between the treatments HCl (M = 7.096, SD = 2.338) and H_2SO_4 (M = 6.543, SD = 2.266), there were no significant differences, indicating that neither of these treatments had a significant advantage over the other ($p = 0.941$). The NaOH treatment was significantly different from all treatments tested (M= 24.896, SD = 5.106, $p < 0.0001$), showing the highest amount of remaining organic content after sample treatment (see figure 2.5 below).

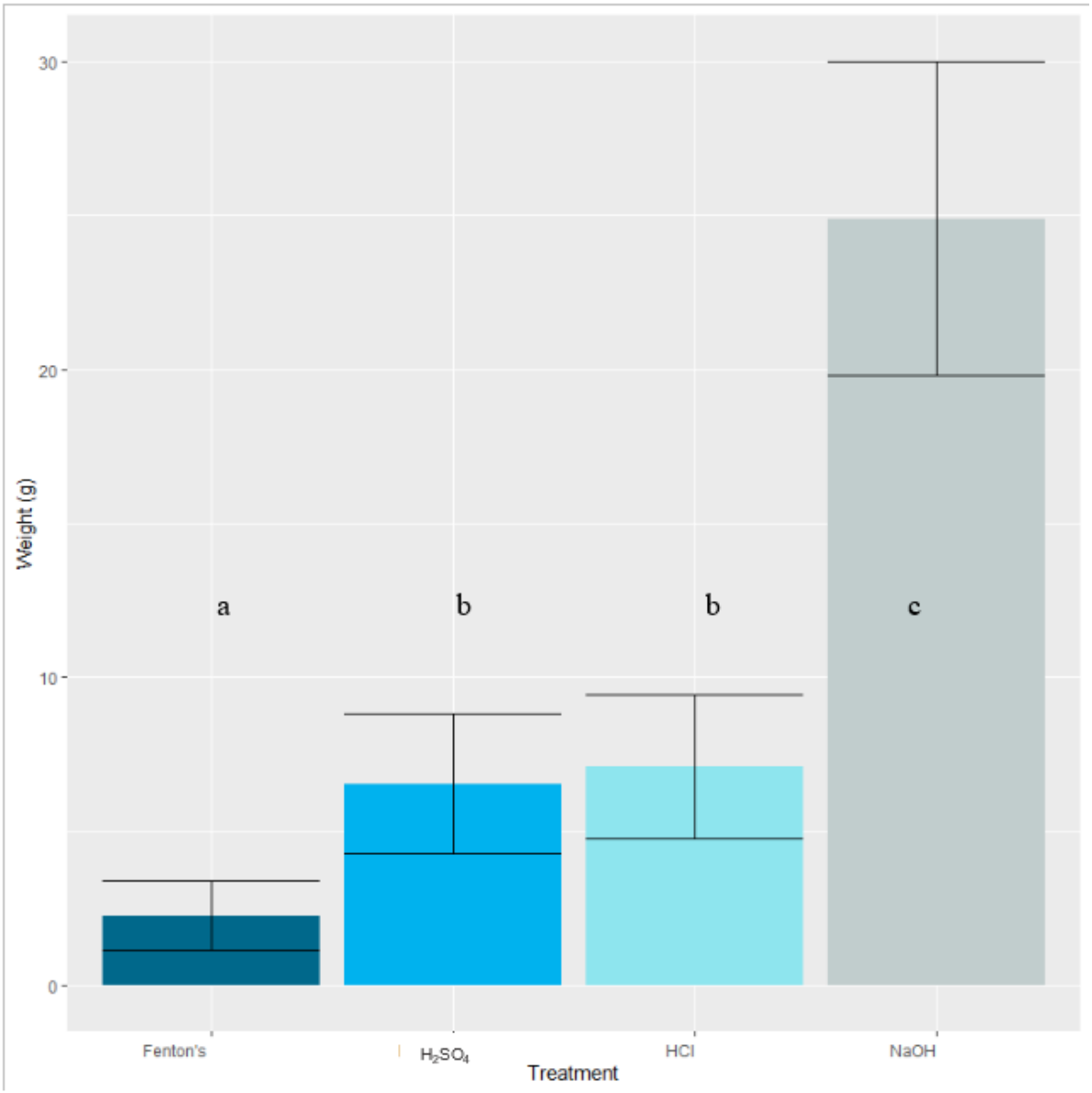


Figure 2.4: The remaining soil matter in g after digestion, density separation and filtration. Results are represented as the mean of n= 20 ± the standard deviation; a, b and c represent the homogenous subsets as determined by Tukey's HSD test, where a represents the group with the least soil remaining, and c represents the group with the most remaining.

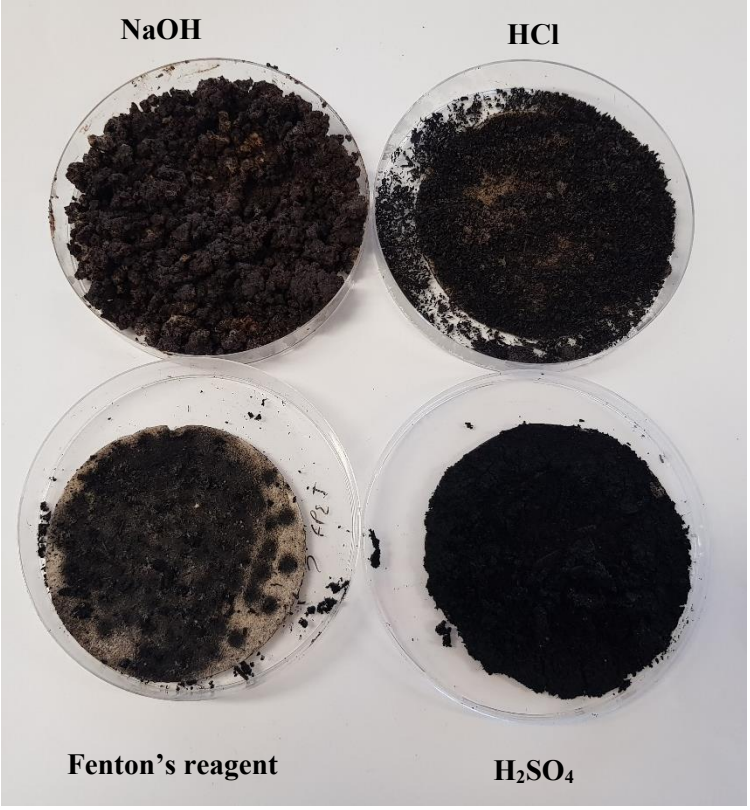


Figure 2.5: Filter papers with the remaining matter after the treatment, density separation, and search for microplastics.

2.4 Discussion

2.4.1 Characterisation of plastics after treatment

In the initial visual grading of the samples to provide a qualitative score, HNO_3 had the lowest score due to the bleaching of the samples, which would make further morphological analysis difficult and thus was the reason this treatment was removed from the second stage of testing. The degradation of plastics by HNO_3 has been noted by Gulizia et al. (2022), who suggested that HNO_3 resulted in the formation of C-N bonds due to electrophilic aromatic substitution. The authors further consider that nitration of the plastics could occur through the addition of HNO_3. Wolkóber (1962) reported that PVC is oxidized by nitric acid, which could help to explain the changes to the match rates for this polymer. Degradation of the polymers by different chemicals is polymer specific, with PVC more affected by the HNO_3 treatment than PP and PET according to spectral changes.

NaOH showed spectral changes for the PET treatment with an average match rate of 17.9 ± 3.5 %, which is likely due to the alkaline hydrolysis, which initiates depolymerization (Gulizia et al., 2022). This is also discussed by Schrank et al, (2022), who suggest that NaOH degrades the outer surface of the PET, causing a reduction in size over time for the polymer. Additionally, they suggested that PET is susceptible to saponification, concurring with Gulizia et al (2022) that alkaline hydrolysis occurs on the organic functional groups. Interestingly, research by Hurley et al. (2018), who also assessed NaOH and its resulting changes to PET, found no differences in the FT-IR spectra but did see changes to the surface of the polymer. In this research, significant changes occurred to the PET compared to the control when analysed with FT-IR; similarly, Schrank et al (2022) also saw changes to the FT-IR spectra for PET when treated with NaOH. The exposure time and the temperature for processing are likely key factors when considering the degenerative effects of alkaline treatments (Hurley et al., 2018).

H_2SO_4 had the lowest match rates for PVC and completed degraded PET, suggesting this is not an applicable method for the extraction of microplastics from soils. Lusher et al, (2017) suggested that strong acids such as nitric acid and sulphuric acid are likely to destroy or damage the majority of polymers, particularly at higher temperatures but did note this was polymer specific. Al Sabagh et al, (2016) described the depolymerisation of PET using H_2SO_4, noting that it results in the production of inorganic salts. The current research has demonstrated that H_2SO_4 has damaging effects on two of the three polymers tested and, thus, is not a suitable methodology for the extraction of microplastics from soils.

The FT-IR match rate data confirmed that HCl and Fenton's reagent had the highest scores across the different plastic types. In addition, Fenton's reagent had consistently high match rates across all three polymer types tested. HCl also demonstrated consistently high match rates; however, Fenton's reagent demonstrated the highest match rates for PET compared to HCl. This suggests that, similar to Hurley et al, (2018), Fenton's reagent was the most suitable approach when considering the match rates for subsequent analysis using FT-IR. Additionally, when considering that Fenton's reagent also removed the most SOM in this study, it appears that Fenton's reagent is the most appropriate technique for the extraction of microplastics from soils.

2.4.2 Efficacy of treatments to remove SOM.

In this study, Fenton's reagent was the most efficient method for removing SOM from samples. Peroxide oxidation is a commonly used method for the removal of matter from sludge and soil samples prior to analysis (Petigara et al., 2002). The completeness of the digestion of organic matter varies based on the composition of organic content; in this study, Fenton's reagent resulted in the least remaining SOM. In addition, Fenton's reagent produces a rapid exothermic reaction (Aramyan, 2017) ($Fe^2 + H_2O_2 \rightarrow Fe^3 + HO + OH$), which helps to contribute to the removal of organic matter. The efficacy of this treatment may have also been enhanced by the low pH of the reagent, which induces

optimal conditions for the removal of organic matter (Kremer, 2003). Fenton's reagent has comparable results to H_2O_2 decomposition at 70 °C (Hurley et al., 2018); however, it may be more efficient for microplastic extraction as Fenton's reagent demonstrated less damage to the plastic particles, potentially due to the shorter treatment times, leading to higher recovery rates (Prata et al., 2019). It was noted in this study that PET samples became more brittle post-treatment compared to the control; however, the treatment itself did not reduce the number of PET samples. Additionally, the FT-IR match rate indicated a match of 98.67 % compared to the control, indicating that any changes did not change the polymer in such a way that it could no longer be identified. Therefore, in terms of the reduction of SOM, Fenton's reagent appears to be the optimum method.

H_2SO_4 and HCl were the second most efficient treatments for the removal of SOM, with mean remaining masses of 6.54 g and 7.09 g. H_2SO_4 is utilised as a method to remove easily oxidised materials from soils; however, it requires heating to improve the efficacy (Matskevich et al., 2022). In this experiment, the H_2SO_4 was diluted previously, added to the beakers, and heated using a water bath. It has been noted that H_2SO_4 may have deleterious effects on PET and has been proposed by Yoshioka et al. (1994) as a means of recycling PET by heating sulphuric acid and PET to 150 °C. When the recovery of PET was treated with H_2SO_4 and soil, PET samples had minimal weight loss, as opposed to the breakdown of the fibre, which was demonstrated when PET was tested alone. This may be due to the H_2SO_4 reacting with the organic matter in the soil; thus, reactions between the PET and H_2SO_4 were not occurring. Therefore, when considering the removal of SOM, H_2SO_4 could be utilised; however, damages to the PET must be regarded.

HCl exhibited comparable reduction rates in SOM to H_2SO_4; HCl is a widely used treatment for soil organic carbon (SOC) decomposition, which can vary significantly across soil types (Wotherspoon et al., 2015). HCl is also beneficial in removing dolomite from samples, which could make it a particularly suitable method in limestone soils (Solihin et al., 2018). Due to the treatment's ability to remove SOC, it may be used as a complementary method alongside other methods tested in this study.

The NaOH resulted in an increase in the soil mass due to the formation of salts. Alkaline hydrolysis is effective at destroying proteins, which is why this technique has been utilised for the extraction of microplastics from biota (Karami et al., 2017). However, cellulosic and chitinous materials are resistant to NaOH treatments (Herrera et al., 2018), both of which are present in soils (Lyon & Rhodes, 1993). Alkali insoluble humins are some of the most abundant organic fractions found in soils (Hurley et al., 2018), which helps to explain the low removal efficacy of NaOH demonstrated in this study and concurring with Hurley et al. (2018).

The higher reductions of SOM resulted in better searching conditions, as samples were easier to identify against the background visually. When considering larger sample volumes which would be expected from field samples, it is imperative to remove as much material as possible to enable the identification of the polymer materials against the background. In this study, Fenton's reagent had the highest efficacy in removing SOM and is considered the optimum SOM method.

2.4.3 Recovery rates

NaOH had the lowest recovery rates of all samples tested, likely due to the high amounts of soil material remaining after the treatment, which caused difficult searching conditions. This further adds to the consideration that NaOH is not an appropriate method for the extraction of microplastics from soils.

HCl, H_2SO_4, and Fenton's reagent exhibited similar recovery rates of the known plastics; however, the higher amounts of soil matter remaining in the HCl and H_2SO_4 treatments will likely make recovery more difficult for unknown samples, like those which would be found in field samples. Fenton's reagent demonstrated the highest recovery rates across all three plastic types with the particular soil type tested. Radford et al, (2021) found that Fenton's reagent was the optimum method for the recovery of plastics from low organic matter soils (12 % organic matter), but H_2O_2 for high organic matter (73 % organic matter). However, considering in soils organic matter constitutes approximately 2 – 10 % w/w of most soils (Dondini et al., 2023), Fenton's reagent would be an

applicable method in most soil types. Peat soils have an organic matter of over 60 % (Scottish Government, 2022); thus, H_2O_2 may be an applicable method if testing soils high in organic matter. Future studies should consider the applicability of digestion techniques across a range of soils to understand optimum methods based on different soil types.

The recovery of PET was lower, and it has been noted that fibres are often difficult to recover due to their irregular shape and the tendency to adhere to the sides of glassware (Carney Almroth et al., 2018). Corradini et al, (2019) also found lower recovery rates for fibres but recommended repeating density separation steps to ensure maximum recover. Recovery rates could be improved by using multiple repeats of the density separation step to minimise the likelihood of plastics adhering to the glassware and enable more particles to be removed from the soils.

Recovery rates are likely to be different in different soil types. Clay and organic matter fractions in soils make the recovery of microplastics more difficult (Li et al., 2019). In clay-heavy soils, micro-sized clay particles are mixed with the soil matrix, which can hinder the quantification and subsequent identification of microplastics. Zhang et al, (2018) found that polypropylene was more challenging to recover from clay soils, compared to sandy soils and loess soils, with pure sand having the highest extraction rates. Sandy soils are likely easier to extract microplastics from compared to clay soils due to a large grain size which can help facilitate the removal of microplastics from the soil sample (Sa'adu & Farsang, 2023). Limited research to date has assessed the applicability of different digestion techniques in different soil types, and this area of research needs further investigation. It is suggested that future studies test the applicability of Fenton's reagent to remove organic matter fractions from different soil types to assess recovery rates in a range of soil types.

The current research has found that Fenton's reagent is the optimum method for recovering microplastics in the soil type tested. The research also found that commonly used methods such as

HNO_3 may result in damage to the plastics which makes further categorisation difficult. The damages to polymers as a result of treatments could result in underestimation of plastics in real-world samples and also lead to difficulties in understanding sources of microplastic pollution.

2.5 Conclusion

Considering the three factors reviewed: SOM removal, recovery rates and characterisation, Fenton's reagent was considered the most suitable method for the extraction of plastics from the tested soil. Fenton's reagent protocol offers an optimum method for the removal of SOM, as initially considered in the hypothesis, which is quick and effective, and beneficial for large-scale environmental studies. The findings of this study will be applied in future studies to enable an evaluation of microplastics present within the terrestrial ecosystem. Future studies should address the applicability of Fenton's reagent for different soil types.

Chapter 3 An assessment of the abundance of microplastic pollution in agricultural soils in the Midlands of England.

3.1 Introduction

This study aimed to determine the amounts and types of microplastic pollution found in agricultural fields in the United Kingdom. This will help to inform the following chapters to determine the effects of a common microplastic type on the development of crop species. In the previous chapter (chapter 2), a method was developed to enable the extraction of microplastics from soils, which was employed during this research.

Research has been undertaken in China, Germany, Chile, Sweden and the UK to assess the extent of microplastic pollution in agricultural soils (Liu et al., 2018; Ding et al., 2020; Huang et al., 2020; Harms et al., 2021; Corradini et al., 2021), but much of this research has focused on soils with high levels of pollution, where agricultural practices such as the application of mulch films or sewage sludge result in increased numbers of microplastic pollution (Bläsing & Amelung, 2018; Mai et al., 2018; Huang et al., 2020). The current research aimed to assess the extent of microplastic pollution in conventionally managed agricultural soils without anthropogenic additions, such as sewage sludge and mulch films and compare this to soils with anthropogenic additions. This enabled an assessment of some of the factors which may influence the abundance of microplastics in agricultural soils in the UK.

It has been increasingly suggested that agricultural soils may act as a sink for microplastic pollution (Nizzetto et al., 2016b; Yu et al., 2022; Sajjad et al., 2022), with agricultural practices such as sewage sludge application, mulching films, and the addition of plastics from fertilizer coats resulting in increasing plastic levels in soils (Sajjad et al., 2022). The agricultural system is critical for human survival, providing the vast majority of the world's food supply (Pawlak & Kołodziejczak, 2020); thus, impacts from pollutants can have significant implications for global food security (Xudong, 2011).

Plastics have been extremely beneficial in modern agriculture, making operations cheaper, safer, and more efficient (Food and Agriculture Organization of the United Nations, 2021). Inputs such as fertilizers and pesticides can be used more efficiently when crops are cultivated with plastic tunnels (Robson et al., 2022), with the tunnels helping to reduce inputs of fertilizers and pesticides into the atmosphere (Theurl et al., 2017). Crop yields are enhanced when plastic mulch films and polytunnels are used to help protect crops from pests and extreme weather conditions (Gao et al., 2019). Plastic containers are utilized in transporting and storing agricultural products, making for more cost-effective (Nikiema & Asiedu, 2022). Plastic pipes are commonly used for irrigation and the drainage of fields (Yannopoulos et al., 2020). Clearly, plastics are an integral part of the agricultural system, but due to their extensive usage, the potential for plastics and microplastics to enter the soil environment is greatly increased (Watteau et al., 2018), and as such, an assessment of the levels of microplastics found in soils is greatly needed.

Currently, limited research exists pertaining to real-world levels of microplastics in soils. Most currently published work pertaining to agricultural soils focuses on lands with increased anthropogenic inputs, such as sewage sludge and mulch films (Huang et al., 2020; van den Berg et al., 2020). This means that risk assessments for flora and fauna are difficult in conventionally managed agricultural soils. Nizetto et al. (2016) estimates that between 63,000 and 430,000 tons of microplastics are added to European farmland soils due to the addition of sewage sludge each year. Zubris and Richards (2005) conducted the first study to assess microplastics in farmland soils in the United States of America; the study used synthetic fibres as an indicator of the past spreading of sewage sludge. The research found that fibres were still detectable in the soils 15 years after the sludge application and reported an average count of 1.21 ± 0.25 fibres per g five years after sewage sludge application. Liu et al. (2018) found that shallow farmland soils in China had an average abundance of 0.078 ± 1.29 microplastics per gram from farms which used mulching and sewage sludge. Zhang et al. (2022) assessed the effects of land use on the occurrence of microplastics in soils in Yunnan province, China. The research assessed three different farmland types (facility, traditional

and orchards) and grasslands and woodlands. Zhang et al. (2022) found that facility farmlands had the highest abundance of microplastics, reporting 1236.36 ± 843.18 items per kg. The traditional farmlands and orchard lands had similar levels of microplastics, finding 695.45 ± 429.83 items per kg in the facility farmland and 640.91 ± 927.32 items per kg in the orchard farmlands. The grassland and woodlands were significantly less polluted than the farmlands, with the grasslands having an average abundance of 200 ± 228.85 items per kg and the woodlands 85 ± 22.91 items per kg. The research suggests that microplastic abundance varies according to land usage type and that lands managed for agriculture have higher levels of microplastics than unmanaged lands.

Research from Spain has found that soils treated with sewage sludge had an increase of MPs by 256% compared to those without sewage sludge containing, on average, 710 microplastics per kg additional compared to the control (van den Berg et al., 2020). However, comparatively little research within Europe has focused on typically managed agricultural soils, instead favouring those with high inputs of microplastics. Piehl et al. (2018) investigated conventionally managed fields (rainfed, ploughed, harrowed, sowed, fertilised, herbicide applications and harvesting) and did not receive sewage sludge applications, organic fertilisers or mulch films. The research investigated 0.5 ha of arable land in southern Germany and reported 0.34 plastics per kg of soil. Harms et al. (2021) assessed microplastic pollution in Northern Germany, focusing on conventionally managed fields and reported 3.7 ± 11.9 microplastics per dry weight soil (DW) kg, with 34% of the sampled sites containing microplastics.

The differences between the research conducted by Liu et al. (2018) and Ding et al. (2020) compared to Harms et al. (2021) and Piehl (2018) suggests that conventionally managed farmlands have significantly less microplastic pollution than those that utilise sewage sludge and mulch films. Additionally, it highlights that even in farmlands that do not utilise inputs considered conventional pathways for microplastics, microplastic contamination is still likely present. Further to this, most research to date has focused on arable lands, as opposed to pasture lands, and thus little understanding

exists as to the levels of plastics in these areas (Corradini et al., 2021; Hernández-Arenas et al., 2021; Weber et al., 2022), despite there being likely pathways for microplastics such as from agricultural feeds (Xu et al., 2022).

The present research aimed to investigate the numbers and types of microplastics found in conventionally managed farms in England, specifically in the North-West and midlands. The research also assessed both arable and grazing lands to see if the usage of the field results in differences in the levels of microplastic pollution. Finally, the research assessed whether acknowledged common sources of microplastics, such as compost and sewage sludge, increased the number of microplastics in agricultural soils. The experiment tested the hypotheses that microplastic concentrations would be increased when exposed to anthropogenic additions such as sewage sludge and that microplastic levels would be higher in arable lands compared to pasture due to increased use of plastics.

3.2 Materials and methods

3.2.1 Study area

Farms were recruited to this study by an information page shared in the National Farmers Unions' (NFU) newsletter for the Midlands region (https://www.nfuonline.com/). The NFU represents over 46,000 farms and businesses in England and Wales; thus, the newsletter the NFU shared has a high level of readership throughout England Landholders were encouraged to contact the research team if they were willing to grant access for sampling. Participating landowners would then be contacted, and an assessment of whether the land was suitable for sampling (distance, land usage, land management including fertilizer usage and plastic usage) was conducted. Eight participants gave permission for sampling to occur, with one in Lincoln, one in Derby, one in Shropshire and five in Staffordshire (see figure 1). On-site questionnaires were also conducted with the landowners recording information such as whether fertilisers were used, sewage sludge application times and planting regimes.

Table 3-1: Field use and anthropogenic additions for each of the sites. Where given, the type of crop is also listed and the use of the land for livestock.

Sample site	Field number	Field usage	Anthropogenic additions
1	1	Arable – used for legumes, however rotation of crops occurs.	Sewage sludge added > 25 years ago
1	2	Grazing field for sheep and cows	Occasionally used to hold events
1	3	Grazing field – Pastureland for sheep	Sewage sludge added > 25 years ago
1	4	Arable – used for legumes.	Sewage sludge was added > 25 years ago. Fertilizer added yearly in low amounts
2	1	Maize field with clay-heavy soil	Artificial fertilizer
2	2	Alfalfa field	Bailing occurs in situ
2	3	Arable field	Compost added
2	4	Grazing field – Pasture for sheep	No additions reported
3	1	Arable field - Winter wheat crop	No additions reported
3	2	Pasture field – sheep grazing	No additions reported
3	3	Arable field – Summer barley crop but used for grazing during winter	No additions reported
3	4	Arable field – Maize crop	Visible contamination
4	1	Arable field	Artificial fertilizer
4	2	Arable field	Directly adjacent to the motorway
4	3	Grazing – pasture for cows	No additions reported
4	4	Arable field	Bailing occurs in-situ
5	1	Grazing – Sheep pasture field – Historic	No additions reported
5	2	Grazing – Sheep pasture field – Historic	No additions reported
6	1	Arable field	Sewage sludge applied every four years since 2011 (three treatments)
7	1	Grazing – Pasture for cows	No additions reported
8	1	Arable field	No additions reported
8	2	Arable field	No additions reported
8	3	Pasture field	No additions reported
8	4	Pasture field	No additions reported

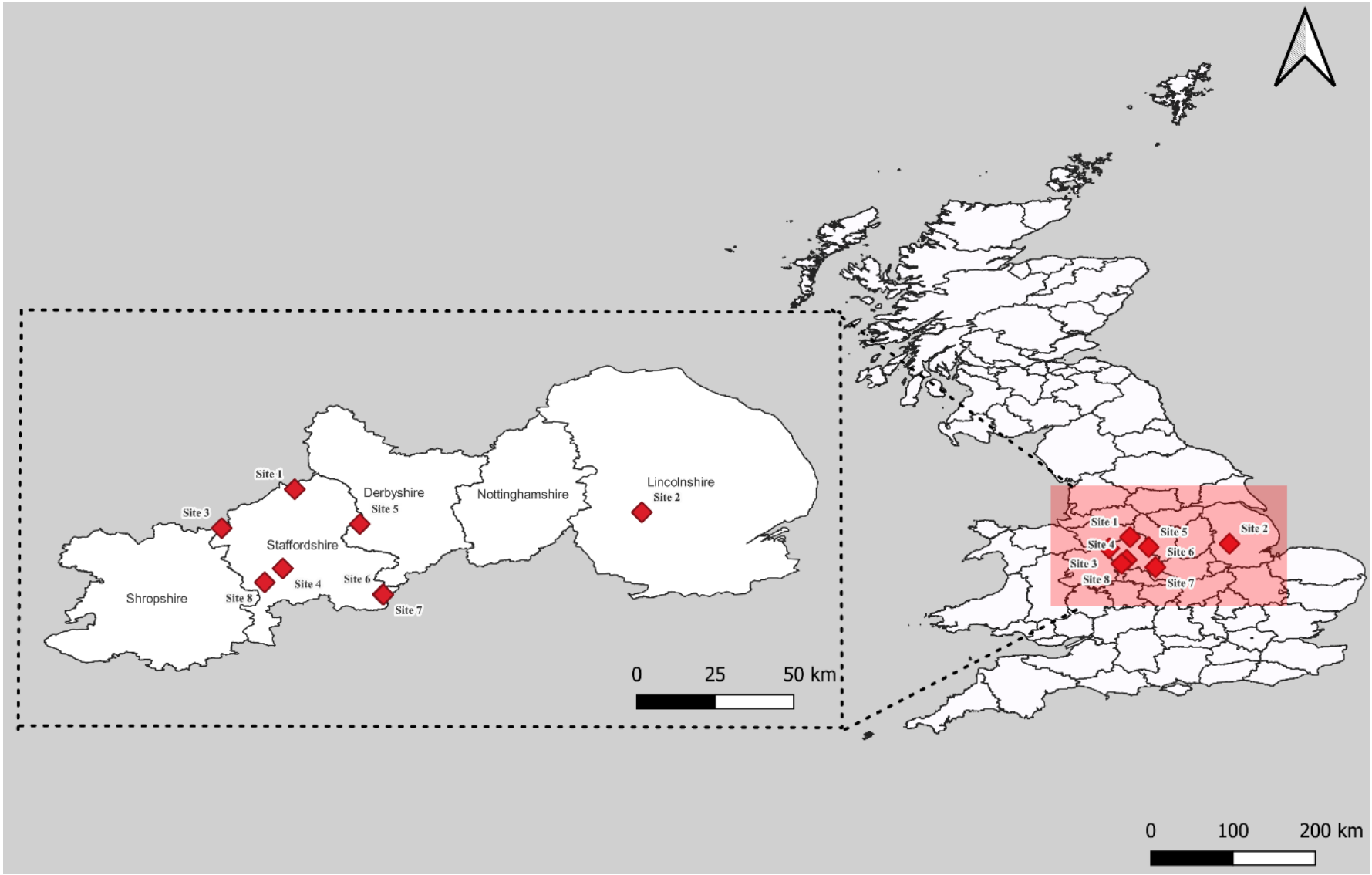


Figure 3.1: shows the sample sites of each farm. The map was created using QGIS (version 3.22.1) (QGIS Development Team, 2019)*. The resolution of this map is at county level to avoid the identification of individual farms.*

3.2.2 Sample collection

A stratified random sampling strategy was employed for the collection of samples. For this, a random number generation app was utilised (Random Number Generator Plus). Two numbers were generated for X and Y between 100 and 1000 for the samples, which gave the number of steps for each sample. If the area was inaccessible, then two new numbers were generated.

Samples were collected between December 2020 and February 2021 from eight sites. The fields sampled were a mix of open field pasture (henceforth referred to as grazing) and arable fields. Five samples were collected from each field, with three in the centrally farmed area and two from the field margins aside from farm two, which had been recently ploughed; thus, the arable soils were collected from within the farmed area (referred to as the central field samples). Soil samples were collected using a steel auger (8 cm width, 20 cm length, 5 cm depth); the surface material was removed from the samples (0 – 2 cm) to remove vegetation. Each sample was packaged into a layer of aluminium foil and then wrapped in two further layers. All samples were labelled, transported to the laboratory, and stored in a – 20 °C refrigerator until analysis was completed. A total of twenty-four individual fields were sampled during the study, ten pasture sites (50 samples, five from each field (2 field margin and 3 central farmed area) and fourteen arable sites (70 samples, five from each field (2 field margin and 3 central farmed area)). The weights of the samples collected varied, with the mean weight of the collected samples being 318 ± 117 g (min = 77 g, max = 576 g, n = 120).

3.2.3 Contamination mitigation whilst sampling

Several in-field contamination mitigation measures were employed. Firstly, all tools used when sampling were stainless steel to avoid contamination from the sampling equipment. All tools (augers and hand trowels) were washed with deionised water between sample collections, and the tools were wrapped with aluminium foil when not in use. When sampling, the researcher wore cotton-based clothing to minimise any contamination from clothing during the sampling process.

3.2.4 Soil type determination

Soil texture was determined using the field texture method (Anderson et al., 1999), where a handful of soil was moistened, and a bolus formed to form a ribbon. Approximately 25 g of soil was used, moistened, and kneaded for between 1 and 2 minutes. The behaviour of the soil during bolus formation and the size of the ribbon produced is then utilised to characterise the field texture. Soil colour was determined using a Munsell colour chart (X-Rite, 2009), and soil type and colour were recorded.

3.2.5 Processing of soil samples

Soil samples were dried in an oven at 40 °C until a consistent weight was achieved. The soil samples were dried in a clean, covered metal tray and then sieved using a soil shaker (Retsch AS20) through a 5 mm sieve (Retsch) to remove large stones and debris. Soils were weighed (A&D Company Ltd, FX1200i), and 250 g of samples were added to beakers which had been cleaned three times using deionised water (blank samples of water were also processed to check for contamination); if soil weights were greater than 250 g (average weight = 317.9 ± 117.3 g, min = 76.9 g, max = 576.1 g), the sample was split into two separate beakers to enable separation of the MPs from the soil.

Microplastics in the sample were then separated from the other substances within the soil by density separation. Zinc bromide ($ZnBr_2$) was prepared as a 25 % saturated solution with a density of 1.7 g/cm^3, following the methodologies of Quinn et al. (2017) (stated in chapter 2, 2.2.6). The samples were stirred using a magnetic stirrer for ten minutes to aid the separation of the plastics. These were then left to settle for an hour to let the heavier particles sink, and the plastics float in the suspension. The supernatant was then sieved through a 63 µm sieve which removed the remaining silt and clay and transferred to a clean 500 ml glass beaker; this process was repeated two times to ensure a more consistent recovery of microplastics (Hurley et al., 2018).

Fenton's reagent ($FeSO_4$), a mixture of 30 % v/v H_2O_2 with an iron catalyst (20 g of iron (II) sulphate heptahydrate in 1 l of RO water – 0.155 m), was used to reduce the remaining organic matter. The

Fenton's reagent was added 10 ml at a time (1:1 ratio of H_2O_2 and the iron catalyst) until reactions (bubbling) stopped; this varied per sample but usually occurred within 6 – 8 hours. Blanks were taken from the reagents used to check for contamination.

After the digestion was completed, the sample was filtered using vacuum filtration with a ceramic Buchner funnel using Whatman 3 cellulose paper (70 mm), which was then added to a clean petri dish. Next, the inside of the Buchner funnel was tape lifted using a single piece of Easylift® tape (Gwinnett et al., 2021). The same piece of tape was used to tape lift the surface of the filter paper by using the adhesive side of the tape repeatedly until the whole filter paper had been tape lifted. If the filter papers contained too much debris, multiple Easylift tapes would be used as this ensured that the tape was not overloaded, making subsequent visual analysis difficult. Finally, the Easylift tape was adhered to a glass microscope slide and labelled with the unique sample number (site number, field number, sample number) using an indelible marker.

3.2.6 Contaminant mitigation during sampling

Measures were taken to ensure the reliability of the results during the treatment process. Samples were always covered with aluminium foil in each experimental step to minimise microplastic contamination from the air. Additionally, ambient contamination was monitored using pieces of Easylift tape with the adhesive side facing upwards. The Easylift tape was left exposed for the duration of the processing and then secured onto glass microscope slides for analysis; these were analysed using polarised light microscopy (PLM – following the same methods as 3.2.8) and then noted as contamination if found in the samples.

Plastic materials were avoided throughout the pre-treatment processes. All glassware and equipment were cleaned in deionised water three times and then wrapped in aluminium foil. Gloves and cotton laboratory coats were used during all experimental steps, with cotton-only clothing worn during processing. Additionally, blank runs were conducted with glassware to assess for contamination, for which no contamination was found in the glassware or deionised water. Before processing the

samples, bench surfaces were cleaned with 2 % Virkon to reduce the likelihood of contamination from the surfaces.

3.2.7 Analysis of microplastics

All samples recovered from the filter papers and the control samples were analysed for their optical, morphological, and chemical properties. All analysis was conducted within the Easylift tape; this minimised the risk of contamination and the loss of samples.

First, slides were searched using a stereomicroscope (Nikon C-Lens) and a grid searching method (Gwinnett & Miller, 2021), and microscopy was conducted in a dark room. Where suspected microplastics were found, a small dot was made on the slide with indelible ink. This enabled easier searching using polarised light microscopy (PLM) (Microtec, RM-1-POL), as any suspected microplastics could be located easier.

The fibres' optical path difference (OPD) was estimated using the interference colours shown using a quarts wedge or a red tint plate (Robertson & Grieve, 1999). Next, the thickness of the fibre was measured in μm using the eyepiece units in the microscope. The OPD and the thickness of the fibre were then used to calculate the birefringence value using the following calculation:

$$\text{OPD (nm)} / (\text{Thickness (μm)} \times 1000)$$

Once the birefringence value is calculated and the sign of elongation determined, this can be compared against published values to provide an identification of the polymer fibre (Robertson & Grieve, 1999b). For fragments, films, spheres and films, the location of the polymer was marked using indelible ink and then analysed using Raman spectroscopy (see section 3.2.9). Plastics were classified as synthetic if they could not be identified via Raman spectroscopy or their birefringence values.

The measurements and photography of microplastics were conducted using a Leica stereo microscope (DM2500P) at x400 magnification, and the morphological features of the microplastic were recorded, including type (fibre, fragment, film, sphere), width (μm), cross-dimensional shape, colour, the presence of delusterant, the sign of elongation and the birefringence. If the fibre type could not be identified using these tables, then Raman spectroscopy was performed to identify the polymer type (see section 3.2.9).

3.2.8 Raman spectroscopy

Raman spectra were collected using a Renishaw inVia confocal Raman Microscope with a Leica microscope attachment. An x20 objective lens was utilised for data collection. A range of different wavelengths was trialled for each microplastic; the excitation wavelength was 514 nm with the laser intensity used including 1%, 5%, 10% and 50 %, the integration time ranging from 5 – 30 seconds. Baseline correction and smoothing of the acquired spectra were performed with the Renishaw WiRE (version 3.4) software. Sample spectra were compared against reference spectra from published literature (An & Flack, 2019; Araujo et al., 2018; Nava et al., 2021).

3.2.9 Statistical analysis

Data were firstly processed using the Waikato Environment for Knowledge Analysis (version 3.8.6) (WEKA), using the random tree algorithm to build a predictive model. The model was trained and evaluated using 120-fold cross-validation. The default settings for the random tree algorithm included using the Gini index as the splitting criterion. After building the predictive model in WEKA, data were processed in R studio version 4.1.2. Data were checked for normality using Shapiro-Wilk tests and for homogeneity of variance using Levene's test from the car package v3.0.12 (Fox & Weisberg, 2021). A Generalized Linear Mixed Model (GLMM) was fitted using the glmmTMB package version 1.1.7 (Brooks et al., 2017). The model assessed the response variable MPs per kg against the predictor

variables field location, field usage and anthropogenic additions with a random intercept term (1 | Sample number) to account for repeated measures of the same field (model equation: MPs per kg + Field location + Field usage + Anthropogenic additions + (1 | sample number). The family was specified as a negative binomial distribution which accounts for overdispersion. Residual distributions were checked to assess model fit.

Pairwise Wilcoxon tests were used from the rstatix package version 0.7.2 (Kassambara, 2023) to perform pairwise comparisons between field usage, anthropogenic additions and field location with respect to MPs per kg. The Pairwise Wilcoxon tests considered the nesting variable sample number to nest the comparisons. The Z statistic was calculated as (test statistic – expected test statistic)/ standard error of the test statistic.

3.3 Results

A total of 285 particles were identified using a mix of PLM and Raman spectroscopy across all the fields sampled (see appendix III for PLM images and spectra). Particle abundances in the sampling units ranged from 0 – 40 MPs per kg DW soil with a mean abundance of 9.21 ± 9.49 MPs per kg (median: 6.42 MPs per kg). Microplastics were found across all sample sites and were found in 85 % (102/120 samples) of the samples across all of the fields. Of these, 86.32 % (246 plastic particles) were microplastics, and 13.68 % (39 plastic particles) were mesoplastics. Of the microplastics, the mean size was 1957 µm ± 1202 µm (min = 149.54 µm, max = 4992.7 µm, median = 1699 µm), the mesoplastics mean size was 9505.9 ± 5611.1 µm (min = 5013.05 µm, max = 30565.64 µm, median = 8027.87 µm). The MP contribution in each size range followed this sequence: < 1000 µm (26.23 %), 1000 – 2000 µm (31.15 %), 2000 – 3000 µm (20.9 %), 3000 – 4000 µm (14.75 %) and 4000 – 5000 µm (6.97 %) (see figure 3.2).

In total, ten different types of polymers were found; of these, polyester was the most common, accounting for 41.05 % (n = 117) of all fibres found (see figure 3.3). The second most common plastic type found was acrylic (20.7 %, n = 59), followed by nylon (8.42 %, n = 23). Variation was demonstrated between the eight farms in relation to the types of plastic found (see Figure 3.4 & 3.5). Of the shape of plastics found, fibres were the dominant shape, making up 95.43 % (n = 272), fragments accounted for 3.16 % (n = 9), and films 1.4 % (n= 4) and no spheres were found within the processed soils.

The majority of the MPs found were colourless (35.44 %, n = 101), with blue being the second most common colour found (22.46 %, n = 63). Grey particles (12.63 %, n = 34), red particles (10.87 %, n = 31) and black particles (10.53 %, n = 30) were also found commonly (see figure 3.6 for a full breakdown of MPs found by colour).

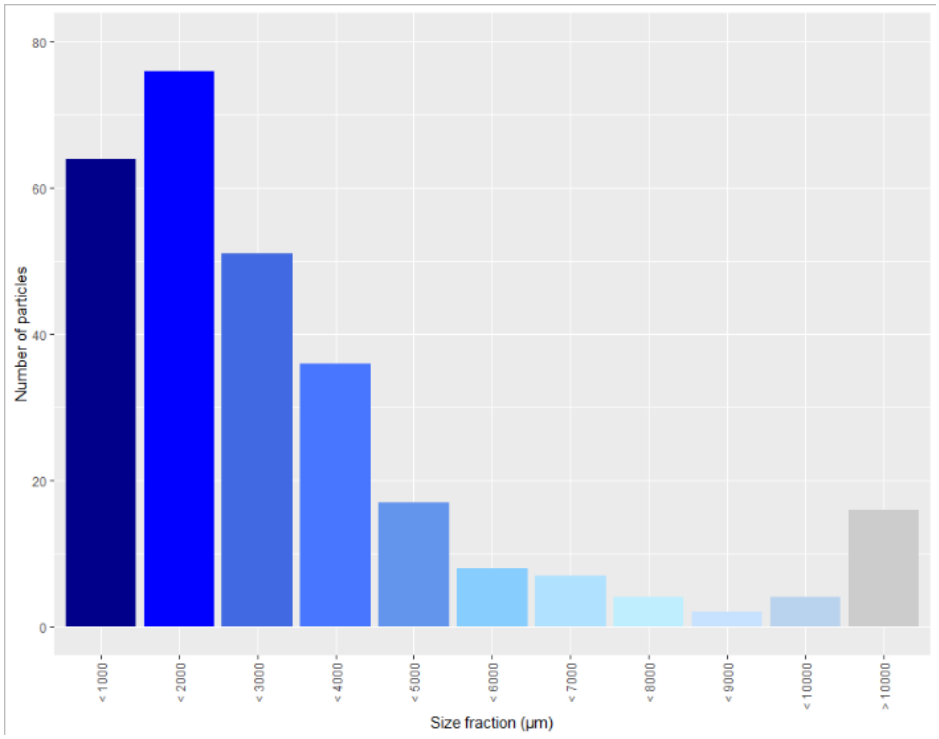


Figure 3.2: The size fractions of the polymers found across the eight sample sites with the number of polymers in each size fraction generally being positively skewed (n = 285).

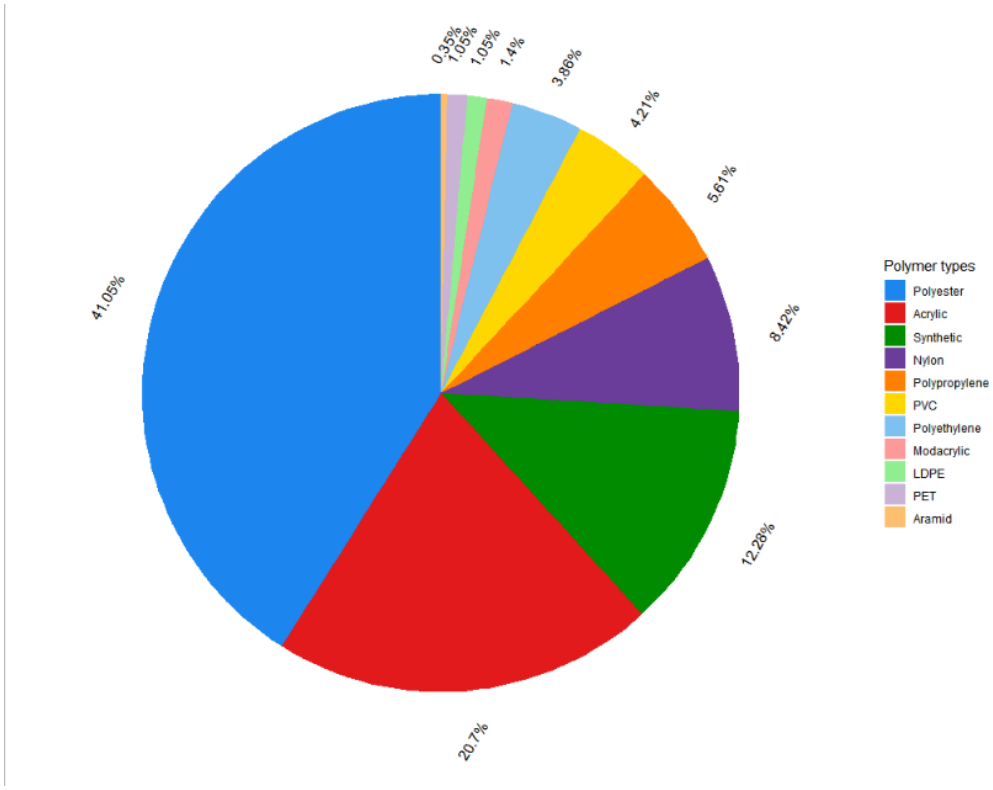


Figure 3.3:The polymer types found across all sample sites as a percentage (n = 285).

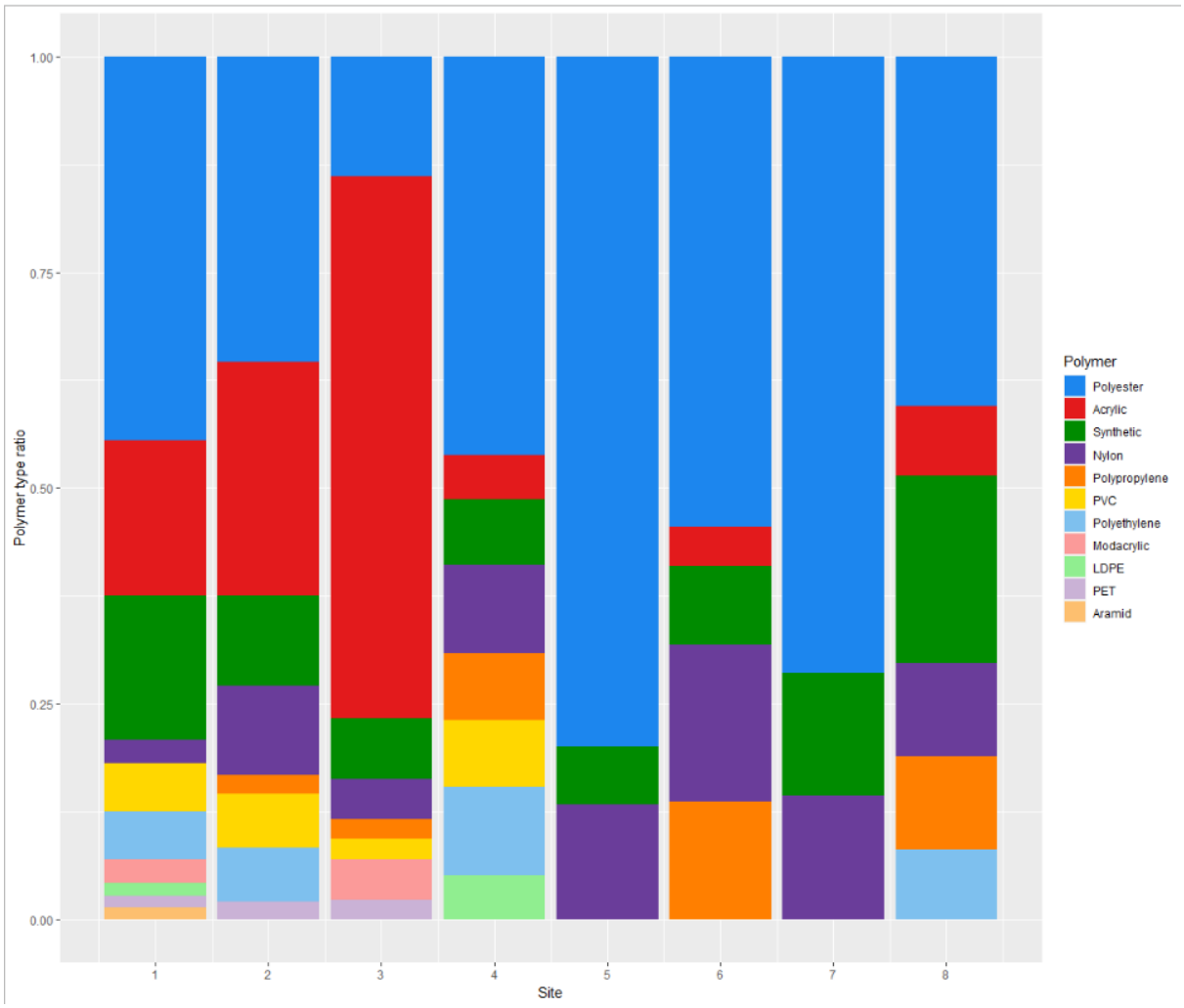


Figure 3.4: demonstrates the occurrence of different polymer types found across the eight sites, n = 285.

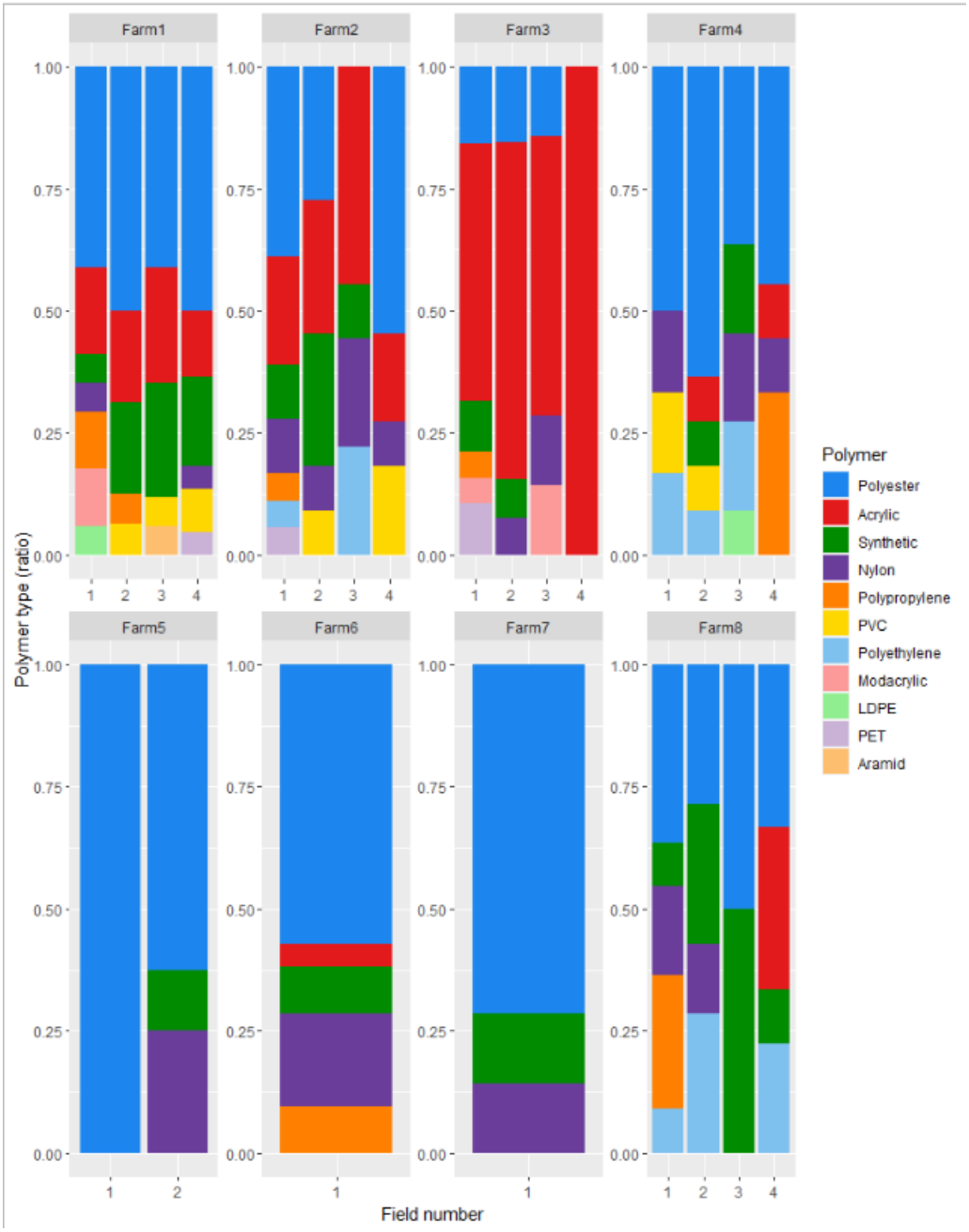


Figure 3.5: The variation of polymers found between fields in each site (N = 5 samples per field). N plastics = 285 and data is displayed as a ratio of the total polymers found in each field.

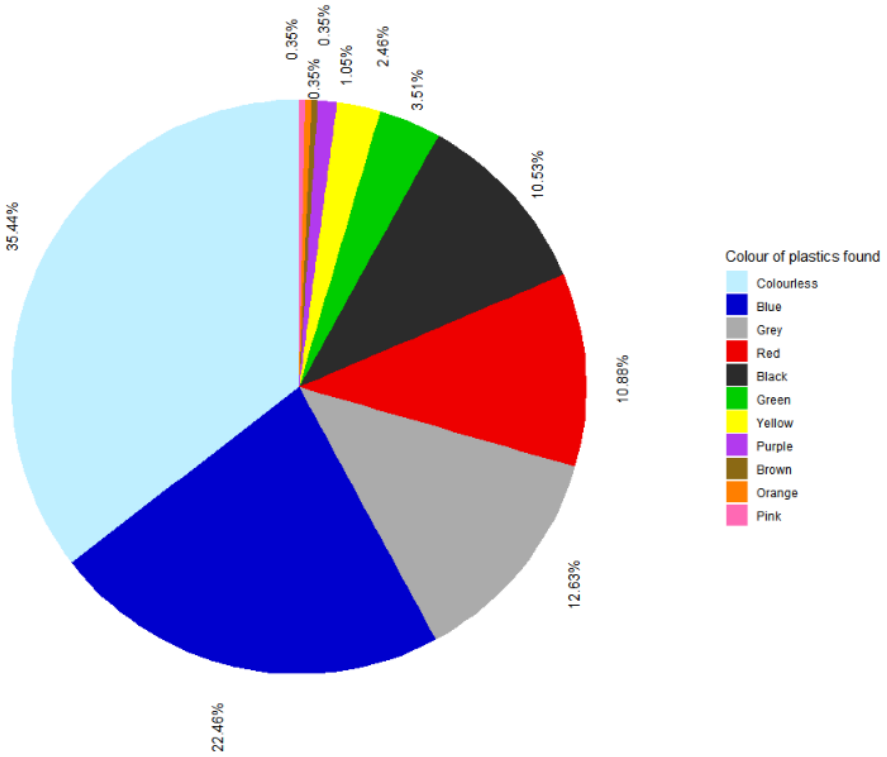


Figure 3.6: the colour of the polymers found across all the sample sites. Colours are ordered from most commonly found to least commonly found (n = 285).

3.3.1 Arable and grazing lands

When considering the two classifications of land usage (arable and grazing), no significant differences were demonstrated between the land usage and abundance of microplastics found (z = - 0.45, p= 0.652, group 1 N = 70, group 2 N = 50) (see figure 3.7). The mean abundance of microplastics per kg was 9.4 ± 10.2 (median = 6.2, min = 0, max = 40) in the arable fields, and the grazing fields' mean abundance of microplastics per kg was 8.9 ± 8.5 (median = 6.5, min = 0, max = 39).

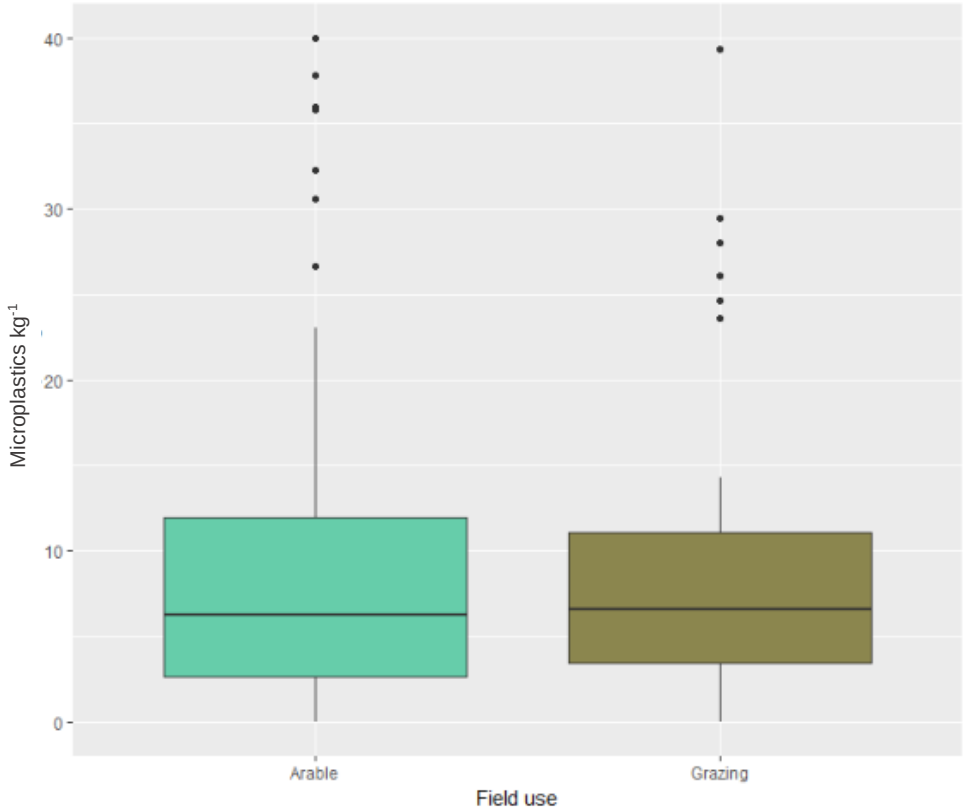


Figure 3.7: the abundance of microplastics found in arable and grazing lands. No significant differences were demonstrated across the two land use types. N for grazing = 50 (5 per field), N for arable = 70 (5 per field).

3.3.2 Farmed land versus field margins

There was a difference between the number of MPs per kg when considering the sampling location within the field; however, this was not statistically significant (z = 1.65, p = 0.098, group 1 N = 78, group 2 N = 42). The farmed areas of the field had an average count of 10.66 ± 10.69 MPs per kg (median = 7.43, min = 0, max = 40), versus the field margins, which had an average count of 6.51 ± 5.93 MPs per kg (median = 5.36, min = 0, max = 26.68).

The difference was more pronounced in the arable fields, which had a mean abundance in the central sampling locations of 11.07 ± 11.32 MPs per kg^{-1} (median = 7.43, min = 0, max = 40) versus the field margins 5.84 ± 5.98 (median = 4.97, min = 0, max = 26.68). In the grazing fields (p = 0.613), the farmed area had a mean abundance of 10.02 ± 9.76 (median = 7.38, min = 0, max = 39.302) and the field margin which had a mean count of 7.25 ± 5.93 (median = 5.83, min = 0, max = 24.68) (See figure 3.8).

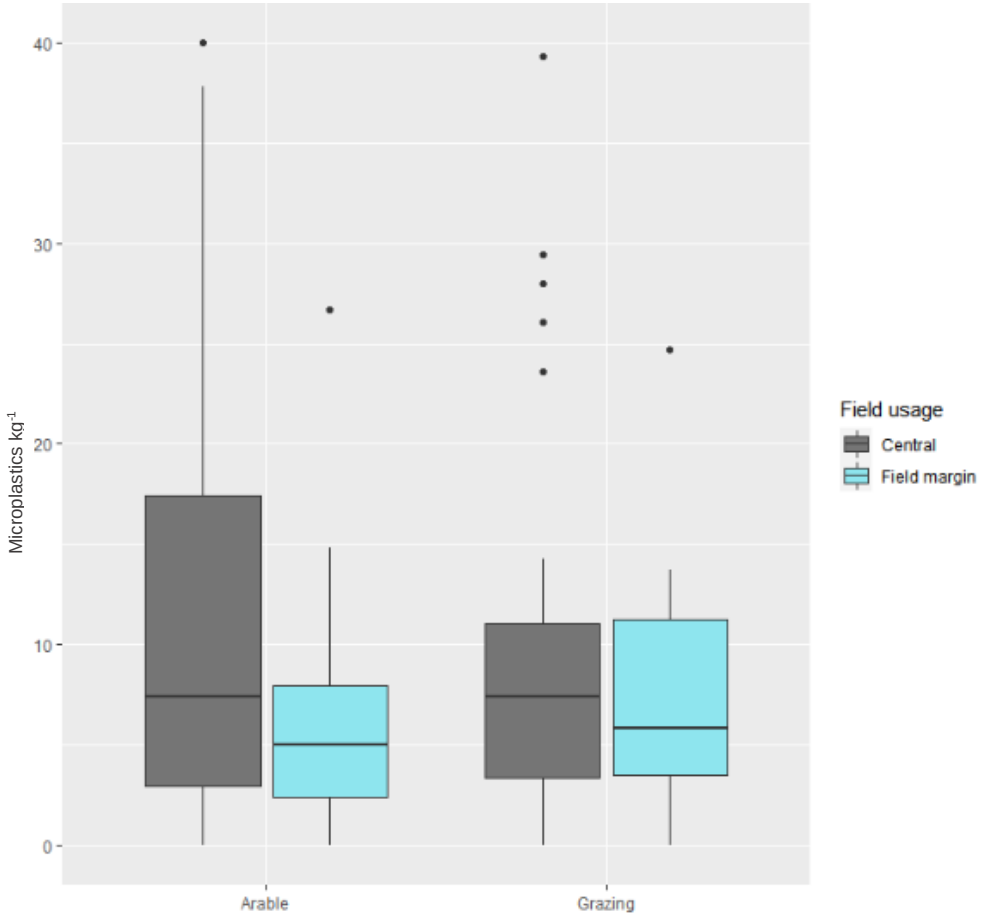


Figure 3.8: The mean abundance of microplastics per kg in the central (farmed areas) versus the field margins across the two land-use types. N for central arable = 48 (3 per field aside from farm 2, which had 5), N for margin arable = 22 (2 per field). N for central grazing = 30 (3 per field), N for margin grazing = 20 (2 per field).

3.3.3 Fields using fertilizers, sludge and compost.

Microplastic counts were significantly higher when the fields had added sewage sludge, fertilisers or compost ($z = -3.49$, $p = 0.0007$, group 1 N = 66, group 2 N = 55) (see figure 3.9). The mean abundance of microplastics in fields which had these additions added was 12.69 ± 11.04 MPs per kg (median = 8.78, min = 0, max = 40 MPs per kg). This was double the abundance of the fields where no known anthropogenic additions were added, with the mean abundance being 6.27 ± 6.72 MPs per kg (median = 5.03, min = 0, max = 39.30 MPs per kg).

In arable fields', those with anthropogenic additions had a mean abundance of 13.01 ± 11.89 MPs per kg DW (median = 8.53, min = 0, max = 40) versus those without had a mean abundance of 4.64 ± 4.03 (median = 4.43, min = 0, max = 14.8). In the grazing fields with anthropogenic additions, there was a mean abundance of 11.84 ± 8.71 (median = 9.68, min = 3.15, max = 29.45 MPs per kg), and those without anthropogenic additions had a mean count of 7.66 ± 8.18 (median = 5.08, min = 0, max = 39.3 MPs per kg).

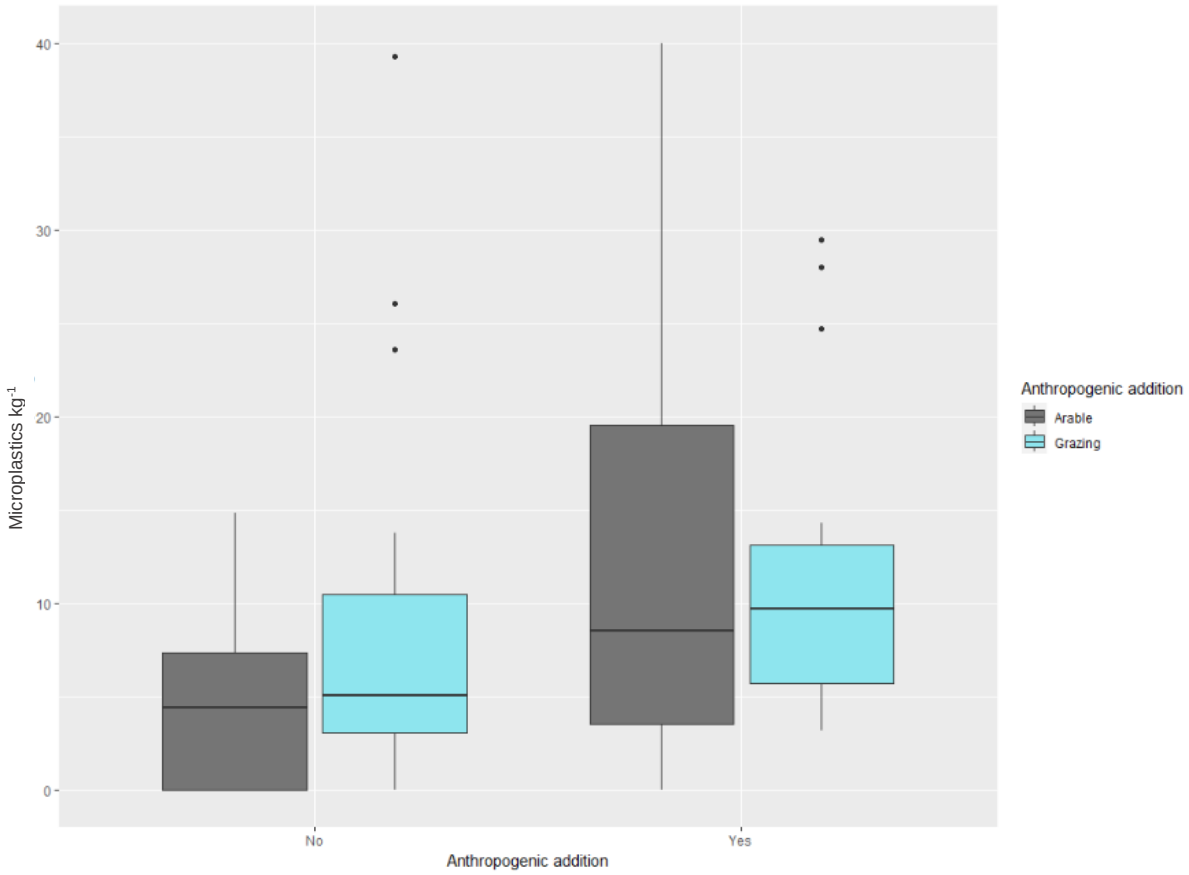


Figure 3.9: The MPs per kg in arable and grazing landscapes with and without anthropogenic additions (sewage sludge, artificial fertilisers and composts). N for arable with anthropogenic additions = 40 (5 per field), N for arable without anthropogenic additions = 30 (5 per field). N for grazing with anthropogenic additions = 15 (5 per field), N for grazing without anthropogenic additions = 35 (5 per field).

3.3.4 Generalised linear models and random forest models

A random forest model was plotted using WEKA (see figure 3.9), which was created to predict the levels of MPs in the soil, which was sampled using three factors (whether there were anthropogenic additions, the sampling location and the field usage). The cross-validation performance was performed 120 times to tune the model, and the model correctly classified instances 33 % of the time.

A GLMM was fitted using the formula: MPs per kg ~ Field location + Field usage + Anthropogenic additions +(1 | Sample number). The model consisted of 120 observations nested within 27 fields. The model had a dispersion parameter of 6.57, estimated using the negative binomial family. The GLMM showed that the intercept was statistically significant (estimate = 1.46, standard error = 0.38, z = 3.83, $p < 0.0001$). Field location was not significant (estimate = 0.29, standard error = 0.17, z = -1.735, p =

0.083), and neither was field usage (estimate 0.27, standard error = 0.22, z = 1.26, p = 0.2). Anthropogenic addition was significant (estimate = 0.72, standard error = 0.22, z = 3.3, p < 0.0001). The Akaike Information Criterion (AIC) for this model was 766.1.

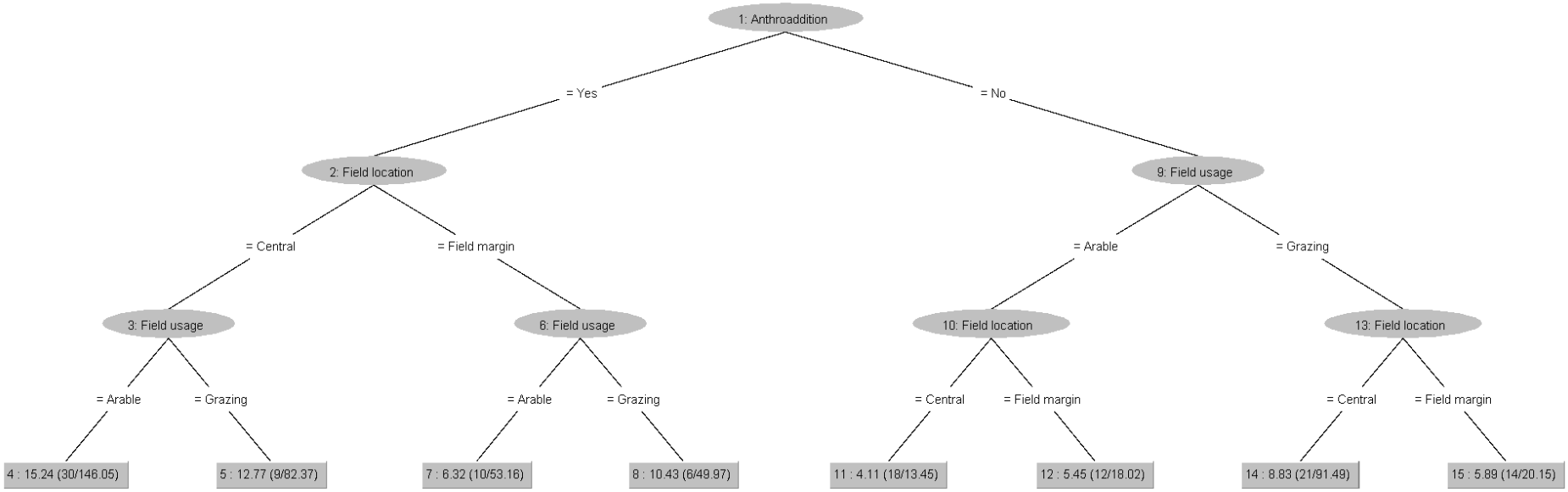


Figure 3.10: A random tree plot produced using WEKA where anthropogenic additions are the best predictor of the level of microplastic pollution in the soils.

3.4 Discussion

3.4.1 Distribution and microplastic abundance

To date, only a few studies have quantified the abundance and composition of microplastics in agricultural soils, with this study representing the first one conducted in the UK considering conventionally managed soils. Microplastics were found in all eight sites; however, the abundance of microplastics varied throughout the sites. The mean abundance of microplastics in conventionally managed lands was eighteen times higher than in studies conducted in Southern Germany in conventionally managed farmland soils (Piehl et al., 2018). This may be partly due to the fact that Piehl et al. (2018) did not assess microplastics < 1 mm, which accounted for 25 % of the microplastics found in this study. As microplastics were found in conventionally managed farmland soils, it is apparent that there are sources other than sewage sludge or mulching films which can account for microplastics in these fields. Inputs of microplastics from runoff from other agricultural lands cannot be discounted in this study and could help to explain some of the polymers found in these conventionally managed fields. Another factor could be the atmospheric deposition of microplastic particles, which Dris et al. (2015) suggested could explain the occurrence of microplastics in urban landscapes. Dris et al. (2015) found that the most common size fraction of microplastics from atmospheric deposition was 200 to 600 μm, which indicates that this cannot account for all the plastic particles in the current study. Another source of microplastics could be the degradation of larger plastic debris (Corcoran, 2021). Ploughing could entrap microplastics into agricultural soils and result in the mechanical degradation of larger particles, resulting in microplastics (Dong et al., 2022). Further research is needed to understand the sources of microplastics in conventionally managed farmland soils.

In China, Liu et al. (2018) studied vegetable farmland in Shanghai, which was cultivated with plastic mulching. This research found 78.0 ± 12.91 MPs per kg DW, which was nearly ten times higher than the average in this study; however, mulch films were not applied to any of the study sites here, and this could account for the much higher volume of plastic in soils. Zhang and Liu (2018) reviewed

croplands in China for the presence of MPs, with concentrations ranging from 7100 to 42,960 particles per kg DW of soil. The study fields were used for intensive vegetable production, with 6 – 8 harvests per year. Due to this, a large number of fertilisers were utilised, and intensive irrigation was practised. Both of these practices are likely major sources of plastics in agricultural soils. Additionally, in Zhang and Liu's (2018) study, greenhouse tunnels made from LDPE were used, which likely contributed to the high numbers of plastics found in agricultural soils, which was far greater than those found in the current study as mulch films and greenhouse tunnels were not used in any fields.

The current study found that soil contamination with MPs was significantly increased when anthropogenic additions (sewage sludge, fertiliser and compost) were applied, which is in agreement with other research (Mahon et al., 2017; Li et al., 2019; Radford et al., 2023). Overall, a 67.72 % increase in MP counts was demonstrated when anthropogenic additions were added. The arable fields showed an increase in MP concentrations by 94.84 % when considering anthropogenic additions versus areas with no anthropogenic additions, suggesting that the management of these fields and the addition of fertilisers and sewage sludge had a significant contribution to the overall plastic load. Grazing fields also demonstrated increases, with an increase of 42.87 % when anthropogenic additions were applied. This suggests that the incorporation of fertilisers, composts and sewage sludge directly contributed to microplastic levels. The variation in the distribution of MPs in the current study could result from the fragmentation of larger plastic debris from ploughing (Campanale et al., 2022). Radford et al, (2023) investigated whether biosolids increased the levels of microplastics found in UK agricultural soils, finding no differences in areas treated with biosolids compared to those without. The authors suggested that this suggested that sewage sludge application was not the sole reason for the increases in microplastics found. However, this is counter to what was found in the current research, which could relate to the fact that our research categorised compost addition, sewage sludge, bailing in the site and artificial fertilizers. These activities could help to explain why there was a significant increase in the concentration of microplastics in these fields.

Additionally, this research found that sample location was important, although not statistically significant. The field margins contained fewer plastics than the central field, indicating that field management leads to an increase in microplastic numbers. Overall, there was a 48.34 % increase in the abundance of plastics in the central field compared to the field margins when arable and grazing fields were combined. In the arable fields, there was a 61.86 % difference in the mean abundance of microplastics per kg in the central sampling locations compared to the field margins, which suggests that management within the fields contributed to the increase in microplastics. There was a 32.08 % increase in microplastics per kg in the central field compared to the field margins in grazing fields. However, the abundance of microplastics was highly variable across fields, which likely explains why the data is not statistically significant; the microplastic abundance is likely strongly influenced by factors not measured in this study, such as time since the last ploughing or local weather conditions.

No significant differences were found across the two land uses (grazing and arable); it was initially hypothesised that arable fields would contain more plastics due to management practices such as ploughing, tilling and fertilisers. The abundance of microplastics in the grazing fields could partly be explained by applying fertilisers, which could explain the 42.87 % increase of microplastics in the grazing fields with anthropogenic additions. Zhang et al. (2022) found that facility farmland soils had the highest abundance of microplastic pollution, finding 1236.36 ± 843.18 items per kg. In orchards, the mean abundance was 640.91 ± 927.32 items per kg, and in traditional farmlands, 695.45 ± 429.83 items per kg. The abundance in traditional farmlands versus orchard farmlands is similar, suggesting similar levels of contamination. This was significantly higher than the numbers found in my study, but this may be due to the area sampled. Yunnan province, where Zhang et al. (2022) conducted this research, had previously been demonstrated to have extremely high levels of microplastic pollution, with Zhang and Liu (2018) finding an average abundance of 18,760 items per kg in the same province.

From this research, it is clear that microplastics are present in UK agricultural soils, and as hypothesised, there is an increase with increased anthropogenic additions. Surprisingly, the levels

between arable fields and grazing fields were similar, as it was initially hypothesised that this would be higher in arable fields due to the increased levels of plastic inputs (Sa'adu & Farsang, 2023). Further information is required regarding the sources of microplastics in agricultural environments, as this may help to elucidate how they are entering agricultural fields. The research adds to the increasing knowledge of concentrations of microplastics in agricultural soils, but further data is needed in the UK to understand the full extent of the problem.

3.4.2 Size type and colour

The most common size of microplastics found was between 1 – 2 mm (31.15 %), and the size distribution of the found plastic debris was left-skewed, indicating an increasing abundance with decreasing size. The fragmentation of larger pieces could explain this into smaller pieces by UV degradation, high temperature and mechanical abrasion; this was also found by Piehl et al. (2018) and Yu et al. (2021). However, it cannot be ruled out that particles may have fragmented into smaller pieces during sample preparation; thus, more single particles may have been detected than were previously present in the soil samples.

Polyester fibres were the most common plastic type found, even in soils without anthropogenic additions. However, the sources for these are unclear; it could be that atmospheric deposition could result in the increase of these lightweight fibres, even in areas without direct anthropogenic additions. Polyester fibres are the most common fibre produced globally and account for approximately 80 % of the synthetic fibre market worldwide (Textile Exchange, 2021); hence it is not surprising that polyester was the most common fibre found in samples. In fibres with anthropogenic additions, it is considered that sewage sludge could have resulted in the addition of these fibres. Other important sources of MPs are considered to be road traffic dust and household waste. Additionally, runoff from the adjacent fields cannot be ruled out, as fields were generally in rural locations with other agricultural properties.

The most common colour of plastics found was colourless (35.44 %); colourless fibres serving as the dominant colour type found was also supported by Piehl et al. (2018) and Yu et al. (2021). Additionally, Yu et al. (2022) also found similar results for both the second (blue – representing 22.46 % in this study) and third (Grey – representing 12.63 %) most common fibre colours found. The different characteristics of colour distribution may imply different sources of MPs, with colourless polymers originating from plastic packaging materials, films and plastic bags and coloured fibres originating from consumer goods such as clothing, ropes and twine used in agriculture and twines (Scarascia-Mugnozza et al., 2012).

3.5 Conclusion

This study has demonstrated that both arable and grazing fields are contaminated with microplastic pollution. Microplastic amounts varied across sites but also varied from other studies. This study reports the first insight into conventionally managed farmland in England, finding that MP contamination here was lower than in China and Chile but at higher levels than within German soils, which are conventionally managed. Additionally, anthropogenic additions could be contributing significantly to the number of microplastics found in soils, as initially hypothesised. Both grazing and arable fields had similar levels of plastic pollution; the most common polymer type found was polyester, with the most common colour being colourless. This data contributes to the establishment of a baseline for the amount, type and size of MP in farmland soils which can help inform future management to help reduce the input of MPs into the agricultural system. However, further research is needed to elucidate the sources of microplastics in UK agricultural soils, which can then be used to help reduce inputs from those sources.

Chapter 4 The impacts of polyester microfibres on the germination and development of agricultural plants

4.1 Introduction

This study was conducted to determine the impacts of polyester microfibres on the germination and early development of crop species. Based on the results of the previous chapter, polyester microfibres were determined to be the most common polymer found in the eight farms surveyed; thus was the polymer used as the focus of this research. This current study aimed to assess whether the addition of a physical contaminant in the soil matrix could result in changes to germination and early plant development.

Studies reviewing the numbers of microplastics in agricultural soils have reported a wide range of concentrations found, with Harms et al. (2021) reporting an average of 3.7 ± 11.9 microplastics per kg, to Ding et al. (2020) finding between 1430 – 3410 microplastics per kg. Some of the highest reported microplastic concentrations in soils come from Fuller and Gautam (2016), who reported levels between 0.3 – 6.7 % w/w microplastics/ soil in industrial soils. Due to the presence of microplastics within agricultural soils, it is important to consider the adverse effects of this pollutant on crop development (Rillig et al., 2017), yet there are a limited number of studies concerning this. In these ~~s~~tudies, the investigation into the effect of microplastics on the germination and development of plants is a common theme. The germination process consists of two main phases. The first phase corresponds to the imbibition of seeds. Phase two is associated with various cellular and biochemical events with increases in metabolic and cellular activity (Wolny et al., 2018). Previous species investigated in terms of the impact of microplastics on their growth and germination include *Triticum aestivum* (Wheat) (Qi et al., 2018), *Lepidium sativum L.* (garden cress) (Bosker et al., 2019; Pignattelli et al., 2020) and *Lolium perenne* (perennial ryegrass) (Boots et al., 2019).

The physiological response of *Lepidium sativum* (garden cress) to different types of microplastics (polypropylene, polyethylene, polyvinyl chloride and a mix of polyethylene and polyvinyl chloride at 0.02 % w/w microplastic to soils) was investigated by Pignattelli et al. (2020). The study found that biometric traits, such as proline production, germination and shoot height, were negatively impacted

by the addition of plastics in the soil. Significant impacts on the germination and root growth of *Lepidium sativum* (garden cress)were also seen by Bosker et al. (2019), who investigated its exposure to polystyrene plastics dyed fluorescent green at varying concentrations (10^3 – 10^7 particles mL^{-1}). This effect was more pronounced with larger size fractions of microplastic (4800 nm). Boots et al. (2019) found that fewer *Lolium perenne* (perennial ryegrass) seeds germinated when in the presence of polylactic acid (PLA), with a reduction of 7 % compared to the control. Additionally, they found that the shoot height of plants exposed to PLA was significantly different, with a 19 % reduction in shoot height compared to the control. Qi et al. (2018) found negative impacts on *Triticum aestivum* (wheat) vegetative and reproductive growth from exposure to polyethylene mulch films and starch-based biodegradable plastics. Research by Wang et al. (2021) found no significant differences in *Vigna radiata* (mung bean) germination rate between the plastic treated and the control group; however, they did find significant differences in the germination rate of *Glycine max* (soybean). Despite these crop studies, the exact mechanisms which cause these impacts are currently not understood, and varied responses are noted throughout the literature; for example, Liwarska-Bizukojc (2022) found no significant differences in *Lepidium sativum* (garden cress) exposed to 0.02 % w/w microplastics, whereas Pignatelli et al., (2020) found *Lepidium sativum (*garden cress) in the same concentration of polypropylene had significantly different germination rates compared to the control. Despite the varied responses noted in the literature, limited explanations as to why responses vary between experiments have been put forward. It has been proposed by Machado et al. (2019) that microfibres may act as a physical soil contaminant and considered here that they may impact the first phase of germination. This may help explain plants' varied responses to microplastics, with the heterogenous distribution causing differences in seed-soil contact. Despite this, no studies have been conducted that investigate whether natural fibres also demonstrate similar impacts. Furthermore, it is currently unknown whether physical blocking responses (Bosker et al., 2019) or physicochemical responses are causing the negative effects demonstrated in many plant species. Thus, further research is needed to investigate whether chemically inert fibres produce similar results to plastic fibres.

Although knowledge is expanding via studies such as those described above, there is still little known about the impact of microplastics on other important European crops, even though they represent a major part of agricultural farming. For example, cereal crops, such as *Hordeum vulgare L*. (Barley), are grown on half of the European Union's (EU) farms, accounting for a quarter of the EU's crop production value. Out of the total production of oilseeds grown in the EU, *Brassica napus L*. (Rapeseed) accounts for nearly two-thirds of the crops grown (58.3%); *Sinapis alba L*. (White mustard) seeds are currently classed as a minor oilseed crop within the EU but are considered as a potential area for economic growth due to its use as a potential bioenergy source (Mitrović et al., 2020).

In this study, we aimed to help fill current knowledge gaps by assessing the impacts of polyester fibres on the germination, root and shoot length and root and shoot weight of four agricultural crop species: *Hordeum vulgare* (Barley), *Triticum aestivum* (wheat), *Brassica napus* (rapeseed) and *Sinapis alba* (mustard). Additionally, we aimed to assess whether any effects demonstrated were solely linked to the polyester microfibres or whether the addition of a relatively inert natural fibre to the soil (with similar morphology to the polyester fibres) acted as a positive control and would also have comparable effects. Finally, this positive control assessed whether a physical response was occurring due to this chemically inert natural fibre changing the structure of the soil. This research hypothesised that microplastics would reduce the germination of the species tested at all concentrations but that when a natural fibre was added, no changes would be demonstrated.

4.2 Materials and methods

Two experiments were conducted for this study:

1. Four crops (*Hordeum vulgare* (Barley), *Triticum aestivum* (wheat), *Sinapis alba* (mustard) and *Brassica napus* (rapeseed)) were exposed to differing concentrations of polyester fibres (the contaminant) to investigate effects on their germination and root and shoot development.
2. Two of the agricultural crop species (*H. vulgare* and *S. alba*) tested in part one were selected (based on results from the first experiment), and the experiment was repeated whilst also utilising a positive control (keratin fibres).

4.2.1 Preparation of fibres

Polyester fibres were obtained from a commercially available source as a polyester fibre spool (Marent Crafts, Marcos Enterprise Ltd, United Kingdom, black polyester) and manually cut using scissors to a mean length of 2.37 mm (maximum = 4.34 mm, minimum = 0.69 mm, thickness = 0.11 mm and n = 100). Polyester fibres were chosen as they represent one of the most abundant types of microplastics found within the environment (Selonen et al., 2020) and account for 52 % of the fibres produced globally in 2020 (Textile Exchange, 2021). The sizes used were based on findings from Zhou (2020) and Yang (2021) that the average size of fibres found in soils are 1 – 3 mm. In addition, Corradini et al. (2021) found that the median length of fibres was 1.6 mm when assessing microplastic occurrence in soils within Chile. This further supports that the sizes utilized in this study are of environmental relevance.

Horsehair was obtained from a commercially available source (Horse Hair extensions, United Kingdom, loose horse hair 18” natural black) and manually cut to a mean length of 2.63 mm (maximum = 4.54 mm, minimum = 0.53 mm, thickness = 0.11 mm, n = 100). Fibres were measured using Moore & Wright callipers (Model number: 110-15DDL) with an accuracy of ± 20 µm. Horsehair was chosen as it is a keratin-based material, which was unlikely to become bioavailable to

plants in the timeframe of the experiment (Vidmar & Vodovnik, 2018). Horsehair fibres will be referred to as keratin fibres henceforth.

4.2.2 Preparation of soil

The soil for experiment one and two were from a commercially available source (Westlands Topsoil, Huntingdon, United Kingdom) and was a clay loam soil with high humus content. The soil was sieved to 2 mm (using a Retsch sieve) to remove any large stones or debris and to homogenise the soil. This was then air-dried at room temperature in a controlled laboratory environment to remove excess water until a constant weight was achieved. Soils were searched manually using the naked eye for any evidence of plastic contamination, and any plastic found was subsequently removed with metal tweezers. Subsequently, 30 g of the soil was weighed into each pot.

4.2.3 Fibre addition to soil

For experiment one, polyester fibres were added to pots at various concentrations (0, 0.1, 0.5, 1, 2 and 5 % w/w). A total of 100 seeds were grown per concentration per species (N = 600 per species, total N = 2400). These concentrations were utilized as they represented the ability to review concentrations of potential environmental relevance based on field survey data with the ability to risk assess high concentrations (Zhang et al., 2018; Zhou et al., 2020; Liu et al., 2018; Huang et al., 2020; Qi et al., 2018). In addition, research by Fuller and Gautam (2016) found concentrations between 0.3 % - 6.7 % w/w plastic in soils; thus, concentrations used in this study were considered environmentally realistic.

The pots were stirred using a clean glass rod and manually shaken; 10 ml of water was added to help homogenise the plastic and soil. As noted by Machado (2019), due to the density of the fibres, true homogenisation of plastics into the soil is difficult but was achieved after approximately 5 minutes of stirring. Samples were stored at room temperature for a maximum of 24 hours before seeds were planted. For experiment two, 5 % w/w polyester and keratin were used based on the concentrations

showing effects on germination in the first experiment, giving a mass of 1.5 g of cut polyester and keratin fibres added for each respective treatment with a total of 10 seeds planted per pot and 10 pots per treatment (N = 100 per treatment, N = 300 per species).

4.2.4 Planting of Seeds and Growth Conditions

S. alba, *H. vulgare,* and *T. aestivum* seeds were sourced from Blades Biological Ltd (Edenbridge, United Kingdom, SKU: ZBB 040, ZBB 005, ZBB 065, respectively). *B. napus* seeds were provided by a local farm. In each treatment, 100 seeds were germinated and grown for four weeks, with ten seeds planted per pot. A total of 600 seeds were planted per species in experiment one, with a total of 2,400 seeds for all treatments and plants. In the second part of the experiment, 100 seeds germinated for each treatment (n = 300 per species, n = 100 per treatment). Plants were grown in an indoor environment, with an average temperature of 16 °C (± 3 °C) and a 10hr:14hr light cycle. All watering was controlled, with plants being watered every two days, ensuring a 60 % water content. Plants were grown for a total of four weeks after the initial germination period (6 weeks total). All plants were grown in biodegradable natural fibre pots purchased from JH Gardening services (Birmingham, UK) with a total volume of 500 ml to eliminate any additional plastics used within the study.

4.2.5 Germination and shoot and root measurements.

Seeds were considered germinated by the emergence of the radicle. Therefore, the number of germinated seeds was recorded every day for the duration of the study. In the case of the monocotyledon seeds (*H. vulgare* and *T. aestivum*), the coleorhiza appeared first; however, seeds were not considered germinated till the appearance of the radicle (Konrad et al., 2020; Luo et al., 2018).

At the end of the four-week growing period, plants were removed from their pots, and each plant was carefully rinsed with distilled water to remove any traces of soil. Next, all surviving plant roots and shoots were measured using Moore and Wright Vernier callipers and recorded for the shoot and root

length prior to drying. Next, plants were separated into the root and shoot portions and air-dried for six weeks before weighing on scales (Mettler Toledo, Pl303) to calculate root and shoot biomass.

4.2.6 Statistical analysis

All statistical analysis was conducted in R v3.6.1 (R Core Team). The data were screened for normality using the Shapiro-Wilks test (stats package v4.1.2, R Core Team) and homogeny of variance using a Levene's test from the car package v3.0.12 (Fox & Weisberg, 2021), as data were not normally distributed the differences in shoot and root lengths and weights were analysed using a Kruskal-Wallis test (stats package) to test with the factor concentration (per plant species). When statistical significance was demonstrated, a Dunn's posthoc test was conducted (with an alpha value of 0.05) using the dunn.test package v1.3.5 (Dinno, 2017). Germination data were tested using a Kruskal-Wallis test for the end point analysis (final germination ratio), and where significance was demonstrated a Dunn's posthoc test was used to assess the difference between the concentrations. All results are presented as the mean ± SD/ SEM (for germination data) and were visualised using ggplot2 v3.4.2 (Wickham, 2016) and arranged using ggpubr version 0.4.0 (Kassambara, 2020).

4.3 Results

4.3.1 Experiment 1:

4.3.1.1 Germination at different concentrations of polyester microfibres

Three species of plant tested showed a statistically significant difference in germination compared to the control at the end point (*B. napus* (χ^2 (5) = 27.397, p < 0.0001), *S. alba* (χ^2 (5) = 23.692, p = 0.00025) and *H. vulgare* (χ^2 (5) = 23.741, p = 0.00025). A Dunn's test was conducted, which indicated that the three species showed a significant reduction in germination when exposed to the highest concentration of polyester microfibres (5 % w/w) compared to the control (p < 0.0001 for all treatments; see figure 4.1). At the highest concentration, *B. napus* germination was reduced by 17 %, and *S. alba* and *H. vulgare* by 8 %. *S. alba* showed effects from doses of 2 % w/w, with *S. alba* showing a reduction of 5 % (p = 0.028).

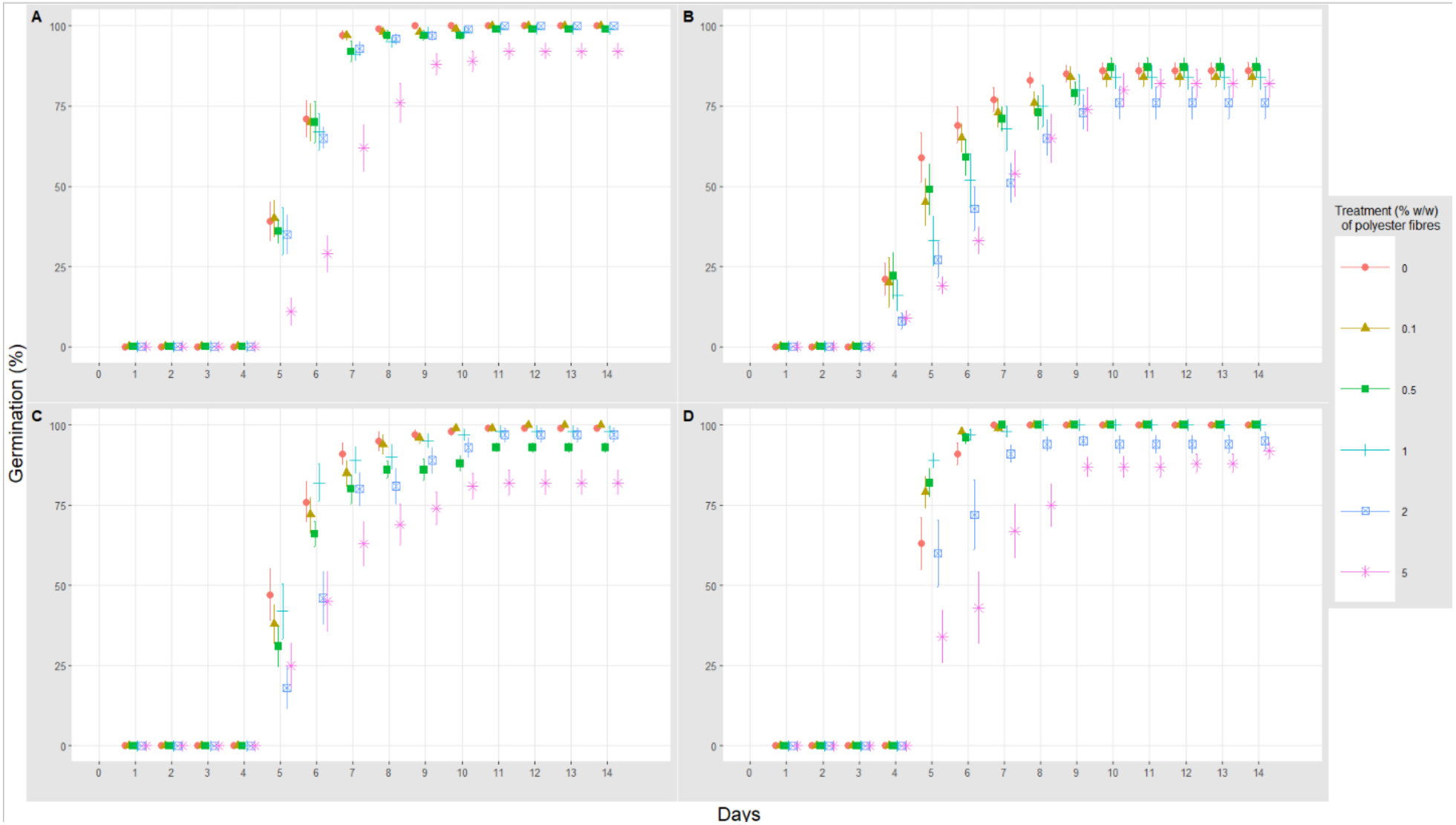


Figure 4.1: The germination of four agricultural crop species in varying concentrations of polyester fibres over time. A shows the germination of H. vulgare. B shows the germination of T. aestivum over time. C represents the germination of B. napus and D represents the germination of S. alba seeds, data is displayed as the mean ± the SEM, n = 100.

4.3.1.2 Shoot and root length

No significant differences were demonstrated in shoot length for the *B. napus* compared to the control ($\chi^2(5) = 9.9691$, $p = 0.076$), *S. alba* ($\chi^2(5) = 8.5062$, $p = 0.13$) or *T. aestivum* ($\chi^2(5) = 5.9546$, $p = 0.31$), a general trend did occur in all groups where the mean shoot length was reduced compared to the control (*T. aestivum* (-4.91 %); *S. alba* (-12.97 %) and *B. napus* (-12.08%). No significant differences were shown in root length for the *B. napus* or *T. aestivum*. However, the *S. alba* showed a statistically significant difference in root length ($\chi^2(5) = 12.407$, $p = 0.03$), with the 0.1 % treatment ($p = 0.004$, 5.34 % decrease in length), the 1 % treatment ($p = 0.004$, 9.63 % decrease in length) and the 5 % treatment ($p = 0.02$, 12.93 % decrease in length) differing from the control.

H. vulgare showed a statistically significant difference in shoot length as determined by a Kruskal Wallis test ($\chi^2(5) = 34.882$, $p < 0.0001$). A Dunn's test indicated significant differences in shoot length at the 5 % polyester fibre concentration, with a shoot length reduction of 12.85 % (26.26 mm) compared to the control. A Dunn's test demonstrated that the 5 % treatment was significantly different to the control ($p = 0.0001$). Additionally, Dunn's test indicated a statistically significant difference between the 0.5 % group and the control ($p = 0.015$), where the length was increased by 4 %.

Root length was also demonstrated to be significantly different for *H. vulgare* ($\chi^2(5) = 13.715$, $p = 0.0175$), where the 0.1 % treatment differed from the control ($p = 0.002$), showing a 9.99 % decrease in length (see figure 4.2 & figure 4.3).

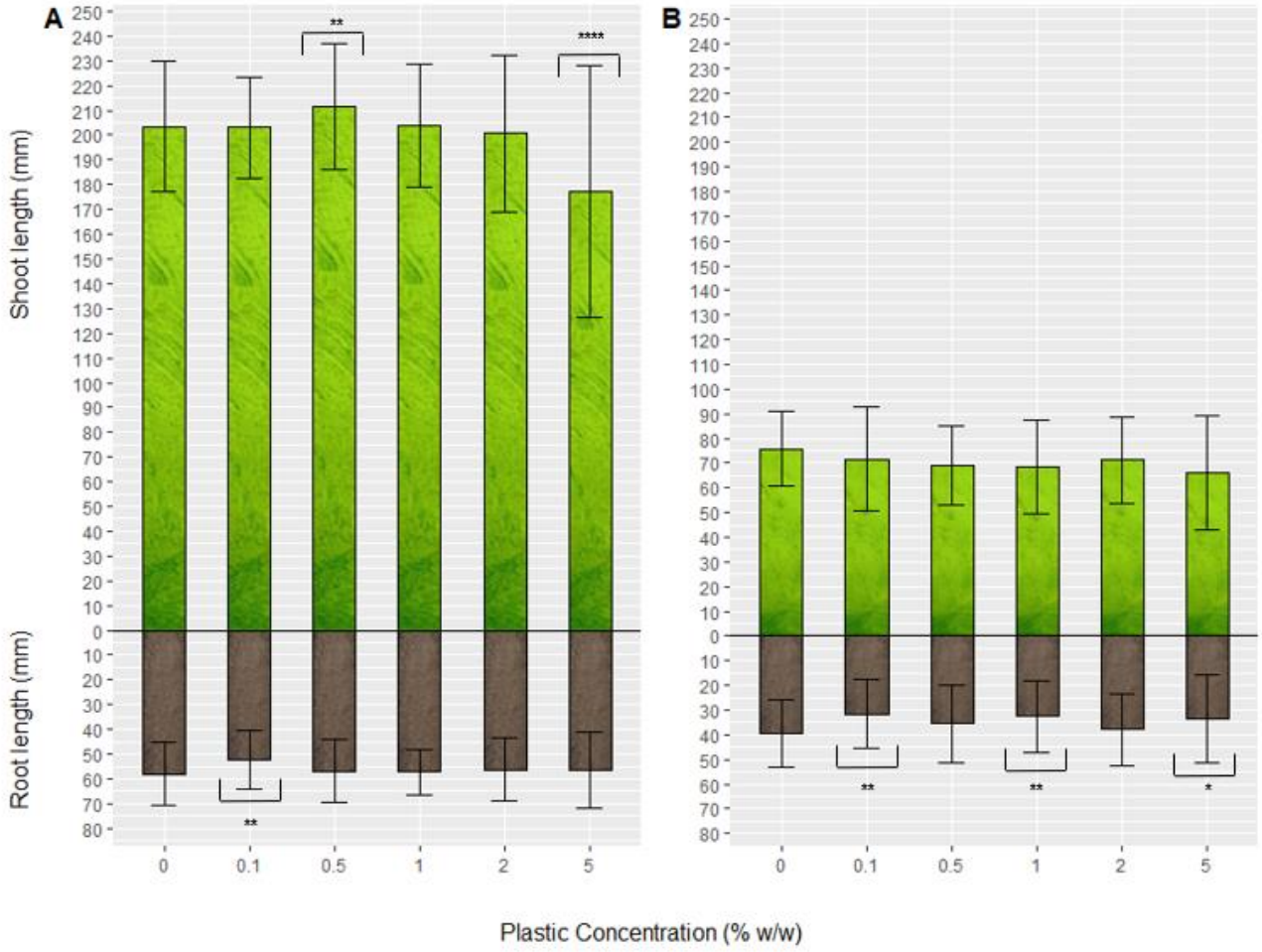


Figure 4.2: A: The shoot and root lengths of H. vulgare at the six concentrations tested. The line intercepting 0 is at soil level, with shoot development above and root development below this line. B demonstrates the root and shoot lengths of S. alba at the six different concentrations. Asterix indicate levels of significance (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001). Data is displayed as the mean ± the standard deviation, N = 100.*

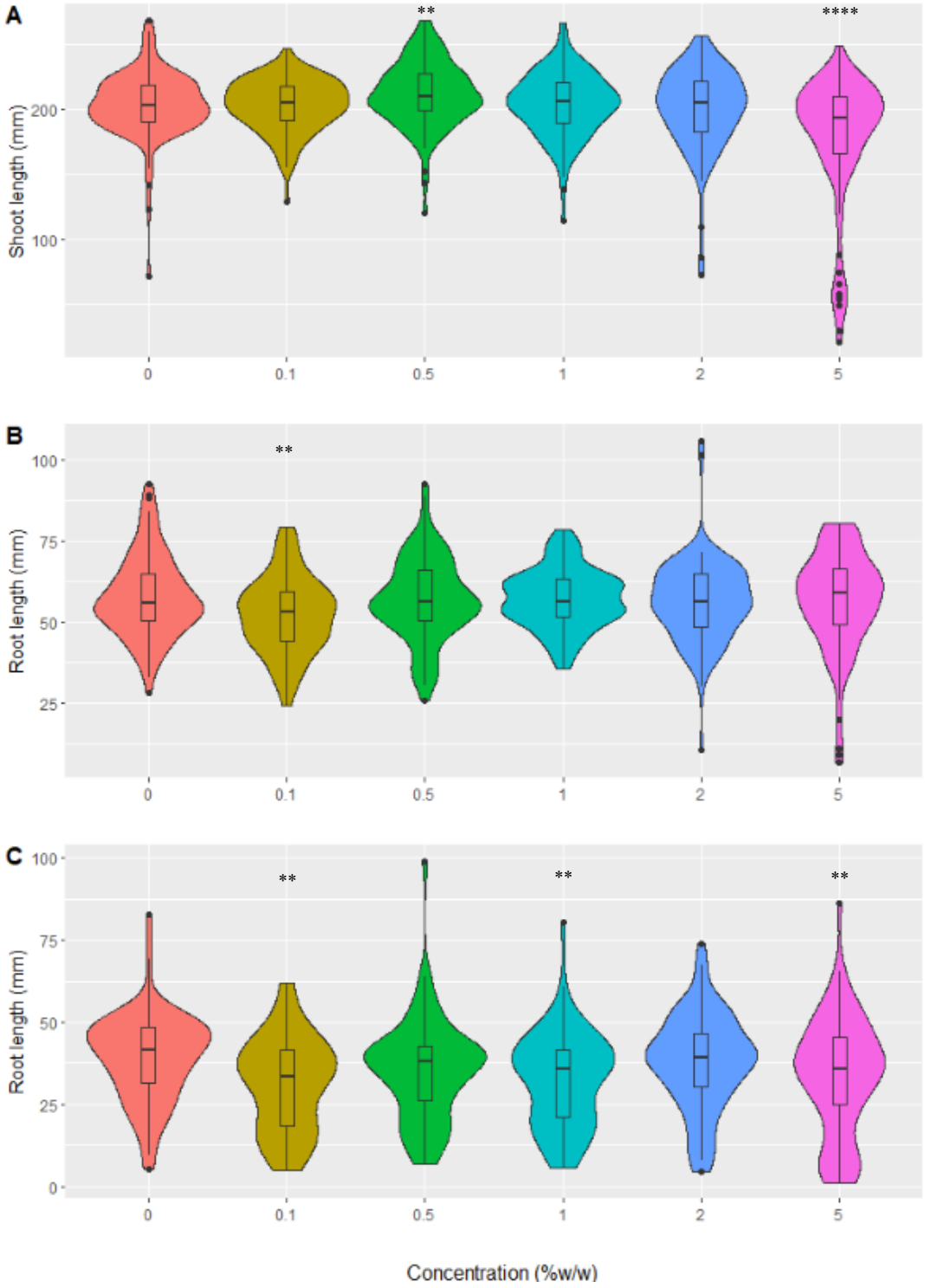


Figure 4.3: Data distribution depicted by violin plots with median, interquartile range and 95% confidence interval overlaid. Dots represent outlying data. A) The shoot length of H. vulgare, B) The root length of H. vulgare, C) The root length of S. alba. Asterix indicate levels of significance (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001). Data is displayed as the mean ± the standard deviation, n = 100.*

4.3.1.3 Root and shoot weight.

A Kruskal Wallis test determined a significant difference in the shoot and root weight of *H. vulgare* compared to the control (Shoot weight, χ^2 (5) = 28.76, p < 0.0001; Root weight, χ^2 (5) = 20.204, p = 0.001). A Dunn's test demonstrated significant differences in shoot weight for the 0.1 % treatment (p = 0.017, with a reduction in weight by 8.032 %) and the 5 % treatment (p < 0.0001, with a reduction in weight by 19.731 %) when compared to the control. The root weight was also significant for the 0.1 % treatment (p = 0.0001, demonstrating a reduction in root weight by 18.498 %) and the 5 % treatment (p = 0.0098, with a reduction in weight by 15.417 %) when compared to the control (see figure 4.4 & 4.5).

T. aestivum dry weight was significant for the root weight (χ^2 (5) = 24.031, p = 0.0002) but not the shoot weight (χ^2 (5) = 9.9314, p = 0.08). For root weight, all concentrations were significantly different from the control. The 0.1 % treatment demonstrated a reduction in weight by 17.652 % (p = 0.0004), the 0.5 % treatment a reduction in weight by 22.014 % (p < 0.0001), the 1 % treatment a reduction of 22.533 % (p < 0.0001), the 2 % treatment 5.012 % (p = 0.0006) and the 5 % a reduction in weight by 5.182 % (p = 0.003).

S. alba root and shoot weights were significantly different (Shoot weight, χ^2 (5) = 17.024, p = 0.00446: Root weight, χ^2 (5) = 27.42, p < 0.0001). *S. alba* showed significant differences in root weight compared to the control for the 0.1 % treatment (p = 0.0043), which saw a 44.19 % increase, and 0.5 % treatment (p = 0.0013) which saw a 37.7 % increase. There were no significant differences in treatments compared to the control for the shoot weights, but the 5 % treatment was significantly different to the 0.1 and 0.5 % treatments (p = 0.0065 and p = 0.0002, respectively).

There were no significant differences in shoot weight (χ^2 (5) = 7.0759, p = 0.22) for *B. napus*; however, root weight was significantly different (χ^2 (5) = 13.332, p = 0.021). A Dunn's test determined that the 5 % group was significantly different to the control (p = 0.0063), showing an 18.9 % decrease in weight.

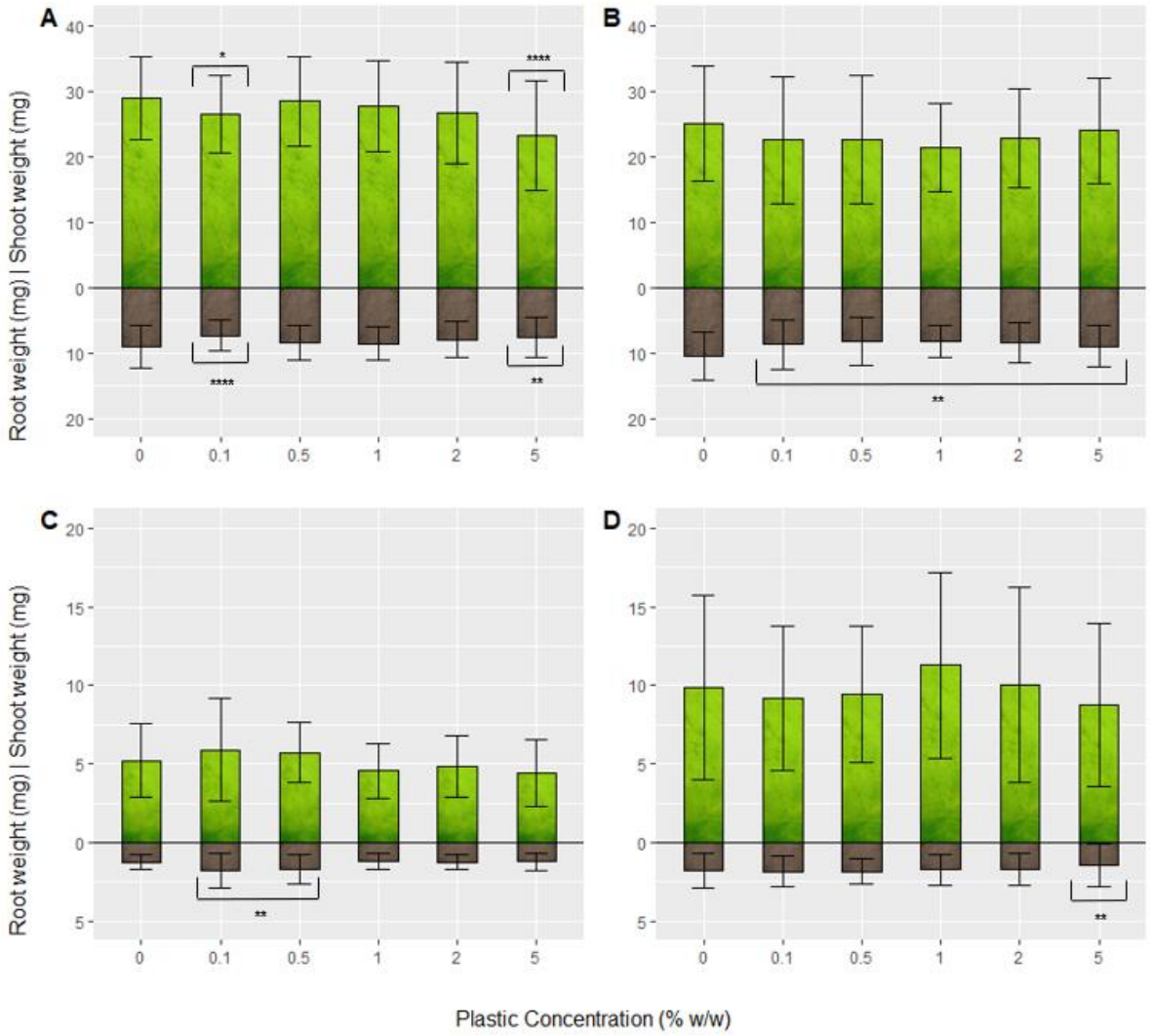


Figure 4.4: The shoot and root biomass of the four species tested with significance highlighted compared to the control. The line intercepting 0 is at soil level, with shoot development above and root development below this line. A demonstrates the root and shoot weight of H. vulgare; B the shoot and root weight of T. aestivum; C root and shoot weights of S. alba and D shoot and root weights of B. napus. Asterix indicate levels of significance compared to the control (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001). Data is displayed as the mean ± the standard deviation, N = 100.*

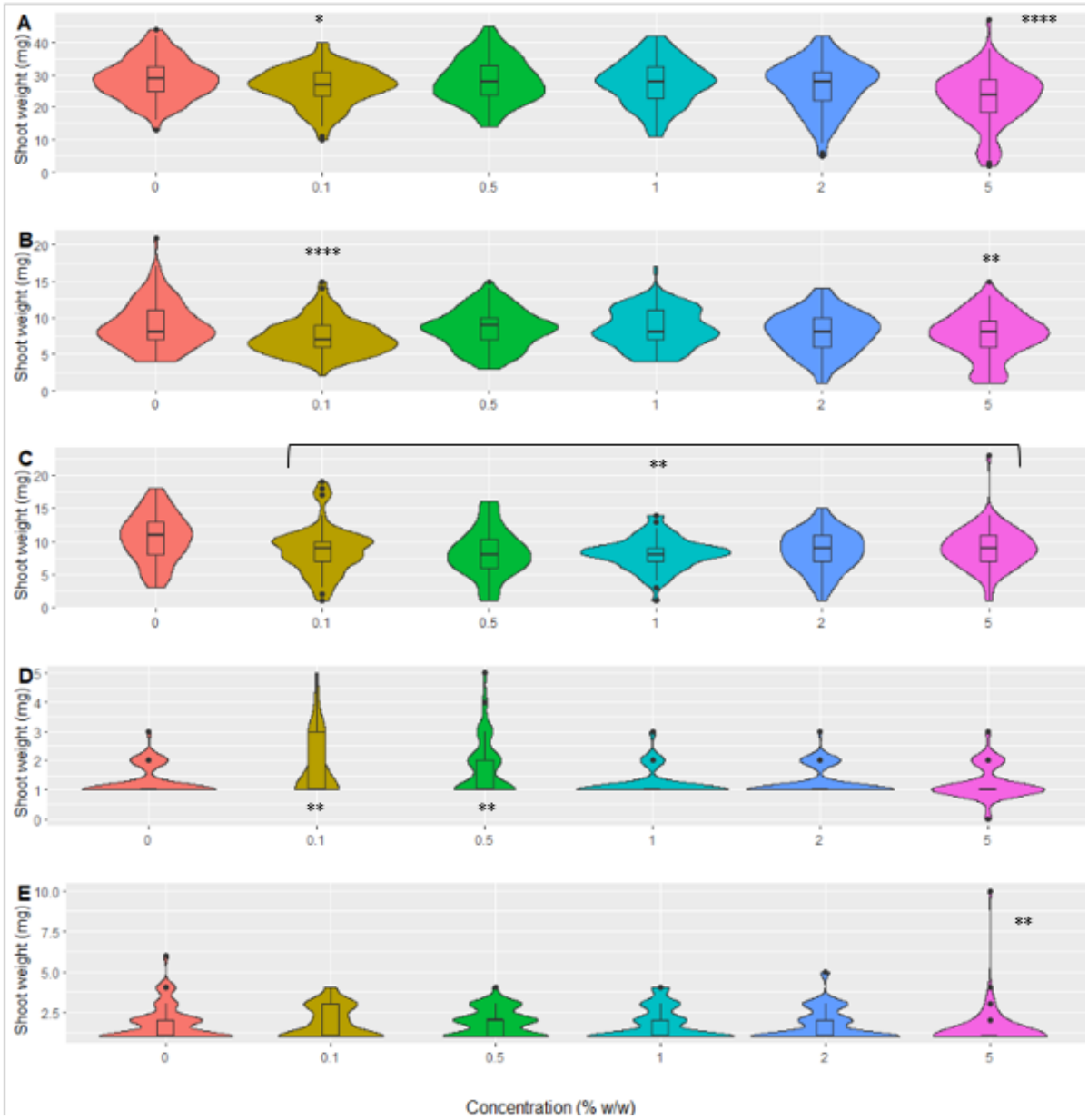


Figure 4.5: Data distribution depicted by violin plots with median, interquartile range and 95% confidence interval overlaid. Dots represent outlying data. A) The shoot length of H. vulgare, B) The root length of H. vulgare, C) The root length of T. aestivum, D) the root length of S. alba and E) the root length of B. napus. Asterix indicate levels of significance compared to the control (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001).*

4.3.2 Experiment 2

4.3.2.1 Germination with polyester and keratin microfibres

S. alba germination was significantly different at the end timepoint (χ^2 (2) = 7.4845, p = 0.02, N = 100 per treatment). Both the keratin treatment (p = 0.0048) and the polyester treatment (p = 0.0198) were significantly different compared to the control. In the final time period, the polyester treatment showed a reduction in germination by 8.79 % and the keratin treatment by 13.19 % (figure 4.6).

H. vulgare showed no significant differences in germination rate compared to the control (χ^2 (2) = 2, p = 0.37, N = 100 per treatment).

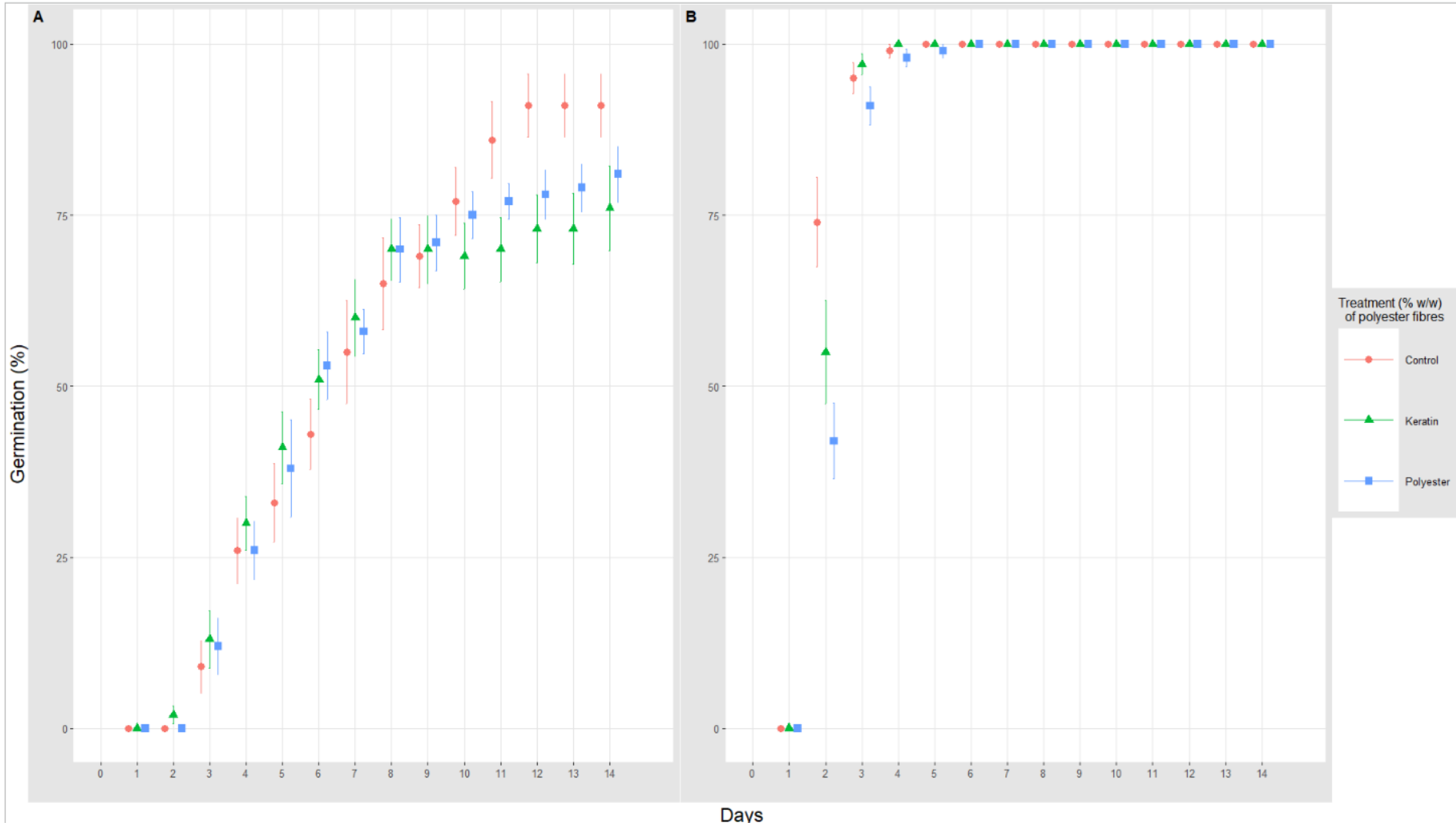


Figure 4.6: the germination of (A) S. alba and (B) H. vulgare when exposed to both polyester and keratin fibres. Data are means ± SEM, N = 100.

4.3.2.2 Root and shoot lengths.

S. alba showed no significant differences to shoot length when exposed to polyester or keratin fibres (χ^2 (2) = 2.3155, p = 0.31). However, *S. alba* root lengths were significantly different as determined by a Kruskal Wallis test (χ^2 (2) = 11.127, p = 0.004). A Dunn's pairwise comparison indicated significant differences between the control and the polyester treatment (p = 0.0097) and the keratin fibre treatment (p = 0.0011). The *S. alba* in the polyester treatment group showed a 54.9 % increase in length compared to the control and a 75.5 % increase compared to the keratin treatment (see figure 4.7 & figure 4.8).

H. vulgare showed no significant differences in shoot length when exposed to either polyester or keratin fibres (χ^2 (2) = 0.48724, p = 0.79). Root lengths were significantly different as determined by a Kruskal Wallis test (χ^2 (2) = 10.847, p = 0.0044). A significant difference was found, indicating that the polyester treatment differed from the control (p = 0.0024) and keratin fibre treatment (p = 0.017). The *H. vulgare* in the polyester treatment group showed a 23.6 % increase in length compared to the control and a 29.5 % increase compared to the keratin treatment.

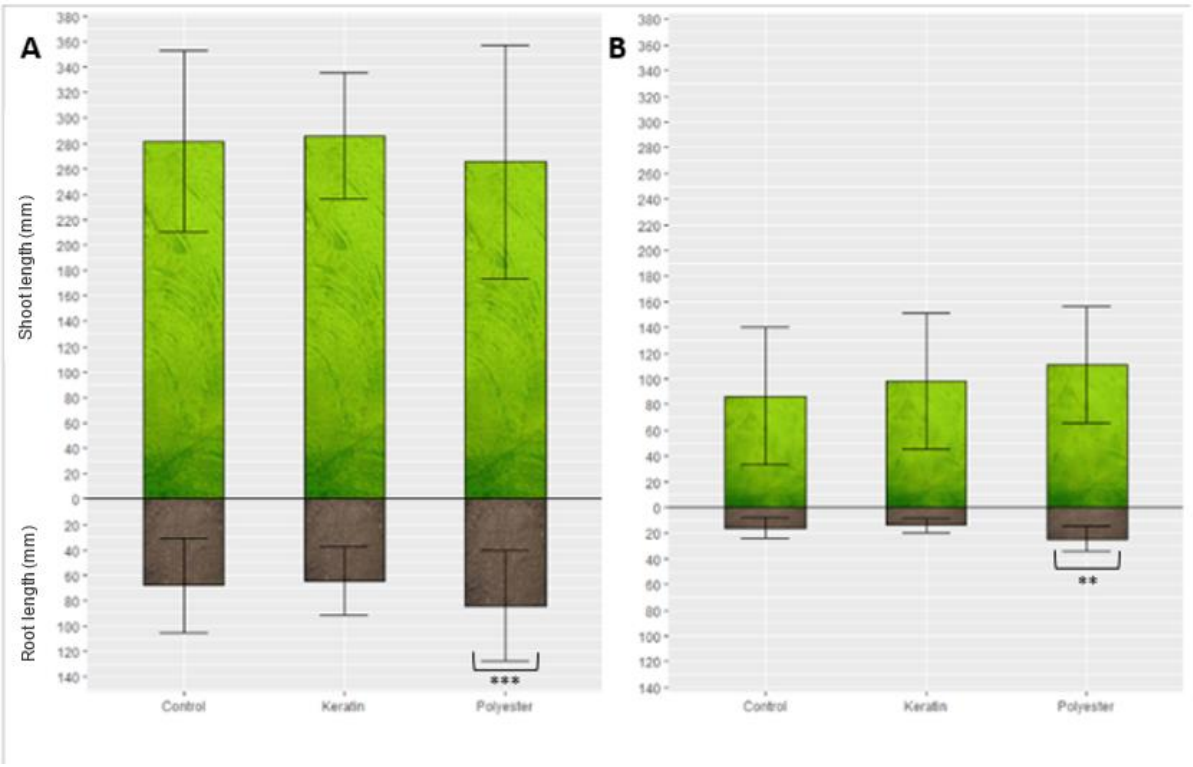


Figure 4.7: The shoot and root lengths of H. vulgare (A) and S. alba (B) with the significance highlighted compared to the control. The line intercepting 0 is at soil level, with shoot development above and root development below this line. Asterix indicate levels of significance (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001). Data are means ± standard deviation, n = 100.*

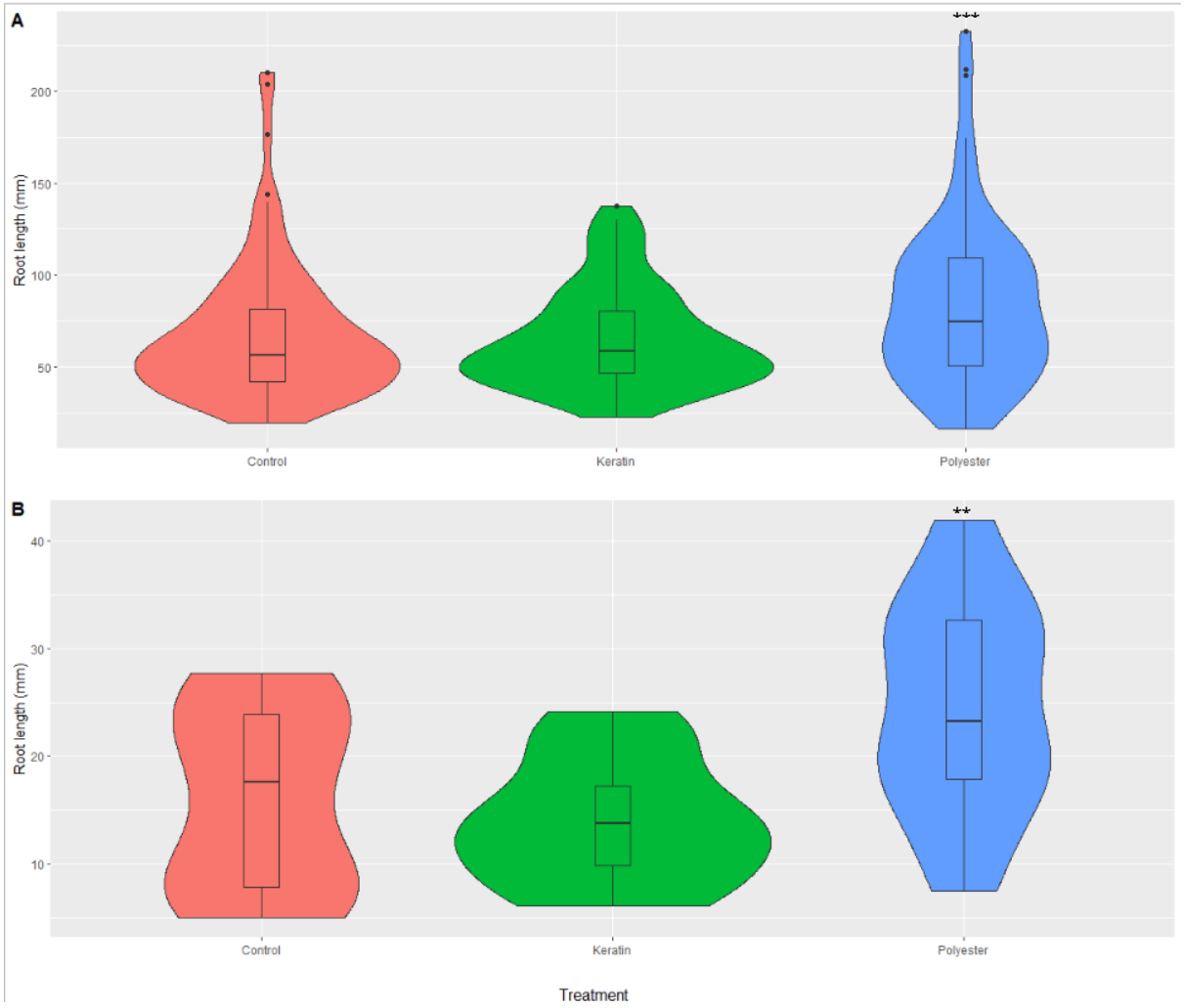


Figure 4.8: Data distribution depicted by violin plots with median, interquartile range and 95% confidence interval overlaid. Dots represent outlying data. A) The root length of H. vulgare, B the root length of S. alba. Asterix indicate levels of significance compared to the control (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001), N = 100.*

4.3.2.3 Root to shoot weight.

H. vulgare showed a significant difference in shoot weight (χ^2 (2) = 10.51, p = 0.0052), where the plastic treatment was significantly different to the keratin treatment (p = 0.0006) but not significantly different compared to the control (p = 0.0714). The control and keratin treatments were significantly different to one another (p = 0.0412). The root weight was significantly different (χ^2 (2) = 21.576, p < 0.0001), with the plastic treatment being significantly different to both the control (p < 0.0001) and the keratin group (p = 0.0017) (see figure 4.9 & 4.10).

There was no significant difference in the shoot weights (χ^2 (2) = 1.0573, p = 0.5894) or root weights (χ^2 (2) = 0.74656, p = 0.6885) of *S. alba* exposed to the different treatments.

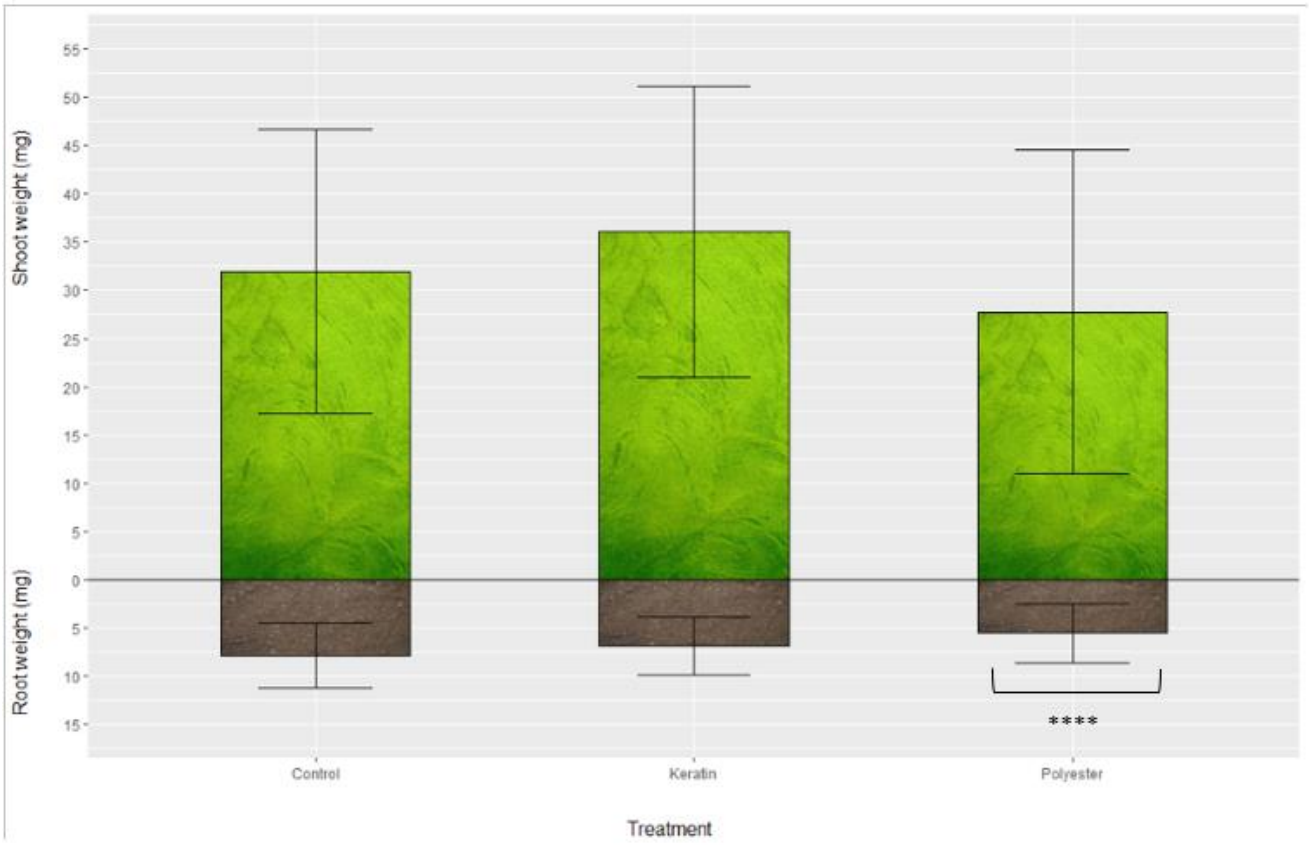


Figure 4.9: The root and shoot weights of H. vulgare with the significance highlighted compared to the control. The line intercepting 0 is at soil level, with shoot development above and root development below this line. Asterix indicate levels of significance (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001). Data are means ± standard deviation, N = 100.*

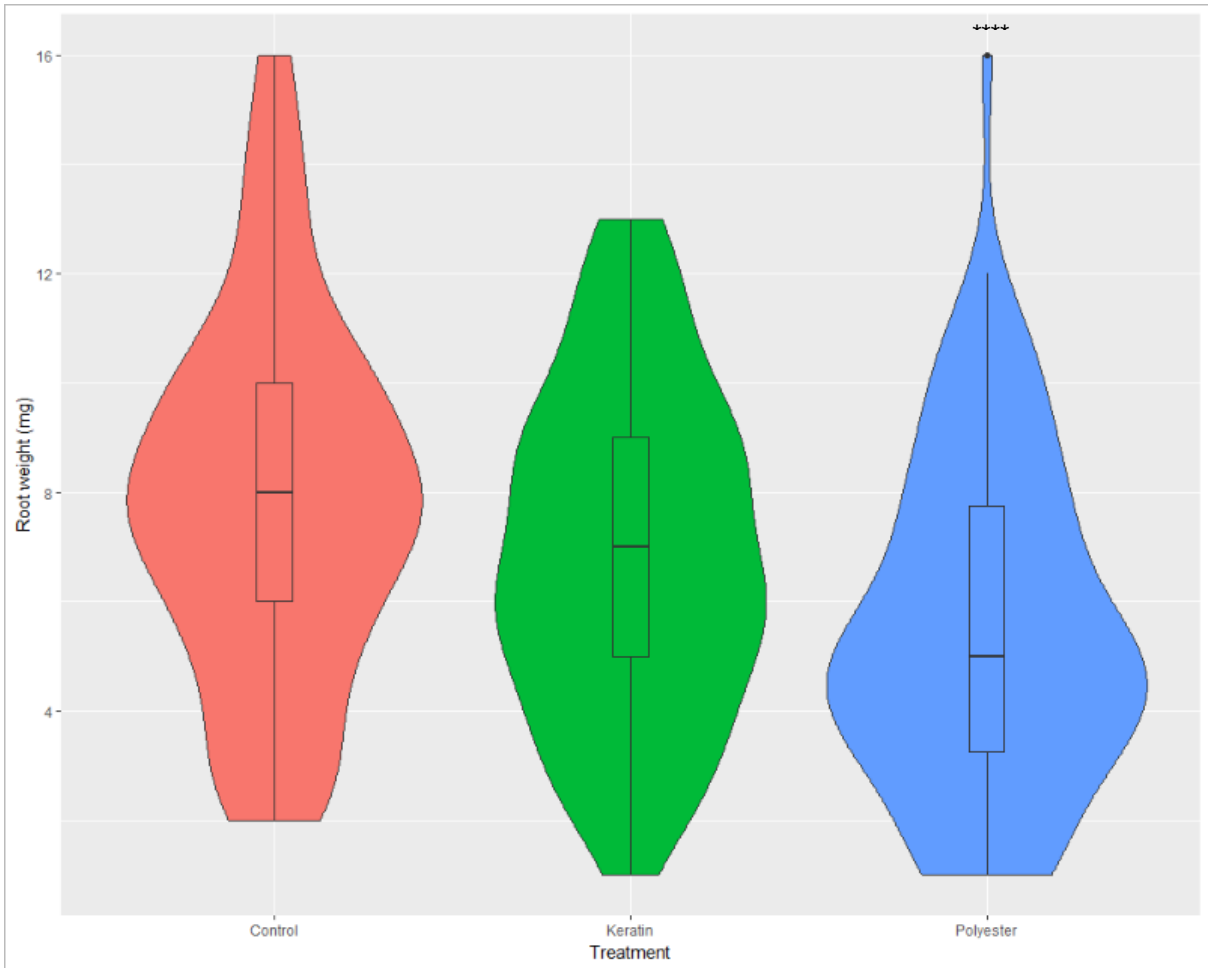


Figure 4.10: The data distribution of the root weight of H. vulgare depicted by violin plots with median, interquartile range and 95% confidence interval overlaid. Dots represent outlying data. Asterix indicate levels of significance compared to the control (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001), N = 100.*

4.4 Discussion

4.4.1 Germination of seeds in different concentrations of polyester fibres

All four species of plants demonstrated significant reductions in germination at the highest concentration of polyester fibres in experiment one. Additionally, in experiment two, *S. alba* germinated in the presence of either polyester or keratin fibres, demonstrating a decrease in germination rate. This indicates that the reduction in germination success was not solely linked to the presence of microplastics but may suggest that changes in the soil matrix from materials of similar structures to the microfibres may lead to reductions in germination success. This study highlights the necessity for appropriate negative/positive controls in microplastic studies and further suggests that future research should endeavour to find a natural material of a similar physical structure to the polymers tested (De Ruijter et al., 2020). This will enable an assessment of whether responses are linked to structural or chemical changes due to the presence of plastics.

Seed germination involves three phases: rapid imbibition of water, reactivation of the metabolism (influenced by external environmental conditions), and the protrusion of the radicle (Ali & Elozeiri, 2017). During germination, seeds are vulnerable to substances in the soil due to the rapid imbibition of water (Parkian 2002). Based on the impact demonstrated in both the polyester and keratin fibre treatments on germination success, changes in the soil structure caused by the addition of fibrous materials are linked to reductions in germination. Changes to soil structure have been demonstrated by Machado et al. (2019), who found that polystyrene fibres decreased soil bulk density and water-stable aggregates. Boots et al, (2019) also found that microplastics changed water-stable aggregates, finding a reduction in the mean weight diameter (MWD) of water-stable aggregates by 24 % due to the addition of microplastic fibres. Boots et al, (2019) also found changes to the profiles of water-stable aggregates, with a reduction in macroaggregates (for HDPE and PLA) and an increase in microaggregates (For fibres, PLA and HDPE) when compared to the control soil.

Conversely, changes in soil bulk density were not demonstrated in research by Zhang et al. (2019), who utilised polyester microfibres. Machado et al. (2019) also found an increase in evapotranspiration by 50 %; however, this was negated due to increased water availability in soils treated with plastic.

Zhang, Zhang and Li (2019) found that polyester fibres of similar sizes to those used in the current study caused a decrease in soil pores of < 30 μm but an increase in larger-sized pores. This research also suggested that the elongated shape of polyester microfibres enabled them to entangle soil particles forming clods, resulting in increased soil macropores. Bosker et al. (2019) noted that the effects on germination were caused by physical blocking, resulting in a slowed uptake of water, which was increasingly pronounced at larger sizes of microplastics.

Another critical factor in the germination of seeds is seed/soil contact, which is necessary for water absorption from soils (Smýkal et al., 2014). The formation of clods and the effects of the linear fibres could result in a reduction in seed-soil contact, which may be more pronounced for smaller seeds; however, clod formation was not measured in this study. This could help explain the differences shown in experiment two for the responses of *S. alba* and *H. vulgare*. Asbestos and microplastic fibres share similar characteristics, such as biopersistence and the ability to fragment into larger sizes (Wieland et al., 2022). Recent research by Charlton-Howard et al, (2023) has even suggested that microplastic fibres can lead to "plasticosis", a fibrotic disease similar to asbestosis. Thus, it is possible that similar effects may be demonstrated to asbestos fibres for the effects on plants. Research by Trivedi and Ahmad (2011) reviewed the impacts of asbestos fibres on the germination of *T. aestivum*, peas and *S. alba*, finding significant reductions for all species as concentrations of asbestos increased. This research suggested a reduction in seed germination due to the partial dehydration of seeds, supporting the idea that a change in available water due to the addition of elongated fibres results in changes in the imbibition phase, thus delaying germination. In the current experiment, the keratin-based fibre showed a lower germination rate than the plastic treatment and the control treatment, further supporting the consideration that changes to the soil matrix may lead to germination delays.

The addition of both natural and polymer fibres resulted in changes to the germination of *S. alba*, suggesting that a physical change in the soil matrix has the capability to impact germination. This demonstrates the importance of using appropriate positive controls to ascertain what impacts are solely linked to microplastics. As far as the author is aware, this is the first time a positive control has been used in germination studies to assess the harm of microplastics.

4.4.2 Varied responses in germination success between experiments

There were differences in the responses of plants tested between the two experiments in terms of germination, with *H. vulgare* showing no changes to the germination success in the second experiment, despite showing a difference in the first experiment. Variable responses have been noted throughout the scientific literature, Liwarska-Bizukojc (2022) found no significant difference in germination in *Sinapis alba* (mustard), of *Lepidium sativum* (Cress) or *Sorghum saccharatum* (sorghum) when exposed to varying concentrations of polypropylene microplastics 0.02, 0.095, 0.48, 2.38 or 11.9 % w/w. This contradicts Pignatelli et al. (2020), who found that *Lepidium sativum* (garden cress) treated with 0.02 % w/w polypropylene had significantly different germination rates. Varied responses could occur due to the fact that seed germination processes are relatively independent of the soil composition as the internal resources can be utilised; however, this still requires that imbibition can occur. Considering different responses throughout studies, it could be that the heterogeneous nature of plastic-contaminated soils could result in varied responses in germination studies as the soil/seed contact is imperative for imbibition. If imbibition is limited, then changes may be demonstrated to the germination rate; however, the heterogeneous nature of soils and the plastic contained within them may lead to differences across experiments. This may also help to explain why more pronounced effects are shown at larger size fractions of microplastics, as demonstrated by Bosker et al. (2019), who found that the larger size fraction of plastic used (4800 nm) had more pronounced effects on relative seed germination than the smaller size fractions (50, 500 nm). However, the particles Bosker et al, (2019) used were at the nanoplastic scale; thus, different responses are expected between nano and microplastics in relation to germination.

4.4.3 Shoot and root development

In experiment one, only *H. vulgare* demonstrated changes to the shoot length compared to the control. Both *H. vulgare* and *S. alba* showed differences in the root length. In terms of biomass, three of the

four species tested demonstrated differences in root weight compared to the control at the highest concentrations. Only *H. vulgare* demonstrated differences in the shoot weights compared to the control. Agathokleous et al. (2019) note that a stimulatory response in one plant trait does not necessarily correlate with a stimulatory response in other traits; hence a change in root biomass and shoot biomass is a response to stress in general.

In experiment two, both *S. alba* and *H. vulgare* showed an elongation of the roots in the indoor environment; this could be linked to the mechanism of root expansion, which plants employ in stressful conditions (Zhang et al., 2020b). The increase in the root system helps to increase water and nutrient uptake, which helps to overcome stress. As the keratin treatment did not show the same effects as the polyester treatment, this could suggest the response is related to changes in the physicochemical properties of the plastic as opposed to the physical contact with the soil, indicating phytotoxicity (Balestri et al., 2019). Alternatively, this could be linked to bulk density changes in the soil, Machado et al. (2019) suggested that polyester fibres lowered bulk density, thus increasing root penetration. However, as keratin fibres and polyester fibres have similar densities (McKittrick et al., 2012; Abbood et al., 2021), it would be expected this would also be found in the keratin treated pots due to similar reductions in soil bulk density (although this was not tested in the current study). Despite plastics being biologically inert, various studies have noted the absorption of organic compounds released from polymers within various crop plants. Fu and Du (2011) found that di-(2-ethylhexyl) phthalate could be up taken by vegetables grown under plastic films. Ma et al. (2014) found that phthalate esters depressed the biomass of mung bean seedlings (*Vigna radiata*) but had no significant effect on germination. Pop et al. (2021) investigated the impacts of Bisphenol A on *Lemna minor* and found that BPA was absorbed in all exposed groups (50 ppm, 100 ppm and 200 ppm), and no BPA was found in the control. Further, the study found that the exposed groups all demonstrated a reduction in chlorophyll levels. This could also impact soil microbiota, as noted by Selonen et al. (2020), who found that enchytraeid (pot worms) reproduction was decreased 30 % in the presence of long polyester fibres in the soil (11,880 µm) at concentrations from 0.17 w/w. This may further impact plant development as soil invertebrates play a crucial role in nutrient cycling.

As this was a pot-based experiment, mechanical or UV degradation is unlikely; however, it is considered that microbial and fungal decomposition could biodegrade plastics, thus making the by-products available to plants. This study did not aim to quantify the degradation of polymers during this experiment; however, this should be further investigated in plant-based studies.

4.5 Conclusion

This study provides evidence of the potentially detrimental effects of microplastics on terrestrial crop plants, with further insights provided as to hypothesised mechanisms in plant germination and growth phases. It has been demonstrated here that a physical response occurs in the germination phase and a physicochemical response during the later phases, though the exact mechanisms by which this occurs are still yet to be understood. In the agricultural setting, such effects may have implications for the production and quality of crop plants via effects to the soil environment and the subsequent effects to plant development.

This study highlights the necessity for appropriate controls to understand where plastic is the primary driver of any effects or whether similar effects could be demonstrated from other natural materials due to changes in the soil matrix. Future studies should also consider what mechanisms these physicochemical changes are occurring and how this leads to stress responses in plants.

Chapter 5 The impacts of plastic leachate on the germination and development of agricultural plants

5.1 Introduction

Following the findings in chapter 4 that polyester microfibres could result in changes to the germination rate and early development of crops when added to the soil matrix, it was decided to assess whether leachates from polyester microfibres could result in similar effects. This enabled an assessment of whether chemical changes or physical changes could be the cause of the results demonstrated in chapter 4.

Plastic polymers are a complex mix of different chemicals, made up of the monomers that form the polymer's backbone and a cocktail of additives used to modify the polymer (Rochman, 2015). Additives can be categorised based on their functional and structural components, with the four recognised classes of additives being fillers, colourants, functional additives, and stabilisers (Gunaalan et al., 2020). Functional additives include substances that modify polymers' physicochemical properties, including plasticisers and flame retardants (Sridharan et al., 2022). Fillers include substances such as pigments and azocolourants, which add colour to the polymer (Quiles et al., 2021). Stabilisers increase the polymers' mechanical resistance and can include substances such as UV stabilisers which reduce the degradation of the polymer when exposed to UV (Yousif & Haddad, 2013). Collectively, these chemicals are termed plastic-related chemicals (PRCs) (Tian et al., 2019). The European Chemical Association has characterised over 400 substances used as plastic additives, some of which are regulated to limit their impacts on human health (ECHA, 2019). Compounds such as phthalate esters, polybrominated diphenyl esters (PBDEs), Bisphenol A (BPA) and hexabromocyclododecane (HBCDs) are currently subjected to bans and restrictions due to their classifications as hazardous substances (Campanale et al., 2020). Thus, the leaching of additives from plastic products discharged into the environment may expose organisms to a complex chemical mixture containing contaminants of emerging concern (CECs) (Zimmermann et al., 2021). Chemicals associated with plastics can be either intentionally added during production, unintentional products from the manufacturing process, by-products of plastic waste recycling or hydrophobic compounds absorbed from surrounding environments (*Gallo et al., 2018*). The fragmentation and degradation of

the polymer may facilitate the release of these additive chemicals, most of which are not covalently bound to the polymers and thus are more prone to be released into the environment (Gunaalan et al., 2020).

Initially, it was assumed that plastic polymers were biologically inert (Verla et al., 2019); therefore, they were considered to have low toxicity, posing no adverse effects to consumers or the environment. However, research has demonstrated that additive chemicals contained within the plastics leach from the polymer as degradation occurs. The leaching of these compounds leads to their accumulation in the water, sediment and soil, which exposes organisms to these compounds in the environment (Campanale et al., 2020). The effects of leachates from plastics on flora and fauna are still unclear, with many of the compounds associated with plastics not yet being subjected to environmental risk assessments (Gunaalan et al., 2020). In recent years, more attention has been focused on toxicity testing leachates from plastics, though much of this pertains to the marine environment (Gandara e Silva et al., 2016; Sarker et al., 2020; Langlet et al., 2020; Mason et al., 2022); thus, further research is needed in sediments and soils.

Gandara e Silva et al. (2016) reviewed the impacts of plastic leachates from virgin polypropylene pellets on the development of larval *Perna perna* (brown mussel) at 0.05, 0.1 and 0.2 % plastic to seawater. This research also reviewed differences between virgin and weathered pellets collected from local beaches; however, all experiments were conducted with leachates and pellets as opposed to leachates alone. The research found that both the virgin pellets and the beached pellet samples resulted in increased dead and abnormal larval mussels, noting that the pellets collected from the beach had higher toxicological effects. The researchers suggested that the difference in toxicity was linked to the different contaminants which may have been adsorbed onto the beached pellets as opposed to just the additives contained within the plastic. However, as the virgin pellets also resulted in in an increase in dead and abnormal mussels it appears that compounds contained within the plastic can lead to adverse toxicological affects in the species tested. The desorbing of compounds from microplastics into plants was noted by Abbasi et al. (2020), who found that zinc, cadmium and lead

absorbed into PET microplastic particles were desorbed into the *T. aestivum* rhizosphere zone. The authors noted that C-heavy metals desorbed more readily than S-heavy metals. Abbasi et al. (2020) did note that desorption rates were low compared to the initially absorbed element and that the microplastics will act as a long-term vector of the pollutant to the plant species. However, it must be considered that although MPs can absorb heavy metals, they are likely reaching an equilibrium with the background levels in the soil (Barus et al., 2021). Thus, unless microplastic particles are transported into different areas (which could happen as a result of sewage sludge application), the absorption and desorption of heavy metals may not result in increased contamination in an area.

Rummel et al. (2022) reviewed the impacts of leachates from virgin PE, PET, PP, PS and additives from electrical waste products (EW) and computer keyboards (KB) produced in artificial seawater on the unicellular green algae *Scenedesmus vacuolatus*. The authors considered Chlorophyll *a* autofluorescence and cell growth to review these impacts, finding that leachate toxicity occurred in the EW and KB groups, which contained high PBDE concentrations, polychlorinated bisphenols (PCBs) and BPA. This suggests that plastic related compounds could result in changes to plant development, causing a physicochemical effect.

Seuront (2018) found that *Littorine littorea* (common periwinkle) exposed to leachates from virgin polypropylene pellets had decreased antipredator responses, suggesting toxicological effects from these leachates. A range of impacts has been demonstrated, from leachates in the marine environment to a range of taxa; however, in comparison to the marine environment, there has been a dearth of research which has considered the impacts of microplastic leachates on the terrestrial system, despite the likelihood of higher levels of plastic contamination.

Research has begun to consider the effects of these leachates on terrestrial flora, with Balestri et al. (2019) assessing the impacts of HDPE and Mater-Bi (biodegradable) bag leachates on the germination of *Lepidium sativum* (cress). No significant differences were found in the total germination of the

seeds; however, the addition of plastic leachates resulted in deformed seedlings. Additionally, inhibitory growth effects were detected in the seedlings, showing differences between the leachate treatments and the controls. The biodegradable polymer resulted in a more significant inhibition in growth than the HDPE. This research suggests that toxicity occurs from the addition of leachates; however, further considerations are needed to understand the physical and chemical toxicity of polymers to various plant species. Additionally, the research highlights that biodegradable polymers may result in higher toxicity, likely due to the increased degradation of the polymer resulting in more compounds being leached from the item.

Menicagli et al. (2019) assessed how biodegradable bags (Mater-bi) leachates affect two coastal dune species, *Thinopyrum junceum* and *Galucium flavum*, reviewing changes to germination and early development. They found that high concentrations of the leachates from the bag materials (low – water to plastic ratio of 100 and high water to plastic ratio of 5) resulted in a higher percentage of abnormalities in the seedlings of *T. junceum* but that low concentrations increased germination rates. In addition, the *G. flavum* seeds germinated earlier in the leachate-treated groups than the control seeds. In terms of early development, this research found that when *T. junceum* seedlings were grown in leachate from virgin plastic bag leachates, they had significantly shorter radicles compared to the control. The same was found in the *G. flavum* seedlings with the leachates from beach-exposed plastic bags. The authors suggested that the increase in germination rates could partly be due to the biological activity of BPA present in the water during the germination phase, which has been shown in low concentrations to promote plant growth. They also suggest that changes to the early development of plants could be related to chemical compounds that had migrated from the bags, which could adversely affect plant growth. This suggests that plastic particles in the environment could have the potential to carry pollutants which result in changes to plant development; this, coupled with the compounds contained within the plastics which could leach, could result in a complex chemical mixture.

Pflugmacher et al. (2020) reviewed the impacts of new and artificially aged microplastics and leachates on the germination of *Lepidium sativum* (cress). In a substrate-free environment, diluted (1:10) polycarbonate leachates caused a 20 % reduction in germination. However, when plants were germinated in a substrate, the research found inhibition of germination by 21 % compared to the controls. This research suggests that there is potential for chemical effects from plastic leachates on terrestrial flora, and further research is required to assess risks from other common plastic polymers.

In the current research, an investigation of the impacts of leachates from polyester fibres on the germination of four agricultural crop species was conducted, reviewing leachate and the use of a positive control (keratin) to assess whether there are effects from both the physical and chemical changes caused by microplastics in soils. First, this research tested the hypothesis that germination would be impacted by the addition of a physical contaminant but not by chemicals released from the plastics. Secondly, the research tested the hypothesis that the growth of the plants could be changed by chemicals contained within the plastics, and there would be a combined effect when considering the addition of a physical contaminant and a chemical contaminant.

5.2 Materials and methods

The following treatments were used in this study (see table one for a summary):

- Soil control (water added to sand, soil, and gravel, then collected once filtered through); henceforth referred to as soil control.
- Soil with polyester leachate (water added to sand, soil, gravel and 5 % polyester fibre, then collected once filtered through); henceforth referred to as soil and polyester.
- Keratin control (water added to sand, soil, and gravel, then collected once filtered through) is referred to as keratin control.
- Keratin fibres and soil with polyester leachate (water added to sand, soil, gravel and 5 % polyester fibre, then collected once filtered through) referred to as keratin and polyester.

This enabled an assessment of both the potential physical and chemical effects of microplastics on the growth and development of the four agricultural crop species tested (*Hordeum vulgare* (barley), *Triticum aestivum* (wheat), *Sinapis alba* (mustard) and *Brassica napus* (rapeseed)).

Table 5-1: The four different treatments used during the experiment.

Treatment	Abbreviation	Description
Soil control	SC	Soil with no keratin fibres. Control leachate used (No plastic in mesocosm set up)
Soil + polyester leachate	SP	Soil with no keratin fibres. Plastic leachate used (Plastic in mesocosm set up).
Keratin control	KC	Soil with 5 % w/w keratin fibres. Control leachate used (No plastic in mesocosm set up).
Keratin + polyester leachate	KP	Soil with 5 % w/w keratin fibres. Plastic leachate used (Plastic in mesocosm set up).

5.2.1 Preparation of fibres

Polyester fibres were obtained from a commercially available source as a polyester fibre spool (Marent Crafts, Marcos Enterprise Ltd, United Kingdom, black polyester) and manually cut using scissors to a mean length of 2890.02 ± 1040.67 μm. Polyester fibres were used due to them being the most prevalent fibre found in Chapter 3 (assessment of microplastics in agricultural fields).

Horsehair, henceforth referred to as keratin, was obtained from a commercially available source (Horsehair extensions, United Kingdom, loose horsehair 18" natural black) and manually cut to a mean length of 2897.44 ± 843.47 µm. Both polyester and keratin fibre measurements were calculated using Leica application suite X (version 3.0.14.23224).

5.2.2 Soil preparation

The soil for this experiment was purchased from a commercially available source (Westlands Topsoil, Huntingdon, United Kingdom) and was a clay loam soil with high humus content. The soil was sieved to 2 mm (using a Retsch sieve) to remove any large stones or debris and to homogenise the soil. This was then air-dried at room temperature in a controlled laboratory environment to remove excess water until a constant weight was achieved. Subsequently, 50 g of the soil was weighed into each pot (N = 20 pots per treatment, N = 240, N of plants = 400 per species). Soils were searched manually using the naked eye for any evidence of plastic contamination, and any plastic found was subsequently removed with metal tweezers.

5.2.3 Leachate preparation

Polyester fibres were used to produce leachate at a concentration of 5 % w/w soil: plastic, which was used to produce the water to water the plants. Plastic was added to terracotta pots with a drainage hole containing a layer of gravel, sand, and soil (see figure 5.1). This provided a similar matrix to real-world conditions to produce leachate. To each terracotta pot, 400 g of gravel, 200 g of sand, and 1 kg of soil were added. For the 5 % plastic leachate, the soil was mixed with 50 g of the cut polyester fibres, which had been exposed to the sun for two weeks prior to the experiment starting and mixed using a clean glass rod for five minutes to homogenise the soil and plastic. Pots were left in full sun and watered weekly with two litres of water per terracotta pot (Four control pots to produce the leachate with soil, sand and gravel and four plastic treatment pots containing 5 % w/w polyester: soil, sand and gravel). Water was collected in 1 l glass beakers, 6 ml was removed and decanted into three

2 ml vials for Liquid Chromatography-Mass Spectrometry (LC-MS). Leachate was produced for six weeks prior to the start of the germination toxicity experiment and produced for the following six weeks giving a total of 12 weeks of collection for LC-MS analysis (N = 72 for total leachate samples collected). Seeds were planted in biodegradable pots (as described in section 2.5.6), and the collected water was used to water each of these pots.

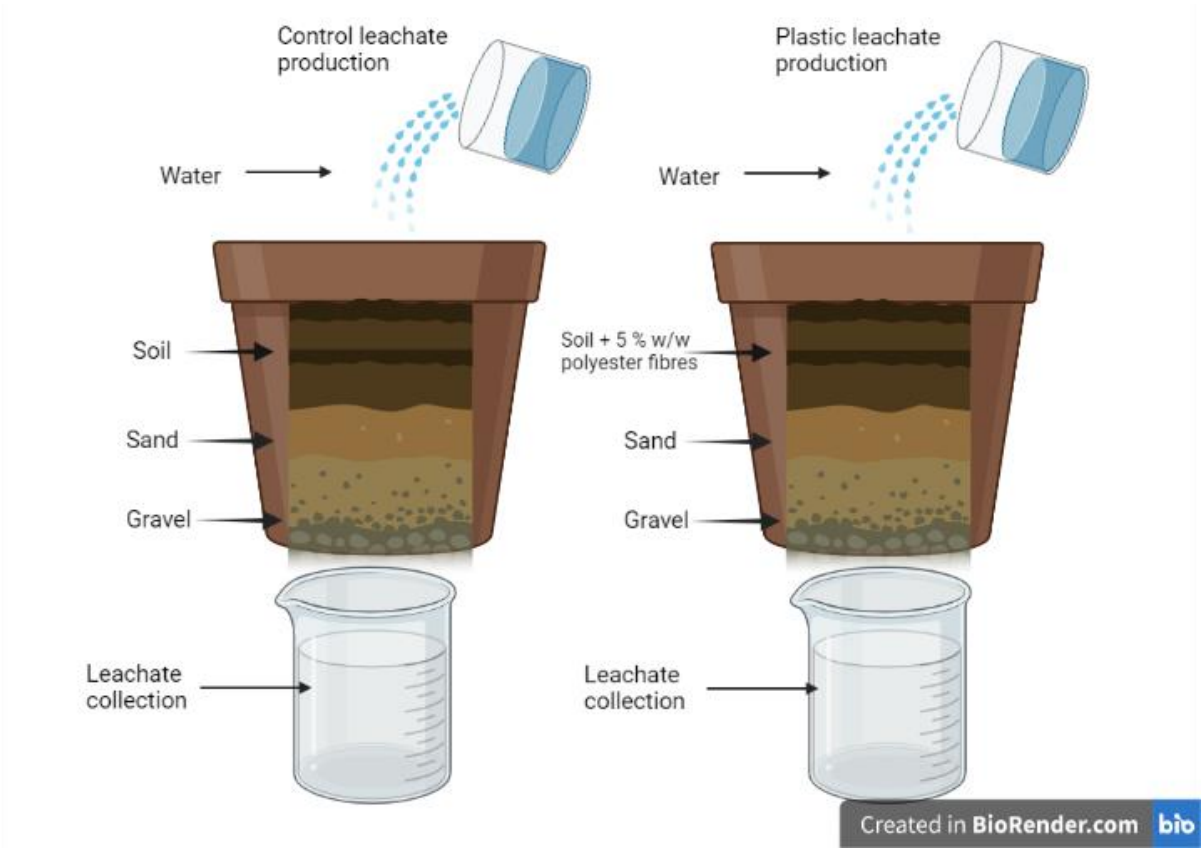


Figure 5.1: Leachate production for the experiment, water was added to the top of the mesocosm containing either soil, sand and gravel (control) or soil with 5 % w/w polyester fibres, sand and gravel (plastic leachate). Water was subsequently collected in a glass beaker after draining through the mesocosm and this was used to water the plants. 2 ml of the collected leachate was collected for LC-MS analysis. Image created with BioRender.com

5.2.4 Liquid Chromatography-Mass Spectrometry

Leachate samples were analysed using an Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, California, USA), coupled to an Agilent 6550A i-Funnel QTOF operating in positive

(ESI+) electrospray ionization modes. The LC separation was conducted on an Agilent Infinitylab Poroshell 120 EC-C18 3mm x 50mm x 2.7 µm column. The mobile phase (0.3 mL min^{-1}) consisted of a mixture of acetonitrile (solvent A) and H_2O + 5 mM ammonium acetate (solvent B), and the flow was set to 0.300 mL/ min^{-1}. The mass range of the instrument varied from 50 to 1300 m/z, and the mass accuracy was less than 5 ppm. The injection volume was set at 10 µL, and the column temperature was maintained at 30 °C. The gas flow was set to 11 L/min.

LC-MS data were analysed using Agilent MassHunter Qualitative analysis (version 10.0); chromatographic data were first aligned, and then features were extracted using the "Extract molecular features" mode, which extracts all the features in the sample, using the parameters which are default within the software. Files were then saved as CEF files and analysed using Agilent Mass Profiler Professional (version 15.1), which enabled a comparison of the samples (control versus plastic); compounds were then identified using the Agilent libraries "Extractables and leachables" and "Environmental water screening".

5.2.5 Fibre addition in soil

Two of the four treatments (KC & KP) were designed to assess the potential for physical impacts from fibre addition to soils. To each pot in the keratin treatments, 5 % w/w of the keratin fibres were added to the soil giving a mass of 2.5 g of cut keratin fibres added for each treatment.

5.2.6 Planting of seeds and growth conditions

Sinapis alba (mustard), *Hordeum vulgare* (Barley) and *Triticum aestivum* (wheat) seeds were sourced from Blades Biological Ltd (Edenbridge, United Kingdom, SKU: ZBB 040, ZBB 005, ZBB 065, respectively). *Brassica napus* (rapeseed) seeds were provided by a local farm. In each treatment, 100 seeds were germinated and grown for four weeks, with five seeds planted per pot (N = 100 per treatment, N = 400 per species). Plants were grown in an indoor environment at an ambient temperature during April and using a natural light cycle.

5.2.7 Germination and shoot and root measurements.

Seeds were considered germinated by the emergence of the radicle. The number of germinated seeds was recorded every day for the first two weeks. In the case of the monocotyledon seeds (*H. vulgare* and *T. aestivum*), the coleorhiza appeared first; however, seeds were not considered germinated till the appearance of the radicle (Luo et al., 2018; Konrad et al., 2020).

At the end of the four-week growing period, plants were removed from their pots, and each plant was carefully rinsed with distilled water to remove any traces of soil. Next, plant roots and shoots were measured using Moore and Wright Vernier callipers and recorded for the shoot and root length prior to drying. Next, plants were separated into the root and shoot portions and air-dried for six weeks before weighing on scales (Mettler Toledo, Pl303) to calculate root and shoot biomass.

5.2.9 Statistical analysis

All statistical analysis was conducted in R v3.6.1 (R Core Team). The data were screened for normality using the Shapiro-Wilks test (stats package v4.1.2, R Core Team) and homogeny of variance using a Levene's test from the car package v3.0.12 (Fox & Weisberg, 2021). Germination data were tested using a Kruskal-Wallis test for the end point analysis (final germination ratio), and where significance was demonstrated a Dunn's posthoc test was used to assess the difference between the concentrations with a Bonferroni correction giving an alpha value of 0.025 due to completing multiple comparisons. All results are presented as the mean ± SD/ SEM (for germination data) and were visualised using ggplot2 v3.4.2 (Wickham, 2016) and arranged using ggpubr version 0.4.0 (Kassambara, 2020). As data were not normally distributed, the differences in shoot and root lengths and weights were analysed using a Kruskal-Wallis test (stats package) to test with the factor treatment (per plant species). When statistical significance was demonstrated, a Dunn's posthoc test was conducted (with an alpha value of 0.05) using the dunn.test package v1.3.5 (Dinno, 2017).

There were significant reductions in the surviving plants of S. alba for the shoot and root lengths by the end of the experiment across all groups (control and keratin); thus, it was decided to remove this set from statistical testing to remove the chance of type II errors.

5.3 Results

5.3.1 Qualitative analysis of LCMS data

All the identified compounds are summarised in table 5.1; confidence level 2a probable structure was assigned to 14 compounds where library spectra were available, and the characteristic fragments could be detected (Schymanski et al., 2014). The vast majority of compounds could not be identified using the libraries available to the researcher, for example, compounds associated with the soils (as the libraries were plastic focused); however, nine compounds unique to the plastic leachate could not be identified. Of the compounds identified from the plastics, the majority are plastic-related compounds either used as surfactants or coatings. Most compounds were found in leachate produced in the first six weeks, with three of the compounds (2,2,6,6-Tetramethylpiperidinol, Calprolactam and Surfynol) found across the course of the study. Considering compounds found in 50 % of the samples across the twelve weeks of the leachate, 23 were unique to the plastic, and 110 were unique to the control (see figure 5.2).

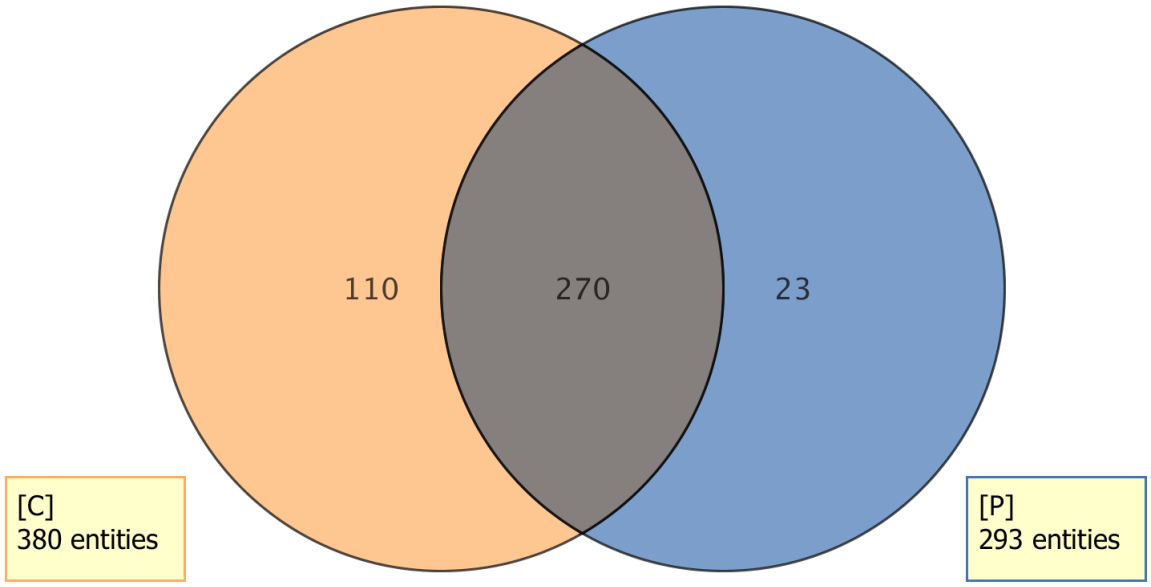


Figure 5.2: The number of compounds found within the control leachate (C) and the plastic leachate (P) in 50% of the samples.

Table 5-2: Suspect compounds identified in this study. All compounds listed were found in the plastic leachate but not in the controls.

Name	Molecular formula	Measured RT	m/z	Use	Cas number
Bisphenol F	$C_{13}H_{12}O2$	7.394	200.0853	Coating	620-92-8
Calprolactam tetramer	$C_{36}H_{66}N_6O_6$	7.761	678.5059	Manufacture	865-14-5
Dibutylamine	$C_8H_{19}N$	2.328	129.152	Emulsifier	111-92-2
2,2,6,6-Tetramethypiperidinol	$C_{11}H_{23}NO_2$	2.878	201.1713	Stabilizer	52722-86-8
Dibutyl sebacate	$C_{18}H_{34}O_4$	12.433	314.2441	Plasticizer	109-43-3
Tetraethylene glycol	$C_8H_{18}O_5$	6.046	216.0978	Stabilizer	3386-18-3
Pentaethylene glycol	$C_{10}H_{22}O_6$	1.873	260.1239		4792-15-8
1,3:2,4-bis(3,4-dimethylbenzylidene)sorbito	$C_{22}H_{26}O_6$	12.396	403.1996	Surfactant	81541-12-0
1-Hydroxycyclohexyl phenyl ketone (Irgacure 184)	$C_{13}H_{16}O_2$	1.414	221.1416	Plasticizer	947-19-3
Caprolactone (6-Hexanolactone)	$C_6H_{10}O_2$	0.875	131.0946	Coating and elastomers	502-44-3
Dipentaerythritol pentaacrylate	$C_{25}H_{32}O_{12}$	6.543	524.1872	Coatings	60506-81-2
DHB / 2,4-Dihydroxybenzophenone (Benzophenone-1)	$C_{13}H_{10}O_3$	6.98	236.048	Stabilizer	131-56-6
Surfynol	$C_{14}H_{26}O_2$	9.724	243.2197	Surfactant	126-86-3
Oxy-phenyl-acetic 2-[2-hydroxy-ethoxy]-ethyl ester	$C_{12}H_{14}O_5$	6.413	238.0821	Stabilizer	442536-99-4

5.3.2 Germination assay

There was a statistically significant difference in germination ratio at the final time point for the *H. vulgare* (χ^2 (3) = 9.43, p = 0.02), *T. aestivum* (χ^2 (3) = 14.202, p = 0.0026), *S. alba* (χ^2 (3) = 14.093, p = 0.0028). *B. napus* did not show any significant differences compared to the control for the final time point (χ^2 (3) = 2.64, p = 0.45) as determined by a Kruskal Wallis test. For *H. vulgare,* a significant difference was demonstrated between the soil control and the keratin and leachate treatment with a reduction in germination by 1 % at the final time period (p = 0.02), but not between the keratin control and soil control (p = 0.27). No significant difference was demonstrated between the soil control and the soil with polyester leachate (p = 0.5).

For the *T. aestivum,* the soil control and soil and leachate were not significantly different from one another (p = 0.14), but there was a significant difference demonstrated between the soil control and the keratin control and keratin + leachate treatments (p < 0.0001 and p = 0.024 respectively). Germination was reduced for the keratin control and keratin and leachate treatment compared to the soil control, with a reduction of 16.5 % for the keratin control; compared to the soil control and 7.5 % for the keratin and leachate at the final time period. A significant difference was also demonstrated between the soil + leachate, and the keratin control (p = 0.0054).

For the *S. alba*, the soil control was significantly different to the keratin and leachate (p = 0.0226) and the keratin control (p = 0.0005). The soil control showed a 13.5 % increase compared to the keratin control and a 7 % increase compared to the keratin with plastic leachate.

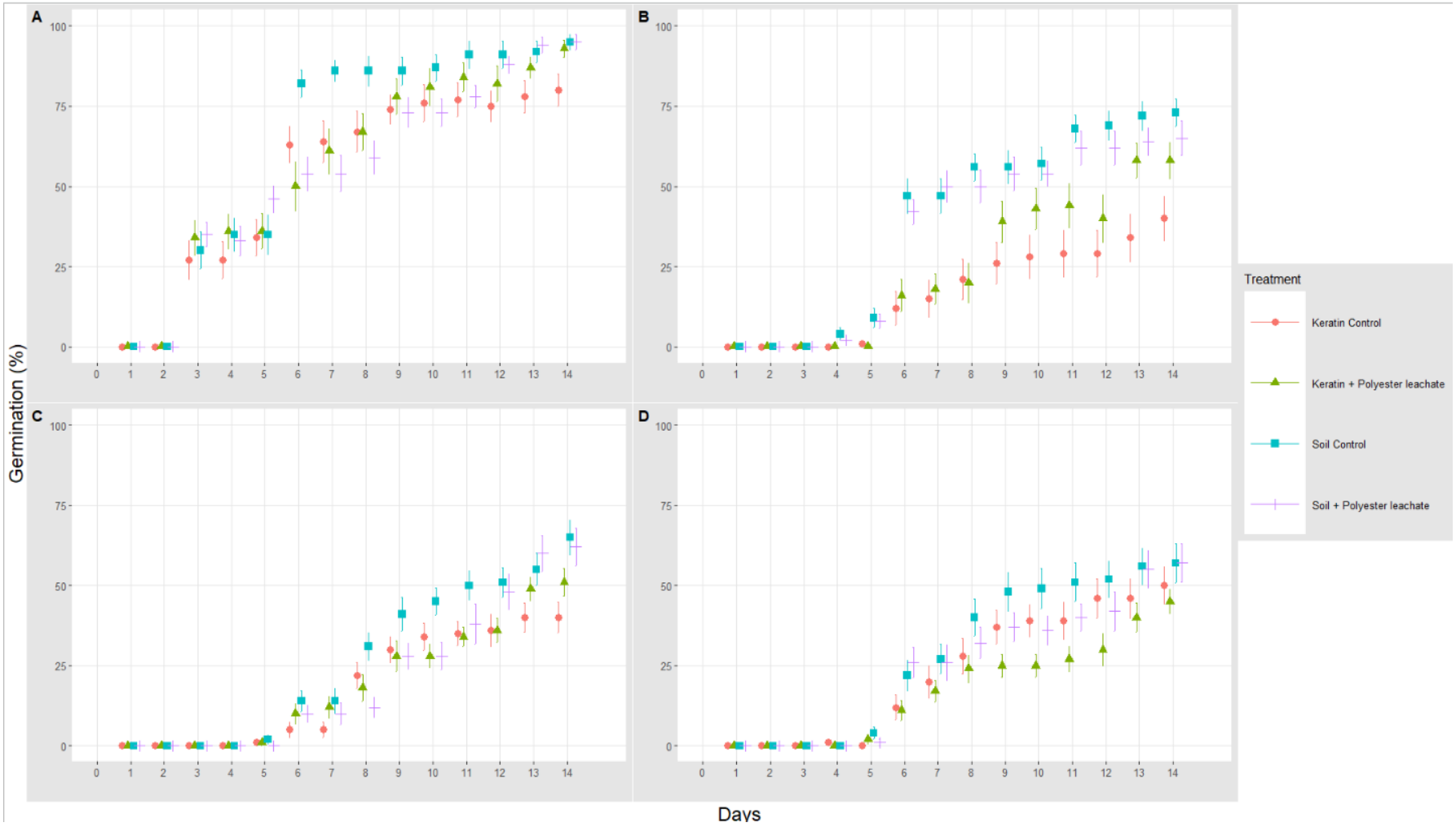


Figure 5.3: The germination of agricultural crop species across the four treatments. A shows the germination of H. vulgare. B shows the germination of T. aestivum, C represents the germination of S. alba and D demonstrates the germination of B. napus. Data is displayed as the mean ± the SEM, N = 100.

5.3.3 Root and shoot length

There was a statistically significant difference demonstrated for both root and shoot lengths for *H. vulgare* (root, χ^2 (3) = 52.731, $p < 0.0001$, shoot, χ^2 (3) = 12.538, p = 0.00575) (See figure 5.4 & 5.5). A Dunn's test indicated that for the shoot lengths, there were no significant differences for the treatments compared to the control; however, a difference was demonstrated between the keratin control and the keratin and plastic leachate (p = 0.0003). There was also a significant difference when comparing the keratin control to the soil and leachate (p = 0.0102). When considering root lengths for *H. vulgare,* all groups were significantly different compared to the control (keratin control, p = 0.0002, soil and leachate, p = 0.0008, keratin and leachate, p = 0.0170). The keratin control group demonstrated a reduction in length compared to the control by 18.21 %. Both of the plastic leachate treatments demonstrated an increase in length compared to the control, with the soil and leachate demonstrating an increase of 19.76 % and the keratin and leachate a 10.47 % increase in length.

There was a statistically significant difference between the *T. aestivum* shoot length (χ^2 (3) = 43.593, $p < 0.0001$) and root length (χ^2 (3) = 93.329, $p < 0.0001$). For *T. aestivum* shoot length, the soil control was significantly different in height to both the keratin control (p = 0.0003) and the keratin with plastic leachate (p = 0.0022), but no significant differences compared to the soil with plastic leachate (p = 0.0253). The keratin control shoots were 10 % larger than the soil control, but the keratin and leachate group showed a 9 % reduction in length compared to the soil control. There was a significant difference between the keratin control and the keratin and leachate for shoot length ($p < 0.0001$). For the root lengths, there was no significant difference between the soil control and the keratin control (p = 0.0262); however, there was a significant difference between the soil control and the plastic leachate treatments (soil and plastic leachate, $p < 0.0001$; keratin and plastic leachate (p = 0.0001). The soil with plastic leachate demonstrated a 33.69 % increase in the root length, and the keratin with plastic leachate demonstrated a reduction in root length by 18.92 % compared to the control.

B. napus showed no significant differences in the shoot length (χ^2 (3) = 7.85, p = 0.04922). However, for the root lengths, there was a significant difference demonstrated (χ^2 (3) = 33.217, p = < 0.0001), there was a significant difference between the soil control and the soil and leachate (p = 0.0249), with a 27 % increase in root length demonstrated for the soil and leachate treatment compared to the control.

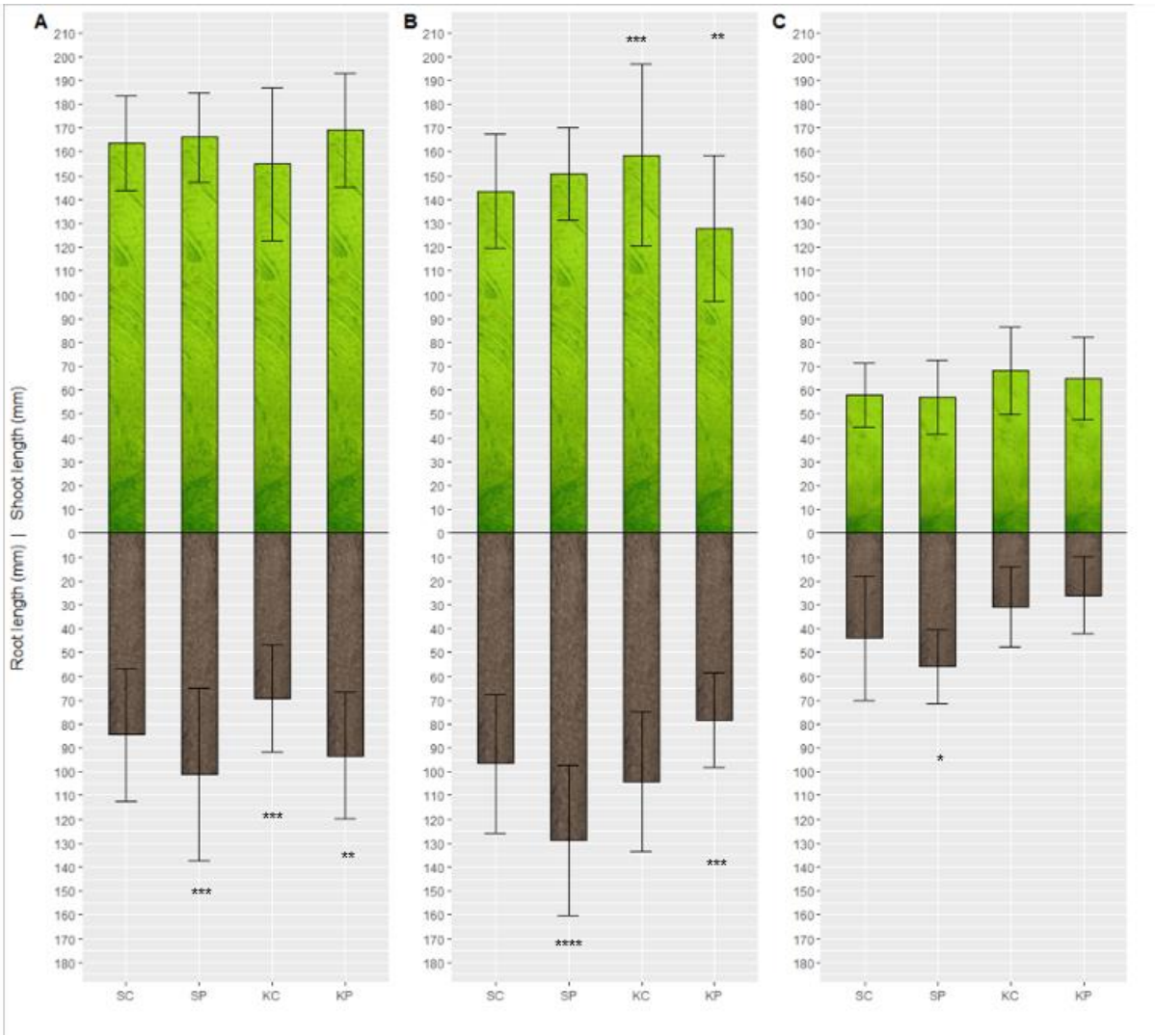


Figure 5.4: The shoot and root lengths of A: H. vulgare, B: T. aestivum and C: B. napus for the four different treatments. The line intercepting 0 is at soil level, with the shoot development above and the root development below. Asterix indicate levels of significance (p = < 0.025, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001) compared to the soil control. Data is displayed as the mean ± the standard deviation, N = 100.*

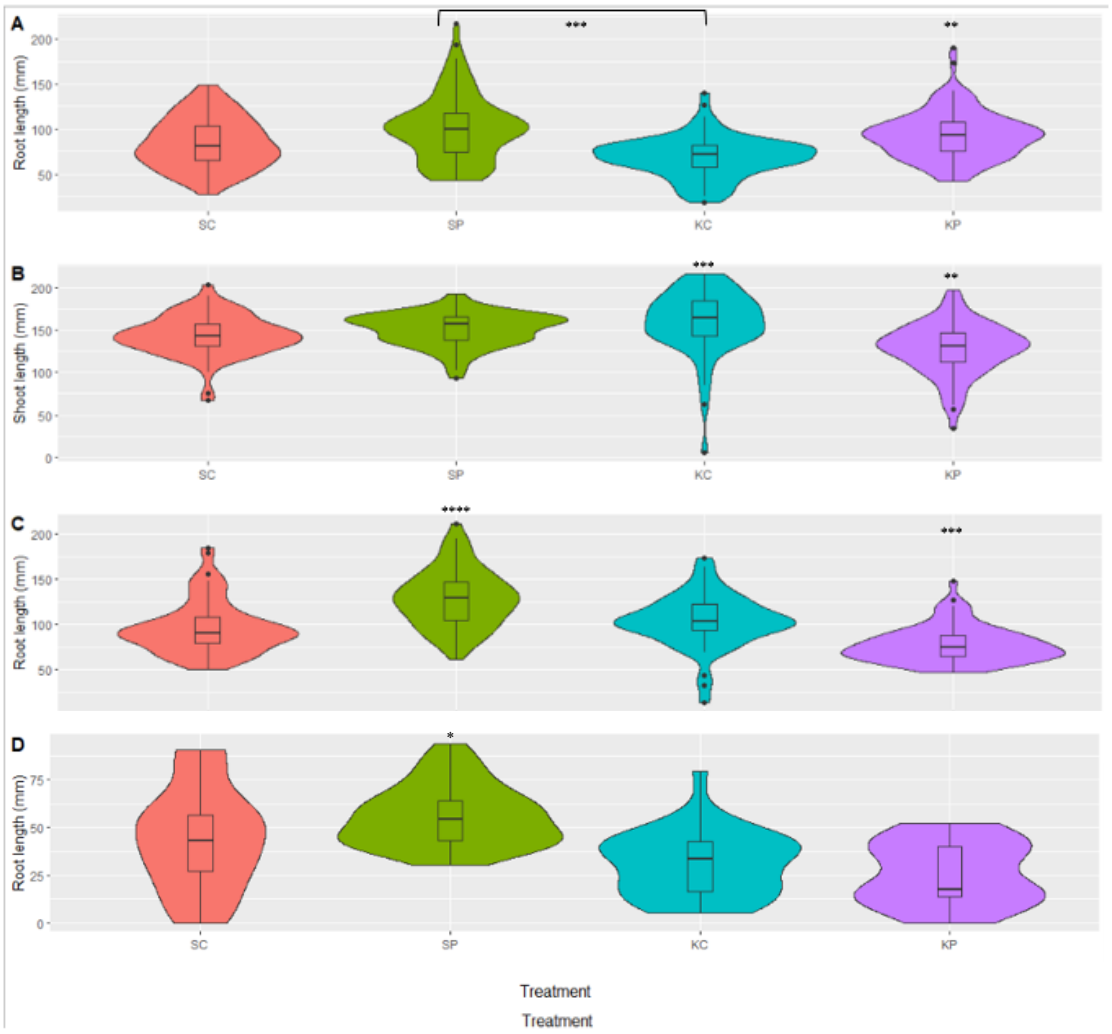


Figure 5.5: Data distribution depicted by violin plots with median, interquartile range and 95% confidence interval overlaid N = 100. Dots represent outlying data. A) The root length of H. vulgare, B) The shoot length of T. aestivum, C) The root length of T. aestivum, D) the root length of B. napus. Asterix indicate levels of significance compared to the soil control (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001).*

5.3.4 Root and shoot weights

There was a statistically significant difference in shoot weight (χ^2 (3) = 38.003, p < 0.0001) and root weight (χ^2 (3) = 46.46, p < 0.0001) as determined by a Kruskal Wallis test for the *H. vulgare* (see figure 5.6 & 5.7). Both the plastic treatments (soil and leachate and keratin and leachate) were significantly different to the soil control (p = 0.0019, p = 0.0187, respectively), with both treatments demonstrating an increase in shoot weight; 17.7% increase in the soil and leachate and a 16.9% increase in the keratin and leachate treatment. The keratin control was also significantly different to

the soil control (p = 0.0027), demonstrating a 17.8% reduction in shoot weight.

Root weight was also increased for the soil and leachate (p = 0.0001) and the keratin and leachate treatments (p = 0.0089), with an increase in weight by 31% in the soil and leachate group and an increase of 40% in the keratin and leachate treatments.

For the *T. aestivum,* there was a statistically significant difference in both the shoot weight (χ^2 (3) = 64.89, p < 0.0001) and root weight (χ^2 (3) = 80.79, p < 0.0001) (see figure 5.5). A Dunn's posthoc test indicated that the soil control was significantly different to all groups for the shoot weight (keratin control; p = 0.0003, keratin and leachate; p = 0.0169 and soil and leachate; p < 0.0001). The soil and leachate treatment showed an increase in shoot weight by 37.66% compared to the control and the keratin control treatment, which demonstrated an increase of 37.76%. The keratin and leachate treatment demonstrated a loss in the overall shoot weight compared to the control of 25.62%.

Root weight was also significantly different for the keratin control (p = 0.0079) and the soil leachate (p < 0.0001); additionally, there was a significant difference between the soil control and the keratin and leachate group (p < 0.0001). Root weight was increased in the soil and leachate treatment compared to the control, demonstrating a 56.9% increase. Root weight was also increased for the keratin control treatment compared to the soil control, with a 68.9% increase in root weight compared to the control. Root weight was reduced in the keratin and leachate treatment, showing a 39.5% decrease compared to the control.

No significant differences in shoot weight (χ^2 (3) = 7.619, p = 0.05458) or root weight (H(3), p = 0.637, p = 0.6376) were demonstrated in the *B. napus* group.

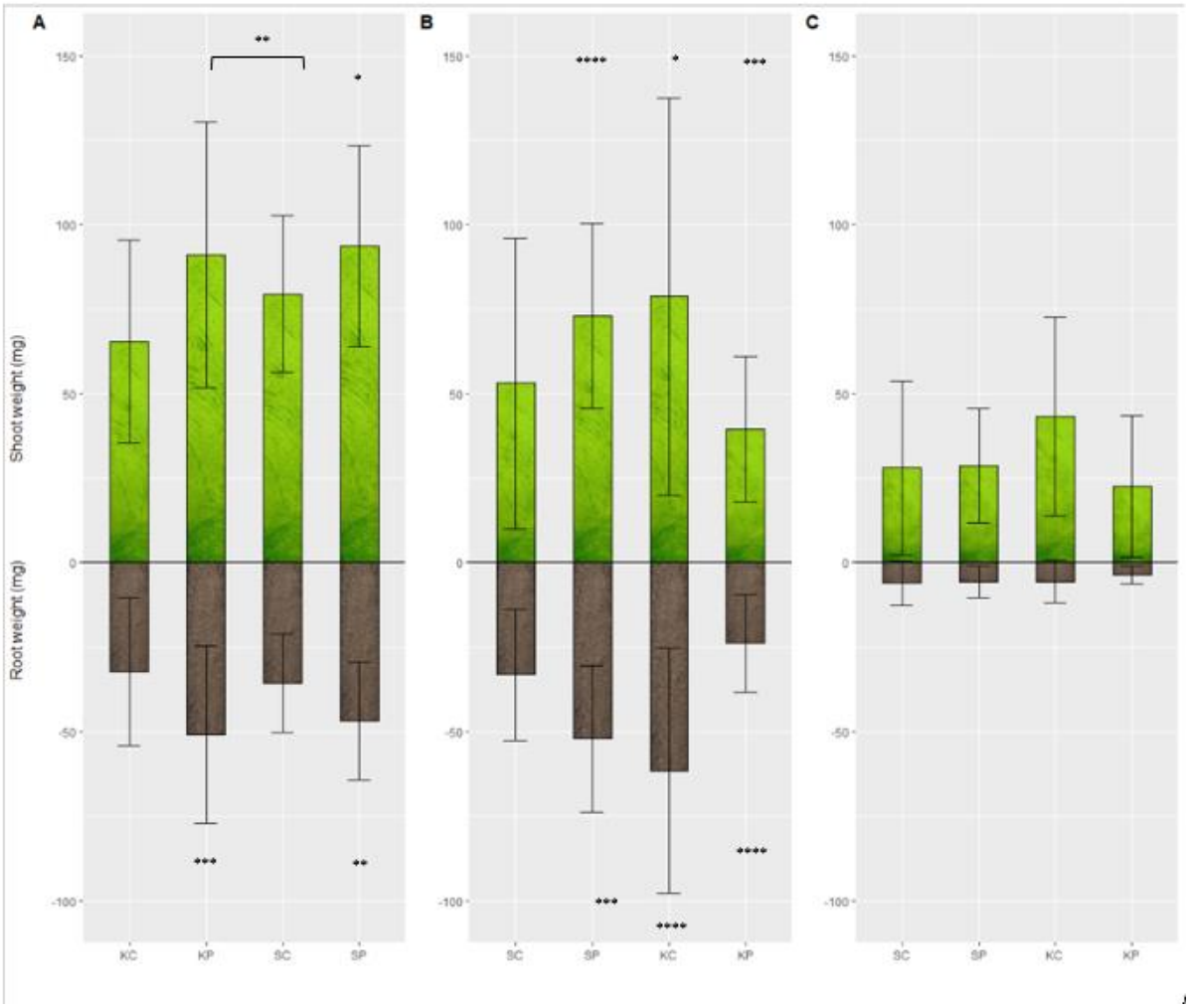


Figure 5.6: The shoot and root weights of A: H. vulgare, B: T. aestivum and C: B. napus for the four different treatments. The line intercepting 0 is at soil level, with the shoot development above and the root development below. Asterix indicate levels of significance (p = < 0.025, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001) compared to the soil control. Data is displayed as the mean ± the standard deviation, N = 100.*

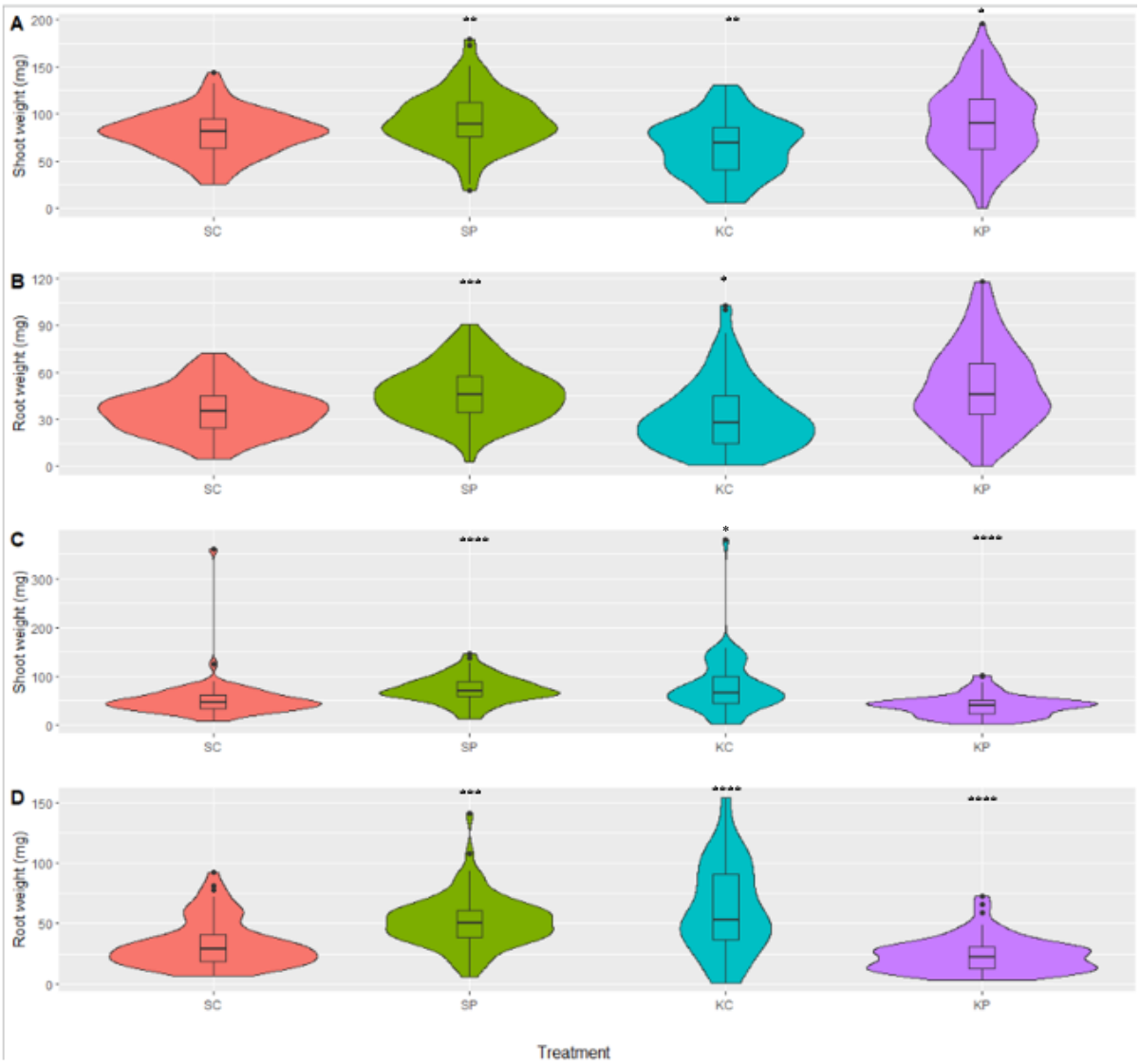


Figure 5.7: Data distribution depicted by violin plots with median, interquartile range and 95% confidence interval overlaid N = 100. Dots represent outlying data. A) The root length of H. vulgare, B) The shoot length of T. aestivum, C) The shoot length of T. aestivum, D) the root length of T. aestivum. Asterix indicate levels of significance compared to the control (p = < 0.05, ** p = < 0.01, *** p = < 0.001 and **** p = < 0.0001).*

Table 5-3: A summary of the results from the growth study, the text highlighted in green demonstrates where a significant increase had occurred, the text in red shows where a significant decrease had occurred and NS refers to non-significant results.

TREATMENT	SPECIES	SHOOT LENGTH	ROOT LENGTH	SHOOT WEIGHT	ROOT WEIGHT
SOIL + POLYESTER LEACHATE (SL)	*H. vulgare*	NS	19.5 % increase.	17.7 % increase.	31.` % increase.
	T. aestivum	NS	NS	37.7 % increase.	56.9 % increase.
	B. napus	NS	26.8 % increase.	NS	NS
KERATIN CONTROL (KC)	*H. vulgare*	NS	18.2 % decrease	17.8 % decrease	NS
	T. aestivum	10.4 % increase.	7.8 % increase.	37.9 % increase.	69 % increase.
	B. napus	NS	NS	NS	NS
KERATIN + POLYESTER LEACHATE (KL)	*H. vulgare*	NS	10.4 % increase.	116.9 % increase.	23.5 % increase.
	T. aestivum	11.1 % decrease.	19 % decrease.	25.6 % decrease.	28.3 % decrease.
	B. napus	NS	NS	NS	NS

5.4 Discussion

The results of the seed germination experiment indicate that the addition of a keratin fibre resulted in changes to the germination rate, as was initially hypothesised. However, no differences were demonstrated between the soil and plastic leachate; this suggests that a physical contaminant is responsible for the changes demonstrated in the germination phase. This could be due to physical changes in the soil matrix, such as those demonstrated by De Souza Machado et al. (2018), who found that polyester fibres decreased soil bulk density and water-stable aggregates. It could be that the addition of the fibres reduced the seed/soil contact, which is necessary for water absorption from soils (Smýkal et al., 2014). Additionally, it could be due to the partial dehydration of seeds due to changes in soil/seed contact, which was found by Trivedi and Ahmad (2011) when reviewing the impacts of asbestos fibres on *Triticum aestivum*, peas and *Sinapis alba* germination. This supports discussions in chapter 4, which considered that a change in available water due to the addition of elongated fibres resulted in changes in the imbibition phase, delaying germination. Research by Menicagli et al. (2019) found that plastic leachates produced from biodegradable plastic bags resulted in increased seed germination for *Glaucium flavum* and *Thinopyrum junceum*. The authors suggested this may be due to the presence of BPA, which in low concentrations has been demonstrated to increase plant growth (Wang et al., 2015). Menicagli et al. (2019) found that leachate produced from plastic bags exposed to the marine environment delayed the germination of *T. junceum*, which the authors suggest could be related to the high amount of salt present in the leachate.

The difference between the current experiment and Menicagli et al. (2019) experiment could be related to the compounds found within the leachate. This highlights some of the difficulties with toxicological testing of plastic related compounds (PRCs) from leachate due to the multidimensionality of plastic particles, which encompass infinite combinations of densities, sizes, shapes and chemical signatures (Koelmans et al., 2022). In the current experiment, only three identified compounds were found in the leachate after the initial six-week leachate production (2,2,6,6-Tetramethylpiperidinol, Caprolactam and Surfynol). 2,2,6,6-Tetramethylpiperidinol is a UV

stabiliser added to polymers; it is classified as harmful to aquatic life, with long-lasting effects; however, as of current, no toxicity data is available for this compound (Synthonix, 2021).

Caprolactam is used in the manufacture of synthetic fibres, where it is used as a plasticiser and could be incorporated into the environment through various waste streams (Sanuth et al., 2013). Caprolactam can also be found naturally as a secondary metabolite in some plants, such as sunflowers. Caprolactam has been found to inhibit the growth of *L. sativum* seedlings, suggesting this effect could occur in other dicotyledon species (Duke, 1986). Conversely, Aikawa et al. (1976) found that in low concentrations (100 – 0.1 ppm) that caprolactam and its derivatives stimulated the growth of *Pisum sativum* (pea); however, inhibition of germination occurred at concentrations higher than 10,000 ppm. Caprolactam is expected to have high mobility in soil (Swann et al., 1983); thus, it is likely to not occur in the soil in high concentrations. This compound could have partially been responsible for the increases in growth demonstrated in the leachate treatments during the course of this study.

Surfynol is a surfactant used in plastic coatings; it is noted to be harmful to aquatic life with long-lasting effects (ECHA, 2021). It is not considered persistent in the environment but has been demonstrated to have toxicological effects on *Pseudokirchneriella subcapitata* with an ErC50 of 82 mg/l (National Center for Biotechnology Information, 2022). Currently, no studies have determined any effects on terrestrial plant development from Surfynol. As there were no impacts to the germination phase from the plastic leachate for this particular polymer type and shape (polyester microfibre), it appears that changes to the soil matrix are more likely to be causing the changes shown in germination, however more testing is needed to elucidate this.

When considering root and shoot lengths, significant differences were primarily shown in the root development. The keratin and leachate treatments for *T. aestivum* and *B. napus* resulted in shorter root lengths than the soil and keratin controls. This could suggest a physical blocking response, as Bosker

et al. (2019) observed in *Lepidium sativum;* however, the plastics used in Bosker et al's study were in the nano plastic range; thus, different effects are likely with larger size plastics. The soil and leachate treatments were significantly longer for root lengths than the soil control and the keratin treatments for all species. This suggests that compounds within the plastic could be promoting the growth of the seedlings, as the leachate without the physical contaminant (keratin) resulted in improved plant growth. Compounds such as caprolactam in low concentrations being demonstrated to promote plant growth (Aikawa et al., 1976). Additionally, as some compounds within the leachate could not be identified, it could be that other growth-promoting chemicals are present. However, it appears that when the leachate is combined with a physical contaminant changing the soil matrix, there is a combined toxicity leading to reduced shoot and root development for *T. aestivum* and *B. napus*. The different species did respond differently, with the *H. vulgare* showing increased root and shoot weight when exposed to the keratin and leachate treatment. Different species often show varied responses to pollutants, with Bakina et al. (2022) finding that *Trifolium repens* (clover) was more resistant to oil than *Arrhenatherum elatius* (false oat grass). Menicagli et al. (2019) found that seedlings of *T. junceum* and *G. flavum* responded differently to the leachates, with the *T. junceum* showing reductions in radicle expansion and *G. flavum* demonstrating root expansion when exposed to plastic leachates. The authors did note that they found increased levels of seedling abnormalities in both species, with some *T. junceum* failing to produce the coleoptile and *G. flavum* seedlings failing to produce the hypocotyl. The seeding abnormalities demonstrated in Menicagli et al. (2019) research could indicate phytotoxicity; however, these abnormalities were not noted in the current experiment.

The differences in response could also relate to the delays demonstrated in the germination assay, where for the *H. vulgare,* the keratin control had the lowest germination rate and, subsequently, the lowest weights in the growth. For *B. napus*, the lowest germination rate was observed in the keratin and leachate treatment, which, although not statistically significant, was the treatment with the lowest weights and lengths for both root and shoot. *T. aestivum* had the lowest germination rate for the keratin control, but the keratin and leachate treatment were also reduced compared to the soil control, which, coupled with modifying the soil structure (Khalid et al., 2020), could explain some of the

differences demonstrated. Short-term germination delays can have significant effects on the subsequent development of plants. Rühl et al. (2016) found that short-term delays decreased the fitness of *Agrostemma* plants, with a delay in the germination of 7 days resulting in 54 % fewer shoots and 57 % less biomass. Yang et al, (2015b) also found that short term delays to germination for *Suaeda corniculata* (common sea blite) also resulted in shorter shoot biomass. Overall, the results of this study suggest that the physical impacts of fibres were more harmful than the chemical effects; however, it is worth considering that due to the multidimensionality of plastics and their associated compounds that, further tests are needed to assess different chemical compounds from polyester microfibres and other polymers.

5.5 Conclusion

The results of the present study indicate that at the germination phase, the addition of a physical material changing the soil matrix is more detrimental to germination and early growth than the addition of leachates. However, there appear to be negative responses for subsequent growth when both a physical contaminant and the chemical leachates are combined. Singularly, the leachate without the addition of a physical contaminant, such as keratin, resulted in improved plant growth, suggesting some compounds found within plastics can act as plant growth promotors. In future, research should assess the impacts of leachates from items which have been in the soil and may have absorbed additional compounds, such as fertilizers and pesticides, to review whether this poses a further risk.

Chapter 6 The impacts of polyester microfibres on the long-term growth and reproduction of agricultural crop species.

6.1 Introduction

This study aims to build on work conducted in chapters four and five, which assessed the germination and seedling development stage, and the current study investigated the impacts of polyester microfibres on the yield of two oilseed plants (*Sinapis alba* – mustard and *Brassica napus* – rapeseed), reviewing any potential changes to the reproductive cycle of the plant. From responses shown in chapters four and five, it appears that microplastics act as a stressor in the initial phase of development; however, for agriculture, it is also important to consider subsequent impacts on seed production. Crop yields are considered one of the most important measures of agricultural performance worldwide (Kosmowski et al., 2021). As such, any impacts microplastics may cause to crop yield is important to measure. Previous research detailed in chapters four and five noted the impacts of microplastic fibres on the germination and early development of plants, finding effects from both microplastics as a physical and chemical stressor. This section of the research will consider any effects post to the long-term growth and reproductive period of *Sinapis alba* (white mustard).

S. alba is a cool season crop, with seed maturity being obtained within 80 – 90 days (Kokotkiewicz & Luczkiewicz, 2015). It is primarily grown in agriculture for the seeds of the crop, which are used in the condiment industry, as a feedstock for biodiesel production (Mitrović et al., 2020), and as a water-binding agent in prepared meats (Saskatchewan Mustard Development Commission, 2019). *S. alba* is cultivated on 60,000 – 80,000 ha annually, producing 685,000 t of seed (Mitrović et al., 2020). The species is insect and wind pollinated (Hemingway, 1976) and is a non-mycorrhizal plant which tends to grow in moderately fertile soils (Lambers & Teste, 2013). Non-mycorrhizal plants have evolved specialist root structures which make them capable of extracting all of the required nutrients from soils without the use of mycorrhiza, using a carboxylate-relating P mining strategy to enable adequate phosphorus for development (Shane & Lambers, 2005). Due to the fact that *S. alba* has a short

cropping cycle and can be grown in a range of soils, it is now a widespread commercial crop globally (Ekanayake et al., 2016). *S. alba* goes through nine life cycle stages (germination, leaf development, side shoot development, stem elongation, vegetable plant development, flowering, fruit development, ripening and senescence) (Saskatchewan Mustard Development Commission, 2019). In chapters four and five, *S. alba* were grown through the germination and leaf development stages. In the current experiment, the *S. alba* were grown through to senescence to review the effects on the full life cycle of the plants, particularly reviewing the effects on seed yield, as with oilseed crops, which is the most important part of the crop.

The germination stage and subsequent emergence of seedlings are two of the most critical phases in plant development and determine the plant's success in the ecosystem (Javaid et al., 2022). It is also when plants are most vulnerable to stress, and small environmental changes during these critical phases in the plants' lifecycle can significantly affect plant survival (Duncan et al., 2019). However, changes to the soil structure and composition may cause effects in the later stages of plant development, and changes in nutrient and water availability can lead to effects in the reproductive stage of the plant lifecycle (Meurer et al., 2020). For some crops, such as cereal and oilseed crops, reproduction and seed production are crucial stages, as the seed is the part of the plant with economic value (Dreccer et al., 2000).

The reproductive and germination stages of plant development are more sensitive to stress than the vegetative phase (Zinn et al., 2010); in the reproductive stage, stress can result in fewer flowers or decreased seed numbers (Rering et al., 2020). Fertilisation, gametogenesis and embryogenesis are impacted, limiting seed development and lowering crop yields (Begcy & Dresselhaus, 2018). Both biotic and abiotic stresses have been noted to interfere with several key reproductive processes, such as germination, vegetative growth, tiller production (for Gramineae species), reproductive organ development, reproduction and grain filling (Sehgal et al., 2019). Abiotic stressors such as drought

and heat have been noted to cause decreases in seed yield in legumes (Farooq et al., 2017) and cereal crops (Dias & Lidon, 2009). Stress has been noted to cause changes to photosynthetic tissues, leading to the inhibition of photosynthesis (Tezara & Lawlor, 1999). Stressors such as high-temperature damage the oxygen-evolving complex of photosystem II (PSII), resulting in disorganisation of the thylakoid membranes (Yamashita et al., 2008). A reduction in photosynthesis ultimately reduces parental resources available for reproduction, subsequently impacting seed yield (Zinn et al., 2010).

To date, limited papers have assessed the effects of microplastics on yield, despite this being a key outcome in the agricultural system and a stage in which plants may be more threatened by additional stressors. For example, Qi et al. (2018) reviewed the impacts of LDPE and biodegradable mulch films on *Triticum aestivum* (wheat) and found no differences compared to the control for the numbers of fruits but did see differences between the biodegradable macroplastic mulch films and the LDPE macro and microplastic films (Bio-macro, 2.8 ± 0.16 fruits, LDPE macro 3.4 ± 0.3 fruits, LDPE micro, 3.6 + 0.3 fruits). There were no significant differences between the LDPE treatments and the controls for seed weight, though a significant difference was demonstrated between the biodegradable treatments and the LDPE. Conversely, Ma et al. (2018) found that plastic film mulching increased crop yield; however, this research did not report the length of time plastic mulch films were applied, making direct comparisons between these works difficult.

Liu et al. (2022) assessed three fields in northwest China that regularly use plastic mulching, one with seven years of mulching, one with seventeen years and one with thirty-two years of plastic mulching. The research reviewed the soil physical properties, finding that soil bulk density was increased along with penetration resistance. Conversely, they found that saturated hydraulic conductivity decreased with increased plastic additions. In regard to yield, the research found that *Gossypium spp.* (Cotton) was reduced by 15.83 % between the seven-year and seventeen-year applications and a reduction by 73.32 % between the seven-year and the thirty-two-year applications.

This study aims to build on work conducted in chapters four and five, which assessed the germination and seedling development stage and investigate the impacts of polyester microfibres on the yield of two oilseed plants (*Sinapis alba* – mustard and *Brassica napus* – rapeseed), reviewing any potential

changes to the reproductive cycle of the plant. However, during this experiment, the *B. napus* did not reach the flowering stage due to the two subsequent heat waves in the UK; thus, this research focuses on impacts to *S. alba*. From responses shown in chapters four and five, it appears that microplastics act as a stressor in the initial phase of development; however, for agriculture, it is also important to consider subsequent impacts on seed production.

The current study was conducted to assess whether microplastics could result in changes to the reproductive stage of plant development, a key outcome for agriculture. In this study, oilseed crops were grown in the presence of polyester microplastics to assess whether changes occurred to seed filling, seed number and seed-to-pod ratio. This research hypothesises that the addition of microplastics will have subsequent impacts on seed production. It also tests the hypothesis that microplastics will result in stress to plants, which is tested using chlorophyll fluorometry.

6.2 Materials and methods

6.2.1 Treatments

Three treatments were conducted for this experiment, a control, 0.1 % w/w and 1 % w/w polyester fibres. Two species of oilseed crops were grown in these conditions, *Sinapis alba* and *Brassica napus* (However, *B. napus* did not reach the fruiting stage during the course of this experiment; thus, only the results for the chlorophyll fluorometry are reported for this species). Polyester fibres were sourced from The Flocking shop (England); fibres were measured using a Leica stereo microscope (DM2500P) with a magnification of x100 and photographed using a Leica Pixel Shift Camera (DMC6200). Measurements were calculated using Leica application suite X (version 3.0.14.23224); the fibres had an average length of 519.34 ± 66.67 µm (min = 402.83 µm, max = 1016.02 µm, n = 100) and an average thickness of 15.5 ± 3.69 µm (min = 8.82 µm, max = 27.35 µm, n = 100) (see figure 6.1).

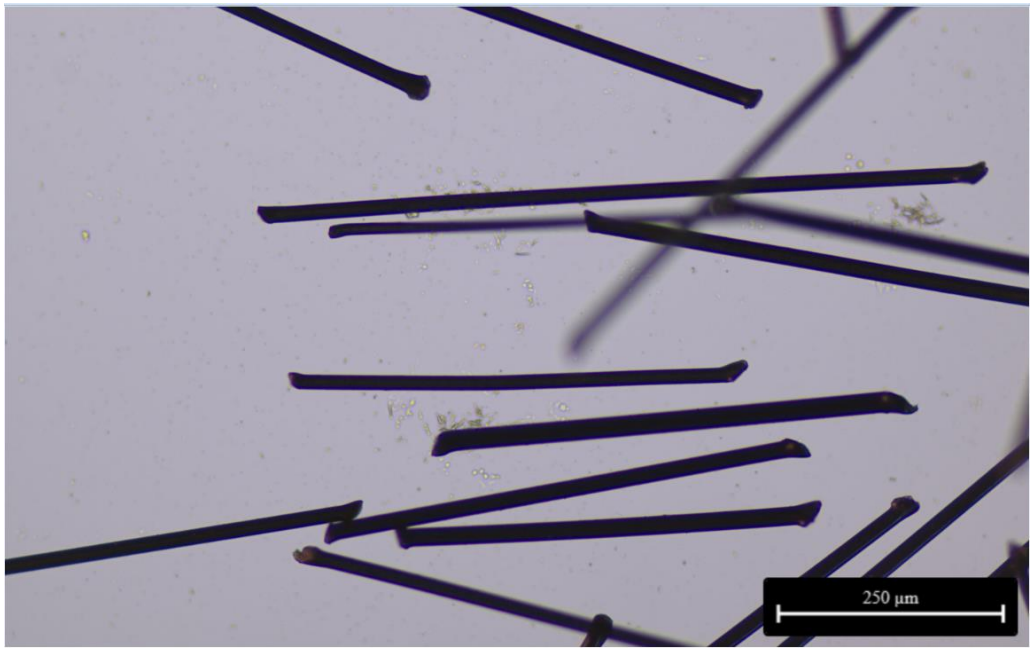


Figure 6.1: The polyester microfibres used in the study.

6.2.2 Seed germination

To negate any losses from germinating plants in the plastic-contaminated soil, seeds were germinated in trays with cotton wool and water and planted once the first leaves had appeared. A total of 200 *S. alba* seedlings and 100 *B. napus* seedlings were planted per treatment (with one seed per pot). A greater amount of *S. alba* seedlings were planted to negate any additional losses, which were demonstrated in chapters four and five.

6.2.3 Planting conditions

Seedlings were planted into plastic pots (Elixir Garden supplies, England; diameter 11 cm, depth 12 cm) with a volume of 1 L. The plant pots were filled with 500 g of John Innes compost No.2. One seedling was planted per pot in one of three concentrations, 0 (control), 0.1 % (0.5 g w/w of polyester fibres) or 1 % (5 g w/w of plastic fibres). Plants were kept thoroughly watered throughout the experimental period. Trays were randomly placed in the glasshouse, and their positions shifted monthly. During the flowering period, fans were placed on timers for four hours per day to enable wind pollination of the species. Due to the location of the greenhouses, it was not possible to encourage insect pollination.

6.2.4 Flowering measurements

The number of flowers were measured from the first flower emergence at eight weeks for the *S. alba* until senescence had occurred. A maximum of thirty flowering plants per treatment were counted for their number of flowers, and the total number of flowering plants was also recorded for each treatment. A random number generator app was used to determine which flowering plants were counted for their flower number, with a mix of plants from different trays used. Due to two subsequent heatwaves in the UK which occurred during the experiment, the *B. napus* plants did not reach inflorescence; therefore, *B. napus* flowers were not measured during this study. See figure 6.2 for images of the *S. alba* plant in flower.

Figure 6.2 A Sinapis alba plant in flower at eight weeks of growth.

6.2.5 Seed pod measurements

Seeds were harvested once the plant had gone through senescence and the pods had turned brown. The seed pods from thirty plants in each treatment were harvested, with the pods for each plant added to a separate envelope. The thirty plants harvested were determined by a random number generator app (Random Number Generator Plus). Subsequently, each plant's total pod number was recorded,

along with the number of seeds per pod and the seed weight. Finally, the seeds were weighed using Mettler Toledo, Pl303.

6.2.6 Shoot measurements

After the seed pods were harvested, plants were removed from their individual pots, the shoots were measured using a 30 m measuring tape, and the lengths of the individual plant were recorded. Root lengths were not recorded in this study because once plants had gone through senescence, the root system was extremely fragile; thus, accurate measurement was not possible.

6.2.7 Chlorophyll fluorescence measurements

Chlorophyll fluorescence is a technique to gain detailed information on the state of photosystem II (PSII) (Murchie & Lawson, 2013), which is the protein super complex that executes the initial reaction of photosynthesis in higher plants (Coe et al., 2015). PSII measurements are commonly applied in plant stress studies, providing rapid insight into the PSII system (Reiling & Davison, 1992; Maxwell & Johnson, 2000). Chlorophyll fluorescence of PSII was recorded every two weeks using a modulated fluorescence system. Thirty fully expanded leaves were selected from individual plants for each treatment (control, 0.1 % w/w, and 1 % w/w) (see figure 6.3 for an image of a leaf with dark adaption clips), and fast fluorescence kinetics were recorded after a 20-minute dark adaption period using an Opti-Sciences OS1P chlorophyll fluorometer (Opti-Sciences, Inc, Hudson, NH, USA). The dark adaption phase is needed to reverse non-photochemical quenching fluorescence before fluorescence can be measured (Maxwell & Johnson, 2000). In the dark-adapted state, it is possible to measure initial fluorescence (Fo) when all PSII reaction centres are open. Maximal fluorescence (Fm), when all PSII reaction centres are closed, is then assessed with saturating light (Murchie & Lawson, 2013). The maximal quantum yield for electron transport by open PSII centres is calculated as Fv/Fm = (Fm – Fo)/ Fm (Murchie & Lawson, 2013). The optimal Fv/Fm measurements in most plant species are between 0.79 – 0.83, with significantly lower values indicating photoinhibition, which can indicate stress (Maxwell & Johnson, 2000). Fv/Fo measurements can be used to assess the number

and size of the active photosynthetic reaction centres, and changes to this can indicate a change in the rate of electron transport from PSII to the primary electron acceptors. This has been reported in plants exposed to different environmental stressors (Kumar et al., 2020a).

Before the experiment, a preliminary study was performed to determine the optimal protocol for chlorophyll fluorescence image acquisition; the dark-adapted time was determined by checking the value of maximum PSII. It was observed that after a 20-minute dark adaption period, the Fv/Fm values were stable, which was considered the optimal dark adaption period.

Figure 6.3 A B. napus leaf with a dark adaption clip attached. Dark adaption clips were left on leaves for 20 minutes prior to the chlorophyll fluorometry measurements being recorded.

6.2.8 Statistical analysis

Statistical analysis was conducted in R version 4.1.2 (R Core Team, 2022), and data were screened for normality using s Shapiro Wilk test and homogeneity of variance using Levene's test from the car package v3.0.12 (Fox & Weisberg, 2021). Differences in Fv/Fm measurements and Fv/Fo, and the shoot height measurements were analysed at the final time period using a Kruskal Wallis test considering the differences between concentrations. The number of plants flowering and the number of flowers at the full flowering stage were analysed using a Kruskal Wallis test investigating the difference between concentrations. Where significance was demonstrated, a Dunn's post hoc test was used to further explore the responses using the dunn.test package v1.3.5 (Dinno, 2017) with an alpha value of 0.05.

The pod-to-seed ratio, seed weight, pod number and seed number were analysed using a one-way ANOVA, and where significance was demonstrated, a Tukey's HSD test was used to test differences in the response variable versus the concentration. The pod number and seed number were tested using a Kruskal Wallis test with a Dunn's post hoc test with an alpha value of 0.05. Data were visualised using ggplot2 (Wickham, 2016) and displayed as the mean ± the standard deviation.

A generalized estimating equation (GEE) with a gamma family was used to analyse Fv/Fm, Fv/Fo and flower number by concentration over time using the package geepack v1.3.9. GEE was used to account for the correlated nature of the data due to the repeated measures, and an exchangeable correlation structure was assumed.

6.3 Results

6.3.1 Chlorophyll fluorescence

There was a statistically significant difference in Fv/Fm measurements for *S. alba* at the final timepoint (χ^2 (2) = 7.62, p = 0.02), with the 1 % treatment being significantly different to both the control (p = 0.0068) and the 0.1 % (p = 0.011). No significant difference was demonstrated between the control and the 0.1 % treatments (p = 0.432) (see figure 6.2). On the final week of measurement (week 16), the Fv/Fm mean measurements for the 1 % treatment were 0.741, compared to the control (0.772) and the 0.1 % (0.773) (see figure 6.4). No significant differences were demonstrated between the *B. napus* treatments (χ^2 (2) = 0.062, p = 0.97). The results of the GEE model showed significant estimates for *S. alba* for the concentration (estimate = 0.019, standard error = 0.0009, Wald = 4.35, p = 0.037) and time (estimate = 0.007, standard error = 0.002, Wald = 9.94, p = 0.0016). The scale parameter for the model was (estimate = 0.0029, standard error = 0.0006).

The Fv/Fo measurements demonstrated a significant difference (χ^2 (2) = 6.27, p = 0.044), and Dunn's post hoc test indicated that the 1 % treatment was significantly different from the control (p = 0.015). However, there were no significant differences between the 0.1 % and the control (p = 0.49) (see figure 6.5). The results of the GEE model showed significant estimates for *S. alba* for the concentration (estimate = 0.015, standard error = 0.007, Wald = 4.47, p = 0.0345) and for time (estimate = 0.0056, standard error = 0.002, Wald = 7.24, p = 0.0071). The scale parameter for the model was (estimate = 0.04, standard error = 0.00853).

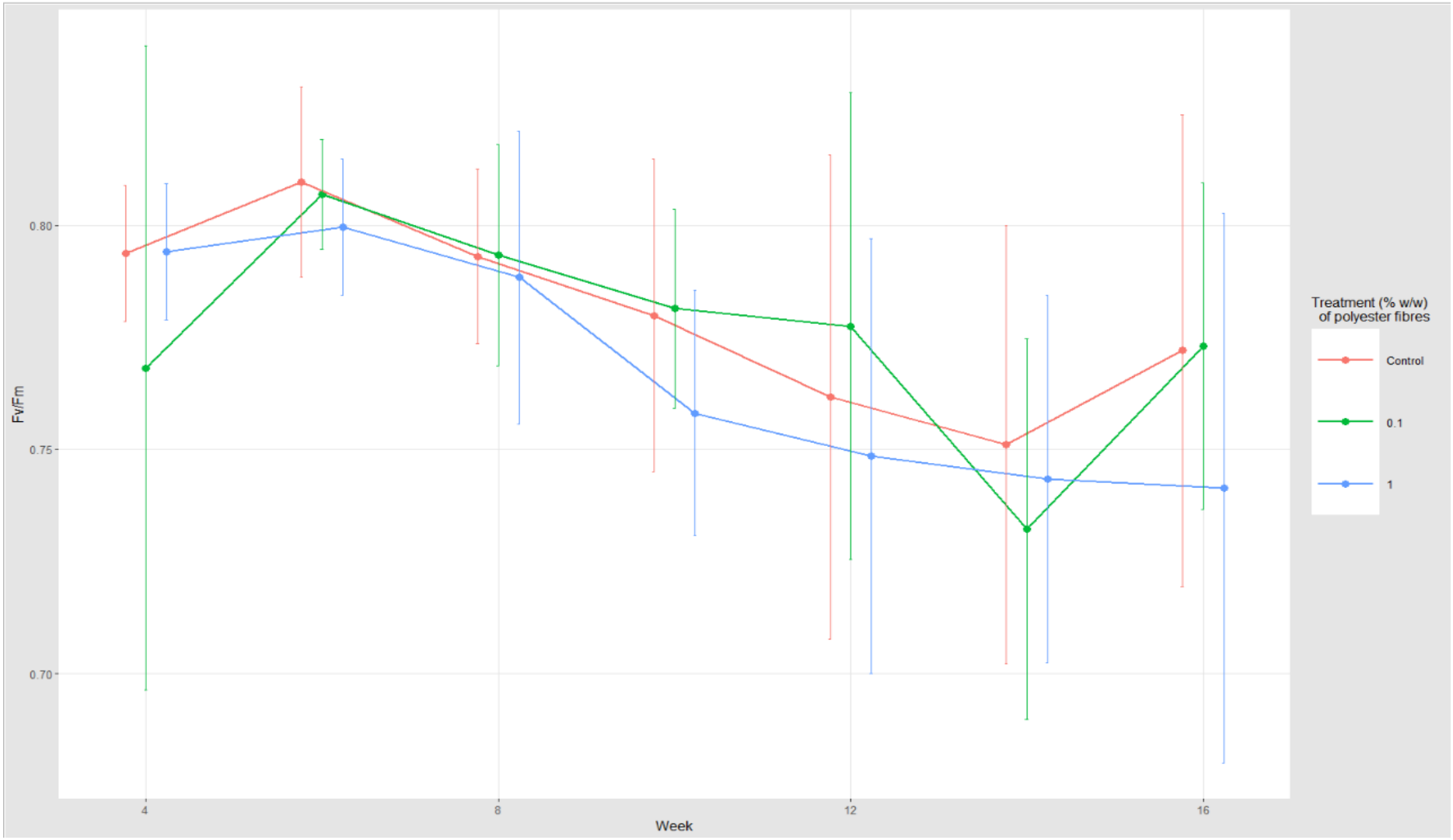


Figure 6.4: The Fv/Fm values of thirty leaves from thirty different S. alba plants across the three concentrations of polyester microfibres at different time periods, measured every two weeks.

Data is presented as the mean ± the standard deviation, N = 30.

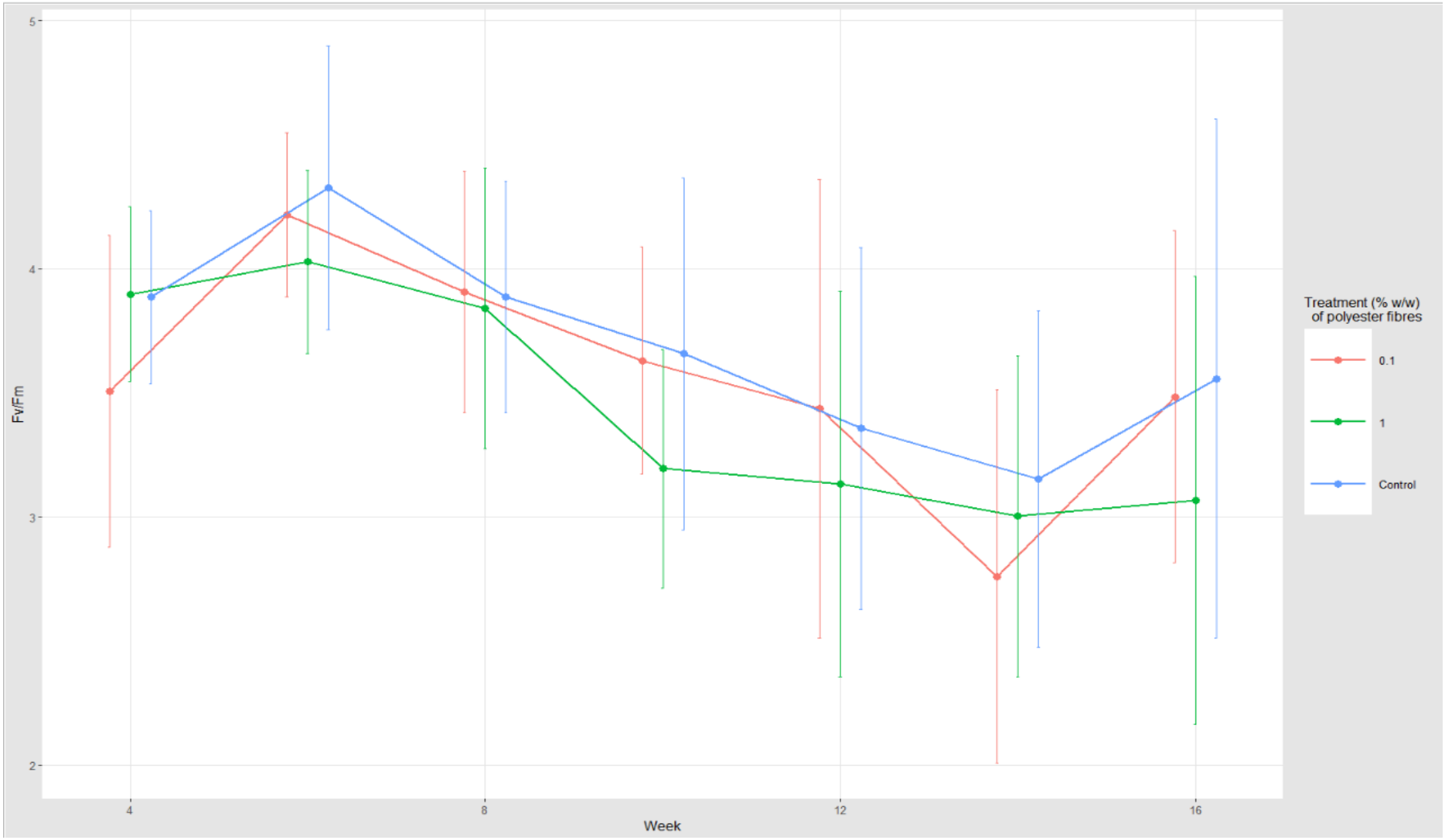


Figure 6.5: The Fv/Fo values of thirty leaves from different S. alba plants across the three concentrations of polyester microfibres at different time periods, measured every two weeks. Data is presented as the mean ± the standard deviation, N = 30.

6.3.2 Flower development

There were no statistically significant differences in the total number of plants flowering (χ^2 (2) = 0.05, p = 0.9753) as determined by a Kruskal Wallis test. However, at week 11, when plants were in the full flowering stage, 50 % of the flowers on the main raceme open (Saskatchewan Mustard Development Commission, 2019); the control had a total of 104 plants flowering, the 0.1 % had 84 plants in flower and the 1 % 100 plants flowering.

There was a statistically significant difference between the number of flowers produced between treatments at week 11 (Full flowering) (χ^2 (2) = 15.526, p = 0.00045) as determined by a Kruskal Wallis test (see figure 6.6). The 0.1 % and the 1 % treatments were significantly different compared to the control (p = 0.0009, p < 0.0001, respectively), but there were no significant differences between the 0.1 % and the 1 % treatments (p = 0.3). The flowers reached the full flowering stage as of week 11, where the control had an average of 74 ± 37 flowers. The control flower number was 43 % higher than the 0.1 % treatment (31 ± 27 flowers) and 30 % higher than the 1 % treatment (44 ± 31 flowers). The control and the 0.1 % treatments began flowering as of week eight, but the 1 % treatment did not begin until one week later, in week nine. The GEE model found that concentration had a significant negative effect on flower numbers (estimate = -0.25, standard error = 0.043, p < 0.001), whilst time did not show a significant effect (estimate = - 0.043, standard error = 0.052, p = 0.4). The estimated scale parameter was 26.4 (standard error = 5.4), and an estimated correlation parameter of 0.245 (standard error = 0.1).

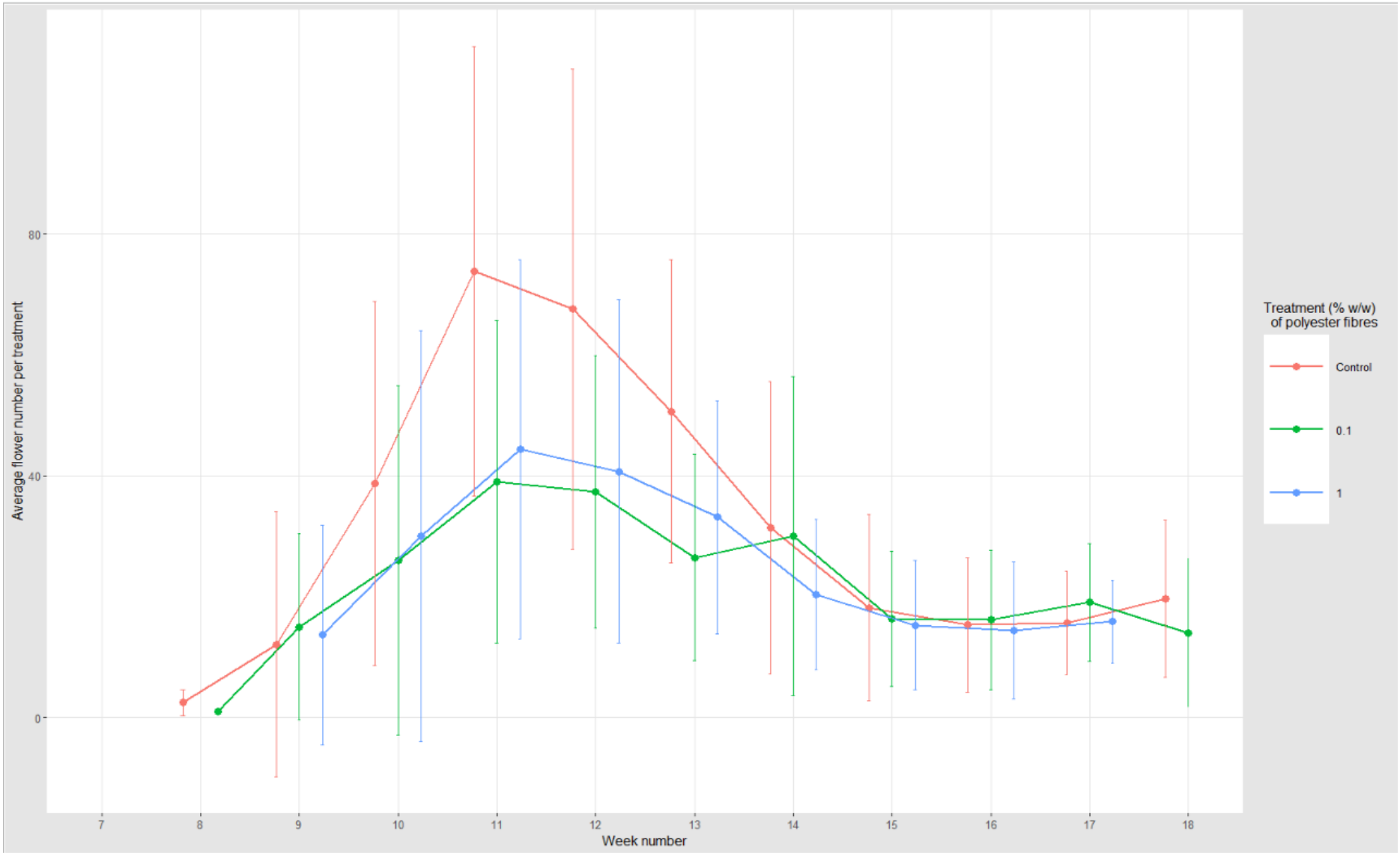


Figure 6.6: The average flower number of thirty S. alba plants, measured weekly, Data is displayed as the mean ± the standard deviation, N = 30 plants.

6.3.4 Shoot development

There were no statistically significant differences in shoot height for *S. alba* as determined by a Kruskal Wallis test (χ^2 (2) = 2.205, p = 0.3). The mean shoot height for the control was 209 ± 61 cm, the 0.1 % w/w 198 ± 45 cm, and the 1 % w/w treatment 203 ± 51 cm (see figure 6.7).

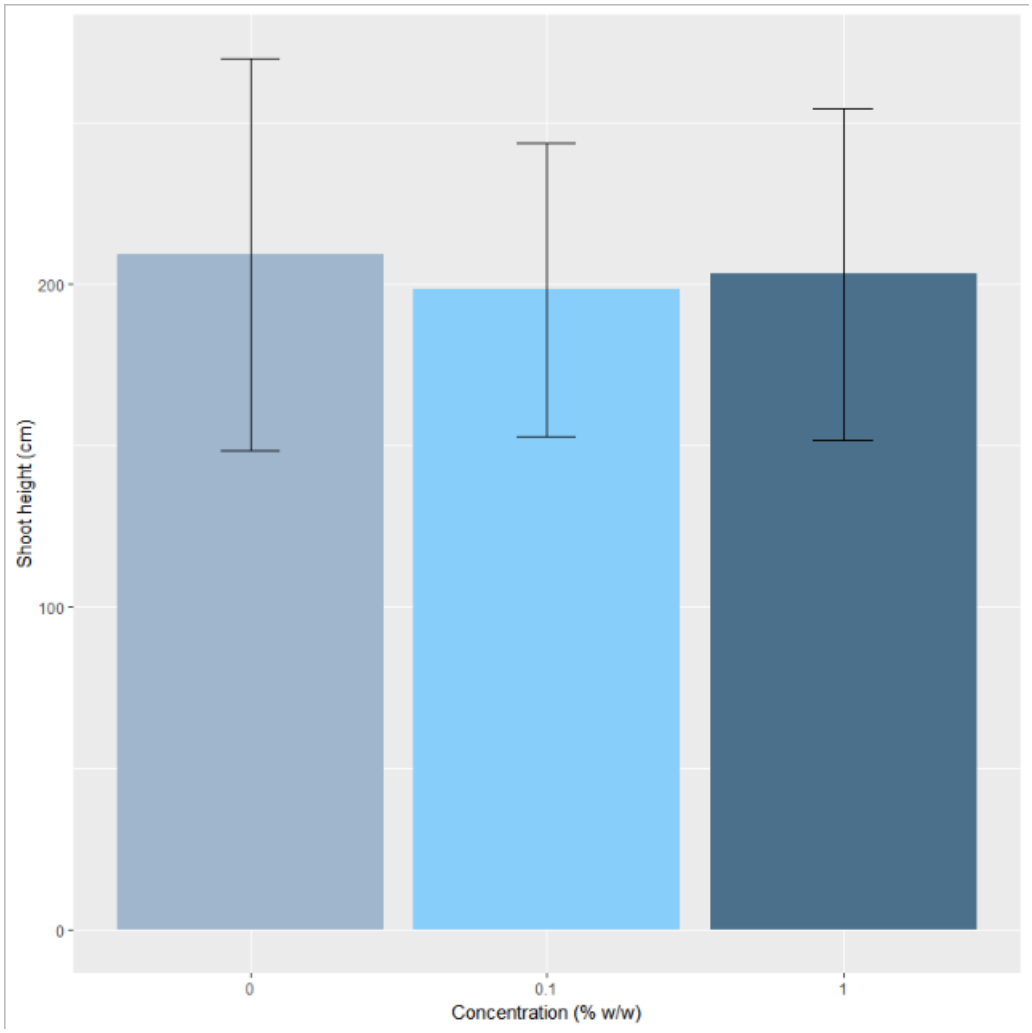


Figure 6.7: The shoot lengths of S. alba across the three different concentrations of polyester microfibres. Data is displayed as the mean ± the standard deviation, n = 78 for the control, n = 80 for the 0.1 and n = 110 for the 1 %.

6.3.5 Seed measurements

There were no significant differences in pod number when compared to the control (χ^2 (2) = 3.346, p = 0.1877) for the *S. alba*; however, the average number of pods per plant was higher in the control (120 ± 100 pods), in the 0.1 % the average pod number was 22 % lower (0.1 %, 94 ± 113) and 19 % lower in the 1 % treatments (1 % 97 ± 103 pods per plant). No significant differences were demonstrated in the total seed number (χ^2 (2) = 5.579, p = 0.06145); however, the average number of seeds was higher for the control (432 ± 371 seeds), the 0.1 % was 32 % lower for the average seed number (294 ± 403 seeds) and 29 % lower in the 1 % treatment (308 ± 354 seeds).

There was a significant difference in the seed-to-pod ratio (F (2,87) = 11.25, p < 0.0001) (see figure 6.8), with the control having an average of 3.5 ± 0.65 seeds per pod. The 0.1 % seed-to-pod ratio was decreased by 21.15 % (2.76 ± 0.5 seeds per pod, p = 0.0001325), and the 1 % treatment decreased by 19.14 % (2.83 ± 0.81 seeds per pod, p = 0.0005688). No significant differences were demonstrated between the 0.1 % w/w and the 1 % w/w treatments (p = 0.9131).

The per 100 seed weight was not significantly different (F(2, 87) = 0.151, p = 0.86), with the control having an average seed weight of 0.462 ± 0.197 g, the 0.1 % 0.482 ± 0.176 g and the 1 % 0.485 ± 0.14 g.

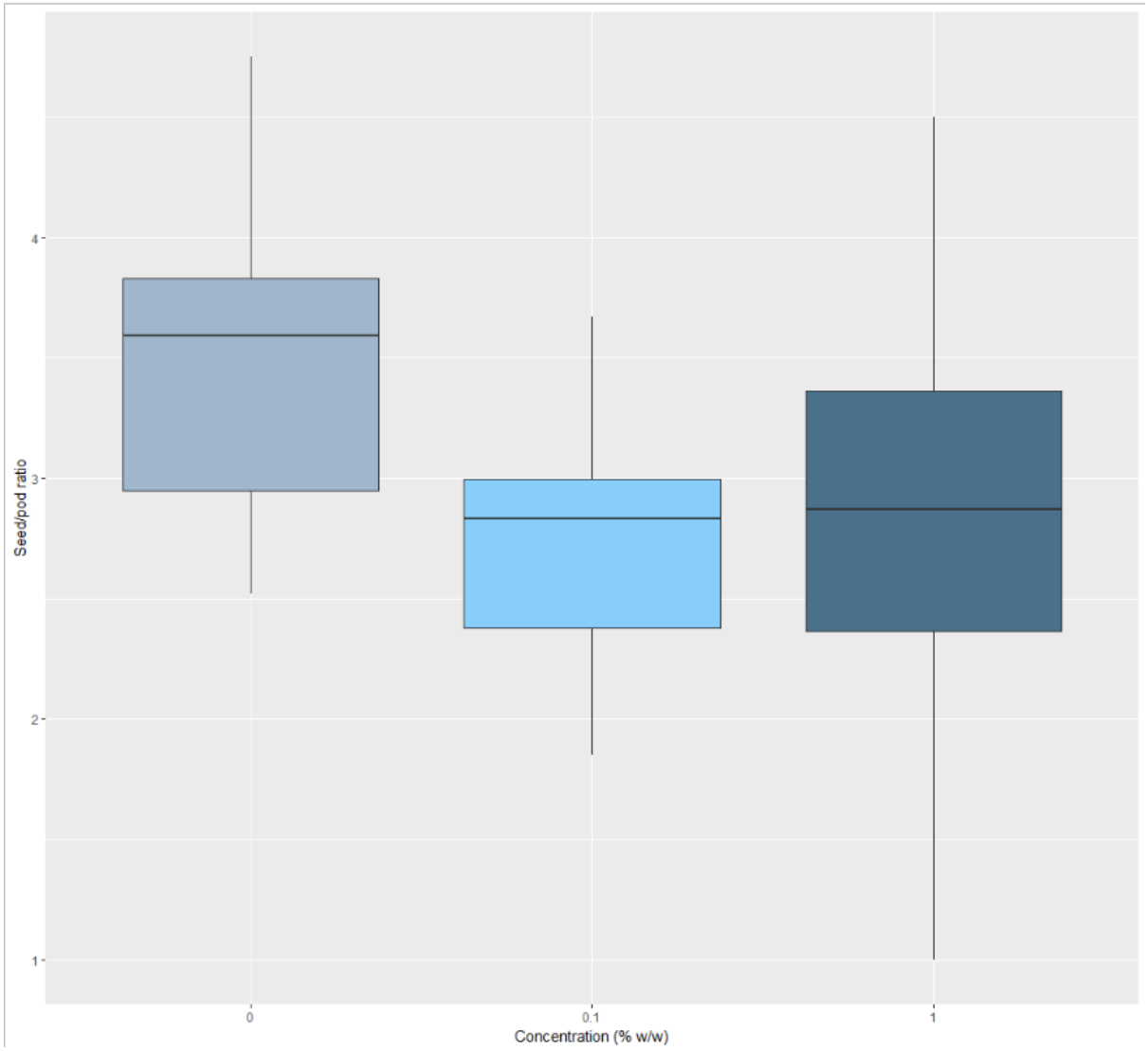


Figure 6.8 shows the seed/pod ratio of S. alba grown in the three treatments (% w/w polyester microfibres). Data is displayed as the mean ± the standard deviation, N = 30 plants total seed pods collected.

6.4 Discussion

Microplastics have been extensively incorporated into agricultural landscapes, either intentionally through agricultural practices such as the application of mulch films or through pollution (Lwanga et al., 2022). In this study, microplastics have been demonstrated to show impacts on the reproductive phase of *S. alba*'s life cycle, from flower number to seed-to-pod ratio. The results from the chlorophyll fluorescence testing indicate that the maximum efficiency of PSII was reduced in the 1 % treatment. Fv/Fm can indicate stress, photoinhibition, and the down-regulation of photosynthesis (Jägerbrand & Kudo, 2016); thus, the lowered values for the 1 % treatment indicate that microplastics were acting as an abiotic stressor for this species. *B. napus* grown in the same conditions did not .demonstrate any differences in the chlorophyll fluorescence values. Plants may not respond to stressors in the same way, with research from Hooks et al. (2019) finding that different genotypes of *B. napus* and *S. alba* raised under salt stress showed significant differences in the Fv/Fm values depending on their phenotype. Research from the marine environment has had similar findings regarding chlorophyll with Prata et al. (2018), who investigated the impacts of microplastics on the marine macroalga *Tetraselmis chuii*, finding that chlorophyll concentrations were reduced when 0.9 and 2.1 mg/L of microplastics were added. Wu et al. (2019) found that polypropylene and polyvinyl chloride microplastics suppressed algal photosynthetic activity in *Chlorella pyrenoidosa* and *Microcystic flos-aquae* by decreasing the quantum yield and inhibiting the PSII reaction centres. Research into terrestrial plants has also found a reduction in the Fm/Fv values Colzi et al. (2022) reviewed the impacts of different types of microplastics on the growth of *Cucurbita pepo*, finding that PVC and PE induced a significant decrease in Fv/Fm measurements from concentrations of 0.02 %, noting that this response was dose dependant. However, in the current research, 0.1 % w/w of polyester microfibres in soil did not induce any changes to the Fv/Fm measurements.

Gao et al. (2019) also found reductions in Fv/Fm measurements in *Lactuca sativa* plants exposed to polyethylene microplastics; their results suggested that the effective pigments involved in photosynthesis were reduced and that exposures damaged the PSII reaction centres to microplastics.

For the *S. alba* in this study, it appears that microplastics impacted the efficacy of photosystem II; however, the *B. napus* showed no differences in the efficiency of photosystem II when grown in microplastic-contaminated soils. This study and the ones discussed above demonstrate that microplastics can act as a stressor on plant species, causing effects on PSII. Changes to PSII change nutrient availability for plants; thus, this will likely impact the nutrient availability for seed production. Teng et al. (2022) found that *Nicotiana tabacum* seedlings exposed to low-density polyethylene at concentrations of 0.1 % had reduced photosynthetic activity after 48 days of culture. The authors suggested that when the MPs were too large to be absorbed by the plants, indirect factors such as changes to soil properties or blocked nutrient transport could explain the changes demonstrated to the photosynthetic performance. Additionally, the authors suggested that accumulation of reactive oxygen species, inhibition of leaf pigment synthesis and the prevention of electron transport between PSII and PSI occurred when exposed to polyethylene microplastics. Wu et al, (2019) hypothesised that stress from nanoplastics slows down the PSII electron transport rate, resulting in a build-up of electrons which amplifies photoinhibition resulting in a rise in reactive oxygen species. Thus, similar interactions could be occurring for *S. alba,* though further testing would be needed to confirm whether this is the mechanism by which changes to PSII are occurring.

Shoot lengths were not significantly different for the *S. alba* grown in the different treatments, which concurs with research by Qi et al. (2018), who found no significant differences in shoot height for *Triticum aestivum* (wheat) grown in soil contaminated with LDPE microplastic films harvested at four months. This is also consistent with research conducted in chapter 4, in which no significant differences were demonstrated in the shoot lengths for *S. alba* or *B. napus*. Ohashi et al. (2009) found that although seed parameters were changed during drought stress, such as decreased pod thickness, no significant differences were demonstrated in stem growth. In the previous studies (chapters four and five), the majority of the changes were demonstrated in root development, likely due to changes in soil properties, such as reductions in soil bulk density. Therefore, any changes to vegetative development were likely to be shown in the root development; however, this was not measured in this

study. Liu et al. (2022) found that increases in plastic resulted in increased root mass density for cotton when grown in soils with the highest levels of plastic contamination. They suggest that the changes in soil conditions resulted in an increase in penetration resistance, resulting in the soil not being suitable for root proliferation or elongation. The changes to root development were also found by Liu et al. (2022), who investigated cotton development and found that plastic films negatively affected the root biomass of soils. However, fibres have been found to decrease soil bulk density, which should help to enhance root development due to decreased penetration resistance (De Souza Machado et al., 2018).

When considering floral development, the total flower number for *S. alba* was significantly different when comparing those grown in plastic-contaminated soils versus the control, with a decrease of 43 and 40 % for the 0.1 % and 1 % treatments. There were, however, no significant changes to the total number of flowering plants. Abiotic stresses can cause floral bud abortion and reduce the flower number, leading to decreased reproductive success (Smith & Zhao, 2016). Stress-related changes can also result in reductions in nectar production, which can result in changes to plant-pollinator interactions (Descamps et al., 2021). Reduced flower numbers have been demonstrated when considering other stressors, such as drought and temperature stress. Su et al. (2013) found that drought stress reduced flower number in *Arabidopsis,* and Descamps et al. (2018) found similar results when *Borago officinalis* (borage) were grown in drought conditions. Barnabás et al. (2008) suggested that stressful environments limit cell division in the meristem and reduce the transport of nutrients to different plant tissues, thus changing overall resource partitioning, which could explain the reduction in flower number. Another strategy that Descamps et al. (2021) suggested is that plants may sacrifice young floral buds and reduce new bud formation to enable nutrients to support the continued development of older flowers and immature seeds. Further research is needed to understand the mechanisms that plants employ in microplastic contaminated soils, which results in the reduction of the total flower number. Nevertheless, it is clear that microplastics are acting as both a direct and indirect stressor on *S. alba* plants, as demonstrated by the changes to the PSII and flower number.

The reduction in flower number is likely to have further impacts on total yield (Pang et al., 2017), which correlates with the results of this study finding a reduction in seed-to-pod ratio. Additionally, although not statistically significant, the pod number and total seed number per plant were lower in the 0.1 % and 1 % treatments compared to the control. As initially hypothesised, the addition of polyester microplastics resulted in changes to the pod-to-seed ratio. Seed filling is regulated by leaf photo assimilation processes, delivering the nutrients needed for seed development, such as sucrose, starch, proteins, and fats (Sehgal et al., 2018). This may explain changes demonstrated in the pod and seed number and the seed/pod ratio for the 1 % treatment, where PSII was disrupted compared to the control (Sehgal et al.2019). However, as changes were also demonstrated to the 0.1 %, where no changes were shown in PSII, other factors not measured in this study, such as stomatal conductance, chlorophyll content, and inhibited carbon fixation enzymes, may be important (Kaushal et al., 2013).

Conversely, if changes were demonstrated in the root biomass, a reduction in vital minerals such as potassium, calcium, iron, or magnesium would result in reduced seed production (Marles, 2017).

Future research should consider how significant the effects demonstrated in seed/pod ratio would be in a typical agricultural landscape; this would enable an understanding of the economic losses that could result from adding microplastics into agricultural lands. In addition, further research is needed into different crops to review whether microplastics have similar effects on crops as on *S. alba.*

6.5 Conclusion

Polyester microfibres act as an abiotic stressor to *S. alba*, changing the plant's reproductive capacity, which could subsequently impact seed yield. Additionally, changes to photosynthesis II occurred, which could have subsequent impacts on yield. In the future, further work should assess seed filling as this is a critical factor for cereal and oilseed crops for their economic value. In addition, research should focus on changes in nutrient status and root development to understand further why changes to PSII occur.

Chapter 7 Discussion

The plastic pollution problem is not new, with accounts of pollution being reported in the 1960s (Ryan, 1987), but the increasing generation of plastic litter since the onset of mass plastic production has resulted in a global crisis of plastic pollution. Soils have been considered a sink for microplastic pollution, particularly agricultural soils (Hurley & Nizzetto, 2018); thus, understanding the extent of microplastic pollution in agricultural soils is necessary. Hence this research aimed to explore the occurrence of microplastics in UK agricultural soils, specifically in the Midlands, whilst reviewing the types and potential pathways of this pollutant of emerging concern.

When this research was started, there were limited studies which assessed the abundance of microplastics in soils, but since 2018, research into microplastics and soils has grown internationally. However, the field still requires more empirical data that can help build a comprehensive understanding of the extent and distribution of microplastic pollution in terrestrial environments, and data is still limited for the abundance and occurrence of microplastics in the UK. Furthermore, due to the high variability of microplastics in the environment, further generations of local datasets are necessary to understand the extent and variability of microplastic pollution. Additionally, an adequate quantification of the abundance of an environmental contaminant is an essential component of toxicological and exposure data and is a key component of risk assessments, which will help to enable an understanding of the effects of plastics in the environment.

A key outcome for agriculture is crop production; thus, understanding any knock-on impacts from the addition of microplastics to soils is crucial. Currently, minimal research exists concerning the impact of microplastics in agricultural soils and their subsequent effects on crop development. However, an understanding of microplastics' toxicological impacts on crops is important due to microplastics being a ubiquitous pollutant. When this research started, only one study had assessed the effects of plastic on an agricultural crop species (wheat) (Qi et al., 2018); thus, further toxicological data was needed on a range of plant species.

At the start of the PhD, the following questions were identified relating to microplastics and terrestrial plant species (1) To create a method to enable the identification and quantification of microplastics from soils (2) To consider what common polymer types were in British agricultural fields (3) To assess whether microplastics cause any changes to the germination and early development of crop species. (4) To elucidate the potential mechanisms of impacts to germination & early development to understand what may cause any changes demonstrated, and finally, (5) To understand whether impacts demonstrated to plant development are localised at the germination phase or whether there are subsequent impacts on plant reproduction and seed development.

This final chapter consolidates the main findings from five empirical chapters according to the specific objectives of this PhD research. A final consideration is given to the main research contributions and the future activity required to resolve some of the remaining research gaps.

7.1 Microplastics in UK agricultural soils

Microplastics have been found in nearly all environments on earth and have been found in soils of many terrestrial ecosystems worldwide (Rillig, 2012; De Souza Machado et al., 2018; Kallenbach et al., 2022). However, very few studies have quantified the abundance and composition of microplastics in agricultural soils (Weber et al., 2022). This is partially because the extraction of microplastics from complex media such as soils remains challenging (Möller et al., 2022). At the beginning of this PhD study, a small number of methods had been developed to extract microplastics from soils, with one of the first studies into the extraction of MPs from soils and sediments being published in 2018 (Hurley et al., 2018). Thus, understanding what methods were applicable to soils was still growing, and no standardised approaches for the extraction of microplastics from soils existed (He et al., 2018). The research in Chapter 2 demonstrated that Fenton's reagent was a suitable method for the removal of organic matter to aid in the extraction of microplastics from soils. Subsequently, a wealth of research

has investigated and validated different methods for the extraction of microplastics from soils (Herrera et al., 2018; Hurley & Nizzetto, 2018; Radford et al., 2021; Möller et al., 2020, 2022).

The second chapter of this PhD aimed to test methods for the removal of organic matter in soils to enable microplastic extraction. This research was conducted when there was limited research into the extraction of microplastics from complex media such as soils, and to date, there are no established standard methods for the sampling, extraction and identification and quantification of microplastics in soil samples (Möller et al., 2022). Thus, at the time, the methods tested were often adapted from aquatic sediment analysis methods, which have been the focus of research for longer than soils (Möller et al., 2020). In addition, at the time, there were few analytical techniques to aid with soil sampling; thus, the method employed in chapter 2 aimed to provide a method which could combine a mixture of visual sorting to enable morphological analysis of the microplastics with the ability to apply analytical methods for microplastic type identification. Despite advances in analytical techniques, Fenton's Reagent is still widely utilised in microplastic extraction (Cunsolo et al., 2021; Möller et al., 2022). The method testing enabled the research in chapter 3 assessing the types and numbers of microplastics in English soils to be conducted, resulting in the finding of polyester being the most common plastic type, which helped to inform the studies into plant development.

The research in chapter 2 concurred with Hurley et al. (2018) that Fenton's Reagent was the most suitable method for the removal of organic matter from soils to aid the extraction of microplastics. Additionally, Fenton's Reagent caused the least chemical changes to the three tested polymers when analysed using FTIR, with this research highlighting that PVC was also not damaged by this treatment, unique to this research. The tested method of Fenton's reagent digestion and subsequent density separation using zinc bromide resulted in an average recovery rate of 84 % across the three different plastic types tested. Subsequently, it was decided to utilise Fenton's reagent to digest organic matter to aid the extraction of microplastics in soils. This work helped to inform chapter 3, which investigated the levels of microplastics in agricultural soils in the United Kingdom. Based on the empirical evidence from chapter 2, the use of Fenton's reagent as a method to remove organic matter

& aid in the extraction of microplastics is recommended in any study employing density separation techniques, as it is a quick and efficient method to achieve organic matter removal.

As far as the author is aware, the study conducted in chapter 3 represents the first conducted investigating microplastic pollution in conventionally managed English agricultural soils. Chapter 3 found that microplastics were present in all the sites sampled and found in 85 % of all samples. However, their abundances varied within and amongst the sites; this varied based on sampling location (centre of the field or field margins) and whether anthropogenic additions, which were known pathways of microplastics, were used, such as sewage sludge, artificial fertilisers and composts. The mean microplastic abundance found in this study was 9.21 ± 9.49 MPs per kg dry weight (DW) across all samples. The study aimed to assess conventionally managed farms (rainfed, ploughed, sowing, fertilisation, herbicide application, and harvesting) and those that used artificial fertilisers and sewage sludge. This enabled an understanding of whether conventionally managed lands could still be contaminated with plastic debris and whether significant differences were shown when anthropogenic additions (known pathways of microplastics) were added. When fields were conventionally managed, there was a mean abundance of 6.27 ± 6.72 MPs per kg DW, which was significantly lower than when sewage sludges, artificial fertilisers, and composts were added, resulting in a mean abundance of 12.69 ± 11.04 MPs per kg DW. Radford et al, (2023) investigated the presence of microplastic in English agricultural soils where biosolids were applied. The research suggested that fields that used biosolids and those that did not have similar numbers of microplastics present. When considering this research alongside the research in chapter three, it is clear that further investigation is needed in relation to the sources of microplastics in UK agricultural soils.

Only one other study has assessed microplastic abundance in conventionally managed farmland soils (Piehl et al., 2018). Microplastic abundance in conventionally managed fields in Southern Germany had a mean abundance of 0.34 ± 0.36 MPs per kg DW (Piehl et al., 2018), which was eighteen times lower than in our study. The difference between our study and the aforementioned study could be that Piehl et al. (2018) did not assess microplastics smaller than 1 mm, which accounted for 25 % of the

microplastics found in our study. This indicates that microplastics in farmland soils can occur from sources other than sewage sludge and mulching films. Inputs from runoff cannot be discounted in our study and could help to explain some of the polymers found in conventionally managed landscapes. The atmospheric deposition of microplastic particles could be a factor in the accumulation of microplastics in conventionally managed soils; however, studies by Dris et al. (2015) found that the most common size fraction for microplastics from atmospheric deposition was 200 to 600 μm, so this cannot account for all of the plastics found. Another source of plastics in the study could be the degradation of microplastic debris (Corcoran, 2021). Ploughing could entrap microplastics and result in mechanical degradation into smaller particles, which would occur at higher rates than those found in landfills due to mechanical degradation (Yu et al., 2022). A large proportion of the polymers found were colourless, which may imply different sources of MPs, with colourless fibres potentially resulting from plastic packaging materials. The second most common colour found was blue, which could come from degraded daily items such as ropes and strings used frequently in agriculture. However, further research is needed to understand the sources of microplastics in conventionally managed farmland soils.

The study found that soil contamination with MPs was significantly increased when anthropogenic additions (sewage sludge, fertiliser and compost) were applied, which is in agreement with other research (Mahon et al., 2017; Li et al., 2019). Overall, a 67.72 % increase in MP counts was demonstrated when direct anthropogenic additions were added. The arable fields showed an increase in MP concentrations by 94.84 % when considering anthropogenic additions versus no additions, suggesting that the management of these fields and the addition of fertilisers significantly contributed to the overall plastic load. Grazing fields also demonstrated increases, with an increase of 42.87 % when anthropogenic additions were applied. This increase suggests that incorporating fertilisers, composts and sewage sludge directly contributed to microplastic levels. Additionally, it could be that microplastics contained within agricultural feeds could be contributing to the total number of plastics found within grazing lands (Ramachandraiah et al., 2022).

When considering differences between the land usage (grazing and arable), no significant differences were shown in the number of microplastic particles found (see chapter 3). This suggests that land usage did not affect the number of plastics found. It is worth considering that for our research, many of the farms rotated their land use, so the grazing lands may have previously been arable fields (within the past ten years), which could have resulted in the similarities between the two land types. Zhang et al. (2022) investigated the occurrence of microplastics in Southern China, concentrating on different land usage and how this affects microplastic abundance. The research suggested that farmlands (traditional, facility and orchards) had a higher abundance of microplastics than grasslands and woodlands. The average abundance of microplastics in the facility farmlands was 1236.36 ± 843.18 items per kg, 695.45 ± 429.83 items per kg in the traditional farmland and 640.91 ± 927.32 items per kg in the orchards. The abundance in traditional farmlands versus orchard farmlands is similar, suggesting similar levels of contamination. This was significantly higher than the numbers found in our study, but this may be due to the area sampled. Yunnan province, where Zhang et al. (2022) conducted this research, had previously been demonstrated to have extremely high levels of microplastic pollution, with Zhang and Liu (2018) finding an average abundance of 18,760 items per kg in the same province. Additionally, in Zhang et al. (2022) study, the land usage classification is different than in the current research; traditional farmlands are more akin to arable land. However, there is no direct comparison to the grazing land in chapter 3, with grassland in Zhang et al. (2022) study appearing similar to unmanaged parklands as opposed to agricultural lands. It is also worth considering that in China, suburban areas often contain agricultural sites, and rural areas can be densely inhabited, which leads to increased anthropogenic contamination (Büks & Kaupenjohann, 2020). This could result in much higher levels of microplastics found in Chinese agricultural soils than in European regions, where there is often a separation between agricultural and urban areas (Walsh et al., 2022).

Compared to studies conducted in China (Liu et al., 2018; Ding et al., 2020), my results indicate significantly lower microplastic particles in UK agricultural soils. Liu et al. (2018) found 78 ± 12.91 MPs per kg DW, which is nearly eight times higher than in our study. Liu et al. (2018) reviewed soils where mulch films were frequently applied; thus, this likely accounts for much of the differences demonstrated. Mulch films are often added to soils to modify soil temperatures, conserve soil moisture and reduce weed pressure (Bandopadhyay et al., 2018), and as disposal is often difficult, films are often ploughed into soils, thus directly incorporated into soils in the long term. Zhang and Liu (2018) reviewed croplands in China for the presence of MPs, with concentrations ranging from 7100 to 42,960 MPs per kg DW of soil. The study fields were used for intensive vegetable production, with 6 – 8 harvests per year. Due to this, a large number of fertilisers were utilised, and intensive irrigation was practised. The authors also reported that plastic mulching was widely used across the sites and plastic tunnels. Practices such as mulching, the use of tunnels, intensive irrigation and the addition of fertilizers are likely major sources of plastics in agricultural soils and thus can help to explain the extremely high numbers of microplastics found in these fields. Corradini et al. (2021) assessed the number of microplastics found in agricultural soils in Chile with sewage sludge additions, finding 400 MPs per kg DW in areas with no sludge applied and between 460 and 3800 MPs per kg DW when three sewage sludge applications had occurred. This was significantly higher than in research conducted in chapter 3, though this study primarily focused on sewage sludge application as opposed to conventionally managed soils.

The research in chapter 3 also found that polyester fibres were the most common plastic type found, though this varied between sites. In addition, the most common plastic shape found was fibres, accounting for 92 % of all the plastic shapes found; this concurs with research by Zhang and Liu (2018) and Corradini et al. (2019), who also reported similar findings on the shape of polymers found. As microplastics derive from a wide range of sources and can be transported into agricultural soils in a wide variety of ways, it is unsurprising that differences were shown across the sites. Unfortunately, this is also what makes risk assessment of microplastics difficult, as plastics can be widely variable in

the environment, with different shapes, sizes, colours, and chemical compositions (Koelmans et al., 2022). Ultimately, the results of this study were utilised to inform what type of plastic should be used for toxicological studies in chapters 4, 5 and 6.

Microplastic data is often reported in different units, which leads to difficulties when comparing results between studies. One limitation of our study is that we did not calculate the weight of the microplastics as this was not possible with the chosen methodology; thus, presenting this as a concentration for weight-to-weight plastic to soil is not possible. Currently, most studies, including our study, report microplastic particles per weight of soil; however, risk assessments generally review the concentration of plastic in soils. This is, to some extent, due to the units being reliant on the methods used; for example, manual and spectroscopic methods rely on counting and characterising individual particles, whereas chemical analysis techniques produce results by the mass of the polymer (Horton, 2019). However, the method employed in chapter 3 has the benefit of being able to fully characterise the particulates, including not only the polymer type but the shape and colour, with is not possible using semi-automated FTIR.

Future work should endeavour to report both the morphological characteristics alongside the estimated mass, which will help to enable greater comparability between the studies; however, it should be noted that this would require the use of microscopy techniques alongside analytical techniques, which can be time-consuming. Whilst methods for detecting microplastics have improved in recent years, this is due to the continually developing analytical capabilities for microplastic detection, quantification, and polymer analysis. However, when this research was conducted, limited methods were available for extracting and subsequent quantifying polymers from the soil matrix. Although ideally, a standardised approach would be applied to all studies quantifying microplastic particles, due to equipment accessibility and variability across samples, it is not reasonable to suggest a strict protocol for all studies.

Additionally, due to the methods used in this research, such as the tape lifting procedure, it was not possible to perform FTIR analysis without dissecting the tapes and removing the polymers, potentially resulting in microplastic loss. In this instance, polymer types were identified using a mixture of polarised light microscopy (PLM) and Raman spectroscopy; however, it was not possible to attain spectrograms for all polymers; thus, some polymers were just labelled as synthetic based on their morphological characteristics using PLM. This may have resulted in an underestimation of some polymer types. Both Raman spectroscopy and FTIR are vibrational spectroscopic methods and are the most common methods for the identification of microplastics (Xu et al., 2019), FTIR would have likely resulted in a similar underestimation of some polymer types. However, the method employed in this study has the benefit of minimising the loss of particles as they are secured on the tape and can be analysed in situ. Additionally, this method reduces the risk of contamination after the sample is secured on the slide. Therefore, the method utilised provided the ability to characterise size, colour and polymer type whilst minimising the loss of microplastics and reducing the risk of contamination once the sample is secured to the slide.

7.2 Microplastics and their impacts on germination

Following the quantification of microplastics within agricultural soils in the UK, it was decided to assess the impacts of polyester microfibres in soils, as these were the most common polymer type and shapes found in chapter 3. In addition, the research aimed to review whether the addition of these microfibres resulted in changes to germination rates. Chapter 4 provided insight into the mechanisms that result in germination delays by utilising a positive control (keratin fibres) with similar morphology to the polyester fibres used in the study. Microplastics were demonstrated to cause delays to germination from concentrations of 2 % w/w in two of the four species tested (*Sinapis alba* (mustard) and *Triticum aestivum* (wheat)), and all four species showed a reduction in germination rate at 5 % w/w (*H. vulgare*, *T. aestivum*, *S. alba* and *B. napus*). Additionally, the addition of a positive

control (keratin fibre) resulted in similar delays to germination as the polyester fibre for *S. alba*. This indicated that a physical change to the soil matrix could be causing delays in germination. To the authors' knowledge, this is the first study regarding germination and microplastics, which utilized positive controls, which can help demonstrate whether the mechanisms that cause germination changes are related to physical changes to the soil or chemical changes.

It has been proposed by Pflugmacher et al. (2020) that a chemical change due to leachate from polymer particles could result in changes in germination. Pflugmacher et al. (2020) assessed whether leachates from aged polycarbonates changed the germination rates of *Lepidium sativum* (cress), finding that plants germinated in a 1:10 diluted polycarbonate leachate that there was a 21 % reduction in germination. However, when the plants were exposed to new polycarbonate granules in the soil, there was a 60 % reduction in germination. The authors suggested that as the polycarbonate granules they used were large (3 ± 1 mm), effects were not related to the physical blocking of the seed pores and that any changes were likely due to the addition of phytotoxic substances from the leachates. In addition, the authors noted that the new polycarbonate granules resulted in lower germination rates than those which had been aged for 160 days. However, in our research in chapter 4, it was noted that physical contaminates could cause changes to the germination rates, which we suggest is due to changes to the seed/soil contact or due to partial dehydration of the seeds, which results in changes to imbibition. Thus chapter 5 was conducted to assess whether potential phytotoxic substances were leaching from the plastics, which resulted in changes to the germination rate. Both chapters 4 and 5 aim to clarify the mechanisms by which changes to germination rates of the seeds are occurring by assessing whether physical changes to the soil or phytotoxic compounds are resulting in these observed changes. The key findings from these studies are that physical changes to the soil matrix result in the changes demonstrated at the germination stage but that chemical changes could result in changes to root and shoot development.

The research presented in chapter 5 found that when considering germination rates, no significant differences occurred when leachate from plastic was utilised to water the seeds (*H. vulgare*, *T.*

aestivum, *S. alba* and *B. napus*). Conversely, when the plants were germinated with the addition of a physical contaminant (horsehair), a change in the germination rate was shown. This indicated that changes in the germination rates are likely to be because of changes to the soil structure due to the addition of fibres to the soil as opposed to phytotoxic compounds released from the plastic. However, this could vary for different plastics, depending on the compounds within that particular polymer. For example, Bisphenol A, a common plastic additive, has been noted to be phytotoxic to plants, resulting in changes to the shoot and root development, the chlorophyll content and the photosynthetic activity of *Vigna radiata* (mung bean) (Kim et al., 2018). Research in chapters 4 and 5 led to the hypothesis that the addition of fibre could result in a change in the potential water availability for seeds, which in turn could reduce imbibition and result in changes to the germination rate; however, further research is needed to investigate this. This was also found by Trivedi and Ahmad (2011), who investigated asbestos fibres and their associated effects on *Triticum aestivum* (wheat), *Pisum sativum* (peas) and *Brassica juncea* (mustard) germination. The authors suggested that the addition of the asbestos fibre resulted in changes to the water availability of the seeds and that the partial dehydration was potentially due to changes in seed-soil contact which led to reductions in germination rates.

Bosker et al. (2019) investigated nanosized PVC particles (50, 500 and 4800 nm) on the germination of *Lepidium sativum* (Cress), finding that the PVC particles resulted in delays in germination at all size fractions. The authors suggested a physical blocking response, with seed pores being blocked due to the addition of the particles. The concentrations of nanoparticles were between 10^3 particles mL^{-1} and 10^7 particles per mL^{-1}. Thus, direct comparisons of the concentrations used in Bosker et al. (2019) research compared to the experiments in chapters 4 and 5 are difficult due to the differences in the reporting of concentrations. Additionally, in my work, the size fractions of fibres used were larger, which means that although in Bosker et al. (2020) experiment, a blocking of seed pores may result in changes to the germination rate, it is unlikely that this explains the differences shown in germination rates in our experiments. Zhang et al. (2021) also found similar results to Bosker et al. (2020) with 200 nm polystyrene nanoplastics, finding a reduction in germination rates for *Oryza sativa* (rice) at

concentrations of 1000 mg/L. It is likely that nanoplastics and microplastics cause very different responses in plants, with nanoplastics able to block seed pores and microplastics able to cause significant changes to soil structures. Boots et al. (2019) found that a mixture of acrylic and nylon fibres (> 2 mm) resulted in a reduction of germination of *Lolium perenne* (perennial ryegrass) by 7 % compared to the control. I, therefore, suggest that changes to the soil structure caused by the microplastics result in changes to the germination rate, which was also found when using keratin as a positive control.

Chapters 4 and 5 have helped to increase understanding of the mechanisms by which germination rates are reduced when microplastics are added to soils, specifically for microfibres. Though, due to the differences shown for different plastic shapes by Machado et al. (2019), who investigated how microplastics change soil properties, it is likely that this would vary for fragments, foams and spheres. For example, Machado et al. (2019) found that bulk density was reduced when polyethylene fragments and polyester fibres were added to soils but found no differences when polyamide beads were added. Additionally, Machado et al. (2019) demonstrated that *Allium fistulosum* (spring onion) had varied responses to different fibre shapes, with root biomass significantly increasing due to the addition of polyester fibres and polystyrene, but not by HDPE particles. This suggests that differential responses occur based on the polymer's shape and/or type. These differential responses to different polymer types and shapes make the risk assessment of microplastic particles difficult; thus, chapters 4 and 5 can only provide insights into the mechanisms by which germination is delayed for polyester microfibres. These results are likely applicable to other microfibres with similar densities; however, different responses may be demonstrated for fragments, foams and spheres. It is important to consider the multidimensionality of polymers to provide accurate risk assessments in the terrestrial system, and thus further work should consider different shapes, size fractions and polymer types. This will add to the literature, enabling future work to understand the plethora of different effects that may be demonstrated.

7.3 Microplastics and the impacts on seedling development

Soil microplastics have been noted to cause direct damage at early crop stages by the physical blockage of pores in the seed capsule or from changes to root development in the seedlings (Pérez-Reverón et al., 2022). Polyester microfibres were found to cause changes to the root development of *S. alba* in chapter 4, with a reduction in length at 12.93 % for the 5 % w/w treatment compared to the control. Additionally, barley showed similar reductions for root length in the 5 % w/w treatment compared to the control, with a 9.99 % decrease. De Silva et al. (2022) found similar reductions in the root length of *Lens culinaris* (lentils) when exposed to polyethylene fibres, which the authors suggested was related to mechanical blockage of the root hairs by microplastics.

In the second experiment in chapter 4, both *S. alba* and *H. vulgare* demonstrated an increase in the root length compared to the control when exposed to polyester microfibres. When root elongation was demonstrated, it was considered that this could be related to the mechanism of root expansion that plants employ in stressful situations. An increase in the root system helps to increase water and nutrient uptake, which can help to overcome stress (Bengough et al., 2011). In chapter 4, it was demonstrated that this occurred for the polyester microfibre treatments but not for the positive control (keratin fibre), which suggests responses could be related to chemical changes in the soil, as opposed to being due to the addition of a physical contaminant; however subsequent research is needed to consider changes to nutrient availability.

Despite plastics being considered biochemically inert (Teuten et al., 2009), studies have noted the absorption and desorption of compounds from polymers in various crop plants. For example, Fu and Du (2011) found that di-(2-ethylhexyl) phthalate could be taken up by vegetables grown under plastic films. Ma et al. (2014) found that phthalate esters depressed the biomass of mung bean seedlings but demonstrated no effects on germination. Thus, chapter 5 aimed to explore whether differences were shown when grown in the presence of plastic leachates but with no physical polymer present.

Additionally, by using a positive control (keratin), this research was able to assess the response of plants to leachates when a physical contaminant was present without providing a double dose of the plastics. In the study, leachates were not collected from keratin pots, which is a limitation of this study. It could be that compounds from the keratin could have resulted in some changes to plant development; however, this was not measured in this study.

In chapter 5, we demonstrated that the addition of leachates from plastic without a physical contaminant resulted in a significant increase in root length but no changes to the shoot lengths for *H. vulgare*, *T. aestivum*, and *B. napus*. However, in two of the plant species tested (*T. aestivum* and *B. napus*), the addition of the plastic leachate and the physical contaminant (keratin) resulted in an overall reduction in the root length. For *H. vulgare*, there was an overall increase in root length when exposed to the leachate and keratin treatment. Varied responses in root and shoot development have been discussed in the literature when exposed to microplastic leachates, with Menicagli et al. (2019) finding that seedlings of *Thinopyrum junceum* and *Glaucium flavum* had varied responses. The authors found that *T. junceum* showed reductions in radicle expansion, and *G. flavum* demonstrated increases in root length when exposed to plastic leachates. The differences in root development demonstrated in our experiment could also relate to delays demonstrated in the germination assay, where for *B. napus* and *T. aestivum*, the germination of the keratin and leachate treatments was significantly different compared to the control. This delay in germination could lead to knock-on impacts in the seedling development, which has been demonstrated by Rühl et al. (2016), who found that short-term delays in germination decreased the fitness of *Agrostemma* plants, resulting in 57 % less biomass.

Interestingly, for all plants tested, the soil and leachate treatment resulted in longer root lengths than the control and keratin treatments. This could suggest that compounds leaching from the plastic could promote the seedlings' root growth. For example, compounds such as caprolactam in low

concentrations (100 – 0.1 ppm), found within the leachate, promote plant growth (Aikawa et al., 1976). Additionally, as there was a range of compounds in the leachate which could not be identified, it could be that other growth-promoting compounds are present. However, when the leachate is combined with a physical contaminant, there was a combined toxicity leading to reduced root and shoot development in two of the three species. Further investigations are needed utilising a positive control to understand how different plastic types and shapes may result in changes to the root and shoot development.

In chapters 4 and 5, I have demonstrated that changes in germination rates were related to the addition of a physical contaminant in the soil but that subsequent developmental changes are related to physicochemical changes in the soil from the addition of plastics. This thesis offers a hypothesis to explain the mechanisms by which changes to plant development have been demonstrated, adding to the literature by utilising positive controls to assess the actual harm of microplastics versus those of a natural fibre within the soil. To the authors' knowledge is the first time the approach of using a positive control to assess potential mechanisms has been conducted.

7.4 Microplastics and their impacts on terrestrial plant reproduction

It was demonstrated in chapters 4 and 5 that microplastics could affect the germination and early development of four different crop plants. However, one key outcome of the agricultural system is the production of seeds and fruit. In chapter 6, we focused on oilseed crops, where the seed is the part of the crop with economic value. Thus, understanding how microplastics may impact the reproductive phase of the plant's lifecycle is crucial. Chapter 6 reviewed the impacts of polyester microfibres on two oilseed crops, *S. alba* and *B. napus*. Unfortunately, during the time this experiment was conducted, the *B. napus* did not reach the fruiting stage; thus, we only report the impacts on chlorophyll fluorescence for this plant. For the rapeseed, the Fv/Fm values (which can be used to indicate plant stress) were not significantly different between the control, the 0.1% or the 1% w/w

polyester fibre treatment. Gentili et al. (2022) investigated PVC (1% w/w) and its impacts on the growth and photosynthetic efficacy of *Senecio inaequidens* (narrow-leaved ragwort) and *Centaurea cyanus* (cornflower). The research indicated that the cornflower's maximum efficiency of PSII was reduced when exposed to the PVC fragments; however, the narrow-leaved ragwort demonstrated no differences in Fv/Fm measurements. The authors suggested that changes to the biophysical and chemical properties of the soil resulted in changes to the absorption of macro and micronutrients, which caused changes to the photosynthetic efficacy. Nutrient availability could be responsible for the changes to Fv/Fm measurements in chapter 6; however, further testing pertaining to the availability of nutrients will be required to test this hypothesis.

S. alba plants exposed to 1 % w/w polyester fibres demonstrated significant changes in the Fv/Fm values, which demonstrates that the maximum efficiency of PSII was reduced. This can indicate stress, photoinhibition and the down-regulation of photosynthesis (Jägerbrand & Kudo, 2016); thus, the lowered values for the 1 % treatment indicate that microplastics were acting as an abiotic stressor for this species. Research has also indicated that PVC and PE induced decreases in the Fv/Fm measurements for *Cucurbita pepo* (field pumpkin) from concentrations of 0.02 % w/w (Colzi et al., 2022). However, chapter 6 did not see changes to Fv/Fm measurements at concentrations of 0.1% w/w polyester fibres. The research conducted in chapter 6 indicated that polyester microfibres could act as an indirect stressor on plant species, resulting in changes to PSII at high concentrations. Changes in PSII can ultimately change a plant's nutrient availability, which will subsequently impact seed production.

Plants are likely to be more impacted by stress in both the germination and reproductive stages. The first part of the reproductive cycle of plants is the development of flowers. We found that *S. alba* flower numbers were significantly reduced when exposed to polyester microfibres for the 0.1 % and the 1 % treatments. Abiotic stresses can induce floral bud abortion and reduce the flowering number,

resulting in decreased reproductive success (Smith & Zhao, 2016). As far as the author is aware, no other studies have considered flower production when exposed to microplastic fibres. However, research into drought stress has found similar results, with Su et al. (2013) finding reduced flower numbers in *Arabidopsis* and Descamps et al. (2018) in *Borago officinalis*. Currently, the mechanisms behind the reduction in flower numbers are unknown, but it is considered that either a reduction in the transport of nutrients is responsible or that plants may sacrifice young floral buds to support the continued development of older flowers and immature seeds.

The reduction in flower number is likely to result in impacts on the total yield. This was found in chapter 6, where a reduction in the seed-to-pod ratio was demonstrated in the plants exposed to polyester microfibres. Additionally, the plants exposed to polyester microfibres demonstrated a lower total seed number per plant and a lower pod number. The seed-filling process is regulated by leaf photo assimilation processes, which deliver the nutrients required for seed development (Sehgal et al., 2018). Therefore, the reduction demonstrated in PSII measurement in the exposed groups may explain the changes demonstrated in the seed/ pod ratio and the reduced pod and seed numbers. It is also possible that other leaf traits, such as stomatal conductance and chlorophyll content, were affected, which could result in changes to the nutrients available for seed production. Future work should consider whether microplastics have impacts on these traits to help explain the mechanisms by which seed filling was affected.

We also consider that changes to the root biomass could ultimately impact the seed-filling process. If the root biomass was reduced, this could result in a reduction in minerals such as potassium, calcium, iron and magnesium, but more research is needed to consider this. Moreno-Jimenez et al. (2022) demonstrated that polyester microfibres at concentrations of 0.4 % w/w could result in reductions in available carbon, nitrogen, potassium, magnesium, iron, copper and manganese in the shoots of *Allium cepa* (onion). The changes in nutrient availability could explain the changes in seed/pod ratio,

seed number and total pod number. Additionally, the nutrient limitation can result in changes to PSII activity, suggesting that changes to the nutrient availability could help to explain the effects demonstrated in this study. Further research is needed to consider how nutrient availability may be affected when microplastics are present in the soil.

Chapters 4, 5 and 6 demonstrated that microplastics could ultimately affect crop germination, early development and/ or reproductive processes. However, it is worth considering that the concentrations used in these experiments are higher than what is typically found in terrestrial environments. To assess the significant effects of microplastics, it is often necessary to apply these higher concentrations, which is often a standard procedure in toxicity testing (Krewski et al., 2010). Although our experiments indicate that polyester microfibres have the potential to cause phytotoxicity, we consider here that in real-world environments at current concentrations of microplastics in soils, there is no immediate ecological risk to crops. However, the plastic pollution problem is not improving, and due to the longevity and durability of plastics, concentrations will likely increase in soils. Ultimately, as concentrations increase, the ecological risk will increase, and as such, crop yields may be lowered in the future due to the presence of microplastics in soils.

7.5 Challenges and limitations

Overall, the challenges and limitations of this research were related primarily to methodology; however, the challenges discussed here are common across studies and emphasise the need for detailed reporting of protocols to inform interpretation, inter-study comparisons and reproducibility of experimental designs.

Firstly, the study on recovery rates of microplastics from soils demonstrated that although the recovery rate was good (84 % across the three plastic types tested), not all particles will be extracted,

leading to an underestimation of microplastics depending on the protocol. Whilst recovery tests were conducted in chapter 2, it was decided not to correct the sample data for the recovery percentage because the particles tested were not observed in the agricultural soil samples. Further testing of particle recovery is necessary using more representative standards, but this is reliant on quantifying the sources of microplastics found in farmland soils.

In chapter 3, it was decided to process the entire soil sample instead of subsampling. This minimised particle loss; however, this did mean that the number of replicates was low for the agricultural soils due to the extended processing time for the samples. However, there are no standardised guidelines on the volume of sample which should be processed but considered here that additional replicates could However, in our study, we prioritised understanding how different land usage impacted microplastic occurrence; thus, further studies should begin to review how variable the occurrence of microplastics is across different sites. Currently, methodologies do not enable rapid processing of soil samples for microplastic analysis, and subsequent research is needed to produce rapid methods for microplastic analysis.

When considering the limitations of the plant studies, limitations which occurred were primarily due to time constraints. In chapter 6, we aimed to quantify the effects of microplastics on the reproductive stage of two agricultural oil seed crops. In the timeframe that the PhD was due to be completed, it was not possible to complete the reproductive assessment for rapeseed, as the plant did not reach inflorescence in this time. Additionally, due to the two subsequent heatwaves in summer, damage occurred to the rapeseed plants due to the high temperatures. Thus, it was decided to end the experiment as it would not be possible to determine stress due to the microplastics or stress due to the high temperatures. This study was undertaken for a 6-month period which is considerably longer than many of the studies undertaken so far assessing the impacts of microplastics on plant development. Further work is needed to assess plant development's reproductive stage, but also generational impacts will enable an understanding of whether microplastics have intergenerational impacts on plant development.

7.6 Implications and impact of this research

It is clear that microplastics are ubiquitous within the environment. There is a significant amount of information available on the fate and occurrence of microplastics in the marine environment, but still a lesser understanding of microplastics in freshwater and terrestrial systems (Kallenbach et al., 2022). This thesis has demonstrated that polyester microfibres can result in changes to the germination phase, early development and reproductive phase in agricultural plant species.

The development of the method in chapter 2 enabled an assessment of the amount and type of microplastics in the tested agricultural fields. Fenton's reagent was the optimum method based on the results of chapter 2, which agreed with findings from Hurley et al, (2018). Future research should consider whether this method applies to a range of soil types or whether different soil compositions require different treatments to reduce organic matter and enable better extraction of microplastics across a range of soil types.

The agricultural system is of critical importance to humanity, providing the majority of our food requirements; thus, changes to this system can ultimately impact food security. Once entering agroecosystems, microplastics are likely to persist in the environment, accumulate and eventually reach levels affecting biodiversity and functioning. Thus, although the levels of microplastics found in chapter 3 are not at a level to yet impact the terrestrial ecosystem, it is possible that in the near future, impacts will be demonstrated due to the incorporation of this pollutant into soils if reduction of plastics into the system and remediation do not occur. Data collected at a local scale provides further opportunities for flux and transport models, which allow the validation of models created. Future work should aim to identify local sources, inputs and hotspots and specific sites, along with the particles' types and characteristics, providing new information to inform models.

When considering microplastics, it is important to remember that the plastic issue is broader than the micro-size fraction. Microplastics should be considered part of the wider debates regarding plastic disposal, manufacture and use. By the time microplastics arrive in soils, it is too late for mitigation;

there is no practical method for the removal of microplastics from soils for remediation; thus, to avoid the potential effects of microplastics in the environment as global concentrations increase, it is important to prevent microplastics entering or forming within the environment in the first place. Reducing or eliminating the use of plastic in everyday life may take decades, and fundamentally as of current, there is no material which can replace plastic, considering its durability, longevity and low cost compared to other materials. Even if plastic production were stopped, secondary microplastics would continue to be produced from the breakdown of plastic litter already in the environment.

Additionally, there are challenges associated with recycling agricultural plastic products, such as the types of films used and the contamination of plastics with pesticides, which results in the agroplastics being costly to recycle (Castillo-Díaz et al., 2021). Therefore, there is an urgent need to begin controlling the inputs of plastics into the environment to start reducing pollution, with further policies needed to encourage the recycling of agricultural plastics. However, reductions in the level of pollution cannot be achieved unless more datasets on actual levels of microplastic pollution in the environment are produced. The research in chapter 3 helps to increase scientific knowledge on the levels of pollution in UK agricultural environments, which is important for advancing the understanding of the extent and variability of microplastic pollution in British agricultural soils.

Large campaigns have made the public aware of the fate of plastic waste in the environment. As a result, there has been a push towards biodegradable plastic products, such as biodegradable mulch films or starch-based polymers. However, research is rapidly suggesting that, in some cases, these products may be more detrimental to the environment than plastic products. For example, Qi et al. (2018) found that wheat grown in soils polluted with biodegradable mulch films resulted in lower plant biomass than those grown in soils contaminated with polyethylene. Fundamentally, regulations are required for plastics in the environment; if and when these regulations are implemented, a risk assessment approach is recommended to compare environmental concentrations to ecologically acceptable concentrations.

The research conducted in chapter 3 can contribute to a risk assessment of microplastics in terrestrial ecosystems by generating information on microplastic abundance. Although microplastics are

considered contaminants of emerging concern, risk assessments of microplastics are limited. As environmental concentrations of microplastics are highly variable, it is difficult to measure risk unless the full extent of contamination can be assessed, which becomes even more difficult at a nanoscale.

This research also generated an understanding of the ecotoxicity of plastic microfibres on the development of plants and helped to understand the mechanisms (whether physical or chemical changes) by which changes to germination rates were demonstrated. Although much more work is needed in this area, this data contributes to the growing understanding of the ecotoxicity of plastics on terrestrial plants. Further work is needed to understand the impacts of other polymer types, shapes and sizes, as these factors may influence the toxicity of these particles on the plants. Thus, although the results of this study can help to provide an understanding of the implications for polyester microfibres in the environment, the mechanisms of impact may be different for fragments, foams and spheres and different plastic types.

The research conducted in chapter 6 provided evidence that polyester microfibres in soils could result in a reduction in crop yield for *S. alba*. However, in chapter 6, the concentrations of microfibres used were higher than those found in agricultural soils (based on research in chapter 3), and as such, it is unclear what effects may be currently occurring in a real-world environment. Additionally, as only one plastic type is considered here, and in the fields, there was a range of polymer types present, future research should consider a mix of polymer types to effectively understand the effects of microplastics in agricultural soils.

The findings presented in this thesis have contributed to the understanding of microplastics as a pollutant in agricultural ecosystems, having particular significance for this work in the UK. In addition, this work has demonstrated that microplastics can have impacts on crop development and has enabled conversations with the National Farmers Union regarding the health of soils. However, further work is needed to understand the impacts of microplastics at real-world concentrations; thus, more data is needed at local scales to assess the level of microplastics occurring throughout UK soils.

Conclusion

In recent years, research into the effects of microplastics on the environment has increased significantly. This PhD research was started when there was little knowledge of plastics in terrestrial

systems and their subsequent effects on crop development. This research presented information pertaining to the most common fibre found in the agricultural soils tested (polyester) and then considered the impacts that polyester could cause to agricultural crop species. The research presented in this thesis has enhanced the understanding of how microplastics impact terrestrial crop species, giving an insight into the mechanisms of these impacts in the germination and early developmental stages. This research found that germination is impacted by the addition of a physical fibre in the soil (whether keratin or polyester) but that the subsequent developmental changes are linked to chemical changes due to the plastics. Finally, this research has demonstrated subsequent impacts on seed production and yield, which is of crucial importance in the agroecosystem. The research also found changes to the PSII system, which needs future research to understand what is causing effects to this system when microplastics are incorporated into soils. Future work should examine whether these impacts occur for different polymers, shapes and sizes. In the future, it will be essential to coordinate research efforts to understand the fates and the effects of microplastics within the natural environment, combined with hazard assessments to determine toxicity and effect thresholds at realistic exposures and timescales.

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Appendix I – NFU Newsletter for sample collection

The following information was sent out by the NFU to members of their newsletter, as sampling was self-selective, farmers had to volunteer their details to the researcher so that further contact could be made to establish information such as farm usage, total acreage and the location of the farm.

"Hello,

My name is Ellie Harrison, and I am a PhD student at Staffordshire University, who is interested in microplastics & their risks to agricultural crop development. To get an understanding of microplastic pollution in the agricultural landscape, we are looking for farms who are willing to allow us to take some soil samples from their land. We are hoping for a multitude of farm types, including pastoral, arable and organic, and can travel to your location to retrieve the samples.

The soil sampling will include taking three small soil samples (approximately a trowel worth) from farming areas, which will subsequently be analysed back at our laboratories. All information from sampling areas will be kept confidential but will be used to inform future work and potentially be used for a future publication. We will also share our results with you to inform you of the amount of plastics we found during this study.

We know that plastic usage is important for farms, and we in no way wish to stop plastic from being used but wish to have a greater understanding of the impacts that agricultural plastics pose and hope you can help us do that.

If you are interested in allowing us to take soil samples from your farm, please see the following survey link, which will allow us to collect some information from you for further contact: https://www.surveymonkey.co.uk/r/W3Z2FQ9

Additionally, if you would prefer to contact me, my email is Eleanor.Harrison@Staffs.ac.uk or my phone number: [redacted]

Please note that all collection of samples will be done in a COVID secure manner.

I thank you for your time and hope to hear from you soon."

Appendix II – Information sheet for landowners

INFORMATION SHEET FOR LAND OWNERS

Title of study : An assessment of microplastic content in agricultural soils

Invitation Paragraph: I would like to invite you to participate in this research project which forms part of my PhD data collection. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information and discuss it with others if you wish. Ask me if there is anything that is not clear or if you would like more information.

What is the purpose of the study? The purpose of this study is to undertake an assessment of the levels of microplastics in agricultural soils in the UK. This study aims to give some insight into the "normal" levels of plastic content and help to understand the current levels of plastic in agricultural soils and to assess if there are any problems posed by this material.

Why have I been invited to take part? You have been invited to take part in this study as you have expressed an interested through the National Farmers Union (NFU). The inclusion criteria is that you are the land owner, or have the land owners permission to grant us access to take soil samples.

What will happen if I take part?

If you take part, we will arrange a time and date which is acceptable with you to come and sample the soil from your farm. This will involve taking small soil samples (approximately 300 g) from multiple locations around your land, which will later be processed back at the Staffordshire University laboratories to quantify the amount of plastic in these soils. The sample collection should take less than three hours; however, this may vary depending on the distance to walk around the land to collect samples.

The data will formulate part of my PhD thesis, and is integral to us in understanding the current levels of plastic and any problems this material may pose to agricultural crop development. The data we will require from you, is a quick questionnaire (attached) which will discuss land usage and whether sewage sludge has been used in the land in the past (to the best of your knowledge) and the GPS co-ordinates of the farm (we will collect these upon sampling). In addition to this we will collect the soils, and this data will be used to formulate a scientific paper, and my PhD thesis.

Do I have to take part? Participation is completely voluntary. You should only take part if you want to and choosing not to take part will not disadvantage you in anyway. Once you

have read this information sheet, please contact us if you have any questions that will help you make a decision about taking part. If you decide to take part we will ask you to sign a consent form (attached) and you will be given a copy of this consent form to keep.

What are the possible risks of taking part? This information is potentially sensitive information, and I am aware that microplastics are currently a contentious issue. As such, all participant information will be linked only to 2 km grid references if used in any scientific publications. Location details will be kept in a separate data store than any experimental data and only I will have the ability to link the two whilst preparing publications.

What are the possible benefits of taking part? I will provide participants with the data from their own farm, to enable you to see the levels of plastic found. As I am aware you are interested in this information, we will endeavour to provide you with this upon completion of the studies.

Data handling and confidentiality: Your data will be processed in accordance with the data protection law and will comply with the General Data Protection Regulation 2016.

Data Protection Statement: The data controller for this project will be Staffordshire University. The University will process your personal data for the purpose of the research outlined above. The legal basis for processing your personal data for research purposes under the data protection law is a 'task in the public interest' You can provide your consent for the use of your personal data (2 km square) in this study by completing the consent form that has been provided to you.

What if I change my mind about taking part? You are free withdraw at any point of the study, without having to give a reason. Withdrawing from the study will not affect you in any way. You are able to withdraw your data from the study up until June 2021, after which withdrawal of your data will no longer be possible due to the processing of results to write up for scientific publication and the formulation of the thesis chapter.

If you choose to withdraw from the study, we will not retain any information that you have provided us as a part of this study.

How is the project being funded? This project is being funded by Staffordshire University.

What will happen to the results of the study? The results will be used to formulate a chapter in my PhD thesis, we also intent to publish this data in a peer reviewed scientific journal. They will also be shared with the NFU who have helped us to facilitate this study and may be disseminated in their monthly news.

Who should I contact for further information? If you have any questions or require more information about this study, please contact me using the following contact details:

Eleanor Harrison – Eleanor.Harrison@Staffs.ac.uk

Or the Principle supervisor Kevin Reiling – K.Reiling@staffs.ac.uk

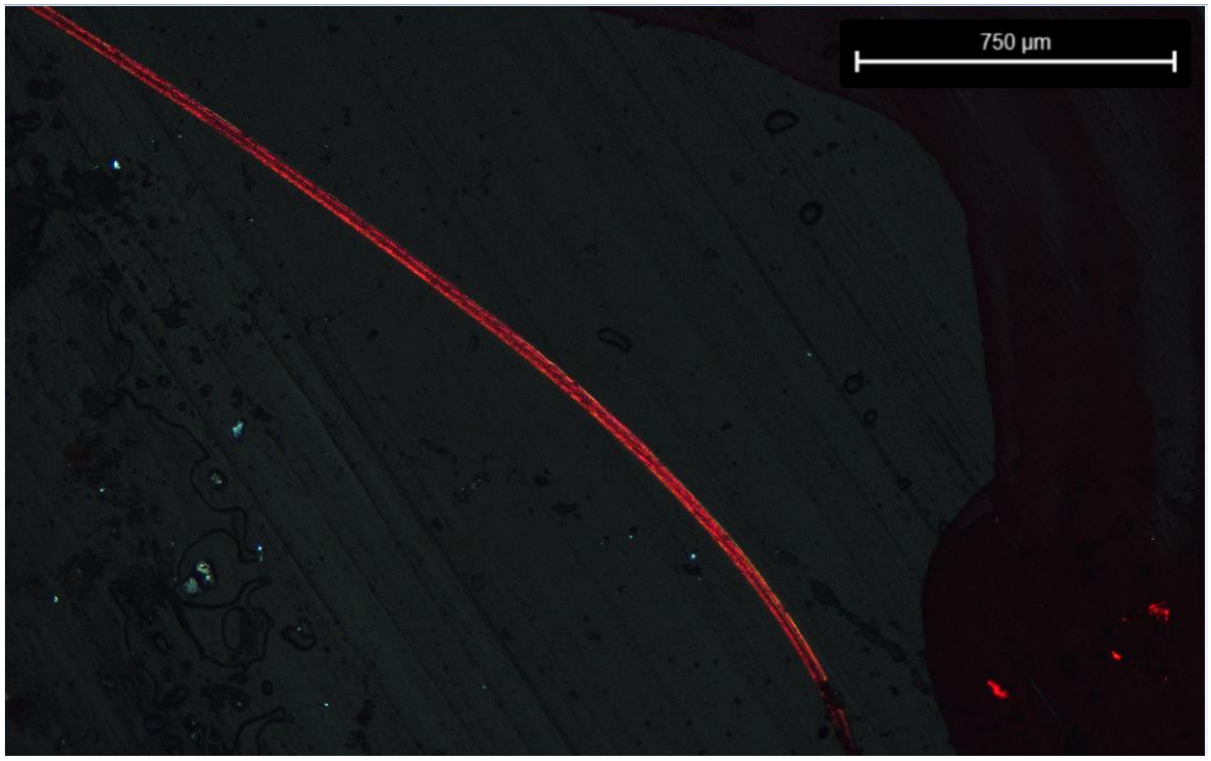
What if I have further questions, or if something goes wrong?

If this study has harmed you in any way or if you wish to make a complaint about the conduct of the study you can contact the study supervisor or the Chair of the Staffordshire University Ethics Committee for further advice and information:

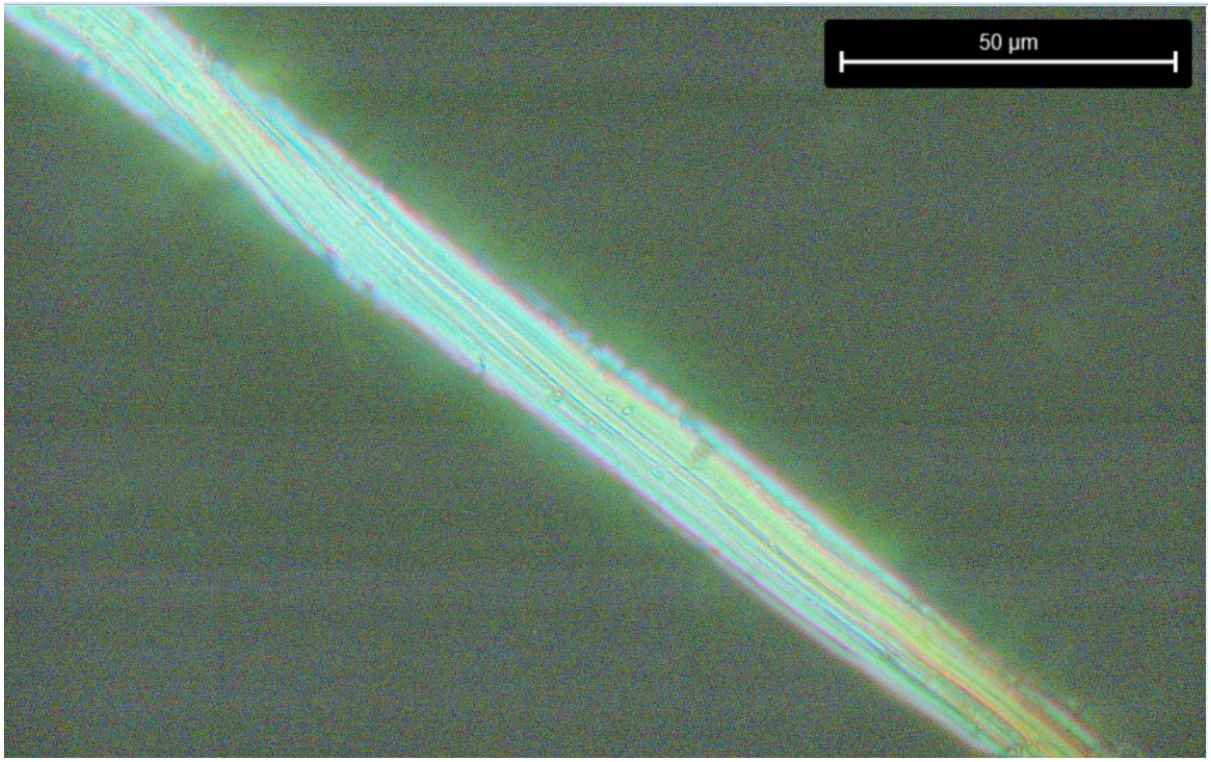
Thank you for reading this information sheet and for considering taking part in this research.

Appendix III – Examples of Fibres under PLM and Raman spectroscopy

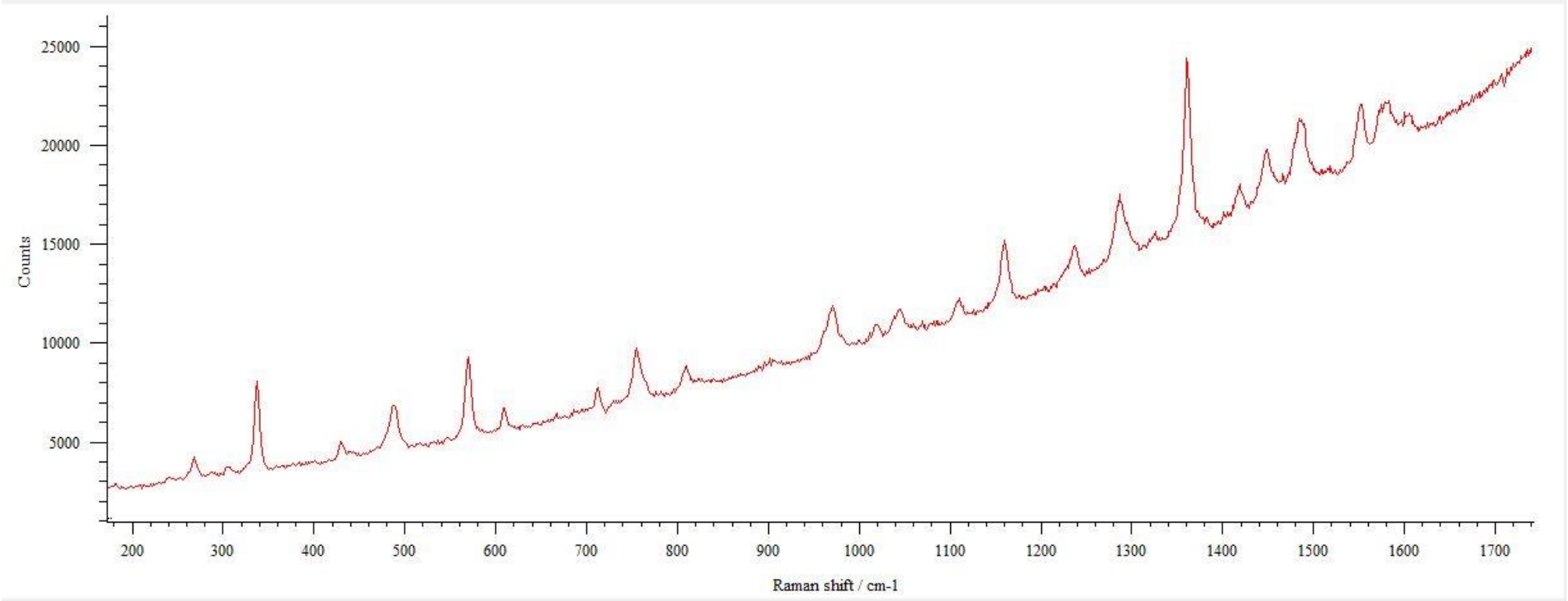
Polarized light microscopy (PLM) & Raman spectroscopy conducted in chapter 3 (section 3.2.8 and 3.2.9 respectively) to assess the types of microplastics found in agricultural soils. These images give an example of what the fibres looked like under PLM and demonstrate the Raman spectra produced.



A red multi-lobal polyethylene fibre at x400 magnification under crossed polars, demonstrating the anisotropic properties of the fibre which gives the birefringent nature. This sample is from farm four and from a grazing pasture used for cows.



A clear polyester fibre at x400 magnification under crossed polars. Note the high birefringence of the fibre, which can help to identify it as polyester. This sample is from the pasture land of farm one, in a field used for legumes.



A polypropylene fibre spectra produced using Renishaw WiRE.

Appendix IV – Questionnaire regarding land usage

The following list is the questions which were asked on site with the farmers who participated in the soil sampling study conducted in chapter 3.

Do you currently use any sewage sludge/ fertilizers/ mulching films?

If so what do you use?

How often are these applied?

What are the crops/ livestock you farm?

Do you rotate these fields?

If so how often

Appendix V - Abstract submission from oral presentation at the SETAC 31st European Meeting

3.14.05 The Impact of Polyester Microfibres and Keratin Fibres on the Germination of Four Agricultural Crop Species

E.G. Harrison, Staffordshire University / Biological Sciences;
K. Reiling, Staffordshire University / Department of Biological Sciences;
R. Halfpenny, Staffordshire University / Department of Biological Science;
C. Gwinnett, Staffordshire University / Criminal Justice and Forensic Science

Microplastics (MPs) (< 5 mm in size) are ubiquitous in all environments and are currently an emerging pollutant of interest in environmental research. Although MPs have been extensively studied in the hydrosphere, there is a dearth of studies which have investigated the terrestrial system. Of specific concern are the agricultural ecosystem where there can be a high load of MPs generated from agricultural practices, such as the application of sewage sludge or mulching films, and as such it is important to understand the impacts microplastics may have on agricultural crop species. Currently there is little known about what impact plastic fibres, a common form of MP pollution, may present to the soil-plant system, by it has been proposed that they may cause a physical change to the soil matrix. This study investigated the hypothesis that plastics are acting as a physical soil contaminant by using both polyester and natural fibres (horsetail hair) of a similar morphology to the polyester fibre. This allowed an assessment of whether physical changes to the soil structure a potential mechanism for any germination differences may be shown in this study. The effects shown in this study have potential consequences for the agro-ecosystem and could ultimately lead to a reduced crop yield.

Appendix VI – Sampling Microplastics For Environmental Forensic Applications – Detritus, Volume 14, 2021.

detritus | Multidisciplinary Journal for Waste Resources & Residues

CISA

Environmental Forensic

SAMPLING MICROPLASTICS FOR ENVIRONMENTAL FORENSIC APPLICATIONS

Claire Gwinnett [1], Eleanor Harrison [1], Amy Osborne [1], Alberto Pivato [2] and George Varghese [3]

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Microplastics – the new challenge to an environmental forensic expert

Environmental forensics involves the investigation of a diverse range of pollutants that have been accidentally or deliberately released into the environment, to understand their origin and aid the courts in attributing responsibility. For decades, pollutants such as oil and heavy metals, have been the focus of investigation. More recently, emerging pollutants such as plastic waste have become of interest to environmental forensic scientists (Aswini and Varghese, 2020). Plastics have revolutionised our daily lives and have provided significant benefits for many industries; however, although the societal benefits have been immense; there is no doubt that plastic has developed into a considerable environmental problem, resulting in calls for plastics to be classed as a hazardous waste (Rochman et al., 2013a).

The extent of this pollution type is vast; far greater than many other pollutants that are typically investigated by environmental forensic scientists. Only 9% to 12% plastic was recycled or incinerated; and 79% was discharged into the natural environment or landfills (He et al., 2020). Plastic pollution has been found to be present in all of our environmental compartments including atmospheric, terrestrial, marine and freshwater ecosystems. Figure 1 shows the abundant nature of microplastics found on a beach in San Diego. Plastic litter has been found to be present in even the most remote locations on the planet, including the Arctic (Bergmann et al., 2019), and the deep sea (Taylor et al., 2016; Woodall et al., 2014). These plastics pose a problem to aquatic biota in both marine and freshwater environments; mega plastic (>1m) and macro plastic items (2.5cm-1m) cause entanglement, suffocation and starvation and microplastics (<5mm) exposure leads to a reduction in fecundity, reduced ability to remove pathogenic bacteria and lower feeding rates (GESAMP, 2019).

Microplastics are released into our environments through a variety of mechanisms, these include both accidental and deliberate release of plastic waste. Primary sources (those which have been deliberately manufactured at this small size, e.g. microbeads in cosmetic products) and secondary sources (formed from the degradation of larger plastic items) transport between environments easily (Arthur, 2008). For example, synthetic fibres are released into the atmosphere through wear and drying of clothes (O'Brien et al., 2020), into water environments via washing (Fontana, G.D, Mossotti, R and Montarsolo, 2020) and into our terrestrial environments via sewage sludge from wastewater treatment plants (Ren et al., 2020). Although microplastics are not a standard contaminant in the remit of an environmental scientist, with other discipline experts from marine science backgrounds taking the lead in these studies, now it is clear that environmental forensic science approaches are beneficial in understanding the source of such contaminants and also in providing robust methods for sampling.

FIGURE 1: Microplastic pollution on San Diego Beach (5-7-18).

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Microplastic Sampling

The microplastic sampling methods used are dependent on the purpose of sampling and the medium being sampled. Air sampling typically uses air pumps which recover particulates onto filter papers, whereby they are easily recovered for analysis. Although air sampling for microplastics is relatively new, it has adopted approaches from other air pollution sampling techniques.

Sampling water is a little more complex depending on the amount of water being sampled, the depth and the microplastic size fraction being targeted. Water sampling can include volume reduction approaches which employs the use of nets with a given mesh size, such as neuston or manta nets that are towed along the surface (Schönlau et al., 2020) or bongo nets for sampling below the surface (Doyle et al., 2011). Alternatively, grab sampling, aka bulk sampling may be employed, where a given amount of water is collected either by using a container, such as a metal bucket (for surface sampling) or niskin bottle (for below surface sampling - Figure 2) or an in-situ pump. Grab sampling is not size selective unlike the use of any mesh or filters which will only capture samples larger than its mesh/pore size. This typically leads to microplastics smaller than 300 micrometers, (a common mesh size) not being collected (Setälä, 2016), which is problematic when trying to understand the extent of this pollution. A critique of the different methods employed for sampling water for microplastics was completed by Prata et al.

Soil sampling utilises metal augers or steel soil samplers (usually trowels) to obtain samples to a given depth (Yang et al., 2021). Figure 3a shows a metal auger used for soil sampling. Sampling in beach sediments (Figure 1) is carried out by scraping out a small depth of sediments from a definite area. The procedure specified by NOAA (Lippiatt et al., 2013) for sampling of micro-debris from shoreline is often adopted. Figure 3b demonstrates mapping out soil sampling locations along a bank of a river.

FIGURE 2: Water sampling using a niskin bottle along the Hudson River with Staffordshire University (UK) and the Rozalia Project (USA). Photo courtesy of the Rozalia Project.

FIGURE 3a-b: Soil Sampling along the Hudson River with Staffordshire University (UK) and Rozalia Project (USA) Photos courtesy of the Rozalia Project.

A specific strategy should be employed for complex biosolid matrices such as composts, anaerobic digestates and sewage sludges where microplastics are widely found nowadays. These end-of-wastes can be directly used as bio-fertilizers in soils and, therefore, a deep investigation on concentrations of MPs is required to avoid their remission into the biosphere.

Because it is not practicable to separate the MPs and the solid matrix before the sampling phase, the sampling should follow several standards developed specifically for the above cited biosolids. The choice of the approach is determined by the research, technical or forensic question and involves considerations of the hypothesized analyte distribution in the field, potential sources, or final destination.

Microplastic sampling for forensic applications

Sampling of contaminants for the context of the courts, requires robust procedures. In environmental forensic sampling, the integrity and continuity of the sample is paramount. In microplastic research studies, the requirements of court are not currently present but as we move towards gaining source level information from microplastic samples, the need for methods that can stand up to scrutiny in court is required. Regardless of the sample type, they should be secured for transportation in a manner that prevents contamination and loss. However, for many years, microplastic studies did not consider contamination of samples during sampling, transportation or processing, likely leading to the overestimation of microplastics present. Since then, protocols from the forensic science industry for minimising contamination have been adapted for microplastic use (Woodall et al., 2015).

Environmental forensic procedures require full labelling of samples and detailed descriptions of the locations they have been obtained from. GPS coordinates, along with local details such as proximity to cities, roads and wastewater treatment plants is required yet not always gathered. In addition, control samples from potential sources of the pollutant are not regularly recovered, for example, agricultural plastics that may have entered the soil and subsequently nearby water environments. Collection of controls in other environmental forensic sampling is standard and it is likely due to the infancy of the analysis of this new pollutant that the sub-discipline has not evolved enough to start investigating source in a meaningful manner. For environmental forensic scientists and those investigating waste, inclusion of plastic pollution into workflows is likely to increase in coming years.

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