Microplastic effects on soil properties: Direct and indirect effects as a single factor and interaction with other global change factors

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Declaration

I hereby declare that this dissertation was written and prepared by me independently. Furthermore, no sources and aids other than those indicated have been used. Intellectual property of other authors has been marked accordingly. I also declare that I have not applied for an examination procedure at any other institution and that I have not submitted the dissertation in this or any other form to any other faculty as a dissertation. The dissertation was reviewed by Prof. Dr. Matthias C. Rillig and Prof. Dr. Britta Tietjen, the defense was conducted on 06.03.2023 at 10:15.

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SUMMARY

Microplastic pollution in terrestrial ecosystems has attracted increasing concern regarding possible impacts on soil functionality. Microplastics can affect soil physicochemical properties, such as aggregation, bulk density, water holding capacity, porosity, pH, etc., and also soil microbial activity measured as respiration and enzymatic activities, with ensuing consequences on plant performance.

This doctoral work firstly summarized the sources, migration, and distribution of microplastics in the soil, their effects on soil physicochemical properties, soil biota and plant performance based on previous studies. Then this work investigated the microplastic effects on soil physicochemical properties and microbial activity. The effects included both direct and indirect effects of microplastics as a single factor, as well as a study including the combined effects of microplastics with other global change factors.

The review study (chapter 2) summarized the microplastic pollution in terrestrial ecosystems including the sources and distribution of microplastics in soil, and the potential migration pathways. Microplastic effects on soil physicochemical properties such as aggregation, water dynamics, pH, and organic matter contents were also included. Finally, this review provided a general understanding of the impacts of microplastics on soil biota including soil fauna and microbes, and their known consequences on plant performance.

The first laboratory study (chapter 3) explored the direct impacts of microplastics with different shapes and polymer types on soil pH and microbial activity, and how these effects may change over incubation time. This work revealed the influences of twelve microplastics (four shapes made of three different polymer types) on soil pH and microbial activities. We specifically found that microplastics could affect soil pH, respiration, and enzymatic activities depending on their shape, polymer type, and incubation time. Specifically, soil pH increased with foams and fragments, and overall soil pH reduced initially and increased afterwards over time. Soil respiration increased with foams, and soil respiration declined with time. Enzymatic activities were impacted by microplastic shapes and polymer types, and fluctuated with incubation time. They were negatively correlated with soil pH, and the presence of microplastics weakened this correlation.

The second laboratory work (chapter 4) revealed the indirect effects of microplastic-contaminated soil layers on water distribution, soil aggregation, and microbial activities of adjacent soil layers without microplastics. This research indicated that microplastic-contaminating soil layers could affect the water flow and distribution, the proportion of different-sized aggregates, and microbial activities in adjacent soil layers. Specifically, microplastic-contaminating soil layers impacted the vertical water flow along the soil profile

surrounding soil layers, with consequences on water contents and distribution in adjacent soil layers. In addition, microplastic-contaminating soil layers changed the proportion of different-sized aggregates in different depths of the adjacent soil layers. These physical changes contributed to the alterations in soil respiration in adjacent soil layers, but not translated to soil enzymatic activities. Interestingly, microplastic fibers showed more pronounced effects than microplastic films on such soil properties.

The third laboratory research project (chapter 5) examined the combined effects of microplastics and drought on a soil-plant system. This study evaluated the microplastics direct effect, and its interaction with drought on soil ecosystem functions and multifunctionality. We found that these effects varied with soil water conditions. That is, microplastic fibers (1) inhibited microbial activity (respiration and enzymatic activities) under well-water conditions, while enhanced microbial activities under drought conditions; (2) promoted litter decomposition under well-water conditions, whereas suppressed it under drought conditions; (4) diminished leachate SO_4^{2-} irrespective of the soil water conditions, decreased leachate NO_3^- only when microplastics combined with drought, increased leachate PO_4^{3-} under well-watered conditions; (5) and increased soil aggregation and soil pH regardless of water conditions; (6) microplastic fibers and drought negatively affected not only single ecosystem functions, but also soil ecosystem multifunctionality.

ZUSAMMENFASSUNG

Die Verschmutzung durch Mikroplastik in terrestrischen Ökosystemen gibt zunehmend Anlass zur Sorge über mögliche Auswirkungen auf Bodenökosysteme. Mikroplastik kann die physikalisch-chemischen Eigenschaften des Bodens wie Aggregation, Schüttdichte, Wasserhaltevermögen, Porosität, pH-Wert usw. sowie die mikrobielle Aktivität des Bodens, gemessen als Atmung und enzymatische Aktivitäten, beeinträchtigen, was sich wiederum auf die Leistung der Pflanzen auswirkt.

In dieser Doktorarbeit wurden zunächst die Quellen, die Migration und die Verteilung von Mikroplastik im Boden sowie ihre Auswirkungen auf die physikalisch-chemischen Eigenschaften des Bodens, die Bodenbiota und die Pflanzenleistung zusammengefasst. Anschließend wurden die Auswirkungen von Mikroplastik auf die physikochemischen Eigenschaften des Bodens und die mikrobiellen Aktivitäten untersucht. Anschließend wurden die Auswirkungen von Mikroplastik auf die physikochemischen Eigenschaften des Bodens und die mikrobielle Aktivität untersucht. Die Auswirkungen umfassten sowohl direkte und indirekte Auswirkungen von Mikroplastik als Einzelfaktor als auch eine Studie, die die kombinierten Auswirkungen von Mikroplastik mit anderen Faktoren des globalen Wandels berücksichtigte.

In der Übersichtsstudie (Kapitel 2) wurde die Verschmutzung durch Mikroplastik in terrestrischen Ökosystemen zusammengefasst, einschließlich der Quellen und der Verteilung von Mikroplastik im Boden sowie der potenziellen Migrationspfade. Die bekannten Auswirkungen von Mikroplastik auf die physikalisch-chemischen Eigenschaften des Bodens wie Aggregation, Wasserdynamik, pH-Wert und Gehalt an organischer Substanz wurden ebenfalls berücksichtigt. Schließlich vermittelte dieser Überblick ein allgemeines Verständnis der Auswirkungen von Mikroplastik auf die Bodenbiota, einschließlich der Bodenfauna und der Bodenmikroben, sowie der bekannten Folgen für die Pflanzenleistung.

Die erste Laborstudie (Kapitel 3) untersuchte die direkten Auswirkungen von Mikroplastik mit unterschiedlichen Formen und Polymertypen auf den pH-Wert und die mikrobielle Aktivität im Boden und wie sich diese Auswirkungen im Laufe der Inkubationszeit verändern können. Diese Arbeit zeigte die Einflüsse von zwölf Mikroplastikarten (vier Formen und drei Polymertypen für jede Form) auf den pH-Wert und die mikrobiellen Aktivitäten im Boden. Wir fanden insbesondere heraus, dass Mikroplastik den pH-Wert, die Atmung und die enzymatischen Aktivitäten im Boden je nach Form, Polymertyp und Inkubationszeit beeinflussen kann. Insbesondere stieg der pH-Wert des Bodens mit Schaumstoffen und Fragmenten an, und der Gesamt-pH- Wert des Bodens sank zunächst und stieg dann mit der Zeit an. Die Bodenatmung nahm mit Schaumstoffen zu, und die Bodenatmung nahm mit der Zeit ab. Die enzymatischen Aktivitäten wurden durch die Form des Mikroplastiks und die Art des Polymers beeinflusst und schwankten mit der Inkubationszeit. Darüber hinaus korrelierten die enzymatischen Aktivitäten

negativ mit dem pH-Wert des Bodens, und das Vorhandensein von Mikroplastik schwächte diese Korrelation ab.

Die zweite Laborarbeit (Kapitel 4) zeigte die indirekten Auswirkungen von mit Mikroplastik verunreinigten Bodenschichten auf die Wasserverteilung, die Bodenaggregation und die mikrobiellen Aktivitäten der angrenzenden Bodenschichten ohne Mikroplastik. Diese Untersuchungen zeigten, dass mit Mikroplastik verunreinigte Bodenschichten den Wasserfluss und die Wasserverteilung, die Anteile unterschiedlich großer Aggregate und die mikrobiellen Aktivitäten in angrenzenden Bodenschichten beeinflussen können. Insbesondere wirkten sich die mit Mikroplastik kontaminierten Bodenschichten auf den vertikalen Wasserfluss entlang des Bodenprofils aus, das die Bodenschichten umgibt, was sich auf den Wassergehalt und die Wasserverteilung in den angrenzenden Bodenschichten auswirkte. Darüber hinaus veränderten die mit Mikroplastik verunreinigten Bodenschichten den Anteil von Aggregaten unterschiedlicher Größe in verschiedenen Tiefen der angrenzenden Bodenschichten. Außerdem veränderten die mit Mikroplastik verunreinigten Bodenschichten den Anteil der unterschiedlich großen Aggregate in den verschiedenen Tiefen der angrenzenden Bodenschichten. Diese physikalischen Veränderungen trugen zu den Veränderungen der Bodenatmung in den angrenzenden Bodenschichten bei, wirkten sich jedoch nicht auf die enzymatischen Aktivitäten im Boden aus. Interessanterweise zeigten Mikroplastikfasern stärkere Auswirkungen auf diese Bodeneigenschaften als Mikroplastikfolien.

Im dritten Laborforschungsprojekt (Kapitel 5) wurden die kombinierten Auswirkungen von Mikroplastik und Trockenheit auf ein Boden-Pflanzen-System untersucht. In dieser Studie wurden die direkten Auswirkungen von Mikroplastik und seine Wechselwirkung mit Trockenheit auf die Funktionen des Bodenökosystems und die Multifunktionalität bewertet. Wir fanden heraus, dass diese Effekte je nach Bodenwasserbedingungen variierten. Das heißt, Mikroplastikfasern (1) hemmten die mikrobielle Aktivität (Atmung und enzymatische Aktivitäten) unter guten Wasserbedingungen, während sie die mikrobielle Aktivität unter Trockenheitsbedingungen erhöhten; (2) förderte die Zersetzung von Streu unter guten Wasserbedingungen, während sie unter Trockenheitsbedingungen unterdrückt wurde; (4) verringerte das Sickerwasser SO_4^{2-} unabhängig von den Bodenwasserbedingungen, verringerte das Sickerwasser NO_3^- nur, wenn Mikroplastik mit Trockenheit kombiniert wurde, und erhöhte das Sickerwasser PO_4^{3-} unter gut bewässerten Bedingungen; (5) und erhöhte die Bodenaggregation und den pH-Wert des Bodens unabhängig von den Wasserbedingungen; (6) Mikroplastikfasern und Trockenheit wirkten sich nicht nur negativ auf einzelne Ökosystemfunktionen, sondern auch auf die Multifunktionalität des Bodenökosystems aus.

THESIS OUTLINE

This thesis is a cumulative work that includes a general introduction (chapter 1), a literature review (chapter 2), three published manuscripts (chapters 3-5), and a general discussion (chapter 6). In the general introduction section, research aims are provided. In the general discussion section, summary figures are presented to show the differences between direct and indirect effects, and between isolated and combined effects. The references of each manuscript are included at the end of the manuscript (chapters 3-5). The references of the general introduction (chapter 1), literature review (chapter 2) and general discussion (Chapter 6) are provided at the end of the thesis.

Chapter 2: Review of microplastic occurrence in and effects on soil

Author contributions: Zhao, T. and Lozano, Y.M. designed the concept of this review. Zhao, T. wrote the first draft with the help of Lozano, Y.M. All the authors contributed to the final version of this chapter.

Chapter 3: **Zhao, T.**, Lozano, Y. M., and Rillig, M. C. (2021). Microplastics increase soil pH and decrease microbial activities as a function of microplastic shape, polymer type, and exposure time. Frontiers in Environmental Science 9:675803, https://doi.org/10.3389/fenvs.2021.675803

Author contributions: All the authors contributed to the conceptualization and design; Zhao, T. established and maintained the experiment and performed the lab analyses; Zhao, T. and Lozano, Y. M. contributed to the data analysis; and all the authors contributed to the writing and final manuscript.

Chapter 4: Kim, S.-W., Liang, Y., **Zhao, T.**, and Rillig, M. C. (2021). Indirect effects of microplastic-contaminated soils on adjacent soil layers: vertical changes in soil physical structure and water flow. Frontiers in Environmental Science 9: 681934, https://doi.org/10.3389/fenvs.2021.681934

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Chapter 5: Lozano, Y. M., Aguilar-Trigueros, C. A., Onandia, G., Maaß, S., **Zhao, T.**, and Rillig, M. C. (2021). Effects of microplastics and drought on soil ecosystem functions and multifunctionality. Journal of Applied Ecology 58:988-996, https://doi.org/10.1111/1365-2664.13839

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analyzed soil enzymatic activities; all the authors contributed to the writing and final manuscript.

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Chapter 1: GENERAL INTRODUCTION

Microplastics have been considered as an emerging global change factor (Rillig et al., 2019a), with multiple consequences on soil ecosystem functions (Lozano et al., 2021). Many studies have suggested that microplastics can affect soil physicochemical properties, soil microbial activities, and soil biota diversity. For instance, microplastic fibers can increase water holding capacity (de Souza Machado et al., 2018; Lozano & Rillig 2020; Chy 2021), while others such as microplastic films can increase soil water evaporation (Wan et al., 2019).

Most studies only examined the direct effects of microplastics on soil properties and water flow behaviour (Jiang et al., 2017), organic matter distribution (Zhang & Zhang 2019), and their toxic additives' effects on soil biota (Kim et al., 2020). However, microplastics also have indirect effects on soil microbial activity by altering different soil properties (Yu et al., 2020). Aligning with this idea, microplastics can leave a legacy effect on soil even when the microplastics have been removed (Lozano & Rillig 2022), or can modify the soil properties of surrounding areas free of microplastics. In any case, both direct and indirect microplastic effects may influence soil ecosystem functionality.

As we know, terrestrial ecosystems are not exposed to only one global change factor but to many factors such as microplastics, or drought that act simultaneously (Rillig et al., 2021c). Although the global change factors always happen in concert, most studies only focused on single-factor effects (Rillig et al., 2021c). It is still unclear how microplastics interact with other global change factors such as drought. However, previous research evidenced that microplastics can help the soils hold water for a longer time (de Souza Machado et al., 2018; Lehmann et al., 2020; Lozano & Rillig 2020; Chy 2021); although they may also increase soil water evaporation rate by creating channels for water movement, which may potentially exacerbate the negative effects of drought (Wan et al., 2019). Despite all of this, our knowledge about how microplastics interact with drought and their consequences on soil ecosystem functionality is still insufficient.

In this doctoral work, I aimed to explore (Figure 1.1):

(1) The direct effects of microplastics of different shapes and polymers on soil properties and microbial activity. Here I will investigate the effects of different microplastics (12 microplastic types) on soil properties and microbial activities in a microplastic-soil test system, and how their effects change along incubation time.
(2) The indirect effects of microplastics on soil properties and microbial activities via a soil test column. Here I will explore how microplastic-contaminating layers impact the soil properties and microbial activities of the control soil layers without microplastics.
(3) The isolated and the combined effects of microplastics and drought on soil properties and microbial activities. Here I will use a plant-soil system in microcosms treated with and without microplastic fibers under drought and well-watered conditions.

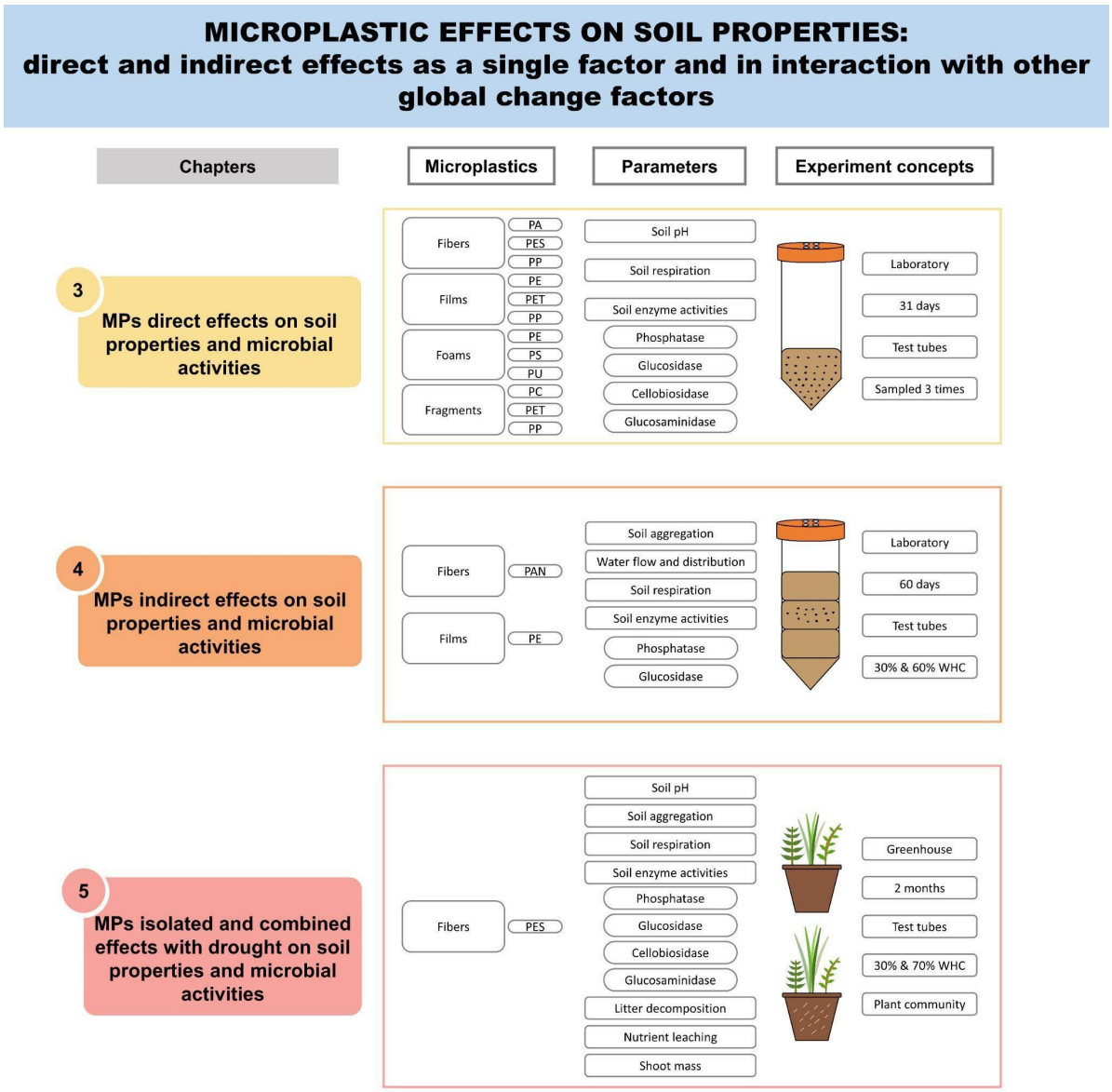

Figure 1.1 Summaries of the purposes and experimental designs of this doctoral thesis.

Abbreviations: MPs represent microplastics.

In chapter 2, we aimed to give a comprehensive overview of how microplastics affect soil properties, soil biota, microbial activity, and plant performance. We firstly reviewed microplastic pollution in terrestrial ecosystems according to the microplastic sources, distribution, and potential migration routes in the environment. Then we reviewed the impacts of microplastics on soil physicochemical properties including aggregation, water dynamics, pH and organic matter contents; and the effects on soil biota and plants.

In chapter 3, we aimed to test the direct effects of various microplastic types on soil properties and microbial activities. We hypothesized that different microplastic types might alter the natural state of soil properties such pH, and microbial activities depending on microplastic shapes,

polymer types, and incubation time. Thus, to test our hypotheses, we designed a 31- day lab experiment that included twelve microplastics with four microplastic shapes, and each shape consisted of three polymer types. We sampled three times (on the 3rd, 11th, and 31st day), and measured soil pH, respiration, and enzymatic activities each sample time.

In chapter 4, we aimed to determine the indirect effects of microplastic on soil properties and microbial activities. We hypothesized that microplastic-contaminated soils would affect soil properties such as soil respiration and soil enzymatic activities of adjacent soils without microplastics. Thus, we designed a soil column test of three-layer soil columns (control layer, microplastic-contaminated layer, and control layer); no microplastics were added to the control layers. The soil was subjected to two levels of water conditions (low: 30% water holding capacity, and high: 60% water holding capacity). We sampled on the 1st day to measure how soil aggregates fraction, water content and flow changed in the short term with the addition of microplastics; and sampled on the 60th day to test how these parameters and soil microbial activity changed in long-term incubation and under different water regimes.

In chapter 5, we aimed to test the isolated (or direct) and combined effects of microplastics and drought on soil properties and microbial activities. We hypothesized that water conditions in the soil could affect the microplastic effects on soil properties, including pH and aggregation, soil respiration, enzymatic activities, litter decomposition and nutrient leaching. Thus, we conducted a microcosm experiment that contained plant communities growing in soil with or without microplastic fibers under drought (30% water holding capacity) or well-water conditions (70% water holding capacity). The parameters mentioned above were measured after a two-month incubation.

Chapter 2: REVIEW OF MICROPLASTIC OCCURRENCE IN AND EFFECTS ON SOIL

Tingting Zhao, Yudi M. Lozano, and Matthias C. Rillig

2.1 ABSTRACT

Plastic pollution has become a global environmental issue due to their wide and intensive use in our daily life. Plastic debris that degrades into particles smaller than 5 mm is defined as microplastics. Soil microplastic pollution has attracted increasing concern recently due to its ubiquity and potential effects on soil ecosystem functions and the ecological environment. They are accountable for many changes in soil properties, soil biota and plant development because of their characteristics including toxicity and hydrophobicity. This review firstly summarizes the microplastic pollution in terrestrial ecosystems including the sources and distribution of microplastics in soil, and the potential migration pathways. Likewise, this review showed the known microplastic effects on soil physicochemical properties such as aggregation, water dynamics, soil pH, and organic matter contents. Finally, this review aims to provide a general understanding of the impacts of microplastics on soil biota including soil fauna and soil microbes, and on plant performance.

2.2 MICROPLASTIC POLLUTION IN TERRESTRIAL ECOSYSTEMS

We currently live in a "plastic age" (Thompson et al., 2019), in which tons of plastics are produced daily to meet the rising demand for plastic-based products. According to PlasticsEurope (2019), global plastic production dramatically increased to 360 million tons in 2018 worldwide, of which 62 million tons were produced in Europe. This tremendous production is due to their wide applicability in agricultural systems, packaging, manufacturing, costumes, medicine, and other fields; added to their advantages in terms of low cost, lightweight nature and resistance among others (Fred-Ahmadu et al., 2020a; Ya et al., 2021). Durability and low recycling rate explain the high accumulation of plastics in terrestrial systems (~79 % in landfills or natural environments) (Rillig 2012; Geyer et al., 2017). Once released into the environment, these plastics can break down into smaller pieces via degradation processes, reaching sizes smaller than 5 mm (Microplastics) (Fred-Ahmadu et al., 2020a; Wang et al., 2022a). According to their origin, microplastics are classified as primary (produced for commercial use such as cosmetic products) and secondary microplastics (derived from the degradation and breakdown of large plastic particles due to environmental factors such light, temperature, and wind) (Lehtiniemi et al., 2018; Leed & Smithson 2019; Guo et al., 2020).

2.2.1 The sources of microplastics in soil

Microplastics released in the soil every year can be over 20 times more than microplastics in

aquatic environments (Horton et al., 2017). They can enter the soil from various sources, such as agricultural mulching films, water irrigation, compost and sludge application, flooding, atmospheric input, and littering or street run-off (Bläsing & Amelung 2018; Wu et al., 2021; Yu et al., 2022b; Kaur et al., 2022). Figure 2.1 shows the potential sources and migration paths of microplastics in soil.

Agricultural mulching films. Agricultural mulching films are one of the most dominant sources of microplastics in terrestrial systems, as they are broadly employed in farmland due to their functions of protecting seeds, maintaining soil moisture, increasing temperature, and overall bolstering crop yields (Bläsing & Amelung 2018; Hu et al., 2021; Zhou et al., 2021; Qi et al., 2022; Roy et al., 2022). Mulching film production was up to 2.695 million tons in China in 2019 (Hu et al., 2021). Such types of plastic could hardly be recovered, recycled, or degraded, thus contributing to large amounts of plastic waste in the soil (Yu et al., 2022b). For example, the detected microplastic concentration of mulching films was 31 to 129.6 particles kg^{-1} in fields with five years of continuous mulching, and it positively correlated with time (Huang et al., 2020). Undoubtedly, agricultural mulching films are important sources of microplastics in soils.

Irrigation water. The irrigation water sources include surface waters (i.e., rivers, lakes, reservoirs), groundwater, and purified sewage (Jiménez 2006; Bläsing & Amelung 2018; Yang et al., 2021a), which may contain high concentrations of microplastics (Su et al., 2016; Mintenig et al., 2019; Yu et al., 2022b), resulting in large amounts of plastic particles entering the soil environments (Okoffo et al., 2021). Evidences indicated that surface waters may contain microplastics ranging from 10^2 to 10^6 particles m^{-3} in lakes and rivers (Dris et al., 2015; Eerkes-Medrano et al., 2015). For example, the concentration of plastic particles in Taihu Lake has been estimated at a range of 1×10^4 to 6.8×10^6 particles km^{-2} (Su et al., 2016). Groundwater provides drinking, agricultural, residential, and industrial water for almost two billion people on Earth (Khant & Kim 2022; Viaroli et al., 2022), such water sources can be strongly affected by microplastics. As microplastics can be transported horizontally or vertically via soil migration, surface runoff, wind erosion, etc., they can contaminate groundwater systems (Khant & Kim 2022). Indeed, the concentration of microplastics in groundwater could vary from 0 to 7 particles m^{-3}, with a mean value of 0.7 particles m^{-3} (Mintenig et al., 2019). Similarly, 3,352 microplastic particles were detected in the groundwater of China Jiaozhou Bay (Su et al., 2021). Wastewater is another source of microplastics in soils. They can be released into the soil at a concentration of 1000 to 627,000 microplastic particles m^{-3} (Bläsing & Amelung 2018; Gies et al., 2018). Sewage treatment processes can concentrate a large proportion of microplastics from domestic and industrial sources (Corradini et al., 2019), making sewage products a significant source of microplastics in water ecosystems (Gao et al., 2020; van den Berg et al., 2020). Personal care products such as cosmetics and detergents, as well as different forms of polyethylene and polypropylene, are also contained in sewage (Leed & Smithson 2019;

Majewski et al., 2016).

Biosolids application. Biosolids are sewage sludges stabilized by aerobic or anaerobic digestion, composting, drying, or liming (Gaylor et al., 2013). Due to their high contents of organic matter and nutrients (i.e., N, P), biosolids are widely applied to improve crop yields (Picariello et al., 2020), but it comes with a price, as large amounts of organic pollutants such as microplastics are contained in biosolids (Gaylor et al., 2013; Mahon et al., 2017; Bläsing & Amelung 2018; Zhang & Liu 2018). Applying biosolids as fertilizer results in microplastic accumulation in soil, exacerbating soil pollution. Indeed, the microplastic contents increased dramatically after the sludge application in a field; for example, the low-density microplastics were up to 280 particles kg^{-1} higher in the soil after sludge application (van den Berg et al., 2020). Biosolids can also contain toxic and harmful substances such as heavy metals, persistent organic compounds, antibiotics, pathogenic bacteria, and parasite eggs (Yu et al., 2022b). Moreover, the amount of microplastics in soils positively correlates with the duration and dosage of biosolid application (Corradini et al., 2019). Therefore, biosolids can be considered as an important source of microplastics in farmland soils.

Organic fertilizers. Organic fertilizers, livestock manure, and bacterial residues produced by aerobic composting, are widely used to improve soil nutritional status and crop yields (Huerta-Lwanga et al., 2022; Zhang et al., 2022a). Organic fertilizers can be a source of soil microplastics as their concentration in mature compost can range from about 20 to122 particles kg^{-1} (Weithmann et al., 2018) or from 0.08 to 6.3 kg ha^{-1} (Zubris & Richards 2005). Furthermore, microplastics in agricultural soils with long-term repeated application of organic manure could be as much as 1.71 to 5.21 × 10^6 particles ha^{-1} $year^{-1}$ (Yang et al., 2021b). Consequently, applying compost products could further contribute to microplastic accumulation in farmland soils.

Atmospheric deposition. Microplastics can enter the soil via atmospheric deposition (Zhang et al., 2020; Zhang & Liu 2018). For example, microplastic concentration in the atmospheric environment near Paris was about 2 to 355 particles m^{-2} d^{-1}, where 3 to 10 tons of microplastic fibers were deposited yearly (Dris et al., 2016). Likewise, the daily deposition of microplastic particles could be up to 512 particles m^{-2} in Hamburg, Germany (Klein & Fischer 2019). In addition, rubber particles from road tire wear could enter the roadside soil via atmospheric deposition or surface runoff. The annual tire dust emissions worldwide were estimated at approximately 3.4 × 10^6 tons (Baensch-Baltruschat et al., 2006). Indeed, a considerable amount of microplastic particles can enter the soil via snow or rain; for instance, the detected microplastic particles in melted snow from Europe and the Arctic were estimated as 190 to 154 × 10^3 particles L^{-1} and 0 to 14.4 × 10^3 particles L^{-1}, respectively (Bergmann et al., 2019).

Tire microplastics. Tire microplastics, including tire wear particles, recycled tire crumb, and tire

repair-polished debris, are mainly made from synthetic rubber tires and are regarded as another significant source of microplastics (Luo et al., 2021; Müller et al., 2022; Worek et al., 2022), because of their polymer structure, solid state, insolubility, and particle sizes (Hartmann et al., 2019; Baensch-Baltruschat et al., 2020). Tire wear particles exhibited toxic effects on water and terrestrial ecosystems such as earthworms, nematodes, and plants (Carrasco-Navarro et al., 2022; Halle et al., 2021; Leifheit et al., 2021a; Kim et al., 2021). These toxic effects are time-dependent at low concentrations (Leifheit et al., 2021a; Kim et al., 2021). Research evidence shows that the annual emission of tire particles is 0.8 kg per capita (cap × a) worldwide (Baensch-Baltruschat et al., 2020), out of which 67% ultimately end up in soils (Kole et al., 2017). Nonetheless, the concentration of tire microplastic in the soil around urban transportation routes could be significantly higher, up to 422 particles kg^{-1} dry soil (Worek et al., 2022).

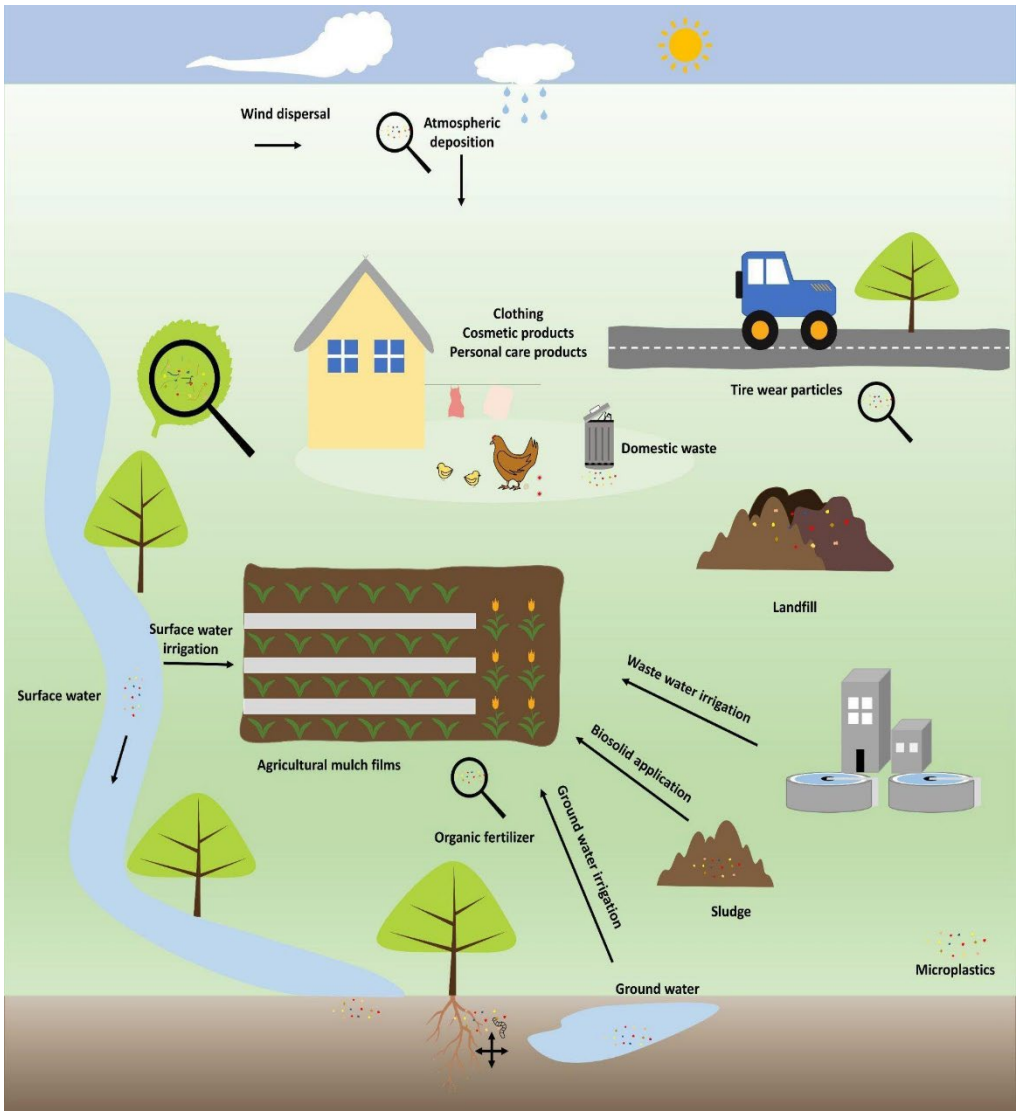

Figure 2.1 The potential sources and migration paths of soil microplastics.

The arrows indicate the potential migration pathways of microplastics in the environment.

2.2.2 The migration of microplastics in soil

Once microplastics enter the soil, they can migrate vertically or horizontally, depending on many factors (Pérez-Reverón et al., 2022). The migration behaviour of microplastics in soil environments is complex and poorly understood (Zhou et al., 2020b). Various factors, including microplastics characteristics (i.e., size, density, and shape), external climate (i.e., wind, rain), soil physical properties (e.g., soil porosity, aggregation, stability), agronomic practices (e.g., ploughing and harvesting), soil fauna and plant root development, were found to influence the vertical and horizontal distribution of microplastics along the soil profile (Dris et al., 2016; Rillig et al., 2017a; Zhou et al., 2020c; Yu et al., 2022a). For instance, microbeads and microplastic fibers interact differently with soil aggregation (de Souza Machado et al., 2018), affecting the movements of microplastics in soil. Such migration could increase with the number of dry-wet cycles compared to single precipitation (O'Connor et al., 2019; Zhou et al., 2020c). Likewise, microplastics on the soil surface, particularly microplastic fibers, can be suspended in the atmosphere for a long time by the upper-air wind before being deposited through rain or dust (Yu et al., 2022a).

Furthermore, there are biotic factors that also influence microplastic migration. For example, the presence of earthworms or collembola contributes to microplastic movements (Rillig et al., 2017b; Maaß et al., 2017). Plant root growth and uprooting could also serve as paths for microplastic migration. For example, corn roots increase soil porosity favouring microplastic movement along the soil profile (Li et al., 2021b). Soil texture, which determines the pore size, soil type, and water status, could directly affect microplastic movement in the soil (Zhou et al., 2020c). Furthermore, regarding agronomic practices, activities such as tilling and ridging could turn over upper and deeper soils, contributing to the transport of microplastics. Lastly, harvesting tubers such as potatoes and yams may facilitate the vertical migration of microplastics in the soil profile (Zhou et al., 2020c).

Another important pathway of microplastic transportation is linked to the atmosphere. Atmospheric transport (i.e., wind) allows microplastics to travel long distances from landfills and roads to remote areas (Bläsing & Amelung 2018; Wang et al., 2021a). For example, about 6.91 to 38.11 kg ha^{-1} of plastic debris was transported by wind erosion in the past 25 years alone (Yang et al., 2022). Plant leaves have also been shown to act as temporary sinks for microplastics (Liu et al., 2020). Indeed, microplastic deposition on leaves surfaces could be of 0.13 trillion pieces, potentially migrating to remote areas by the action of wind (Liu et al., 2020).

2.2.3 The distribution of microplastics in soil

The horizontal distribution of plastics (or geographical distribution) may be affected by

atmospheric transport (You et al., 2022, Table 2.1). For example, the geographical distribution of microplastics showed that their abundance differed from each other among 30 farmlands across China. Concerning the vertical distribution, the number of plastic particles varied vertically (i.e., with soil depth) (Okoffo et al., 2021; Hu et al., 2022). In that sense, microplastic concentration in soil declines with soil depth (Hu et al., 2021, 2022; Yang et al., 2022a). In addition, soil dynamics driven by animals, plants, and soil microbes may also affect the distribution of microplastics in soils (Rillig et al., 2017a; Okoffo et al., 2021). For example, the amount of microplastics in 0 to 10 cm deep soil layers was significantly more considerable than those in the 20 to 30 cm deep soil layers (Liu et al., 2018; Yang et al., 2022a). Additionally, mulching film was mainly preserved in shallow than deep soil layers. In contrast, microplastics from composting sludge were mainly concentrated in the middle layer of the soil (10 to 20 cm) (Zhang et al., 2020a). The spatial distribution of microplastics in the soil profile is also related to soil morphology and aggregate size (Hu et al., 2022).

Table 2.1 The distribution and abundance of microplastics in the soils of different regions worldwide.

"/" indicates that the related information was not mentioned in the references.

Country	Location	Sources	depth	microplastics abundance	Reference
America	Washington	Tidal freshwater wetland	0-5 cm	334-3068 particles kg^{-1}	Helcoski et al., 2020
Australia	Victoria	Roadside dust	/	20.6-529.3 particles kg^{-1}	Su et al., 2020
Canada	Ontario	Agricultural soil	0-15 cm	4-541 particles kg^{-1}	Crossman et al., 2020
Chile	Melipilla	Agricultural soil	0-25 cm	18,000-41,000 particles kg^{-1}	Corradini et al., 2019
China	Beijing	Construction land soils	0-2 cm	272-13,752 particles kg^{-1}	Chen et al., 2021
China	Guangdong	E-waste dismantling zone	0-20 cm	70-18,970particles kg^{-1}	Chai et al., 2020
China	Hubei	Agricultural soil	0-20 cm	647-2,840 particles kg^{-1}	Zhang et al., 2021a
China	Hubei	woodland	0-5 cm	4.1×10^5 particles kg^{-1}	Zhou et al., 2019
China	Hubei	Agricultural soil	0-5 cm	1.6×10^5 particles kg^{-1}	Zhou et al., 2019
China	Hubei	Vacant land	0-5 cm	1.2×10^5 particles kg^{-1}	Zhou et al., 2019
China	/	Agricultural soil	/	2,879-4,941 particles kg^{-1}	Wang et al., 2021c
China	/	Paddy land	/	4,917-6.063 particles kg^{-1}	Wang et al., 2021c
China	/	Agricultural soil	/	2,793-3,979 particles kg^{-1}	Wang et al., 2021c
China	/	Mulching land	/	4,533-6,239 particles kg^{-1}	Wang et al., 2021c
China	/	Greenhouse land	/	4,429-5,756 particles kg^{-1}	Wang et al., 2021c
China	Jiangsu	Sediments	/	11-234.6 particles kg^{-1}	Su et al., 2016
China	Jiangsu	Urban Soil	0-20 cm	239-683 particles kg^{-1}	Zhou et al., 2022
China	Jiangxi	Manure amended soil	0-20 cm	16.2-60.0 particles kg^{-1}	Yang et al., 2021b
China	Shaanxi	Agricultural soil	0-10 cm	1,430-3,410 particles kg^{-1}	Ding et al., 2020
China	Shaanxi	Mu Us Desert	0-20 cm	1,360-6,940 particles kg^{-1}	Ding et al., 2021
China	Shandong	Agricultural soil	0-5 cm	648-3,072 particles kg^{-1}	Yu et al., 2021a
China	Shandong	Agricultural soil	5-10 cm	130- 3,322 particles kg^{-1}	Yu et al., 2021a
China	Shandong	Agricultural soil	10-25 cm	113-2,007 particles kg^{-1}	Yu et al., 2021a

China	Shanghai	Agricultural soil	0-10 cm	8.1-12.5 particles kg^{-1}	Lv et al., 2019
China	Tibet	Sediments	0-2 cm	656-1,782 particlesm^{-2}	Zhang et al., 2016
China	Tibet	Sediments	0-5 cm	17-2644 particles kg^{-1}	Liang et al., 2022
China	Qinghai-Tibet Plateau	Agricultural soil	0-3 cm	23.5-82.9 particles kg^{-1}	Feng et al., 2021
China	Qinghai-Tibet Plateau	Mulching land	3-6 cm	21.6-66.2 particles kg^{-1}	Feng et al., 2021
China	Tibet	soil	0-5 cm	5-340 particles kg^{-1}	Yang et al., 2022b
China	Tibet	Agricultural soil	0-6 cm	20-110 particles kg^{-1}	Feng et al., 2020
China	Xinjiang	Agricultural soil	0-40 cm	31-129.6 particles kg^{-1} (5 years)	Huang et al., 2020
China	Xinjiang	Agricultural soil	0-40 cm	169.9-446.1 particles kg^{-1} (15 years)	Huang et al., 2020
China	Xinjiang	Agricultural soil	0-40 cm	728.8-1,422.4 particles kg^{-1} (24 years)	Huang et al., 2020
China	Xinjiang	Agricultural soil	0-5 cm	1,655.7-1,792.3 particles kg^{-1}	Hu et al., 2021
China	Xinjiang	Agricultural soil	0-30 cm	1,563-1,667 particles kg^{-1}	Hu et al., 2021
China	Xinjiang	Agricultural soil	40-80 cm	101-123 particles kg^{-1}	Hu et al., 2021
China	Yunnan	Agricultural soil	0-10 cm	13,470-42,960 particles kg^{-1}	Zhang and Liu 2018
China	Zhejiang	Agricultural soil	0-10 cm	263-571 particles kg^{-1}	Zhou et al., 2020a
China	Shanghai	Sediments	0-5 cm	208-1,396 particles kg^{-1}	Peng et al., 2018
China	Across the country	Agricultural soil	0-5 cm	226.72-544.62particles kg^{-1}	Hu et al., 2022
China	Across the country	Agricultural soil	5-10 cm	263.51-471.37 particles kg^{-1}	Hu et al., 2022
China	Across the country	Agricultural soil	10-15 cm	207.1-336.9 particles kg^{-1}	Hu et al., 2022
Germany	Rhine River	Sediments	2-3 cm	228-2,763 particles kg^{-1}	Klein et al., 2015
Germany	Main River	Sediments	2-3 cm	786-1,368 particles kg^{-1}	Klein et al., 2015
Germany	Middle Franconia	Agricultural fields	0-5 cm	0.02-0.7 particles kg^{-1}	Piehl et al., 2018
Germany	Schleswig-Holstein	Agricultural soil	0-10 cm	0-217.8 particles kg^{-1}	Harms et al., 2021
Germany	/	Soil near the roads	/	65,400 tone a^{-1}	Baensch-Baltruschat at al., 2020
Germany	/	Sewage sludge	/	1,400-2,800 tone a^{-1}	Baensch-Baltruschat at al., 2020
Iran	Fars Province	Agricultural soil	0-10 cm	67-400 particles kg^{-1}	Rezaei et al., 2019
Mexico	Campeche	Garden	0-20 cm	870-1900 particles kg^{-1}	Huerta-Lwanga et al., 2017

Nigeria	Lagos	Beaches sediments	0-2 cm	324.4 particles kg^{-1}	Fred-Ahmadu et al., 2020b
South Korea	Tanchon Stream	Sediments (upstream)	/	357.1-629.1 MPs m^{-3}	Park et al., 2020
South Korea	Tanchon Stream	Sediments (downstream)	/	235.8-524.2 MPs m^{-3}	Parket al., 2020
South Korea	Yong-In	Paddy land	0-5 cm	20-325 particles kg^{-1}	Kim et al., 2021
South Korea	Yong-In	Mulching land	0-5 cm	10-265 particles kg^{-1}	Kim et al., 2021
South Korea	Yong-In	Greenhouse land	0-5 cm	57-7,630 particles kg^{-1}	Kim et al., 2021
South Korea	Yeoju	Forest, urban, agricultural soil	5-10 cm	625-775 particles kg^{-1}	Choi et al., 2021
Spain	Valencia	Agricultural soil	0-10 cm	540-2,970 particles kg^{-1}	Van den Berg et al., 2020
Spain	Valencia	Agricultural soil	0-30 cm	440-3040 particles kg^{-1}	Van den Berg et al., 2020
Spain	Valencia	Sewage sludge	/	2,060-85,090 particles kg^{-1}	Van den Berg et al., 2020
Spain	Madrid	Wastewater treatment plant	/	169,000-459,000 particles kg^{-1}	Edo et al., 2020
Switzerland	/	Floodplains	0-5 cm	593 particles kg^{-1}	Scheurer and Bigalke, 2018
UK	River Tame	Sediments	5-10 cm	165 (mean) particles kg^{-1}	Tibbetts et al., 2018

2.3 MICROPLASTIC EFFECTS ON SOIL PROPERTIES

Once entering the soil environments, microplastics can impact the function and health of soil ecosystems (e.g., soil structure, respiration, and enzymatic activities). The effects of microplastics on soil physicochemical properties depend on soil type, microplastic concentration, shape, polymer type, and fertilization history (de Souza Machado et al., 2018; Li et al., 2021c; Inubushi et al., 2022). Figure 2.2 shows the effects of microplastics on soil properties, soil microbes, and plant performance.

2.3.1 Effects on soil physical properties

Microplastics could change the soil physical characteristics, including soil aggregate formation, bulk density, porosity, and water dynamics (de Souza Machado et al., 2019).

Soil aggregation. Microplastics can affect soil aggregation by altering the formation and stability of soil aggregates (de Souza Machado et al., 2018; Rillig & Lehmann 2020; Lehmann et al., 2021). For example, polyester fibers can suppress the formation of macro-aggregates in the soil (Zhang & Liu 2018) as a function of microplastic shape and polymer type (Lehmann et al., 2021; Lozano et al., 2021). Moreover, the presence of plants, soil biota (i.e., AMF, saprobic fungi), and organic matter can also impact the microplastic effects on soil aggregation (de Souza Machado et al., 2019; Lehmann et al., 2019; Liang et al., 2019; 2021).

Soil bulk density. The effects of microplastics on soil bulk density depend on the type, shape, and concentration of microplastics (de Souza Machado et al., 2018; Zhang et al., 2019a). For example, the addition of polyester and polyacrylic microplastic fibers, polyethylene fragments, and polyamide beads reduce soil bulk density, with polyester microplastic fibers having the most significant effects and a dose-dependent response (de Souza Machado et al., 2018), although some of them may not affect soil bulk density (de Souza Machado et al., 2019). Spherical microplastics could resemble the shape of natural soil particles, which may affect soil physical properties. For example, a decreased soil bulk density generally leads to an increase in soil macroporosity and aeration, which may further facilitate root penetration (de Souza Machado et al., 2019; Rillig et al., 2019a), enrich the population of aerobic microorganisms (Rubol et al., 2013) and speed up aerobic processes such as nitrification and mineralization of organic matter in the soil (Wang et al., 2022a).

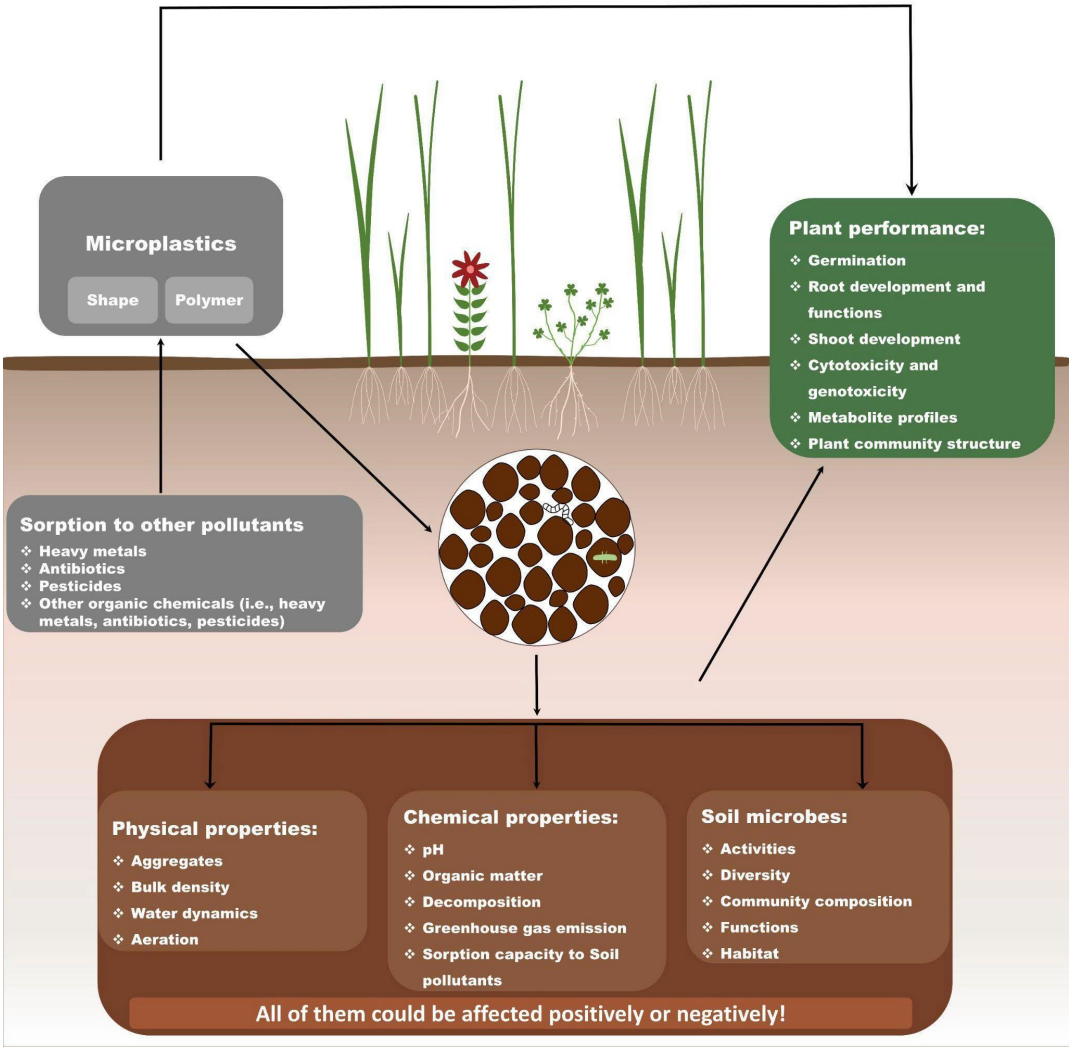

Figure 2.2 The effects of microplastics on soil properties, soil microbes, and plant performance.

Soil porosity. Microplastics significantly impact soil porosity (Sajjad et al., 2022). Soil porosity essentially regulates air conditions and water dynamics in the soil (Pagliai & Vignozzi 2003), thus indirectly altering the diversity and composition of anaerobic and aerobic microorganisms in the soil (Borowik & Wyszkowska 2016; Li et al., 2020a). Polyester microplastic fibers could improve soil porosity with consequences on soil aeration and root penetration, ultimately promoting plant growth (Lozano & Rillig 2020). While microplastic films and fragments could improve soil macroporosity with potential consequences on soil respiration (Lozano et al., 2021), results also showed that polyamide could promote oxytetracycline mobility under soil aeration conditions (Li et al., 2021d). In addition, microplastics in the shapes of pellets, beads, spheres, and particles may easily enter soil pore space affecting soil pore volume. Thus, changes in soil aeration can be one of the mechanisms by which microplastics affect the migration and dispersion of other pollutants (Wang et al., 2022a).

Water dynamics. Soil water determines the availability of nutrients and contaminants and

affects the survival and reproduction of soil organisms and plants. Microplastics can alter water holding capacity, distribution, and availability directly through their hydrophobic surfaces and indirectly through their impacts on soil physical structures (Kumar et al., 2020b; Guo et al., 2022). Changes in water holding capacity may impact soil moisture content and evapotranspiration, consequently affecting water availability. For example, polyester microplastic fibers have been proved to increase water holding capacity (de Souza Machado et al., 2019) and promote plant growth and drought tolerance by improving water retention and root water uptake (de Souza Machado et al., 2019; Lozano & Rillig 2020). By contrast, microplastic films enhanced the water evaporation rate by creating water flow channels (Wan et al., 2019). This indicates that microplastics could affect water distribution in the soil (Jiang et al., 2017) as a function of their type, shape, size, and concentration.

2.3.2 Effects on soil chemical properties

Soil pH. Microplastics can alter soil pH, a critical soil characteristic that can impact various microbial functions (Higashida & Takao 1986). Low-density polyethylene (LDPE) films, for example, may raise soil pH (Qi et al., 2020), whereas high-density polyethylene (HDPE) may have the opposite effects (Boots et al., 2019), but see that HDPE may also increase soil pH (Wang et al., 2020b). Microplastics with varying compositions are expected to have contrasting impacts on soil pH. Indeed, the same type of microplastics can affect soil pH depending on their concentration and size (Boots et al., 2019; Li et al., 2021c; Yang et al., 2021c). For example, increasing polystyrene and polytetrafluorethylene concentrations declined soil pH, demonstrating a dose-dependent relationship between soil pH and microplastics. Nevertheless, it seems that small microplastic particles had more significant impacts than larger ones (Dong et al., 2021a).

Microplastic effects on soil pH might be linked to the chemical compounds released by them as a result of their decomposition and degradation. For example, HDPE declined pH after being exposed to photo-oxidation (Bandow et al., 2017). Likewise, microplastics may vary the amount of soil cation exchange and allow the free exchange of protons in soil water due to their large surface area (Boots et al., 2019). They may indirectly alter soil pH by modifying soil microbial community structure. For example, LDPE can change the abundance of N-cycling bacteria (Fei et al., 2020; Seeley et al., 2020), with consequences on soil pH, which in turn may affect soil microbial community and structure (Kim et al., 2020; Zhou et al., 2021). Such changes in soil pH are another environmental stress for crops. For example, by decreasing soil pH, biodegradable microplastics like fabric and laminate plastics may suppress maize germination (Inubushi et al., 2022).

Biogeochemical cycles. Microplastics could also impact biogeochemical cycles, organic matter decomposition, and nutrient levels (Ren et al., 2020; Gao et al., 2021; Meng et al., 2022). For instance, microplastics could affect nitrification and denitrification by increasing NH_4^+ and

decreasing NO_3^- concentrations (Gao et al., 2021; Han et al., 2022), which are the substrates of nitrification and denitrification. Microplastics can also affect N_2O emissions positively, negatively, or negligibly (Gao et al., 2021; Rillig et al., 2021a; Yu et al., 2021b; Inubushi et al., 2022; Yu et al., 2022c; Song et al., 2019b), depending on microplastics type and size (Iqbal et al., 2020). Similarly, microplastics could affect other greenhouse gasses, including CH_4 and CO_2 emissions, and the effects might be dose-dependent (Ren et al., 2020; Gao et al., 2021; Rillig et al., 2021a; Han et al., 2022; Yu et al., 2022c). One example is that a low dose of microplastics in soil (<1%) had negligible effects on CO_2 production, while a high dose (1%) may accelerate it (Zhang et al., 2022b). In addition, microplastics may alter soil organic matter (Ren et al., 2020; Meng et al., 2022; Zhang et al., 2022b), including dissolved organic matter and soil organic carbon (Zhang et al., 2022b). Since microplastics might increase soil aeration, which enhances microbial respiration and consumption of soil organic matter (Zhang et al., 2022b), a lower soil organic matter content can be found. In addition, microplastics could alter soil organic matter distribution, resulting in changes in soil microbial diversity and composition (Guo et al., 2021). Polyester fibers could absorb soil fine mineral and organic particles, reducing soil organic matter accumulation (Guo et al., 2021), thus declining nutrient levels, as observed with available nitrogen and phosphorus (Zhang and Zhang, 2020; Dong et al., 2021a). Microplastics can also indirectly suppress nutrient uptake for plants as for example, fibers can be attached to the root surface hindering that process (van Weert et al., 2019; Zeb et al., 2022).

2.3.3 Effects on other soil pollutants

Microplastics in soil could serve as vectors for some soil pollutants, including heavy metals, antibiotics, pesticides, and herbicides (Sunta et al., 2020), due to their adsorption capacity linked to their chemical and structural composition (Wang et al., 2019b; Wang et al., 2020b), which may have negative effects on plant-soil interactions (Li et al., 2020c; Li et al., 2020d).

Microplastics could affect the bioavailability of heavy metals either negatively (Dong et al., 2021a) or positively (Wang et al., 2020a; Yu et al., 2020b; Feng et al., 2022; Jia et al., 2022). In addition, they could decrease the soil adsorption capacities to metals and increase their mobility into the soil matrix (Li et al., 2021e; Feng et al., 2022). The microplastic type, dose, particle size, and soil pH may determine these effects (Wang et al., 2020a; Wang et al., 2020b; Zhang et al., 2020c; Dissanayake et al., 2022). The co-existence of microplastics and heavy metals could change the abundance of soil microbial taxa and enzymatic activities. For example, Cd showed slight but strong interactions with microplastics on the AMF community structure and diversity (Wang et al., 2020b). Likewise, microplastics can absorb As in on their surface, declining the

abundance of Proteobacteria while increasing the abundance of Chloroflexi and Acidobacteria. Likewise, the combined effects of microplastics and heavy metals may inhibit enzymatic activities, including urease, acid phosphatase, dehydrogenase, and peroxidase (Dong et al.,

2021a). However, microplastics could also inhibit the adverse effects of As on microbial and chemical properties of rhizospheric soil (Dong et al., 2021a).

Microplastics might affect the distribution, spread, mobility, and bioavailability of these harmful pollutants (Gao et al., 2021; Mo et al., 2021; Tang 2021; Xu et al., 2021a), altering the ecotoxicity of harmful pollutants on soil microbes and fauna (Xu et al., 2021b; Xu et al., 2021d). For example, polystyrene markedly decreased the negative effects of sulfamethazine on bacterial diversity, and composition (Xu et al., 2021d); although it may promote phenanthrene accumulation and trigger DNA damage in earthworms. In addition, the combined effects of microplastics and organic pollutants might affect nutrient status in soil. For instance, polystyrene and sulfamethazine showed antagonistic effects on soil available nitrogen, and impacted root exudates, clay content, and substrate availability, influencing organic compound degradation (Xu et al., 2021d).

2.4 MICROPLASTICS EFFECTS ON SOIL BIOTA AND PLANTS

2.4.1 Effects on soil fauna

Microplastics could also impact soil fauna, as observed in earthworms and nematodes (Boots et al., 2019; Ju et al., 2019; Jiang et al., 2020; Kim et al., 2020). This is because microplastics may be adhered to the external surface or ingested, affecting their motility and damaging animal tissues (Huerta-Lwanga et al., 2016; Zhu et al., 2018a; Zhu et al., 2018b), with consequences on their growth and development. In addition, microplastics can also damage DNA in earthworms (Jiang et al., 2020), organisms that play dynamic roles in transforming plastics into microplastics (Rodriguez-Seijo et al., 2017).

2.4.2 Effects on soil microbes

Microplastics in soil could alter soil microbial activities, diversity, and community composition. They can either positively or negatively affect soil enzymatic activities, which might be linked to changes in soil nutrient substrates, physicochemical properties, and/or the sorption of microplastics to toxic contaminants such as heavy metals (Huang et al., 2019; Wang et al., 2019a; Fei et al., 2020; Wang et al., 2020b; Yi et al., 2020; Yu et al., 2020a). Futhermore, they could also affect soil microbial diversity and composition. For example, microplastics could alter bacterial taxa involved in nitrification and denitrification processes (Seeley et al., 2020; Han et al., 2022; Lee et al., 2022), impact arbuscular mycorrhizal fungi structure (Wang et al., 2020a; Wang et al., 2020b; Yang et al., 2021c), and colonization to their host partners (Leifheit et al., 2021b; de Souza Machado et al., 2019; Lehmann et al., 2020). The effects of microplastics on soil microorganisms would depend on microplastics' origin, type, texture, and chemical properties. For instance, biodegradable microplastics have more significant impacts on bacterial diversity associated with the Triticum aestivum rhizosphere than polyethylene microplastics,

which are highly influenced by microplastic size (Qi et al., 2020). Additionally, microplastics can absorb soil fine mineral and organic particles, inducing greater microbial activities (Guo et al., 2021).

Microplastics could act as vectors for some pathogens (Huang et al., 2019; Zhang et al., 2019b), including Fusarium and Alternaria (Gkoutselis et al., 2021), potentially inhibiting plant growth (Lozano & Rillig 2020). In addition, microplastics significantly increased the relative abundance of Cucurbitariaceae (common pathogenic fungi) in multiple fertilization treatments (Li et al., 2021c). Likewise, polyethylene microplastics could increase the abundance of animal parasites, human pathogens, and plant pathogens in arable soil systems (Zhu et al., 2021).

2.4.3 Effects on plants

The persistence and migration of microplastics in plant-soil systems would directly affect plant growth from negative to positive) throughout their life cycle. This includes effects on the processes of germination, nutrient uptake, physiological activity, tissue development, etc. (Bosker et al., 2019; Jiang et al., 2019; Khalid et al., 2020; Pignattelli et al., 2020; Zeb et al., 2022).

Effects on seed germination and root development. Microplastics could accumulate onto the seed surface, causing a physical obstruction of the seed pores, potentially reducing seed water and nutrient uptake (Bosker et al., 2019; Yuan et al., 2019). Thus, environmental stress on seeds produced by microplastics in the soil might be influenced by microplastic properties (i.e., size, shape, age, polymer), and the plant species identity (Rillig et al., 2019a; Lozano et al., 2021). In addition, tiny microplastics could accumulate in the plant root system, and some of them (0.1-5 μm) can even be absorbed by the roots and translocated to the aerial parts of the plants via apoplastic and symplastic pathways (Dong et al., 2021b), which might suppress the nutrient uptake and then restrict plant growth (Urbina et al., 2020). For instance, microplastics were observed to cling to the radicle and root hairs that are sprouting after germination, due to root mucilage secretion of hydrophobic connections among the cell wall and microplastics (Bosker et al., 2019; Taylor et al., 2020).

Microplastics could influence root traits, i.e., length, weight, activity, viability, biomass, and lateral root formation. Indeed, microplastics had a substantial influence on root growth by reducing root length in crops (i.e., *Lactuca sativa, Glycine max,* and *Hordeum vulgare*) (Gao et al., 2019; Li et al., 2021a; Li et al., 2021f), an effect that depends on microplastics concentration (Meng et al., 2021). By contrast, microplastics boosted root biomass of *Zea mays* and *Elodea species*, without affecting *Spirodela polyrhiza, Lemna minor, Arabidopsis,* and *Triticum aestivum* (Dovidat et al., 2019; Judy et al., 2019; van Weert et al., 2019; Taylor et al., 2020; Mateos-Cardenas et al., 2021). Microplastics also decrease root transpiration and affect water and nutrient uptake. For example, mulching films could influence nutrition uptake through root

distribution (Liu et al., 2021), or impact the radicle morphology (Lopez et al., 2022).

Effects on plant growth and tissue development. Microplastics affect plant growth as observed in a wide range of species such as *Lactuca sativa*, *Vicia faba*, *Glycine max*, *Triticum aestivum*, *Allium fistulosum*, *Daucus carota*, *Zea mays*, and *Oryza sativa* (Gao et al., 2019; Jiang et al., 2019; Li et al., 2021a; Li et al., 2021f, Lozano et al., 2021). Their effects can be negative as they may affect the leaf interface with the antioxidant defense system, suppressing chlorophyll fluorescence (Chen et al., 2022; Colzi et al., 2022; Zeb et al., 2022), and thus photosynthesis, one of the most fundamental physiological activities in plants. In addition, microplastics may affect soil moisture content and enzymatic activities, with potential consequences on plant growth (Dong et al., 2020; Colzi et al., 2022; Wu et al., 2022). Microplastics might cause cytotoxicity and genotoxicity in plants, as they could cause cell wall destruction and cell maturation breaking (Mao et al., 2018). The mitotic index of *Vicia faba* root tip cells considerably decreased following exposure to microplastics, which was confirmed by the amount of micronuclei suggesting that microplastics influenced cell division (mitosis) (Jiang et al., 2019; Dong et al., 2021b). Furthermore, the reduction in hydroxybenzoic acid caused by microplastics changed in plant cell wall composition (Zhang et al., 2020c). Nonetheless, microplastic effects on plant growth can also be positive mainly due to their positive effects on soil properties (Lozano et al., 2021).

Moreover, microplastics could affect the impacts of organic pollutants on plants; thus, their interactions might influence plant development. For example, polystyrene decreased phenanthrene uptake in *Glycine max* (Xu et al., 2021c). By contrast, polystyrene microplastics exacerbated the toxicity of dibutyl phthalate on *Lactuca sativa*, reducing their biomass, and enriched the concentration of dibutyl phthalate in lettuce roots and leaves, thus increasing oxidative stress and subcellular damages, with negative consequences on root development and soil enzymatic activities (Gao et al., 2021). Furthermore, these absorbed organic pollutants can be easily released into the soil matrix during the degradation of plastic products, which poses a significant hazard to the ecosystem and human health (Xiang et al., 2022).

Effects on plant metabolite profiles. Microplastics can enhance the production of reactive oxygen species, which can cause oxidative stress in plants, thus affecting metabolic processes, damaging cell structures and functionality in plant tissues (Li et al., 2020e; Mateos-Cardenas et al., 2021; Colzi et al., 2022; Zeb et al., 2022). Enhanced production of reactive oxygen species mostly deals with diminished production of amino acids, nucleic acids, lipids, and other secondary metabolites resulting in decreased cell membrane function (Wu et al., 2020).

Microplastics could interfere with metabolic pathways in plants, including carbohydrate and amino acid metabolisms (Lian et al., 2020; Lopez et al., 2022; Wu et al., 2022; Zeb et al., 2022; Zhang et al., 2022c). Likewise, microplastics can affect related gene expression, enhancing plant

adaptation to stressors (Wu et al., 2022). Indeed, plants can modify the metabolisms of galactose, pentose phosphate, starch, and sucrose in some crops as a tolerance strategy to microplastic stress (Lian et al., 2020; Fu et al., 2022; Zhang et al., 2022c). For example, plants stimulating galactose production may enhance their tolerance to external pressures (Wu et al., 2022; Zhang et al., 2022c). Additionally, changes in the contents of these carbohydrates might affect pathways such as the citrate cycle (Zhang et al., 2022c), altering amino acid and lipid metabolisms (Wu et al., 2022). Furthermore, microplastics may affect other metabolisms such as alanine, aspartate, and glutamate, as observed in *Zea mays*, *Triticum aestivum*, and *Oryza sativa* (Zhang et al., 2022c; Lian et al., 2020; Wu et al., 2022).

Chapter 3: MICROPLASTICS INCREASE SOIL PH AND DECREASE MICROBIAL ACTIVITIES AS A FUNCTION OF MICROPLASTIC SHAPE, POLYMER TYPE, AND EXPOSURE TIME

Tingting Zhao, Yudi M. Lozano, and Matthias C. Rillig This chapter has been published as an open-access paper:

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Keywords: Fibers, films, foams, fragments, pH, soil respiration, acid phosphatase, β-D-glucosidase, cellobiosidase and N-acetyl-β-glucosaminidase.

3.1 ABSTRACT

Microplastic pollution is a topic of increasing concern, especially since this issue was first addressed in soils. Results have so far been variable in terms of effects, suggesting that there is substantial context-dependency in microplastic effects in soil. To better define conditions that may affect microplastic-related impacts, we here examined effects as a function of microplastic shape and polymer type, and we tested if effects on soil properties and soil microbial activities change with incubation time.

In our laboratory study, we evaluated twelve different secondary microplastics representing four microplastic shapes: fibers, films, foams and fragments; and eight polymer types: polyamide (PA), polycarbonate (PC), polyethylene (PE), polyester (PES), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyurethane (PU). We mixed the microplastics with a sandy soil (0.4 % w/w) and incubated at 25 ℃ for 31 days. Then, we collected soil samples on the 3rd, 11th, and 31st day, and measured soil pH, respiration and four enzyme activities (acid phosphatase, β- D-glucosidase, cellobiosidase and N-acetyl-β-glucosaminidase).

Our results showed that microplastics could affect soil pH, respiration and enzymatic activities depending on microplastic shape and polymer type, effects that were altered with incubation time. Soil pH increased with foams and fragments and overall decreased in the first days of incubation and then increased. Soil respiration increased with PE foams and was affected by the incubation time, declining over time. Overall, acid phosphatase activity was not affected by shape or polymer type. β-D-glucosidase activity decreased with foams, cellobiosidase activity decreased with fibers, films and foams while N-acetyl-β-glucosaminidase activities decreased with fibers and fragments. Enzymatic activities fluctuated during the incubation time, except N-acetyl-β-glucosaminidase, which showed a declining trend with incubation time. Enzymatic activities were negatively correlated with soil pH and this relationship was less strong when microplastics were added to the soil.

Our study adds to the evidence that research should embrace the complexity and diversity of microplastics, highlighting the role of microplastic shape and polymer type in influencing effects; additionally, we show that incubation time is also a parameter to consider, as effects are dynamic even in the short term.

3.2 INTRODUCTION

Large amounts of plastics have been produced worldwide due to the widespread use of these materials in our daily life (Geyer et al., 2017), to the point that plastic is now becoming an important threat to terrestrial systems (Rillig 2012; Bläsing and Amelung 2018). Microplastics,

plastic particles smaller than 5 mm, and their effects on soil systems, have received increasing attention in recent years (Rillig 2012; Mai et al., 2018). They can pollute terrestrial systems through a variety of pathways, including soil amendments, mulching, sludge, irrigation, flooding, atmospheric input and littering or street runoff (Rillig et al., 2017; Mai et al., 2018; Boots et al., 2019).

As a result of their manufacturing origin and environmental degradation, microplastics may occur in many shapes and a variety of physical and chemical properties (Rillig and Lehmann, 2020; Helmberger et al., 2020). The accumulation of microplastics in soil may impact soil characteristics (Liu et al., 2017; Yi et al., 2020), depending on microplastic properties (Lozano et al., 2021a). Indeed, microplastic shape may determine how microplastics interact with soil particles (de Souza Machado et al., 2018; Rillig et al., 2019a; Lehmann et al., 2020; Rillig and Lehmann 2020). For instance, fibers due to their linear shape, may destabilize soil structure once they are incorporated into soil aggregates (de Souza Machado et al., 2018).

In addition, the chemical properties of microplastics, such as molecular chain arrangement and functional group, could impact their capacity of absorption to other chemicals like heavy metals or antibiotics (Fred-Ahmadu et al., 2020a), with potential consequences on soil properties and microbial activities (Pathan et al., 2020). For example, polyethylene (PE) had high sorption capacity for phenanthrene (Wang & Wang 2018), which along with its nitrogen heterocyclic analogues could inhibit microbial activities in soil (Anyanwu & Semple 2016). Likewise, studies have shown that different polymer types (e.g., PE, PP and PVC) may have different sorption capacities for certain chemicals (Teuten et al., 2009; Brennecke et al., 2016; Wang et al., 2018). For example, PE had greater sorption capacity for hydrophobic organic compounds such as pesticide and solvents (Teuten et al., 2009; Fred-Ahmadu et al., 2020a), while PS had larger sorption capacity for Polycyclic Aromatic Hydrocarbons than PET, PVC, PE or PP (Rochman et al., 2013). In the same way, PVC could absorb more Cu than PS (Brennecke et al., 2016). Therefore, the effects of microplastics on soil enzymatic activities may be also influenced by their polymer type.

Among soil properties, little is known about microplastics effects on soil pH, a key soil parameter that could impact a range of microbial processes (Higashida & Takao 1986). Some research has been done regarding the effects of polyethylene on soil pH. For instance, low density polyethylene (LDPE) films may increase soil pH (Qi et al., 2020); while high density polyethylene (HDPE), may have the opposite pattern (Boots et al., 2019), however, a study by Wang et al. (2020) suggested that HDPE may also cause an increase in soil pH. Yet, how other types of microplastics (shapes or polymers) present in terrestrial systems (Piehl et al., 2018; Bläsing & Amelung 2018; Rillig et al., 2019b) may affect this soil property is currently unknown.

In addition, our knowledge of microplastic effects on soil respiration is still rudimentary. Soil respiration, an indicator of the total soil microbial activity (Rousk et al., 2009), is very sensitive to environmental factors, such as soil texture, porosity, moisture, and pH (Luo & Zhou 2006), soil properties that can be potentially altered by microplastics addition (de Souza Machado et al., 2019; Rillig et al., 2019b, Lozano et al., 2021a). Indeed, recent research has observed that microplastics could alter the soil microbial community (Huang et al., 2019; Fei et al., 2020), suggesting potential effects on soil respiration (Lozano et al., 2021a; b).

Microplastics could alter soil microbial communities (Fei et al., 2020; Wiedner et al., 2020; Yi et al., 2020), affecting enzymatic activities (Hargreaves & Hofmockel 2014). Indeed, recent research has showed that microplastics could affect nutrient and/or substrate availability (Yu et al., 2020; Zhou et al., 2020, Lozano et al., 2021b), likely due to microplastic absorption or its competition for physicochemical niches with microorganisms (Yu et al., 2020). Microplastic shape and polymer type may also play a role. For instance, polyethylene (PE) and polyvinyl chloride (PVC) microplastics could enhance enzymes such as urease and acid phosphatase (Huang et al., 2019; Fei et al., 2020) while PP, PES and PVC could inhibit or enhance soil fluorescein diacetate hydrolase activity, respectively (Liu et al., 2017; de Souza Machado et al., 2019; Liang et al., 2019; Fei et al., 2020), depending on the polymer type. Likewise, enzymes such as β-D-glucosidase and cellobiosidase (involved in cellulose degradation), N-acetyl-β-glucosaminidase (involved in chitin degradation), and phosphatase which are related to C, N, P-cycling, could be negatively affected by microplastics (Liang et al., 2021; Lozano et al., 2021b).

Depending on the shape, polymer type and exposure time, microplastics can have different effects on soil properties, adding to the strong context dependency of microplastic effects as reported in the literature. To systematically test this, we established a lab experiment that included four microplastic shapes (fibers, films, foams and fragments), each of them made of three different polymer types, in order to determine the effects of microplastics on soil pH and microbial activity. We hypothesized that soil pH, respiration and enzymatic activities may be affected by microplastic addition as a function of microplastic shape and polymer type; in addition, we examined effects of exposure time during our short-term laboratory incubation.

3.3 MATERIALS AND METHODS

3.3.1 Soil and microplastics preparation

Test soil. We selected a loamy sandy soil from a dry grassland community located in Dedelow, Brandenburg, Germany (53º 37’ N, 13º 77’ W). Dry soil was sieved through a 2-mm mesh sieve, homogenized and mixed with microplastics. The detailed properties of test soil are shown in Table 3.1.

Table 3.1 Physical and chemical properties of test soil.

pH	~ 6.0
N	0.07%
C	0.77%
F^-	0.76 ± 0.03 (mg kg^{-1})
Cl^-	4.9 ± 0.08 (mg kg^{-1})
NO_3^-	0.26 ± 0.03 (mg kg^{-1})
PO_4^{3-}	1.73 ± 0.14 (mg kg^{-1})
SO_4^{2-}	5 ± 1.31 (mg kg^{-1})
Electrical conductivity	61.6 ± 4.9 µS cm^{-1}

Microplastics. Primary microplastics are produced on purpose and used in cosmetic products and various industries, while secondary microplastics are obtained from degradation of larger plastics (Wang et al., 2018). We selected twelve different secondary microplastics, representing four microplastic shapes: fibers, films, foams and fragments and eight polymer types: polyamide (PA), polycarbonate (PC), polyethylene (PE), polyester (PES), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyurethane (PU). See additional details on the plastics in Table 3.2. We manually cut the fibers and films with scissors. The length for fibers was 1.26 ± 0.03 mm, and the size of films was 1.55 ± 0.03 mm × 2.26 ± 0.04 mm. Plastic fragments and foams were cut into small pieces using a Philips HR3655/00 Standmixer (1400 Watt, ProBlend 6 3D Technologie, Netherlands), and then sieved through a 4- mm mesh sieve. The sizes for the fragments were 1.28 ± 0.05 mm × 1.72 ± 0.07 mm, while for the foams were 1.28 ± 0.04 mm × 1.76 ± 0.06 mm. To minimize microbial contamination, microplastics were exposed in an oven at 101 °C for 24 hours, as previous assays using different temperatures, showed that at this temperature, microplastics did not present any type of distortion. Then, a sample of each microplastic was placed on PDA plates (PDA X931.2, Roth, Germany), which were incubated at 25 ℃ for one week. No microbial colonies were observed.

Microplastic addition to soil. The soil was mixed with each of the microplastic types at a concentration of 0.4 % (w/w), as this simulates higher levels of microplastic pollution (Scheurer & Bigalke 2018; Xue et al., 2020; Zhu et al., 2019a), since we should be more concerned about the future than the current levels of microplastic contamination, just like is the case of other factors of global change. Therefore, 80 mg of each microplastic type were mixed into 20 g of soil by stirring with a metal spoon for 3 min in a large container before transferring the mixture into a 50-mL polypropylene centrifuge tube (Corning 431720, Corning Incorporated), the caps of which had 4 vents to provide gas exchange. We had 12 microplastic types (4 shapes × 3 polymer

types) × 9 replicates = 108 tubes. Fifteen additional tubes were included as control without microplastics. Soil was stirred in the same way that in the control samples, to provide the same disturbance. All tubes were randomly distributed in the incubator chamber.

Throughout the incubation period, in order to maintain soil moisture at ~70 % water holding capacity, every four days we pipetted distilled water into the tubes according to their weight loss due to evaporation. Tubes were kept at 25 ℃ throughout the experiment. Soil samples were randomly collected on the 3rd, 11th and 31st day. To avoid disturbance which could be a confounding factor, 1/3 of the samples were collected (three replicates for each microplastic treatment and five replicates for control), destructively harvested and analyzed for every harvest time (on the 3rd, 11th and 31st day). At harvest, soil respiration was measured, and then samples were collected and kept at 4℃ prior to measuring enzymatic activities and soil pH.

3.3.2 Measurements

Soil pH. Soil pH was measured following the procedure described by Hendershot and Lalande (2007). That is, air-dried soil samples were mixed with distilled water at the ratio of 1:2 (w:v), i.e. 10 g soil : 20 mL water. The tubes were shaken for 30 min and the suspensions were allowed to settle for 1 h. Then, 20 mL of each suspension was pipetted into a 50-mL tube (Sarstedt AG & Co. KG, Nümbrecht, Germany, item number 62.548.004) and centrifuged at 3000 rpm for 5 min. The supernatants were filtered, and the pH was determined with a pH-meter 766 (Knick, Germany).

Enzyme activities. Acid phosphatase (EC3.1.3.2), β-D-glucosidase (EC3.2.1.21), cellobiosidase (EC3.2.1.91) and N-acetyl-β-glucosaminidase (EC3.2.1.52) were measured from 5 g of soil by using high throughput microplates assays following the methods described by Jackson et al. (2013). Briefly, 5 g soil was mixed with 10 mL 50 mM acetate buffer (pH 5.0-5.4) in a 50-mL falcon tube. Then, 150 uL of soil slurry was pipetted into each of six wells (six wells per sample) on a 96-deep well plate after vortexing. Then, 150 uL acetate buffer was added into the last two wells of each sample (sample buffer control), and 150 uL substrate solutions (5 mM 4-*p*-nitrophenyl- phosphate disodium salt hexahydrate, 5 mM 4-*p*-nitrophenyl-β-glucopyranoside, 2 mM 4-*p*-nitrophenyl-β-D-cellobioside and 2 mM 4-*p*-nitrophenyl-β-N-acetylglucosaminide, Sigma, Germany, item no.: N71768, N7006, N5759, and N9376) to the first four wells. Then, the plates were incubated at 25 °C in dark for 2 h (for acid phosphatase and β-D- glucosidase) or 4 h (for cellobiosidase and N-acetyl-β-glucosaminidase). After the incubation, plates were centrifuged at 3000 rpm for 5 min, and then 100 uL supernatant from each well was transferred into new microplates containing 10 uL 1M NaOH and 190 uL distilled water in each well. Finally, the absorbance at 410 nm was recorded by a microplate reader (Benchmark Plus Microplate Spectrophotometer System, BioRad Laboratories, Hercules, CA, US).

Table 3.2 List of plastic products used in the experiment.

Shapes, polymer types, providers, item number, products and sources of the plastic products are included.

Shape	Polymer	Abbreviation	Provider	Item no.	Product
Fibers	Polyamide	PA	Hornbach.de	6702575	Rope
	Polyester	PES	Hornbach.de	8442172	Rope
	Polypropylene	PP	Hornbach.de	8442182	Rope
Films	Polyethylene	PE	Frischhalte folie		silo film black
	Polyethylene terephthalate	PET	Toppits		Bratschlauch
	Cast Polypropylene	PP	STYLEX		transparent folders
Foams	Polyethylene	PE	Lab storage		black low density closed cell etha foam
	Polystyrene	PS	Lab storage	EPS70	Insulation Packing Board SLABS
	Polyurethane	PU	Hornbach.de	3838930	gray foam sheet
Fragments	Polycarbonate	PC	Verbatim		CD-R
	Polypropylene	PP	treppens.de		black plastic pots
	Polyethylene terephthalate	PET	stationary shop EDEKA		Water bottle

Soil respiration. Soil respiration was measured on undisturbed soil samples. That is, each time soil respiration was measured before sample collection for enzymes and pH measurements. Therefore, on the 3rd day, soil respiration was measured on all replicates tubes; on the 11th day, it was measured on the six microplastic and ten control replicates remaining; and on the 31st day, it was measured on the three microplastic and five control replicates left. The CO_2 concentration (ppm) was used to indicate the soil respiration. To control gas exchange, we used modified tube caps that had a rubber septum (VWR, Germany, item no. 548-3369) to provide a seal. Then, we flushed the tubes with CO_2-free air for 3 min to normalize the experimental units and kept the tubes in the incubator at 25 ºC for 3 h under dark conditions. After the 3-h incubation, we took a 1-mL air sample from each tube and injected it

into the infrared gas analyzer (LiCOR- 6400XT).

3.3.3 Statistical analyses

The effects of microplastic shape, polymer type and exposure time on soil pH, respiration and enzymatic activities were analyzed using linear models and multiple comparisons. First, the residuals of linear models were checked to validate assumptions of normality and homogeneity. When necessary, we implemented the functions "varIdent" from the "vegan" R package to account for heterogeneity in variances. Then, we implemented the function "glht" and "Dunnett" test from the "multcomp" R package, to compare each microplastics treatment with the control (without microplastics). Respiration and enzymatic activities were log-transformed and correlated with soil pH by using the Pearson method. Plots were generated with the "ggplot2" R package (Wickham, 2016). Results shown throughout the text and figures are mean values ± SE. All analyses were conducted using R software version 3.6.3 (R Core Team, 2020).

3.4 RESULTS

3.4.1 Soil pH

Soil pH was affected by microplastic shapes, polymer types, and incubation time (Table 3.3-5, Figure 3.1). Soil pH increased with foams and fragments and a slight increase was observed with films (Table 3.4; Figure 3.1A). We found that pH was higher in the soil mixed with all the polymers used for foams and fragments than in control soils without microplastics (Table 3.4; Figure 3.1A). Regarding exposure time, overall, soil pH declined in the first eleven days and then increased (Table 3.5, Figure 3.1B). This pattern was observed for PA and PES fibers, all the films, PU foams, PC and PP fragments. However, pH of the soil treated with PE foams showed a contrary trend (Table 3.5, Figure 3.1B). Soil pH tended to increase over time with PS foams and PET fragments addition (Table 3.5, Figure 3.1B). Overall, pH was higher in soil mixed with foams and fragments polymers for each time of measurement, than in soils without microplastics (Table 3.5, Figure 3.1B).

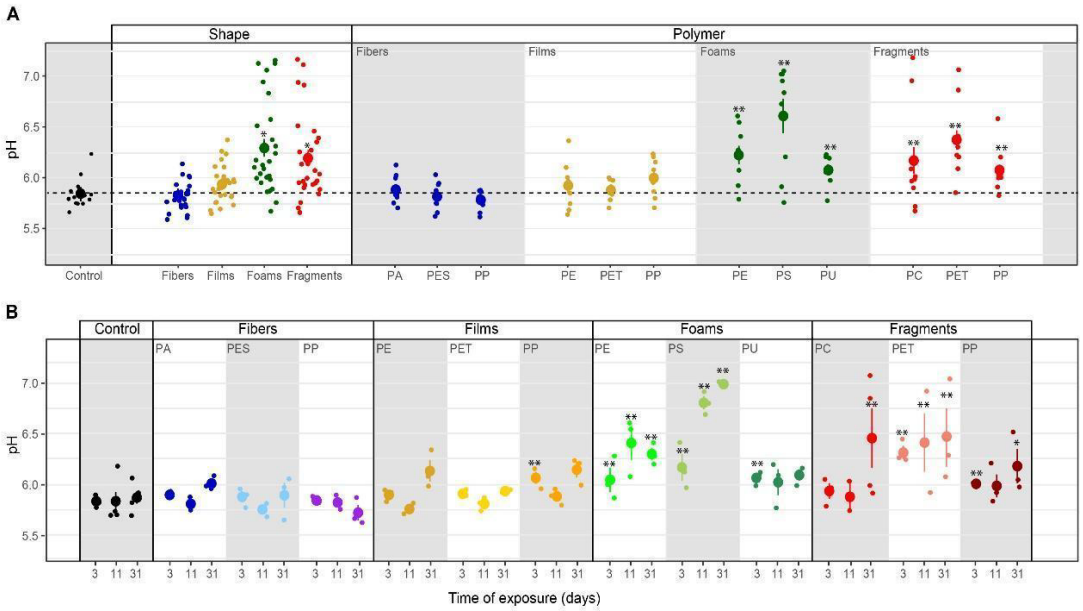

Figure 3.1 Microplastic effects on soil pH.

Effects of shape, polymer type A) and incubation time B) on soil pH. Mean and standard errors are shown. n = 5 (control); n = 3 (microplastic treatments). Significance * $p \leq 0.1$, ** $p \leq 0.05$ compares each microplastic with its respective control treatment for each time of measurement.

3.4.2 Soil respiration

Soil respiration was not affected by microplastic shapes, although it slightly increased with foams (Table 3.3-5, Figure 3.2). Only PE foams increased soil respiration within all the polymer types (Table 3.4, 5, Figure 3.2). Overall, soil respiration declined over time, being more evident in soils with than without microplastics (Table 3.5, Figure 3.2B). Soil respiration was lower in soil mixed with each of all the microplastic types at the last measurement time (day 31st), than in soils without microplastics (Table 3.5, Figure 3.2B).

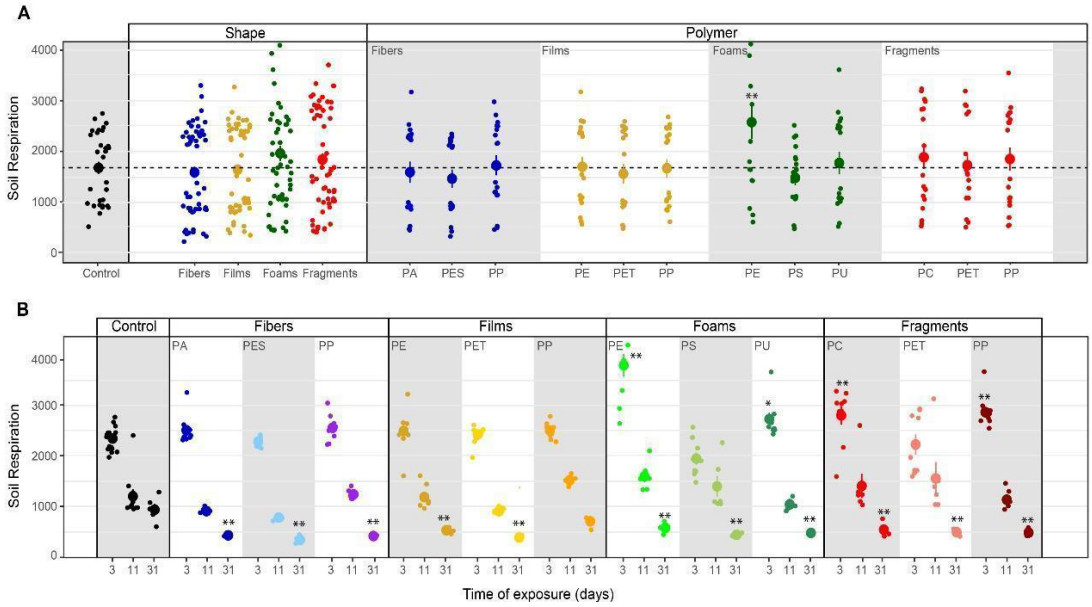

Figure 3.2 Microplastic effects on soil respiration.

Effects of shape, polymer type A) and incubation time B) on soil respiration. Mean and standard errors are shown. (n = 15 for control; n = 9 for microplastic treatments, day 3); n = 10 (control, day 11); n = 6 (microplastic treatments, day 11); n = 5 (control, day 31); n = 3 (microplastic treatments, day 31). Soil respiration is measured as CO_2 unit (ppm). Significance * $p \leq 0.1$, ** $p \leq 0.05$ compares each microplastic with its respective control treatment for each time of measurement.

3.4.3 Soil enzymatic activities

Acid phosphatase activity. Overall, acid phosphatase activity was not affected by microplastic shape although it tended to be higher with fibers, films and foams than in control samples without microplastics (Table 3.3-5, Figure 3.3). We observed that this enzyme increased with PA fibers and PE foams (Table 3.4, Figure 3.3A). Overall, acid phosphatase activity tended to decline during the first eleven days and then increased (Table 3.5, Figure 3.3B). This pattern was evident for PA and PP fibers, PP films, PE foams and PET fragments. Over time, acid phosphatase activity tended to decline with PES fibers, PS and PU foams, while tended to increase with PET films and PP fragments (Table 3.5, Figure 3.3B). Likewise, acid phosphatase activity was negatively correlated with soil pH when microplastics in the soil were absent ($R = -0.55$, $p = 0.034$) or present ($R = -0.47$, $p < 0.01$, Figure 3.4).

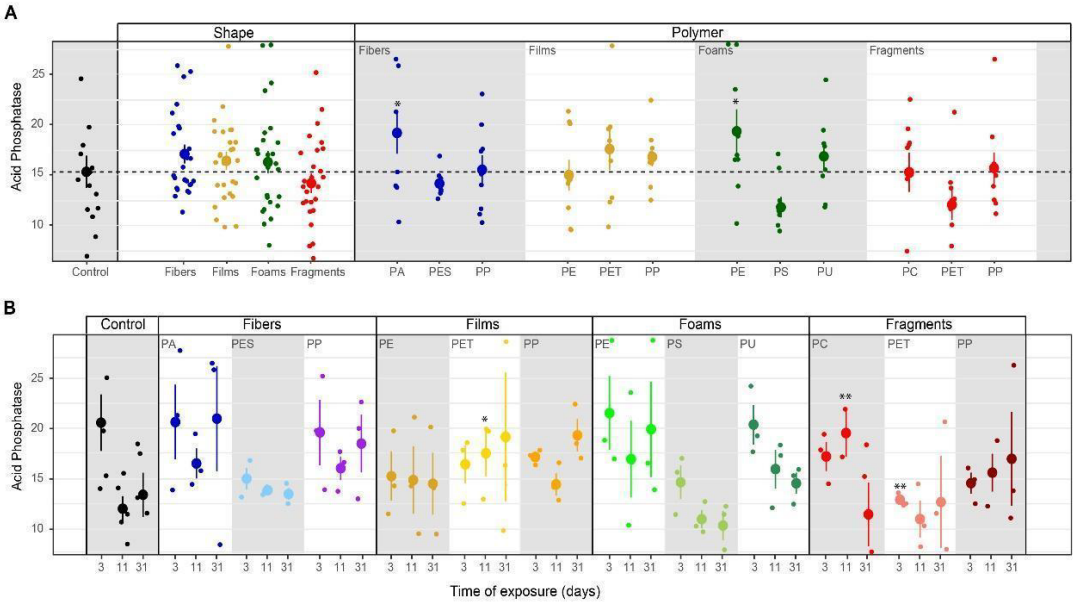

Figure 3.3 Microplastic effects on acid phosphatase.

Effects of shape, polymer type A) and incubation time B) on acid phosphatase. Mean and standard error are shown. n = 5 (control); n = 3 (microplastic treatments). Unit: µmol mg^{-1} h^{-1}. Significance * $p \leq 0.1$, ** $p \leq 0.05$ compares each microplastic with its respective control treatment for each time of measurement.

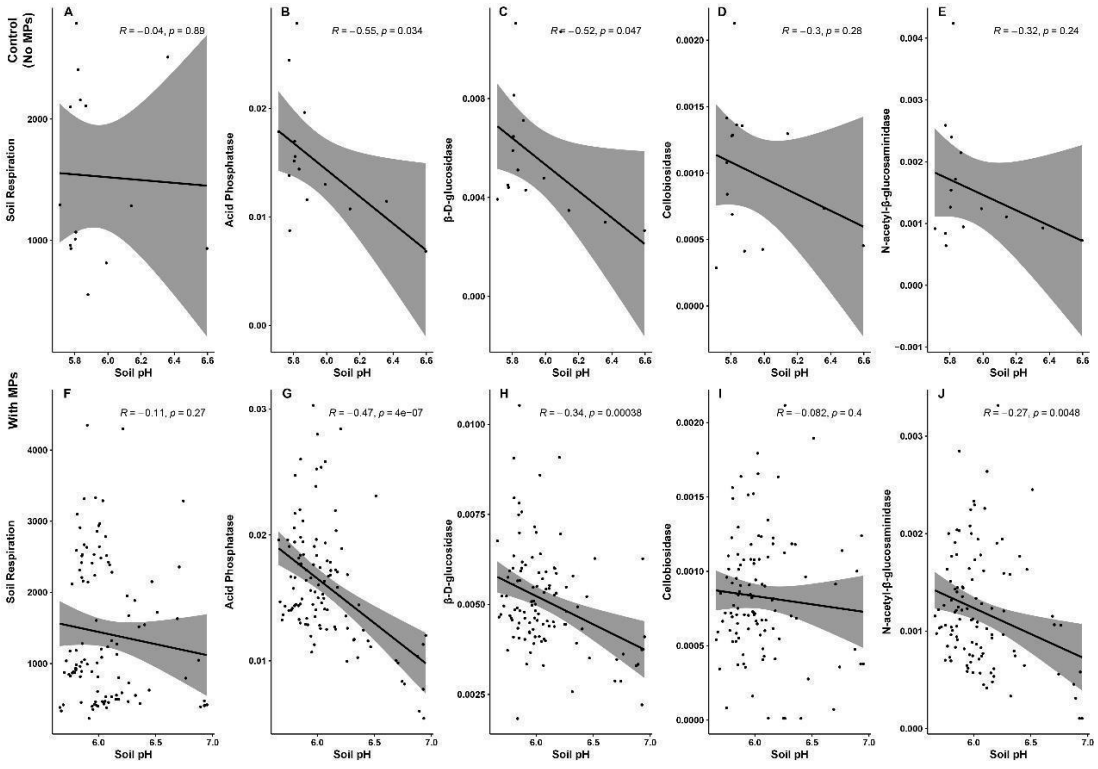

Figure 3.4 The negative correlation between microplastic effects on soil pH and microbial activities.

The data for enzymatic activities were log-transformed to generate the figures. Negative correlation between soil pH and microbial activities for the control (A-E), and the microplastic treatment (F-J).

β-D-glucosidase activity. β-D-glucosidase activity decreased with foams although it also tended to decrease in the presence of the other microplastic shapes (Table 3.3-5, Figure 3.5). Specifically, β-D-glucosidase activity decreased with PS foams (Table 3.4; Figure 3.5A). Over time, β-D-glucosidase activity declined with PA fibers, PE and PET films, and PS foams, while tended to increase with PP fragments. Overall, β-D-glucosidase activity was lower in soil mixed with foams, films and fragments polymers for the first time of measurement (PE and PP films, PS foams and all fragments) than in soils without microplastics, while it was higher in soil with PE foams than control for the last time of measurement (Table 3.5, Figure 3.5B). This enzymatic activity was negatively correlated with soil pH without or with microplastics in the soil (R = -0.52, p = 0.047, R = - 0.34, p <0.01, respectively, Figure 3.4).

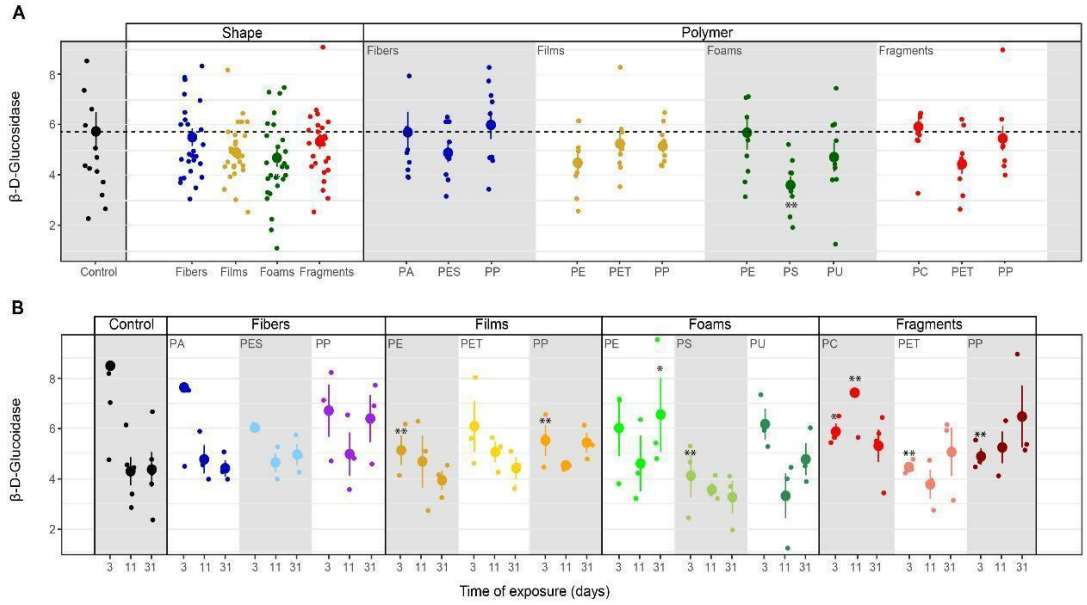

Figure 3.5 Microplastic effects on β-D-glucosidase.

Effects of shape, polymer type A) and incubation time B) on β-D-glucosidase. Mean and standard error are shown. n = 5 (control); n = 3 (microplastic treatments). Unit: µmol mg^{-1} h^{-1}. Significance * $p \leq 0.1$, ** $p \leq 0.05$ compares each microplastic with its respective control treatment for each time of measurement.

Table 3.3 Microplastic type and exposure time effects on soil pH, respiration, and enzymatic activities.

Results of linear models. F and *p*-values are shown. Values in bold indicate $p < 0.05$.

	df	**pH**		**Soil Respiration**		**Acid Phosphatase**		**β-D-glucosidase**		**Cellobiosidase**		**N-acetyl-β-Glucosaminidase**	
		F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value
Microplastic type	12	43.6	**<0.01**	12.81	**<0.01**	4.63	**<0.01**	3.10	**<0.01**	14.93	**<0.01**	3.23	**<0.01**
Time	2	45.2	**<0.01**	2012.42	**<0.01**	7.20	**<0.01**	16.44	**<0.01**	15.00	**<0.01**	162.52	**<0.01**
Microplastic: Time	24	5.8	**<0.01**	3.02	**<0.01**	1.31	**0.19**	1.98	**0.01**	6.35	**<0.01**	3.21	**<0.01**

Table 3.4 Microplastic shape and polymer type effects on soil pH, respiration, and enzymatic activities.

Results of multiple comparisons by using the Dunnett test. Values in bold and italic and in bold indicate $p \leq 0.05$ and $p \leq 0.1$, respectively.

Multiple comparisons (Dunnett)		**pH**		**Soil Respiration**		**Acid Phosphatase**		**β-D-glucosidase**		**Cellobiosidase**		**N-acetyl-β-glucosaminidase**	
Treatment-control = 0		z value	p-value	z value	p-value	z value	p-value	z value	p-value	z value	p-value	z value	p-value
shapes	Fibers - control = 0	-0.22	0.99	-0.46	0.96	1.28	0.46	-0.40	0.59	-1.80	**0.09**	-1.78	**0.09**
	Films - control = 0	1.12	0.57	-0.23	1.00	0.85	0.77	-1.45	0.18	-1.67	**0.10**	-1.37	0.20
	Foams - control = 0	6.01	***< 0.01***	1.11	0.58	0.60	0.91	-1.88	**0.08**	-1.69	**0.10**	-1.33	0.21
	fragments - control = 0	4.68	***< 0.01***	0.53	0.94	-0.55	0.93	-0.76	0.44	-0.02	0.75	-2.08	***0.05***
polymers	Fibers (PA) - Control = 0	0.46	0.86	-0.35	1.00	2.15	**0.10**	-0.10	0.94	-0.97	0.56	-1.04	0.62
	Fibers (PES) - Control = 0	-0.33	0.99	-86	0.99	-0.15	0.99	-1.26	0.51	-1.62	0.26	-1.70	0.30
	Fibers (PP) - Control = 0	-0.72	0.99	0.14	1.00	1.49	0.39	0.40	0.99	-1.62	0.26	-1.42	0.42
	Films (PE) - Control = 0	0.94	0.67	0.03	1.00	-0.13	0.97	-1.75	0.27	-1.02	0.54	-1.129	0.58
	Films (PET) - Control = 0	0.27	0.91	-0.55	1.00	1.30	0.49	-0.81	7.33	-2.10	0.11	-1.80	0.25
	Films (PP) - Control = 0	1.78	0.26	-0.02	1.00	0.90	0.69	-0.87	0.71	-1.06	0.52	-0.52	0.92
	Foams (PE) - Control = 0	4.43	***<0.01***	3.04	***0.03***	2.24	**0.10**	-0.06	0.96	-0.14	0.94	0.85	0.99
	Foams (PS) - Control = 0	9.03	***<0.01***	-0.75	0.99	-1.72	1.00	-3.08	***0.01***	-1.62	0.27	-2.11	0.14
	Foams (PU) - Control = 0	2.55	***0.05***	2.86	1.00	0.948	0.70	-1.41	0.43	-3.05	**0.01**	-1.84	0.23
	Fragments (PC) - Control = 0	3.78	***<0.01***	0.62	1.00	<0.01	0.96	0.38	0.99	1.46	0.99	-1.23	0.53
	Fragments (PET) - Control = 0	6.18	***<0.01***	0.017	1.00	-1.63	1.00	-1.90	0.21	-1.38	0.37	-2.13	0.14
	Fragments (PP) - Control = 0	2.53	***0.05***	0.58	1.00	0.27	0.91	0.27	0.91	-0.45	0.97	-1.48	0.40

Table 3.5 Microplastic exposure time effects on soil pH, respiration, and enzymatic activities.

Results of multiple comparisons using the Dunnett test. Values in bold and italic and in bold indicate $p \leq 0.05$ and $p \leq 0.1$, respectively.

Multiple comparisons (Dunnett)		**pH**		**Soil Respiration**		**Acid Phosphatase**		**β-D-glucosidase**		**Cellobiosidase**		**N-acetyl-β-glucosaminidase**	
Treatment-control >= 0		z value	p-value	z value	p-value	z value	p-value	z value	p-value	z value	p-value	z value	p-value
Day 3	Fibers (PA) - Control >= 0	0.55	0.83	1.11	0.59	0.04	0.96	-0.67	0.79	-2.75	***0.03***	-1.44	0.42
	Fibers (PES) - Control >= 0	0.42	0.88	-0.35	0.99	-1.90	0.20	-2.17	0.12	-3.07	***0.01***	-2.86	***0.02***
	Fibers (PP) - Control >= 0	0.06	0.95	1.42	0.42	-0.25	0.91	-1.62	0.32	-2.37	**0.08**	-2.97	***0.02***
	Films (PE) - Control >= 0	1.78	0.26	0.93	0.68	-1.79	0.25	-2.96	***0.02***	-3.60	***<0.01***	-2.39	**0.08**
	Films (PET) - Control >= 0	0.79	0.74	0.50	0.85	-1.42	0.42	-2.08	0.15	-5.26	***<0.01***	-2.75	***0.03***
	Films (PP) - Control >= 0	3.83	***<0.01***	0.98	0.66	-1.17	0.55	-2.67	***0.04***	-4.43	***<0.01***	-1.10	0.60
	Foams (PE) - Control >= 0	3.66	***<0.01***	9.00	***<0.01***	0.35	0.99	-2.16	0.13	-4.80	***<0.01***	-0.61	0.81
	Foams (PS) - Control >= 0	5.23	***<0.01***	-2.21	1.00	-2.03	0.16	-3.86	***< 0.01***	-4.03	***<0.01***	-1.63	0.32
	Foams (PU) - Control >= 0	3.83	***<0.01***	2.40	**0.07**	0.003	0.96	-2.04	0.16	-4.53	***<0.01***	-2.77	***0.03***
	Fragments (PC) - Control >= 0	2.16	0.13	2.84	***0.02***	-1.15	0.57	-2.30	**0.09**	-2.76	***0.03***	-1.42	0.42
	Fragments (PET) - Control >= 0	8.17	***<0.01***	0.47	0.99	-2.65	***0.04***	-3.47	***<0.01***	-7.46	***<0.01***	-2.06	0.15
	Fragments (PP) - Control >= 0	3.29	***<0.01***	3.06	***0.01***	-2.03	0.16	-3.10	***0.01***	-3.78	***<0.01***	-2.01	0.17
Day 11	Fibers (PA) - Control >= 0	-0.71	1.00	-1.60	0.64	1.93	0.20	0.67	0.80	-0.38	0.89	-0.20	0.98
	Fibers (PES) - Control >= 0	-1.08	1.00	-1.67	0.59	0.88	0.71	0.51	0.85	-0.39	0.89	-0.42	0.99
	Fibers (PP) - Control >= 0	-0.67	1.00	-0.004	1.00	1.76	0.26	0.88	0.7**1**	1.70	0.29	-0.30	0.99
	Films (PE) - Control >= 0	-1.01	1.00	-0.11	1.00	1.30	0.49	0.44	0.87	-1.32	0.48	1.17	0.56
	Films (PET) - Control >= 0	-0.75	1.00	-1.61	0.64	2.36	**0.08**	0.97	0.66	-1.14	0.58	0.27	0.92
	Films (PP) - Control >= 0	-0.31	0.99	-0.85	0.99	1.10	0.60	0.39	0.89	-0.79	0.75	2.15	0.13
	Foams (PE) - Control >= 0	4.29	***<0.01***	1.93	0.39	2.17	0.12	0.59	0.83	0.15	0.97	3.13	***<0.01***

	Foams (PS) - Control >= 0	8.060	***<0.01***	0.95	0.98	-0.46	0.99	-0.78	1.00	-0.10	0.95	-0.43	0.99
	Foams (PU) - Control >= 0	1.30	0.49	-0.76	1.00	1.72	0.28	-1.05	1.00	-1.70	0.29	0.37	0.89
	Fragments (PC) - Control >= 0	0.27	0.92	0.92	0.99	2.86	***0.02***	3.47	***<0.01***	1.86	1.00	0.49	0.86
	Fragments (PET) - Control >= 0	4.26	***<0.01***	1.79	0.49	-0.47	0.99	-0.56	1.00	*-0.09*	0.95	-0.89	1.00
	Fragments (PP) - Control >= 0	1.00	0.65	-0.50	1.00	1.53	0.37	1.20	0.54	1.39	1.00	-0.19	0.98
Day 31	Fibers (PA) - Control >= 0	1.13	0.58	-5.14	***<0.01***	1.80	0.49	0.03	0.95	1.05	0.62	-1.16	0.56
	Fibers (PES) - Control >= 0	0.08	0.94	-6.01	***<0.01***	0.11	1.00	-0.51	0.99	-0.46	0.99	-0.54	0.83
	Fibers (PP) - Control >= 0	-0.76	1.00	-5.09	***<0.01***	1.20	0.91	2.09	0.15	0.32	0.90	0.63	0.99
	Films (PE) - Control >= 0	1.75	0.27	-3.92	***<0.01***	0.32	1.00	-0.53	0.99	3.15	***<0.01***	-1.18	0.55
	Films (PET) - Control >= 0	0.72	0.77	-5.34	***<0.01***	1.41	0.78	1.00	0.96	2.43	**0.068**	-2.17	0.12
	Films (PP) - Control >= 0	1.79	0.25	-2.06	0.30	1.40	0.79	1.06	0.61	2.99	***0.015***	-1.38	0.44
	Foams (PE) - Control >= 0	2.91	***0.02***	-3.44	***<0.01***	1.58	0.65	2.28	**0.09**	5.61	***<0.01***	1.08	1.00
	Foams (PS) - Control >= 0	7.35	***<0.01***	-4.93	***<0.01***	-0.69	1.00	-1.18	1.00	-0.42	0.99	-5.02	***<0.01***
	Foams (PU) - Control >= 0	1.88	0.21	-4.53	***<0.01***	0.33	1.00	0.39	0.88	0.78	0.74	-2.42	**0.07**
	Fragments (PC) - Control >= 0	4.56	***<0.01***	-4.08	***<0.01***	-0.43	1.00	1.00	0.64	4.68	***<0.01***	-2.35	**0.08**
	Fragments (PET) - Control >= 0	4.15	***<0.01***	-4.47	***<0.01***	-0.14	1.00	0.68	0.7**9**	3.00	***<0.01***	-3.62	***<0.01***
	Fragments (PP) - Control >= 0	2.31	**0.09**	-4.13	***<0.01***	0.89	0.99	2.18	0.11	3.18	***<0.01***	-1.78	0.25

Cellobiosidase activity. Cellobiosidase activity was reduced by all microplastic shapes except fragments, whose effects were similar to control (Table 3.3-5, Figure 3.6). In particular, cellobiosidase activity decreased with PET films and PU foams (Table 3.4; Figure 3.6A). Over time, this enzyme tended to decline with PA and PP fibers, while showing a contrary trend with PET, PP films and PE foams. Overall, cellobiosidase activity was lower in soils mixed with microplastics of different polymer type (for the first time of measurement) than in soils without microplastics, while promoted by PE films and foams, and fragment polymers for the last time of measurement (Table 3.5, Figure 3.6B). Cellobiosidase activity was not correlated with soil pH when microplastics in the soil were absent (R = - 0.3, p = 0.28) or present (R = - 0.08, p = 0.4, Figure 3.4).

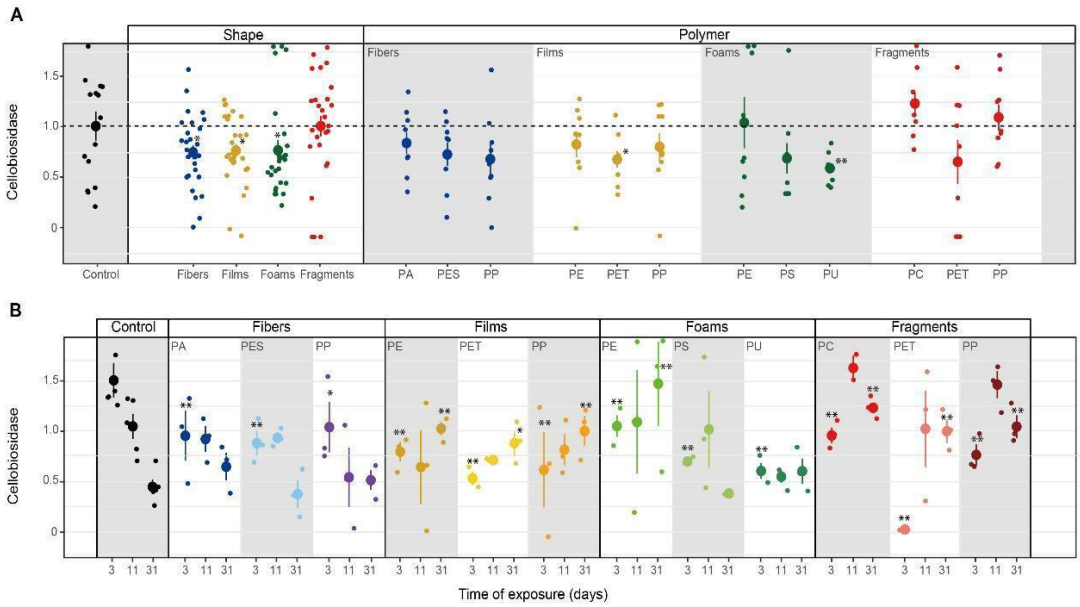

Figure 3.6 Microplastic effects on cellobiosidase.

Effects of shape, polymer type A) and incubation time B) on cellobiosidase. Mean and standard error are shown. n = 5 (control); n = 3 (microplastic treatments). U Unit: µmol mg^{-1} h^{-1}. Significance * p ≤ 0.1, ** p ≤ 0.05 compares each microplastic with its respective control treatment for each time of measurement.

N-acetyl-β-glucosaminidase activity. N-acetyl-β-glucosaminidase activity was lower in the presence of microplastic fibers and fragments compared to the control and was neutrally or slight negatively affected by all the polymers (Table 3.3-5; Figure 3.7). N-acetyl-β-glucosaminidase activity steadily decreased with films, foams and fragments over time (Table 3.5; Figure 3.7B). Overall, this enzyme activity was lower in soils mixed with microplastics of different polymer types for the first time of measurement, than in soils without microplastics (Table 3.5, Figure 3.7B). Likewise, N-acetyl-β-glucosaminidase activity was not correlated with soil pH when microplastics in the soil were absent (R = - 0.32, p = 0.24) but it was negatively correlated when the microplastics were present (R = - 0.27, p = <0.01, Figure 3.4).

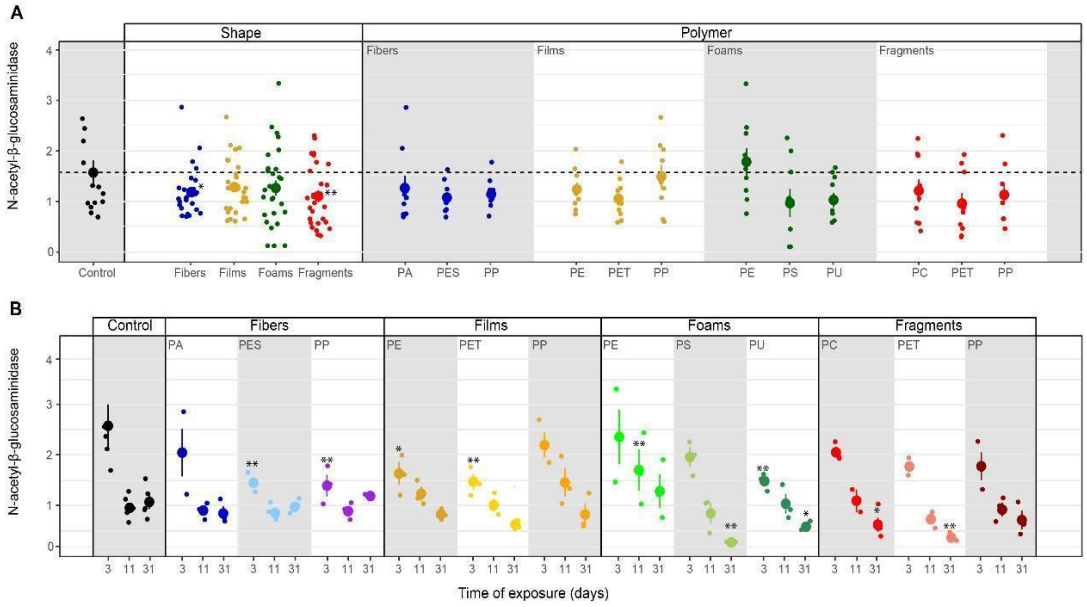

Figure 3.7 Microplastics effect on N-acetyl-β-glucosaminidase.

Effects of shape, polymer type A) and incubation time B) on N-acetyl-β-glucosaminidase. Mean and standard error are shown. n = 5 (control); n = 3 (microplastic treatments). Unit: μmol mg^{-1} h^{-1}. Significance * $p \leq 0.1$, ** $p \leq 0.05$ compares each microplastic with its respective control treatment for each time of measurement.

3.5 DISCUSSION

Our results showed that microplastic effects on soil pH, respiration, and enzymatic activities depended on microplastic shape, polymer type, and the effects changed with incubation time.

3.5.1 Microplastics increased soil pH

Our results indicated that microplastic foams and fragments increased soil pH, which can be due to the increase in soil aeration and porosity when these microplastics were added into the soil (de Souza Machado et al., 2019; Lozano et al., 2021a). This along with the leaching of microplastic chemical compounds into the soil (Waldman & Rillig 2020; Kim et al., 2020), may alter soil biota with consequences for soil pH. Likewise, although slightly, microplastic films increased soil pH. In this regard, it has observed that PE films may alter the diversity of nitrogen fixation bacteria taxa in the soil (Fei et al., 2020), which would alter the contents of soil NH_4^+, increasing soil pH, as the conversion of organic N to NH_4^+ would consume H^+ (Butterly et al., 2010; You et al., 2015). Notably, PE foams increased soil pH more than PE films, this may be due to the shape or additives differences. Our results showed that soil pH increased with PE polymers, which agrees with previous research on that topic (Qi et al., 2020; Wang et al., 2020). However, recent research indicates that soil type may also play a role (Boots et al. 2019; Yu et al., 2020); for example, depending on the soil organic matter content acid buffering and retention of major cations may change (Jiang et al.,

2018). In addition to the soil type, the presence of plant species in the system may also influence microplastics effects on soil pH (Lozano et al., 2021b), as plants could potentially mitigate the effects of microplastics on soil pH in comparison to bare soil. Finally, we found a negative correlation between soil pH and enzymatic activities.

3.5.2 Microplastics affected soil respiration after long incubation time

Our results also showed that soil respiration decreased over time for all microplastic treatments and the control, a situation that can be linked to the reduction of labile substrates (Chen & Wu 2019). However, we observed that after longer incubation time (i.e., 31 days), the decrease in respiration was more pronounced in soils with microplastics than without. This sharp decrease would be linked to the potential harmful effects of microplastics leachates on soil biota (Kim et al., 2020), a situation that was only evident after several days of soil subjected to microplastics. Added to this, our results showed that soil respiration was higher with PE foams than with the control (without microplastics). This positive effect may be due to their loose spongy structure that may increase soil aeration (Lehmann et al., 2020). This may could be the reason that PE foams caused higher respiration than PE films. Films and fragments had neutral effects on soil respiration, while positive effects have been observed on this property when a plant species was included in the system (Lozano et al., 2021a). The latter as the presence of roots in the soil matrix contributes to soil aggregation and facilitates water uptake and its redistribution through the soil profile, which in the end promotes soil microbial activity (Lozano et al., 2021a).

3.5.3 Microplastics had negative effects on most soil enzymatic activities

Microplastics in the soil inhibited most of the enzymatic activities. That is, β-D-glucosidase, cellobiosidase and N-acetyl-β-glucosaminidase activities.

Fibers negatively affected cellobiosidase and N-acetyl-β-glucosaminidase activities which can be linked to the negative effects that fibers may have on soil aggregation as they may prevent macroaggregates formation (Zhang & Liu 2018) and/or introduce fracture points into aggregates, affecting aggregate stability. As soil aggregation is positively correlated with soil microbial activity (Bronick & Lal, 2005), the negative effects of microplastics on soil aggregation may have consequences for soil microbial activity. Reduction in oxygen diffusion within the soil pores and the effects on water flows (Six et al., 2004) may explain the decrease in enzymatic activities. Likewise, changes in physicochemical niches, which provide space for growth and activity of soil microorganisms (Yu et al., 2020), would be altered with the presence of microplastics. In addition, N-acetyl-β-glucosaminidase activity could be reduced as macro-aggregates (>2mm) where this enzyme is highly active (Wang et al., 2015), were affected in their formation due to the presence of microplastic fibers (Lehmann et al., 2020). On the other hand, foams decreased β-D-glucosidase, cellobiosidase and

N-acetyl-β-glucosaminidase activities, which can be linked with the sorption capacities of microplastics. Microplastics can carry toxic chemicals serving as vectors of transport for different pollutants (Wang et al., 2018) and in addition, different hazardous chemicals are voluntarily added during their production such as additives to increase polymer properties and prolong their life (Lithner et al., 2011). All these substances can be released into the soil matrix with negative effects on soil biota (Kim et al., 2020) and potentially on soil enzymatic activities.

Likewise, films caused lower activities of cellobiosidase and N-acetyl-β-glucosaminidase, which may be influenced by their negative effects on soil water evaporation (Wan et al., 2019), a soil condition that negatively affects soil microbial activity (Six et al., 2004). Films might also increase N-cycling microorganisms, and thus N-fixation (Fei et al., 2020), which could decrease N-acetyl-β-glucosaminidase activity as observed early in the incubation time (day 3) for PE and PET films. Similar to Yu et al., (2020), microplastic films had negative effects on β-D-glucosidase, however this was only evident at early stages of incubation (day 3).

Our results also showed that microplastics in the soil can stimulate enzymatic activity. Specifically, we observed that after some time of incubation, microplastic foams (e.g., PE) increased cellobiosidase, β-D-glucosidase (31 days) and N-acetyl-β-glucosaminidase (11 days) activities. This can be linked to the loose spongy structures of this plastic, which may increase soil pore space and thus water and air flows, promoting soil microbial activity. Indeed, PE foam was the microplastic that promoted the most soil respiration.

We observed that three enzymes (acid phosphatase, β-D-glucosidase and cellobiosidase) showed fluctuation trends during the incubation time. As recently observed, microplastics may have toxicity effects on soil biota after 24 h, although at higher concentration (1 %) (Kim et al., 2020), which would have negative effects on microbial activity, thus causing reductions of the enzyme activities during the first days of exposure. Later, the microbiota may have adjusted to the new environmental conditions (Yi et al., 2020), and/or some of the toxic additives may have been inactivated or degraded, causing a rebound of enzyme activities. In that sense, previous studies indicated that microplastic effects on enzymatic activities might differ with incubation time i.e., 30 vs. 150 days (Liu et al., 2017; Yu et al. 2020).

Finally, we found that negative correlation between enzymatic activity and soil pH, which is consistent with previous studies (Adetunji et al., 2017; Ullah et al., 2019). However, this relationship was weakened in the presence of microplastics, as the increase in soil pH may promote the abundance, diversity, biomass and activity of certain bacterial groups (Zhalnina et al., 2014, Rousk et al., 2009), as observed for members of *Acidobacteria*, *Nitrospira* or *Proteobacteria* phyla (Rousk et al., 2010).

In this way, microplastic may have weakened the negative correlation between soil pH and enzymatic activities.

3.6 CONCLUSION

Our study contributes to a better understanding of the effects that microplastics have on soil microbial activities, which can be linked among others, to the changes in soil pH. Likewise, our results suggest that microplastics can affect soil enzymes with potential consequences on C, N and P cycles. We found that in addition to including shape and polymer type as microplastics properties that affect soil systems (Lehmann et al., 2020; Rillig & Lehmann 2020; Wiedner & Polifka 2020; Lozano et al., 2021b), the exposure time of soil to the microplastics is another experimental parameter to consider, especially when studies report diverging effects. As the presence of plants, the type of soil and its content of organic matter (Lozano et al., 2021b, Liang et al., 2021) would influence the effects of microplastics on soil pH, respiration, and enzymatic activities, future specific research on this area is needed.

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Chapter 4: INDIRECT EFFECTS OF MICROPLASTIC-CONTAMINATED SOIL ON ADJACENT SOIL LAYERS: VERTICAL CHANGES IN SOIL PHYSICAL STRUCTURE AND WATERFLOW

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Keywords: aggregates, fibers, heterogeneous pollution, water path

4.1 ABSTRACT

Previous microplastic research under laboratory conditions has focused on microplastics that are homogeneously mixed into test media, in order to maximize test reproducibility and uniform bio-accessibility. Here we specifically focused on testing the idea that microplastics in soil could affect adjacent soil layers not containing microplastic themselves. We included two different microplastics (low-density polyethylene films and polyacrylonitrile fibers) and carried out a soil column test consisting of three different vertical layers (0-3 cm, top, control soil; 3-6 cm, middle, microplastic-containing soil; 6-9 cm, bottom, control soil). Our study shows that microplastic-containing soil layers can act as an anthropogenic barrier in the soil column, interrupting the vertical water flow. These changes directly affected the water content of adjacent layers, and changes in the proportion of soil aggregate sizes occurred for each depth of the soil columns. We also observed that these physical changes trigger changes in soil respiration, but do not translate to effects on enzyme activities. These results imply that the soil environment in non-contaminated parts of the soil can be altered by microplastic contamination in adjacent layers, as might occur for example during plowing on agricultural fields. More generally, our results highlight the need to further examine effects of microplastic in experiments that do not treat this kind of pollution as uniformly distributed.

4.2 INTRODUCTION

Scientists estimate that less than 5% of plastic production is recycled (Sutherland et al., 2019), and a considerable amount of plastic waste is accumulating in the environment (Jambeck et al., 2015; Rillig and Lehmann, 2020). One of the main concerns about plastic pollution is that plastic waste can be slowly fragmented into smaller sizes under environmental conditions such as UV-radiation and mechanical weathering (Arthur et al., 2009). These tiny particles (<5 mm), defined as "microplastics," are ubiquitously observed in freshwater (Sarijan et al., 2021), oceans (Andrady 2011), atmospheres (Chen et al., 2020), and soils (Rillig 2012). An annual input rate of microplastics into European agricultural lands has been estimated to be 125-850 tons per million inhabitants, and 427 thousand tons of plastic mulch films are used every year in European farmlands (Nizzetto et al., 2016). Previous studies have reported that 300-67,500 mg kg^{-1} or 40-18,760 particles kg^{-1} of microplastics are observed in agricultural (Liu et al., 2018; Piehl et al., 2018; Zhang et al., 2018; Zhang and Liu, 2018; Ding et al., 2020), coastal (Zhou et al., 2018), floodplain (Scheurer and Bigalke, 2018), and industrial lands (Fuller & Gautam 2016).

Research on microplastics effects has been mainly conducted under highly controlled laboratory conditions since this provides more accurate results, and many studies have

mixed microplastic into test media as homogeneously as possible to keep variability of results low. In liquid media, homogeneous dispersion of insoluble test substances (e.g., nanomaterials and microplastics) is an important requirement to reduce agglomeration or sedimentation, and the use of dispersants is often adopted as an efficient strategy (Potthoff et al., 2017). For soil, it is also recommended for target material to be mixed thoroughly and homogenized (Thomas et al., 2020). A recent study explained that the "homogeneity of exposure" is a crucial criterion to guarantee the reproducibility and uniform bio-accessibility during laboratory tests in microplastic research (de Ruijter et al., 2020).

Here, we were specifically interested in testing if microplastics in soil can affect adjacent soil layers not even containing microplastic themselves. Microplastics can induce changes in soil physicochemical and biological parameters, and these effects have been well established in previous studies (Rillig & Lehmann 2020). For instance, microplastic fibers can interfere with soil aggregate formation due to their linear shape (de Souza Machado et al., 2018; de Souza Machado et al., 2019; Zhang et al., 2019), and microplastic films influence soil tensile strength (Wan et al., 2019). It is likely that such physical changes in microplastic-containing soils would become more intense with time (de Souza Machado et al., 2018; de Souza Machado et al., 2019; Lehmann et al., 2020b), and that flows of water and nutrients into adjacent soil layers can be influenced. This would be important, because such indirect effects would suggest that previous work might have underestimated the extent of microplastic effects in soil. To capture this situation, we designed an experiment in which we added microplastic in a layer of a soil column, and this afforded us the opportunity to study effects on adjacent soil layers that are themselves not contaminated. We selected two different microplastics as target materials; low-density polyethylene (LDPE) films and polyacrylonitrile (PAN) fibers. The soil column was constructed with three layers (control soil; microplastic-containing soil; control soil), and two different levels of water addition (low and high) were included in the experimental design. To evaluate biophysical parameters at each depth of the soil columns, water content, water flow, soil aggregates sizes, soil respiration, and enzyme activities were measured after short- (1 day) and long-term (60 days) incubation periods.

4.3 MATERIALS AND METHODS

4.3.1 Preparation and characterization of microplastics

LDPE films and PAN fibers were prepared using commercial mulching films (thickness, 13.66 ± 2.32 μm, Ihlshin Chemical Co., Ltd., Ansan, South Korea) and knitting wool (100% PAN, DIKTAS Sewing & Knitting Yarns Co., Turkey) (Kim et al., 2020). Each material was cut using sterilized scissors, and then passed through a 630 μm-sieve. Each microplastic was observed under a microscope, and close-up photographs were captured to determine average sizes using image analysis (ImageJ,

1.52a, National Institutes of Health, United States) (Appendix Figure A4.1). The average area of LDPE films was calculated as 1.5 ± 0.8 mm^{-2} (n = 100), and the average length of PAN fibers was 2.4 ± 0.6 mm (n = 100). Target microplastics were stored at room temperature before main experiments. To characterize the actual nature of each material, a spectrophotometer (Jasco, model FT/IR-4100, ATR mode) was used, and each sample was scanned 32 times from 4000 to 600 cm^{-1}, with a resolution of 4 cm^{-1} (Appendix Figure A4.2).

4.3.2 Soil column test

Test soil was collected from a grassland site of the Institute of Biology of Freie Universität, Berlin, Germany (52.45676N, 13.30240E) on January 20, 2020. The soil was passed through a 2 mm-sieve, and then dried at 60°C for 24 h. The texture of test soil was a sand (sand 93.3%, silt 5.0%, and clay 1.7%), and pH and water holding capacity (WHC) were 6.7 ± 0.2 and 0.34 ± 0.10 ml g^{-1}, respectively (n = 3). In order to prepare microplastic soils (LDPE films and PAN fibers), 100 mg of each microplastic and 99.9 g of dry test soil were mixed using laboratory tweezers and a spatula, and each mixture was shaken using an overhead shaker (Reax 2, Heidolph, Germany) for 5 min (0.1% based on dry weight). The control soil was treated by an equivalent process (shaking), but not containing microplastics, and each soil was directly used for the soil column test. To prepare the soil column, 10 g of test soil was placed into 50 ml-test tubes (bottom layer), and 10 g of each microplastic-containing soil (LDPE films and PAN fibers, 0.1%) were added (microplastic-containing soil layer), after which additional test soil (10 g) was placed into the test tube (top layer) (n = 3). A control treatment was prepared with no microplastic-containing soil layer, but using an otherwise equivalent process (n = 3). The total soil depth of the soil column was approximately 9 cm, and the depth of each layer (top, microplastic-containing soil, and bottom) was 3 cm (Figure 4.1). To moisten the soil columns, 3 ml (low level of irrigation) or 6 ml (high) of deionized water was carefully injected into surface soil (<1 cm) using a syringe needle, and these water levels were regarded as 10 and 20% of total soil weight. Each soil column was covered by a vented cap and incubated at 20°C laboratory incubator (PP110 plus, Memmert GmbH, Schwabach, Germany) in the dark for 1 day or 60 days, respectively. Changes in biological parameters are expected to be observed after long term incubation, while the water infiltration occurs within 1-2 days (Schneider et al., 2018). We determined two test periods (1 and 60 days) to check both parameters in the soil columns. Since the different water content in soil can influence our measurement, parameters such as soil respiration and enzyme activities, water content was replenished every 3 days to keep uniform moisture during incubation periods.

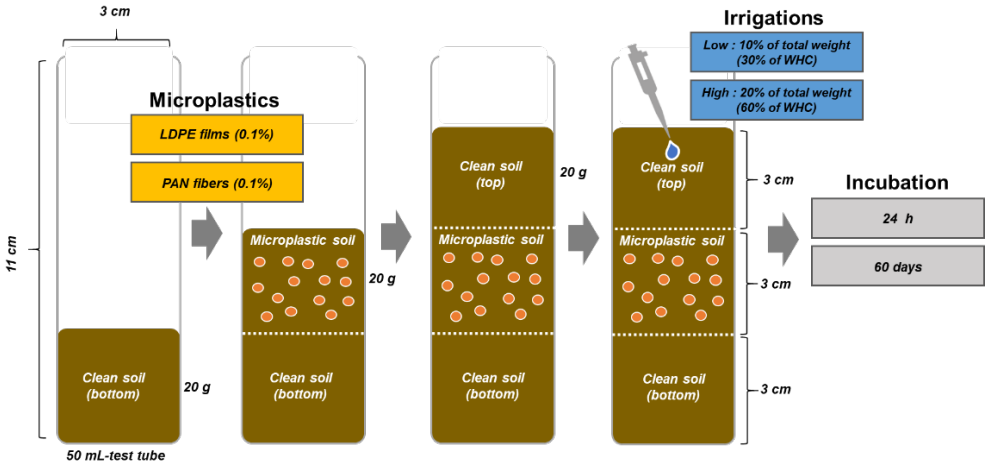

Figure 4.1 The diagram of the soil column test in this study.

At the end of each incubation period, soil samples of each depth (1 cm) were carefully collected using laboratorial spatula. The weights of each soil sample were recorded before and after drying at 60°C for 24 h to calculate water content (%). Soil structures of each depth were assessed as reported in previous studies (de Souza Machado et al., 2019; Lehmann et al., 2020a). Shortly, the whole soil was gently passed through a set of stacked sieves (4,000, 2,000, 1,000, and 212 µm), and the weights of four separated fractions were recorded to determine the proportions (%) of each soil aggregate size class. Bulk density was computed by measuring the volume of soils within the plastic pot and soil dry weight (g cm^{-3}). We measured the soil respiration of three layers (top, microplastic-containing soil, and bottom), as CO_2 production rate (ppm h^{-1}) after 60 days of the experiment. Before the measurement, we flushed each of the tubes with CO_2-free air for five minutes to standardize among experimental units (Rillig et al., 2019c). After 18 h, we sampled 1ml of air from the headspace of each tube and injected this sample into an infrared gas analyzer (LiCOR-6400x). Extracellular soil enzyme activities, acid phosphatase and β-D-glucosidase were measured after 60 days of incubation (Jackson et al., 2013). Briefly, 5 g of each soil sample (top, microplastic-containing soil, and bottom) was placed into a 50ml test tube and mixed with 10 ml of 50mM acetate buffer (pH 5.0-5.4), and 150 µl soil slurry was pipetted into each of six wells on a 96-well plate after vortexing. Then 150 µl acetate buffer was added into the last two wells of each sample (sample buffer control), and 150 µl substrate solutions (p-nitrophenyl-phosphate and p-nitrophenyl- β-glucopyranoside; Sigma, Germany) to the first four wells. Then the plates were kept in an incubator at 25°C for 2-4 h. After incubation, the microplates were centrifuged at 3000 x g for 5 min, and then 100 µl supernatant from each well was added into the new microplates with 10 µl 1M NaOH and 190 µl distilled water in each well. Finally, the absorbance was recorded at 410 nm by a microplate reader (Benchmark Plus Microplate Spectrophotometer System, BioRad Laboratories, Hercules, CA, United States).

4.3.3 Dye tracer test

To observe the spatial patterns of water flow in the soil columns, dye tracer experiments were conducted with starting and 60 days incubated soil columns. The starting soil columns (0 days, before irrigation) were directly used for dye tracer test, and the 60 days incubated columns were dried at 60°C for 48 h. We employed Brilliant Blue dye as a tracer since it is highly visible (Schneider et al., 2018). Although dye transport is slower than the advance of infiltrating water, dye-stained soil patterns are generally considered to reasonably reflect flow patterns in soil experiments (Cey & Rudolph 2009). We dissolved 100 mg of Brilliant Blue powder in 100 ml of deionized water, and 3 ml or 6 ml of dye solutions were applied to each soil column. After 24 h, the soil was carefully separated from the soil column, and vertically excavated to observe the soil profiles. To study the distribution of dye tracer in the soil profiles, photographs were captured. For each profile, the close-up photographs were adjusted for analyzing the relative pixel intensity of Brilliant Blue dyed path using ImageJ software (ImageJ, 1.52a, National Institutes of Health, United States).

4.3.4 Statistical analyses

Data were analyzed using the SPSS statistical software (Ver. 24.0, SPSS Inc., Chicago, IL, United States). One-way analysis for variance (ANOVA) and Turkey's tests were conducted to determine the significance ($p < 0.05$) of multiple comparisons.

4.4 RESULTS AND DISCUSSION

4.4.1 Effects on water contents and flows

We observed each soil sample at each depth to examine the potential migration of microplastics during the soil column tests. As shown in Appendix Figures A4.3-4.6, microplastic-contaminated soil layers contained numerous LDPE films and PAN fibers, while only a few microplastic particles were found in top and bottom layers. We assume that the microplastics ended up in adjacent layers during the layer separation or soil analysis steps. Although the microplastics may migrate to the adjacent soil layers for longer time or under different conditions, we concluded here that the microplastics were not transported in our soil column tests. Water contents of each depth were considerably different already after the 1 day incubation. In the control treatment, after low-level irrigation, water content of the top layer (0-3 cm) was relatively higher than the bottom layer (6-9 cm), and this difference significantly increased in microplastic-containing soil layer treatments. Water contents increased to 13.03 ± 0.29 (LDPE films) and 12.98 ± 0.28 (PAN fibers) % in the top transition layer (3-4 cm), while the control treatment had a water content of 11.32 ± 0.19%. In the bottom transition layer (6-7 cm), water contents were 1.67 ± 0.19 (LDPE films) and 1.57 ± 0.23 (PAN fibers) %, while control treatment had 4.72 ± 0.57% (Figure 4.2A).

The gaps of water contents between top and bottom layers were reduced after the 60 days of incubation, but significant differences among depths remained in the soil column containing PAN fiber layer (Figure 4.2B). In high-level irrigation treatments, only the soil layer with PAN fibers significantly influenced the vertical water distribution (Figure 4.2C), and the difference of water content in the top layer disappeared after the 60 days incubation, but remained in the bottom layer (Figure 4.2D).

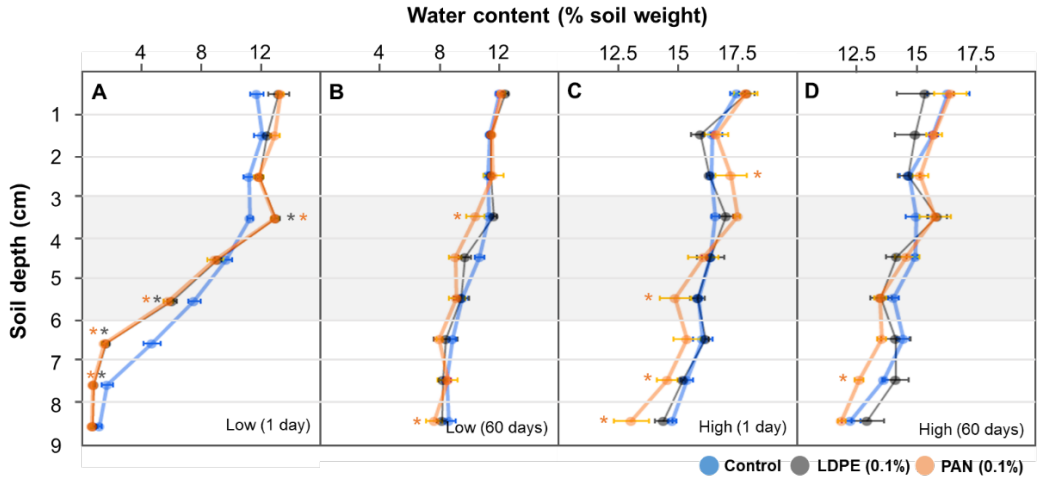

Figure 4.2 Water contents at each soil depth of each soil column.

Each soil column was irrigated either at a low level (A, B) or with higher water volume (C, D), and incubated for 1 day (A, C) and 60 days (B, D). Asterisk represents significance at the level of 5% (p = 0.05) between control and microplastic-containing soil layer treatments.

The infiltrated dye stain patterns for vertical soil profiles are shown in Figure 4.3. In the control treatment after low-level irrigation, the dye tracer solution had uniformly infiltrated into soil depth 3-4 cm for both incubation periods (1 and 60 days), while uneven dye distributions and several discontinuities were observed in microplastic-containing soil layer treatments (yellow arrows in Figures 4.3A, B). After high level irrigation, the maximum depth of dyed soil in the control treatment increased to 4-5 cm for both incubation periods (1 and 60 days) (Figures 4.3C, D). Paths of preferential flow appeared in microplastic-containing soil layer treatments (LDPE films and PAN fibers), and these patterns were observed below a soil depth of 6 cm (yellow arrows in Figure 4.3C). Although the dye transport does not exactly match the infiltrating water volume, the preferential flow indicates that the microplastic-containing soil layer might block and influence the water flow path in the soil column. These preferential flows were not observed after 60 days of incubation, and uneven dye distributions were observed in the top layers (yellow arrows in Figure 4.3D).

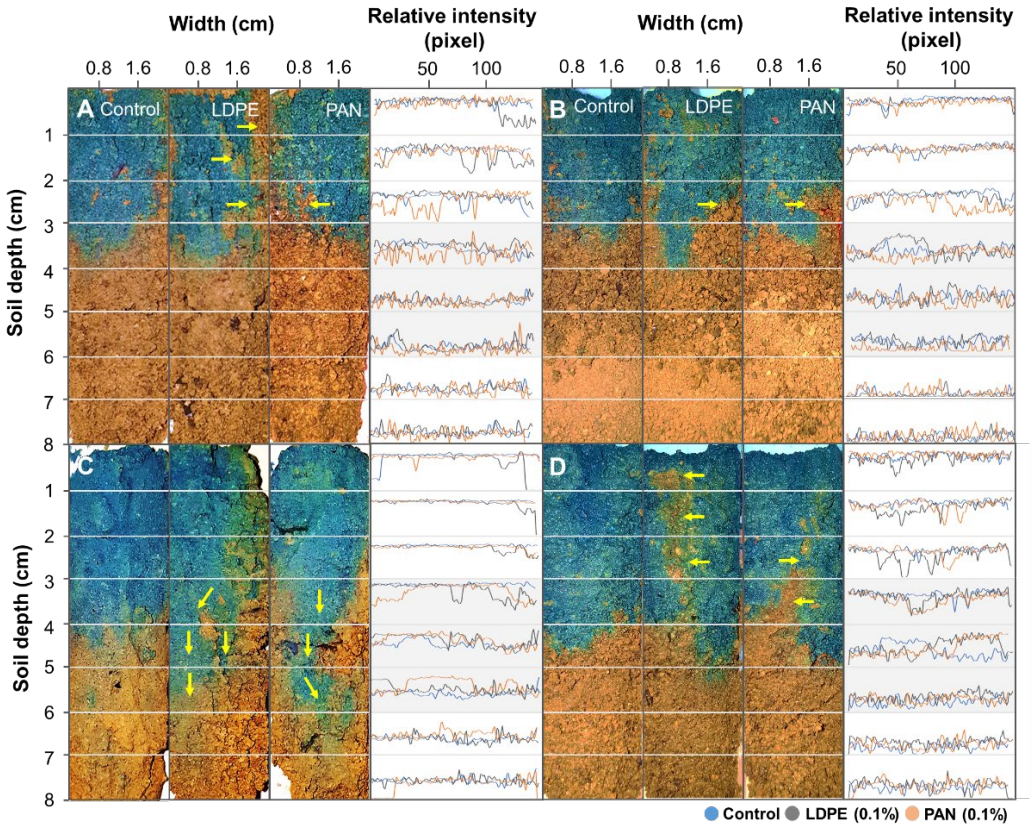

Figure 4.3 Infiltrated dye stain patterns for vertical soil profiles.

Each soil column was incubated for 0 days (A, C) and 60 days (B, D), and irrigated either at a low level (A, B) or with a higher water volume (C, D).

Soil water content plays an important role in hydrological and biological processes, and spatial variability, both horizontally and vertically, is typically present in soil profiles (Wang & Liu 2013). In anthropogenically modified soils, a high heterogeneity of substrates and unique patterns of water infiltration are often observed, such as in mine spoil soils, tilled soils, and biochar-containing soils (Andreini & Steenhuis 1990; Badorreck et al., 2010; Schneider et al., 2018). A high spatial heterogeneity of pore volumes can be associated with anthropogenic (e.g., relict charcoal hearths) or natural fragments (e.g., organic matter and plant roots), and these can affect water flows in soil profiles (Schneider et al., 2018). There are several previous studies reporting that microplastics can influence water dynamics (de Souza Machado et al., 2018; de Souza Machado et al., 2019; Wan et al., 2019). Polyethylene films and polyester fibers induced changes in soil aggregation and pore sizes, and these phenomena can be directly or indirectly linked with water evaporation and soil cracking (Wan et al., 2019; Zhang et al., 2019). Alterations in soil structure can affect pore space in soils, which can simultaneously alter water holding capacity and water availability (de Souza Machado et al., 2019). Our study here shows that microplastic-containing soil layers can affect water contents and flows in adjacent soil layers, even if total water contents in the soil columns were kept the same in each

treatment (control, LDPE films, and PAN fibers) (Appendix Figure A4.7).

4.4.2 Effects on soil physical structure

With low-level irrigation (1 day incubation) in the control treatment, large soil aggregate size fractions (2-4 mm) decreased with increasing soil depth, while intermediate sized fractions (1-2 and 0.1-1 mm) increased. This difference was more pronounced in microplastic-containing soil layer treatments, and mainly occurred in the microplastic-containing soil and bottom layers (< 4 cm soil depth) (Figures 4.4 A-C). After 60°days, the differences in soil aggregate size fractions between each soil depth were noticeably reduced in the control treatment, but significant differences among soil depths were still observed in the microplastic-containing soil layer treatments (Figures 4.4 E-G). With high-level irrigation, large and intermediate sized soil aggregate fractions (2-4 and 1-2 mm) showed similar levels at each soil depth, but the PAN fiber layer influenced other size fractions (0.2-1 and < 0.2 mm) (Figures 4.4 A-D). After 60°days, the proportion of small soil aggregate size fractions (< 0.2 mm) was dramatically changed by LDPE films and PAN fiber layers. The proportion of the small size fraction significantly increased in the top layers and decreased in the bottom layers of PAN fiber treatment, and LDPE film layers had a significantly lower level than the control treatment (Figure 4.5H). Soil bulk density in the bottom layer seemed to be slightly influenced by microplastic-containing soil layers, but overall levels were similar in each treatment and depth (Figure 4.6).

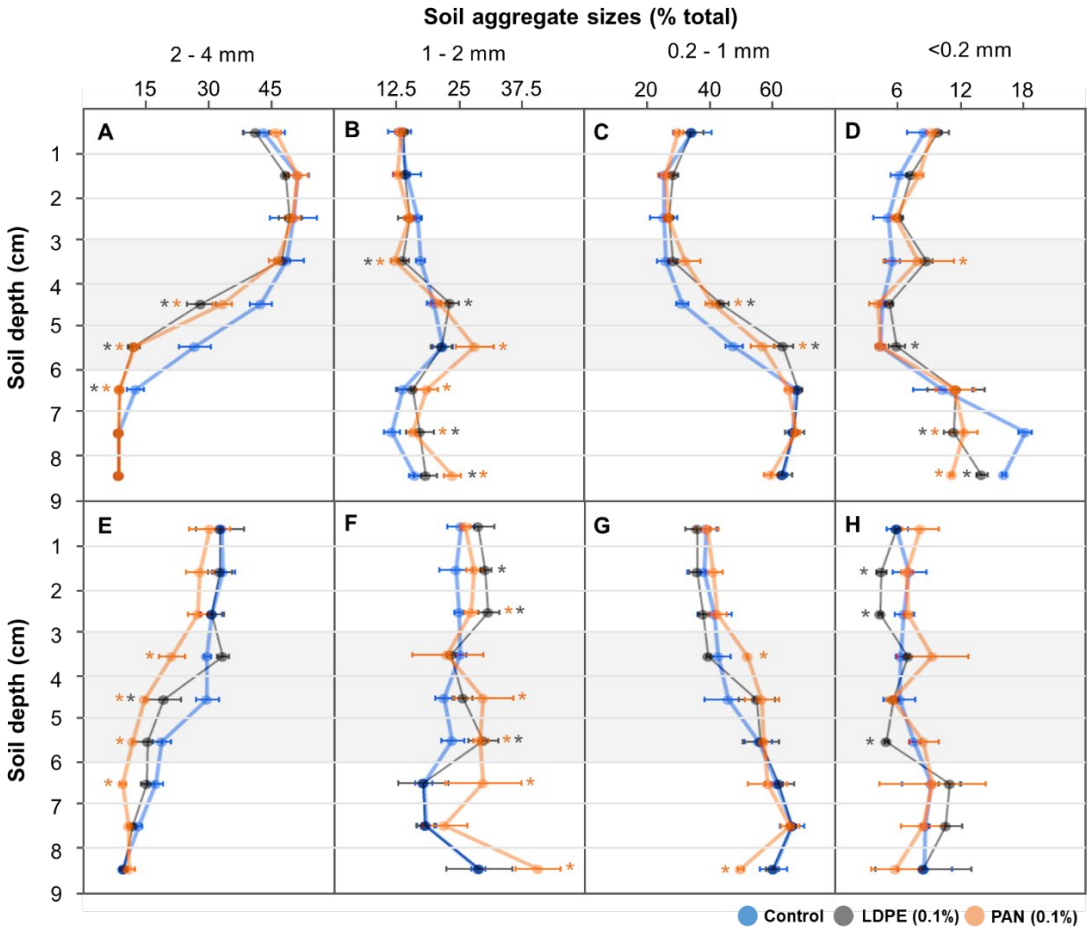

Figure 4.4 Soil aggregate size fractions at each depth after low-level irrigation.

The soil aggregate size fractions were defined as 2-4; 1-2; 0.2-1; < 0.2 mm. Each soil column was incubated for 1 day (A-D) and 60 days (E-H). Asterisk represents significance at the level of 5% (p = 0.05) between control and microplastic-containing soil layer treatments.

The relative proportion of micro- (< 0.2 mm) and larger macro-aggregates (2-4 mm) is crucial for pore size distribution (Horn & Smucker 2005), and thus directly and indirectly influence the movement of water, gas, and nutrients (Jayarathne et al., 2021). We observed that the differences in size fractions between adjacent layers were less pronounced after the 60 days incubation since the water started to slowly infiltrate into the whole soil column from the soil surface (Figures 4.2, 4). In microplastic-containing soil treatments with low-level irrigation, the significant differences in large and intermediate sized soil aggregate fractions (2-4, 1-2, and 0.2-1 mm) were still observed after 60 days of incubation (Figures 4.4E-G). With high-level irrigation, each size fraction in the soil columns after the 60 days incubation showed similar levels in control and microplastic treatments due to relatively homogenous water contents (Figures 4.5 E-G), but fluctuations were observed in small soil aggregate size fractions (< 0.2 mm) (Figure 4.5 H). Since more intense irrigation can increase the dispersion of water and the mobility of clay particles (Horn & Dexter 1989), the soil fraction in the size range of micro-aggregates seems to be influenced

by both clay contents (Schweizer et al., 2019) and microplastics (Rillig & Lehmann 2020).

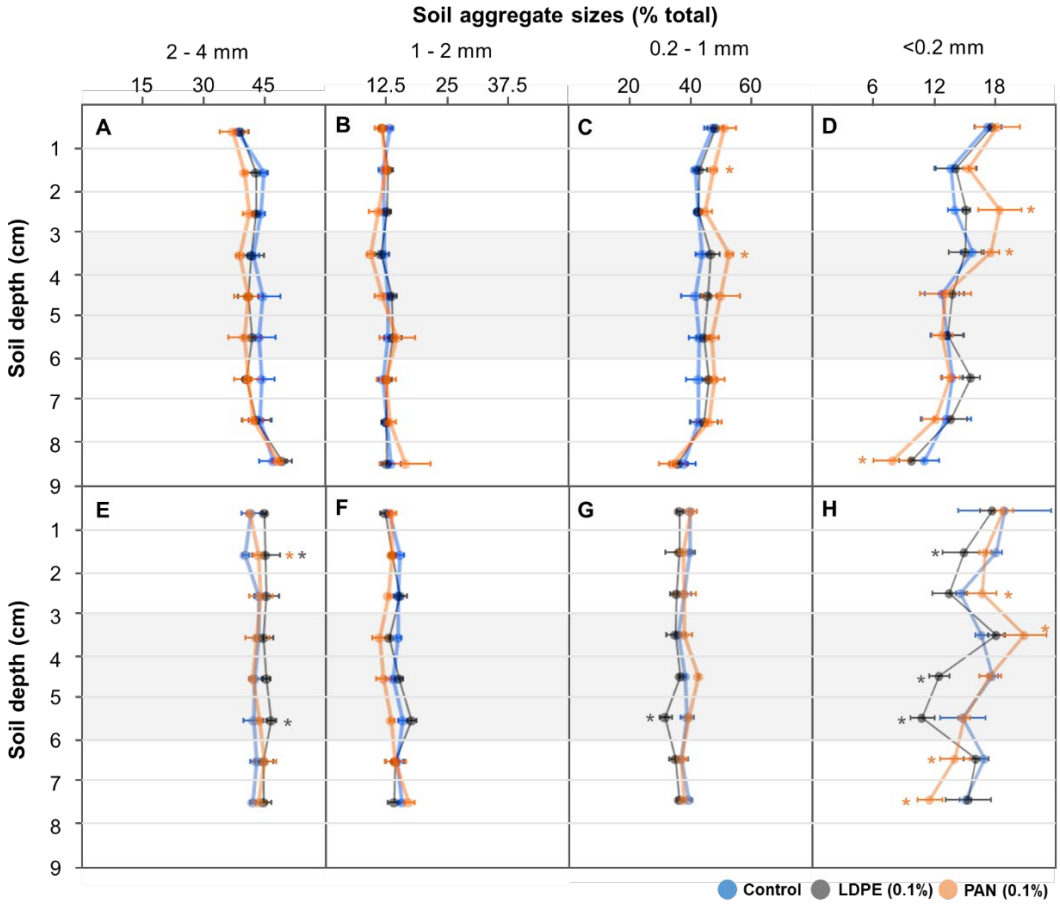

Figure 4.5 The fractions of each soil aggregate size at each depth after high-level irrigation.

The soil aggregate size fractions were defined as 2-4; 1-2; 0.2-1; < 0.2 mm. Each soil column was incubated for 1 day (A-D) and 60 days (E-H). Asterisk represents significance at the level of 5% ($p = 0.05$) between control and microplastic-containing soil layer treatments.

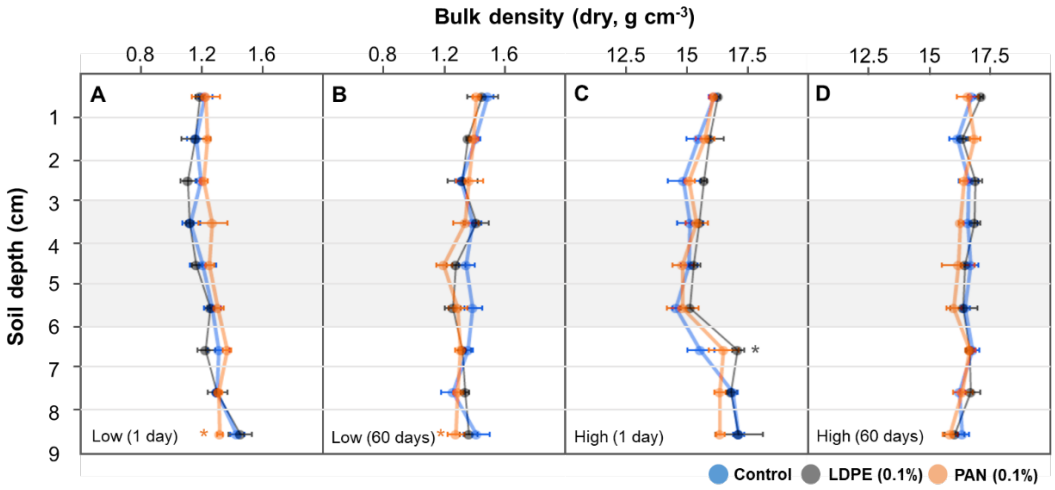

Figure 4.6 Bulk density at each soil depth of each soil column.

Each soil column was irrigated with either at a low level (A, B) or with a higher water volume (C, D), and incubated for 1 day (A, C) and 60 days (B, D). Asterisk represents significance at the level of 5% (p = 0.05) between control and microplastic-containing soil layer treatments. The unit for soil bulk density is dry, g cm^{-3}.

The effects of microplastic fibers on soil aggregation have been well established in previous studies (de Souza Machado et al., 2019; Rillig & Lehmann 2020). Aggregate water stability decreased by polyamide and polyester fibers in sandy loam soil (de Souza Machado et al., 2018; de Souza Machado et al., 2019; Lehmann et al., 2019), however, the contrary result that macro-aggregate fractions increased by polyester fibers addition in clayey soil (Zhang et al., 2019) was also observed. Films, which is one of the two microplastic shapes we use here, reduce tensile strength of soil, and desiccation shrinkage and cracking can be induced, depending on film particle size (Wan et al., 2019). In the present study, changes in each soil aggregate size fraction occurred in both microplastic-containing soil and bottom layers, and larger macro-aggregate fractions (2-4 mm) decreased while micro-aggregates (< 0.2 mm) were more variable.

Our results here show that the microplastic-containing soil layer acts as an anthropogenic barrier, disrupting water flow paths into the bottom soil layer, and the different water contents in each layer seemed to be highly linked with the changes in the soil aggregate size fraction. Regarding microplastic target concentration, the changes in soil aggregate fractions by microplastic addition were induced in the ranges of 0.1-0.4% in previous reports (de Souza Machado et al., 2018; de Souza Machado et al., 2019; Lehmann et al., 2019). In our study, the soil aggregate size fractions were influenced not only in 0.1% of LDPE films or PAN fibers containing soil layers, but also in adjacent layers. Since many previous studies have focused on the homogeneous microplastic distribution in test soil and the effects in themselves, the observed changes in non-contaminated

adjacent layers might mean that the effects of microplastics have been underestimated.

4.4.3 Effects on biological parameters

The results for soil respiration and enzyme activities are shown in Appendix Table A4.1. A part of β-galactosidase data are missing due to experimental errors during measurements. With low-level irrigation, soil respiration rates (CO_2 production) in the top layers (0-3 cm) were 5.10-5.25 ppm h^{-1}, and those of the middle layers (3-6 cm) were 3.78-4.45 ppm h^{-1}. Significant changes were observed in the bottom layer (6-9 cm), as LDPE films and PAN fibers treatments had lower respiration (2.54 ± 0.12 (LDPE films), 2.75 ± 0.36 (PAN fibers) ppm h^{-1}) than control (3.27 ± 0.45 ppm h^{-1}). With high-level irrigation, soil respiration rates increased in the bottom layers with microplastic-containing soil layers, with 6.26 ± 0.80 (control), 7.64 ± 0.79 (LDPE films), and 7.86 ± 0.38 (PAN fibers) ppm h^{-1}, respectively. Enzyme activities in the bottom layers showed no significant differences between control and microplastic-containing soil layer treatments. Although acid phosphatase in soil columns containing PAN fibers tended to have higher activity (8.90 ± 5.02 and 8.32 ± 6.06 μmol mg^{-1} h^{-1} for low- and high-level irrigations, respectively), these values were not significantly different from the control (5.40 ± 3.05 and 4.09 ± 0.35 μmol mg^{-1} h^{-1}). The activities of β-D-glucosidase in each treatment were calculated as 1.92-3.68 (for low-level irrigation) and 2.10-3.08 (for high-level irrigation) μmol mg^{-1} h^{-1}, and there were no significant differences compared with control treatment.

Broad and extensive microbial responses to microplastic exposure have been reported in many previous studies (Liu et al., 2017; Yang et al., 2018; Huang et al., 2019). LDPE films and PAN fibers, the target microplastics in this study, can affect the rate of fluorescein diacetate hydrolysis (Huang et al., 2019; Liang et al., 2019). Microplastic fibers could provide more porosity, and their effects on soil respiration and enzyme activities can depend on soil water conditions (Lozano et al., 2021). Microplastic films can strongly influence soil respiration (Ng et al., 2020), and could reduce activity of aerobic microbes by affecting soil aeration due to their planar shape (Lehmann et al., 2020b). Previous studies have suggested that changes in soil structure can be a trigger for a series of events (de Souza Machado et al., 2018; de Souza Machado et al., 2019). Changes in soil structure can influence pore spaces, which can alter water dynamics and soil aeration, and this microplastic-driven physical change is particularly linked to biological or chemical processes. In our study, microplastic-containing soil layers interrupted water flow in soil and changed soil physical structure. These differences would be directly or indirectly linked with microbial activities: water content in soil has a linear relationship with soil respiration (Cook & Orchard 2008), and soil aggregate size class is highly correlated with biological soil parameters since each size fraction has a different available organic matter content and C-N ratio (Ashman et al., 2003). We found evidence that microplastic-containing soil layers can affect a biological parameter (soil respiration) in the non-contaminated bottom layer. Despite the changes

in soil respiration in the bottom layer, these changes did not translate to overall changes in the rate of enzyme activities.

4.5 CONCLUSION

Microplastics have unique properties compared with more traditional pollutants, such as heavy metals or organic chemicals, and many previous studies have reported effects of microplastic on soil properties. We here examined that microplastic-containing soil can affect adjacent soil layers not containing microplastic. We conducted a simple soil column test taking a phenomenological approach. Our results provide crucial evidence that microplastic-containing soil layers could act as an anthropogenic barrier, leading to vertically interrupted soil water flows and changes in physical structure. These effects occurred not only in microplastic-containing soil layers, but also in adjacent layers (top and bottom). Our results imply that the indirect effects on adjacent soils might be underestimated, and soil systems can be altered by microplastic contamination in unexpected ways. While our study was intended as a proof-of-concept, it also has relevance to real world situations, for example when plastic-containing soil surface layers are flipped during certain plowing operations in agricultural systems. Overall, we argue that future research should also consider heterogeneous distribution of microplastic pollutants in ecosystems.

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Chapter 5: EFFECTS OF MICROPLASTICS AND DROUGHT ON SOIL ECOSYSTEM FUNCTIONS AND MULTIFUNCTIONALITY

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Keywords: enzymatic activities, grasslands ecosystem, litter decomposition, nutrient cycling, nutrient leaching, soil aggregation, soil pH, soil respiration

5.1 ABSTRACT

Microplastics in soils have become an important threat for terrestrial systems as they may potentially alter the geochemical/biophysical soil environment and can interact with drought. As microplastics may affect soil water content, this could exacerbate the well-known negative effects of drought on ecosystem functionality. Thus, functions including litter decomposition, soil aggregation or those related with nutrient cycling can be altered. Despite this potential interaction, we know relatively little about how microplastics, under different soil water conditions, affect ecosystem functions and multifunctionality.

To address this gap, we performed an experiment using grassland plant communities growing in microcosms. Microplastic fibers (absent, present) and soil water conditions (well-watered, drought) were applied in a fully factorial design. At harvest, we measured soil ecosystem functions related to nutrient cycling (β-glucosaminidase, β-D-cellobiosidase, phosphatase, β-glucosidase enzymes), respiration, nutrient retention, pH, litter decomposition and soil aggregation (water stable aggregates). As terrestrial systems provide these functions simultaneously, we also assessed ecosystem multifunctionality, an index that encompasses the array of ecosystem functions measured here.

We found that the interaction between microplastic fibers and drought affected ecosystem functions and multifunctionality. Drought had negatively affected nutrient cycling by decreasing enzymatic activities by up to ~39%, while microplastics increased soil aggregation by ~18%, soil pH by ~4% and nutrient retention by up to ~70% by diminishing nutrient leaching. Microplastic fibers also impacted soil enzymes, respiration and ecosystem multifunctionality, but importantly, the direction of these effects depended on soil water status. That is, under well-watered conditions, these functions decreased with microplastic fibers by up to ~34% while under drought they had similar values irrespective of the microplastic presence, or tended to increase with microplastics. Litter decomposition had a contrary pattern increasing with microplastics by ~6% under well-watered conditions while decreasing to a similar percentage under drought.

Synthesis and applications. Single ecosystem functions can be positively or negatively affected by microplastics fibers depending on soil water status. However, our results suggest that microplastic fibers may cause negative effects on ecosystem soil multifunctionality of a similar magnitude as drought. Thus, strategies to counteract this new global change factor are necessary.

5.2 INTRODUCTION

Microplastics are a group of polymer-based particles with a diameter under 5 mm (Hidalgo-Ruz et al., 2012), which occur in many shapes, and possess a high physical and chemical diversity

(Helmberger et al., 2020; Rillig, Ryo, Lehmann, et al., 2019). These particles can originate from many sources, including tire abrasion, the loss of fibers from synthetic textiles during washing or the environmental degradation of larger plastic objects (Boucher & Friot 2017). In addition, many plastics are already produced as microplastics (primary microplastics), for example, for use in the cosmetics industry (Boucher & Friot 2017). Therefore, microplastics are ubiquitous around the globe and may pollute not only oceans but also terrestrial systems through soil amendments, plastic mulching, irrigation, flooding, atmospheric input and littering or street run-off (Bläsing & Amelung 2018; Rillig, 2012; de Souza Machado et al., 2018).

Our knowledge about microplastic effects on ecosystem functions is limited (Rillig & Lehmann 2020) and potential interactive effects of microplastics with soil water availability are unknown. Among microplastics, fibers are considered one of the most abundant microplastic types in the soil (Dris et al., 2015; Zhang & Liu 2018), and due to their linear shape, size and flexibility, can potentially affect soil-water dynamics mainly through links with soil aggregation. Fiber shape, which roughly mimics that of the roots, may entangle soil particles promoting aggregation. They also might form large pores between aggregates allowing the water to enter the soil profile, and could create small pores within aggregates helping to hold the water. Likewise, the hydrophobicity of microplastic fibers (Prorokova et al., 2012) may contribute to the soil aggregation (Zheng et al., 2016) as they would serve as a binding agent. However, microplastic fibers could also decrease soil aggregation (Lozano, Lehnert, et al., 2021) by preventing microaggregates from being integrated into macroaggregates (Zhang & Liu 2018) and, as soil biota enhance soil aggregation by providing mucilages and extracellular compounds that bind particles together (Bronick & Lal 2005), the presence of fibers could reduce the stability of soil aggregates by affecting soil biota (Lehmann et al., 2019; Liang et al., 2019; de Souza Machado et al., 2019).

Therefore, microplastic fibers through their effects on soil aggregation can potentially alter soil water holding capacity and so lead to differential retention of water, thus altering soil water conditions, and potentially influencing other ecosystem functions. Indeed, microplastic fibers may promote plant growth and other processes (de Souza Machado et al., 2019), and this could alleviate drought conditions promoting plant productivity at the community level (Lozano & Rillig 2020). All this evidence suggests that drought effects on ecosystem functionality may be altered when other global change factors, such as microplastics, come into play.

This potential interaction between microplastics in the soil and drought can affect multiple ecosystem functions involved in nutrient cycling, litter decomposition and soil aggregation. However, research on how microplastics and drought affect such functions is limited. For example, nutrient cycling and energy flows are closely related to soil enzymes produced by microbes and plants (Stark et al., 2014), and enzymatic activity is highly influenced by

environmental factors such as soil pH, nutrient availability and soil water content (Paul & Clark 1989). By altering these factors, microplastics may potentially affect soil enzymatic activities. Indeed, there is evidence for microplastic influencing some enzymes, depending on the microplastic polymer type. For instance, polyamide (PA), polyester fibers (PES) and polypropylene (PP) can stimulate the activity of fluorescein diacetate hydrolase (Liu et al., 2017; de Souza Machado et al., 2019), while polyethylene (PE) and polyvinyl chloride (PVC) can show the opposite effect (Fei et al., 2020). Likewise, PP, PE and PVC can stimulate phenol oxidase, urease and acid phosphatase activities (Fei et al., 2020; Liu et al., 2017). In contrast, data on the effect that microplastic may have on key enzymes related to C, N, P-cycling (such as β-glucosidase and β-D-cellobiosidase involved in cellulose degradation, or β-glucosaminidase involved in chitin degradation) are missing or limited (as in the case of phosphatases).

Litter decomposition is also a key ecosystem function with a crucial role in carbon cycling (Schmidt et al., 2011). This process depends on many factors including soil water content, litter quality and the decomposer community (Paul & Clark 1989). Microplastics may directly affect decomposition by modifying some of these factors, or indirectly through its effects on soil aggregation (a function that is highly correlated with decomposition). So far, empirical evidence of the effect of microplastics on litter decomposition is sparse (Barreto et al., 2020; Lehmann et al., 2020), and we know even less about how decomposition might be affected under different water regimes (e.g., well-watered, drought conditions).

The trends summarized above not only illustrate the scarce knowledge on the effects of microplastic on terrestrial ecosystem functions but also highlight the potential link between microplastics and drought, as the addition of microplastics may exacerbate the magnitude of the drought effects and its direction (positive or negative depending on the function measured). In addition, the net effect of each ecosystem function can alter the overall functioning of the soil. Given this heterogeneity of effects, and that ecosystem functioning is inherently multidimensional, addressing how microplastics influence multifunctionality (defined as the ability of an ecosystem to deliver multiple functions simultaneously (Hector & Bagchi 2007), could generate an integrative understanding of the terrestrial systems response to this global change factor.

Thus, in this study, we determined the potential interactive effects that microplastics and drought have on ecosystem functions linked to nutrient cycling, litter decomposition and soil aggregation. To do that, we established microcosms of plant communities, on which we measured the effect of microplastic fiber addition and a drought treatment in a factorial design on different ecosystem functions such as nutrient cycling (e.g., soil enzymatic activities, respiration, nutrients and soil pH), soil aggregation and litter decomposition (Giling et al., 2019) and on ecosystem multifunctionality. We expected that microplastic fibers would affect single ecosystem functions

and ecosystem multifunctionality in a positive or negative way depending on soil water conditions.

5.3 MATERIALS AND METHODS

5.3.1 Microplastics and soil preparation

In Dedelow, Brandenburg, Germany (53°37′N, 13°77′W), we collected dry sandy loam soil from grasslands communities (0.07% N, 0.77% C, pH 6.66). Soil was sieved (4 mm mesh size), homogenized and mixed with microplastic fibers at a concentration of 0.4% w/w (0.4 g of microplastic fibers for each 100 g of dry soil). This concentration aimed to simulate higher levels of microplastic pollution (Scheurer & Bigalke, 2018; Xu et al., 2020; Zhu et al., 2019), while in soils of strongly polluted areas, a microplastic concentration up to ~7% (w/w) was observed (Fuller & Gautam, 2016). To do so, we manually cut with scissors polyester fibers (Rope Paraloc Mamutec polyester white, item number, 8442172, Hornbach.de) to generate microplastic fibers that had a length of 1.28 ± 0.03 mm and a diameter of 0.030 ± 0.0008 mm. Polyester fibers are made to at least 80% of polyethylene terephthalate (PET; Council Directive, 2011). See details in Table 5.1 about polyester fibers properties. Twelve grams of microplastic fibers (~763,333 fibers g^{-1} microplastic) was mixed into 3 kg of soil for each pot (16 cm diameter, 16.5 cm height, 3,000 ml). For each experimental unit, fibers were separated manually and mixed with the soil in a large container before placing into each individual pot, to help provide a homogeneous distribution of microplastic fibers throughout the soil and the intended concentration. Twenty experimental units (pots) were established. Half had soil with microplastic fibers, while the other half had soil without added microplastic fibers. Soil was mixed in all experimental units in order to provide the same level of disturbance.

5.3.2 Experimental setup

In May 2019, we established the experiment in a temperature-controlled glasshouse with a daylight period set at 12 hr, 50 klx, a temperature regime at 22/18°C day/night and a relative humidity of ~40%. We selected seven grassland plant species (*Festuca brevipila*, *Holcus lanatus*, *Calamagrostis epigejos*, *Achillea millefolium, Hieracium pilosella*, *Plantago lanceolata* and *Potentilla argentea*) frequently co-occurring in Central Europe. Seeds were obtained from a commercial supplier in the Brandenburg region (Rieger-Hofmann GmbH) in order to shape a plant community consisting of three individuals per species. We will refer to plant species by their generic names from now on. For additional details, see Lozano and Rillig (2020).

Pots were well watered (100 ml twice a week) during the first 3 weeks of growth. Then, half of them were kept at ~70% of soil water holding capacity (WHC) by adding 200 ml of water, while the other half were kept at ~30% WHC by adding 50 ml of water. Pots were watered from the top twice a week for 2 months with distilled water. We thus had a design that includes two

microplastic fiber treatments (with and without added microplastic fibers, also called 'present' and 'absent') and two drought treatments (with and without drought, also called 'drought' and 'well-watered'), with five replicates each (n = 5). Pots were randomly distributed in the chamber and their position was shifted twice to homogenize environmental conditions during the experiment.

We measured 11 variables that capture aspects of nutrient cycling (β-glucosidase, β-glucosaminidase, β-D-cellobiosidase, phosphatase, soil respiration, leaching of NO_3^-, SO_4^{2-}, PO_4^{3-}), decomposition (litter decomposition), soil aggregation (water stable aggregates) and soil pH, functions thereafter. At harvest, pots were watered to saturate the soil to roughly 10% beyond the water holding capacity to induce leaching; then soil samples for enzymes and respiration measurements, and litter bags used for litter decomposition were collected. Finally, soil was dried at ~22°C for 1 month and a sample for water stable aggregates measurement was obtained.

5.3.3 Measurement of soil ecosystem functions

We measured the functions related to soil nutrient cycling by fluorometry as described in Bell et al. (2013). Soil respiration was determined via an infrared gas analyzer, while litter decomposition was measured by using a composite sample that reflected the proportion of plant biomass of each plant species in the field. Water stable soil aggregates, a proxy of soil aggregation, were measured following a modified version of the method of Kemper and Rosenau (1986). Soil nutrients were analyzed using ion chromatography (Dionex ICS-1100, AS9-HC, Thermo Scientific) while soil pH was determined with a Hanna pH meter (Hanna Instruments GmbH). For additional details, see Appendix 5.1.

5.3.4 Assessing ecosystem multifunctionality

To calculate ecosystem multifunctionality, we followed the ecosystem function multifunctionality method proposed by Manning et al. (2018). Briefly, four clusters were identified for the 12 ecosystem functions, and ecosystem multifunctionality was calculated by using the threshold approach. See details in Appendix 5.2.

5.3.5 Statistical analyses

The experimental design was a fully crossed orthogonal design where microplastic fibers, drought and the interaction were considered fixed factors. Each function was analyzed using linear models. Model residuals were checked to validate normality and variance homogeneity assumptions. We implemented the 'varIdent' function to account for heterogeneity in the microplastic fiber treatment for β-D-cellobiosidase, soil aggregation and in the water treatment for soil respiration. The effect of microplastics and drought on the ecosystem multifunctionality index was analyzed using generalized linear models with a quasibinomial distribution and a logit

link function to avoid overdispersion. We also assessed the contribution of each function to multifunctionality by using the down weighting data after clustering and the metric 'pmvd' from the package RELAIMPO (Grömping, 2006). Statistical analyses were done with R version 3.5.3 (R Core Team, 2019).

5.4 RESULTS

Ecosystem functions were affected by microplastic fibers, drought and their interaction (Table 5.1). While enzymatic activities and soil respiration were on average higher under well-watered than under drought conditions, these trends changed in the presence of microplastics, decreasing under well-watered conditions but increasing under drought. As for enzymatic activity, β-glucosaminidase decreased by ~35% with drought and was not affected by microplastic fibers (Table 5.1; Figure 5.1). β-D-cellobiosidase decreased by ~39% with drought (p = 0.02), while soil respiration was marginally affected by microplastic fibers and drought (p = 0.1). Phosphatase and β-glucosidase were affected by the interaction between microplastic fibers and drought (p = 0.03, p = 0.1 respectively). Both decreased with microplastic fibers in soil by 27% and 17% under well-watered while increasing by 75% and 40% under drought conditions respectively (Table 5.1; Figure 5.1). By contrast, litter decomposition increased with microplastic fibers by 6.4% under well-watered conditions while decreasing by 6.6% under drought conditions (p = 0.09, Figure 5.1). Likewise, soil aggregation increased with microplastic fibers under both well-watered and drought conditions by 15% and 21.7% respectively (p = 0.07). Overall, soil leachate nutrients increased with drought and decreased with microplastic fibers in the soil. Specifically, leachate NO_3^- decreased by 70% with microplastic fibers under drought conditions (p = 0.01, Figure 5.1), a similar trend was found under watered conditions. Leachate SO_4^{2-} decreased with microplastic fibers under either well-watered or drought conditions by 52% and 37% respectively (p = 0.01). PO_4^{3-} in leachate was not clearly affected by drought or microplastic fibers, while soil pH increased both with drought and microplastic fibers in the soil (p < 0.01, Figure 5.1).

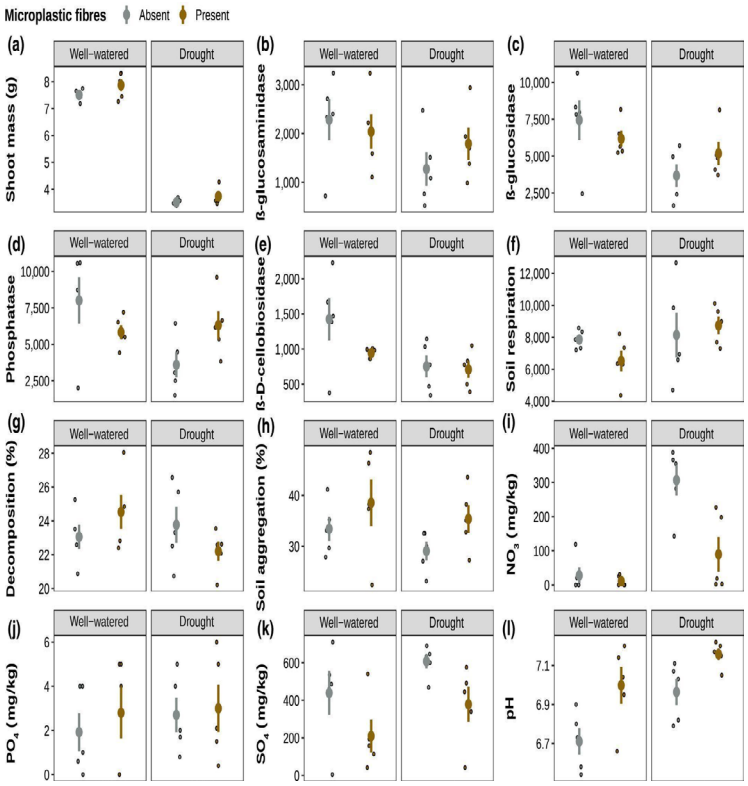

Figure 5.1 Microplastic fibers and drought effects on 12 ecosystem functions.

(a) shoot mass, (b) β-glucosaminidase, (c) β-glucosidase, (d) phosphatase, (e) β-D-cellobiosidase, (f) soil respiration, (g) litter decomposition, (h) soil aggregation, (i) NO_3^-, (j) PO_4^{3-}, (k) SO_4^{2-}, (l) soil pH. Mean and standard error are represented. Data points are shown as circles. Enzymes and soil respiration units (μmol g^{-1} dry soil hr^{-1}, ppm). p values in Table 5.1; n = 5.

Ecosystem multifunctionality was affected by the interaction between microplastic fibers and drought (Table 5.1; Figure 5.2). That is, the effect of microplastics on ecosystem multifunctionality strongly depended on the drought treatment (p = 0.01), a treatment that alone tended to decrease ecosystem multifunctionality (p = 0.10). Regarding the interaction, under well-watered conditions, microplastic fibers addition to the soil decreased multifunctionality, while under drought conditions, microplastic addition did not affect multifunctionality (Figure 5.2). Different thresholds when calculating multifunctionality showed similar trends (Appendix Figure A5.2, see Appendix Table A5.1 for statistical results). The analysis of the relative importance of each function showed that β-glucosidase (31.87%), soil respiration (25.65%), phosphatase (11.14%), pH (9.16%), SO_4^{2-} (8.84%), β-D-cellobiosidase (3.03%), β-glucosaminidase (2.88%), shoot mass (1.88%), PO_4^{3-} (1.67%), soil aggregation (1.63%), litter decomposition (1.56%), NO_3^- (0.62%) contributed in this order to multifunctionality (R^2 = 91.53%, Figure 5.3).

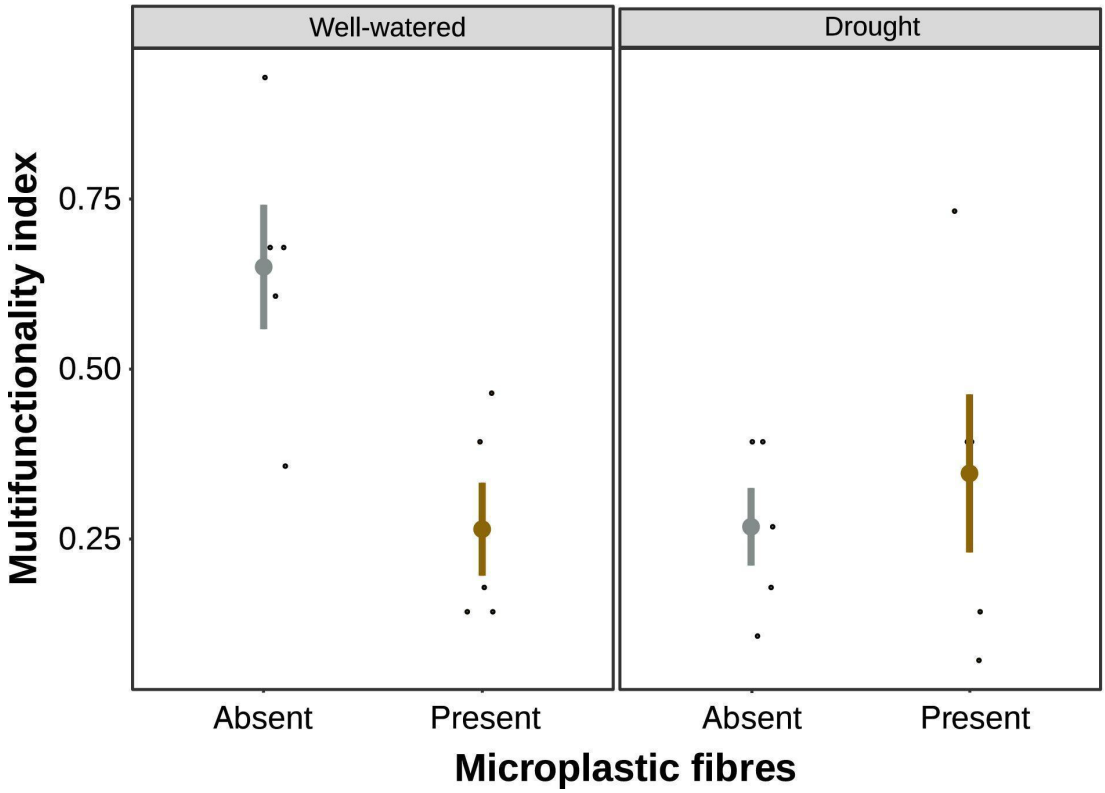

Figure 5.2 Microplastic fibers and drought effects on soil ecosystem multifunctionality.

Mean and standard error are represented. Multifunctionality was calculated based on the threshold approach in which each function that exceeds 70% of the standardized maximum contributes to the multifunctionality score. Data points are shown as circles; *p* values in Table 5.1; n = 5.

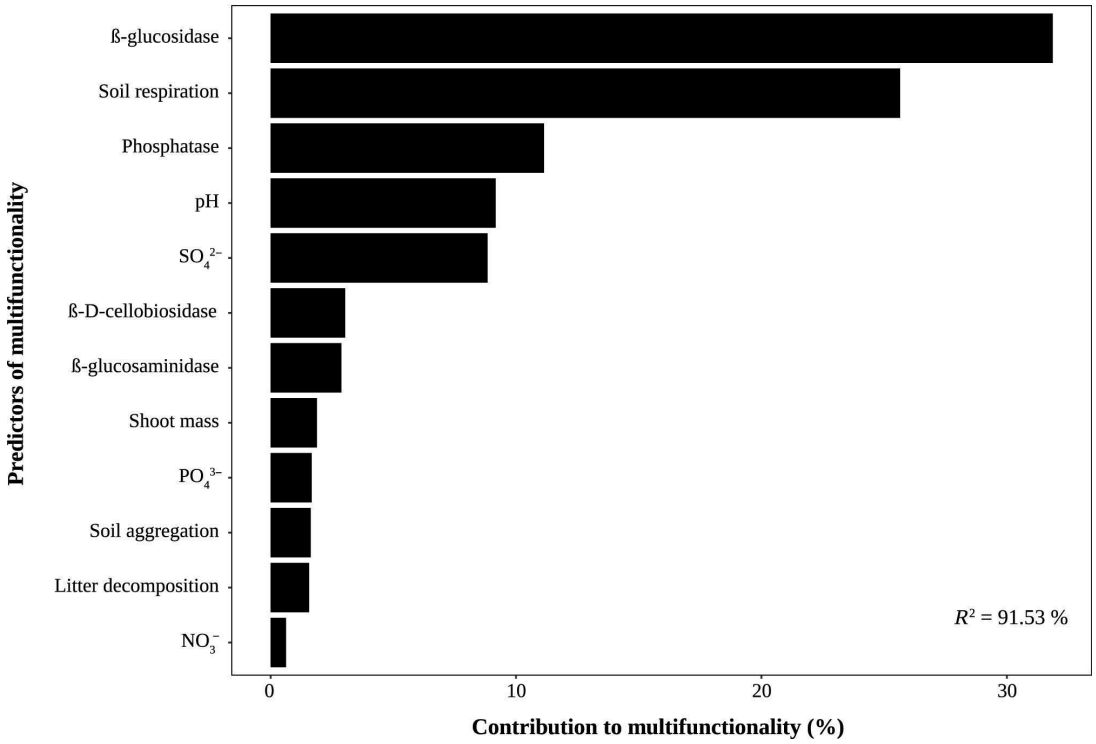

Figure 5.3 Relative importance of each predictor to multifunctionality.

The proportionate contribution of each function considered both its direct effect (i.e., its correlation with multifunctionality) and its effect when combined with the other variables in the regression equation. The metrics 'pmvd' was used for the calculation and the down-weighting via the cluster was taken into account.

Table 5.1 Results from linear models on 11 ecosystems functions and multifunctionality response to microplastic fibers (M), drought (D) and their interaction (M × D).

Multifunctionality also included shoot mass (data extracted from Lozano & Rillig 2020). Degrees of freedom of each factor (df = 1). F values and *p* values (in parentheses) are shown; $p < 0.1$ in bold; n = 5.

Ecosystem functions	**Microplastic fibers (M)**	**Drought (D)**	**M × D**
β-glucosaminidase	0.14 (0.70)	2.98 (0.10)	1.08 (0.31)
β-glucosidase	0.02 (0.89)	**6.88 (0.01)**	2.31 (0.14)
Phosphatase	0.07 (0.79)	**3.55 (0.07)**	**5.53 (0.03)**
β-D-cellobiosidase	2.14 (0.16)	**6.32 (0.02)**	1.49 (0.23)
Soil respiration	2.49 (0.13)	2.29 (0.14)	1.37 (0.25)
Litter decomposition	0.002 (0.95)	0.88 (0.36)	**3.13 (0.09)**
Soil aggregation	**3.54 (0.07)**	2.51 (0.13)	0.03 (0.84)
NO_3^-	**10.66 (0.004)**	**24.93 (0.0001)**	**7.85 (0.01)**
PO_4^{3-}	0.36 (0.55)	0.25 (0.62)	0.08 (0.77)
SO_4^{2-}	**6.75 (0.01)**	**3.66 (0.07)**	0.00 (0.99)
pH	**12.38 (0.002)**	**9.14 (0.008)**	0.47 (0.50)
Multifunctionality	3.16 (0.09)	3.02 (0.10)	**7.23 (0.01)**

5.5 DISCUSSION

As hypothesized, microplastic fibers and drought affected ecosystem functions linked with soil aggregation, nutrient cycling and decomposition as well as ecosystem multifunctionality. Overall, drought had a negative impact on ecosystem functions, while the impact of microplastic fibers depended on the soil water status and the function considered. Below, we discuss likely mechanisms behind these complex outcomes.

5.5.1 Soil aggregation increased with microplastic fibers irrespective of drought

Microplastic fibers promoted soil aggregation either under well-watered or drought conditions, likely due to positive effects of fibers on soil bulk density, aeration and water retention (de Souza Machado et al., 2019), which may promote root growth (Lozano & Rillig 2020) and hyphal extension (Elliot & Coleman 1988; Wang et al., 2017). Therefore, roots, hyphae and microplastic fibers might together have helped entangle soil particles, promoting soil aggregation. In addition, microplastic fibers are generally hydrophobic (Prorokova et al., 2012), a property that is positively correlated with soil aggregation (Zheng et al., 2016). As soil aggregation may help hold water, thus enhancing soil microbial activity, the provision of extracellular compounds that help to bind soil particles could have been promoted (Bronick & Lal 2005), which in turn may also have contributed to the observed soil aggregation response.

5.5.2 Microplastic fibers reduced soil enzyme activity and soil respiration only under well-watered conditions

We observed that microplastic fibers affected potential enzymatic activities and soil respiration depending on soil water conditions. That is, under drought, enzymes and soil respiration increased when microplastic fibers were added, probably because soil water content and aeration (Rillig, Lehmann, de Souza Machado, et al., 2019; Rillig, Lehmann, Ryo, et al., 2019; de Souza Machado et al., 2019), increase with microplastic fibers which in turn may promote microbial activity (Alster et al., 2013; Nannipieri et al., 2002; Sanaullah et al., 2011). By contrast, under well-watered conditions, enzymes and soil respiration decreased with microplastic fibers in the soil, probably linked with a decline in soil microbial community richness and diversity as seen by Fei et al. (2020). Changes in soil porosity and soil water content with microplastic fibers may alter the flow of oxygen in the soil, with consequences on the relative distribution of anaerobic and aerobic microorganisms (Rubol et al., 2013). Alterations in pore space may also lead to their habitat loss. Likewise, as microplastic fibers may potentially release harmful contaminants into the soil in the form of additives (Kim et al., 2020) or organic pollutants associated with fiber manufacturing (Hermabessiere et al., 2017), specific microorganisms could have been affected by these new environmental conditions (Rillig, de Souza Machado, et al., 2019).

5.5.3 Microplastic fibers increase litter decomposition only under well-watered conditions

Litter decomposition increased under well-watered Conditions when microplastic fibers were added. Our results suggest that the increase in litter decomposition may be related to an increase in soil aggregation. Soil aggregation promotes oxygen diffusion within larger soil pores and regulates water flow, which in turn stimulates microbial activity (Six et al., 2004) promoting litter decomposition. In addition, soil pH, a parameter influenced by soil aggregation (Jiang et al., 2013), that affects soil microbial community structure (Fierer & Jackson 2006) could also have played a role. In fact, recent research found that an increase in litter decomposition was linked with better soil aggregation (Yang et al., 2019). By contrast, the combined effect of drought and microplastic in soil decreased litter decomposition, which can be related to a decrease in microbial activity as water becomes limiting (Six et al., 2004). Our results suggest that microplastics in interaction with drought may have large consequences for ecosystem C stocks and fluxes, as changes in litter decomposition may influence the feedback to the atmosphere from terrestrial ecosystems.

5.5.4 Microplastic fibers reduced soil nutrient leaching

Nutrient leaching, after a simulated rain event, increased under drought but decreased when microplastic fibers were added to the soil. Drought conditions might have led to the formation of cracks as preferential flow paths in the soil, increasing the leaching of nutrients when the soils were rewetted. In support of this, in fertilized soils, the leachate NO_3^- was threefold higher under drought than under non-drought conditions (Klaus et al., 2020). Nutrient leaching is also known to be related to change in the structure of plant and microbial communities (Mueller et al., 2013), biotic factors that are indeed affected by drought (Fitzpatrick et al., 2018; Lozano, et al., 2020). Likewise, we observed that leachate PO_4^{3-} was not affected by drought, most likely because phosphates are more strongly bound to soil particles than nitrate or sulfate (Paul & Clark, 1989). By contrast, nutrient leaching decreased with microplastic fibers (i.e., more nutrient retention). This can be related to the positive effect that microplastic fibers had on soil aggregation, which may have increased the soil capacity to retain nutrients. This positive relation between soil nutrients retention and soil aggregation has been reported by Liu et al. (2019).

5.5.5 Microplastic fibers and drought effects on ecosystem multifunctionality and ecosystem services

Our results showed that microplastic fibers and drought impacted not only single functions but also multifunctionality, and that such impact depended on the interaction between these two global change factors. Specifically, with the addition of microplastic fibers, ecosystem multifunctionality decreased under well-watered

conditions, while it was maintained at similar levels under drought conditions. This trend mirrors the one observed for nutrient cycling functions (i.e., β-glucosidase, soil respiration), as they are the ones that contribute most to multifunctionality. This highlights the importance of considering nutrient cycling functions when managing microplastics in soils. Drought and microplastic fibers under well-watered conditions had similar negative effects on ecosystem multifunctionality, suggesting that microplastics in soils may negatively impact ecosystem functionality as much as drought.

Microplastic effects on ecosystem functions and multifunctionality can be related with their shape (Lozano, Lehnert, et al., 2021; Rillig, Ryo, Lehmann, et al., 2019) and very likely with the leaching of additives to the soil matrix. Indeed, recent research showed that microplastic fibers of polyacrylonitrile may cause toxicity in the soil inducing negative effects on soil biota due to their extractable additives (Kim et al., 2020). Polyester fibers contain different water soluble hazardous additives (Appendix Table A5.1), which can potentially be released into the soil, affecting soil biota communities and therefore ecosystem functionality.

Our results showed that two global change factors (i.e., microplastics and drought) influence ecosystem functions and multifunctionality, which in turn may affect ecosystem services (Díaz et al., 2018; Manning et al., 2018) and thus impact various aspects of human well-being. In the short term, microplastic fibers may contribute to plant productivity or soil aggregation; however, we do not currently know what the long-term responses will be, as additional factors could come into play. Indeed, microplastic fibers may release harmful chemical substances into the soil (Fred-Ahmadu et al., 2020a) and affect nutrient cycling processes, with consequences for soil quality, and thus on the provision of different services, such as food and water (MEA, 2005). This becomes relevant as agricultural lands are often managed with sewage sludge or compost, which contains a large amount of microplastic fibers (Wang et al., 2019; Weithmann et al., 2018). Indeed, it has been estimated that between 125 and 850 tons of microplastics per million inhabitants are added annually to European agricultural soils through the application of sewage sludge (Nizzetto et al., 2016), whose concentrations in the soil can range from 1,500 to 56,400 particles kg^{-1} (Zhu et al., 2019). Few studies describe the degree of microplastic pollution in terms of mass concentration (weight of microplastic per kilogram of soil) (Xu et al., 2020; Zhu et al., 2019), which would allow the comparison with other microplastic types and in different soil environments. Nonetheless, microplastics in soil can be found in a wide range of concentrations including the one we used in the study. For example, in floodplain and agricultural soils, both low (~0.0055%-0.00129%) and medium (0.022%-0.03%) levels of microplastics concentration have been reported (Scheurer & Bigalke, 2018; Xu et al., 2020; Zhu et al., 2019), while high levels of microplastic

concentration (~7%) can be found in industrial soils (Fuller & Gautam 2016). Likewise, it is not necessarily the current levels of microplastic contamination that we should be most concerned about, but future levels—just like is the case for other factors of global change. Our results showed that relatively high levels of microplastic concentration in soil (i.e., 0.4%), as may occur in the future more widely if plastic use is not curtailed, may affect different soil ecosystem functions and multifunctionality.

As soils are increasingly polluted with microplastics worldwide, it is becoming more necessary to understand how the properties of this material (including shape and polymer type) interact with other global change factors such as drought. This experiment conducted in microcosms suggests that microplastic fibers in soil may cause effects on ecosystem multifunctionality of a size comparable to drought. Further research under field conditions has to be performed in order to test the applicability of these results. Our findings also highlight the potential of microplastic to affect Earth system feedbacks of terrestrial ecosystems, especially via observed changes in litter decomposition, respiration fluxes and soil aggregation.

5.6 ACKNOWLEDGEMENTS

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Chapter 6: GENERAL DISCUSSION

6.1 DISCUSSION

Microplastics as an emerging global change factor have been studied as a single factor, and most studies only take into account their direct effects on soil ecosystem functions, which may not be the real pictures, as we may overlook the indirect effects. In addition, terrestrial ecosystems are mostly exposed to multiple global change factors acting simultaneously and not just to the effect of a single factor. Hence, this doctoral work aimed to explore the direct and indirect effects of microplastics on soil properties and microbial activities, and the isolated (or direct) vs combined effects of microplastics with drought, another global change factor, on soil properties and microbial activities.

6.1.1 The effects of microplastics on soil pH

Our results showed that the direct effects of microplastic fibers (in bare soil) on soil pH were negative, while the effect was positive with the presence of plants; as for the combined effects of microplastics with drought were slightly positive (Figure 6.1).

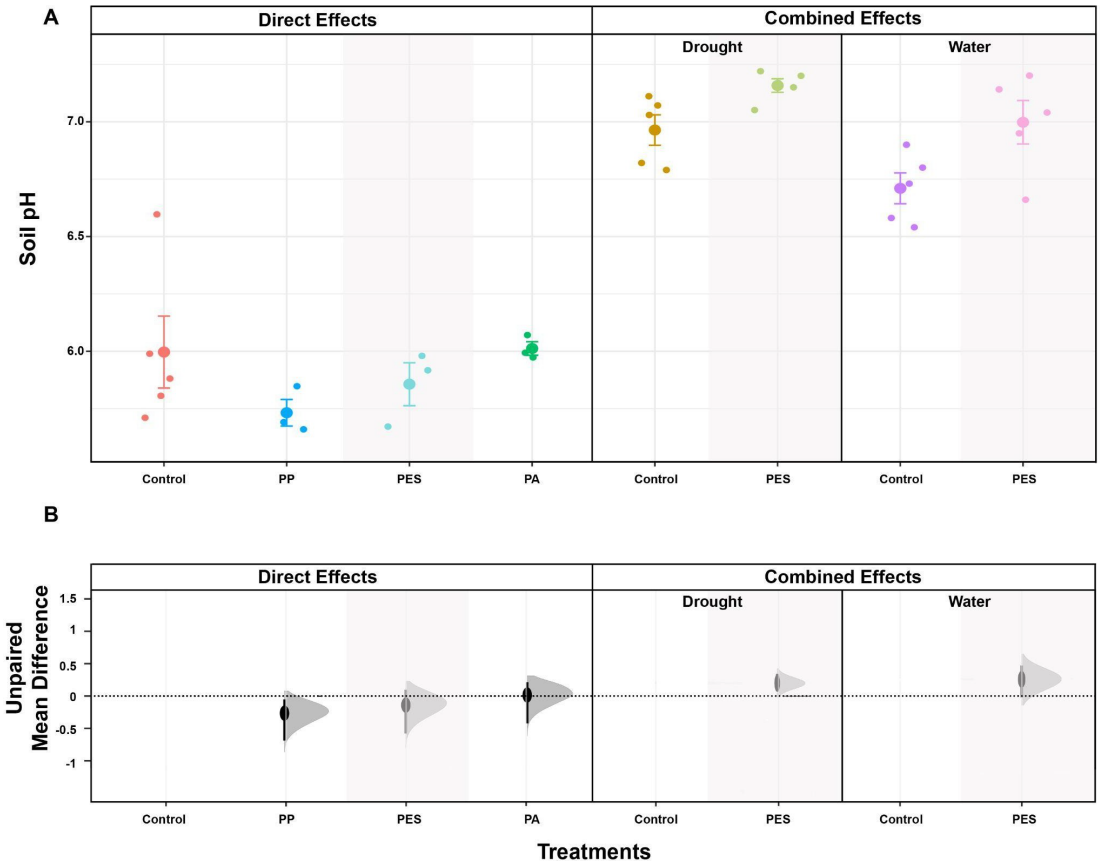

Figure 6.1 Microplastic fibers effects on soil pH.

Direct and combined effects of microplastics on soil pH (A). Mean and standard errors are shown. Effects sizes (B)

are displayed as mean, and 95% confidence intervals; the horizontal dotted line indicates the mean difference value between each treatment and control. Control: soil treated without microplastics; polymers: PA (polyamide), PES (polyester), PP (polypropylene). This figure integrates data from chapters 3 and 5. Note that the direct effects did not include a plant in the system, contrary to the combined effects.

The direct effects of microplastic fibers on soil pH varied with polymer types. Microplastics of different polymer types displayed various effects on soil pH as observed elsewhere (Boots et al., 2019; Blöcker et al., 2020; Qi et al., 2020; Wang et al., 2020b; Chen et al., 2022; Feng et al., 2022; Inubushi et al., 2022; Li et al., 2022a; Medynska-Juraszek & Jadhav 2022); this may result from the fact that soil biota could be affected by the chemical compounds leached from microplastics (Kim et al., 2020; Waldman & Rillig 2020). For example, fabric and laminate plastics decreased soil pH, which might be due to the release of organic acids into the soil (Inubushi et al., 2022).

The direct effects of polyester (PES) microplastic fibers on soil pH showed different patterns when plants were included. That is, without plants, the PES fibers decreased soil pH; while when plants were present PES fibers increased soil pH. As we used the same soil and microplastic fibers in these two experiments, our results suggest that the presence of plant species may alter the effects of microplastics on soil pH. This, as without plants, PES fibers may increase soil porosity which could promote the denitrification process, a process that will consume NH_4^+, thus lowering soil pH (Nye 1981); whereas, with plants, on the one side, root exudates can affect nitrification, a process that consumes NO_3^- (Maurer et al., 2021); while on the other side, plant roots can absorb NO_3^- causing an increase in soil pH (Nye 1981).

Indeed, a recent study showed that the presence of plants weakened the negative effects of microplastic on soil pH (Gharahi & Zamani-Ahmadmahmoodi 2022), but another research observed that plants might not modify microplastic effects on soil pH (Boots et al., 2019). Such different results may be linked to the differences in soil types which could also impact microplastic effects on soil pH (Blöcker et al., 2020; Chen et al., 2022); for example, microplastics decrease pH in acid soil, while increasing pH in alkaline soil (Li et al., 2021c). This may explain why PE microplastics could either increase or decrease soil pH (Gao et al., 2021; Palansooriya et al., 2022). In addition, the organic matter content in the soil may also play a role, due to the retention of major cations and acid buffering (Jiang et al., 2018). Indeed, applying organic fertilizers mitigate the microplastic effects on soil pH (Li et al., 2021c; Chen et al., 2022). Furthermore, a recent study showed that microplastic effects on soil pH could be also affected by plant growth stages or fertilization periods (Chen et al., 2022).

With the presence of plants, the combined effects of PES microplastic fibers with drought increased soil pH, less pronounced than under well-watered condition. This may be ascribed to the root biomass being higher under well-watered than under drought conditions (Lozano & Rillig 2020), thus more NO_3^- could be adsorbed on the roots (Nye 1981). Similarly,

polyethylene (PE) microplastic addition contributed to reductions in soil pH differently under drought (30% water holding capacity, WHC) and well-watered conditions (70% WHC) (Dissanayake et al., 2022).

As discussed, some mechanisms why microplastics affect soil pH have been reported. For example, microplastics may have developed slightly negatively charged surfaces, impacting soil cation exchange capacity, and ultimately changing soil pH (Boots et al., 2019; Blöcker et al., 2020; Li et al., 2021c; Palansooriya et al., 2022). Chemicals released from microplastic such as organic acid might also cause alteration in soil pH (Inubushi et al., 2022). Likewise, microplastics could impact nitrification and denitrification processes (Ren et al., 2020; Seeley et al., 2020; Feng et al., 2022; Lee et al., 2022; Wang et al., 2022b). However, further research on this topic is still needed.

6.1.2 The effects of microplastics on soil respiration

Our results showed that the direct effects of microplastic fibers and films on soil respiration were negative irrespective of the presence of plants; the indirect effects were neutral, while the combined effects with drought were slightly positive (Figure 6.2).

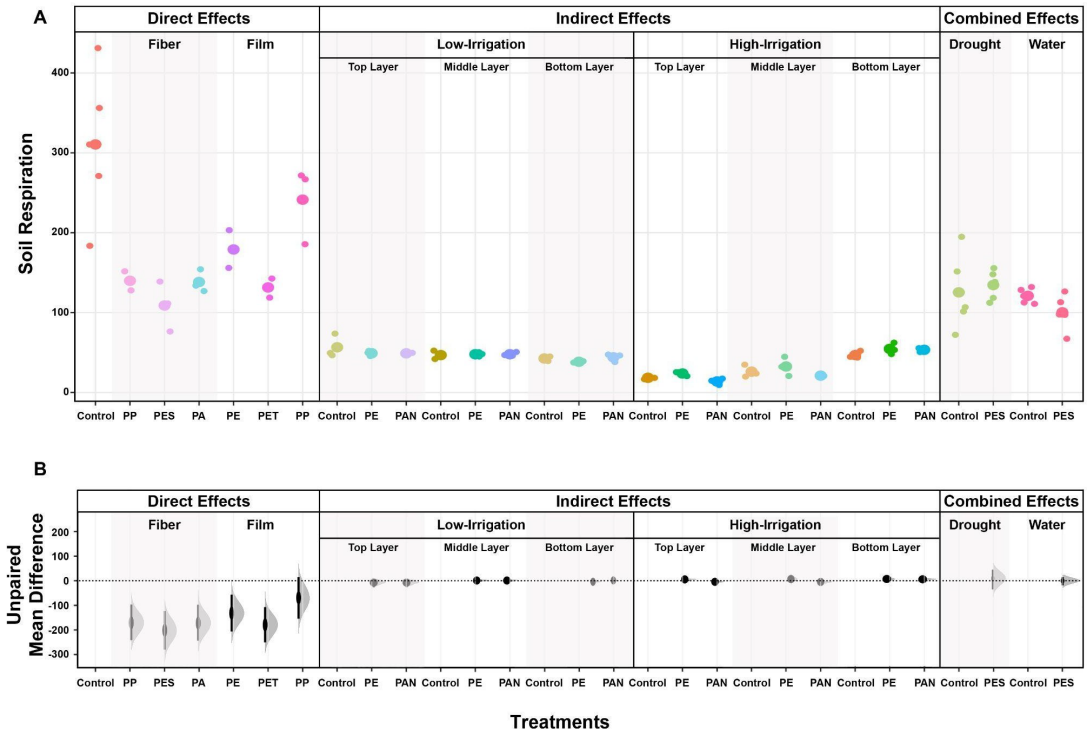

Figure 6.2 Microplastic fibers and films effects on soil respiration.

Soil respiration was measured as CO_2 unit (ppm hr^{-1}). Direct, indirect and combined effects of microplastics on soil respiration (A). Mean and standard errors are shown. Effects sizes (B) are displayed as mean, and 95% confidence intervals; the horizontal dotted line indicates the mean difference value between each treatment and control. Control: soil treated without microplastics; polymers: PA (polyamide), PAN (polyacrylonitrile), PE

(polyethylene), PES (polyester), PET (polyethylene terephthalate), PP (polypropylene). This figure integrates data from chapters 3, 4 and 5. Note that the direct effects did not include a plant in the system, contrary to the combined effects.

Under well-watered conditions, the direct effects of microplastics on soil respiration depended on microplastic shape, and polymer type. Microplastic fibers rather than films contributed to lower soil respiration. This may be because microplastic films could increase aeration (Lehmann et al., 2021), and microplastic fibers could reduce oxygen diffusion by blocking the soil pore spaces (Yu et al., 2020a); therefore, the soil treated with microplastic films displayed higher soil respiration than the soil with microplastic fibers. Another reason may be that microplastic fibers have larger surface area than microplastic films (Yi et al., 2020), consequently adsorbing more fine soil particles and soil organic particles (Guo et al., 2021), declining to a greater extent soil nutrient availability (Yu et al., 2020a; Gao et al., 2022; Li et al., 2022b; Xiao et al., 2022; Zhang et al., 2022b), with consequences on microbial activities, growth and biomass (Gao et al., 2021), and finally affecting soil microbial respiration. Furthermore, there may be competition between soil microbes and microplastics for physicochemical niches, and microplastic fibers may outcompete soil microbes due to their larger surface area (Yu et al., 2020a), thus inhibiting microbial activities (Li et al., 2022b), and potentially suppressing the enzyme activities involved in C cycling (Yu et al., 2020a).

Microplastic effects on soil respiration vary with microplastic polymer types (Blöcker et al., 2020; Lozano et al., 2021; Gharahi & Zamani-Ahmadmahmoodi 2022; Inubushi et al., 2022), which may be ascribed to the toxic chemicals released into the soil that are harmful to soil microbes (Kim et al., 2020; Li et al., 2022b). In addition, PES fibers reduced soil respiration more than PP and PA fibers, likely as PP leachates can be highed in organic carbon (Romera-Castillo et al., 2018) and as the nitrogen contained in PA fibers can be utilized by soil microbes (de Souza Machado et al., 2019). Another reason may be that changes in soil pH due to microplastics could affect the decompositions of soil organic matter (Gao et al., 2021; Hou et al., 2021; Inubushi et al., 2022; Li et al., 2022b; Zhang et al., 2022b), thus impacting soil respiration. Specifically, under well-watered conditions, PES fibers inhibited soil pH in bare soil more than when plants were present. This may be linked to plants mitigating microplastic effects on soil pH as discussed above, thus weakening the effects on soil respiration. Another reason may be that root exudates could enhance soil respiration (Adamczyk et al., 2021), alleviating the negative effects of PES fibers.

The indirect effects indicated that under low-water irrigation, the respiration in each soil layer were similar; this as the low level of soil moisture may limit soil microbial activities (Stark & Firestone 1995). Under high-water irrigation, PE films slightly increased soil respiration, whereas the PAN fibers reduced it. This may be due to the shape differences causing different impacts on soil aeration and oxygen diffusion as discussed above; or due to the toxic effects of the PAN fibers on microbes, as PAN showed the highest toxicity on soil biota among several tested microplastics (Kim et al., 2020).

The combined effects of microplastic and drought suggested that PES fibers slightly enhanced soil respiration under drought conditions, which may be linked to fibers increasing soil water holding capacity (de Souza Machado et al., 2019; Rillig et al., 2019b; Rillig et al., 2019a), as discussed earlier, under drought conditions. Overall, soil water content may be the major limiting factor for soil microbial activity. Likewise, the presence of plants and root-microbe linkages may account for the slight increase in soil respiration.

Even though recent papers have illustrated the microplastic effects on soil respiration, it is still necessary to conduct more research in the future to better understand the potential mechanisms by which microplastic can affect soil microbial respiration.

6.1.3 The effects of microplastic on soil enzymatic activities

Our results showed that without plants the direct effects of microplastic fibers and films on enzyme activities depended on the microplastic shape and polymer type; while with the presence of plants the effects were slightly negative on all the enzyme activities; the indirect effects of microplastics on enzyme activities varied with microplastic types, soil layers, and water conditions; and generally, microplastics increased enzyme activities when combined with drought (Figure 6.3-6.6).

Microplastic effects on phosphatase activity. Phosphatase, the enzyme that transforms organic phosphorus into inorganic forms, is determined by soil microbial activity, C, N, and P contents, pH, and moisture (Fei et al., 2020; Yi et al., 2020; Dissanayake et al., 2022). Figure 6.3 shows the effects of microplastics on phosphatase activity.

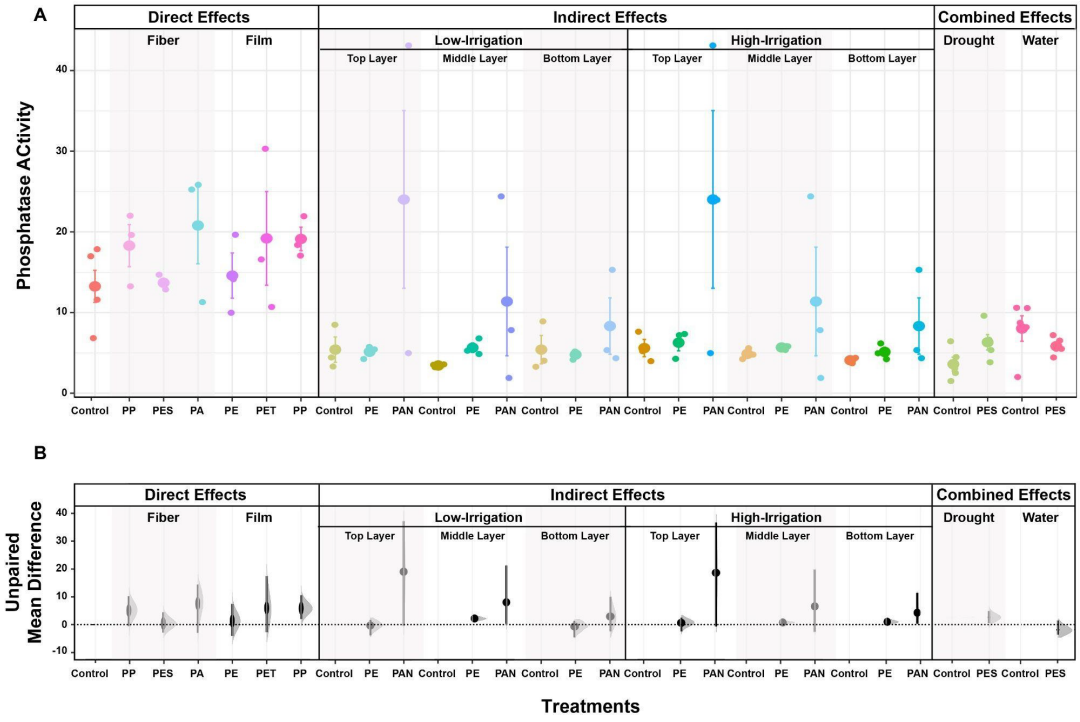

Figure 6.3 Microplastic fibers and films effects on phosphatase activity.

Unit: μmol $mg^{-1}hr^{-1}$. Direct, indirect and combined effects of microplastics on soil phosphatase activity (A). Mean and standard errors are shown. Effects sizes (B) are displayed as mean, and 95% confidence intervals; the horizontal dotted line indicates the mean difference value between each treatment and control. Control: soil treated without microplastics; polymers: PA (polyamide), PAN (polyacrylonitrile), PE (polyethylene), PES (polyester), PET (polyethylene terephthalate), PP (polypropylene). This figure integrates data from chapters 3, 4 and 5. Note that the direct effects did not include a plant in the system, contrary to the combined effects.

Overall, without plants, the direct effects of microplastic fibers and films on phosphatase were positive or neutral, which was determined by microplastic shapes and polymer types. The shape determined how microplastics change soil aeration, and oxygen diffusion, as we discussed earlier, thus impacting microbial activities differently. Likewise, the polymer types influenced the toxic effects of microplastics on soil microbes and consequently impact the enzyme activities. Besides, these microplastics triggered changes in soil pH, affecting phosphatase activity as the last is sensitive to soil pH (Yi et al., 2020; Yu et al., 2020a; Dissanayake et al., 2022). Another reason may be that microplastic addition contributed to significant changes in soil available phosphorus content (Yan et al., 2021; Pinto-Poblete et al., 2022; Wang et al., 2022b), resulting in changes in phosphatase activity. Particularly, PES microplastic fibers exerted neutral effects on this enzyme activity in bare soil, whereas slightly enhanced it when plants existed. This may result from the regulation of plants to available soil P (Manzoor et al., 2022).

Regarding the indirect effects, under both low- and high-water irrigation conditions, PE films had negligible effects on phosphatase activity, whereas the PAN fibers increased it. However, phosphatase activity decreased with soil depth. PAN fibers enhanced phosphatase activity while PE films exerted negligible effects; such differences may be triggered by both shape and polymer differences; specifically, PAN polymers contain N, which may attribute to the positive effects of PAN fibers on phosphatase which is positively correlated with soil carbon and nitrogen (Yi et al., 2020). The effects of PAN fibers on phosphatase activity decreased with soil depth, which may be linked to their effects on water flow pathways and distribution in the soil profile (Jiang et al., 2017; Chy 2021; Xing et al., 2021).

As for the combined effects (microplastics and drought), PES fibers enhanced this enzyme activity. This may be due to PES fibers increasing water holding capacity (de Souza Machado et al., 2019; Lozano & Rillig 2020), thus alleviating the negative effects of drought on the metabolic performance of soil organisms (Dissanayake et al., 2022).

Microplastic effects on β-D-glucosidase. β-D-Glucosidase is involved in the degradation of cellulose in soils (Turner et al., 2002); it is greatly dependent on soil organic matter, and influenced by soil depth, moisture and pH (Adetunji et al., 2017). The effects of microplastics on glucosidase activity are shown in Figure 6.4.

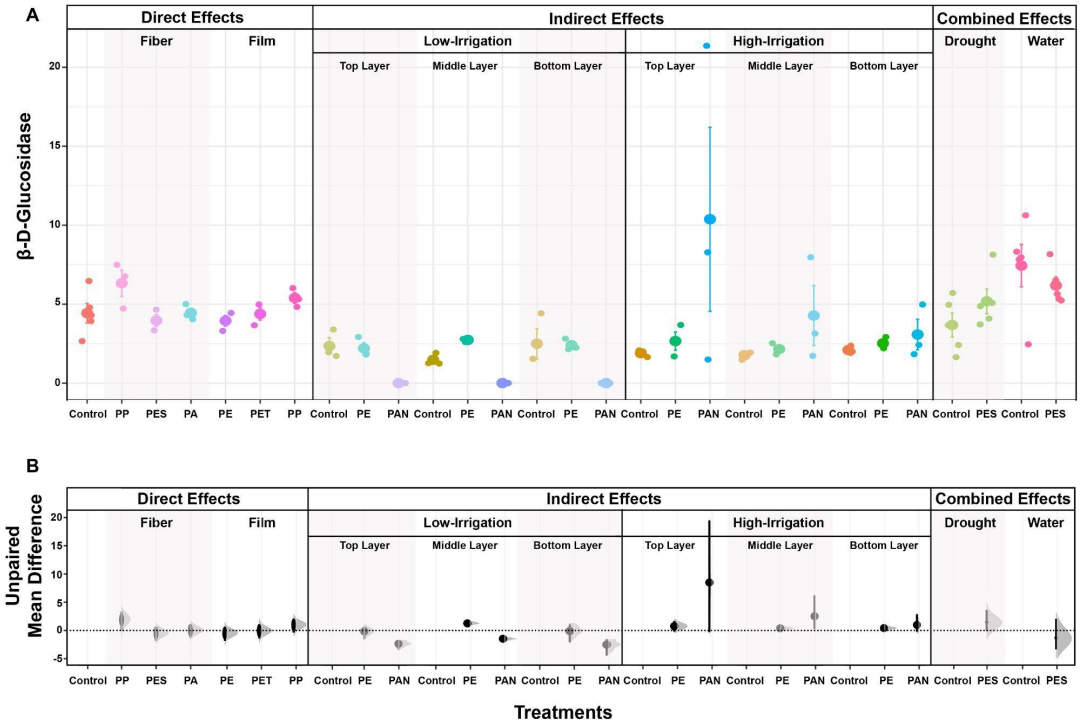

Figure 6.4 Microplastic fibers and films effects on β-D-glucosidase activity.

Unit: μmol $mg^{-1}hr^{-1}$. Direct, indirect and combined effects of microplastics on soil β-D-glucosidase activity (A). Mean and standard errors are shown. Effects sizes (B) are displayed as mean, and 95% confidence intervals; the horizontal dotted line indicates the mean difference value between each treatment and control. Control: soil treated without microplastics; polymers: PA (polyamide), PAN (polyacrylonitrile), PE (polyethylene), PES (polyester), PET (polyethylene terephthalate), PP (polypropylene). This figure integrates data from chapters 3, 4 and 5. Note that the direct effects did not include a plant in the system, contrary to the combined effects.

Overall, the direct effects of microplastic fibers and films on β-D-glucosidase were neutral, except for PP fibers, which increased β-D-glucosidase activity. This may be due to PP releasing dissolved organic carbon which can stimulate glucosidase activity (Romera-Castillo et al., 2018).

Regarding the indirect effects, under low-water irrigation, PE films had no discernible effects on glucosidase activity, while PAN fibers inhibited it. Such differences may be caused by PAN fibers containing and releasing more toxic substances than PE films (Kim et al., 2020), contributing to more deleterious effects on soil microbes, and thus suppressing glucosidase activity. However, under both low- and high-water irrigation conditions, PE films slightly increased this enzyme activity, showed no discernible differences among the three soil layers. By contract, under low-water irrigation conditions, PAN fibers decreased glucosidase activity in a similar way among the soil layers; while under high-water irrigation conditions, PAN fibers promoted this enzyme activity being higher in upper than in lower soil layers, which may be due to the differences of their surface areas playing an important role in substrate adsorption (Yu et al., 2020a; Guo et al., 2021), thus on the enzyme activity.

As for the combined effects (MPs and drought), PES fibers increased this enzyme activity, while reducing the enzyme activity under well-watered conditions. Drought negatively affected microorganisms' metabolic performance; as discussed above, the increase of water holding capacity caused by PES fibers migrated the negative effects of drought on microbial activity. However, under well-watered conditions, soil organic matter could have been consumed faster in the soil with microplastics than in the control due to better aeration triggered by microplastics. This could have caused less liable substrates in soil with microplastics than in control soils, which in the end could help explain the lower glucosidase activity.

Microplastic effects on β-D-cellobiosidase. β-D-cellobiosidase, another enzyme involved in degrading cellulose (Sanaullah et al., 2011), is vulnerable to soil pH, soil organic matter content and bacterial and fungal communities (Ullah et al., 2019). Microplastic effects on β-D-cellobiosidase activity are shown in Figure 6.5.

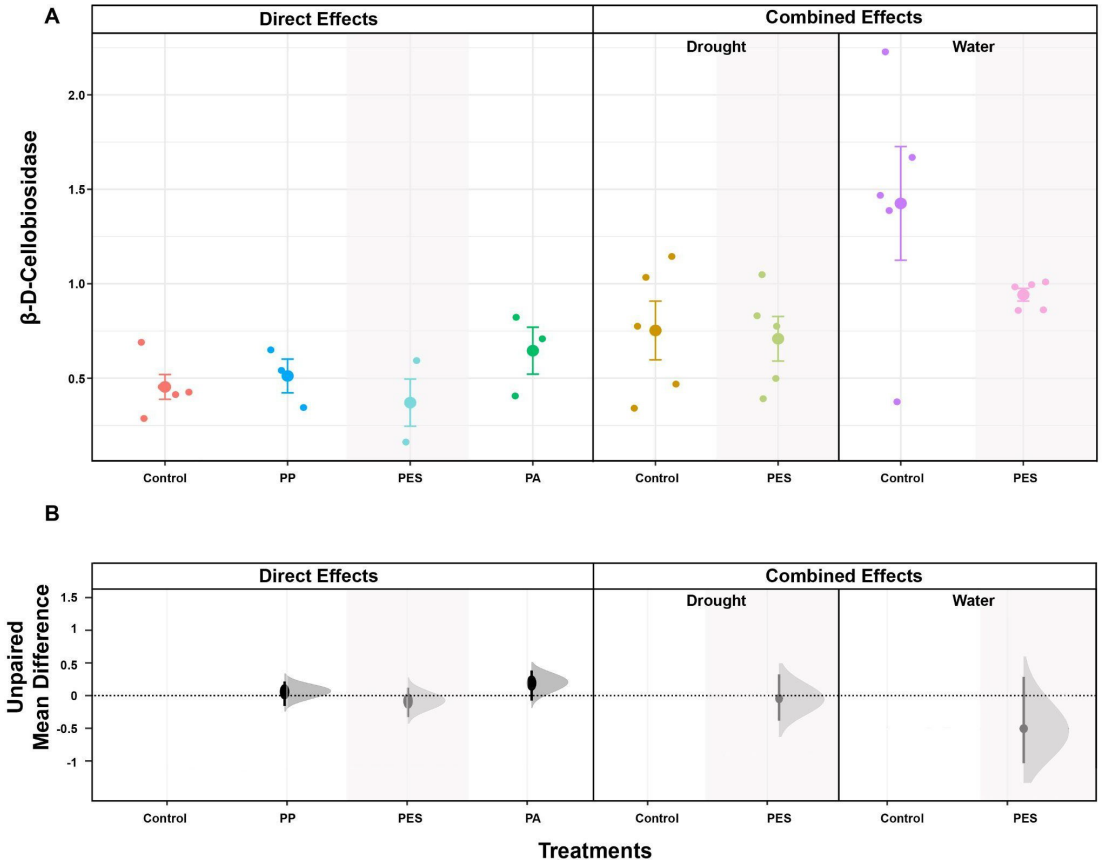

Figure 6.5 Microplastic fibers effects on β-D-cellobiosidase activity.

Unit: μmol $mg^{-1}hr^{-1}$. Direct and combined effects of microplastics on soil β-D-cellobiosidase activity (A). Mean and standard errors are shown. Effects sizes (B) are displayed as mean, and 95% confidence intervals; the horizontal dotted line indicates the mean difference value between each treatment and control. Control: soil treated without microplastics; polymers: PA (polyamide), PES (polyester), PP (polypropylene). This figure integrates data from chapters 3 and 5. Note that the direct effects did not include a plant in the system, contrary to the combined effects.

Overall, the direct effects of microplastic fibers on β-D-cellobiosidase activity were almost negligible, although PA and PP fibers triggered an increase in β-D-cellobiosidase activity. This may be linked to the PA containing nitrogen (de Souza Machado et al., 2019), and PP releasing dissolved organic carbon (Romera-Castillo et al., 2018), enhancing microbial activities.

As for the combined effects (microplastics and drought), PES fibers did not affect the enzyme activity; while causing negative effects on this enzyme under well-watered conditions. Except for the negative effects of drought on microbe metabolic performance, and different consumption levels of substrates caused by PES fibers; more fine soil and organic mineral particles might be absorbed on the surfaces of the fibers under well-watered conditions than under drought conditions (Guo et al., 2021), thus attributing to the more significant negative effects on this enzyme activity under well- watered than under drought conditions.

Microplastic effects on N-acetyl-β-D-glucosaminidase. N-acetyl-β-D-glucosaminidase, one of the N-degrading enzymes, is highly correlated with substrate concentration, incubation time, soil N content, C-cycling enzymes, soil pH, and fungal biomass (Parham & Deng 2000; Ullah et al., 2019). Microplastic effects on N-acetyl-β-D-glucosaminidase activity are shown in Figure 6.6.

Overall, the direct effects of microplastic fibers and films on N-acetyl-β-D-glucosaminidase were negative. This may be ascribed to the carbon consumption by soil microbes, as N-cycling enzymes declined with the decrease in carbon availability (Yi et al., 2020). Likewise, microplastic toxic effects on soil microorganisms may have caused decrease (Fred-Ahmadu et al., 2020). Microplastic fibers showed more pronounced effects than microplastic films, which may be due to the former having larger surface areas than the latter (Yi et al., 2020), impacting microplastic adsorption to soil organic particles (Guo et al., 2021). Specifically, PP fibers exerted high positive effects on this enzyme activity, which may be linked to their positive effects on soil organic carbon availability (Romera-Castillo et al., 2018).

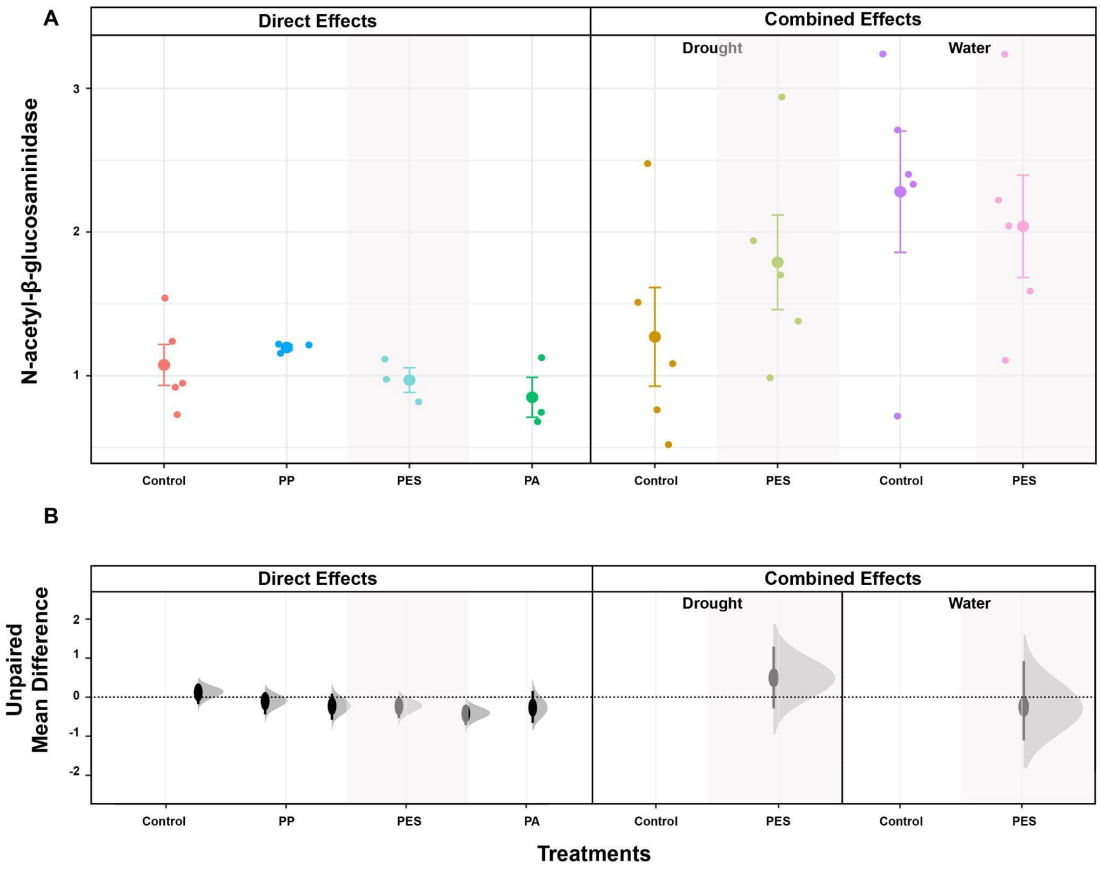

Figure 6.6 Microplastic fibers effects on N-acetyl-β-D-glucosaminidase activity.

Unit: μmol $mg^{-1}hr^{-1}$. Direct, indirect and combined effects of microplastics on N-acetyl-β-D- glucosaminidase activity (A). Mean and standard errors are shown. Effects sizes (B) are displayed as mean, and 95% confidence intervals; the horizontal dotted line indicates the mean difference value between each treatment and control. Control: soil treated without microplastics; polymers: PA (polyamide), PAN (polyacrylonitrile), PE (polyethylene), PES (polyester), PET (polyethylene terephthalate), PP (polypropylene). This figure integrates data from chapters 3 and 5. Note that the direct effects did not include a plant in the system, contrary to the combined effects.

As for the combined effects (microplastics and drought) on glucosaminidase activity, the pattern was similar to that on other enzymes. That is, the PES fibers increased glucosaminidase activity under drought; on the contrary, PES fibers reduced this enzyme activity under well-watered conditions. The reasons for such effects may be similar to those discussed above. Added to what was discussed earlier, root exudates could also alleviate the negative effects of drought on soil microbial activity.

The mechanisms of microplastics affecting soil enzyme activities are complex. For example, soil types could play a role, as soil pH and nutritious status vary, i.e., microplastic fibers can show negative effects on enzymatic activities depending on the soil organic matter (Liang et al., 2021). As most enzymes are sensitive to soil pH, and negatively correlated with it (Adetunji et al., 2017; Ullah et al., 2019; chapter 3), the changes in soil pH caused by microplastics could

also affect enzymatic activities. In addition, microplastics could change soil nutritious availability through effects on dissolved organic carbon (Romera-Castillo et al., 2018; Yu et al., 2020a), and the decomposition of organic matter (Guo et al., 2021). Furthermore, due to their adsorption capacity to substrates, microplastic addition could reduce substrate availability (Yu et al., 2020a; Guo et al., 2021); this may be the reason why microplastics of different shapes altered enzyme activities differently.

6.2 CONCLUSIONS

This work reviewed the effects of microplastic pollution on terrestrial ecosystems, in particular, their effects on soil physicochemical properties, soil biota and plants. Due to the wide application of plastics in our daily life and agricultural systems, there are many sources of microplastics in soil, such as agricultural mulching films, water irrigation, biosolids, etc., which can enter the soil and migrate vertically or horizontally influenced by many environmental factors. The horizontal distribution of plastics differs with their location which can be affected by for instance, atmospheric transport; the vertical distribution occurs with soil depth which can be affected by soil biota, plants, and agronomic practices. Regarding microplastic effects on soil physicochemical properties, soil biota and plants, the effects are determined by many factors including microplastic characteristics (i.e., polymer type, shape, size, exposure concentration and time), soil properties (i.e., organic matter content, water condition, soil porosity, and soil aggregation), soil biota and the presence of plants in the system.

However, most of these review findings are based on single-factor studies, which only surveyed the direct effects of microplastics. This can mask the real total effects of microplastics on terrestrial systems, as the hidden indirect effects can be overlooked. In addition, microplastics, as a new global change factor, together act in concert with other global changes factors such as drought. Scarce research has been done on the combined effects of microplastics with other global change factors.

Therefore, after a careful review of previous research, this work investigated some gaps of knowledge regarding microplastic effects on terrestrial systems. That is, this work aimed to study the effects (direct and indirect as a single factor, and combined with another global change factor, drought) of microplastics on soil properties and microbial activity. Our findings revealed that (1) the diverse direct effects of microplastics on soil pH and microbial activities vary with microplastic shapes and polymer types. The presence of plants could mediate these microplastic effects; (2) under low-water irrigation conditions, the indirect effects of microplastic exerted no important differences among the microplastic types or soil layers; whereas, under high-water irrigation, the indirect effects of PAN fibers had more pronounced effects than the PE films on soil microbial activity (respiration and enzymatic activities), and the effects decreased with soil depth; (3) the combined effects of microplastics and drought

were positive for soil pH and microbial activities. Plants may also influence the direct and combined effects of microplastics on soil properties and microbial activity.

6.3 DATA AVAILABILITY

Data availability for chapter 3: Data available via the figshare: https://doi.org/10.6084/m9.figshare.14546985.v1 (Zhao, et al., 2021).

Data availability for chapter 4: the original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author. Or Data available via the figshare: https://doi.org/10.6084/m9.figshare.20737621.v1 (Kim, et al., 2021)

Data availability for chapter 5: Data available via the Dryad Digital Repository https://datadryad.org/stash/dataset/doi:10.5061/dryad.nvx0k6drc (Lozano, Aguilar-Trigueros, et al., 2021).

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APPENDIX

APPENDIX A4: CHAPTER 4

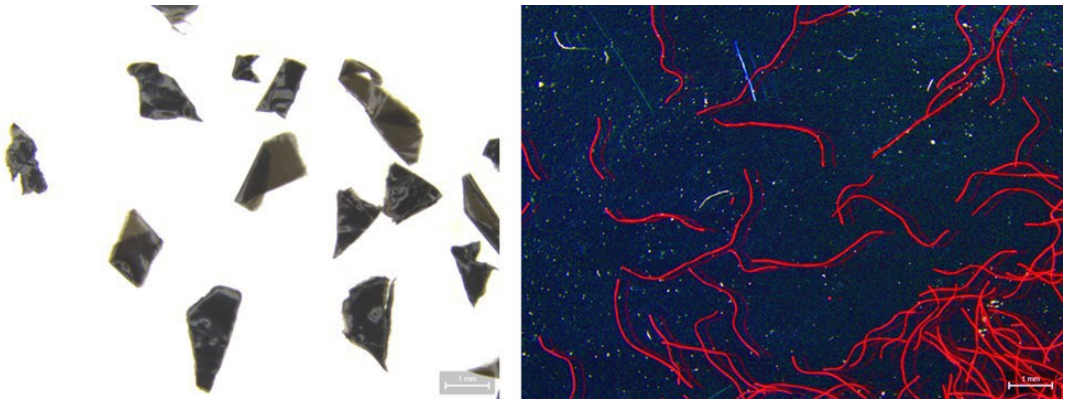

Figure A4.1 Target microplastics used in Chapter 4.

Low-density polyethylene (LDPE) films (left) and polyacrylonitrile (PAN) fibers (right).

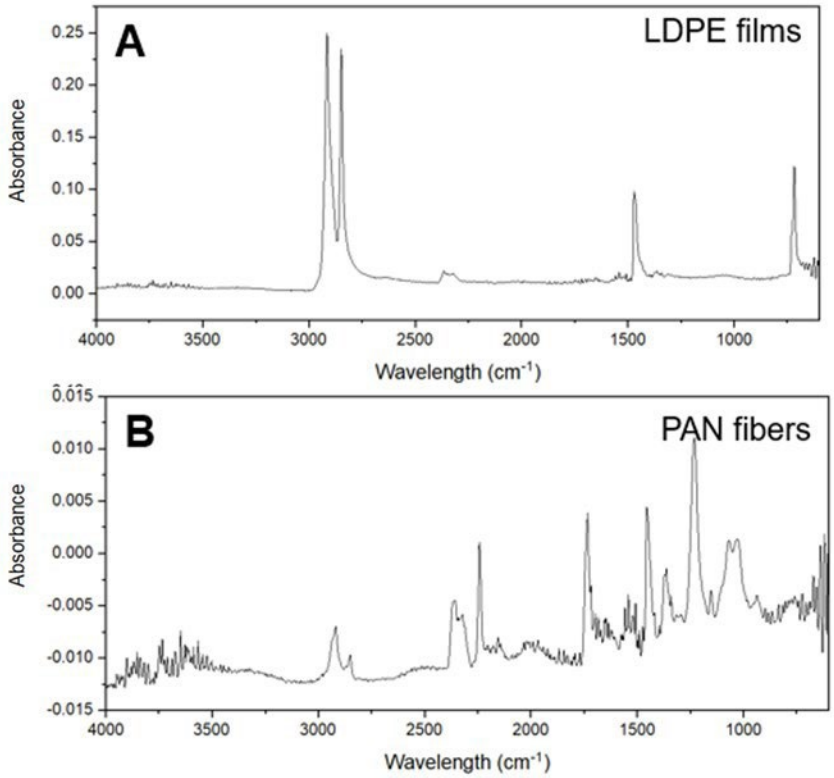

Figure A4.2 FTIR spectra, ATR mode, of all polymers tested in Chapter 4. (A) Low-Density Polyethylene (LDPE) and (B) Polyacrylonitrile (PAN).

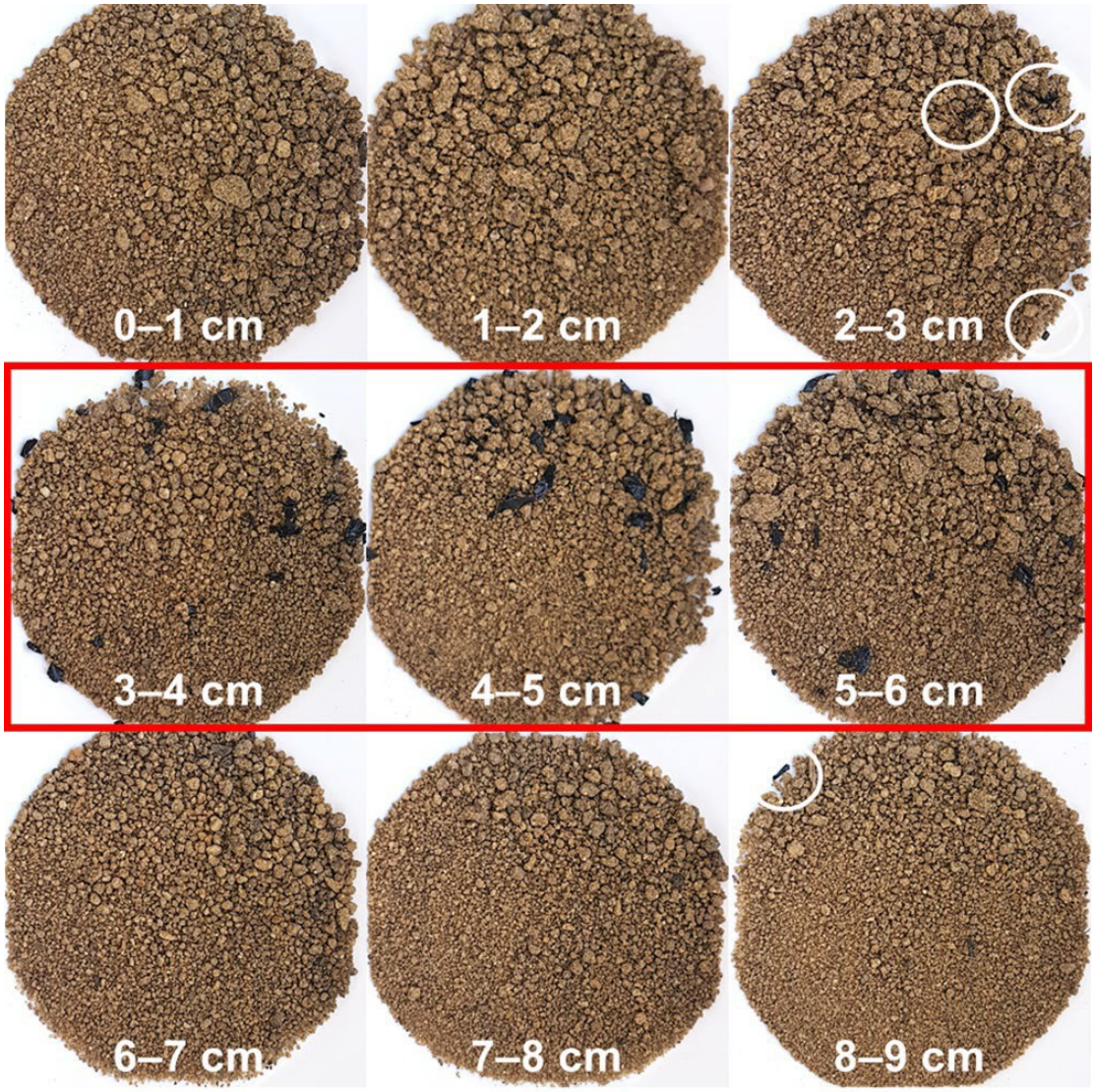

Figure A4.3 The close-up photographs of soil samples in each depth of LDPE film treatment with low- level irrigation.

The red border indicates microplastic-containing soil layer (3-6 cm), and LDPE films observed in the top (0-3 cm) and bottom (6-9 cm) layers were marked in the white circles.

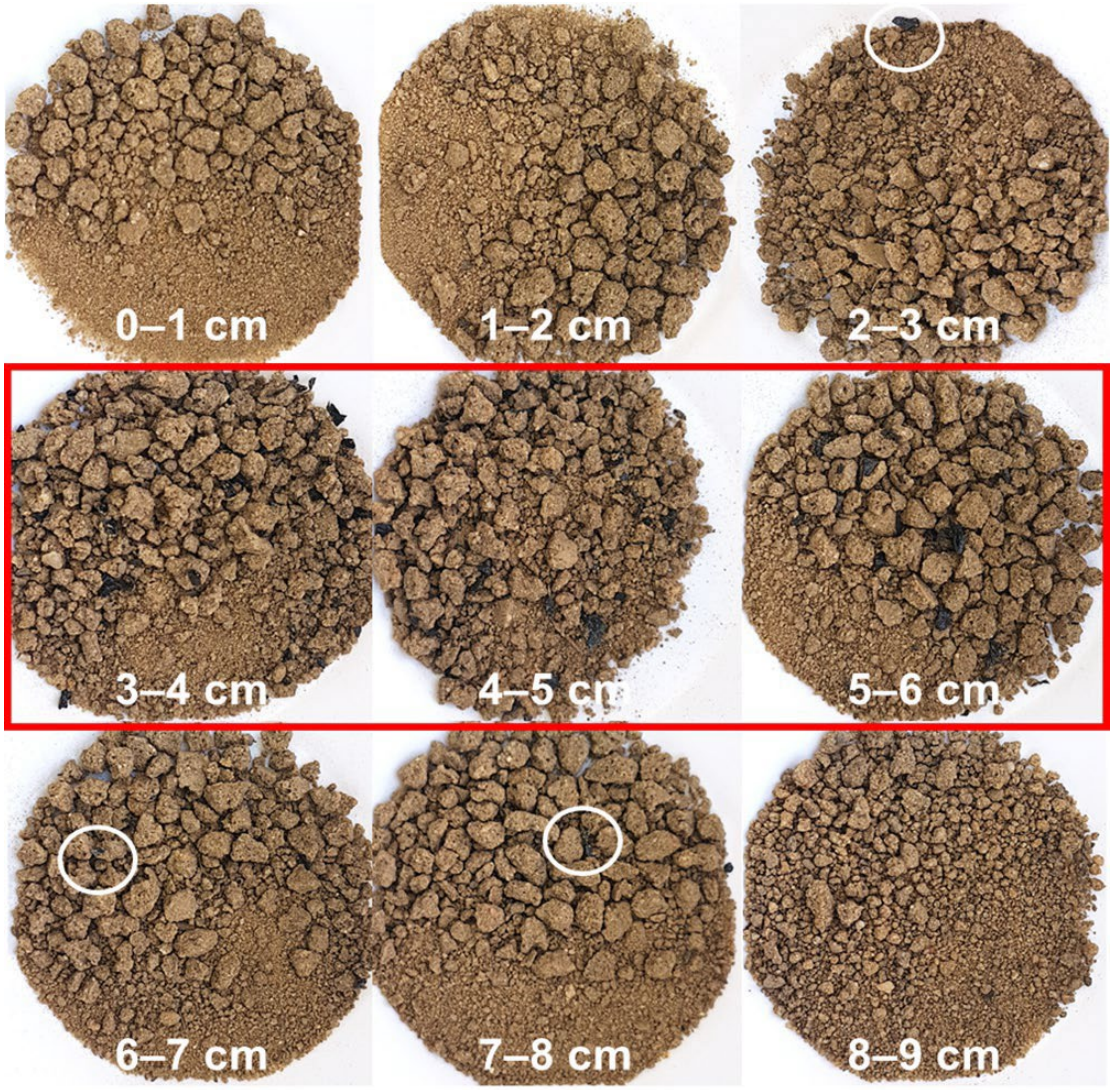

Figure A4.4 The close-up photographs of soil samples in each depth of LDPE film treatment with high- level irrigation.

The red border indicates microplastic-containing soil layer (3-6 cm), and LDPE films observed in the top (0-3 cm) and bottom (6-9 cm) layers were marked in the white circles.

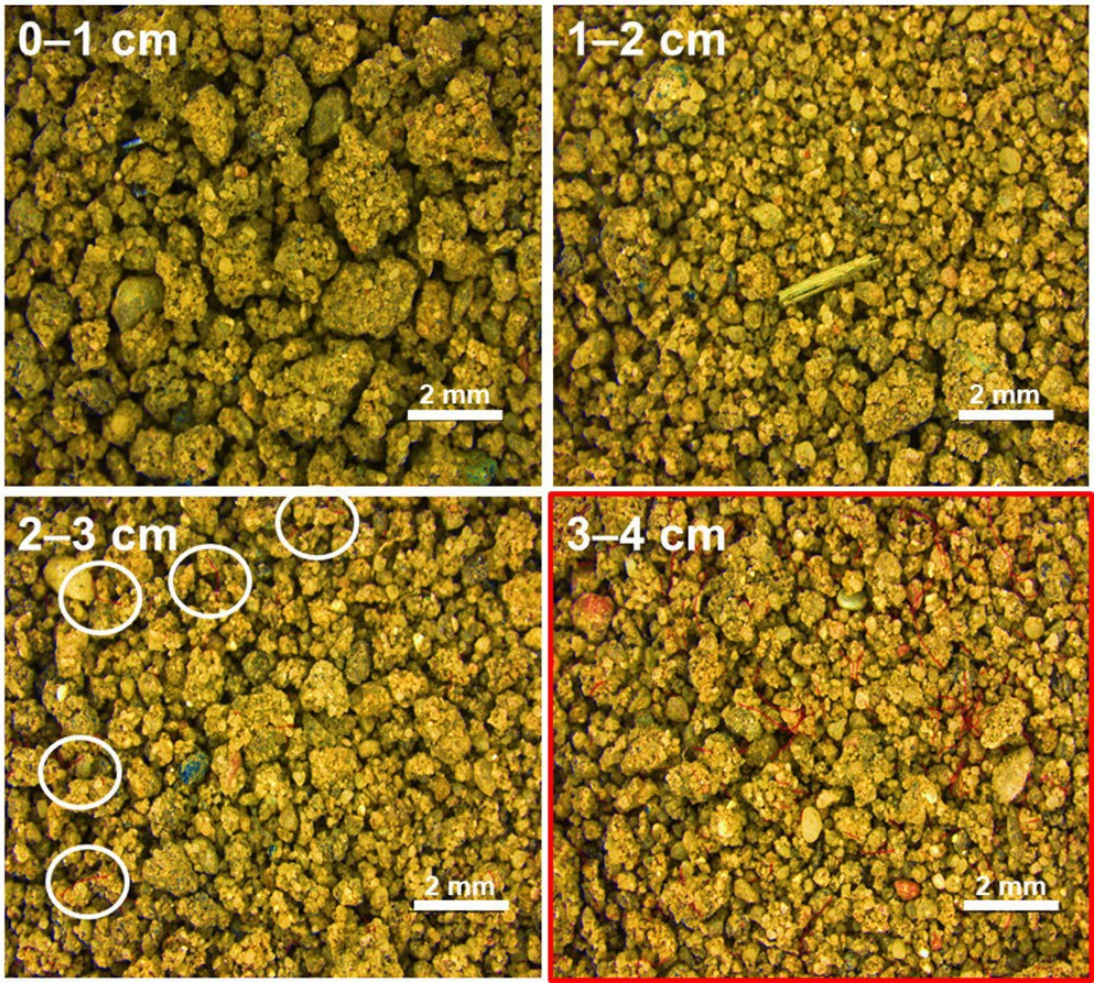

Figure A4.5 The close-up photographs of soil samples in each depth of PAN fiber treatment with low- level irrigation.

The red borders indicate microplastic-soil layer (3-6 cm), and PAN fibers observed in the top (0-3 cm) and bottom (6-9 cm) layers were marked in the white circles.

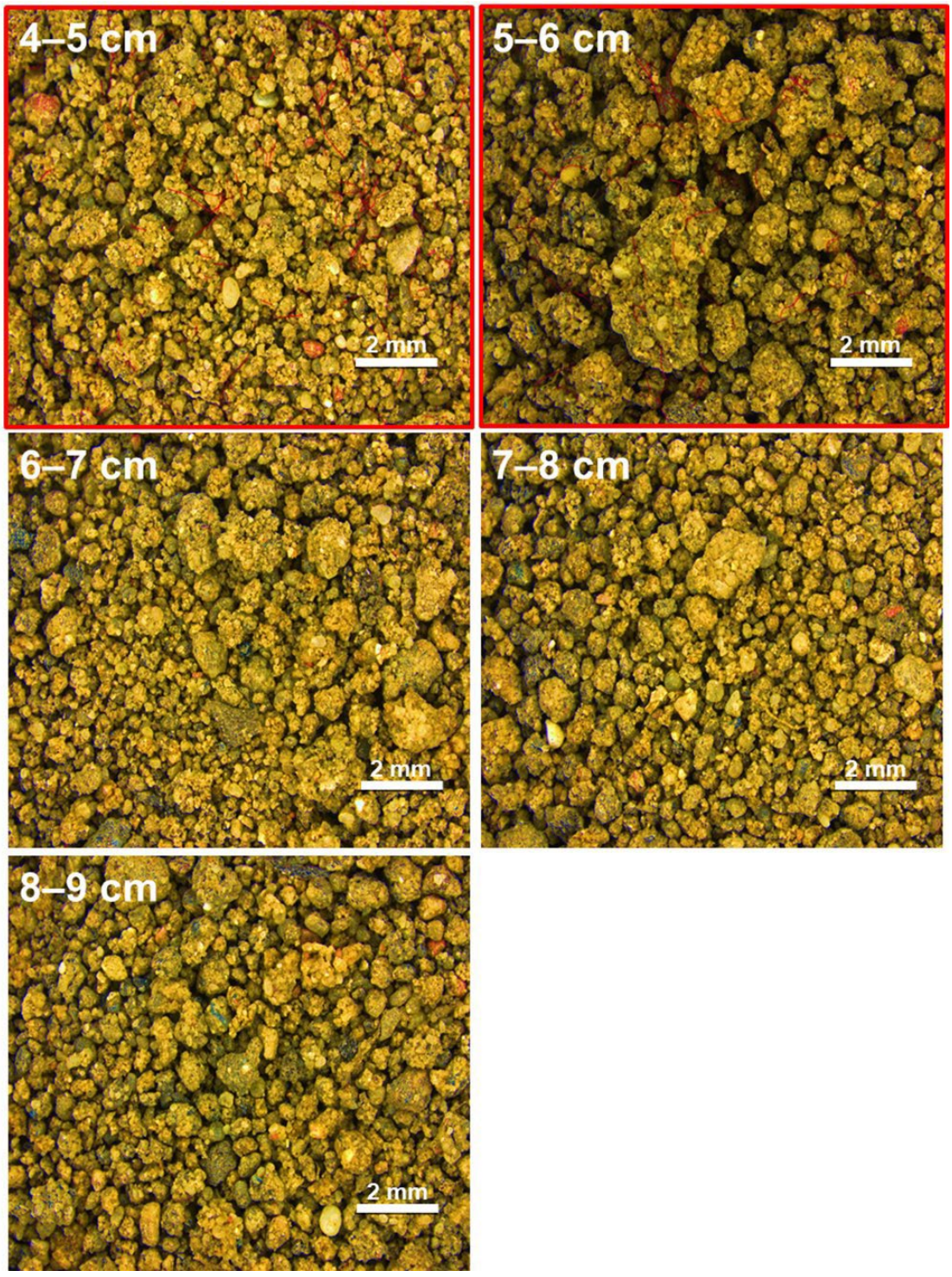

Figure A4.5 Continued.

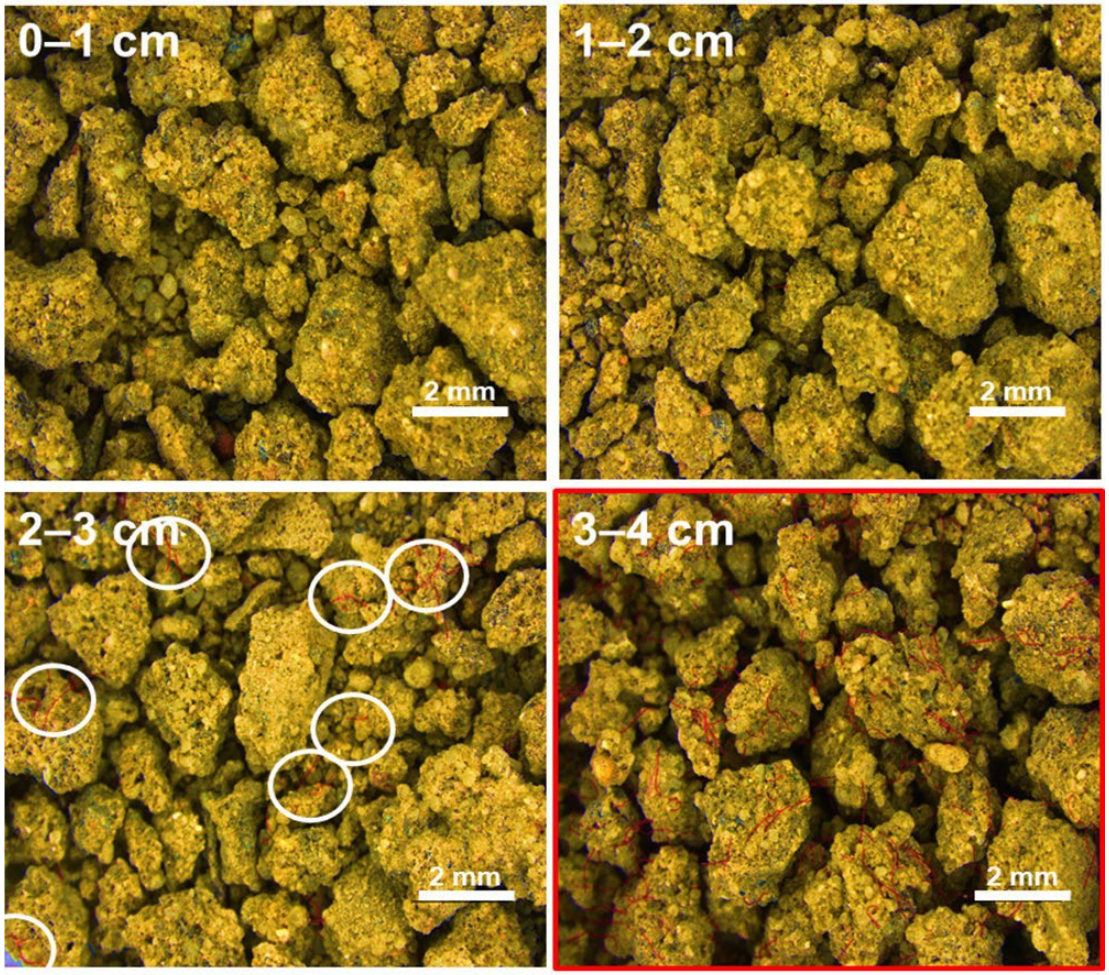

Figure A4.6 The close-up photographs of soil samples in each depth of PAN fiber treatment with high- level irrigation.

The red borders indicate microplastic-soil layer (3-6 cm), and PAN fibers observed in the top (0-3 cm) and bottom (6-9 cm) layers were marked in the white circles.

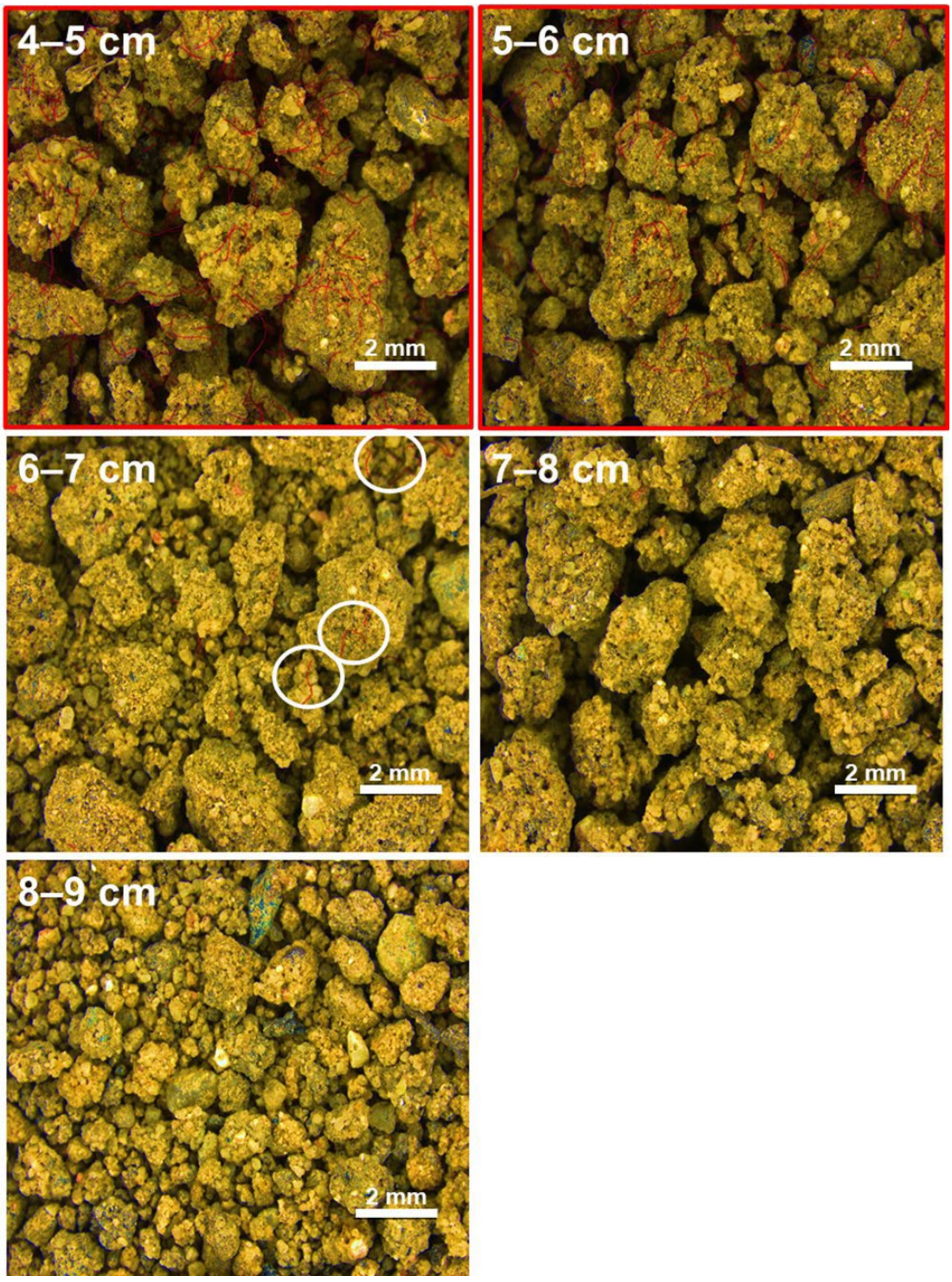

Figure A4.6 Continued.

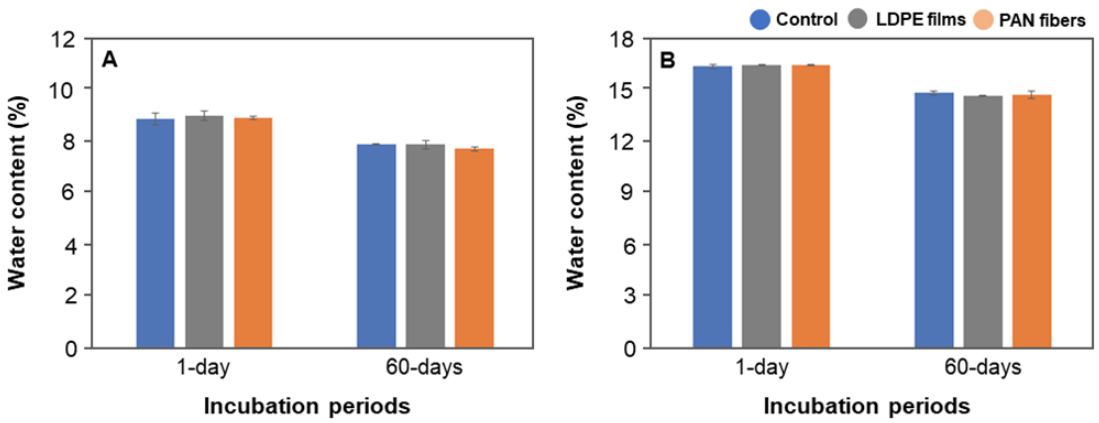

Figure A4.7 Total water contents in each soil column.

(control, LDPE films, and PAN fibers) after (A) low- and (B) high-level irrigations.

Table A4.1 Results of one-way ANOVA and Tukey's test for soil respiration and enzyme activities (acid phosphatase and β-D-glucosidase). Asterisk represents significance at the level of 5% (p = 0.05) between control and microplastic-containing soil layer treatments.

	Soil depth	**Top (0-3 cm)**			**Middle (3-6 cm)**			**Bottom (6-9 cm0**		
	Treatment	Control	LDPE	PAN	Control	LDPE	PAN	Control	LDPE	PAN
Low-level irrigation	Soil respiration (ppm h^{-1})	5.22 ± 1.09	5.25 ± 0.43	5.10 ± 0.18	4.45 ± 0.69	4.07 ± 0.36	3.78 ± 0.18	3.27 ± 0.45	2.54 ± 0.12*	2.75 ± 0.36*
	Acid phosphatase (μmol mg^{-1} h^{-1})	5.41 ± 2.72	5.14 ± 0.79	4.35 ± 1.29	3.44 ± 0.19	5.64 ± 1.02	4.13 ± 0.73	5.40 ± 3.05	4.79 ± 0.55	8.90 ± 5.02
	β-D-glucosidase (μmol mg^{-1} h^{-1})							2.96 ± 1.44	1.92 ± 0.48	3.68 ± 2.30
		NC	NC	NC	NC	NC	NC			
High-level irrigation	Soil respiration (ppm h^{-1})	3.77 ± 0.58	4.33 ± 0.37	2.63 ± 0.78	4.60 ± 1.43	5.12 ± 1.81	3.73 ± 0.30	6.26 ± 0.80	7.64 ± 0.79*	7.86 ± 0.38*
	Acid phosphatase (μmol mg^{-1} h^{-1})	5.60 ± 1.86	6.26 ± 1.74	24.02 ± 19.07	4.87 ± 0.68	5.67 ± 0.27	11.37 ± 11.67	4.09 ± 0.35	5.12 ± 0.98	8.32 ± 6.06
	β-D-glucosidase (μmol mg^{-1} h^{-1})							2.10 ± 0.24	2.52 ± 0.36	3.08 ± 1.67
		NC	NC	NC	NC	NC	NC			

APPENDIX A5: CHAPTER 5

Appendix 5.1 Measurement of soil ecosystem functions

Soil nutrient cycling: In fresh soil, we measured four functions related to C, N and P cycling: activity of ß-glucosidase and ß-D-cellobiosidase (cellulose degradation), N-acetyl-ß-glucosaminidase (chitin degradation) hereafter ß-glucosaminidase, and phosphatase (organic phosphorus mineralization). Extracellular potential soil enzyme activities were measured from 1.0 g of soil by fluorometry as described in Bell et al. (2013).

Soil respiration: We took 25 g of fresh soil from each pot to measure soil respiration via an infrared gas analyzer. To do this, we placed the subsamples in individual 50 ml centrifuge tubes (Sarstedt AG & Co. KG, Nümbrecht Germany, number item 62.548.004) whose lids were modified in order to control gas exchange via a rubber septum (Supelco, Darmstadt, Germany, number item 27235 U). We measured CO_2 concentration (ppm) at two time points from these tubes as described in Rillig, Ryo, et al. (2019). The first time point was obtained after we flushed the tubes with CO_2 free air for five minutes thus reflecting CO_2 concentration at time 0. The second point was obtained after letting the tubes with the soil samples incubate at 25°C for 65 h. At both time points, we took a 1-mL air sample and injected it to an infrared gas analyzer (LiCOR- 6400XT). We reported soil respiration as the net CO_2 production (in ppm) after the incubation period by subtracting the measurement from the first time point from that of the second.

Litter decomposition: We collected plant material from dry grasslands where our species naturally grow (see Onandia et al., 2019 for methodological details) and obtained a composite sample that reflected the proportion of plant biomass of each plant species in the field. Plant material was oven-dried at 60 ºC for 72 h, milled, and 0.75 mg were placed in 6×6 cm polyethylene terephthalate (PET, Sefar PET 1500, Farben- Frikell Berlin GmbH, Germany) bags with a mesh size of 49 µm. One litter bag was buried in each pot at 8 cm depth prior to seedling transplanting, and retrieved at harvest. Litter bags were stored at 4°C and processed within 2 weeks. Soil attached to the bags was carefully washed away using tap water and then, litter decomposition was estimated as mass loss after each bag was oven-dried at 60°C until constant weight (i.e., 72 h).

Soil aggregation: Water stable soil aggregates are a proxy measure of soil aggregation and were measured following a modified version of the method of Kemper and Rosenau (1986), as described in Lehmann et al., 2019. Briefly, 4.0 g of dry soil (<4 mm sieve) was placed on small sieves with a mesh size of 250 µm. Soil was re-wetted with deionized water by capillarity and inserted into a sieving machine (Agrisearch Equipment, Eijkelkamp, Giesbeek, Netherlands) for 3 min. Agitation and re-wetting causes the treated aggregates to slake. We collected the soil left

on the sieve (coarse matter + water stable fractions, also called dry matter) and then separated the coarse matter by crushing the aggregates and pushing the soil through the sieve. Coarse matter refers to the sand and soil particles >250 µm that were left on the sieve. Dry matter and then coarse matter were dried at 60 °C for 24 h. Soil aggregation (i.e., water stable aggregates) was calculated as:

WSA (%) = (Dry matter - coarse matter) / (4.0 g - coarse matter) (Eq1)

Soil nutrient leaching and pH. At harvest, after inducing leaching by watering the pots ~10 % beyond water holding capacity, leachate percolating through the soil column was collected from small outlets at the bottom of the pot and assessed for nutrient concentrations (NO_3^-, SO_4^{2-}, PO_4^{3-}) using ion chromatography (Dionex ICS-1100, AS9- HC, Thermo Scientific Massachusetts, USA). Air-dried soils were extracted in deionized water for 1 h to achieve a 1:5 (v:v) soil: water solution and soil pH was determined with a Hanna pH-meter (Hanna Instruments GmbH, Vöhringen, Deutschland).

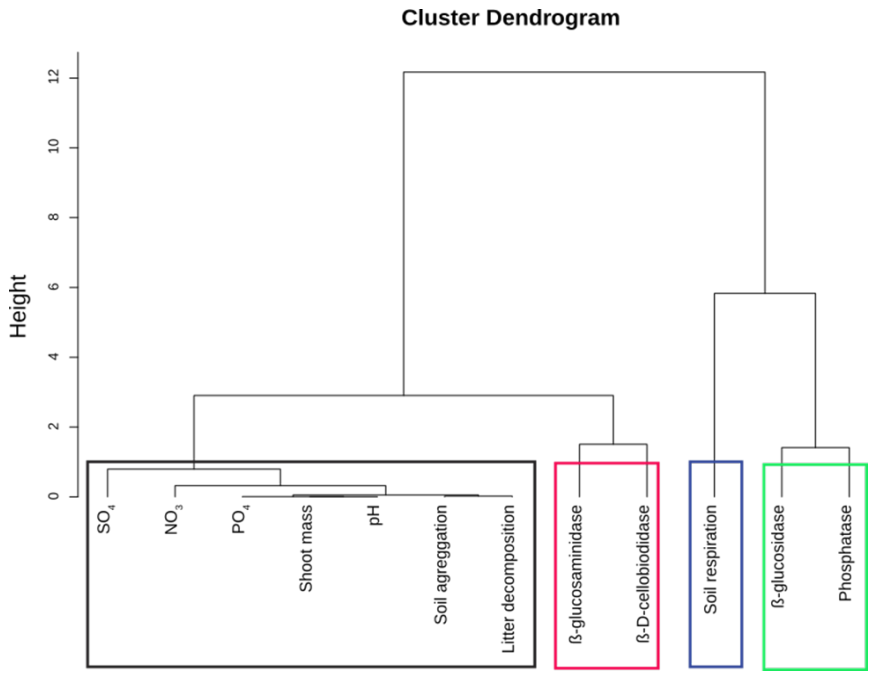

Figure A5.1 Dendrogram of twelve ecosystem functions showing four main clusters affected by microplastics.

Functions were used to measure ecosystem multifunctionality. Cluster in black shows soil nutrients, shoot mass and litter decomposition, clusters in red and green group the enzymatic activities while cluster in blue indicates soil respiration.

Appendix 5.2 Assessing ecosystem multifunctionality

To calculate ecosystem multifunctionality we followed the ecosystem function multifunctionality method proposed by Manning et al. (2018). Briefly, we identified 12 ecosystem functions (Figure A5.1), which included the soil functions measured in this study and total shoot mass (raw data obtained from Lozano and Rillig (2020)). This cluster analysis allowed us to give more even weights to the ecosystem functions as they are interrelated and shared drivers. We determined the number of clusters by the Elbow method (Kassambara & Mundt, 2017), and weighted each of them equally, irrespective of the number of functions within each cluster. Four clusters were determined. Then, we calculated the standardized maximum for each function and placed the function data on a standardized scale. Thus, we standardized by the average of the top 10% values within the data and calculated ecosystem multifunctionality for each experimental unit using the threshold approach, in which each ecosystem function that exceeds 70% of the standardized maximum contributed to the ecosystem multifunctionality score with its respective weighted value obtained after clustering. The medium range of thresholds (50 - 70%) is a conservative choice with high responsiveness (Van der Plas et al., 2016; Byrnes et al., 2014). Additional calculations of ecosystem multifunctionality were done using a threshold of 30% and 50% (Figure A5.2, Table A5.2).

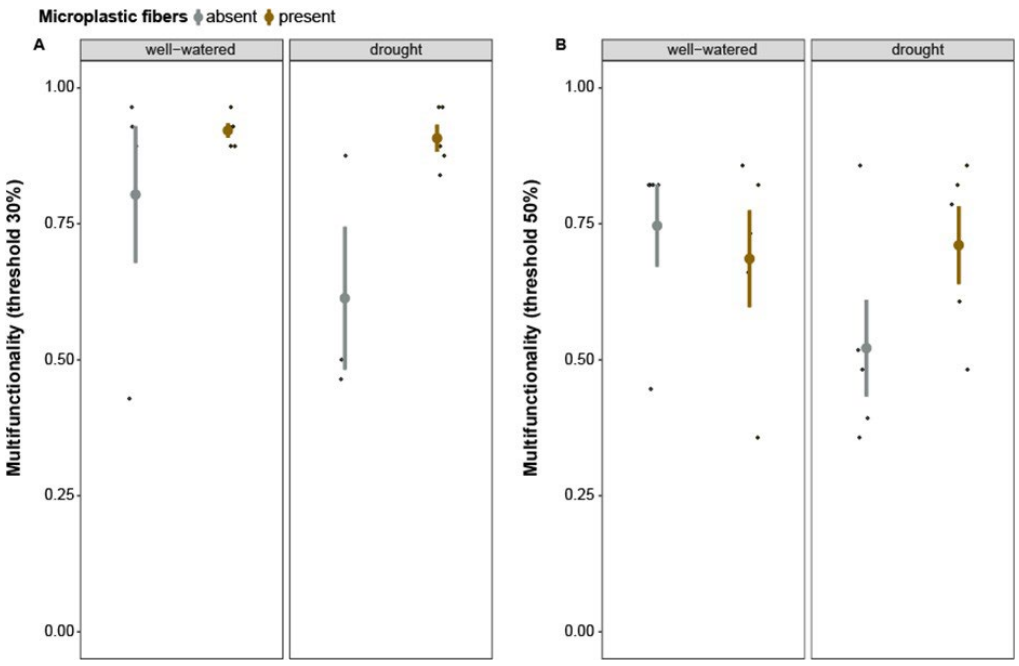

Figure A5.2 Microplastic fibers and drought effects on ecosystem multifunctionality.

Mean and standard error are represented. Threshold at 30 and 50%. See statistical results in Table A5.1.

Data points are shown as circles; n = 5.

Table A5.1 List of polyester fiber additives and their characteristics based on Polyester Additives Thiele Polyester Technology, Bruchköbel/Germany.

Additive substance	Property target	Solubility in water	Hazardous	References
TiO_2 –(anatase type)	Dulling agent, stretching aid, pigment	Insoluble	Reproductive toxicity - Category 2 Aspiration hazard - Category 1 Hazardous to the ozone layer - Category 4	National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 26042, Titanium dioxide. Retrieved October 16, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Titanium-dioxide.
5-sulpho-isophthlic acid, sodium salt NaSiP	Cationic dying	Slightly soluble	It can cause skin or eye irritation.	National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 80714, 5-Sulfoisophthalic acid. Retrieved October 16, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/5-Sulfoisophthalic-acid.
diethylene glycol (DEG)	Plasticizing	Soluble	Contact may slightly irritate skin, eyes and mucous membranes. Harmful if swallowed.	https://echa.europa.eu/documents/10162/13626/clh_rep_methoxyethoxy_ethanol_en.pdf/f054406f-de9c-7083-c83e-7663d8fe4b93
Siloxanes	Low pill	Soluble	Some siloxanes are classified as irritant, corrosive and acute toxic.	National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 57939932. Retrieved October 18, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/57939932.
Phosphinates	Flame retardant	Soluble	According to the classification provided by companies to ECHA in REACH registrations this substance causes severe skin burns and eye damage, causes serious eye damage and may be corrosive to metals.	European Chemicals Agency (ECHA) https://echa.europa.eu/substance-information/-/substanceinfo/100.026.001

Pentaerythritol	Melt viscosity modifier fire retardant	Soluble	It can cause irritation of eyes and affections in the respiratory system	National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 8285, Pentaerythritol. Retrieved October 16, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Pentaerythritol.
$BaSO_4$	Reduce fiber breakage rate, increase fiber dyeability, spinnability and tensile strength. It makes polyester fiber reflect excellent optical performance.	Slightly soluble	Aspiration hazard - Category 1 (respiratory organs) Hazardous to the aquatic environment (Long-term) - Category 3 Hazardous to the ozone layer - Category 3	http://www.hutongglobal.com/e_showproducts.asp?id=398 National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 24414, Barium sulfate. Retrieved October 18, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Barium-sulfate.
H_3PO_4, H_3PO_3, P-ester of different kind	Stabilizer, color improvement.	Soluble	Causes severe skin burns and eye damage. Corrosive	National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 1004, Phosphoric acid. Retrieved October 18, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Phosphoric-acid.
Polyacrylates	Spinnability	Soluble	It can be flammable, corrosive, irritant, and carcinogenic. It is very toxic to aquatic life.	National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 6581, Acrylic acid. Retrieved October 18, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Acrylic-acid.

Table A5.2 Microplastic, drought and their interactive effects on soil ecosystem multifunctionality (results from linear models). At a threshold of 30 % at 50 %. n = 5.

		Threshold 30 %		**Threshold 50 %**	
	df	F value	P value	F value	P value
Microplastic fibers (M)	1	3.28	0.08	0.62	0.44
Drought (D)	1	0.004	0.94	1.50	0.23
M x D	1	0.34	0.56	2.35	0.14

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