From plastic mulching to microplastic pollution: an effect assessment of microplastics in the soil-plant system

Fanrong Meng

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Propositions

1. Focusing only on the time period of plastic mulching is far from illustrating the plastic residues accumulation.
   (this thesis)

2. Phytotoxicity of biodegradable plastic materials is underestimated.
   (this thesis)

3. Science is a vicious circle: using new problems to solve old problems.

4. Data transformation is like plastic surgery, opinions vary.

5. All new invented materials will damage the environment, it's just a matter of time.

6. 10 not recommend reviewers will be more efficient than "5 recommended and 5 unrecommended.

7. Dating apps provide therapy for the pressure during the PhD journey.

8. The campus is a big room and the lack of Asian researchers in senior positions, is the elephant.

Propositions belonging to the thesis, entitled
From plastic mulching to microplastic pollution: an effect assessment of microplastics in the soil-plant system

Fanrong Meng
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Fanrong Meng

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1. General introduction

1.1 When plastic met agriculture – plastic mulching and plastic pollution

The compelling story of plastic film mulches (PFM) begins with the "awkward hand tight situation" of Prof. E.M. Emmert, the man who is now also considered the "Father of plastic mulch" due to his dedicated efforts to the development of agricultural plastic technology (Emmert, 1954, 1955, 1957; Kasirajan and Ngouajio, 2012). Back in 1948, Prof. E.M. Emmert, a horticulturist and instructor from the University of Kentucky, was trying to find a less expensive material to replace the glass in his "glass house". At the time, a glasshouse was used to protect horticultural crops against the cold. Professor Emmert first applied a cellulose acetate film to the soil and later replaced this with a polyethylene (PE) plastic film mulch (PE-PFM). This was the first time that PE was introduced to agriculture, and ever since then, PE and agriculture have been inseparable during their long lasting "honeymoon" (Anderson and Emmert, 1994; Emmert, 1957; Espí et al., 2016). Over the years, the use of PE-PFM proved to be more effective than other mulches (straw, paper and aluminium films) in controlling soil microclimatic changes and was gradually recognized by other scientists (Waggoner et al., 1960). In the 1960s, PFM began to be commercially used for vegetable production in the US (Hussain and Hamid, 2003; William James Lament, 1993). The initial reason for using PFM in agriculture was to increase soil temperature but farmers soon realized that PFM could protect crops from less ideal weather conditions and increase crop yield in cold regions. It wasn't long before the use of plastic mulch technology exploded (Garnaud, 1974; Scarascia-Mugnozza et al., 2011; Wan and El-Swaify, 1999; Zhang et al., 2013).

Over the years, PFM has provided a range of benefits including increased soil temperature, moisture conservation, reduced weed and pest pressure, enhanced fertilizer use efficiency, and improved crop yield and quality. PFM has made a substantial contribution to key Sustainable Development Goals, such as improving global food security and reducing poverty. A review from Yan et al. (2014b) pointed out that plastic mulch has extended the boundary of planting regions for thermophilic crops by 2-5 latitudes and 0.5-1 altitudes, which in turn has allowed for yield increases of 20%-80% for vegetable and fruit production. PFM has also proved superior in soil moisture conservation and weed and pest control as well as helped to improve nutrient cycling and soil microbe activities (Kader et al., 2017a; Moreno and Moreno, 2008; Qin et al., 2015; Tarara, 2000). Over a span of 7 decades, the development of plastic mulch technology has been effectively promoted and advanced. Many techniques have been especially designed and tailored for different soil conditions, crops and climates. There have been special mulch methods have been designed, e.g. conventional flat mulching, ridge-furrow mulching and mulching with drip irrigation (Abd

El-Wahed et al., 2017; Gosar et al., 2009; Gu et al., 2017) (see Figure 1.1). Due to the countless benefits, this technology was rapidly transferred from the US to Europe and the Far East. It is now used extensively within a specific range of arable and horticultural cropping systems throughout the world (Kasirajan and Ngouajio, 2012). According to Díaz-Hernández and Salmerón (2012), in 2011 in Europe, 427059 ha of agricultural fields were covered with PFM. It was introduced to China in 1977 from Japan and initially covered a mere 50 ha (Cai et al., 2013; Ma et al., 2018; Wittwer, 1993). The usage of PFM in China has risen from 6,000 tons used on 117,000 hectares of farmland in 1982 to about 1.38 million tons used on 17.6 million hectares of farmland in 2019 (NBSC, 2020).

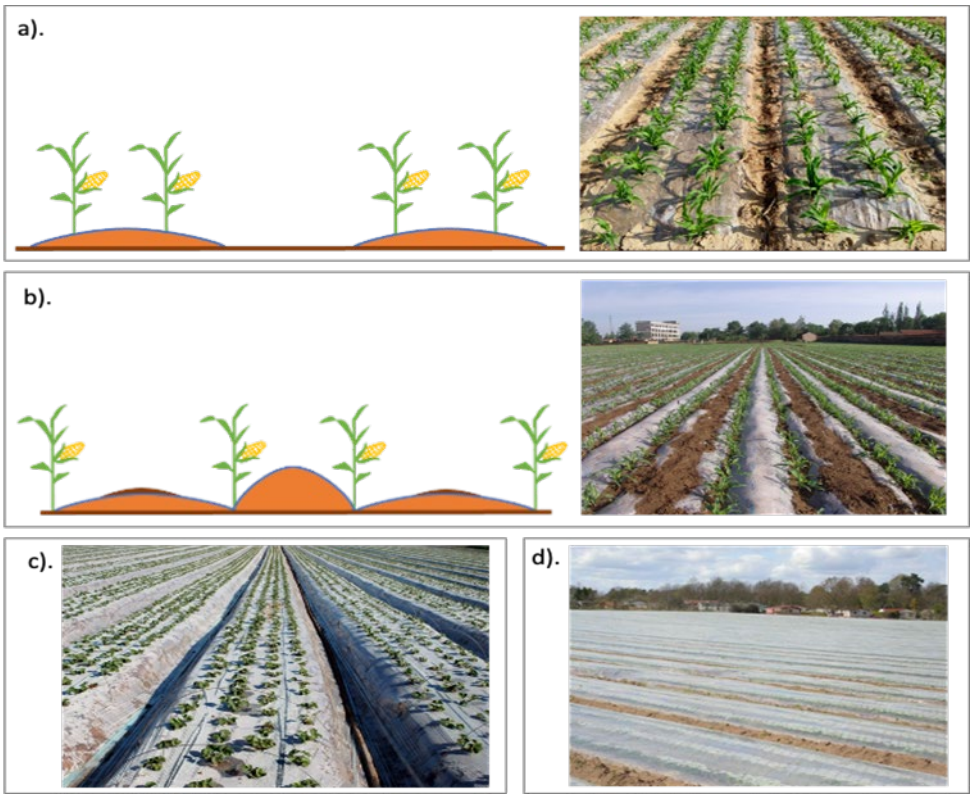

Figure 1.1 Schematic of two plastic mulching techniques.

Note. *a). Half film ridge-furrow mulching; b). Full film ridge-furrow mulching, photo taken in Qingyang, Gansu Province, China; c). Plastic mulch with drip irrigation in a strawberry field in Israel, free copyright. Photo obtained from Wikimedia; d). Plastic mulch in British carrot fields. free copyright. Photo obtained from Wikimedia.*

In order to be considered suitable mulching materials for the actual environmental conditions, the proposed mulching materials should meet three main mechanical, optical

and thermal requirements which are defined by American and/or European standards. For example, the European Regulation UNI EN 13206 [EUROPEAN COMMITTEE FOR STANDARDIZATION] requires that thermoplastic covering films used in agriculture and horticulture should be within the thickness range of 20 µm - 250 µm, based on polyethylene and/or ethylene copolymer materials. Other standards can be referenced from EN ISO 527-3 and ASTM 2002 (Dilara and Briassoulis, 1998; Mormile et al., 2017; Scarascia-Mugnozza et al., 2011). Currently, the dominant mulch film material used in agriculture is low density polyethylene (LDPE) due to its outstanding performance in mechanical stretch, chemical resistance, optical properties and affordability (Briassoulis et al., 2004; William James Lament, 1993). Over the years, researchers and commercial producers have made numerous efforts to tailor LDPE to enhance its performance in agricultural fields by adding various additives. For example, pigments (red/yellow/black) were added to LDPE film to increase the soluble solid content and total phenolics of wine grapes and plums (Coventry et al., 2003; Kim et al., 2008; William James Lament, 1993). Antioxidants and ultraviolet (UV) inhibitors/stabilizers were added to prevent ageing and provide UV-blocking (Espí et al., 2016). Nowadays, LDPE and LDPE-based mulch materials have become an "oligarch" in the agricultural plastic mulch market (Dilara and Briassoulis, 2000). Figure 1.2 shows an overview of agricultural plastic consumption in several regions with intense application of PFM. Do you wonder how many LDPE films are used in agriculture today? According to PlasticEurope (2020), in 2018-2019, 368 million tons of plastics were produced worldwide. Previous reports indicated that about 2% (2010) of the global production of plastic was destined for agriculture, and LDPE plastic films alone accounted for 60% of the agricultural plastic (Briassoulis et al., 2013a; Picuno, 2014). If PFM use in agriculture remained at this level, 4.42 million tons of LDPE film were used in 2018 as plastic mulch. To put this into perspective, the weight of Eiffel Tower is 10100 tons, which means that from 2018 to 2019, the weight of LDPE films applied to agricultural fields worldwide was equivalent to around 437 Eiffel Towers.

According to a report from the "Food and Agriculture Organization of the United Nations (FAO)", the world population is expected to reach 9.7 billion by 2050. This population growth is creating an unprecedented challenge for the global food demand (Godfray et al., 2010). Agricultural plastic mulching is an effective way of improving crop production therefore, we can expect that its use will continue to increase. However, even though plastic mulching provides indisputable advantages for modern agriculture, as the time goes by, the drawbacks of the intensive application of LDPE plastic mulch to agricultural soils will gradually come to light. Over the last decade, the sustainability of PFM technology has been questioned as long-term PFM use may cause severe unexpected environmental problems. During the growing and harvesting period, agricultural films are usually mechanically broken or weathered into smaller pieces due to exposure to UV light, thus creating large amounts

of plastic wastes (Hurley et al., 2020; Steinmetz et al., 2016; Vox et al., 2016). Recycling of these plastic wastes usually entails considerable financial cost and is incredibly labour-intensive, resulting in plastic wastes being piled in fields (Briassoulis et al., 2013b; Brodhagen et al., 2017; Levitan and Barros, 2003; Shen and Worrell, 2014). Some of these

Figure 1.2 *L(L)DPE films used in the agricultural section during 2018-2019.*

Note.

- *Australian data obtained from 2018–19 Australian Plastics Recycling Survey, total L(L)DPE consumption was 351 900T, 15.5% were in the agricultural section;*
- *US data obtained from: US Recovering Agricultural Plastics: Obstacles and Opportunities, link https://wasteadvantagemag.com/recovering-agricultural-plastics-obstacles-and-opportunities/;*
- *Brazilian data was estimated based on the report from pastemart.com, total demand of plastic in 2018 was 8 000 000 tons, 60% was films and sheets in agricultural and livestock production. Link: Imports from USA to meet Latin America's growing PE demand, Europe to continue with high PE imports from Middle East (plastemart.com);*
- *China data obtained from "China Rural Statistical Yearbook 2020";*
- *European data obtained from "APE statitics, Statistics - APE Europe".*

plastic wastes are usually tilled directly into soil profiles causing the plastic to accumulate in agricultural soils. A remote sensing survey by Blanco et al. (2018) in Barletta-Andria-Trani Province-Apulia Region (Italy) reported that plastic mulching generated 627 kg·ha^{-1} of plastic waste per year. He et al. (2018) in Xinjiang (China) found 121.85 to 352.38 kg·ha^{-1} of plastic waste at 0-30 cm soil depth in fields with 5-19 years of plastic mulch film use and plastic waste accumulated at an annual rate of 15.69 kg·ha^{-1}. Ironically, LDPE was chosen as a predominant mulch material due to its superior performance in stability and durability,

however, when LDPE becomes waste, these properties greatly restrict the control of plastic pollution. Due to its chemically inert structure $(C_2H_4)_n$, once LDPE is released and accumulates in the soil profile, the main degradative forces that break it down, namely photodegradation and increased temperatures, cease to play a role (Dilara and Briassoulis, 2000; Fotopoulou and Karapanagioti, 2019). "Plastics buried in soil are like diamonds, they last forever" (Barnes et al., 2009; Goldberg, 1994). The accumulation of plastic debris in the soil profile could significantly reduce soil gravimetric water mass, and bulk density. It also has the potential to release chemical additives, such as phthalic acid esters (PAEs) and polycyclic aromatic hydrocarbons (PAHs), and decrease soil enzymatic activities and thus, reduce soil quality and fertility (Hahladakis et al., 2018; Wang et al., 2013; Wang et al., 2016; Yin et al., 2008). As a result, plastic residues can seriously affect crop productivity and food security (Gao et al., 2019; Wang et al., 2016). Recently, scientists stated that the agricultural plastic debris buried in soils may gradually be fragmented into smaller particles, such as micro-sized plastic particles measuring under 5 mm. The ecological effects and fate of these particles, called microplastics, in the soil matrix remain largely unknow and have quickly become a major concern for scientists (Barnes et al., 2009; Rillig, 2012). These issues negatively affect the goals that the development of PFM set out to solve, i.e., food security, soil health and the creation of more sustainable agricultural systems. Is there any solution that can address the plastic pollution that arises as a consequence of plastic mulching used on agricultural soil? For many years, more attention has been paid to designing plastic films that either could maintain the functionality of traditional LDPE plastic mulching films or could require less post-harvest management, including being completely degraded under natural conditions. Biodegradable plastic mulches offer one potential solution for eliminating waste caused by the use of polyethylene plastic mulches.

1.2 Biodegradable plastics: solution or delusion

Before addressing biodegradable plastic, we would like to discuss the related terminologies: bio-based plastic, biodegradable plastic and bioplastic. Bio-based plastic, according to European standard EN 16575, is a product wholly or partly derived from biological origin, such as starch, polylactic acid (PLA) and Polyhydroxyalkanoates (PHA). The definition of bio-based depends on the origin of the original materials of which the plastics are made, mainly plant biomass. Bio-based does not mean that a plastic is necessarily biodegradable. Biodegradable plastics are defined as materials that could be degraded by microorganisms into water, carbon dioxide and methane (Bandopadhyay et al., 2018). The definition of biodegradable plastics does not depend on the source of a polymer but is rather linked to its molecular structure. Biodegradable material formation could either be based on fossil fuels or plant biomass (i.e., corn starch, sugarcane or cellulose). "In other words, 100%

biobased plastics may be non-biodegradable, and 100% fossil-based plastics can biodegrade." (European Bioplastic 2020, https://www.european-bioplastics.org/bioplastics/). Hence, Bio-based and Biodegradable are not synonymous. In Europe, bioplastic refers to plastics that are characterised with both bio-based and biodegradable features (Riggi et al., 2011). We present some commonly used plastics and their classifications in Figure 1.3, hoping to clear up any confusion surrounding the definitions of bio-based plastic, biodegradable plastic and bioplastic. The term biodegradability is often misused by commercial retailers and producers. Materials that are conventional petroleum-based plastics (i.e., PE, PVC) mixed with additives that initiate the "oxo-", "hydro-", "chemo-", or "photo-"degradable processes of conventional materials are defined as "Pro-oxidant Additive Containing plastic (PAC)" (Hann et al., 2017; Koutny et al., 2006). PAC materials can quickly fragment into smaller pieces, but they do not break down at the molecular or polymer level (Hann et al., 2017). Thus, PAC materials are not considered to be biodegradable materials (Narayan, 2017; Siwek et al., 2019). For this thesis, we will only focus on biodegradable material.

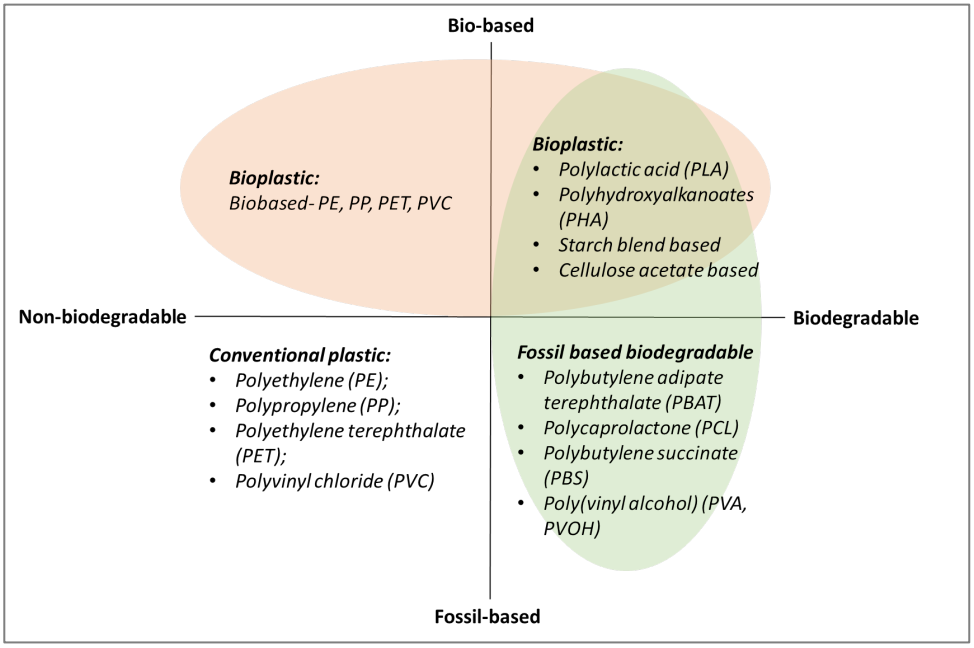

Figure 1.3 *Diagram indicating fossil-based plastic, biodegradable plastic, bio-based plastic and bioplastic. Data from (Riggi et al., 2011) and European bioplastic 2020.*

Recently, the rising concerns surrounding plastic pollution have led to a booming market for biodegradable plastic film. Research concerning biodegradable plastic film began in the early 1970s and remains a significant topic of research (Albregts and Howard, 1972;

Brodhagen et al., 2015; Otey et al., 1974). Since the 1990s, biodegradable materials have been increasingly used in agricultural fields in order to improve environmental conditions as well as to provide better end-of-life options for agricultural plastic waste (Blackwell, 2011; Siwek et al., 2019). However, there are still many concerns regarding the development of biodegradable agricultural materials. Firstly, biodegradable plastic performs poorly under field conditions. According to current international standards, biodegradable plastic films should (1) be ≥ 90% biodegraded into CO_2, H_2O and biomass within 2 years or less; and (2) not contain heavy metals or elicit any ecotoxicological effects from residues that remain in the environment (ASTM, 2003; Briassoulis and Degli Innocenti, 2017; Hayes and Flury, 2018; ISO 17556, 2012). Unfortunately, most of the biodegradable films on the market can only meet the biodegradation requirements under laboratory conditions due to the fact that biodegradation rates are highly dependent on soil water and temperature in the field. For example, polylactic acid (PLA) is one of the most promising materials used for mulching film due to its high resistance to UV radiation, which is very important during the growing season. However, PLA requires temperatures between 55 and 60 °C to degrade, which is impossible in normal field conditions, not to mention after it is buried and incorporated into the soil profile (Guerrini et al., 2017; Siwek et al., 2019). Secondly, there is still no strict regulation of the market. Many products currently on the market are made by adding low levels (1-2%) of oxo, organic or photo additives to conventional plastics (PE, PP, PS). These products are then labelled as "(partially) biodegradable materials" by referencing a standard test method that doesn't require any further details from the producers. However, the truth is that these products can often only be quickly fragmented into smaller plastic pieces and not 'biodegraded', which essentially means that even more plastic fragments are created (Narayan, 2017). The third barrier is the high cost, which prevents the wide use of biodegradable film. According to a report by Oeve et al. (2017), starched blends of plastic cost between 2000 and 4000 euro per ton and PLA plastic is around 2000 euro per ton, while LDPE is about 1250-1450 euro per ton. Scientists and policy makers estimate that traditional LDPE film could end up being more expensive once recycling is taken into consideration. However, when it comes to reality, biodegradable films are just simply more expensive from the perspective of the farmer (Goldberger et al., 2013). Last but not least, the major concern is its ecological impact on terrestrial ecosystems. "Degraded mulch products can be invisible to the naked eye, but out-of-sight does not mean they are safe", stated by Sintim and Flury (2017). With the growing concern for plastic pollution, especially microplastics in soil, biodegradable material in the agricultural sector has received extensive attention. Extensive literature related to the breakdown of biodegradable material in laboratory conditions and the effects of biodegradable films on vegetable, crop and fruit production have been published (Kasirajan and Ngouajio, 2012; Martín-Closas et al., 2017; Moreno and Moreno, 2008). Unfortunately, there is yet very little relevant research on the effects of bio-microplastics on soil-plant systems and ecosystem functioning. Given the fact

that the so-called biodegradable materials currently available on the market tend to break down into smaller plastic particles rather than completely biodegrade, which leads to the accumulation of bio-microplastic in soils, it is vitally important that more scientific attention be focused on the effects of bio-microplastic accumulation on soil quality (Sintim et al., 2019).

1.3 Microplastics in agricultural soils

Carpet-bombing propaganda often portrays plastic pollution as mounds of plastic accumulating in fields, along shorelines or in landfill sites. But this is not an accurate picture of the plastic problem. Social media tends to grab your attention by using "jaw-dropping" figures and emotionally spurring pictures. Plastic waste has always been a headache to deal with because its distribution in the environment is usually very complicated. Plastic debris is often sparsely dispersed in the ocean or buried deep in soils, making it hard to find much less recycle (Figure 1.4). Once it enters the environment, plastic debris gradually degrades into smaller pieces, such as microplastics (< 5mm) and nanoplastics (< 1 μm) (Barnes et al., 2009; Gaylarde et al., 2021; Thompson et al., 2004). In general, environmentally occurring microplastics fall into one of two categories: primary and secondary. Primary microplastics are industrially manufactured plastics that are used in personal care products like cosmetics or made into raw virgin plastic pellets for textile products. Secondary microplastics refer to plastic particles that originate from larger particles broken down by solar radiation, mechanical processes or even from clothing fibres (Fendall and Sewell, 2009; GESAMP, 2015). No other pollutants have ever been so ubiquitous or persisted like microplastics. These tiny compounds can be detected everywhere from bottomless marine systems to remote mountain catchments, from low-lying agricultural fields to the Alps, from secluded beaches to the Arctic, from pet food and animal faeces to human stools and placentas (Allen et al., 2019; Bergmann et al., 2019; Huerta Lwanga et al., 2017; Ivar do Sul and Costa, 2014; Mohajerani and Karabatak, 2020; Ragusa et al., 2021; Schwabl et al., 2019; Zhang et al., 2019b).

So, you may now have a good understanding of what microplastics are but you may still wonder why microplastic pollution has become one of the most widespread anthropogenic threats to terrestrial ecosystems (Barnes et al., 2009). Briefly, there are two main reasons: (1) microplastics are chemically inert and present relatively large specific surface areas, which allows them to sorb and enrich environmental pollutants, thus posing threats to indigent microbial communities and soil health; and (2) the small sizes of microplastics means that they can be ingested by micro-mega sized animals, transferred into food chain, thus threating food safety (Andrady, 2011; Huerta Lwanga et al., 2017; Rillig, 2012).

Scientific focus on microplastics was initiated by Thompson et al. (2004) who reported on the fate of plastic debris lost in the sea and on microscopic plastic fragments that accumulated in the pelagic zone and sedimentary habitats. Since then, the fate and ecological effects of microplastics in aquatic/marine systems have gained more and more

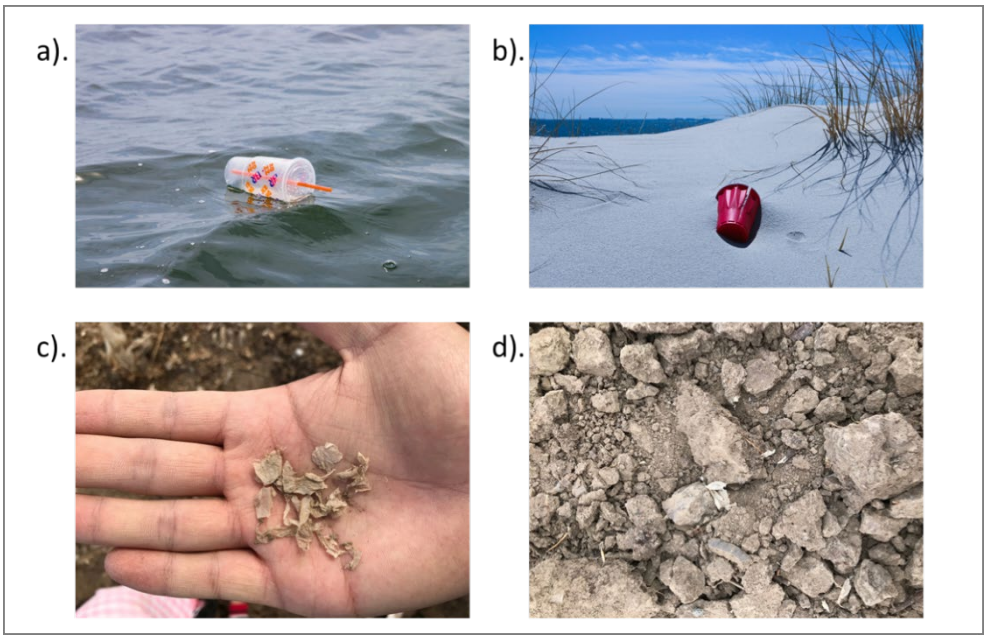

Figure 1.4 *Plastic residues scarcely distributed. a) and b) are taken by Brian Yurasits, free copyrights; c) and d) are taken by the author of this thesis.*

Attention due to the fact that aquatic/marine systems are considered to be the end points for plastic debris (Andrady, 2011; Ashton et al., 2010; Wright et al., 2013). However, this idea fails to address the fact that terrestrial ecosystems are in fact the biggest sink and source of plastic debris and microplastics. Nizzetto et al. (2016) estimated that all farmlands in the EU received a yearly input of 63 000−430 000 tons of microplastics, while all farmlands in North American received 44 000−300 000 tons of microplastics, the total amount of microplastics in the farmlands of these two regions has already exceeded the estimation of the total accumulation of microplastics in the surface water of oceans globally (93 000-236 000). Agricultural soils receive both primary and secondary microplastics via various pathways, i.e., wastewater irrigation, compost, wind transportation and plastic mulching (Corradini et al., 2019b; Rezaei et al., 2019; Van den Berg et al., 2020). Wastewater contains primary plastics from personal care products and secondary microplastics from washing machines along with several others (Barnes et al., 2009; Ziajahromi et al., 2017). Plastic mulch films that are left in fields can fragment into secondary microplastics due to UV

radiation, ploughing, diurnal temperature variations or a combination of any of these things (Briassoulis et al., 2004; Huang et al., 2020). In addition, rubber tire abrasion, landfills and atmospheric deposition all have the high probability of releasing microplastic pollution in soil (Allen et al., 2019; Chae and An, 2018). Considering the huge amount of microplastics that have accumulated in the terrestrial ecosystems, more attention needs to be paid to the microplastic in soils.

One of the main barriers hampering the investigation of microplastics in terrestrial ecosystems is the lack of effective methods to extract and quantify microplastics in soil (Huang et al., 2020; Rillig, 2012). Unlike aquatic systems, soil is a complex organo-mineral matrix, it contains not only a soil-sand mixture, but also organic residues, soil organisms, etc., making the microplastic extraction procedures for soil much more complicated than for aquatic systems. The situation has changed since 2017, more and more new identifying and quantifying methods have been established based on heating methods, attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), micro-FT-IR imaging, and Raman spectroscopy (Beriot et al., 2021; Corradini et al., 2019a; Mintenig et al., 2017; Piehl et al., 2018; Scheurer and Bigalke, 2018b; Zhang et al., 2018). Using these methods, more and more field surveys for measuring the occurrence of microplastics in agricultural fields have been conducted. Van den Berg et al. (2020), using a floatation method, found 18 000 to 32 070 microplastics·kg^{-1} soil in Spanish agricultural soils that received sewage sludge treatment. Corradini et al. (2019b) found 1.1-3.5 microplastics·g^{-1} soil in Chile. Liu et al. (2018) found 78.0 microplastics·kg^{-1} soil in the top 0-3 cm soil layer and 62.5 microplastics·kg^{-1} soil in the 3-6 cm soil layer in vegetable fields in the suburbs of Shanghai, China. However, in contrast to the rising evidence, the data and information concerning the ecological impacts of microplastics on agricultural soils remain inadequate (Mohajerani and Karabatak, 2020; Rillig, 2018; Rillig and Bonkowski, 2018).

After entering soils, microplastics have the potential to threaten soil health (Figure 1.5). The “whistle-blower” for microplastics in agricultural soils is Rillig (2012) who expressed his concerns surrounding microplastics in agricultural soils and suggested that even though soil and aquatic systems are different ecosystems, some of the same principles could still be extrapolated to terrestrial ecosystems, such as microplastics ingested by multiple organisms, release of additives/plasticizers added during manufacturing, absorbing agrichemicals and then acting as vectors (Koelmans et al., 2013; McCormick et al., 2014; Nizzetto et al., 2016). After raising the concerns for microplastics in soil, Rillig and his team conducted a series of experiments related to the ecological effects of microplastic on soils. For example, Maass et al. (2017) and Rillig et al. (2017b) found that microplastics were able to be transported by earthworms. de Souza Machado et al. (2019) conducted two pot experiments and revealed that microplastics are capable of shifting soil biophysiochemical

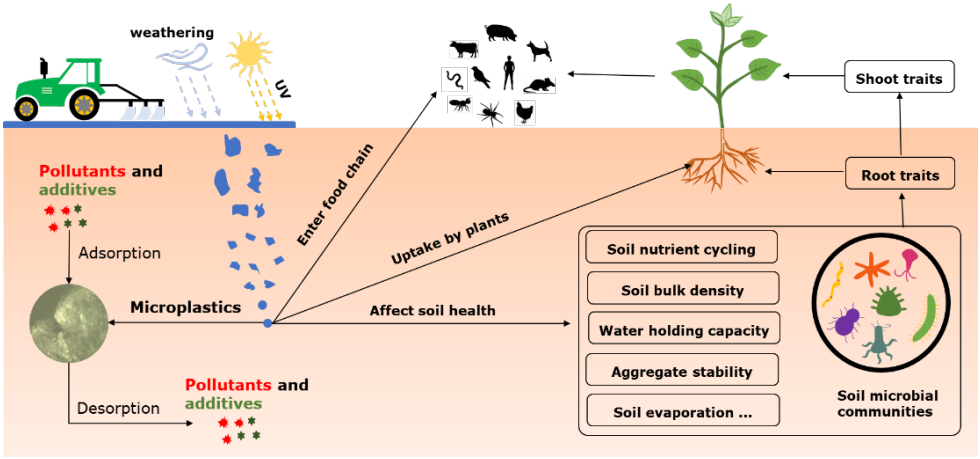

Figure 1.5 *Potential threats of microplastics to agricultural soils.*

properties and affect plant growth. In addition to experiments, Prof. Rillig also wrote profound review papers concerning the long-term implications of microplastic pollution, which has been extremely valuable and shed light on further research (Rillig, 2020; Rillig and Bonkowski, 2018; Rillig et al., 2019). Prof. Violette Geissen is also one of the first soil scientists who focused on microplastics in the agroecosystem. With her team, they established efficient microplastic quantification methods (Corradini et al., 2019a; Zhang et al., 2018), investigated the effects of microplastics on plant growth and soil biophysiochemical properties (Qi et al., 2020b; Qi et al., 2020c; Qi et al., 2018), examined microplastic interaction with agrochemicals (Beriot et al., 2020; Yang et al., 2018), and investigated microplastic interactions with earthworms (Huerta Lwanga et al., 2016a, b). Along with these scientists, other soil scientists have also made tremendous contributions to reveal the fate of microplastics in terrestrial ecosystems. Li et al. (2020c) found efficient uptake of microplastics by wheat (*Triticum aestivum*) and lettuce (*Lactuca sativa*) via crack-entry. Liu et al. (2017) reported 7% and 28% (w/w weight ratio between microplastics to soil) increased soil nutrient content. Fei et al. (2020) found that microplastics of PE and PVC inhibited fluorescein diacetate hydrolase activity and reduced the richness and diversity of the bacterial communities. Even though the effects of microplastics in soil plant systems have received increasing attention in recent years, the existing information is still scant. A thorough understanding of the potential threats posed by microplastics in soil-plant systems remains a challenge and requires urgent scientific research.

1.4 Research scope and research objectives

Although the use of LDPE-based plastic mulching films has brought countless benefits to agriculture, it has also brought unrecoverable plastic pollution to agricultural fields. Biodegradable mulching films were invented as an alternative to traditional LDPE mulching films in an attempt to alleviate the increasing plastic pollution. Unfortunately, current biodegradable products tend to breakdown into smaller plastic fragments rather than fully biodegrade in fields (de Souza Machado et al., 2018a; Whitacre, 2014). Both conventional LDPE-based films and biodegradable films are contributing to microplastic accumulation in soil. A thorough investigation is needed, paying particular attention to fragmentation of agricultural plastic residues from macro- and microplastics (Sintim and Flury, 2017). Although microplastic pollution in agricultural soils has received increasing attention, the impacts of microplastics on plant growth and soil quality are still limited (Rillig, 2012; Rillig et al., 2017a; Rillig et al., 2019). Therefore, this PhD study aims to strengthen the understanding of plastic residues in agricultural fields and to assess the potential impacts of microplastics on soil-plant systems. The research objectives are as follows:

1. Investigate plastic residues and microplastic pollution in different farming systems under long-term plastic mulching - a case study in Northwest China;
2. Elaborate the impacts of two types of microplastics, based on traditional low-density polyethylene (LDPE-MPs) and biodegradable plastic film (Bio-MPs, 85% PBAT, 10% PLA and 5% calcium carbonate), on plant growth;
3. Study the effects of LDPE-MPs and Bio-MPs on soil labile carbon fractions and nitrogen cycling;
4. Interpret the responses of rhizosphere microbe communities to LDPE-MPs and Bio-MPs pollution;

1.5 Thesis outline

This PhD thesis comprises 6 chapters. Chapter 1 has given an overview of plastic mulching and microplastics in agricultural soils and defined research objectives. Results of the field observations were presented in Chapter 2; net house experiments and laboratory analysis were presented in Chapters 3 to 5. In Chapter 6, we synthesized all the results of the current thesis and explored the potential implications of this research. The outline of this PhD thesis is summarized in Figure 1.6.

Chapter 2 describes the accumulation and distribution of plastic residues in agricultural soils under two different farming systems with long-term plastic mulch history in Northwest

China. Characteristics of plastic residues in 0-30 cm of agricultural soils were analysed. The most relevant factors for the different distribution patterns of plastic residues were analysed using observed data and face-to-face interviews with the famers.

After identifying the occurrence of LDPE microplastics in soil, we started focusing on the effects of microplastics on plant growth, soil carbon and nitrogen dynamics as well as the rhizosphere microbial community in the soil-plant system. In the meantime, using biodegradable material as an alternative for traditional LDPE mulching film has drawn increasing attention. Considering the fact that biodegradable materials tend to generate

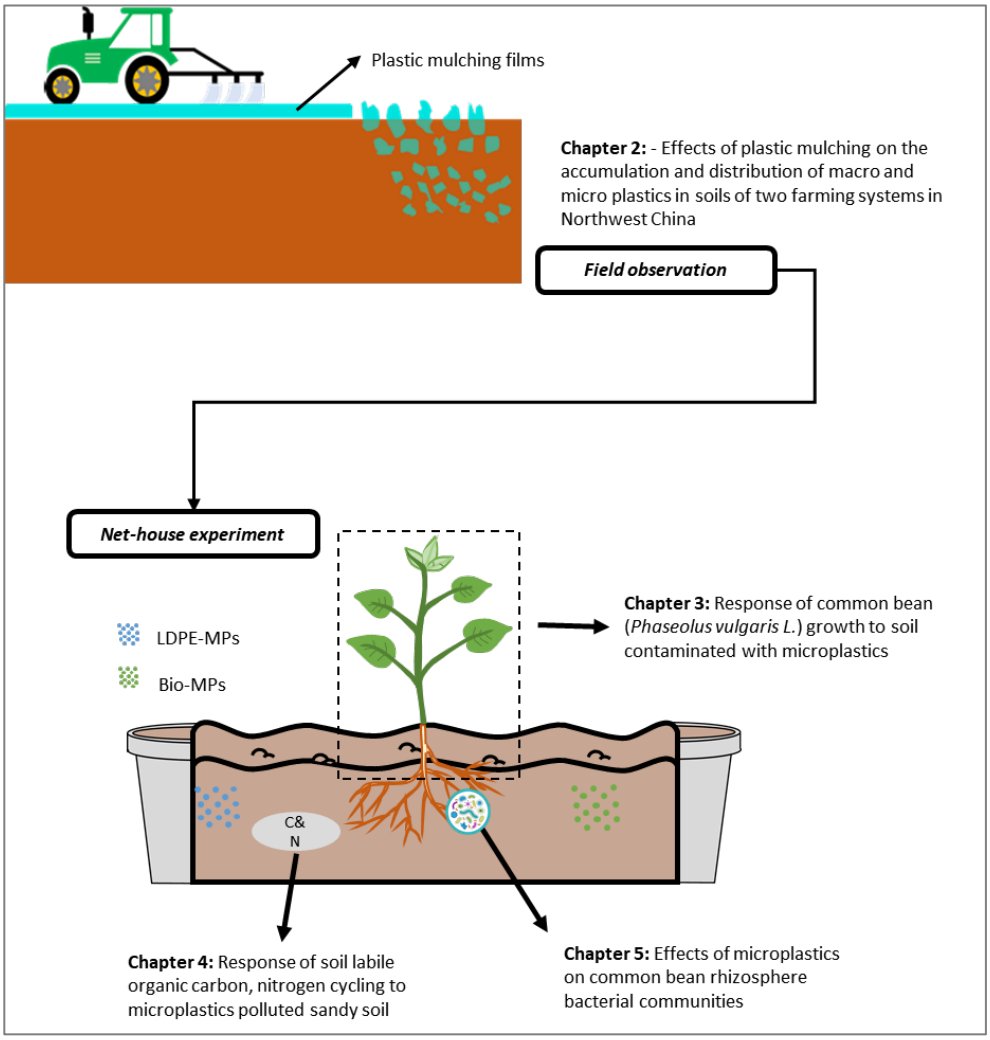

Figure 1.6 *Outline of this PhD thesis.*

bio-microplastics in field conditions and the toxicity and effects of these bio-microplastics on the soil-plant systems during the degradation process remains unclear, we decided to incorporate biodegradable microplastics into our study. The biodegradable plastic pellets were obtained from a local factory.

Chapter 3 investigates the responses of plant growth to microplastic polluted soil using the common bean (*Phaseolus vulgaris L.*) as the model plant. Sandy soils were polluted with two types of microplastics (LDPE and biodegradable) at a range of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w dry soil weight). A suite of proxies of plant shoot and root performance were analysed.

Chapter 4 further explores the effects of microplastic pollution on soil organic carbon fractions and soil nitrogen cycling based on chapter 3.

Chapter 5 investigates the effects of microplastics on soil rhizosphere microbe communities.

Chapter 6 summarizes the major findings of this research and places them in a broader context, combining the current knowledge on microplastics with the findings of this thesis. This chapter ends with implications, recommendations and outlooks for future research regarding microplastics in soil-plant systems.

1.6 Study area (Chapter 2)

The field observations were conducted in North-western China, which has a dry climate and low precipitation (Figure 1.7). Two typical rural regions which both have a long history (dating back to the mid-1980s) of plastic mulching application. Two different farming systems were selected for the study.

The first study region (S1, N107.2°, E35.68°) is in Wutong Village, Shangxiao Township, Zhenyuan County, Qingyang City of Gansu, with the soil type Gypsisols. S1 is a Loess hilly-gully landscape where double or triple cropping per year are carried out on small-scale farmlands (usually smaller than 1 ha according to farmers) and low levels of agricultural mechanization are used. The second region selected (S2, N 43'26"-45'20", E 84'58"-86'24) is in the Shihezi reclamation area, Xinjiang Province, with the soil type Chernozems. S2 is an alluvial plain landscape with only a single cropping per year on large-scale farmlands (larger than 3 ha per field) where high levels of agricultural mechanization are used. The outdoor net-house experiment was performed at Unifarm, Wageningen University and Research.

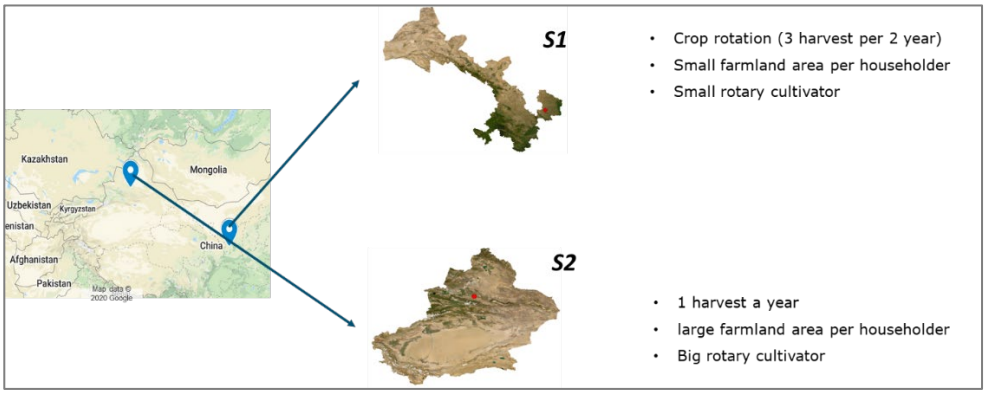

Figure 1.7 *Study area in North-western China.*

2. Effect of plastic mulching on the accumulation and distribution of macro and micro plastics in soils of two farming systems in Northwest China

Inappropriate disposal of the plastic mulching debris could create macroplastics (MaPs) and microplastics (MPs) pollution in agricultural soil. To study the effects of farming systems on accumulation and distribution of agricultural plastic debris, research was carried out on two farming systems in Northwest China. Farming in Wutong Village (S1) is characterized by small plots and low-intensity machine tillage while farming in Shihezi (S2) is characterized by large plots and high-intensity machine tillage. In September 2017, we selected 6 fields in S1, 3 fields with 6-8 years of continuous plastic mulching (CM) as well as 3 fields with over 30 years of intermittent mulching (IM). In S2, we selected 5 cotton fields with 6, 7, 8, 15 and 18 years of continuous mulching. In both regions, MaPs and MPs from soil surface to 30 cm depth (0-30 cm) were sampled. The results showed that in S1, MaPs mass in fields with 6-8 years CM (i.e. 97.4 kg·ha^{-1}) were significantly higher than in fields with 30 years IM (i.e. 53.7 kg·ha^{-1}). MaPs in size category of 10-50 cm^2 accounted for over 40% (46.9% in fields of CM and 44.5% in fields of IM) of total collected MaPs number. In S2, MaPs mass ranged from 43.5 kg·ha^{-1} to 148 kg·ha^{-1}. MaPs in size category of 2-10 cm^2 account for 41.1% of total collected MaPs number while 0.25-2 cm^2 accounted for 40.6%. MPs in S1 were mainly detected in fields with over 30 years of intermittent mulching (up to 2200 particles·kg^{-1} soil), whereas in S2 were detected in all fields (up to 900 particles·kg^{-1} soil). The results indicated farming systems could substantially affect the accumulation and distribution of agricultural plastic debris. Continuous plastic mulching could accumulate higher mass of MaPs than intermittent plastic mulching. High-intensity machine tillage could lead to higher fragmentation of MaPs and more severe MPs pollution. These results suggest that agricultural plastic regulations are needed.

Based on:

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2.1 Introduction

Plastic mulching is a widespread agricultural practice in arid and semi-arid agricultural areas. Plastic mulching has been proved to be beneficial in conserving water (Ingman et al., 2017), increasing surface soil temperature, modifying microclimates (Tarara, 2000), reducing weeds, discouraging pests (Díaz-Hernández and Salmerón, 2012), and improving crop productivity (Scarascia-Mugnozza et al., 2011). Plastic mulching has experienced a rapid growth in China since it was first introduced in the 1980s (Cai et al., 2013; Ma et al., 2018), growing from 6000 tons used on 117,000 hectares of land in 1982 to about 1.5 million tons used on 18.4 million hectares of land in 2016 (NBSC, 2020). Due to the high labour-intensity and costs of removal, plastic films were usually left in fields after crops were harvest. There is a growing concern about the impacts of these discarded plastics on soil health and food security (Blanco et al., 2018; Briassoulis et al., 2013a).

Macro-size plastics (MaPs) in agricultural fields have been reported could significantly reduced the gravimetric water mass and bulk density of soils, decreases macro-pores and alters soil water distribution(Jiang et al., 2017). Zhang et al. (2017) indicated that soil enzyme activity and soil fertility could be significantly decreased when plastic debris mass reached up to 450 $kg \cdot ha^{-1}$. Plastic debris may act as potential pesticide vehicles in soil and lead to unpredictable migration of pesticides in the soil matrix (Ramos et al., 2015; Teuten et al., 2009). Furthermore, agricultural plastic mulching has been reported as a source of microplastics (MPs) in terrestrial environment (de Souza Machado et al., 2018a; Huang et al., 2020; Scheurer and Bigalke, 2018a). Rillig (2012) reported that MPs could be ingested by soil mesofauna and microfauna and thus, bio-accumulate in the food chain. MPs could also negatively affect the growth and survival rate of soil organisms and influence soil function (Cao et al., 2017; Huerta Lwanga et al., 2016a). In addition, plastic debris could be easily migrated into surrounding ecosystems (Rezaei et al., 2019; Vermeiren et al., 2016). It has been widely reported that plastic debris poses considerable threats by choking and starving wildlife (Barnes et al., 2009) and by transferring and releasing chemicals into aquatic ecosystems (Teuten et al., 2009). Hence, it is of vital importance to monitor the dynamic of plastic debris.

Previous research that document agricultural plastic debris accumulation mainly attributed it to the mulching time. Ma and Yang (2013) reported that plastic debris accumulated in Xinjiang fields at a rate of 27.6, 30.8 and 42.3 $kg \cdot ha^{-1}$ with < 10, 10-20 and 20-30 years of mulching, respectively. He et al. (2018) observed that the annual rate of plastic debris accumulation was 15.69 $kg \cdot ha^{-1}$ in Xinjiang. However, other factors such as the size of plastic debris, continuous or intermittent mulching and debris recycling activities could also affect the accumulation of MaPs in agricultural soils (Briassoulis et al., 2004; Ma and Yang, 2015;

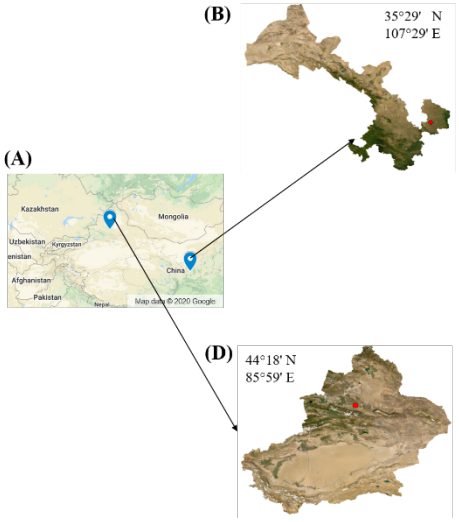
Qi et al., 2020a; Steinmetz et al., 2016). In different farming regions, different farming practices (mechanical tillage intensity, plastic mulching techniques and etc.) were applied due to the local soil type and climate, thus resulting in different accumulation patterns of plastic debris. Yan et al. (2008) conducted a field observation in Xinjiang (Northwest China) and found that highest amount of MaPs reached up to 308 $kg \cdot ha^{-1}$, MaPs were mainly concentrated in 0-10 cm soil. They also found that 80% of MaPs detected in their study were in the size category of 1-25 cm^2. Li et al. (2017) conducted a field observation in Qingdao (Middle China) and found the amount of MaPs in agricultural fields was ranging between 11-69 $kg \cdot ha^{-1}$. MaPs were mainly concentrated in 0-20 cm soil. However, in their study, the detected MaPs were mainly in the size category of > 100 cm^2. Therefore, farming system plays an important role in agricultural plastic pollution. Unfortunately, the effects of different farming systems on plastic accumulation remained inadequate addressed.

In this current work, we assumed that different farming systems could affect the accumulation and distribution of plastic debris in agricultural soil. We hypothesized that (1). Under the same farming system, continuous plastic mulching could accumulate more MaPs mass than intermittent plastic mulch; (2) farming system of higher mechanical intensity could lead to higher fragmentation of MaPs and create more MPs than farming system of lower mechanical intensity. To test our hypothesis, we selected two regions in Northwest China that both have a long history (dating back to the mid-1980s) of plastic mulching application but with different farming systems (Figure 2.1). First study region is characterized by small-scale farmlands with low levels of agricultural mechanization. Second study region is characterized by large-scale farmlands and high levels of agricultural mechanization. We examined the accumulation and distribution of MaPs and MPs in 0-30 cm soil of two study regions. In our paper, MaPs were defined as plastic particles with a size area of > 0.25 cm^2 (which was the smallest MaPs size we collected from field, Supplementary Figure S2.3a). MPs were defined as plastic particles derived from LDPE plastic mulching film with a diameter of < 2 mm and a density smaller than 1 $g \cdot cm^{-3}$ due to plastic mulching was considered as the main source for plastic pollution in the selected two study regions. We hope to provide a basis information for future efforts aimed at controlling and managing plastic pollution in agricultural soils.

2.2 Materials and methods

2.2.1 Study area description

The first study region located in Wutong Village (S1, 35°29' N, 107°29' E), Gansu Province, where the cultivated area is 2 779 ha. S1 is characterized by small-scale farmlands (usually



(C)

Field	Mulching Pattern	Mulching Duration	Area (ha)	Crop (Rotation)
Control_S1	No mulching	--	0.13	Wheat
S1-1	Continuously FF	2012 - 2017	2.27	Maize Monoculture
S1-2	Continuously FF	2010 - 2017	1.87	Maize Monoculture
S1-3	Continuously FF	2012 - 2017	0.53	Maize Monoculture
S1-4	Intermittently FF	Mid-1980s - 2017	0.27	Rape/Wheat/Soybean
S1-5	Intermittently FF	Mid-1980s - 2017	0.13	Maize/Wheat
S1-6	Intermittently FF	Mid-1980s - 2017	0.27	Maize/Soy Bean

(E)

Field	Mulching Pattern	Mulching Duration	Area (ha)	Crop (Rotation)
Control_S2	No mulching	--	2	Fallow
S2-1	Continuously FCDI	2012 - 2017	5.3	Cotton
S2-2		2011 - 2017	4	
S2-3		2010 - 2017	3.3	
S2-4		2003 - 2017	7.2	
S2-5		2000 - 2017	7.2	

Figure 2.1 *Sampling site. (A). Field measurements were conducted in two regions in Northwest China (Map data @ 2020 Google); (B). Study site Gansu (S1) was characterized by small plots and low intensity tillage; (C). Mulching pattern, duration, field area and crop rotation of the selected fields in S1; (D). Study site Xinjiang (S2) was characterized by large plots, lower plastic input and intensive machine tillage; (E). Mulching pattern, duration, field area and crop rotation of the selected fields in S2.*

smaller than 1 ha according to farmers) and low levels of agricultural mechanization. Tillage is performed using small rotary cultivators at a depth of 30 cm and harvesting is mainly done manually. In S1, farmers predominantly practiced full film flat mulching (FF, Figure 2.2A). The plastic film was transparent and made from low-density polyethylene (LDPE) (Supplementary Table S2.1) and there was an annual usage of 150 kg·ha^{-1} in this area. Plastic mulching had been intermittently applied to the fields over a span of 30 years at 3- or 4-year intervals. Maize (*Zea mays L.*) was the main crop for which plastic mulching was used. After the maize had been sown, the land was covered with the plastic much. The maize plants grew through the plastic mulch. After harvesting the maize, the plastic films were manually removed from the soil before preparing the land for the next crop. The common practice in study area S1 was to rotate maize with soybean (*Glycine max*), oilseed rape (*Brassica napus*) and winter wheat (*Triticum aestivum L.*). For these other crops, plastic mulching was not used. The common cultivation pattern was three harvests every two years. However, in recent years, some farmers have switched to a monoculture of maize due to its increasing economic value. In S1, we selected 6 fields to investigate the impacts of monoculture and crop rotation on agricultural plastic debris accumulation and distribution (Figure 2.1A). Fields S1-1 (contact: Shangzhong Li), S1-2 (contact: Yi Dang) and S1-3 (contact: Lei Wang) were monocultured with maize, with 6, 8 and 6 years of continuous mulching, respectively.

In fields S1-4, S1-5 and S1-6, crops were rotated. In S1-4 (contact: Limin Wang), the crop rotation was oilseed

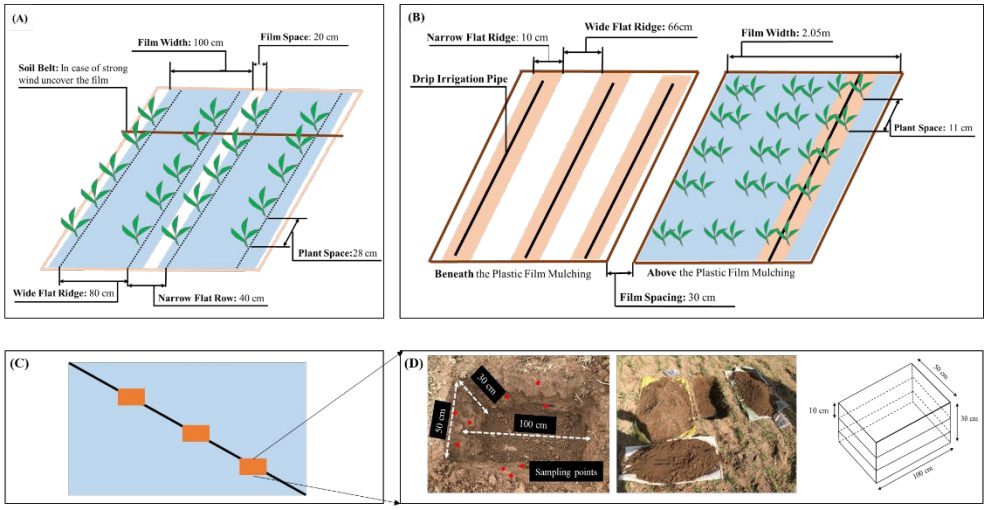


Figure 2.2 *Schematic of plastic mulching patterns; (A) Full film flat mulching (FF) in S1; (B) Flat cover with drip irrigation under plastic film (FCDI) in S2; (C). Sampling quadrats location in the field; (D). Sampling activity, the red dots represent the sampling points for extraction of Microplastics.*

rape (Early September 2015 to mid-June 2016), Winter Wheat (late September/early October 2017 to the end of May 2018), and Soybean (mid-June 2017 to late September 2017). In S1-5 (contact: Sanzhi Li), the crop rotation was Maize (mid-April 2016 to mid-September 2016) and Winter Wheat (late September/early October 2017 to the end of May 2018). In S1-6 (contact: Limin Wang), the crop rotation was Maize (mid-April 2016 to mid-September 2016), left Fallow (mid-September 2016 to mid-June 2017), and Soybean (mid-June 2017 to late September 2017).

The second study region located in Shihezi City (S2, 44°18' N, 85°59' E), Xinjiang, where the cultivated area is 971 301 ha. S2 is characterized by large-scale farmlands (larger than 3 ha per field) and high levels of agricultural mechanization. Tillage is performed using large rotary cultivators at a depth of 30 cm and harvesting is done using a cotton picker. In S2, the mulching pattern was flat cover combined with drip irrigation (FCDI, Figure 2.2B). The use of plastic mulching coupled with irrigation was initiated in the 1990s. The annual usage of plastic film in S2 was 60 kg·ha^{-1}. The plastic film was transparent and made from LDPE & LLDPE (linear low-density polyethylene) (Supplementary Table S2.1). We selected 5 fields with 6 (contact: Yu Liu), 7 (contact: Yu Liu), 8 (contact: Gongmao Wang), 15 (contact: Jihong Shi), and 18 (contact: Jiancheng Liu) years of continuous FCDI mulching (Figure 2.1B). All the fields were planted with the same type of cotton cultivar and the same fertilization practices

were followed. After harvesting, the plastic films were machinery removed from the fields along with the cotton stalks.

The farming system chosen in each region was representative of the typical situation for the local farmers. Both regions have a temperate continental climate. The climate data from 2017 of two study region and the soil information are shown in Supplementary Table S2.1. The climate data was recorded by a local weather station. For this research, we assumed that the mixing procedure mainly depended on the different farming systems prevalent in the two study regions. Hence, only farming systems and crops from these regions were taken into account in current work.

2.2.2 Field sampling

MaPs were manually collected using a quadrat sampling method. In each selected field, on the diagonal line (Figure 2.2C), we randomly dug 3 quadrats (each quadrat was 100 cm long, 50 cm wide and 30 cm deep and covered two crop rows, Figure 2.2D). Each sampling quadrat was then separated into three depth layers: 0-10 cm, 10-20 cm and 20-30 cm. The entire soil mass from each layer were then put onto a "flat polypropylene (PP) wire weaved mesh sheet" (Supplementary Figure S2.1). To prevent further fragmentation of the MaPs during the collection process, visible MaPs were gently picked out by hand from the entire mass of each layer of the sampled quadrat. Each layer of the sampling quadrat was carefully checked three times. The collected MaPs from each layer were then stored in a PP bag. All the collected MaPs samples then transferred to the laboratory for further analysis. Totally, resulted in 54 MaPs samples in S1 (6 fields × 3 quadrats × 3 layers) and 45 MaPs samples in S2 (5 fields × 3 quadrats × 3 layers).

2.2.3 Macroplastic quantification and residual ratio

In the laboratory, all MaPs were cleaned thoroughly. First, plant roots, soil, sand, etc. were separated manually from the MaPs. Next, MaPs were unfolded and washed with tap water three times in a PP basin (solid colour) until the films were transparent. Then, the cleaned MaPs from each layer of soil quadrat were stored in a 500 mL glass beaker that was filled with 300 mL of tap water. The beaker was put into an ultrasonic cleaner (KQ 3200DA, KUNSHAN ULTRASONIC INSTRUMENTS CO., LTD) for 1h to remove any fine sands attached to the MaPs. The MaPs then were stored in a PP mesh bag (12*15 cm, diameter 1 mm) and air-dried for 2-days. The collected MaPs were present in arbitrary shapes, i.e. curved together and flake shape (Supplementary Figure S2.2). All the collected MaPs were gently

spread and measured by using graph paper. The smallest size of collected MaPs was measured 0.25 cm^2 (Supplementary Figure S2.3a). We separate the plastic debris into five size groups: 0.25-2 cm^2, 2-10 cm^2, 10-50 cm^2, 50-100 cm^2, and > 100 cm^2 (Supplementary Figure S2.3b-f). For each size group, MaPs were weighed using an analytical balance (METTLER AE 200, METTLER AE 200, MARSHALL SCIENCE, accuracy of 0.1 mg) and the number of particles (p) was counted. The concentrations of MaPs were recorded as mass **(**$kg \cdot ha^{-1}$) and number ($p \cdot ha^{-1}$). The mass and number were calculated as follows:

$$(M_i / S) * 100 = \text{Mass } (kg \cdot ha^{-1}) \quad (1)$$

$$(N_i / S) * 100 = \text{Number } (p \cdot ha^{-1}) \quad (2)$$

Where, $\mathbf{M}_i$ (mg) is the total weight of collected MaPs from each of the 10 cm sampling depths. **S** (cm^2) is the surface area of each sampling quadrat. The conversion coefficient from $mg \cdot cm^{-2}$ to $kg \cdot ha^{-}1$ is **100**. $\mathbf{N}_i$ (p) is the total number of collected MaPs from each of the 10 cm sampling depths.

The residual ratio of MaPs per selected field, which referred to as the amount of MaPs found in soil in relation to the total applied biofilm amount, was calculated with the following equation:

$$U*t = \text{Total input} \quad (3)$$

$$\text{Mass / Total input } *100 = \text{Residual ratio (\%)} \quad (4)$$

where, **U** is the annual plastic film usage, S1 is 150 $kg \cdot ha^{-1}$, and S2 is 60 $kg \cdot ha^{-1}$. **t** is the number of years of plastic mulching application. **Mass** is the total weight of MaPs collected from the selected fields. For fields where mulching was used continuously, **t** is equal to the mulching year. However, for the intermittent use of plastic mulching in the S1 region, **t** is assumed to be 7 years, which was calculated using the plastic mulching interval of every 4 years during the 30 years of mulching history.

2.2.4 Microplastic extraction and identification

Soil samples for MPs extraction were collected from the sides of the pits that were dug out of the soil quadrats (Figure 2.2C). For each 10 cm depth, 1 kg of soil sample from three randomly selected sampling points were directly collected in situ and homogenized. The soil samples were extracted using a metal augur and transferred to the laboratory in PP plastic bags (Supplementary Figure S2.4). Once in the lab, the soil samples were air dried in open paper trays in a room without visible plastic materials. The soil was then sieved through a 2 mm steel sieve for the further MPs extraction. A control field had never been applied with plastic mulching was selected in each study region (Contact of control field in S1: Jianjun

Zhang; in S2: Yu Liu). A soil sample from each control field was collected to check whether the PP plastic containers polluted the soil samples with plastic.

MPs extraction was carried out following a float method published by Zhang et al. (2018). This method was specially developed for the extraction of LDPE-MPs. According to Zhang et al. (2018), the recovery rates were > 90% and the lower limit of detection for this method is 20 µm. Before the extraction procedure, the MaPs in the soil samples were collected until no more plastic could be seen with the naked eye. Then, 10 g of the air-dried soil samples were added to 100 ml centrifuge tubes (PP). 50 mL of distilled water was added to each tube and a glass stick was used to stir the soil and water together in order to get a homogeneous suspension. The glass stirrer was rinsed off using distilled water and the water was then collected in the same centrifuge tube. Next, soil samples were spun 4 times using high speed centrifugation (GL-21MC/GL21MC, CENCE XIANGYI, CHINA) at 14 400 g for 10 min to separate the soil particles from the floating materials. The resulting supernatant was filtered using filter paper (pore diameter < 3 µm). After that, 50 mL of distilled water was added again to each centrifuge tube and then placed in an ultrasonic cleaner (KQ 3200DA, KUNSHAN ULTRASONIC INSTRUMENTS CO., LTD) for 2 h in order to isolate any MPs that might have still been adsorbed on soil micro aggregates. The samples were then centrifuged for a 5th time. Finally, the filter papers (pore diameter < 3 µm) with the extracted MPs were dried in an oven (TYPE A 1500-145, KEMA KEUR) at 60 °C to a constant weight and stored in glass Petri dishes for optical inspection. The soil samples from the control fields were also put through the same procedure for extracting MPs.

As a quality control measure, each set of soil samples (n) from each study site (n = 18 in S1, n = 15 in S2) contained three blank samples of distilled water. This measurement was used to account for any contamination which could have occurred inside the lab (Mahon et al., 2017; Scheurer and Bigalke, 2018a). White cotton lab coats were worn during analysis and sample manipulations.

The extracted MPs were inspected using a microscope (Leica wild M3C, Type S, simple light) at 6.4 X Zoom. The MPs collected from each filter were placed on glass slides. The glass slides were then inspected using a microscope and a picture ***"I"*** was taken. In order to get rid of any organic material from the soil samples that might have interfered with the counting, the glass slides were placed gently on top of an electric heating plate. (TYPE A 1500-145, KEMA KEUR) and heated for 5-7s at 130 ℃ in order to melt the MPs. The MPs were transformed into transparent shiny surfaces which could be easily distinguished from soil particles. The glass slides were then inspected again using the microscope and a second picture ***"II"*** was taken. By comparing pictures **"*I*"** and **"*II*"**, the melted MPs could be identified. The smallest microplastic particle detected in our work was 0.49 mm (44 pixels),

calculated by image J, 1 pixel = 0.585/60 mm (microscope at 6.4 X Zoom). The picture of the setup for identification MPs is presented in supplementary Figure S2.5.

2.2.5 Limitation of microplastic extraction method

Only MPs from LDPE or MPs with density < 1 $g \cdot cm^{-3}$ were able to be extracted due to the water reagent. MPs with densities higher than 1 $g \cdot cm^{-3}$ (e.g. PVC 1.45 $g \cdot cm^{-3}$) were not able to be extracted (Nuelle et al., 2014). However, this method provides a validated method for estimating the presence of LDPE-MPs in the soil.

2.2.6 Data analysis

The arcsine square root transformation was applied to the mass and number of MaPs pieces to avoid violating the underlying assumptions of normality. One-way analyses of variances (ANOVAs) were applied to compare the mass and number of MaPs pieces between different fields within the same selected region and different soil layers within the same field, followed by the application of an LSD post hoc test at the $p < 0.05$ level. MaPs mass ($kg \cdot ha^{-1}$) and number ($p \cdot ha^{-1}$) were presented as "means ± standard deviations". MPs that were detected in the fields were presented in raw data in the unit of $p \cdot kg^{-1}$ soil due to the highly random distribution of the particles and no statistical test was performed.

2.3 Results

2.3.1 Accumulation and distribution of macroplastics in selected agricultural fields

In S1, across the 6 selected fields, MaPs number varied from 56.7×10^4 $p \cdot ha^{-1}$ to 264.7×10^4 $p \cdot ha^{-1}$ and MaPs mass varied from 53.7 $kg \cdot ha^{-1}$ to 108 $kg \cdot ha^{-1}$ (Table 2.1). Fields with 6 to 8 years of continuous plastic mulching use (S1-1, S1-2 and S1-3) showed significant higher MaPs number (one-way ANOVA, $F_{5,12} = 20.9$, $p < 0.01$) and MaPs mass (one-way ANOVA, $F_{5,12} = 4.24$, $p = 0.02$) than fields with more than 30 years of intermittent plastic mulching use (S1-4, S1-5), except S1-6, where fields showed similar numbers of MaPs as compared to S1-2. The residual ratios varied from 5.11% to 12.0% across the selected fields (Table 2.2). Fields where continuous mulching was practiced (S1-1, S1-2 and S1-3) showed significantly higher residual ratios as compared to fields with intermittent mulching (S1-4, S1-5 and S1-

6) (Table 2.2, one-way ANOVA, $F_{5,12} = 6.89$, $p = 0.03$). The distribution pattern of MaPs in each 10 cm of 0-30 cm soil layer across the 6 fields in S1 are presented in Table 2.3. The results showed that MaPs were mainly concentrated in the first 0-10 cm soil layer, followed by 10-20 cm, and then 20-30 cm. The number of MaPs in the 0-10 cm layer was significantly higher (one-way ANOVA, $p < 0.01$, Supplementary Table S2.6) than the 10-20 cm and/or 20-30 cm soil layers. However, for the mass of MaPs, there were significant differences (one-way ANOVA, more detail showed in Supplementary Table S2.6) found between soil layers 0-10 cm and 10-20 cm and between soil layers 10-20 cm and 20-30 cm, except for S1-1 and S1-6. In addition, we also compared the number and

Table 2.1 *Macroplastics number and content in 0-30 cm (one-way ANOVA and followed by LSD test at the $p < 0.05$ level).*

Study region	Sampling Site	MaPs number ($\times 10^4$ p·ha^{-1})	MaPs mass (kg·ha^{-1})
S1	S1-1	235±45.8a	105±20.1a
	S1-2	170±40.8b	97.4±22.0a
	S1-3	265±12.9a	108±13.2a
	S1-4	88.0±22.3c	56.1±37.3b
	S1-5	56.7±4.2c	57.1±16.4b
	S1-6	155±58.2b	53.7±12.4b
S2	S2-1	502±201c	43.5±9.3c
	S2-2	650±136c	88.9±12.2b
	S2-3	461±79.1c	80.6±18.6b
	S2-4	2,016±188a	148±28.1a
	S2-5	991±163b	81.1±3.93b

Note. Lowercase letters (a,b,c) indicate significant difference between different selected fields.
In S1: S1-1: 6 years of FF mulching; S1-2: 8 years of FF mulching; S1-3: 6 years of FF mulching; S1-4, S1-5 and S1-6: 30 years history of intermittent FF mulching.
In S2: S2-1: 6 years of FCDI mulching; S2-2: 7 years of FCDI mulching; S2-3: 8 years of FCDI mulching; S2-4: 15 years of FCDI mulching; S2-5: 18 years of FCDI mulching.

mass percentage of MaPs in different size categories (Figure 2.3 and Supplementary Table S2.4). Continuous (S1-1, S1-2 and S1-3) and intermittent (S1-4, S1-5 and S1-6) mulching fields showed similar composition patterns. For MaPs number, size category of 10-50 cm^2 accounted for highest of the total collected MaPs number (46.9% for continuous mulching fields and 44.5% for intermittent mulching fields). Size category of 0.25-2 cm^2 accounted for lowest of the total collected MaPs number (3.55% for continuous mulching fields and 4.20% for intermittent mulching fields) (Figure 2.3A, Figure 2.3B). Significant differences were observed between different size groups (one-way ANOVA, $F_{4,40} = 148$, $p < 0.01$ for continuous mulching fields; $F_{4,40} = 35.9$, $p < 0.01$ for intermittent mulching fields). As for MaPs mass, MaPs in size categories > 100 cm^2 and 10-50 cm^2 contributed highest (34.8% and 35.8% in continuous mulching fields; 42.9% and 34.2% in intermittent mulching fields)

to the total mass while size category of 0.25-2 cm^2 contributed lowest (0.16% for continuous mulching fields and 0.26% for intermittent mulching fields) (Figure 2.3D, Figure 2.3E). Significant differences were observed between different MaPs size groups (one-way ANOVA, $F_{4,40}$ = 217, $p < 0.01$ for continuous mulching fields; $F_{4,40}$ = 28.4, $p < 0.01$ for intermittent mulching fields).

In S2, across the selected fields, MaPs number varied from 461×10^4 p·ha^{-1} to $2\,016\times10^4$ p·ha^{-1} and MaPs mass varied from 43.5 kg·ha^{-1} to 148 kg·ha^{-1} (Table 2.1. Fields exposed to 15 years of plastic mulching use (S2-4) showed significant higher MaPs number (one-way ANOVA, $F_{4,10}$ = 61.7 $p < 0.01$) and mass (one-way ANOVA, $F_{4,10}$ = 17.1 $p < 0.01$) than other selected fields. The residual ratios varied from 7.51% to 21.2% (Table 2.2). The field exposed to 18 years of plastic mulching use (S2-5) showed the lowest residual ratio, which was only significantly lower than field S1-2 (Table 2.2, one-way ANOVA, $F_{4,10}$ = 2.68, $p = 0.09$). The distribution pattern of MaPs in each 10 cm soil 0-30 cm across the 5 fields in S2 are presented in Table 2.4. MaPs were mainly concentrated in the first 0-10 cm soil layer, followed by 10-20 cm and 20-30 cm. For the numbers of MaPs, the significant differences were mainly found between the 0-10 cm and 20-30 cm soil layers (one-way ANOVA, one-way ANOVA, more detail showed in Supplementary Table S2.7). For the mass of MaPs, the significant differences (one-way ANOVA, more detail showed in Supplementary Table S2.7) were found between soil layers 0-10 cm and 10-20 cm and between soil layers 10-20 cm and 20-30 cm, except for S2-1 and S2-5. In S2, we also compared the number and mass percentage of MaPs in different size categories (Figure 2.3, Supplementary Table S2.4). For MaPs number (Figure 2.3C), the highest contributors were size categories of 0.25-2 cm^2 (40.6%) and 2-10 cm^2 (41.1%). The lowest contributor was size category of > 100 cm^2 (1.09%). Significant differences were observed between different groups (one-way ANOVA, $F_{4,70}$ = 18.4, $p < 0.01$). For MaPs mass (Figure 2.3F), the highest contributor was size category of 10-50 cm^2 (36.4%), the lowest contributor was size category of 0.25-2 cm^2 (26.2%). The significant differences between each group were observed (one-way ANOVA, $F_{4,70}$ = 172, $p < 0.01$).

Table 2.2 *Residual ratios of MaPs in Wutong Village, Gansu Province (S1) & Shihezi City, Xinjiang Province (S2).*

Study site	Sampling Site	Mulching Pattern	Input per application ($kg \cdot ha^{-1}$)	Mulching Duration	Total input ($kg \cdot ha^{-1}$)	Collected MaPs ($kg \cdot ha^{-1}$, average)	Residual ratio (%)
S1	S1-1	Continuously FF	150	2012 - 2017	900	105	11.7±2.23a
	S1-2	Continuously FF		2010 - 2017	1200	97.4	8.12±1.83a
	S1-3	Continuously FF		2012 - 2017	900	108	12.0±1.47a
	S1-4	Intermittently FF		Mid-1980s - 2017	1050	56.1	5.34±3.56b
	S1-5	Intermittently FF		Mid-1980s - 2017	1050	57.1	5.44±1.56b
	S1-6	Intermittently FF		Mid-1980s - 2017	1050	53.7	5.11±1.19b
S2	S2-1	Continuously FCDI	60	2012 - 2017	360	43.5	12.1±6.76ab
	S2-2			2011 - 2017	420	88.9	21.2±7.64a
	S2-3			2010 - 2017	480	80.6	16.8±3.87ab
	S2-4			2003 - 2017	900	148	16.5±5.62ab
	S2-5			2000 - 2017	1080	81.1	7.51±0.36b

Note. Total input was calculated using equation 3 and residual ratio was calculated using equation 4. Lowercase letters (a,b,c,d) indicate significant differences between different selected fields within each selected study region ($p < 0.05$, $n = 3$). Removal rate estimated based on farmers' information.

Table 2.3 *Macroplastics number and mass in different soil layer in S1 (one-way ANOVA and followed by LSD test at the p < 0.05 level).*

Sampling Site	Soil Layer	MaPs number $\times 10^4$(p/ha)	MaPs mass kg·ha^{-1}
S1-1	0-10 cm	134±25.1a	70.1±1.98a
	10-20 cm	78.7±37.2a	30.5±15.9b
	20-30 cm	22.7±15.5b	4.26±3.69c
S1-2	0-10 cm	111±38.2a	64.7±12.7a
	10-20 cm	43.3±13.3b	28.2±16.5b
	20-30 cm	16.0±12.2b	4.50±3.19c
S1-3	0-10 cm	173±27.3a	82.3±10.6a
	10-20 cm	69.3±21.9b	17.9±2.25b
	20-30 cm	22.0±11.1c	8.13±7.93b
S1-4	0-10 cm	50.0±10.4a	42.2±19.5a
	10-20 cm	33.3±29.1a	13.0±17.2b
	20-30 cm	4.67±3.06b	0.82±0.77b
S1-5	0-10 cm	30.0±7.2a	33.6±18.4a
	10-20 cm	19.3±2.31a	14.7±1.11ab
	20-30 cm	7.33±5.03b	6.79±6.97b
S1-6	0-10 cm	71.3±17.9a	30.2±5.63a
	10-20 cm	53.3±5.03ab	16.1±1.42b
	20-30 cm	30.7±19.0b	7.30±5.84c

Note. Lowercase letters (a,b,c) indicate significant difference between different layers within same selected field. S1-1: 6 years of FF mulching; S1-2: 8 years of FF mulching; S1-3: 6 years of FF mulching; S1-4, S1-5 and S1-6: 30 years history of intermittent FF mulching.

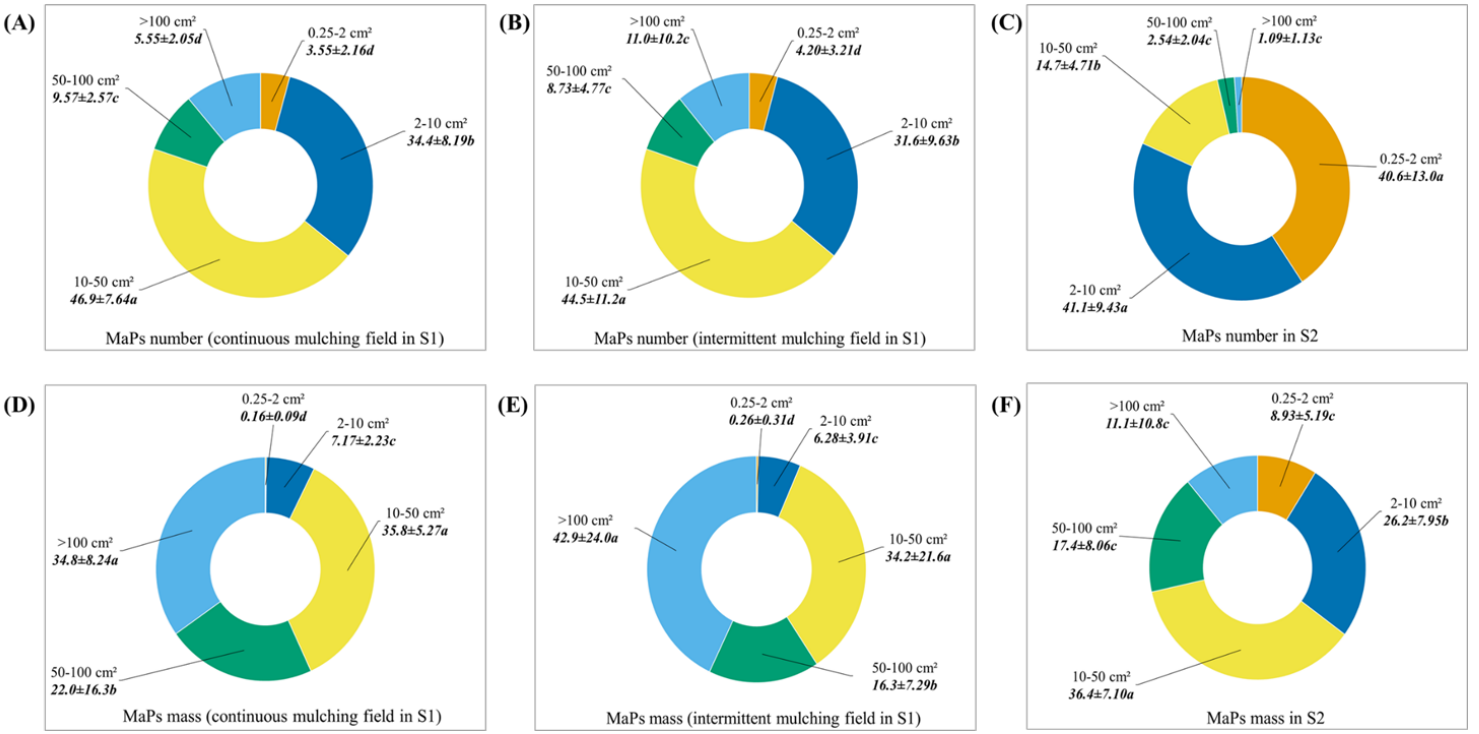


Figure 2.3 The percentages of number and mass of MaPs in different size categories. (A). The number percentages of MaPs in different size categories in 6-8 years continuous mulching fields (S1-1, S1-2 and S1-3) in S1; (B). The number percentages of MaPs in different size categories in over 30 years of intermittent mulching fields (S1-4, S1-5 and S1-6) in S1; (C). The number percentage of MaPs in different size categories in S2; (D). The mass percentage of MaPs in different size categories in continuous mulching fields (S1-1, S1-2 and S1-3) in S1; (E). The mass percentage of MaPs in different size categories in continuous mulching fields (S1-4, S1-5 and S1-6) in S1; (F). The mass percentage of MaPs in different size categories in S2. Lowercase letters (a,b,c,d**)** indicate significant differences between different size groups ($p < 0.05$).

Table 2.4 *Macroplastics number and content in different soil layer in S2 (one-way ANOVA and followed by LSD test at the p < 0.05 level).*

Sampling Site	Soil Layer	MaPs number $\times 10^4$(p·ha^{-1})	MaPs mass (kg·ha^{-1})
S2-1	0-10 cm	241±24a	21.4±1.3a
	10-20 cm	159±72.5ab	11.6±4.53b
	20-30 cm	101±44.6b	10.4±5.05b
S2-2	0-10 cm	278±47a	51.7±4.6a
	10-20 cm	216±55.2ab	25.4±8.03b
	20-30 cm	156±40.8b	11.7±5.21c
S2-3	0-10 cm	251±68.2a	52.0±9.07a
	10-20 cm	162±118ab	24.7±14.6b
	20-30 cm	48±29.5b	3.86±3.03c
S2-4	0-10 cm	1,011±185a	78.1±15.2a
	10-20 cm	685±41b	50.9±8.13b
	20-30 cm	320±76c	19.1±11.2c
S2-5	0-10 cm	467±34.5a	49.6±5.00a
	10-20 cm	336±130ab	20.9±9.91b
	20-30 cm	188±87.1b	10.6±4.77b

Note. Lowercase letters (a,b,c) indicate significant difference between different layers within same selected field. S2-1: 6 years of FCDI mulching; S2-2: 7 years of FCDI mulching; S2-3: 8 years of FCDI mulching; S2-4: 15 years of FCDI mulching; S2-5: 18 years of FCDI mulching.

2.3.2 Occurrence of microplastics in agricultural soils

In two study regions, the occurrence of MPs was highly random and only the raw data were presented in the form of p·kg^{-1}. No MPs were detected in the control sites and quality controls.

In S1, MPs were mainly detected in intermittent mulching fields (Table 2.5). In continuous mulching fields, MPs were only detected in S1-1, no MPs were detected in S1-2 or S1-3. The highest MPs concentration of 2200 p·kg^{-1} was detected in the 20-30 cm layer of S1-5, which with 30 years intermittent mulching history. In S2, MPs were detected in all the selected fields while not all the soil layers (Table 2.6). In S2-5, MPs were detected in all the soil samples. The highest MPs concentration (900 p·kg^{-1}) was detected in the 0-10 cm soil layer of S2-3 (8 years mulching) and S2-5 (18 years mulching).

Table 2.5 *Microplastics (MPs) number p·kg^{-1} soil in S1.*

Soil layer	Replicates	Control	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6
0-10 cm	1	nd	nd	nd	nd	nd	200	200
	2	nd	200	nd	nd	nd	100	800
	3	nd	nd	nd	nd	200	nd	nd
10-20 cm	1	nd	nd	nd	nd	nd	nd	200
	2	nd	nd	nd	nd	nd	nd	nd
	3	nd	nd	nd	nd	100	nd	nd
20-30 cm	1	nd	1	nd	nd	100	2200	1000
	2	nd	nd	nd	nd	100	nd	nd
	3	nd	100	nd	nd	nd	200	nd

Note. S1-1: 6 years FF mulching; S1-2: 8 years FF mulching; S1-3: 6 years FF mulching; S1-4, S1-5 and S1-6: 30 years history of intermittent FF mulching.

Table 2.6 *Microplastics (MPs) number p·kg^{-1} soil in S2.*

Soil layer	Replicates	Control	S2-1	S2-2	S2-3	S2-4	S2-5
0-10 cm	1	nd	600	300	300	nd	900
	2	nd	nd	400	Nd	100	700
	3	nd	nd	nd	900	800	400
10-20 cm	1	nd	nd	100	Nd	nd	800
	2	nd	nd	800	Nd	400	100
	3	nd	nd	nd	100	400	100
20-30 cm	1	nd	100	nd	Nd	nd	300
	2	nd	nd	600	nd	nd	300
	3	nd	100	nd	200	700	200

Note. S2-1: 6 years FCDI mulching; S2-2: 7 years FCDI mulching; S2-3: 8 years FCDI mulching; S2-4: 15 years FCDI mulching; S2-5: 18 years FCDI mulching. nd = not detected.

2.4. Discussion

In current research, we aimed to examine the characteristics of the MaPs and MPs accumulation and distribution under two farming systems. Many previous research attributed the accumulation solely to the mulching year (He et al., 2018; Ma and Yang, 2015). Understanding the impacts from other factors of different farming system is essential for regulating agricultural plastic film management. However, relevant knowledge is still limited.

2.4.1 Accumulation and distribution of macroplastics in agricultural soils

In S1, fields with 6-8 years of continuous mulching (S1-1, S1-2 and S1-3) contained significant higher MaPs numbers and mass than fields with 30 years of intermittent mulching (S1-4, S1-5 and S1-6). One possible explanation might be attributed to the removal activity by farmers. In S1, according to local farmers, 80% of applied plastic films (remained intact and could be easily collected) were manually removed after the harvesting of mulched crop and before sowing of the next rotated crop. In addition, the remained smaller particles (could still be picked up by hand) were constantly collected during the seedling and weeding stages. Therefore, fields with 30 years of intermittent mulching, as compared to fields with 6-8 years of continuous mulching, were subject to more plastic debris removal activities. On the contrary, the plastic films in continuous mulching fields were only collected once after the harvest of maize. The smaller particles were remained in soils and experienced freeze-thaw cycles during the winter and spring, which also posed more difficulties for manually removal. As a result, fields with 30 years intermittent mulching accumulated fewer MaPs than continuous mulching fields. Another possible explanation for this might be attributed to wind dispersion. Zylstra (2013) provided evidence that wind action could spread substantial plastic debris between different ecosystems. Strong winds are very common in the Gansu province (Guan et al., 2017). Hence, in the fields with over 30 years of intermittent mulching, wind could have dispersed more agricultural plastic debris into other environments and thus, lead to the significant lower accumulation of MaPs.

Crop rotation could have also affected the accumulation of MaPs. Looking closer at our results of fields with 30 years intermittent mulching history in S1, S1-6 showed a higher number of MaPs than S1-4 and S1-5. According to farmers, in S1-4, no plastic mulching was applied to the field from 2015 to 2017 due to the crop rotation of oilseed rape (Early September 2015 to mid-June 2016), winter Wheat (late September/early October 2016 to the end of May 2017), and soybean (mid-June 2017 to late September 2017). In S1-5, the rotation of maize (mid-April 2017 to mid-September 2017, when plastic film was applied) and winter Wheat (late September/early October 2017 to the end of May 2018) required farmland to be ploughed in September. However, in S1-6, with a rotation of maize (mid-April 2016 to mid-September 2016, when plastic was applied) , left fallow (mid-September 2016 to mid-June 2017) and Soybean (mid-June 2017 to late September 2017), farmland was ploughed in May while still some plastic debris incorporated into the soil. In addition, the winter could have accelerated the weathering and aging of MaPs. Any of these things could have led to the higher MaPs number seen in S1-6 as compared to S1-4 and S1-5.

In S2, we detected lower MaPs mass (ranging from 43.5 $kg \cdot ha^{-1}$ to 148 $kg \cdot ha^{-1}$) as compared to other researches. In the same study region, Yan et al. (2008) discovered plastic residues

of 259.9 kg·ha^{-1} (10 years) and 307.9 kg·ha^{-1} (20 years) in the soils of monocultural cotton. He et al. (2018) found that plastic residues (LDPE, LLDPE) ranged from 121.9 to 352.4 kg·ha^{-1} in fields where there were 5-19 years of mulching use. This discrepancy might be explained by the differences between the plastic debris sampling methods. In our field observations, the plastic films found on the surface of the soils were not taken into consideration for measurements since farmers claimed that these films would normally be removed along with the cotton stalk. In addition, in S2, the collected MaPs number and mass were not linearly increased with the mulching year, the highest accumulation was observed at 15 years mulching field. This emphasized that years of mulching use was not the main factor affecting the accumulation of agricultural plastic debris (Dong et al., 2015; Yan et al., 2014a). As we mentioned in ***Material and method***, fields in Xinjiang were subjected to high intensity machinery tillage, which lead to higher fragmentation of MaPs (Figure 2.3). The smaller particles were difficult to be collected and could also move into deeper soil layer, posed difficulties for MaPs recycle. In addition, combined with the strong winds in Xinjiang (Xiong et al., 2019), highly fragmented plastic debris in fields could be easily transferred to other environments by the wind (He et al., 2018; Rezaei et al., 2019; Steinmetz et al., 2016). The residual ratios measured in our two study regions suggested that the longer the plastic debris remained in the fields, the more likely that the plastic would disperse to other environments, which would affect the plastic debris accumulation and pose a threat to the environment. Overall, the accumulation pattern of MaPs in Xinjiang, a high machinery intensity region, subjected to many factors. Thus, the nonlinear increase of MaPs raises an important question: do other natural factors have significant effects on agricultural plastic accumulation? If so, what is the relative importance of these different factors?

According to our results, in both two study regions, in general, MaPs number in 0-10 cm soil showed no or less significant difference compared to 10-20 cm, however, as for MaPs mass, 0-10 cm and 10-20 cm layers usually showed significant difference. This result indicated that even though 10-20 cm soil contained less amount MaPs compared to 0-10 cm soil, it still contained a significant MaPs number. Previous studies in China (Ma et al., 2008; Yan et al., 2008) have indicated that long-term tillage and intense machine tillage/ploughing might have homogenized the soil, especially in the top 0 to 20 cm, thus leading to the insignificant differences seen for MaPs number among the various layers. This result also suggested that MaPs number (p·ha^{-1}) should also be an indicator for plastic pollution in future research. Machinery tillage intensity can also affect the size of the MaPs in soils. In our research, the majority size categories of the MaPs collected in S1 were 10-50 cm^2 and 2-10 cm^2, while for S2, the majority of collected MaPs were 0.25-2 cm^2 and 2-10 cm^2. These results indicate that the MaPs in S2 were more fragmented as compared to S1. These results agree with previous research findings that the sizes of plastic debris found in regions where applied

with low-intensity machinery tillage are usually bigger than in regions where applied with high-intensity machinery tillage (Li et al., 2017; Ma et al., 2008; Yan et al., 2008).

2.4.2 Microplastics in agricultural soils

In current research, MPs were mainly detected in the soils exposed to 30 years of mulching history in S1 and were detected in all the selected fields in S2. These results are in agree with the MaPs number percentage results that in S2, MaPs were more fragmented than in S1. These results also indicated that long-term exposure of plastic debris in agricultural fields and high-intensity machine tillage could create more ubiquitous MPs. Our MPs results were far more less than reported in other studies. Research conducted in southwestern China reported that MPs were detected in the range of 71 to 429 p·10 g^{-1} in the 0-10 cm layer of soil in a vegetable production system housed in a plastic greenhouse (Zhang and Liu, 2018). They attributed the higher MPs to the intense use of wastewater and sewage irrigation needed for the intensive vegetable rotation (6 to 8 crops per year). However, in our research, the cropping rotation and irrigation intensity were less than those vegetable fields.

The rare MPs detected in current research might be attributed to the extraction method limitation mentioned in Material and method. However, the MPs data in our research could be regarded as a minimum estimation of accumulation of LDPE originated MPs in agricultural fields, our work has made the attempt to connect MPs pollution to plastic mulching use in a real in situ study. More detailed research with better detection methods need to take place for a good estimation of the amount of MPs in the soil profile.

2.5. Conclusions

In this paper, we have shown that different farming systems can affected accumulation and distribution of agricultural plastic debris (both MaPs and MPs). Our study confirmed our hypothesis that 1) under the same farming system (low-intensity machinery tillage), continuous mulching could accumulate more MaPs than intermittent mulching; 2) high-intensity machinery tillage farming system (S2) could lead to higher fragmentation of MaPs and lead to higher fragmentation of MaPs and a create severer MPs pollution as compared to low-intensity machine tillage farming systems (S1). We also found that in S1, crop rotation system could affect ploughing time (Spring or Autumn), thus affecting the accumulation of MaPs. The residual ratios were lower for fields with a long mulching history. However, it remains unclear if this is due to wind and/or water transportation or due to further degradation of MaPs into smaller particles or even MPs, which are difficult to recycle.

Further research on the degradation process of agricultural plastic debris are needed, which could also provide a better understanding of the risk of agricultural MaPs and MPs and its effects on soil health and food quality.

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Supplementary Material

Table S2.1 *Climate data and soil information.*

Indicators	S1	S2
Annual average temperature	7-10 ℃	6.5-7.2 ℃
Accumulate temperature (> 10 ℃)	2 722 ℃	2 950 ℃
Annual average precipitation	540 mm	210 mm
Annual sunshine duration	2 262 h	2 865 h
Frost-free days	160 days	160 days
Annual average wind speed	2.16 m/s	1.75 m/s
Soil type	Calcarid Regosol	Calcaric Fluvisol
Soil pH	7.98±0.14	7.90±0.05
Soil organic matter content (SOM)	13.1±1.76 g·kg^{-1}	14.3±0.44 g·kg^{-1}

Note. SOM measured by method:H_2SO_4-$K_2Cr_2O_7$.Soil pH and soil organic matter (SOM) content was the average of selected fields in each study region.

Table S2.2 *Plastic specification used in S1 and S2.*

Plastic film	Colour	Thickness (mm)	Density (g·cm^{-3})	Materials	Standard	Manufacturer
S1	Transparent	0.01	0.9	LLDPE	GB 13735-1992(II)	Gansu Swift Management Consulting Co., Ltd., China
S2	Transparent	0.01	0.9	LLDPE & LDPE	GB 13735-1992(IV)	Xinjiang Tianshili Plastic Industry Co., Ltd.

Note. Low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE).

Table S2.3 *Summary of the one-way ANOVA for macroplastics number and content in 0-30 cm.*

MaPs accumulation	Study region	df	F	Sig
MaPs number	S1	5,12	20.9	0.00
	S2	4,10	61.7	0.00
MaPs mass	S1	5,12	4.24	0.02
	S2	4,10	17.1	0.00

Table S2.4 *The number and mass percentage of MaPs in different size categories in two study regions (one-way ANOVA and followed by LSD test at the p <0.05 level).*

MaPs accumulation	Fields	0.25-2 cm²	2-10 cm²	10-50 cm²	50-100 cm²	> 100 cm²
MaPs number	S1 Continuous	3.55±2.16d	34.4±8.19b	46.9±7.64a	9.57±2.57c	5.55±2.05d
	S1 Intermittent	4.20±3.21d	31.6±9.63b	44.5±11.2a	8.73±4.77c	11.0±10.2c
	S2	40.6±13.0a	41.1±9.43a	14.7±4.71b	2.54±2.04c	1.09±1.13c
MaPs mass	S1 Continuous	0.16±0.09d	7.17±2.23c	35.8±5.27a	22.0±4.50b	34.8±8.24a
	S1 Intermittent	0.26±0.31d	6.28±3.91c	34.2±21.6a	16.3±7.29b	42.9±24.0a
	S2	8.93±5.19c	26.2±7.95b	36.4±7.1a	17.4±8.06c	11.1±10.8c

Note. Lowercase letters (a,b,c) indicate significant difference between different MaPs size categories within same fields.

Table S2.5 *Summary of the one-way ANOVA for the number and mass percentage of MaPs in different size categories in two study regions. Data have been transformed using arcsine square root.*

MaPs accumulation	Fields	df	F	Sig.
MaPs number	S1 Continuous	4,40	148	0.00
	S1 Intermittent	4,40	35.9	0.00
	S2	4,70	18.4	0.00
MaPs mass	S1 Continuous	4,40	217	0.00
	S1 Intermittent	4,40	28.4	0.00
	S2	4,70	172	0.00

Table S2.6 *Summary of the one-way ANOVA for macroplastics number and mass in different soil layer in S1. Data have been transformed using arcsine square root.*

MaPs accumulation	Study region	df	F	Sig
MaPs number	S1-1	2,6	12.1	0.01
	S1-2	2,6	16	0.00
	S1-3	2,6	39.4	0.00
	S1-4	2,6	7.74	0.02
	S1-5	2,6	11.7	0.01
	S1-6	2,6	4.88	0.06
MaPs mass	S1-1	2,6	28.9	0.001
	S1-2	2,6	22.2	0.002
	S1-3	2,6	46.7	0.000
	S1-4	2,6	9.19	0.015
	S1-5	2,6	4.38	0.067
	S1-6	2,6	12.6	0.007

Table S2.7 *Summary of the one-way ANOVA for macroplastics number and mass in different soil layer in S2. Data have been transformed using arcsine square root.*

MaPs accumulation	Study region	df	F	Sig
MaPs number	S2-1	2,6	4.51	0.064
	S2-2	2,6	4.83	0.056
	S2-3	2,6	4.5	0.064
	S2-4	2,6	32.5	0.001
	S2-5	2,6	6.79	0.029
MaPs mass	S2-1	2,6	5.55	0.043
	S2-2	2,6	24.6	0.001
	S2-3	2,6	16.9	0.003
	S2-4	2,6	18.7	0.003
	S2-5	2,6	19.2	0.002

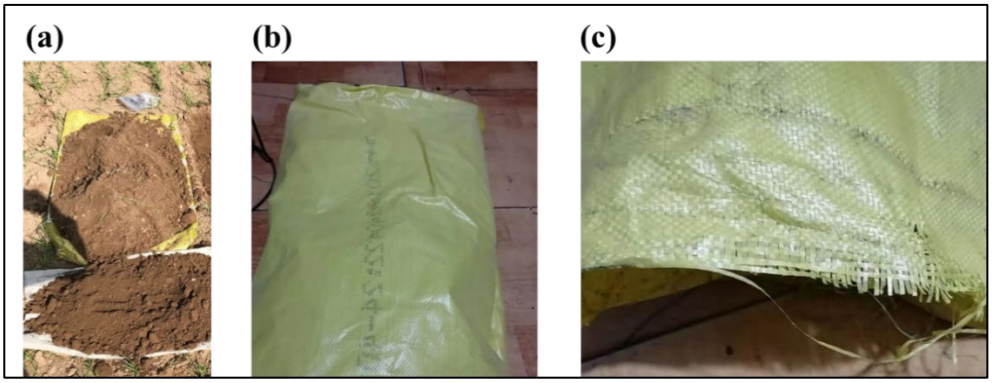

Figure S2.1 *Flat PP wire weaved mesh sheet. (a), (b) are the Flat PP wire weaved mesh sheet used in the field sampling, (c) is the flat wire used for weaving the bag.*

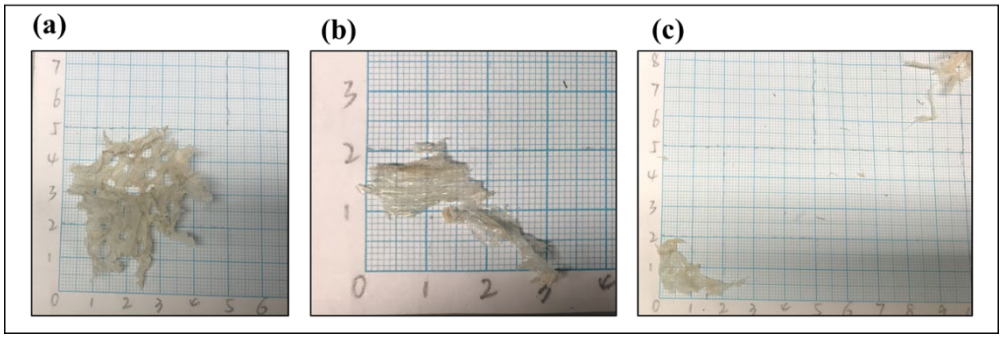

Figure S2.2 *The shapes and smallest size of collected Macroplastics. (a) arbitrary shape; (b) curved shape; (c) flake shape and curved shape; (d) the macroplastic buried in soil layers and collected macroplastics.*

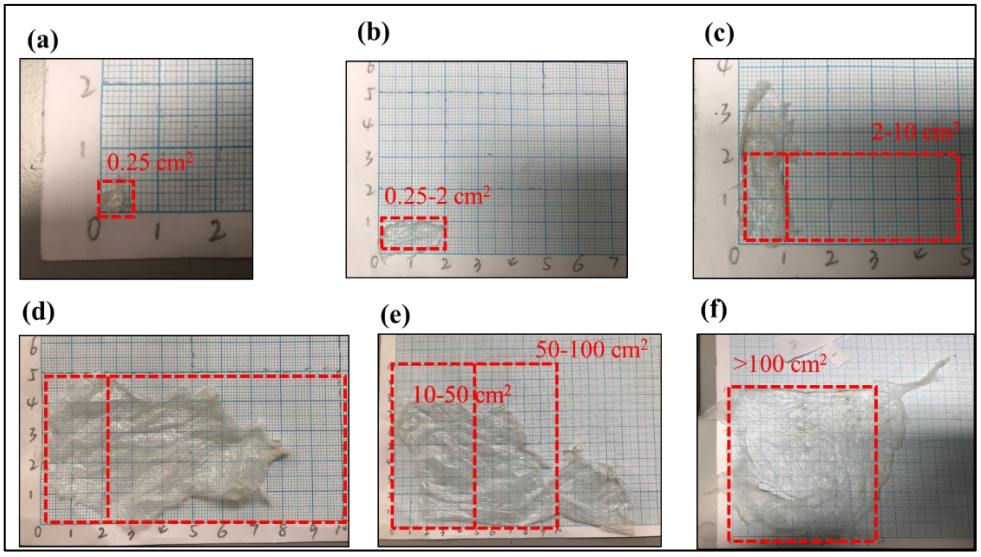

Figure S2.3 *Macroplastics different size group; (a) the smallest size of detected MaPs (approximately 0.25 cm²); (b) Macroplastics size 0.25-2cm²; (c) Macroplastics size 2-10 cm²; (d) Macroplastics size 10- 50 cm²; (e) Macroplastics size 50- 100 cm²; (f) Macroplastics size > 100 cm².*

Figure S2.4 *PP bag for collecting soil samples for microplastic extraction.*

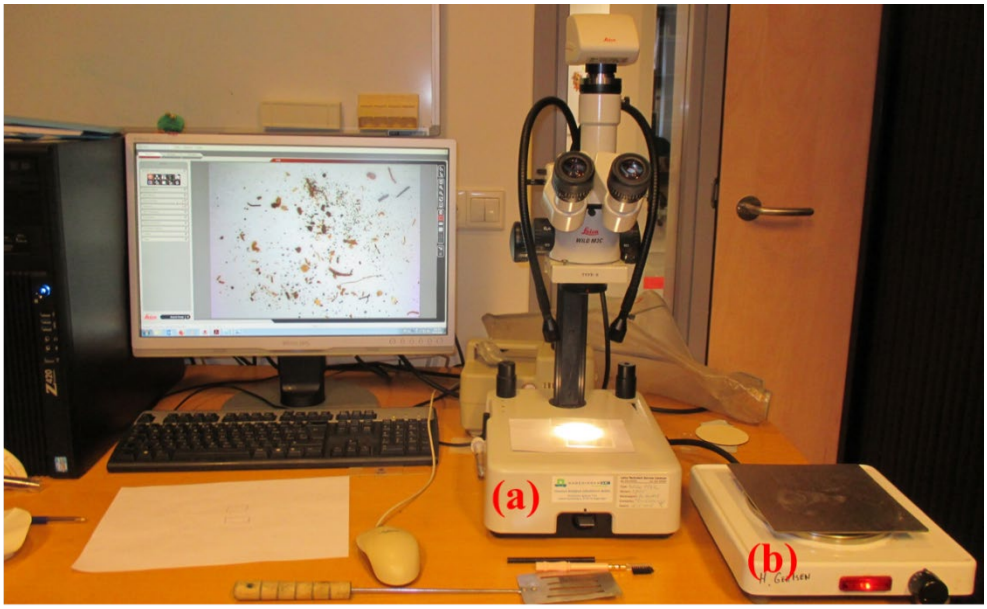

Photo credit: Nicolas Beriot

Figure S2.5 *Microplastics identification set up; (a) microscope, Leica wild M3C, Type S, simple light; (b) hot plate, TYPE A 1500-145, KEMA KEUR.* ***Copyright:*** *Beriot, Nicolas nicolas.beriot@wur.nl.*

3. Response of common bean (*Phaseolus vulgaris L.*) growth to soil contaminated with microplastics

Although concerns surrounding microplastics (MPs) in terrestrial ecosystems have been growing in recent years, little is known about the responses of plant growth to MPs pollution. Here, we conducted a pot experiment in a net house under natural condition by adding two types of MPs, low-density polyethylene (LDPE-MPs) and polylactic acid (PLA) mixed with poly-butylene-adipate-co-terephthalate (PBAT, Bio-MPs), to sandy soil at 5 doses (0.5%, 1.0%, 1.5%, 2.0%, 2.5% w/w dry soil weight). The effects of LDPE-MPs and Bio-MPs on common bean (Phaseolus vulgaris L.) were tested. Compared to control (no MPs addition), LDPE-MPs showed no significant effects on shoot, root and fruit biomass while ≥ 1.0% LDPE-MPs showed significant higher specific root nodules ($n \cdot g^{-1}$ dry root biomass) and only 2.5% LDPE-MPs showed significant higher specific root length ($cm \cdot g^{-1}$ dry root biomass). 1.0% LDPE-MPs caused significant higher leaf area and 0.5% LDPE-MPs caused significant lower leaf relative chlorophyll content. For Bio-MPs treatment, compared to control, ≥ 1.5% Bio-MPs showed significant lower shoot and root biomass. ≥ 2.0% Bio-MPs showed significant lower leaf area and fruit biomass. All Bio-MPs treatments showed significant higher specific root length and specific root nodules as compared to control. The results of the current research show that both MPs induced the responses of common bean growth, and ≥ 1.5% Bio-MPs exerted stronger effects. Further studies of their ecological impacts on soil-plant systems are urgently needed.

Based on:

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3.1 Introduction

In arid and semi-arid regions, plastic mulching is widely used in farming to control weeds, conserve water and improve soil temperatures (Kader et al., 2017b; Ma et al., 2018; Qin et al., 2015). Unfortunately, the plastic mulches are not always removed from the soil after harvest. As a result of machinery tillage and natural degradation, the mulches left on the fields were fragmented into microplastics (MPs, < 5 mm) (Andrady, 2017; Briassoulis, 2004; Palsikowski et al., 2017a; Sintim et al., 2019; Steinmetz et al., 2016). These plastic mulches derived MPs have been identified as one of the main sources of agricultural MPs pollution (Ng et al., 2018; Wierckx et al., 2018). After entering the soil, MPs could pose serious potential threats to soil health and ultimately damage the environment (Ng et al., 2018; Piehl et al., 2018).

MPs have been reported that could be ingested by soil organisms, i.e. snails (Achatina fulica) and earthworm *Lumbricus terrestris* (Oligochaeta, Lumbricidae), thus affecting their growth, activities, gut microbiota and immune systems (Huerta Lwanga et al., 2016a; Song et al., 2019; Zhu et al., 2018b). Considering the important role of soil organisms in soil organic matter decomposition and nutrient cycling, the occurrence of MPs will pose threats to soil ecosystem (Chae and An, 2018; Rillig et al., 2017b). In addition, due to the chemical inertia and structural characteristics, MPs have been proven to have the capacity to adsorb toxic chemicals onto the surface (Ivar do Sul and Costa, 2014; Koelmans et al., 2013; Wright et al., 2013). After entering the soil, these MPs can be considered as vectors for agrichemicals and heavy metals, thus posing threats to soil health (Chae and An, 2018; Huerta Lwanga et al., 2017; Li et al., 2020a).

The increasing concerns surrounding plastic pollution in agriculture have led to the development of biodegradable materials (Bandopadhyay et al., 2018; Sintim et al., 2019). Biodegradable plastic films (BDFs) have been developed as an alternative for conventional low-density polyethylene (LDPE) films. BDFs could be left in agricultural fields after use and then degraded into CO_2 and H_2O by soil microorganisms (Bandopadhyay et al., 2018; Bettas Ardisson et al., 2014). However, the total degradation of BDFs in farmland conditions is rarely observed (Li et al., 2014; Palsikowski et al., 2017b). In addition, Sintim and Flury (2017) expressed their concerns about the toxicity of biodegradable material and indicated that “out-of-sight does not mean they are safe”. Qi et al. (2020c) found starch based biodegradable MPs could shift the soil bacterial communities and volatiles emitted in the rhizosphere. Research by Wang et al. (2020a) also indicated that polylactic acid (PLA) MPs exhibited a noticeable phytotoxicity to maize growth. BDMs have been suggested as the most promising solution for agricultural plastic pollution. Unfortunately, the knowledge

about its ecological impacts on soil-plant systems are still insufficient and require further study.

The growing body of literature have indicated that MPs could affect the soil biophysical environments, i.e. decreased soil bulk density and soil microbial activities, increased soil evaporation and desiccation cracking (de Souza Machado et al., 2018b; Wan et al., 2019). However, little information is available on the effects of MPs on plant growth (Rillig et al., 2019). It has been reported that changes in soil properties by the occurrence of MPs could enhance plant performance. For example, de Souza Machado et al. (2019) found onion growth affected as a result. Qi et al. (2018) found starch-based MPs led to the reduction of wheat biomass. A recent study by Li et al. (2020b) observed an effective uptake of micrometre-sized (2.0 μm) and submicrometre-sized (0.2 μm) polystyrene (PS) by wheat and lettuce root via a crack-entry mode and the translocation of 0.2 μm PS within roots, shoots and leaves of wheat and lettuce. Considering the important role of plant in terrestrial ecosystems and increasing accumulation of MPs in agricultural soils. Understanding the effects of MPs on plant thus is crucial. LDPE is the most commonly applied plastic mulching material, PLA blended with PBAT has been suggested as one of the most promising materials as an alternative for agricultural plastic film due to its durability and environment friendliness (Palsikowski et al., 2017a; Zhang et al., 2019c). Therefore, a better understanding of the effects of LDPE microplastics (LDPE-MPs) and biodegradable microplastics (Bio-MPs) on plant growth will provide deeper insight into the impacts of these particles on the soil-plant systems.

In our present study, according to the previous research, we hypothesized that both LDPE-MPs and Bio-MPs affect plant growth, and that Bio-MPs have stronger impacts than LDPE-MPs. To test our hypothesis, we conducted a pot experiment by using common bean (*Phaseolus vulgaris L.*), a Leguminosae crop, as a model plant due to it often being cultivated with plastic mulching and sensitive to changes in soil conditions, such as water deficiency and soil nitrogen (Abd El-Wahed et al., 2017; Chekanai et al., 2018; Fenta et al., 2019). Common bean was exposed to two types of MPs, LDPE-MPs and biodegradable bioplastics derived from PLA/PBAT (Bio-MPs), at gradient doses (0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/w dry soil weight). Several commonly applied growth parameters were used to assess the impacts of the MPs on the growth of common bean, i.e. shoot and root biomass, shoot to root ratio, specific root length, specific root nodules etc.

3.2 Materials and Methods

3.2.1 Experimental setup

We conducted a two-factorial pot experiment from the 28th of June 2019 until the 18th of October 2019 in an outdoor net house (diameter 0.25 mm) at Unifarm, Wagenigen University & Research (WUR), the Netherlands (Supplementary Figure S3.1A). Supplementary Figure S3.2 shows the monthly temperatures in Wageningen during the experiment.

In the experiment we applied two types of microplastics: low-density polyethylene (LDPE-MPs) and biodegradable plastic (Bio-MPs). The industrial pellets of biodegradable (Bio) plastic consisted of 85% PBAT, 10% PLA and 5% calcium carbonate. The pellets of LDPE and Bio materials were first frozen with liquid nitrogen and then ground using a grinding machine into smaller particles, the particles were sieved manually using steel sieves with pore sizes of 53 μm, 125 μm, 250 μm, 500 μm and 1000 μm to ensure the particle size ranging from < 53 μm to 1000 μm. The MPs used in this experiment were comprised of 250~500 μm (60% of total MPs weight) and 500~1000 μm (40% of total MPs weight). These two size categories were chosen based on Scheurer and Bigalke (2018a) and Zhang and Liu (2018). The ratio was chosen to simulate the heterogeneity of sizes of MPs in terrestrial ecosystems. The MPs used in our research were arbitrarily shaped particles (scanned by Laser Direct Infrared system, Agilent, US), the shape and flourier transform infrared spectroscopy (FTIR) are shown in supplementary files (Supplementary Figure S3.3).

LDPE-MPs and Bio-MPs were applied in 5 different doses: 0.5%, 1.0%, 1.5%, 2.0% and 2.5% dry soil weight. In addition, a control treatment (CON) without MPs was prepared. The doses of MPs were chosen based on the current knowledge of MPs concentrations in soil (Corradini et al., 2019b; de Souza Machado et al., 2019; Ng et al., 2018). The gradient and high doses could amplify the potential side effects that might otherwise be overlooked and also determine a potential threshold (van Weert et al., 2019). Totally, 11 treatments with 8 replicates were included (Figure 3.1), so that a total of 88 pots were cultivated (Supplementary Figure S3.1).

The substrate used in this study was a sandy soil (87% sand, 12% silt and 1% clay with an organic matter content of 4%, and pH 6.0. More details can be found in the Supplementary Figure S3.4). The soil was collected from an agricultural field near Wageningen, the Netherlands on June 14th, 2019. The soil was immediately sieved to 4 mm to remove large roots and gravel, air-dried and homogenized. MPs were manually mixed into homogenized

air-dried soil using a wooden stick for 10 min in an iron tank until achieving target doses. Then, a 7L polypropylene (PP) pot (21 cm height, 16 cm bottom diameter and 21 cm top diameter) was filled with 6 kg of homogenized soil-MPs until 5 cm below the top of the pot,

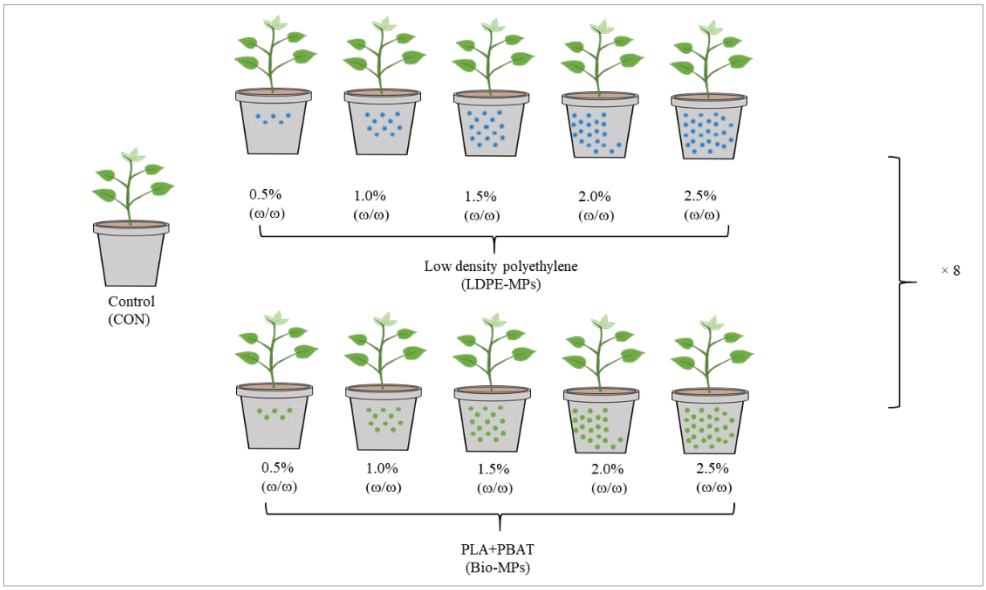


Figure 3.1 *Pot experimental design. All 11 treatments were repeated 8 times (4 replicates per harvesting moment).*

resulting in a bulk density of approximately 1.16 g·cm^{-3}. The bottom of the PP pots was covered with a piece of geotextile to prevent soil loss. After all the pots were filled, the soil moisture was unified to 10% (gravimetric water content). Pots were then placed in the outdoor net house for one week to allow interactions between the soil microbiome, soil and microplastics (the 28th of June to the 5th of July 2019).

Common bean (*Phaseolus vulgaris L.; Cultivar: Bruine Noordhollandse*) seeds were obtained from Unifarm, Wagenigen University & Research. The seeds were surface sterilized for 5 min using 10% sodium hypochlorite and then washed several times with deionized water. Five seeds were sown in each pot. Germination occurred within 14 days. 2 seedlings per pot were kept for the experiment and the rest were removed from the pots. During the growing period, 100 mL of a diluted nutrient solution (Supplementary Figure S3.5) was added to each pot in the 4th (26th of July) and 5th (2nd of August) week. The diluted nutrient solution contained 1/3 of the nitrogen of the original nutrient solution and served as a starter nitrogen to stimulate early growth (Chekanai et al., 2018). From the 6th to the 12th week, 100 mL of the nutrient solution was added to each pot once a week to ensure the fully

development of common bean. Pots were randomly placed within the net house and their positions were shifted once a month. The water content of the pots during the whole growth period was maintained at 10(±1) % by watering twice a week.

3.2.2 Measurements of Phaseolus vulgaris L. growth parameters

During the growing period, the height and stem diameter of the common bean were measured once a week from the 14th to the 105th day. Plant height was measured using a steel ruler and stem diameter was measured using a Vernier caliper (Data recorded in Supplementary Figure S3.6).

The plants were harvested twice based on the common bean development stage (Supplementary Table S3.1). The first harvest was performed on the 15th of August 2019, 46 days after seeding, near the end of the vegetative stage (VS) when plant root and leaves finished the early development stage. During the first harvest, plant shoot biomass (SB_VS), root biomass (RB_VS), relative leaf chlorophyll content (Chlor_VS), leaf area (LA_VS), and root traits were measured. Root samples were only collected once at vegetative stage due to most of the roots having decayed after full maturation. A second harvest was performed on the 18th of October 2019, 105 days after seeding, after full maturation (FM). During the second harvest, fruit biomass (FruitB), number of fruits (FruitNb), number of pods (PodNb) were recorded. At each time point, 4 replicates were harvested. All the measured parameters and their abbreviations are shown in Table 3.1.

3.2.2.1 Shoot measurements

At the end of the vegetative stage (15th of August 2019), Chlor_Vs was measured using a hand-held automated chlorophyll meter (SPAD-502plus, Minolta, USA) before the first harvest. Then, plants were cut 10 mm above the soil and separated into shoot and roots. Plant shoots were transported to the laboratory, fresh shoot biomass was weighed using a digital balance (DK-6200-C-M), then the leaves were cut off and measured using a Leaf Area Meter (LI-3100C Laboratory, LICOR Biosciences, USA). Thereafter shoots and leaves were dried in an oven (TYPE A 1500-145, KEMA KEUR) at 60 °C to a constant weight to determine the SB_Vs. After plant shoots had been removed, the pots were stored in a 4 °C cooling room before the root samples were collected. After full maturation (18th of October 2019), FruitB, FruitNb and PodNb were recorded.

Table 3.1 *Measured growth parameters and their abbreviation.*

Sampling time	Growth Parameters	Abbreviation
Vegetative stage	Shoot biomass (g)	SB_VS
	Leaf area (cm2)	LA_VS
	Relative leaf chlorophyll content	Chlor_VS
	Root biomass (g)	RB_VS
	Root average diameter (mm)	RAD
	Specific root length ($cm \cdot g^{-1}$)	SRL
	Specific root surface area ($cm^2 \cdot g^{-1}$)	SRSA
	Specific root volume ($cm^3 \cdot g^{-1}$)	SRV
	Specific root nodules ($n \cdot g^{-1}$)	SRN
	Fine root length proportion (%)	FRL
	Fine root surface area proportion (%)	FRS
	Fine root volume proportion (%)	FRV
	Shoot to root ratio	S:R_VS
Fully mature	Fruit biomass (g)	FruitB
	Pod number (n)	PodNb

3.2.2.2 Root traits

To collect the root samples, each pot was carefully rinsed with tap water to remove any traces of soil. Then, the roots were carefully placed in a steel sieve (410 μm) and gently rinsed again to remove any fine sand. The recovered roots from each pot was placed in a steel container (20 cm wide, 30 cm long and 5 cm deep) and immersed in tap water. Floating organic debris (Supplementary Figure S3.7A) was picked out using tweezers. After that, the roots from each pot was homogenized in the new steel container and three subsamples were randomly selected to examine the root traits. Each subsample consisted of the roots in a sample area of 8 cm length and 5 cm width (Supplementary Figure S7B). The roots were cut off using a pair of scissors. The retrieved subsample was then stored in 100 ml centrifuge tubes (polypropylene, PP) and soaked with 25% ethanol. The rest of the root sample was oven dried at 60 °C to a constant weight and recorded as root $biomass_1$ (RB_1).

To obtain the root traits, each root subsample from each pot was placed on a transparent tray (19 cm wide, 25 cm long and 2 cm deep, Supplementary Figure S3.7C) and evenly spread out by hand with distilled water. The Imagery Scan Screen (EPSON Expression V700XL) was used to scan the root samples to create a black and white image (600 dpi,

tagged image file format [TIF], white background) (Supplementary Figure S3.7D). The scanned image was then analysed using "WinRHIZO" software (Regent Instruments Inc., Quebec), which was specially designed for root architecture measurements: root length, root surface area, root volume, average diameter and proportion of fine root (roots with diameter < 0.4 mm) length (FRL,%), fine root surface area (FRA,%), and fine root volume (FRV,%) (Fenta et al., 2019; Sofi et al., 2018). After scanning, the number of nodules per subsample was manually counted. Each subsample was then oven dried at 60 °C to a constant weight and recorded. The total weight of 3 subsamples then recorded as root biomass$_2$ (RB_2). Total dry root biomass (RB) was calculated as RB_1+RB_2.

Specific root length (SRL, $cm \cdot g^{-1}$), specific root surface area (SRSA, $cm^2 \cdot g^{-1}$), specific root volume density (SRV, $cm^3 \cdot g^{-1}$) and specific root nodules were calculated as root length (cm), surface area (cm^2), root volume (cm^3) and root nodule number (n) divided by biomass of each scanned root subsample, respectively (Araújo et al., 2004; Pérez-Jaramillo et al., 2017). The biomass of shoot to root ratios (S: R_VS) were calculated by dividing the dry weight of the shoot by the dry root biomass.

3.2.3 Data analysis

All the measured growth parameters were normalized using arcsine square root transformation to avoid violating the underlying assumptions of normality. For each type of microplastic material (LDPE-MPs and Bio-MPs treatments), comparisons of each growth parameter in different MP concentrations in contaminated soil were performed using one-way ANOVAs, growth parameters that were significant affected ($p < 0.05$) by the occurrence of MPs then tested by the LSD test (Supplementary Table S3.2). Comparisons between LDPE-MPs and Bio-MPs were performed using the Independent-Samples t-Test (Supplementary Table S3.3). In all the analyses, the significance levels were considered at $p < 0.05$ and all the plant growth parameters were presented as "Means ± Standard deviations" (Supplementary Table S3.4).

3.2.4 Correlation analysis

To identify the relationships between the microplastics (types and concentrations) and the plant growth parameters, three multivariate statistical methods including correlation analysis (CA), factor analysis (FA) and redundancy analysis (RDA) were employed in this study. Firstly, correlation analysis (Supplementary Table S3.5) was performed to explore the collinearity among measured growth parameters. The growth parameters whose

correlation coefficient values with other growth parameters were larger than 0.9 or smaller than 0.35 were screened out. According to the CA (Supplementary Table S3.5), SRL had a high collinearity with SRSA and SRV. Since SRL correlated strongly with other growth parameters, SRSA and SRV were removed from the CA while SRL was retained. FRL was retained and FRS and FRV were removed for the same reason. Growth parameter of relative Chlor_Vs, and SRN were excluded because of the low correlation (Pearson correlation r < 0.35) with other parameters (Supplementary Table S3.5). Growth parameters of S: R and RAD were excluded because no significant effects were observed in the microplastic materials and microplastic concentrations and their interactions.

Table 3.2 *Variable loading coefficients (eigenvectors) of the first four factors extracted using 7 common bean growth parameters, their eigenvalues, and individual and cumulative percentage of total variance explained by each factor.*

Growth indicator	Factor 1	Factor 2	Factor 3	Cumulative
RB_VS	**0.851**	0.039	-0.018	0.871
LA_VS	**0.843**	0.165	-0.218	0.791
SB_VS	**0.823**	0.204	-0.346	0.680
PodNb	0.083	**0.927**	-0.088	0.922
FruitB	0.191	**0.924**	-0.044	1.072
FRL	-0.112	-0.013	**0.919**	0.794
SRL	-0.252	-0.122	**0.843**	0.470
Eigenvalue	3.194	1.462	1.107	11.4
Variance	45.6	20.9	15.8	82.3
Cumulative variance (%)	39.1	66.5	82.3	

Note. Bold face values loadings (> 0.70) are considered highly weighted.
SB_VS: Shoot biomass at the end of vegetative stage.
RB_VS: Root biomass at the end of vegetative stage.
LA_VS: Leaf area at the end of vegetative stage.
SRL: Specific root length at the end of vegetative stage.
FRL: Proportion of fine root (diameter < 0.4 mm) length at the end of vegetative stage.
FruitB: Fruit biomass after fully mature.
PodNb: Pod number after fully mature.
Factor 1: Plant shoot and root biomass.
Factor 2: Plant production.
Factor 3: Root characteristics.

In order to recognize the comprehensive effects of microplastics on common bean growth, FA was applied to classify the latent factors. All meaningful loadings (i.e. loadings > 0.70) were included in the interpretation of factor analysis results. The statistical data analyses were performed using IBM SPSS Statistics 23. The factor analysis results of bean growth parameters are shown in Table 3.2. Finally, we used RDA to identify the relationships among

microplastics and plant growth parameters. The three extracted factors by FA and the growth parameters of Chlor_Vs and SRN, which showed low correlation with other parameters, were included in the RDA (Figure 3.4). The arrows represent the different plant growth parameters, and the direction of the arrows represents the correlations between each parameter and the axes as well as the relationships among the parameters. The length of the arrows represents the relative contribution of the parameters to the axes and the parameter factor relationships. RDA was performed using CANOCO 5.

3.3 Results

3.3.1 Effects of LDPE-MPs on common bean growth and root traits

In our study, LDPE-MPs showed no significant (one-way ANOVA, $p > 0.05$) impact on shoot biomass, root biomass, fruit biomass (Figure 3.2A, Figure 3.2B and Figure 3.2D) or pod number as compared to control treatment (Supplementary Figure S3.8A). However, leaf area (Figure 3.2F and Supplementary Table S3.4) in 1.0% LDPE-MPs (724±56.0 cm^2) was significantly higher (one-way ANOVA, $p = 0.034$) than control (626±80.0 cm^2). Leaf relative chlorophyll content (Figure 3.2E and Supplementary Table S3.4) in 0.5% LDPE-MPs (27.2±2.34) was significantly lower (one-way ANOVA, $p = 0.004$) than control (33.1±1.16).

For root traits, the significant impacts were mainly observed from 2.5% LDPE-MPs treatment. For example, specific root length in 2.5% LDPE-MPs treatment (20047±989 $cm \cdot g^{-1}$) was significantly higher (one-way ANOVA, $p < 0.05$) than control treatment (16604±1082 $cm \cdot g^{-1}$, Figure 3.3A and Table S3.4). Besides, 2.5% LDPE-MPs also showed highest fine root surface area proportion (64.5±2.36%), which is significantly higher than control (57.1±2.03%, Supplementary Figure S3.8F and Supplementary Table S3.4). In addition, except 0.5% LDPE-MPs, all LDPE-MPs led to higher specific root nodules as compared to control treatment (510±58.4 $n \cdot g^{-1}$), while only 2.0% showed no significant difference (Figure 3.3B). Other doses of LDPE-MPs showed no significant effects on root traits. Specific root volume (Supplementary Figure S3.8D and Supplementary Table S3.4) and root average diameter (Supplementary Figure S3.8E and Table S3.4) were not significantly affected by LDPE-MPs.

3.3.2 Effects of Bio-MPs on common bean growth and root traits

For Bio-MPs addition, shoot and root biomass were significantly affected by 1.5%, 2.0% and 2.5% Bio-MPs compared to control (Figure 3.2A and Figure 3.2B). For example, shoot

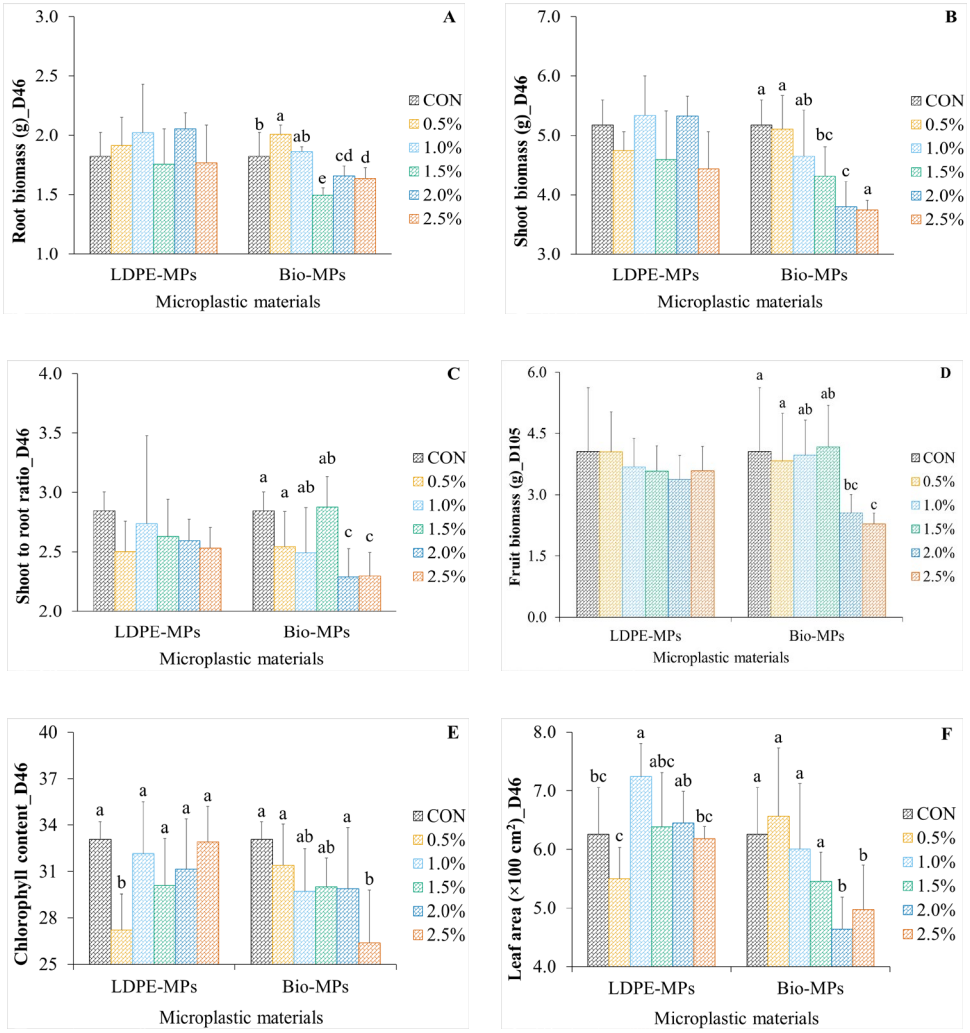


Figure 3.2 *(A). Shoot biomass (SB_VS) at D46 (the end of vegetative stage); (B). Root biomass (RB_VS) at D46 (the end of vegetative stage); (C). Shoot to root ratio of biomass (S: R_VS) at D46 (the end of vegetative stage); (D). Fruit biomass (FruitB) at D105 (fully mature); (E). Relative leaf chlorophyll content (Chlor_VS) at D46 (the end of vegetative stage); (F). Leaf area (LA_VS) at D46 (the end of vegetative stage) in LDPE microplastic (LDPE-MPs) and biodegradable microplastic (Bio-MPs) contaminated soil (0.5-2.5 % w/w soil, uncontaminated control CON). Legend indicates the microplastic contamination level, including control (dark), 0.5% (orange), 1.0% (light blue), 1.5% (green), 2.0% (blue) and 2.5% (Vermillion). Error bars represent*

standard deviation; and the lowercase letters (a and b) indicate significant differences between control treatment and microplastic contamination treatment within each microplastic material. Post-hoc test was only performed when growth parameters were significantly affected by the occurrence of MPs.

biomass in 1.5%, 2.0% and 2.5% Bio-MPs treatments were 4.31±0.49 g, 3.80±0.43 g, and 3.75±0.16 g, respectively (Supplementary Table S3.4), which were significantly lower (one-way ANOVAs, $p < 0.05$) than in control treatment (5.18±0.42 g). Root biomass in 1.5%, 2.0% and 2.5% Bio-MPs treatments were 1.50±0.06 g, 1.66±0.08 g and 1.64±0.09 g, respectively (Table S3.4), which were significantly lower than control treatment (1.82±0.20 g). Correspondingly, shoot to root ratio (Figure 3.2C and Supplementary Table S3.4) in 2.0% and 2.5% Bio-MPs treatments were 2.29±0.24 and 2.30±0.20, respectively, which were significantly lower than control treatment (2.85±0.16). Fruit biomass (Figure 3.2C) and leaf area (Figure 3.2F) were also observed significantly lower in 2.0% and 2.5% Bio-MPs treatments, e.g. fruit biomass in 2.0% and 2.5% Bio-MPs were 2.55±0.45 g and 2.28±0.27g, respectively, which were significantly lower than control treatment (4.06±1.57 g). Leaf area in 2.0% and 2.5% Bio-MPs were 463±54.8 cm^2 and 497±75.9 cm^2, respectively, which values were significantly lower than control treatment (625±80.0 cm^2, Supplementary Table S3.4). In addition, in 2.5% Bio-MPs treatment, leaf relative chlorophyll content (Figure 3.2E) and pod number (Supplementary Figure S3.8A) were also significantly higher than the control treatment.

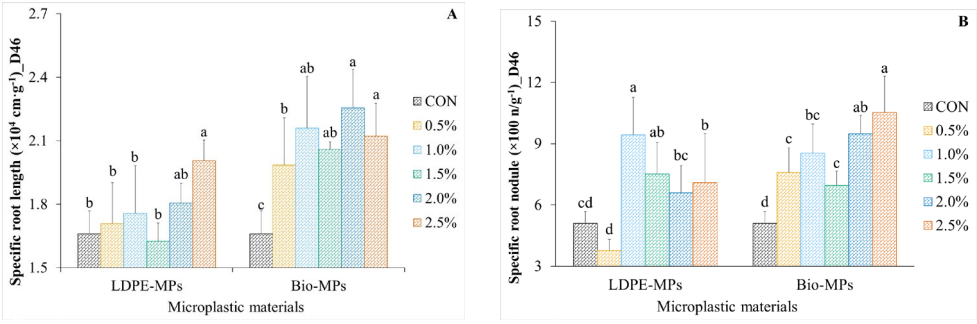


Figure 3.3 *(A). Specific root length at D46 (the end of vegetative stage); (B). Specific root nodules at D46 (the end of vegetative stage) in LDPE microplastic (LDPE-MPs) and biodegradable microplastic (Bio-MPs) contaminated soil (0.5-2.5 % w/w soil, uncontaminated control CON). Legend indicates the microplastic contamination level, including control (dark), 0.5% (orange), 1.0% (light blue), 1.5% (green), 2.0% (blue) and 2.5% (Vermillion) Error bars represent standard deviation; and the lowercase letters (a and b) indicate significant differences between control treatment and microplastic contamination treatment within each microplastic material. Post-hoc test was only performed when growth parameters were significantly affected by the occurrence of MPs.*

Contrary to the negative effects observed on root biomass and shoot biomass, compared to the control treatment, Bio-MPs treatments showed significantly higher values on specific

root length (Figure 3.3A), specific root nodules (Figure 3.3B) at all concentrations. The highest specific root length (22 550±1 816 $cm \cdot g^{-1}$, Figure 3.3A and Supplementary Table S3.4) was observed in the 2.0% Bio-MPs treatment, significantly higher than control treatment (16 604±1 082 $cm \cdot g^{-1}$). The highest specific root nodules were observed at 2.5% Bio-MPs treatment (1053 ± 178 $n \cdot g^{-1}$), which is significantly higher than control treatment (510±58.4 $n \cdot g^{-1}$). Specific root volume (Supplementary Figure S3.8D) and root average diameter (Supplementary Figure S3.8E) were not significantly affected by the Bio-MPs addition (more data showed in Supplementary Table S3.4).

3.3.3 Comparison of the effects between LDPE-MPs and Bio-MPs

The impacts on growth parameters from LDPE-MPs and Bio-MPs were compared using the Independent-Samples t-Test (Supplementary Table S3.3). In general, for shoot and root biomass, leaf area and relative chlorophyll content, growth parameters showed lower values in Bio-MPs treatments compared to LDPE-MPs treatments, while for root traits parameters, specific root length and specific root nodules showed higher value in Bio-MPs treatment compared to LDPE-MPs. However, the differences between the two types of materials were not always significant. For shoot and root biomass, significant differences between LDPE-MPs and Bio-MPs were only observed at 2.0% contamination level (Supplementary Table S3.3). For specific root nodules, significant differences between LDPE-MPs and Bio-MPs were observed at 0.5% and 2.5% contamination level.

3.3.4 Factor analysis results and RDA analysis

Factor analysis results showed three axes (factors) with eigenvalues > 1 and collectively explained about 82.3% of the variance in the original data (Table 3.2). This means the corresponding 7 measured growth parameters were related and that three factors effectively expressed the overall changes in the common bean growth: Factor 1 explained the highest variance (45.6%) in the results, while Factor 2 accounted for 20.9% and Factor 3 accounted for 15.8%. Factor 1 (F_1) included SB_ VS, RB_ VS and LA_VS. This group of parameters implied that Factor 1 was mainly associated with total plant biomass, thus F_1 was defined as shoot and root biomass. Factor 2 (F_2) included PodNb and FruitB, for this reason, F_2 was defined as plant production. Factor 3 included FRL and SRL. This group of parameters implied that Factor 3 was mainly associated with the root development, for this reason, F_3 was defined as root characteristics. The relationships among the measured parameters of the common bean growth and treatment factors are illustrated in a redundancy analysis diagram (Figure 3.4). The first axis explains 62.6% of the variation in

the parameter-factor relationships according to the Monte Carlo permutation tests (Supplementary Table S3.6). The diagram indicates that Pure soil and LDPE_0.5 were positively correlated to common bean production (F2). LDPE_2.0 and Bio_0.5 were positively correlated to plant biomass (F1) and Chlor_VS. While Bio_1.0, Bio_2.0 and Bio_2.5 are positively related with SRN and root characteristics (F3).

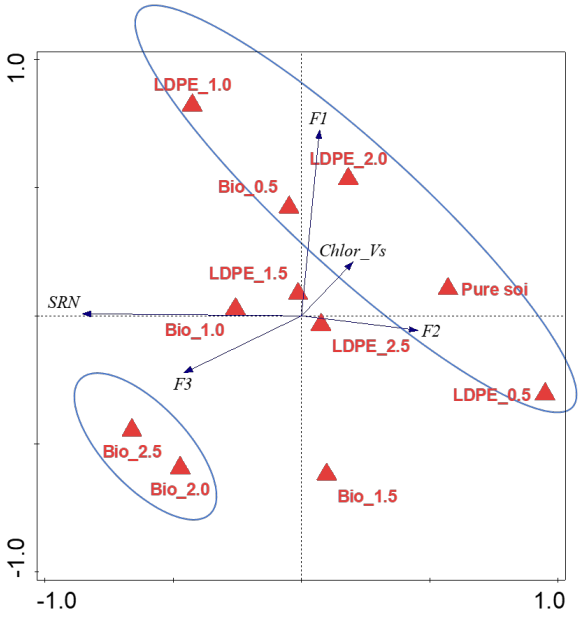


Figure 3.4 *Redundancy analysis ordination diagram of common bean growth parameters with treatment factors.*

Pure soil: soil without microplastics;

LDPE_0.5: soil with LDPE microplastics of 0.5% w/w.

LDPE_1.0: soil with LDPE microplastics of 1.0% w/w.

LDPE_1.5: soil with LDPE microplastics of 1.5% w/w.

LDPE_2.0: soil with LDPE microplastics of 2.0% w/w.

LDPE_2.5: soil with LDPE microplastics of 2.5% w/w.

Bio_0.5: soil with biodegradable microplastics of 0.5% w/w.

Bio_1.0: soil with biodegradable microplastics of 1.0% w/w.

Bio_1.5: soil with biodegradable microplastics of 1.5% w/w.

Bio_2.0: soil with biodegradable microplastics of 2.0% w/w.

Bio_2.5: soil with biodegradable microplastics of 2.5% w/w.

Chlor_Vs: relative leaf chlorophyll content at the end of vegetative stage.

SRN: specific root nodules at the end of vegetative stage.

F1 defined as plant shoot and root biomass.

F2 defined as plant production.

F3 defined as root characteristics.

3.4 Discussion

In our experiment we looked at the effects of LDPE-MPs and Bio-MPs in the soil on the growth of common bean. We will first discuss the effects of both types of MPs on common bean growth separately and then followed by the limitations and implications of current research.

3.4.1 Effects of LDPE-MPs on common bean growth

Our experiment showed that LDPE-MPs had limited effects on common bean growth. We found no significant effect on plant shoot and root biomass. This was also observed in a study by van Weert et al. (2019), in which they exposed *Myriophyllum spicatum* and *Elodea sp.* to sediments amended with polystyrene (PS) nanoplastic (nano-PS, 50–190 nm, up to 3% sediment dry weight) and PS microplastic (micro-PS, 20–500 μm, up to 10% dry weight) under laboratory conditions. They found that micro-PS did not significantly affect shoot and root biomass while nano-PS did. They suggested the observed difference between nano-PS and micro-PS might be related to the difference in surface area, in which nano-PS could efficiently bind the nutrient, activate competition for nutrients between roots and microbial communities, thus reducing the nutrient status. Consequently, enhanced competition or reduced nutrient status triggered the root biomass growth. Our result is also in line with a study conducted by Wang et al. (2020a), who reported that 1% w/w polyethylene high density (PEHD, 100-154 μm) had no significant effect on maize growth. A possible explanation for this is given by de Souza Machado et al. (2019), who found that up to 2% PEHD (w/w 2000-3000 μm) in the soil had limited effects on soil structure and onion growth. They assumed the less pronounced effects of PEHD on the changes of soil properties due to the PEHD chemical structure: $(C_2H_4)_n$, which is structurally stable and contained no nutritional elements that could have elicited soil nutrient dynamics. In our research, we used LDPE, which has also a $(C_2H_4)_n$ structure but has a lower molecular weight. As for the observed variability in the leaf area and relative chlorophyll content, we have no conclusive explanations, the effects might be attributed to the common biological variability in the LDPE-MPs treatments (van Weert et al., 2019).

However, our research showed that all the LDPE-MPs treatments, except 0.5% LDPE-MPs, resulted in significant higher specific root nodules compared to control treatment except the treatment of 2.0%, which showed no significant difference. It seems that the presence of LDPE-MPs in the soil stimulates the forming of root nodules. Nodule number has been suggested as a proxy for biological nitrogen fixation (de Oliveira et al., 1998). Haase et al.

(2007) found N-deficiency treatments could induce the formation of a significantly higher number of nodules in common bean. Therefore, the higher specific root nodules might be explained by the effect of the LDPE-MPs treatments on available N in the soil. As soil nutrient and microbial activities were not measured in current research, further research is needed to fully understand the mechanism of how LDPE-MPs affects the common bean root traits.

3.4.2 Effects of Bio-MPs on common bean growth

Contrary to LDPE-MPs, the Bio-MPs of PBAT+PLA exerted stronger negative effects on common bean. 1.5%, 2.0% and 2.5% w/w showed significantly lower root and shoot biomass, 2.5% w/w showed significant lower leaf chlorophyll content. Several factors might account for this. Qi et al. (2018) exposed wheat to 1% w/w of starch-based MPs, thus resulting in a plant total biomass of 3.71±0.67 g, significantly lower than the control treatment of 5.59±0.47 g. A later study from Qi et al. (2020c) suggested that the shifted rhizosphere bacterial communities and increased volatile compounds like dodecanal might account for the decreased total wheat biomass. Another study by Wang et al. (2020a) found that soil with a concentration of 10% w/w PLA-MPs (100-154 μm) also had significant phytotoxic effects on maize growth as compared to PEHD, i.e. lower dry shoot and root biomass and lower chlorophyll content. They suggested that the intermediate and final metabolites degraded from PLA-MPs, which may have directly and/or indirectly affected soil properties, soil biota and soil nutrient availability, which may account for the inhibition on the plant biomass and leaf chlorophyll content. While contrast to the lower plant shoot and root biomass, all Bio-MPs treatments showed significantly higher specific root length and specific root nodules. As we mentioned previously, the number of common bean nodules has been suggested as an estimate of biological nitrogen fixation and positively related to N-deficiency (de Oliveira et al., 1998; Haase et al., 2007). PBAT material has been reported could increase soil rhizobacterial growth and thus competing for nutrients with plant roots (Kuzyakov and Xu, 2013; Muroi et al., 2016; van Weert et al., 2019). Therefore, in our experiment, it is plausible that in Bio-MPs treated soil, in order to overcome the competition with the soil communities, common beans produced more specific root length and specific root nodules to allow for better nutrient transportation. However, judging by the observed decreased root and shoot biomass, the nutrient status in Bio-MPs treatments might be reduced.

3.4.3 Limitations and Implications

In this study a wide range of MPs concentrations (0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/w dry soil weight) was used to study their effect on the growth of common bean. However, MPs concentrations reported under normal field conditions are much lower. To depict the potential subtle effects caused by MPs, it is necessary to use these relatively high concentrations as was also stated by van Weert et al. (2019). Another limitation of our study is that it was not tailored to identify degradation of MPs in soil or nutrient cycling in the soil. Of all the responses, we observed no clearer consistent dose-effects with the increased doses of MPs, which revealed the uncertainties and complexities to predict the impacts of MPs in soil-plant systems. Considering the native properties of the two materials, the effects of Bio-MPs probably come from the degraded by-products while the less pronounced effects of LDPE-MPs might attribute to its stable structure. It should also be noticed that species-species effects, i.e. micro-PS (20–500 μm, up to 10% dry sediment weight) showed no significant impacts on macrophytes in sediments (van Weert et al., 2019), while common bean specific root nodules responded to the occurrence of LDPE-MPs in sandy soil (250–1000 μm, up to 2.5% dry soil weight) in current research, which highlights that different root traits may be susceptible to different mechanisms caused by the occurrence of MPs in soil (Rillig, 2020). In addition, even though LDPE-MPs were structural stable, other properties (i.e. type, size, shapes and surface properties) should also be taken into consideration in future studies since they could also pose threat to plant growth (Rillig, 2020). A recent study by Li et al. (2020b) has evidenced uptake of 0.2 μm and 2 μm PS MPs by wheat and lettuce root. Thus, urgent ecological assessments for those petroleum-based polymers are crucial as those particles will eventually degrade into smaller particles (Ng et al., 2018; Rillig et al., 2017a).

3.5 Conclusion

In this study we tested the hypotheses that Bio-MPs have a stronger effect on the growth of common bean (*Phaseolus vulgaris L.*) than LDPE-MPs. From the results we can conclude that this is indeed the case. LDPE-MPs showed no significant effects on shoot and root biomass, while Bio-MPs, especially at 1.5%, 2.0% and 2.5% w/w significantly inhibited the root and shoot biomass. Bio-MPs produced higher specific root length and specific root nodules while LDPE-MPs also showed significant impacts on specific root nodules, suggesting a potential threat of MPs to soil-plant systems. The results presented have demonstrated that the occurrence of MPs in soil are capable of changing the plant growth, this is a fundamental understanding for future efforts to assess risks of agricultural MPs pollution in soil-plant systems. This current research, therefore, has highlighted the

necessity to gain more insight into the mechanisms (i.e., dynamics of nutrient status and soil bacterial communities) underlying MPs effects on plant growth and the fate of MPs with different properties (types and size) in soil-plant systems.

Acknowledgements
Many thanks to Andre Maassen, Sean Geurts, Bertus van der Laan and Gerard Derks from Wageningen Unifarm for helping to manage the experiment in the net-house. Thanks to Peter van der Putten, for providing the method for root morphology measurement and sharing the apparatus for analysing the root. Thanks to Tamara ten Den for sharing the chlorophyll meter SPAD 502 plus.

Supplementary Material

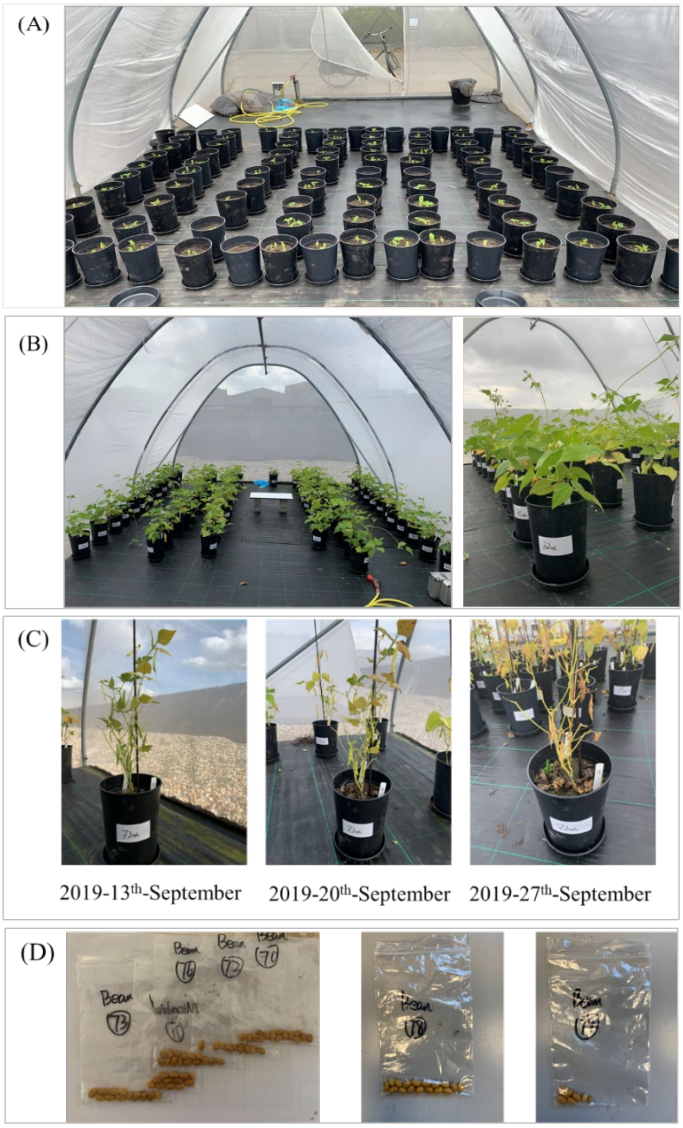


Figure S3.1 *The experiment set up of the common bean growth for LDPE-MP and Bio-MP. (A) The pots at the beginning of the experiment, plastic film was partially placed during the germination to prevent seed rotten by the excessive rainfall; (B) Common bean at the end of vegetative stage; (C) Common bean development from13th September to fully mature stage; (D) Harvest beans.*

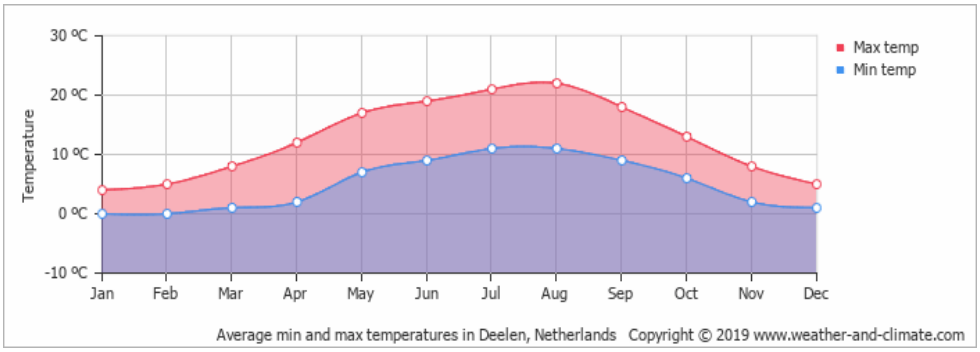


Figure S3.2 *The average temperature in Wageningen during 2019.*

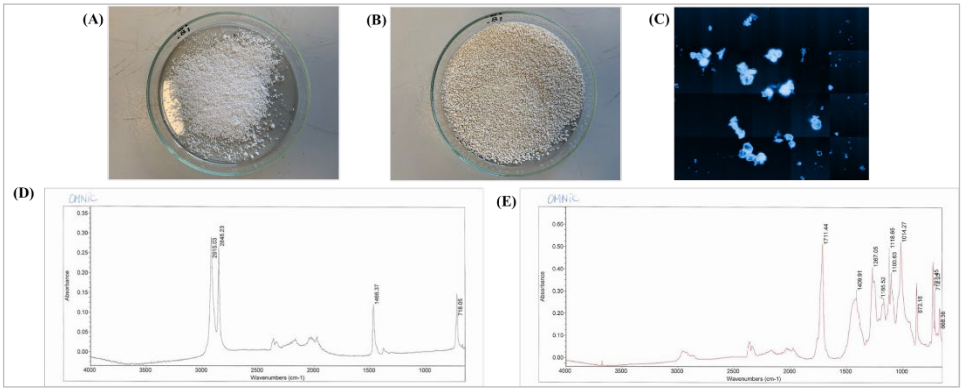
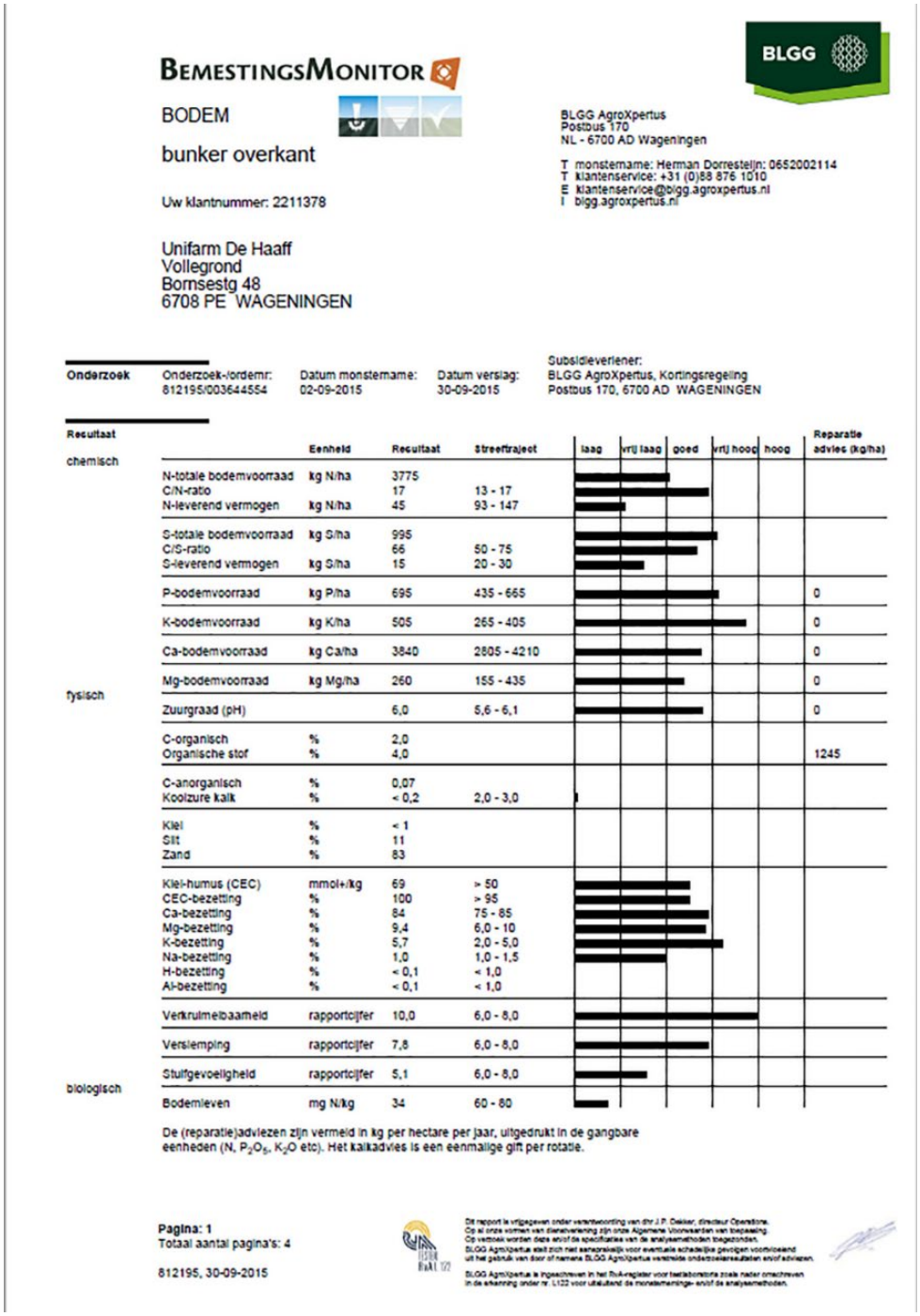


Figure S3.3 *The MPs applied in current research. (A) LDPE-MPs; (B) Bio-MPs; (C) scanned PLA and Bio MPs by Laser Direct Infrared (LDIR) system (Agilent Cross Lab); (D) FTIR of LDPE-MPs; (E) FTIR of LDPE-MPs.*



BEMESTINGSMONITOR

BODEM

bunker overkant

Uw klantnummer: 2211378

Unifarm De Haaff
Vollegrond
Bornsestg 48
6708 PE WAGENINGEN

BLGG

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Onderzoek	Onderzoek-/ordernr:	Datum monstername:	Datum verslag:	Subsidieverlener:
	812195/003644554	02-09-2015	30-09-2015	BLGG AgroXpertus, Kortingsregeling Postbus 170, 6700 AD WAGENINGEN

Resultaat		Eenheid	Resultaat	Streeftraject	laag	vrij laag	goed	vrij hoog	hoog	Reparatie advies (kg/ha)
chemisch	N-totale bodemvoorraad	kg N/ha	3775							
	C/N-ratio		17	13 - 17						
	N-leverend vermogen	kg N/ha	45	93 - 147						
	S-totale bodemvoorraad	kg S/ha	995							
	C/S-ratio		66	50 - 75						
	S-leverend vermogen	kg S/ha	15	20 - 30						
	P-bodemvoorraad	kg P/ha	695	435 - 665						0
	K-bodemvoorraad	kg K/ha	505	265 - 405						0
	Ca-bodemvoorraad	kg Ca/ha	3840	2805 - 4210						0
	Mg-bodemvoorraad	kg Mg/ha	260	155 - 435						0
fysisch	Zuurgraad (pH)		6,0	5,6 - 6,1						0
	C-organisch	%	2,0							
	Organische stof	%	4,0							1245
	C-anorganisch	%	0,07							
	Koolzure kalk	%	< 0,2	2,0 - 3,0						
	Klei	%	< 1							
	Slit	%	11							
	Zand	%	83							
	Klei-humus (CEC)	mmol+/kg	69	> 50						
	CEC-bezetting	%	100	> 95						
	Ca-bezetting	%	84	75 - 85						
	Mg-bezetting	%	9,4	6,0 - 10						
	K-bezetting	%	5,7	2,0 - 5,0						
	Na-bezetting	%	1,0	1,0 - 1,5						
	H-bezetting	%	< 0,1	< 1,0						
	Al-bezetting	%	< 0,1	< 1,0						
	Verkruimelbaarheid	rapportcijfer	10,0	6,0 - 8,0						
	Verslemping	rapportcijfer	7,8	6,0 - 8,0						
	Stuifgevoeligheid	rapportcijfer	5,1	6,0 - 8,0						
biologisch	Bodemleven	mg N/kg	34	60 - 80						

De (reparatie)adviezen zijn vermeld in kg per hectare per jaar, uitgedrukt in de gangbare eenheden (N, P_2O_5, K_2O etc). Het kalkadvies is een eenmalige gift per rotatie.

Pagina: 1
Totaal aantal pagina's: 4

812195, 30-09-2015

Dit rapport is vrijgegeven onder verantwoording van dhr J.P. Dekker, directeur Operations.
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Figure S3.4 *Detailed information about soil used in the experiment.*

OVERZICHT BEREKENING TOMAAT-N

Datum : Woensdag 29 December 2010 (Overzicht)

	uitgang-schema	□ correcties	teeltfase correcties	na teeltf. correcties	EC correctie	NaCl correctie	water correcties	drainwater correctie	Na water correcties	optie correctie	concentr correctie
H+	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EC	2.000	0.000		2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
pH											
NH4	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	8.000	0.000		8.000	8.000	8.000	0.000	0.000	8.000	8.000	8.000
Na											
Ca	4.000	0.000		4.000	4.000	4.000	0.000	0.000	4.000	4.000	4.000
Mg	2.000	0.000		2.000	2.000	2.000	0.000	0.000	2.000	2.000	2.000
NO3	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CL	8.000	0.000		8.000	8.000	8.000	0.000	0.000	8.000	8.000	8.000
SO4	5.000	0.000		5.000	5.000	5.000	0.000	0.000	5.000	5.000	5.000
HCO3											
P	2.000	0.000		2.000	2.000	2.000	0.000	0.000	2.000	2.000	2.000
Si	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fe	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mn	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Zn	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cu	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mo	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

```
Gewas : tomaat-n

EC-voorregeling (a+b+c+d)  0.0 EC

  0.00% drainwater          0.00 EC
100.00% Regenwater          0.00 EC
        A 00.00.00.00.00.00
  0.00% Bronwater           0.00 EC
        A 00.00.00.00.00.00
  0.00% Leidingwater        0.00 EC
        A 00.00.00.00.00.00

NatriumChloride Corr.: Uit
CalciumChloride Corr.: Aan
Vrije drainage
```

```
SUBSTRAFEED Pakket : E1
A-bak  1000 liter 100 x geconc.
CALSAL                    0.00 liter
MAGNITRA                  0.00 liter
AMNITRA                   0.00 liter
CALCIUMCHLORIDE         102.56 liter

B-bak  1000 liter 100 x geconc.
SALPETERZUUR              0.00 liter
SULFAKAL                 72.15 liter
FOSFORZUUR               23.47 liter
BASKAL                   73.44 liter
MAGNESUL                 92.92 liter

extra dosering per 100.000 liter bemest water
```

spoorelementen :

IJzerchelaat DTPA 3.0%	0 gr	(0 ml)
IJzerchelaat EDDHSA 3.0%	0 gr	(0 ml)
Mangaansulfaat 32,5%	0 gr	
Zinksulfaat 22,7%	0 gr	
Borax 11,3%	0 gr	
Kopersulfaat 25,5%	0 gr	
Natriummolybdaat 39,6%	0 gr	

```
Bedrijfsnaam : Unifarm
Adres        : Bornsesteeg 48
Plaatsnaam   : 6708PB WAGENINGEN
Telefoonnr.  : 031785345
Faxnummer    : 0
```

Hydro Agri 010 445321RR
Hydro Agri 010 445
HydroQuest@hydro

Hydro Agri Specialities
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Figure S3.5 *Reagents and concentrations of the nutrient solution.*

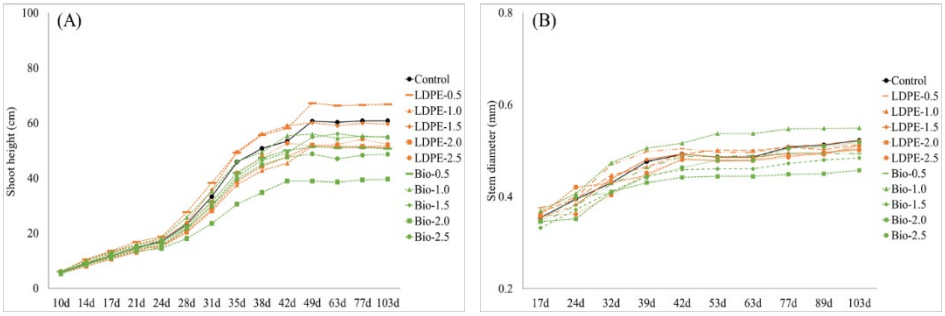

Figure S3.6 *Plant shoot height and shoot diameter during the whole growing phase of the common bean. (A) Plant root height; (B) Plant diameters.*

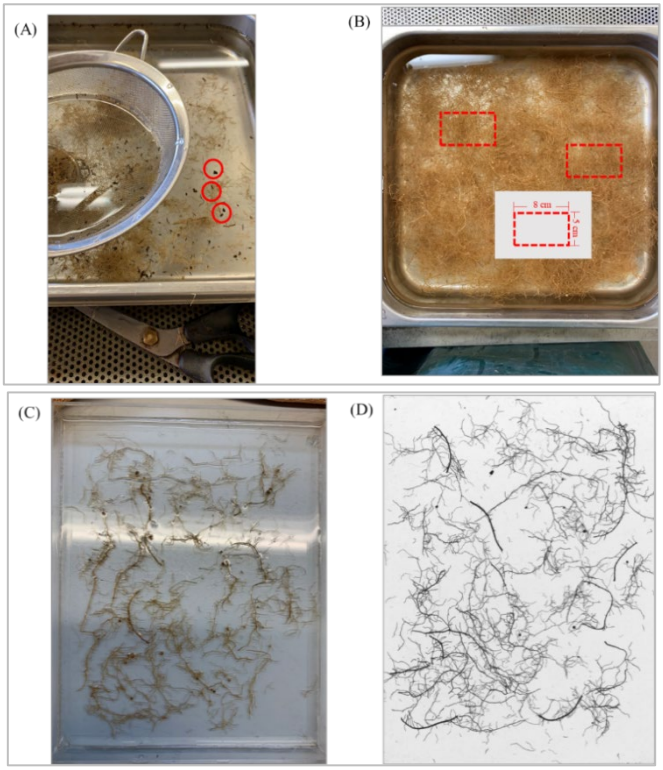

Figure S3.7 *The root sampling procedure. (A) Organic debris in the root system; (B) Subsample size; (C) Root subsample for scanning; (D) The scanning result of WinRHIZO.*

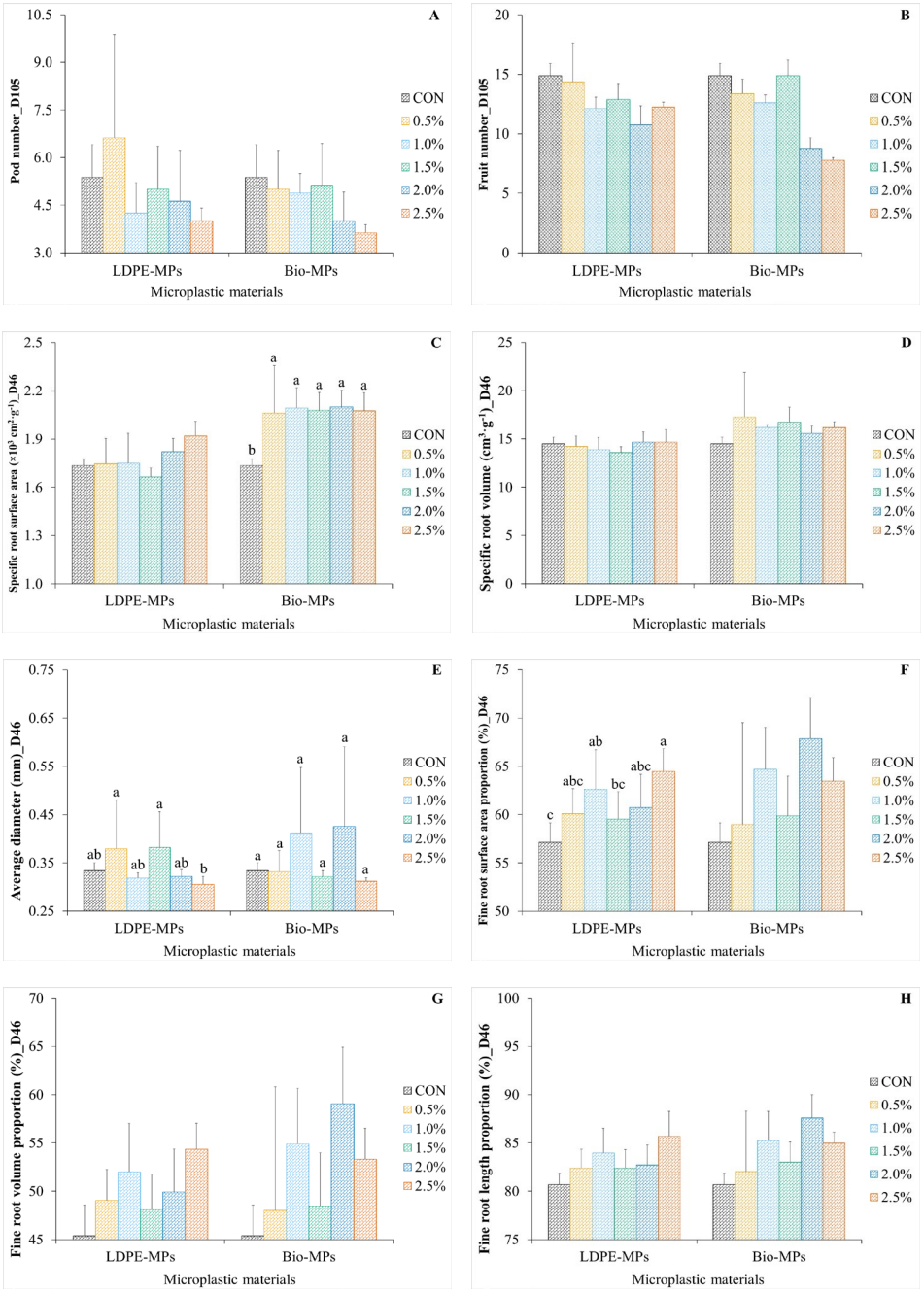

Figure S3.8 *Plant growth parameters. (A) Pod number at the fully mature stage; (B) Fruit number at the fully mature stage; (C). Specific root surface area at the end of vegetative stage (Vs); (D). Specific root volume at the end of vegetative stage; (E). Root average diameter at the end of vegetative stage; (F) Fine root surface area proportion at the end of vegetative stage; (G) Fine root volume proportion at the end of vegetative*

stage; (H) Fine root length proportion at the end of vegetative stage. Legend indicates the microplastic contamination level, including control (dark), 0.5% (orange), 1.0% (light blue), 1.5% (green), 2.0% (blue) and 2.5% (Vermillion). Error bars represent standard deviation; and the lowercase letters (a and b) indicate significant differences between control treatment and microplastic contamination treatment within each microplastic material. Post-hoc test was only performed when growth parameters were significantly affected by the occurrence of MPs.

Table S3.1 *Development stage of common bean*

Stage development	Stage Number	Days from planting (Approximately)	General Description
Vegetative stage	VE	7-8	Hypocotyl Emergence (crook stage)
	VC	8-9	Cotyledon (seed leaves) and unifoliolate leaves visible
	V1	10	First fully developed trifoliate at the third node
	V2	19	Second trifoliate (count when leaf edges no longer touch)
	V3	29	Third trifoliate (secondary branching begins to show in leaf axils)
	V(n)	A new node every 3 to 5 days	Nth trifoliate, but with blossom clusters still not visibly opened
	V5	50	Plants may begin to exhibit blossom and become stage R1
Reproductive stage	R1	50	One blossom opens at any node
	R2	53	Pods 1/2-inch-long at first blossom position (usually node 2 to 3)
	R3	56	Pods 1 inch long at first blossom position; secondary branching at all nodes, so plant is becoming denser but not taller, 1/2 bloom
	R4	59	Pods 3 inches long (seeds not discernible; bush types may be shorter)
	R5	64	Pods 3 to 4 inches (seed discernible)
	R6	66	Seeds at least 1/4 inch over long axis
	R7	72	Oldest pods have developed seeds (other parts of plant will have full-length pods with seeds almost as large as first pods; pods will be developed over the whole plant)
	R8	90	Leaves yellowing over half of plant, very few small pods may be drying (point of maximum production has been reached)
	R9	105	Mature, at least 80% of pods showing yellow and mostly ripe; only 40% of leaves still green

Note. Information provided by North Dakota State University: https://www.ag.ndsu.edu/crops/dry-bean-articles/stages-of-development.

Table S3.2 *Summary of the one-way ANOVA applied to the growth parameters.*

Materials	**Parameters**	df	F value	P-value
LDPE-MP	SB_VS	5,18	2.000	0.127
	RB_VS	5,18	0.846	0.535
	S:R_VS	5,18	0.827	0.547
	LA_VS	5,18	3.112	***0.034***
	Chlor_VS	5,18	4.071	***0.004***
	FruitB	5,18	0.367	0.864
	FruitNb	5,18	0.777	0.576
	PodNb	5,18	1.258	0.324
	SRL	5,18	3.459	***0.023***
	SRSA	5,18	2.377	0.080
	SRV	5,18	0.762	0.589
	SRN	5,18	7.822	***0.000***
	FRL	5,18	2.57	0.064
	FRSA	5,18	2.97	***0.04***
	FRV	5,18	2.65	0.06
Bio-MP	SB_VS	5,18	5.938	***0.002***
	RB_VS	5,18	12.817	***0.000***
	S:R_VS	5,18	3.251	***0.029***
	LA_VS	5,18	28.286	***0.000***
	Chlor_VS	5,18	3.244	***0.014***
	FruitB	5,18	2.855	***0.045***
	FruitNb	5,18	2.746	0.052
	PodNb	5,18	2.06	0.118
	SRL	5,18	6.254	***0.002***
	SRSA	5,18	3.746	***0.017***
	SRV	5,18	0.942	0.478
	SRN	5,18	12.08	***0.000***
	FRL	5,18	2.36	0.082
	FRSA	5,18	2.10	0.11
	FRV	5,18	2.02	0.12

Note.

SB_VS: shoot biomass at D46, vegetative stage.

RB_VS: root biomass at D46, vegetative stage.

S:R_VS: biomass of shoot to root ratio at D46, vegetative stage.

LA_VS: leaf area at D46, vegetative stage.

Chlor_VS: relative leaf chlorophyll content at D46, vegetative stage.

FruitB: Fruit biomass at D105, fully mature stage.

FruitNb: Fruit number at D105, fully mature stage.

PodNb: Pod number at D105, fully mature stage.
SRL: specific root length at D46, vegetative stage.
SRSA: specific root surface area at D46, vegetative stage.
SRV: specific root volume at D46, vegetative stage.
SRN: specific root nodule at D46, vegetative stage.
FRL: the proportion of fine root (diameter < 0.4 mm) length at D46, vegetative stage.
FRSA: the proportion of fine root surface area at D46, vegetative stage.
FRV: the proportion of fine root volume at D46, vegetative stage.

Table S3.3 continued. *Independent-Samples T Test between groups for common bean growth parameters. Data have been transformed using arcsine square root.*

Growth parameter	MPs dose	Levene's Test for Equality of Variances			t-test for Equality of Means		
		Equal variances	F	Sig.	t	df	Sig.
Shoot biomass at vegetative stage	0.5	assumed	0.724	0.427	-1.111	6	0.309
		not assumed			-1.111	4.686	0.32
	1.0	assumed	0.004	0.95	1.352	6	0.225
		not assumed			1.352	5.861	0.226
	1.5	assumed	0.458	0.524	0.592	6	0.575
		not assumed			0.592	4.931	0.58
	2.0	assumed	0.168	0.696	5.576	6	0.001
		not assumed			5.576	5.715	0.002
	2.5	assumed	2.64	0.155	2.146	6	0.075
		not assumed			2.146	3.401	0.11
Root biomass at vegetative stage	0.5	assumed	2.462	0.168	-0.777	6	0.467
		not assumed			-0.777	3.612	0.485
	1.0	assumed	4.847	0.07	0.798	6	0.455
		not assumed			0.798	3.072	0.482
	1.5	assumed	50.46	0	1.789	6	0.124
		not assumed			1.789	3.367	0.161
	2.0	assumed	0.386	0.557	5.019	6	0.002
		not assumed			5.019	5.297	0.003
	2.5	assumed	2.787	0.146	0.703	6	0.508
		not assumed			0.703	3.426	0.527
S:R at vegetative stage	0.5	assumed	0.63	0.458	0.392	6	0.708
		not assumed			0.392	4.552	0.713
	1.0	assumed	0.059	0.816	-0.474	6	0.652
		not assumed			-0.474	5.505	0.654
	1.5	assumed	0.19	0.678	1.452	6	0.197
		not assumed			1.452	5.115	0.205
	2.0	assumed	0.3	0.604	-2.049	6	0.086
		not assumed			-2.049	5.441	0.091
	2.5	assumed	0.129	0.732	-1.505	6	0.183
		not assumed			-1.505	5.975	0.183

Table S3.3 continued. *Independent-Samples T Test between groups for common bean growth parameters. Data have been transformed using arcsine square root.*

Growth parameter	MPs dose	Levene's Test for Equality of Variances			t-test for Equality of Means		
		Equal variances	F	Sig.	t	df	Sig.
Leaf area at vegetative stage	0.5	assumed	0.947	0.368	-1.673	6	0.145
		not assumed			-1.673	4.239	0.166
	1.0	assumed	1.58	0.255	1.982	6	0.095
		not assumed			1.982	4.416	0.112
	1.5	assumed	0.913	0.376	1.774	6	0.126
		not assumed			1.774	4.606	0.141
	2.0	assumed	0.121	0.74	4.694	6	0.003
		not assumed			4.694	5.999	0.003
	2.5	assumed	3.436	0.113	3.073	6	0.022
		not assumed			3.073	3.447	0.045
Relative leaf chlorophyll content at vegetative stage	0.5	assumed	0.555	0.469	-2.995	14	0.01
		not assumed			-2.995	13.551	0.01
	1.0	assumed	0.017	0.899	1.697	14	0.112
		not assumed			1.697	13.778	0.112
	1.5	assumed	10.282	0.006	0.685	14	0.504
		not assumed			0.685	10.033	0.509
	2.0	assumed	0.836	0.376	0.187	14	0.854
		not assumed			0.187	12.828	0.855
	2.5	assumed	0.819	0.381	4.397	14	0.001
		not assumed			4.397	11.467	0.001
Fruit biomass at fully mature stage	0.5	assumed	0.209	0.664	0.292	6	0.78
		not assumed			0.292	5.828	0.781
	1.0	assumed	0.754	0.419	-0.532	6	0.614
		not assumed			-0.532	5.776	0.615
	1.5	assumed	0.659	0.448	-0.992	6	0.359
		not assumed			-0.992	4.952	0.367
	2.0	assumed	0.514	0.5	2.216	6	0.069
		not assumed			2.216	5.644	0.071
	2.5	assumed	1.82	0.226	4	6	0.007
		not assumed			4	4.141	0.015

Table S3.3 continued. *Independent-Samples T Test between groups for common bean growth parameters. Data have been transformed using arcsine square root.*

Growth parameter	MPs dose	Levene's Test for Equality of Variances			t-test for Equality of Means		
		Equal variances	F	Sig.	t	df	Sig.
Pod number at fully mature stage	0.5	assumed	1.905	0.217	0.951	6	0.378
		not assumed			0.951	4.281	0.392
	1.0	assumed	1.43	0.277	-1.09	6	0.317
		not assumed			-1.09	4.952	0.326
	1.5	assumed	0.153	0.71	-0.15	6	0.886
		not assumed			-0.15	5.861	0.886
	2.0	assumed	0.497	0.507	0.664	6	0.531
		not assumed			0.664	5.192	0.535
	2.5	assumed	0.148	0.713	1.57	6	0.168
		not assumed			1.57	5.089	0.176
Specific root length at vegetative stage	0.5	assumed	0.004	0.953	-1.923	6	0.103
		not assumed			-1.923	5.983	0.103
	1.0	assumed	0.102	0.761	-2.42	6	0.052
		not assumed			-2.42	5.998	0.052
	1.5	assumed	2.785	0.146	-8.962	6	0
		not assumed			-8.962	3.868	0.001
	2.0	assumed	2.368	0.175	-4.543	6	0.004
		not assumed			-4.543	4.786	0.007
	2.5	assumed	0.337	0.583	-1.268	6	0.252
		not assumed			-1.268	5.168	0.259
Specific surface root area at vegetative stage	0.5	assumed	3.747	0.101	-1.918	6	0.104
		not assumed			-1.918	4.763	0.116
	1.0	assumed	5.425	0.059	-3.044	6	0.023
		not assumed			-3.044	4.978	0.029
	1.5	assumed	0.409	0.546	-6.787	6	0.001
		not assumed			-6.787	4.719	0.001
	2.0	assumed	1.245	0.307	-4.224	6	0.006
		not assumed			-4.224	5.82	0.006
	2.5	assumed	0.038	0.852	-2.156	6	0.074
		not assumed			-2.156	5.796	0.076

Table S3.3 continued. *Independent-Samples T Test between groups for common bean growth parameters. Data have been transformed using arcsine square root.*

Growth parameter	MPs dose	Levene's Test for Equality of Variances			t-test for Equality of Means		
		Equal variances	F	Sig.	t	df	Sig.
Specific root volume at vegetative stage	0.5	assumed	3.229	0.122	-1.31	6	0.238
		not assumed			-1.31	3.461	0.27
	1.0	assumed	5.042	0.066	-3.487	6	0.013
		not assumed			-3.487	3.202	0.036
	1.5	assumed	1.07	0.341	-3.925	6	0.008
		not assumed			-3.925	4.093	0.016
	2.0	assumed	0.783	0.41	-1.405	6	0.209
		not assumed			-1.405	5.249	0.216
	2.5	assumed	3.227	0.123	-2.067	6	0.084
		not assumed			-2.067	4.146	0.105
Specific root nodule at vegetative stage	0.5	assumed	2.238	0.185	-6.398	6	0.001
		not assumed			-6.398	5.219	0.001
	1.0	assumed	0.384	0.558	0.732	6	0.492
		not assumed			0.732	5.765	0.493
	1.5	assumed	1.64	0.248	0.646	6	0.542
		not assumed			0.646	4.341	0.551
	2.0	assumed	1.032	0.349	-3.408	6	0.014
		not assumed			-3.408	4.619	0.022
	2.5	assumed	0.242	0.64	-2.319	6	0.06
		not assumed			-2.319	5.096	0.067
Root average diameter at vegetative stage	0.5	assumed	1.972	0.21	0.861	6	0.422
		not assumed			0.861	4.257	0.435
	1.0	assumed	12.248	0.013	-1.387	6	0.215
		not assumed			-1.387	3.093	0.257
	1.5	assumed	75.977	0	1.6	6	0.161
		not assumed			1.6	3.282	0.2
	2.0	assumed	29.553	0.002	-1.223	6	0.267
		not assumed			-1.223	3.085	0.306
	2.5	assumed	0.6	0.468	-1.095	6	0.315
		not assumed			-1.095	4.412	0.329

Table S3.3 continued. *Independent-Samples T Test between groups for common bean growth parameters. Data have been transformed using arcsine square root.*

Growth parameter	MPs dose	Levene's Test for Equality of Variances			t-test for Equality of Means		
		Equal variances	F	Sig.	t	df	Sig.
Fine root length proportion at vegetative stage	0.5	assumed	3.475	0.112	0.149	6	0.886
		not assumed			0.149	3.55	0.889
	1.0	assumed	0.102	0.76	-0.625	6	0.555
		not assumed			-0.625	5.881	0.556
	1.5	assumed	0.142	0.72	-0.416	6	0.692
		not assumed			-0.416	5.896	0.692
	2.0	assumed	0.031	0.866	-3.032	6	0.023
		not assumed			-3.032	5.863	0.024
	2.5	assumed	1.163	0.322	0.454	6	0.666
		not assumed			0.454	4.059	0.673
Fine root surface area at vegetative stage	0.5	assumed	4.055	0.091	0.272	6	0.795
		not assumed			0.272	3.324	0.802
	1.0	assumed	0.02	0.893	-0.709	6	0.505
		not assumed			-0.709	5.982	0.505
	1.5	assumed	0.841	0.394	-0.154	6	0.883
		not assumed			-0.154	5.324	0.884
	2.0	assumed	0.016	0.904	-2.647	6	0.038
		not assumed			-2.647	5.892	0.039
	2.5	assumed	0.125	0.736	0.593	6	0.575
		not assumed			0.593	5.996	0.575
Fine root volume at vegetative stage	0.5	assumed	4.055	0.091	0.272	6	0.795
		not assumed			0.272	3.324	0.802
	1.0	assumed	0.02	0.893	-0.709	6	0.505
		not assumed			-0.709	5.982	0.505
	1.5	assumed	0.841	0.394	-0.154	6	0.883
		not assumed			-0.154	5.324	0.884
	2.0	assumed	0.016	0.904	-2.647	6	0.038
		not assumed			-2.647	5.892	0.039
	2.5	assumed	0.125	0.736	0.593	6	0.575
		not assumed			0.593	5.996	0.575

Table S3.4 Data for all growth parameters values are displayed as (mean ± standard deviation) for the different microplastic concentrations.

Growth parameters	Microplatic type	Control	0.5	1.0	1.5	2.0	2.5
SB-Vs	LDPE-MiP	5.18±0.42ab,A	4.75±0.31ab	5.34±0.66a	4.59±0.82ab	5.32±0.34a	4.44±0.62b
	Bio-MiP		5.10±0.56A	4.65±0.77AB	4.31±0.49BC	3.80±0.43C	3.75±0.16C
RB-Vs	LDPE-MiP	1.82±0.20a,B	1.91±0.24a	2.02±0.41a	1.76±0.30a	2.05±0.14a	1.77±0.32a
	Bio-MiP		2.01±0.08A	1.86±0.04AB	1.50±0.06E	1.66±0.08CD	1.64±0.09D
LA_Vs	LDPE-MiP	626±80.0bc,A	549±54.1c	724±56.0a	638±92.1abc	645±54.2ab	618±20.8bc
	Bio-MiP		657±116A	601±112A	546±49.6A	464±54.8B	497±75.9B
Chl.	LDPE-MiP	33.07±1.16	27.19±2.34	32.15±3.36	30.09±3.05	31.15±3.25	32.91±2.29
	Bio-MiP		31.40±2.67	29.72±2.75	30.01±1.86	29.88±3.96	26.38±3.41
RS_ratio	LDPE-MiP	2.85±0.16a,A	2.50±0.26a	2.74±0.74a	2.63±0.32a	2.60±0.18a	2.53±0.17a
	Bio-MiP		2.54±0.30A	2.49±0.38AB	2.88±0.26AB	2.29±0.24C	2.30±0.20C
SRL	LDPE-MiP	16,604±1,082b,C	17,090±1,934b	17,568±2,252b	16,250±862b	18,049±937ab	20,047±989.4a
	Bio-MiP		19,859±2,213B	21,590±2,442AB	20,582±367AB	22,550±1,816A	21,219±1,539A
SRSA	LDPE-MiP	1,735±40.5b,B	1,746±157b	1,750±186b	1,663±56.5b	1,823±80.8ab	1,920±90.0a
	Bio-MiP		2,061±295A	2,094±125A	2,079±112A	2,100±104A	2,076±113A
SRV	LDPE-MiP	14.5±0.71a,A	14.2±1.10a	13.9±1.26a	13.6±0.62a	14.7±1.04a	14.7±1.27a
	Bio-MiP		17.3±4.61A	16.2±0.26A	16.7±1.55A	15.6±0.73A	16.2±0.61A

Note. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP contamination level and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP contamination level and the control treatment. Only growth parameters with $p < 0.05$ (one-way ANOVAs) were checked with post-hoc test. Data have been transformed using arcsine square root. SB_VS: shoot biomass at the end of vegetative stage.
RB_VS: root biomass at the end of vegetative stage. **S:R:** biomass of shoot to root ratio at the end of vegetative stage; **LA_VS:** leaf area at the end of vegetative stage. **Chlor_VS:** relative leaf chlorophyll content at the end of vegetative stage. **SRL:** specific root length at the end of vegetative stage. **SRSA:** specific root surface area at the end of vegetative stage. **RAD:** root average diameter at the end of vegetative stage. **SRV:** specific root volume at the end of vegetative stage.

***Table 3.4* Continued.** Data for all growth parameters values are displayed as (mean ± standard deviation) for the different microplastic concentrations.

Growth parameters	Microplatic type	Control	0.5	1.0	1.5	2.0	2.5
SRN	LDPE-MiP	510±58.4cd,B	377±55.0d	943±184a	752±153ab	658±133bc	709±239b
	Bio-MiP		760±119AB	853±143AB	695±70.0AB	947±91.0B	1,053±178A
RAD	LDPE-MiP	0.33±0.02ab,A	0.38±0.10a	0.32±0.01ab	0.38±0.07a	0.32±0.01ab	0.31±0.02b
	Bio-MiP		0.33±0.04A	0.41±0.14A	0.32±0.01A	0.43±0.16A	0.31±0.01A
FRL(%)	LDPE-MiP	80.7±1.21c	82.4±1.94bc	84.0±2.55ab	82.4±1.91bc	82.7±2.08abc	85.7±2.56a
	Bio-MiP		82.1±6.23B	85.3±3.00AB	83.0±2.10AB	87.6±2.42A	85.0±1.15AB
FRA(%)	LDPE-MiP	57.1±2.03c,B	60.1±2.57abc	62.6±4.09ab	59.5±2.85bc	60.7±3.46abc	64.5±2.36a
	Bio-MiP		59.0±10.5B	64.7±4.34AB	59.9±4.11AB	67.9±4.24A	63.5±2.43AB
FRV(%)	LDPE-MiP	45.4±3.16c	49.1±3.21abc	52.0±5.01ab	48.1±3.68bc	49.9±4.44abc	54.4±2.70a
	Bio-MiP		48.0±12.8B	54.9±5.76AB	48.5±5.52AB	59.1±5.84A	53.3±3.20AB
FruitB	LDPE-MiP	4.06±1.57a	4.05±0.98a	3.68±0.70a	3.58±0.62a	3.37±0.59a	3.59±0.60a
	Bio-MiP		3.83±1.16A	3.97±0.86AB	4.17±1.02AB	2.55±0.45BC	2.28±0.27C
PodNb	LDPE-MiP	5.38±1.03a	6.63±3.25ab	4.25±0.96ab	5.00±1.35ab	4.63±1.60ab	4.00±0.41b
	Bio-MiP		5.00±1.22AB	4.88±0.63AB	5.13±1.31A	4.00±0.91AB	3.63±0.25B

Note. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP contamination level and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP contamination level and the control treatment. Only growth parameters with $p < 0.05$ (one-way ANOVAs) were checked with post-hoc test. Data have been transformed using arcsine square root. SB_VS: shoot biomass at the end of vegetative stage.

FRL: the proportion of fine root (diameter < 0.4 mm) length at the end of vegetative stage. **FRSA:** the proportion of fine root surface area at the end of vegetative stage. **FRV:** the proportion of fine root volume at the end of vegetative stage. **SRN:** specific root nodule at the end of vegetative stage. **FruitB:** Fruit biomass at fully mature stage. **PodNb:** Pod number at fully mature stage.

Table S3.5 Pearson correlations matrix for the all growth parameters.

Growth parameters	SB_Vs	RB_Vs	LA_Vs	Chlor_Vs	SRL	SRSA	SRV	FRL	FRS	FRV	SRN	FruitB	PodNb
SB_VS	1												
RB_VS	.593**	1											
LA_VS	.780**	.552**	1										
Chlor_VS	0.242	-0.067	0.188	1									
SRL	-.497**	-.304*	-.363*	-0.275	1								
SRSA	-.373**	-0.269	-0.266	-0.264	.894**	1							
SRV	-0.112	-0.151	-0.065	-0.171	.508**	.840**	1						
FRL	-.438**	-0.128	-.311*	-0.191	.674**	.287*	-0.265	1					
FRS	-.419**	-0.098	-.351*	-0.151	.635**	0.248	-.294*	.974**	1				
FRV	-.417**	-0.1	-.364*	-0.145	.635**	0.248	-.294*	.964**	.998**	1			
SRN	0.252	0.141	.300*	.328*	-0.077	-0.083	-0.057	-0.028	-0.053	-0.062	1		
FruitB	.374**	0.082	.357*	.324*	-0.186	-0.235	-0.226	-0.1	-0.095	-0.079	0.244	1	
PodNb	0.247	0.17	0.158	0.235	-0.225	-0.257	-0.225	-0.126	-0.109	-0.09	0.103	.770**	1

Note. Bold italic growth parameters are considered weakly correlated with other growth parameters (|r| < 0.35).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

SB_VS: shoot biomass at the end of vegetative stage.

RB_VS: root biomass at the end of vegetative stage.

S:R_VS: biomass of shoot to root ratio at the end of vegetative stage;

LA_VS: leaf area at the end of vegetative stage.

Chlor_VS: relative leaf chlorophyll content at the end of vegetative stage.

SRL: specific root length at the end of vegetative stage.

SRSA: specific root surface area at the end of vegetative stage.

RAD: root average diameter at the end of vegetative stage.

SRV: specific root volume at the end of vegetative stage.

FRL: the proportion of fine root (diameter < 0.4 mm) length at the end of vegetative stage.

FRSA: the proportion of fine root surface area at the end of vegetative stage.

FRV: the proportion of fine root volume at the end of vegetative stage.

SRN: specific root nodule at the end of vegetative stage.

Table S3.6 Statistical summary of Redundancy analysis

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.642	0.028	0.011	0.005
Explained variation (cumulative)	64.2	67.0	68.1	68.6
Pseudo-canonical correlation	0.86	0.77	0.59	0.36
Explained fitted variation (cumulative)	93.1	97.2	98.8	99.6

Permutation Test Results:
On First Axis: pseudo-F = 5.5, P = 0.002
On All Axes: pseudo-F = 7.3, P = 0.002

4. Effect of different polymers of microplastics on soil organic carbon and nitrogen – a mesocosm experiment

Agricultural microplastic pollution has become a growing concern. Unfortunately, the impacts of microplastics (MPs) on agricultural soil carbon and nitrogen dynamics have not been sufficiently reported. In an attempt to remedy this, we conducted a 105-day outdoor mesocosm experiment in a soil-plant system using sandy soils amended with two types of MPs, low-density polyethylene (LDPE-MPs) and biodegradable (Bio-MPs), at concentrations of 0.0% (control), 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w, weight ratio of microplastics to air-dry soil). Soil organic carbon (SOC), dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), available nitrogen (AN) of N-NH_4^+ and N-NO_3^-, and dissolved organic nitrogen (DON) were measured on day 46 (D46) and 105 (D105) of the experiment. SOC was also measured after microplastics were mixed into soils (D0). For LDPE-MPs treatments, SOC on D0, D46 and D105 showed no significant differences, while for Bio-MPs treatments, SOC significantly ($p < 0.05$) decreased from D0 to D46, which might be attributable to the rapid biodegradation of Bio-MPs. Compared to the control, soil POXC was significantly ($p = 0.001$) lowered by 0.5%, 1.0% and 2.5% LDPE-MPs and ≥ 1.0% Bio-MPs on D105. LDPE-MPs showed no significant effects on soil DOC and nitrogen cycling. 2.0% and 2.5% Bio-MPs showed significantly higher ($p < 0.001$) DOC and DON (D46 and D105) and ≥ 1.5% Bio-MPs showed significantly lower ($p = 0.02$) AN (D46). Overall, Bio-MPs exerted stronger effects on the dynamics of soil carbon and nitrogen cycling. In conclusion, microplastics might pose serious threats to agroecosystems and further research is needed.

Based on:

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4.1 Introduction

Microplastics (MPs) are plastic particles with a diameter < 5 mm. MPs pollution in the agroecosystem has received increasing attention globally (Andrady, 2017; Barnes et al., 2009; Qi et al., 2020a). Mounting evidence has shown that agricultural soils receive microplastics in various ways. For example, a field survey conducted in four different agricultural areas in southwestern China, where plastic mulching and sewage sludge was applied to agricultural fields, found MPs ranging between 7100 to 42,960 particles·kg^{-1} soil (Zhang and Liu, 2018). Corradini et al. (2019b) found microplastic accumulation in agricultural fields that received sewage sludge irrigation. Inappropriate disposal of conventional plastic mulching films (low density polyethylene, LDPE) has been identified as one of the major contributors to agricultural microplastic pollution (Huang et al., 2020). To combat the growing plastic pollution caused by LDPE films used in agriculture, biodegradable (Bio) plastic mulches were developed as alternative solutions. However, recent research has suggested that most biodegradable materials currently available on the market tend to break down into smaller plastic particles rather than completely biodegrade, which leads to the accumulation of bio-microplastic in soils (de Souza Machado et al., 2018a; Li et al., 2014). Therefore, considering that agricultural microplastic pollution is likely to continue to be a problem in the future, uncovering the impacts of microplastics in agricultural soils deserves more attention.

The accumulation of microplastics in soil profiles could affect soil physical, chemical and biological processes (Iqbal et al., 2020; Ng et al., 2018). Numerous studies have shown that microplastics can significantly alter soil porosity, bulk density, water holding capacity and soil water repellency (de Souza Machado et al., 2018b; Qi et al., 2020b). In addition, the small size and large specific area of microplastics allow them to interact with the soil microbiome, affecting the soil microbial community and nutrient dynamics (Fei et al., 2020; Torres et al., 2021). A study from Liu et al. (2017) found that 28% polypropylene (PP) MPs stimulated the soil microbial activity and enhanced decomposition of organic matter while also suppressing the accumulation of soil available nitrogen content. The suppressive effects of microplastics on nitrification and denitrification processes have also been observed in other ecosystems. Seeley et al. (2020) conducted an incubation experiment in a sedimentary system and found that polyvinylchloride (PVC)-MPs and PLA-MPs could alter the microbial community composition, inhibit sediment nitrification and denitrification processes and lower the content of available nitrogen. Although there have been many efforts to study the effects of microplastics on terrestrial ecosystems, the effects of

microplastics on the dynamics of nitrogen in soil-plant systems remains largely unknown (de Graaff et al., 2010; Li et al., 2016a).

Another concern is the effect of microplastics on the soil organic pool (Rillig, 2018; Rillig et al., 2021). Owning to the carbon-based composition, microplastics might have already made hidden contributions to current carbon storage (Rillig, 2018). Until now however, the effects of microplastics on the soil organic matter (SOM) pool has only received limited attention (Zhang and Zhang, 2020). Soil labile organic carbon and nitrogen are sensitive and play important roles in soil ecosystem functions (Blanco-Moure et al., 2016; Muqaddas et al., 2019). For example, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) are more sensitive to soil microbial activity than total SOM (Bongiorno et al., 2019; Straathof et al., 2014). DOC and DON are small soluble fractions of SOM that mainly originate from the exudates of root and soil microorganisms. Soil permanganate oxidizable carbon (POXC) is mainly composed of polysaccharides and lignin originating from SOM decomposition and has been found to be closely related to soil microbial biomass and soil phospholipid fatty acid (Bongiorno et al., 2019; Jokela et al., 2009; Weil et al., 2003). As such, considering the current knowledge gaps in the effects of microplastics on soil fertility, a detailed study of the effects of microplastics on the dynamics of soil labile organic carbon and nitrogen cycling is necessary.

In our previous study, we observed that the occurrence of MPs in soil-plant systems alters the common bean growth (Meng et al., 2021). We speculated that the responses of common bean growth might be related to soil nutrient dynamics. Therefore, the general objective of this study was to investigate the effect of MPs on soil nutrient dynamics in a soil-plant system. Specifically, we measured soil labile C and N pools as indicated by (i) soil dissolved carbon and nitrogen (DOC, DON) and soil POXC; as well as (ii) available nitrogen content of soil N-NH_4^+ and N-NO_3^- in an outdoor mesocosm experiment that used two types of microplastic polymers: low-density polyethylene (LDPE-MPs) and biodegradable plastic of PBAT mixed with PLA (Bio-MPs). We hypothesized that both LDPE-MPs and Bio-MPs could affect the dynamics of soil labile carbon fractions and nitrogen, and that Bio-MPs would have stronger impacts than LDPE-MPs. The findings of this study will provide basic information for understanding the interactive effects of MPs and soil-plant systems.

4.2 Materials and methods

4.2.1 Experimental setup and soil sampling

4.2.1.1 Experiment setup

An outdoor net house mesocosm experiment was conducted (the side length of each square mesh was 0.25 mm) at Unifarm at Wagenigen University & Research (WUR, the Netherlands) from the 28th of June 2019 until the 18th of October 2019. Sandy soil with 87% sand, 12% silt and 1% clay, and an organic matter content of 4% and an organic carbon content of 2% was used (more details on Supplementary Table S4.1). Common bean (*Phaseolus vulgaris L.; Cultivar: Bruine Noordhollandse, P.vulgaris*) was selected as the model plant. The microplastics used in the research were LDPE-MPs and Bio-MPs. LDPE-MPs is obtained from Agrotechnolody & Food Science group of Wageningen University. Bio-MPs is 10% Polylactic acid (PLA), 85% polybutylene adipate terephthalate (PBAT), 5% calcium. The infrared spectroscopy (FTIR) of LDPE-MPs and Bio-MPs is presented in Supplementary Figure S4.1. For the manufacturing process, please refer to our previous publication Meng et al. (2021). The mesocosm experiment consisted of 11 treatments including a control treatment (CON) with only sandy soil and sandy soils polluted with two types of microplastics in five different doses, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w, weight ratio of microplastic to air-dry soil). There were 8 replicates for each treatment.

To achieve the target doses of soil-MPs mixtures for each treatment, 50 kg of homogenized air-dried sandy soil was manually mixed with the target amount of MPs (0.25 kg, 0.50 kg, 0.75 kg, 1.00 kg and 0.25 kg) in an iron tank using a wooden stick for 10 min. 6 kg of the homogeneous soil-MPs mixture was then placed in a 7 L polypropylene (PP) pot (21 cm high, 16 cm bottom diameter and 21 cm top diameter). The rest of the soil-MPs mixtures were stored for initial soil sample measurements for the soil organic carbon (SOC). The cultivation of the plants followed the same protocols as previously described (Meng et al., 2021). Two types of nutritive solutions were applied. At week 4 (26th of July) and 5 (2nd of August), 100 mL of Tomaat-N nutritive solution (Supplementary Table S4.2) was added to each pot. From the 6th to the 12th week, 100 mL of Hoagland 2.0 nutritive solution (Supplementary Table S4.2) was added to each pot once a week to ensure full development. Tomaat-N nutritive solution contained 1/3 of the nitrogen of the Hoagland 2.0, which served as a starter nutrient solution to initiate early growth of common bean (Chekanai et al., 2018). The nutritive solutions were prepared by Wageningen Unifarm. The PP pots used in the experiment were resistant and did not degrade during such a short time (105-day) of use. All treatments were treated in the same way. Hence, cross contamination could be ignored.

4.2.2.2 Soil sampling

Soil samples were collected twice. The first time was on the 15th of August 2019, 46 days after seeding (D46), near the end of the vegetative stage when the plant roots and leaves completed the early development stage. The second sampling was carried out on the 18th of October 2019 (105th days, D105), after plants were harvested. For each sampling time, four pots were harvested per treatment and plants were completely removed from the pots. Soil mass from each pot was thoroughly mixed. For each pot, 5 subsamples (50~60g/per sample) of bulk soil were randomly collected and mixed to form a composite sample. The soil samples were air-dried and passed through a 2 mm steel sieve for measuring SOC, soil permanganate oxidizable carbon (POXC), available nitrogen (AN), including nitrate nitrogen (N-NO_3^-) and ammonium nitrogen (N-NH_4^+), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON) and DOC (All abbreviations are shown in Table 4.1).

Table 4.1 *Abbreviations of measured soil parameters*

Abbreviation	Measured parameters
SOM	Soil organic matter
DOC	Dissolved organic matter
POXC	Soil permanganate oxidizable carbon
TDN	Soil total dissolved nitrogen
N-NH_4^+	Soil ammonium nitrogen
N-NO_3^-	Soil nitrate nitrogen
NO_3^-/ NH_4^+	Ratio of nitrate nitrogen to ammonium nitrogen
AN	available nitrogen, total amount of ammonium nitrogen and nitrate nitrogen
AN%	the proportion of AN to TDN
DON	Soil dissolved organic nitrogen
TC/TDN	Ratio of total dissolved carbon (TC) to TDN

4.2.2 Soil physiochemical parameter measurements

4.2.2.1 SOC content

SOC was measured following the loss on ignition (LOI) method. The method has long been used to estimate SOC content (Howard and Howard, 1990; Nakhli et al., 2019). First, the empty crucible cups were placed into a 95℃ muffle furnace for 1 h and were cooled to room temperature and weighed (METTLER AE 200, METTLER AE 200, MARSHALL SCIENCE, accuracy of 0.1 mg). Then, 6.0 g of the air-dried soil samples were weighed into crucible cups and dried at 105℃ in a muffle furnace for 24 h to a constant weight. After oven-drying, the cups were then placed into a 550 ℃ muffle furnace for 4 h to combust the organic matter.

$$\text{SOC} = \frac{W2-W3}{W2-W1} \times 100\%$$

where: ***W1*** = the weight of each crucible cup; ***W2*** = total weight of crucible cup and soil after dried at 105℃ in a muffle furnace for 24 h; ***W3*** = total weight of crucible cup and soil after placed at 550℃ in a muffle furnace for 4 h.

4.2.2.2 SOC dynamics

SOC was measured 3 times, after initial mixing (0 day, D0) and at sampling times D46 and D105. Considering that there was no external organic matter added to the soil-plant systems during the growing period of *P.vulgaris* (apart from the decayed plant roots), we compared the SOC across D0, D46 and D105 for each treatment using one-way ANOVA (Detailed in data analysis section). For the treatments where SOC showed significant differences among D0, D46 and D105, the dynamics of SOC were calculated as follows:

$$\text{SOC dynamic (D0 to D46)} = \frac{(SOC_{D46}-SOC_{D0})}{SOC_{D0}} \times 100\%$$

$$\text{SOC dynamic (D46 to D105)} = \frac{(SOC_{D105}-SOC_{D46})}{SOC_{D46}} \times 100\%$$

$$\text{SOC dynamic (D0 to D105)} = \frac{(SOC_{D105}-SOC_{D0})}{SOC_{D0}} \times 100\%$$

where: $\boldsymbol{SOC_{D0}}$= SOC at day 0; $\boldsymbol{SOC_{D46}}$= SOC at the end of vegetative stage; $\boldsymbol{SOC_{D105}}$= SOC after harvest. The carbon dynamic was calculated by using the mean value of the replicates and is referred to as a minimum estimation thus, no statistical comparison was applied.

4.2.2.3 Soil permanganate oxidizable carbon (POXC)

Soil POXC was measured using the adaption method of Weil et al. (2003). The procedure followed Bongiorno et al. (2019). Specifically, 2.5 g (accuracy of 0.1 mg) of the air-dried soil sample was weighed into a 50 mL polypropylene tube. 18 ml of demineralized water and 2 ml of 0.2 mol·L^{-1} $KMnO_4$ was added to each tube. The tubes were vigorously shaken by hand for 30s and then shaken at 120 rpm for 2 min. After shaking, the tubes were placed in a dark cabinet to settle for 8 min while the $KMnO_4$ continued to react with the soil. Then, 0.5 ml of the supernatant solution from a tube was transferred into a second tube as soon as possible and diluted with 49.5 ml of demineralized water. The second tube was inverted to mix the final solution sample. Soil POXC was determined by measuring the absorbance of the sample solution at 550 nm (Abs) in a spectrophotometer (Abs, GENESYS 10S UV–VIS Spectrophotometer). Soil POXC was calculated using the following equation:

POXC mg·kg^{-1} = [0.02 mol·L^{-1} - (a + b×Abs)] × (9000 mg·C·mol^{-1}) × (0.02 L solution Wt^{-1})

where: 0.02 mol·L^{-1} = initial concentration of the $KMnO_4$ solution; a = intercept of the standard calibration curve; b = the slope of the standard calibration curve; Abs = the absorbance of final sample solution; 9000 mg = the amount of carbon oxidized by 1 mol of MnO_4^- changing from Mn^{7+} to Mn^{2+}; 0.02 L = the volume of the $KMnO_4$ reacting with the samples; Wt = weight of air-dried soil sample (kg).

As a quality control measure, each set of soil samples (10) contained two blank samples of distilled water and two standard soil samples (ISE-989, International Soil-Analytical Exchange). This measurement was used to account for any contamination which could have occurred inside the lab.

4.2.2.4 Soil carbon and nitrogen analysis

Soil N-NH_4^+, N-NO_3^-, TDN, soil inorganic carbon (IC) and total dissolved carbon (TC) were measured using the Segmented flow analyser system (SKALAR). Quality control using blank samples of distilled water and a standard soil sample (ISE-989, International Soil-Analytical Exchange) was also included. Soil available nitrogen (AN) and its percentage of TDN (AN%), dissolved organic nitrogen (DON) and DOC were calculated as follows:

AN = N-NO_3^- + N-NH_4^+;
AN% = AN/TDN*100%;
DOC = TC-IC;
DON = TDN-AN;

4.2.3 Data and correlation analysis

All the collected data were checked for normality with Q-Q plots and the Shapiro-Wilk test and checked for homogeneity of variances with Levene's test to meet the assumptions for ANOVA. To meet the requirement of the assumptions for ANOVA, the transformation of some data was performed. Specifically, once the assumptions were met with the raw data, the difference in soil properties were tested with two independent one-way ANOVAs (LDPE-MPs and Bio-MPs) with the factor of microplastic concentration. When the significance level of $p < 0.05$ was met, a post-hoc test using the least significant difference method (LSD) at 95% confidence level was carried out. In the cases where the assumptions were not met, data were transformed using the square root and checked again following the method above. If the assumptions were not met after this transformation, a non-parameter analysis of Kruskal-Wallis H test with pairwise comparison was carried out. The results of one-way ANOVA are shown in Supplementary Table S4.3. Statistical analysis of current research was carried out using SPSS version 23.0 (SPSS Incorporated, USA) and results are presented as "mean ± standard deviation" (Supplementary Table S4.4). Comparisons between LDPE-MPs and Bio-MPs were performed using the Independent-Samples t-Test and Manny-Whitney U-test (Supplementary Table S4.5). All figures were generated using Microsoft Excel 365.

To identify the relationships between the soil properties and microplastics, soil properties at the vegetative stage were subjected to correlation analysis (CA) and redundancy analysis (RDA). Firstly, a correlation analysis was performed to exclude the collinear soil properties (Pearson correlation > 0.9). According to the CA results (Supplementary Table S4.6), AN% had a high collinearity with DON%. Since AN% correlated strongly with other properties, it was used for the further analysis while DON% was removed. $N\text{-}NO_3$/$N\text{-}NH_4$, TC and TC/TDN were removed for the same reason. For the remaining soil properties, we used RDA to identify the relationships among soil properties and experimental treatments (different microplastic types and doses). RDA was performed using CANOCO 5.

4.3 Results

4.3.1 Dynamics of soil organic carbon

The SOC of all treatments were measured on D0, D46 and D105 (Table 4.2). Compared with the control treatment, for every measured time point, the addition of LDPE-MPs and Bio-MPs linearly increased SOC with the increasing MPs doses, significant differences ($p < 0.05$) were observed between each microplastic dose and the control treatment.

Table 4.2 *Soil organic carbon content (mg·kg^{-1}) at different sampling time after expose to different types of microplastics with L. Phaseolus vulgaris.*

Treatment	D0	D46	D105	C dynamic (%) D46-D0	C dynamic (%) D105-D46	C dynamic (%) D105-D0
CON	40.2±1.35 f,B	39.3±0.52 f,B	43.3±0.69 f,A	nd	10.0	7.78
LDPE-0.5	45.5±1.98 e	45.9±1.33 e	45.4±1.45 e	nd	nd	nd
LDPE-1.0	48.2±1.16 d	48.1±1.35 d	47.5±0.77 d	nd	nd	nd
LDPE-1.5	52.2±1.99 b	51.9±1.02 c	52.3±1.00 c	nd	nd	nd
LDPE-2.0	55.6±1.15b B	55.7±1.90 b,B	57.6±0.68 b,A	nd	3.34	3.54
LDPE-2.5	59.5±4.50 a	60.5±1.37 a	61.6±1.90 a	nd	nd	nd
Bio-0.5	44.1±1.33 e,A	42.6±0.77 e,B	44.8±0.94 e,A	-3.30	5.01	nd
Bio-1.0	46.4±1.25 d,A	44.7±0.54 d,B	46.4±0.72 d,A	-3.72	3.86	nd
Bio-1.5	49.0±1.98 c,B	47.4±1.32 c,C	50.6±0.98 c,A	-3.31	6.70	3.16
Bio-2.0	53.5±1.82 b,A	51.9±1.31 b,B	51.5±1.16 b,B	-2.96	nd	-3.60
Bio-2.5	56.2±2.46 a,A	53.7±0.92 a,B	53.8±2.18 a,B	-4.41	nd	-4.23

Note. LDPE-0.5: soil with LDPE microplastics of 0.5% w/w; LDPE-1.0: soil with LDPE microplastics of 1.0% w/w; LDPE-1.5: soil with LDPE microplastics of 1.5% w/w; LDPE-2.0: soil with LDPE microplastics of 2.0% w/w;LDPE-2.5: soil with LDPE microplastics of 2.5% w/w; Bio-0.5: soil with biodegradable microplastics of 0.5% w/w; Bio-1.0: soil with biodegradable microplastics of 1.0% w/w; Bio-1.5: soil with biodegradable microplastics of 1.5% w/w; Bio-2.0: soil with biodegradable microplastics of 2.0% w/w; Bio-2.5: soil with biodegradable microplastics of 2.5% w. Lowercase letters (a, b, c, d) within the same column mean significant differences among MPs doses in each sampling time; capital letters (A,B,C) within the same row mean significant differences in each individual treatment throughout D0, D46 and D105. **nd** means not detected.

We also compared the SOC dynamics throughout D0, D46 and D105 for each treatment. For the control treatment, SOC on D0 and D46 showed no significant difference, which was significantly lower than on D105 ($p < 0.001$). For LDPE-MPs, SOC across D0, D46 and D105 showed no significant differences in treatments of 0.5%, 1.0%, 1.5% and 2.5% LDPE-MPs. Only for the 2.0% LDPE-MPs treatment was SOC on D105 (57.6 mg·kg^{-1}) significantly higher ($p < 0.05$) than on D0 (3.34%) and on D46 (3.54%). For Bio-MPs treatments, SOC on D0 was significantly higher ($p < 0.05$) than on D46, ranging between 2.96% and 4.41% (Table 4.2). From D46 to D105, SOC showed significant increments in 0.5%, 1.0% and 1.5% Bio-MPs treatments, while no significant difference was observed for 2.0% and 2.5% Bio-MPs treatments.

4.3.2 Impacts of MPs on soil DOC and POXC

The effects of LDPE-MPs and Bio-MPs on soil DOC and POXC are shown in Figure 4.1. For soil DOC, as compared to the control treatment (137 mg·kg^{-1} on D46 and 115 mg·kg^{-1} on D105), the addition of LDPE-MPs showed no significant effects on DOC on either D46 (Figure 4.1A) or D105 (Figure 4.1B, Supplementary Table S4.3 and Table S4.4). As for Bio-MPs, the addition of 2.0% and 2.5% Bio-MPs measured significantly higher ($p < 0.05$) DOC on D46 (153 mg·kg^{-1} and 159 mg·kg^{-1}) and D105 (137 mg·kg^{-1} and 148 mg·kg^{-1}) (Figure 4.1A, Figure 4.1B and Supplementary Table S4.4).

In terms of soil POXC, as compared to the control treatment (585 mg·kg^{-1} on D46 and 610 mg·kg^{-1} on D105), on D46, the addition of LDPE-MPs and Bio-MPs showed no significant effects on soil POXC (Figure 4.1C, Figure 4.1D). On D105, in general, the addition of LDPE-MPs and Bio-MPs led to lower POXC values, except for 0.5% Bio-MPs, which was slightly higher than the control but showed no significant difference (Figure 4.1D). Significant differences ($p < 0.05$) were observed for LDPE-MPs treatments of 0.5% (570 mg·kg^{-1}), 1.0% (550 mg·kg^{-1}) and 2.5% (575 mg·kg^{-1}) and Bio-MPs treatments of 1.0% (552 mg·kg^{-1}), 1.5% Bio-MPs (572 mg·kg^{-1}), 2.0% Bio-MPs (540 mg·kg^{-1}), and 2.5% Bio-MPs (567 mg·kg^{-1}).

4.3.3 Impacts of MPs on soil nitrogen cycling and TC/TDN

Soil AN (including N-NH_4^+ and N-NO_3^-) and its proportion to TDN (AN%), DON, the ratio between nitrate and ammonium (NO_3^-/NH_4^+), and TC/TDN were measured in soil on D46 (Figure 4.2) and D105 (Figure 4.3). On D46, soil N-NH_4^+, N-NO_3^-, NO_3^-/NH_4^+, DON, AN% and

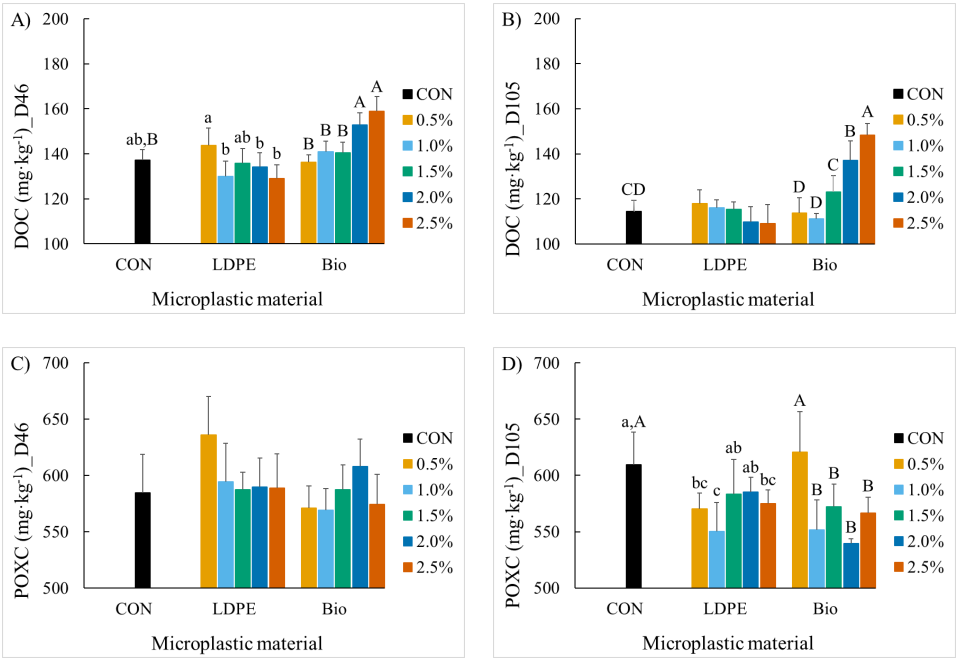

Figure 4.1 *Soil labile carbon fraction at the end of the vegetative stage (D46) and fully mature stage (D105). (A) Dissolved organic carbon (DOC) on D46; (B) Dissolved organic carbon (DOC) on D105 (C) Permanganate oxidizable carbon (POXC) on D46; (D) Permanganate oxidizable carbon (POXC) on D105. Error bars are standard deviation (SD). LDPE indicates LDPE microplastics, Bio indicates biodegradable microplastics. CON (black column) is the control treatment, 0.5% (yellow column), 1.0% (light blue column), 1.5% (green column), 2.0% (blue column) and 2.5% (orange column) are the weight percentage of microplastic to dry soil weight. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP doses and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP doses and the control treatment. Data were plotted as "Mean ± SD".*

TC/TDN in the control treatments were 3.55 mg·kg^{-1}, 1.30 mg·kg^{-1}, 0.37, 8.15 mg·kg^{-1}, 37.2% and 10.7, respectively. On D105, soil N-NH_4^+, N-NO_3^-, NO_3^-/NH_4^+, DON, AN% and TC/TDN in the control treatments were 8.43 mg·kg^{-1}, 5.70 mg·kg^{-1}, 0.83, 7.13 mg·kg^{-1}, 65.9% and 5.55, respectively (More details shown in Supplementary Table S4.4).

The addition of LDPE-MPs showed no significant ($p > 0.05$) effects on measured soil nitrogen cycling indicators (Figure 4.2 and Figure 4.3), except for soil N-NH_4^+ on D46 (Figure 4.2A). However, on D46, we observed that the addition of LDPE-MPs led to a slight accumulation of N-NH_4^+, N-NO_3^- and NO_3^-/NH_4^+ from 0.5% to 1.0% and then dropped at > 1.0% LDPE-MPs doses (Figure 4.2A), while there were no significant differences found.

As for Bio-MPs, on D46, the addition of Bio-MPs significantly ($p<0.05$) affected all the measured indicators except for soil N-NH_4^+ (Figure 4.2). Overall, as compared to the control, soil N-NO_3^- (Figure 4.2B), NO_3^- / NH_4^+ (Figure 4.2C) and AN% (Figure 4.2E) showed a decreasing trend with the increasing Bio-MPs doses, while TC/TDN showed a rising trend. Significant differences ($p < 0.05$) were observed at 1.5% and 2.5% Bio-MPs for soil N-NO_3^-; 2.5% Bio-MPs for soil N-NH_4^+, ≥ 1.5% for AN% and ≥ 1.5% for TC/TDN. DON was significantly higher in 2.5% (Figure 4.2, Figure 4.3 and Supplementary Table S4.4). While on D105, the addition of Bio-MPs only significantly ($p < 0.05$) affected soil N-NO_3^- and DON (Figure 3). Significant differences ($p < 0.05$) were observed at 0.5%, 2.0% and 2.5% Bio-MPs for N-NO_3^- and 2.0% and 2.5% for DON (Figure 4.3B and Figure 4.3D).

4.3.4 Comparison of the effects of LDPE-MPs and Bio-MPs on soil labile carbon and nitrogen

The impacts of LDPE-MPs and Bio-MPs on soil physiochemical properties were compared using the Independent-Samples t-Test (Supplementary Table S4.5). Overall, as compared to LDPE-MPs, Bio-MPs showed significantly lower ($p < 0.05$) SOC and significantly higher ($p < 0.05$) soil DOC at 2.0% and 2.5% doses. LDPE-MPs and Bio-MPs showed no significant differences in terms of soil POXC, except on D46 where LDPE-MPs were significantly higher than Bio-MPs for the 0.5% dose. On D105, the Bio-MPs were significantly higher than the LDPE at the 0.5% dose while LDPE-MPs were significantly higher than Bio at the 2.0% dose. For nitrogen cycling, as compared to LDPE-MPs, Bio-MPs showed significantly lower N-NH_4^+ and AN% for the 1.0%-2.5% doses on D46, while it showed significantly higher DON at 2.0% (D46) and 2.5% (D46 and D105, more details showed in Supplementary Table S4.4).

4.3.5 Correlations of MPs to soil carbon and nitrogen

The relationships among the measured soil properties and common bean growth parameters are depicted in a redundancy analysis diagram (Figure 4.4). The first four axes explain 52.4% of the variation according to the Monte Carlo permutation tests (Supplementary Table S4.7). In Figure 4.4, soil AN, TDN and POXC values are on the left side of diagram while DOC, DON and DOC/DON are on the right side of the diagram. The treatments for the control, all LDPE doses and Bio-0.5 are found on the left side of the diagram while Bio-MPs treatments are on the right side. For LDPE-MPs, LDPE_1.5, LDPE_2.0 and LDPE_2.5 are close to each other and the control treatments, which can be found close

to the origin point. LDPE_0.5 is positively correlated to POXC and LDPE_1.0 is positively correlated to AN%. Bio-MPs treatments, especially Bio_2.0 and Bio_2.5, lay in the positive direction of soil organic matter (DOC, DON and DOC/DON) and in the negative direction of AN (AN%, N-NO_3^- and N-NH_4^+) and TDN.

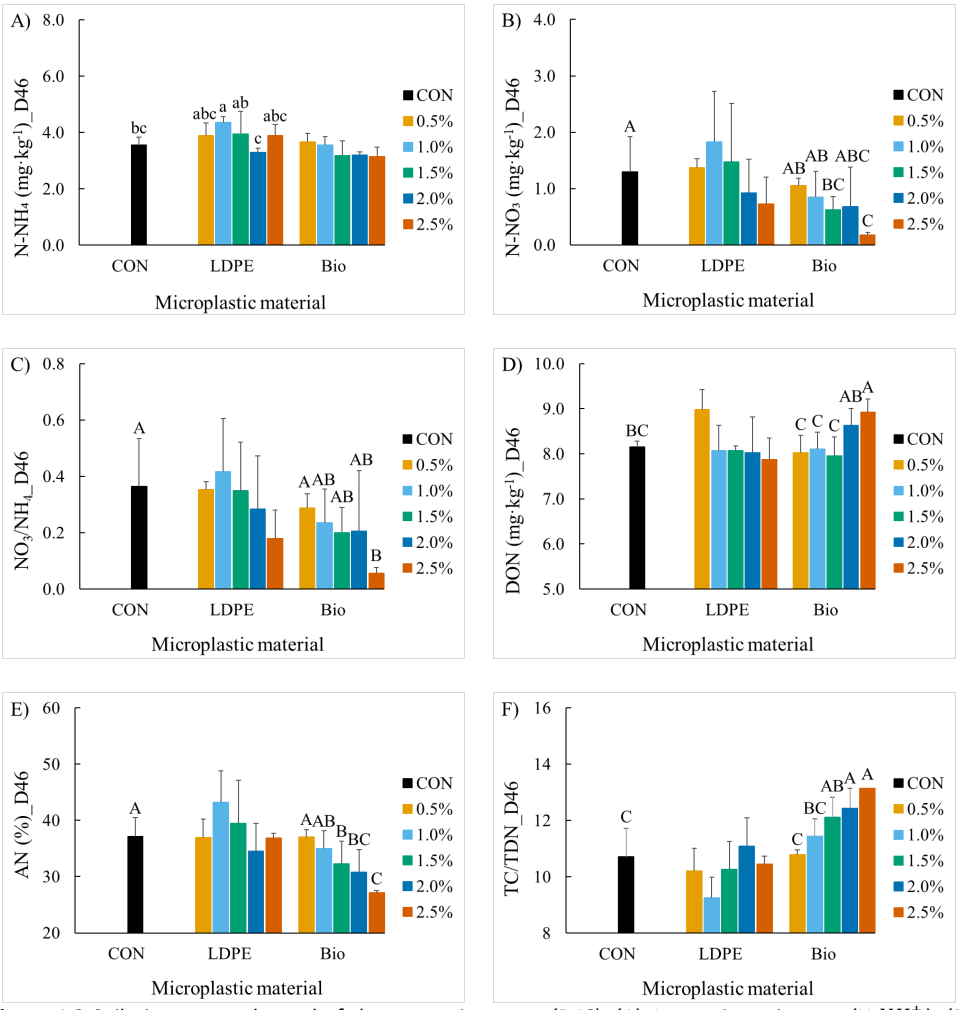

Figure 4.2 *Soil nitrogen at the end of the vegetative stage (D46). (A) Ammonium nitrogen (N-NH_4^+); (B) Nitrate and nitrite nitrogen N-NO_3^-; (C) The ratio of nitrate and nitrite nitrogen to ammonium nitrogen NO_3^-/NH_4^+; (D) Dissolved organic nitrogen (DON); (E) Percentage of AN ($NO_3^- + NH_4^+$) to total dissolved nitrogen content (TDN); (F) Ratio of total dissolved carbon (TC) to total dissolved nitrogen (TDN). Error bars are standard deviation (SD). LDPE indicates LDPE microplastics, Bio indicates biodegradable microplastics. CON (black column) is the control treatment, 0.5% (yellow column), 1.0% (light blue column), 1.5% (green column), 2.0% (blue column) and 2.5% (orange column) are the weight percentage of MPs to dry soil weight.*

Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP doses and the control treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP doses and the control treatment. No post-hoc was performed when p > 0.05 in ANOVA test. Data is shown as "Mean ± SD".

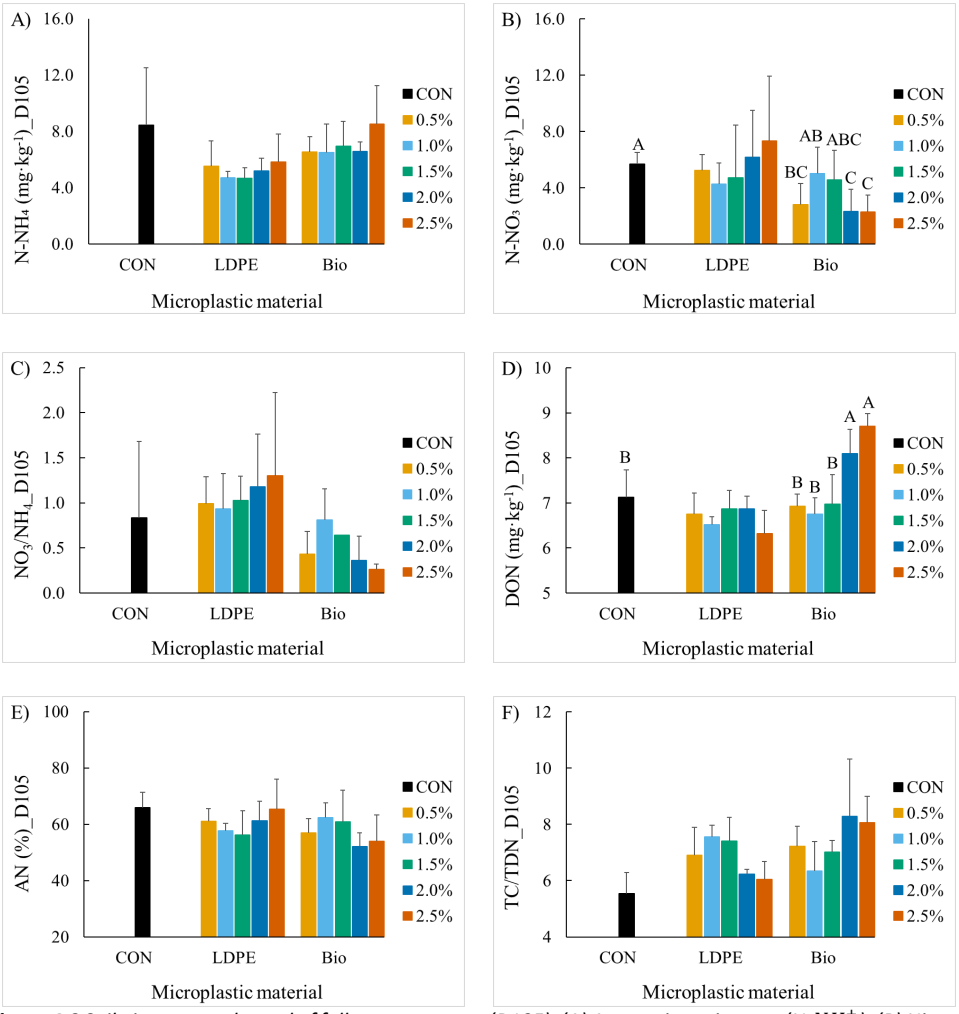

Figure 4.3 *Soil nitrogen at the end of fully mature stage (D105). (A) Ammonium nitrogen (N-NH_4^+); (B) Nitrate and nitrite nitrogen N-NO_3^-; (C) The ratio of nitrate and nitrite nitrogen to ammonium nitrogen NO_3^-/NH_4^+; (D) Dissolved organic nitrogen (DON); (E) Percentage of AN ($NO_3^- + NH_4^+$) to total dissolved nitrogen content (TDN); (F) Ratio of total dissolved carbon (TC) to total dissolved nitrogen (TDN). Error bars are standard deviation (SD). LDPE indicates LDPE microplastics, Bio indicates biodegradable microplastics. CON (black column) is the control treatment, 0.5% (yellow column), 1.0% (light blue column), 1.5% (green column), 2.0% (blue column) and 2.5% (orange column) are the weight percentages of MPs to dry soil weight. Lowercase letters (a, b, c, d) indicate significant differences between the LDPE-MP doses and the control*

treatment; Capital letters (A, B, C, D) indicate significant differences between the Bio-MP doses and the control treatment. No post-hoc was performed when p > 0.05 in ANOVA test. Data is shown as "Mean ± SD".

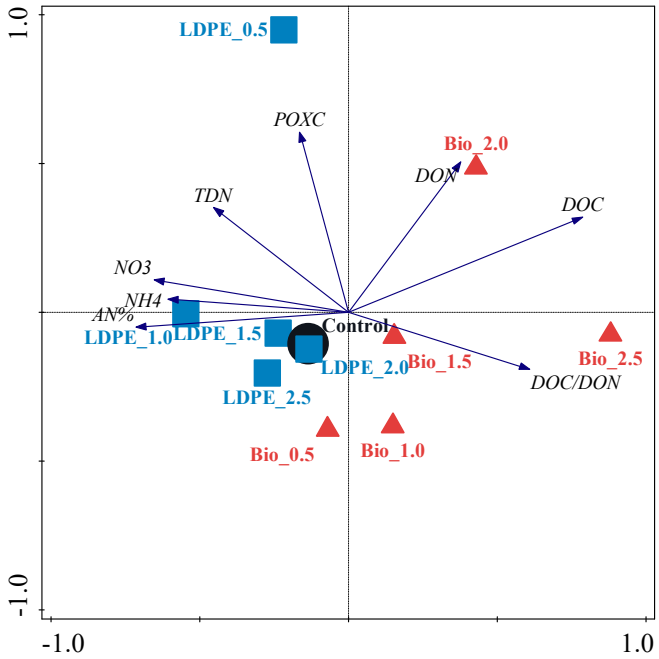

Figure 4.4 *Redundancy analysis ordination diagram of soil properties with treatment factors. LDPE-MPs treatments are indicated by the blue squares, Bio-MPs treatments are indicated by the red triangles, and the control treatment is indicated by the black circle. Soil properties are indicated by the arrows and the angles between the two arrows represent the correlations between each of the soil properties. The smaller the angle between two arrows, the stronger the correlation between the two corresponding parameters; the longer the arrow, the more important the corresponding properties are. The projected distances between the blue square/red triangles/black circle and the arrows represent the relative contribution of the treatment factors to the soil properties.*

Pure soil: Control treatment.
LDPE_0.5: soil with LDPE microplastics of 0.5% w/w.
LDPE_1.0: soil with LDPE microplastics of 1.0% w/w.
LDPE_1.5: soil with LDPE microplastics of 1.5% w/w.
LDPE_2.0: soil with LDPE microplastics of 2.0% w/w.
LDPE_2.5: soil with LDPE microplastics of 2.5% w/w.
Bio_0.5: soil with biodegradable microplastics of 0.5% w/w.
Bio_1.0: soil with biodegradable microplastics of 1.0% w/w.
Bio_1.5: soil with biodegradable microplastics of 1.5% w/w.
Bio_2.0: soil with biodegradable microplastics of 2.0% w/w.
Bio_2.5: soil with biodegradable microplastics of 2.5% w/w.

Blue squares indicate LDPE-MPs.
Red triangles indicate Bio-MPs.
Black circle indicates control treatment.

4.4 Discussion

4.4.1 Effects of microplastics on SOC

In the current study, the LOI method was applied to measure SOC. Because the carbon-base properties of the added LDPE-MPs and Bio-MPs, for all the treatments, the reported losses consisted of two fractions: SOC of the soil-plant system and added LDPE-MPs and Bio-MPs. According to our results, for each LDPE-MPs treatment, the loss mass across D0, D46 and D105 showed no significant difference among each other. This might be attributed to the property of LDPE polymers. LDPE has a linear hydrocarbon structure with stable C=C double bonds, which are relatively resistant to degradation under natural field conditions (Dilara and Briassoulis, 2000; Miranda et al., 2020). As a result, the SOC of each LDPE-MPs treatment remained stable during the experiment. The mass loss for each Bio-MPs treatment significantly dropped from D0 to D46. This might be attributed to the biodegradation of Bio-MPs polymers. Bio-MPs applied in the current research contained heteroatomic polymers (i.e., PLA is an aliphatic polymer and PBAT is an aliphatic–aromatic polymer). Compared to LDPE, Bio-MPs presented low susceptibility to microbial attack and natural degradation (Palsikowski et al., 2017a). This could account for the drop in mass losses between D0 and D46. However, considering the fact that the mass loss for each of the Bio-MPs treatments was still significantly higher compared to the control treatment at both D46 and D105, we have to conclude that the biodegradation of Bio-MPs was incomplete. In contrast, we observed that mass losses of 0.5%, 1.0% and 1.5% Bio-MPs on D105 were significantly higher than on D46, while for 2.0% and 2.5% Bio-MPs, mass losses on D105 and D46 showed no significant differences. One possible explanation for this might be the decayed roots. Our previous paper showed that Bio-MPs on D46 led to significantly higher specific root length (SRL), while 0.5% and 1.0% showed higher root biomass and ≥ 1.5% showed lower root biomass (Meng et al., 2021). Our current results, coupled with previous findings of the effects of microplastics on plant growth, suggest that 0.5%, 1.0% and 1.5% Bio-MPs might enhance the turnover of the roots, thus contributing the higher mass losses on D105 as compared to D46. 2.0% and 2.5% Bio-MPs exerted phytotoxicity to plants and supressed growth of common bean roots and as a result, roots failed to contribute the mass losses on D105 as compared to D46. These findings emphasize the

importance of exploring the potential effects of microplastics on soil carbon cycling (Rillig, 2018; Rillig et al., 2021).

4.4.2 Response of labile fractions of soil organic matter to microplastics

According to our results, LDPE-MPs showed no significant effects on soil DOC and DON, while 2.0% and 2.5% Bio-MPs significantly increased soil DOC and DON. Previous research by Liu et al. (2017) found that 28% PP microplastic significantly stimulated the soil enzymatic activity and enhanced soil DOC concentration. However, the microplastic concentration used in Liu's study was much higher than our research, indicating that up to 2.5% LDPE-MPs were not strong enough to elicit soil DOC and nitrogen cycling. As for Bio-MPs treatments, we noticed that 0.5%-1.5% Bio-MPs treatments showed no significant effects on soil DOC and DON, while Bio-MPs of 2.0% and 2.5% significantly increased soil DOC and DON at both D46 and D105. Our SOC results suggested that Bio-MPs might have experienced a rapid degradation from D0 to D46. The Bio-MPs used in our study contained large amounts of labile carbon and nitrogen elements, which might account for the increased soil DOC and DON in 2.0% and 2.5% Bio-MPs treatments. However, the degradation did not contribute to the DOC and DON in 0.5%, 1.0% and 1.5% Bio-MPs treatments. One possible explanation for this might be attributed to the fact that, in 0.5%, 1.0% and 1.5% Bio-MPs treatments, the biodegraded fraction from Bio-MPs polymers were totally catabolized by soil microorganisms and converted to microbial biomass, CO_2 and water (Bandopadhyay et al., 2018; Bettas Ardisson et al., 2014). For 2.0% and 2.5% Bio-MPs, the organic carbon and nitrogen fractions that leached/disintegrated from Bio-MPs were too overwhelming for microorganisms to catabolize, thus resulting in the significantly higher content of DOC and DON. Naturally, soil DOC and DON polymers were soluble fractions of decomposed SOM as well as roots and microbial exudates, which played important roles in soil quality and plant growth (Bongiorno et al., 2019; Straathof et al., 2014). However, so far, the effects of DOC and DON fractions that originating from bio-microplastic polymers on dynamics of SOM are rarely studied, and its impacts on and on soil-plant systems still needed more research.

Soil POXC is part of the labile fraction of SOM, which consists of mainly small-sized (53-250 mm), heavy organic particles (> 1.7 $g \cdot cm^{-3}$) and a portion of soil microbial biomass (Culman et al., 2012; Li et al., 2018). POXC has also been identified as a labile carbon fraction that is closely related to soil physical, chemical and biological processes (Bongiorno et al., 2019). Unfortunately, to our knowledge, no publications have reported the dynamic of soil POXC in microplastic-contaminated soil. In the current research, soil POXC was significantly

lowered by LDPE-MPs and Bio-MPs on D105. The longer response time of POXC as compared to DOC suggested that the effects of microplastics on soil organic carbon pool cycling persist for a relative long period. Considering the composition of POXC, one explanation might be that the presence of LDPE-MPs and Bio-MPs altered soil biological processes, thus resulting in lower POXC content. Qi et al. (2020c) found that starch-based biodegradable MPs induced high amounts of decanal in the rhizosphere, which is known to have negative effects on fungal growth. Research by Cluzard et al. (2015) indicated that PE possessed antimicrobial additives and could regulate soil microbial taxa and affect soil microbial biomass. There were also studies showing that bioavailable carbon from biodegradable materials can increase microbial biomass (Zhou et al., 2021; Zumstein et al., 2018b). In the current study, soil microbial biomass was not measured, as such, the decrease of soil POXC in microplastic polluted soil remains unexplained. Therefore, further studies related to soil microbial dynamics are needed to fully understand the effects of microplastics on the soil-plant system.

4.4.3 Responses of available nitrogen to microplastics

Nitrogen (N) is essential to manage agricultural soil health and crop productivity (LeBauer and Treseder, 2008). However, there are limited studies about the effects of microplastics on the dynamics of soil available nitrogen (AN) in soil-plant systems. Overall, LDPE-MPs exerted no significant effects on soil AN, while Bio-MPs significantly lowered the AN% and the ratio of N-NO_3^-/N-NH_4^+ with the increasing doses on D46. Previously, our findings showed that LDPE-MPs exerted no significant effects on root development, while 2.0% and 2.5% Bio-MPs resulted in higher specific root length (SRL, root length per gram of dry root weight) and specific root nodules (SRN, number per gram of dry root weight), but significant lower root biomass. We therefore hypothesized that soil available N content was greatly limited by addition of 2.0% and 2.5% Bio-MPs, but not by addition of LDPE-MPs (Meng et al., 2021). Here, we confirmed that that indeed was the case.

The insignificant effects of PE-based microplastics on soil properties have also been observed in other studies. Previously, de Souza Machado et al. (2019) found that PE-MPs were less capable of triggering biogeochemical changes in the soil. They attributed the insignificant effects to the stable C=C bones structure, which is resistant to degradation. It should be mentioned that on D46, LDPE-MPs treatments showed an accumulating trend of AN from 0.5% to 1.0% and then a decreasing trend from 1.0% to 2.5%, even though there were no significant changes observed. LDPE-MPs have been reported to increase soil

porosity and allow for greater diffusion of soil N-NH_4^+, thus facilitating the nitrification process (de Souza Machado et al., 2018b; Huang et al., 2019b; Wan et al., 2019; Zhang et al., 2019a). However, the increased soil porosity could also allow more N leaching. Thus, our data suggest that LDPE-MPs might act as a dual-direction regulator in the soil-plant system depending on the concentrations of microplastics. This highlights the fact that robust investigations focusing on the effects of LDPE-MPs on soil nitrogen cycling are urgently needed.

For Bio-MPs, the decreasing trend of AN% and N-NO_3^-/N-NH_4^+ with the increasing doses of Bio-MPson D46 indicated that Bio-MPs not only lowered nitrogen availability, but also suppressed the nitrification process of soil N-NH_4^+ to soil N-NO_3^-. This might be attributed to the sequestration of N-NH_4^+ by Bio-MPs polymers. Chen et al. (2019a) observed a significant decrease in N-NH_4^+ when soils were amended with 2% PLA-MPs. They ascribed the decrease to the adsorption of negative charge functional groups carboxyl (COOH) of PLA and PBAT to the cations of N-NH_4^+ (Green et al., 2016; Zumstein et al., 2018a). An alternative explanation might be the microbial N immobilization. In our research, we have observed a clear increasing trend of soil TC:TDN ratio in Bio-MPs treatments on D46. This is in line with previous research by Qi et al. (2020c), who also reported that incorporating starch-based Bio-MPs into soils can substantially increase soil C:N ratio. Higher C:N ratio via microplastic addition could lead to soil nitrogen immobilization (Rillig et al., 2019). Another report by Zhou et al. (2021) concluded that carbon source supply from biodegradable material of PHBV (poly-(3-hydroxybutyrate-co-3-hydroxyvalerate)-$[COCH_2CH(CH_3)O]_m$ $[COCH_2CH(C_2H_5)-O]_n$)) can stimulate the growth of microbial biomass and intensify the nitrogen limitation. Thus, in our study, we suggested that the lower availability of nitrogen might have joint effects: 1). the absorption of Bio-MPs to cation N-NH_4^+ suppressed nitrification processes from N-NH_4^+ to N-NO_3^-; 2). The C supply from Bio-MPs to microorganisms stimulated the microbial N immobilization.

4.4.4 Limitation and implications

The wide range (0.5%, 1.0, 1.5%, 2.0% and 2.5% w/w dry soil weight) of LDPE-MPs and Bio-MPs used in current study was aimed to investigate actual environmental thresholds as well as to depict the subtle effects of microplastics on the soil-plant ecosystem (van Weert et al., 2019). RDA analysis showed LDPE-MPs and Bio-MPs were stand in the opposite directions of Y axis (except 0.5% Bio-MPs), LDPE-MPs were stand in the positive direction of soil available nitrogen, while Bio-MPs treatments were stand in the positive direction of soil

DOC and DON (Figure 4.4). Indicating they might affect soil C and N dynamics via different ways. Considering the stable C-C structure of LDPE-MPs, LDPE-MPs most likely affected soil nitrogen cycling by altering soil porosity (de Souza Machado et al., 2019). While the Bio-MPs, on the one hand, contained carbonyl (=O) and hydroxyl (-OH) groups that can absorb cation like N-NH_4^+, on the other hand, it can also provide more bioavailable C to microorganisms to increase microbial biomass and intensify soil restriction (Boots et al., 2019; Chen et al., 2019a; Wan et al., 2019; Yan et al., 2020; Zhou et al., 2021). However, the dynamics of microbial communities were not measured, lowering the connection between carbon and nitrogen cycling and soil microorganisms. As such, biological mechanisms affecting the decrease in soil nitrogen availability in microplastic-treated soil remain unexplained and require further study.

4.5 Conclusion

In this study, we verified our hypothesis that Bio-MPs exerted stronger effects on soil DOC, DON and soil available nitrogen (N-NH_4^+ and N-NO_3^-) than LDPE-MPs. Significant decreases in SOC in Bio-MPs treatments from D0 to D46 were observed, while the SOC of LDPE-MPs treatments on D0, D46 and D105 showed no significant differences, suggesting that Bio-MPs experienced a rapid biodegradation from D0 to D46. Exposure to LDPE-MPs (0.5%, 1.0% and 2.5%) and Bio-MPs (≥ 1.0%) led to a reduction in soil POXC content on D105. LDPE-MPs showed no significant effects on soil labile organic carbon cycling, while Bio-MPs of 2.0% and 2.5% showed significantly higher soil DOC and DON (at D46 and D105) and lower soil available nitrogen (at D46). Even LDPE-MPs showed no significant effects on soil nitrogen cycling. This does not mean that LDPE-MPs pose no ecological risks. The dynamics of carbon and nitrogen cycling in LDPE-MPs still showed deviations from the control treatment, indicating potential threats from LDPE-MPs to soil ecological function. Great expectations have been placed on biodegradable materials to solve the agricultural plastic pollution problem; however, our results suggest that using biodegradable materials in agricultural soils needs to be reconsidered and thoroughly investigated. Taken together, the impacts from different types of microplastics on soil-plant systems still require careful attention and long-term field observations focused on the safety of biodegradable materials regarding soil health.

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Supplementary Material

Table S4.1 Detailed information about soil used in the experiment.

Soil parameters	Unit	Results
Total nitrogen (N)	$kg \cdot ha^{-1}$	3775
C/N ratio		17
Available nitrogen	$kg \cdot ha^{-1}$	45
Total sulfur (S)	$kg \cdot ha^{-1}$	995
C/S ratio		66
Available sulfur	$kg \cdot ha^{-1}$	15
Total phosphorus (P)	$kg \cdot ha^{-1}$	695
Total potassium (K)	$kg \cdot ha^{-1}$	505
Total calcium (Ca)	$kg \cdot ha^{-1}$	3840
Total magnesium (Mg)	$kg \cdot ha^{-1}$	260
pH		6
Organic carbon (SOC)	%	2
Organic matter (SOM)	%	4
Inorganic carbon	%	0.07
Carbonated lime	%	< 0.2
Clay	%	< 1
Silt	%	11
Sand	%	83

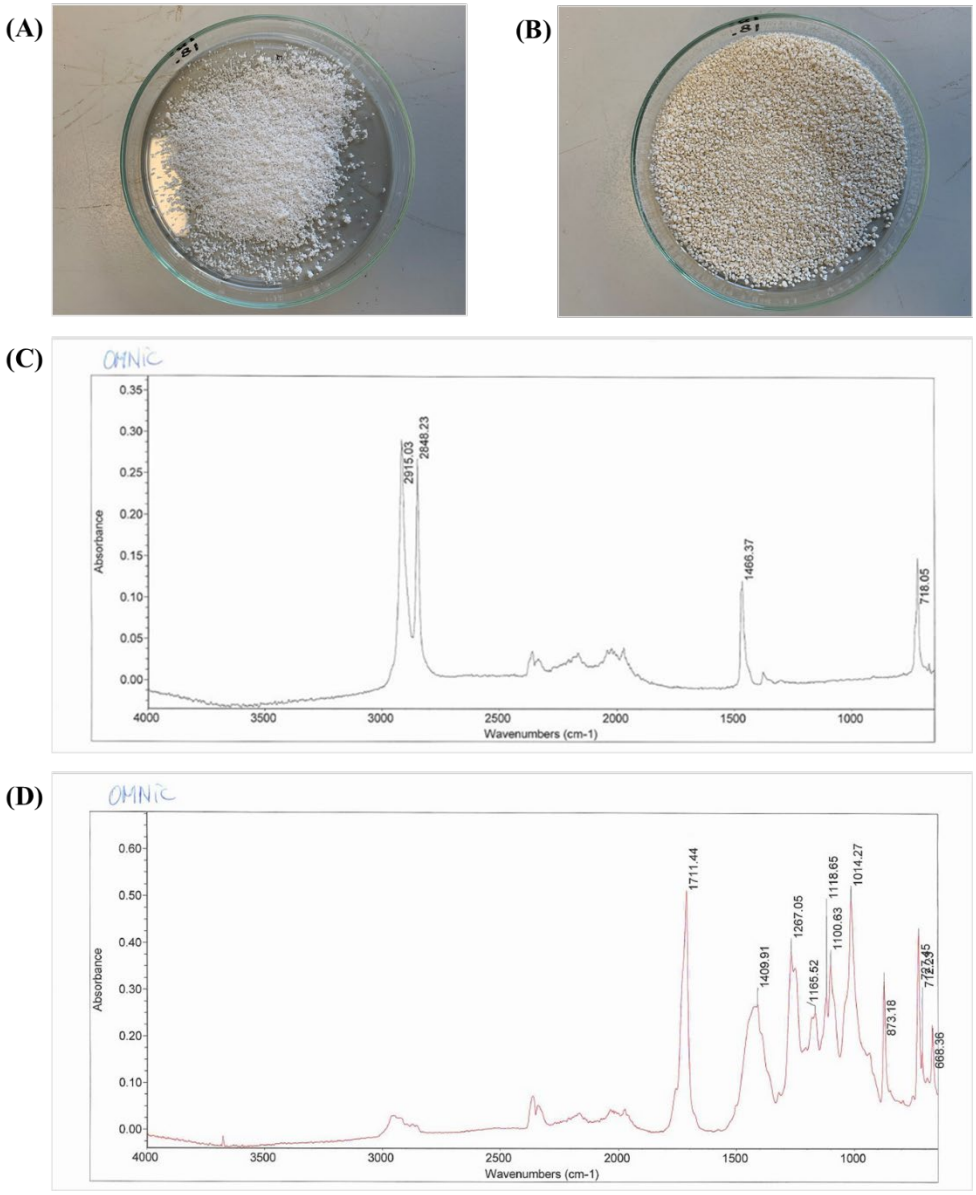


Figure S4.1 *The MPs applied in current research. (A) LDPE-MPs; (B) Bio-MPs; (C) FTIR of LDPE-MP; (D) FTIR of Bio-MP.*

Table S4.2 *Detailed information about applied nutrient in this experiment.*

Indicators	Tomaat-N(modified)	Hoagland 2.0
EC	2	2
Macronutrients (mmol/liter)		
NH_4	2.0	1.0
K	8.0	6.0
Ca	4.0	3.6
Mg	2.0	2.0
NO_3	2.0	11
SO_4	5.0	2.6
P	2.0	2.0
Micronutrients (mmol/liter)		
Fe	25,0	25.0
Mn	11,0	11,8
Zn	1,75	1,75
B	44,0	43,0
Cu	0,125	0,13
Mo	0,52	0,52

Table S4.3 *Statistic for all soil properties at D46 and D105. One-way ANOVAs followed by LSD test.*

Sampling time	Microplastic type	Soil properties	df	F value/ Chi-square	p-value
D46	LDPE	***DOC[a]***	5,18	2.908	***0.043***
		POXC[a]	5,18	1.976	0.131
		N-NH$_4$[a]	5,18	2.810	***0.048***
		N-NO$_3$[b]	5,18	1.665	0.194
		NO$_3$/NH$_4$ [a]	5,18	1.171	0.361
		DON[a]	5,18	2.662	0.057
		AN%[b]	5,18	1.170	0.362
		TC/TDN[a]	5,18	2.259	0.093
	Bio	***DOC[a]***	5,18	13.844	***0.000***
		POXC[a]	5,18	1.453	0.254
		N-NH$_4$[a]	5,18	1.974	0.132
		N-NO$_3$[a]	5,18	3.081	***0.035***
		N-NO$_3$/N-NH$_4$ [c]	5	11.913	***0.036***
		DON[a]	5,18	5.086	***0.004***
		AN%[a]	5,18	6.295	0.002
		TC/TDN[a]	5,18	6.867	0.001

Note. DOC: dissolved organic carbon; **POXC:** permanganate oxidizable carbon; **N-NH$_4$:** ammonium nitrogen; **N-NO$_3$:** nitrate and nitrite nitrogen; **NO$_3$/NH$_4$:** the ratio of nitrate and nitrite nitrogen to ammonium nitrogen; **DON:** dissolved organic nitrogen; **AN%:** percentage of available nitrogen to total dissolved nitrogen; **TC/TDN:** the ratio of total dissolved carbon to total dissolved nitrogen. Superscript: **a**: Raw data; **b**: data were square rooted transformed; **c**: Kruskal-Wallis test. **Bold and italic** properties were significantly affected by the occurrence of MPs.

Table S4.3 continued. *Statistic for all soil properties at D46 and D105. One-way ANOVAs followed by LSD test.*

Sampling time	Soil properties	Microplastic type	df	F value/ Chi-square	p-value
	DOC[a]	LDPE	5,18	1.538	0.228
	POXC[a]	LDPE	5,18	3.110	***0.034***
	$N\text{-}NH_4$ [c]	LDPE	5	6.585	0.253
	$N\text{-}NO_3$[b]	LDPE	5,18	0.557	0.732
	NO_3/NH_4 [b]	LDPE	5,18	0.421	0.828
	DON[a]	LDPE	5,18	1.734	0.178
	DON/Nts[a]	LDPE	5,18	1.342	0.292
	TC/TDN[a]	LDPE	5,18	1.761	0.172
D105					
	DOC[a]	Bio	5,18	22.987	***0.000***
	POXC[a]	Bio	5,18	7.127	***0.001***
	$N\text{-}NH_4$[b]	Bio	5,18	0.565	0.726
	$N\text{-}(NO_3+NO_2)$[a]	Bio	5,18	3.205	**0.030**
	$N\text{-}NH_4$ / NO3[b]	Bio	5,18	2.677	0.056
	DON[a]	Bio	5,18	10.730	***0.000***
	AN%[a]	Bio	5,18	2.097	0.113
	TC/TDN[a]	Bio	5,18	2.224	0.097

Note. DOC: dissolved organic carbon; **POXC:** permanganate oxidizable carbon; **$N\text{-}NH_4$:** ammonium nitrogen; **$N\text{-}NO_3$:** nitrate and nitrite nitrogen; **NO_3/NH_4:** the ratio of nitrate and nitrite nitrogen to ammonium nitrogen; **DON:** dissolved organic nitrogen; **AN%:** percentage of available nitrogen to total dissolved nitrogen; **TC/TDN:** the ratio of total dissolved carbon to total dissolved nitrogen. Superscript: **a**: Raw data; **b**: data were square rooted transformed; **c**: Kruskal-Wallis test. **Bold and italic** properties were significantly affected by the occurrence of MPs.

Table S4.4 *Data for all soil properties at D46 (vegetative stage) and D105 (fully mature stage). Values are displayed as treatment mean ± standard deviation for the different microplastic concentrations.*

Sampling time	Soil properties	Microplastic type	Control	0.5	1.0	1.5	2.0	2.5
Vegetative stage	DOC	LDPE	137.34±3.97	143.78±6.58	130.09±5.73	135.92±5.49	134.28±5.29	129.13±5.13
		Bio		136.24±2.94	140.91±4.13	140.61±3.90	152.99±4.58	158.86±5.75
	POXC ($mg \cdot kg^{-1}$)	LDPE	585±34.2	636±20.1	594±34.2	588±15.4	590±25.9	589±30.2
		Bio		571±10.6	569±19.2	588±21.8	608±24.5	574±26.4
	$N\text{-}NH_4$ ($mg \cdot kg^{-1}$)	LDPE	3.55±0.25	3.90±0.37	4.35±0.18	3.95±0.69	3.30±0.12	3.90±0.34
		Bio		3.68±0.25	3.55±0.27	3.18±0.45	3.20±0.10	3.15±0.29
	$N\text{-}NO_3$ ($mg \cdot kg^{-1}$)	LDPE	1.30±0.54	1.38±0.13	1.83±0.78	1.48±0.90	0.93±0.52	0.73±0.41
		Bio		1.05±0.11	0.85±0.39	0.63±0.20	0.68±0.61	0.18±0.04
	$N\text{-}NO_3/N\text{-}NH_4$	LDPE	0.37±0.15	0.35±0.02	0.42±0.16	0.35±0.15	0.28±0.16	0.18±0.09
		Bio		0.29±0.04	0.23±0.10	0.20±0.08	0.21±0.18	0.06±0.02
	DON	LDPE	8.15±0.11	8.98±0.39	8.08±0.48	8.08±0.08	8.03±0.69	7.88±0.41
		Bio		8.03±0.33	8.10±0.33	7.95±0.36	8.63±0.33	8.93±0.25
	AN%	LDPE	37.15±2.92	36.99±2.80	43.19±4.84	39.46±6.63	34.55±4.29	36.86±4.62
		Bio		37.07±1.12	35.03±2.69	32.27±3.47	30.80±3.49	27.12±1.28
	TC/TDN	LDPE	10.71±0.87	10.22±0.69	9.25±0.64	10.26±0.86	11.09±0.36	10.45±0.73
		Bio		10.79±0.15	11.43±0.55	12.11±0.62	12.43±0.91	13.14±0.36

Note. DOC: dissolved organic carbon; **POXC:** permanganate oxidizable carbon; **$N\text{-}NH_4$:** ammonium nitrogen; **$N\text{-}NO_3$:** nitrate and nitrite nitrogen; **$N\text{-}NO_3/N\text{-}NH_4$:** the ratio of nitrate and nitrite nitrogen to ammonium nitrogen; **DON:** dissolved organic nitrogen; **AN%:** percentage of available nitrogen to total dissolved nitrogen content; **TC/TDN:** the ratio of total dissolved carbon to total dissolved nitrogen.

Table S4.4 continued. *Data for all soil properties at D46 (vegetative stage) and D105 (fully mature stage). Values are displayed as treatment mean ± standard deviation for the different microplastic concentrations.*

Sampling time	Soil properties	Microplastic type	Control	0.5	1.0	1.5	2.0	2.5
Fully mature	DOC	LDPE	114.54±4.13	118.03±5.22	116.09±2.97	115.31±2.94	109.83±5.79	109.14±7.30
		Bio		113.75±5.88	111.12±2.16	123.06±6.34	137.05±7.55	148.20±4.60
	POXC ($mg \cdot kg^{-1}$)	LDPE	610±29.1	570±13.8	550±25.3	584±30.8	585±12.8	575±11.9
		Bio		621±36.1	552±26.5	572±20.1	540±4.19	567±13.8
	$N\text{-}NH_4$ ($mg \cdot kg^{-1}$)	LDPE	8.43±3.55	5.53±1.54	4.70±0.39	4.68±0.65	5.20±0.78	5.83±1.70
		Bio		6.53±0.93	6.50±1.73	6.95±1.53	6.58±0.59	8.50±2.36
	$N\text{-}(NO_3+NO_2)$ ($mg \cdot kg^{-1}$)	LDPE	5.70±0.70	5.23±0.98	4.28±1.28	4.70±3.27	6.18±2.89	7.35±3.97
		Bio		2.80±1.31	5.00±1.64	4.58±1.81	2.33±1.37	2.30±1.04
	$N\text{-}NO_3/N\text{-}NH_4$	LDPE	0.83±0.38	0.99±0.26	0.93±0.34	1.03±0.73	1.18±0.51	1.30±0.80
		Bio		0.43±0.22	0.81±0.30	0.64±0.23	0.36±0.23	0.26±0.05
	DON	LDPE	7.13±0.53	6.75±0.41	6.53±0.15	6.88±0.35	6.88±0.24	6.33±0.44
		Bio		6.93±0.24	6.75±0.32	6.98±0.57	8.10±0.46	8.70±0.24
	AN%	LDPE	65.93±4.66	60.98±3.93	57.74±2.27	56.22±7.49	61.17±6.12	65.42±9.14
		Bio		56.87±4.47	62.31±4.66	60.95±9.64	52.13±4.26	53.91±8.13
	TC/TDN	LDPE	5.55±0.80	6.90±0.82	7.56±0.40	7.41±1.20	6.24±0.94	6.05±1.65
		Bio		7.22±0.91	6.34±0.97	7.01±1.62	8.28±0.85	8.07±1.69

Note. DOC: dissolved organic carbon; **POXC:** permanganate oxidizable carbon; **$N\text{-}NH_4$:** ammonium nitrogen; **$N\text{-}NO_3$:** nitrate and nitrite nitrogen; **$N\text{-}NO_3/N\text{-}NH_4$:** the ratio of nitrate and nitrite nitrogen to ammonium nitrogen; **DON:** dissolved organic nitrogen; **AN%:** percentage of available nitrogen to total dissolved nitrogen content; **TC/TDN:** the ratio of total dissolved carbon to total dissolved nitrogen.

Table S4.5 *Independent-Samples t-Test between soil properties.*

Growth parameters (D46, Vegetative stage)	MPs dose	t	df	Sig. (2-tailed)
SOC	0.5	6.040	14	***0.000***
	1.0	6.599	14	***0.000***
	1.5	7.751	14	***0.000***
	2.0	4.661	14	***0.000***
	2.5	11.611	14	***0.000***
DOC	0.5	1.813	6	0.120
	1.0	-2.653	6	***0.038***
	1.5	-2.626	6	***0.039***
	2.0	-2.653	6	***0.038***
	2.5	-6.681	6	***0.001***
POXC	0.5	5.687	6	***0.001***
	1.0	1.277	6	0.249
	1.5	0.337	6	0.748
	2.0	1.277	6	0.249
	2.5	0.715	6	0.501
N-NH_4	0.5	0.867	6	0.419
	1.0	4.276	6	***0.005***
	1.5	4.160	6	***0.006***
	2.0	4.276	6	***0.005***
	2.5	2.923	6	***0.027***
N-NO_3	0.5	3.284	6	***0.017***
	1.0	1.938	6	0.101
	1.5	2.580	6	***0.042***
	2.0	1.938	6	0.101
	2.5	2.285	6	0.062
NO_3/NH_4	0.5	2.294	6	0.062
	1.0	1.620	6	0.156
	1.5	2.070	6	0.084
	2.0	0.534	6	0.612
	2.5	2.385	6	0.054
DON	0.5	3.204	6	***0.019***
	1.0	-0.075	6	0.943
	1.5	0.361	6	0.730
	2.0	-0.075	6	0.943
	2.5	-3.803	6	***0.009***
AN%	0.5	0.132	6	0.899
	1.0	2.691	6	***0.036***
	1.5	3.117	6	***0.021***
	2.0	2.691	6	***0.036***
	2.5	3.365	6	***0.015***
TC/TDN	0.5	-1.393	6	0.213
	1.0	-4.493	6	***0.004***
	1.5	-5.517	6	***0.001***
	2.0	-2.354	6	0.057
	2.5	-5.724	6	***0.003***

Note. SOC: soil organic carbon; **DOC:** dissolved organic carbon; **POXC:** permanganate oxidizable carbon; **N-NH_4 :** ammonium nitrogen; **N-NO_3:** nitrate and nitrite nitrogen; **NO_3/NH_4:** the ratio of nitrate and nitrite nitrogen to ammonium nitrogen; **AN%**: percentage of available nitrogen to total dissolved nitrogen content; **DON/TDN:** percentage of dissolved organic carbon to total dissolved nitrogen. **Bold and italic:** $p < 0.05$.

Table S4.5 continued. *Independent-Samples t-Test between soil properties.*

Growth parameters (D105, fully mature)	MPs dose	t	df	Sig. (2-tailed)
SOC	0.5	0.986	14	0.341
	1.0	2.988	14	***0.010***
	1.5	3.540	14	***0.003***
	2.0	12.645	14	***0.000***
	2.5	7.679	14	***0.000***
DOC	0.5	0.942	6	0.382
	1.0	2.343	6	0.058
	1.5	-1.922	6	0.103
	2.0	-4.955	6	***0.003***
	2.5	-7.844	6	***0.000***
POXC	0.5	-2.602	6	***0.041***
	1.0	-0.072	6	0.945
	1.5	0.617	6	0.560
	2.0	6.795	6	***0.000***
	2.5	0.923	6	0.391
N-NH_4	0.5	-0.959	6	0.374
	1.0	-1.758	6	0.129
	1.5	-2.374	6	0.055
	2.0	-2.428	6	0.051
	2.5	-1.590	6	0.163
N-NO_3	0.5	2.572	6	***0.042***
	1.0	-0.603	6	0.569
	1.5	0.058	6	0.956
	2.0	2.086	6	0.082
	2.5	2.133	6	0.077
NO_3/NH_4	0.5	2.890	6	***0.028***
	1.0	0.484	6	0.646
	1.5	0.872	6	0.417
	2.0	2.546	6	***0.044***
	2.5	2.269	6	0.064
DON	0.5	-0.640	6	0.546
	1.0	-1.105	6	0.311
	1.5	-0.260	6	0.804
	2.0	-4.069	6	***0.007***
	2.5	-8.197	6	***0.000***
AN%	0.5	1.241	6	0.261
	1.0	-1.631	6	0.154
	1.5	-0.670	6	0.528
	2.0	2.114	6	0.079
	2.5	1.607	6	0.159
TC/TDN	0.5	-0.447	6	0.671
	1.0	2.029	6	0.113
	1.5	0.339	6	0.746
	2.0	-2.787	6	***0.032***
	2.5	-1.472	6	0.191

Note. SOC: soil organic carbon; **DOC:** dissolved organic carbon; **POXC:** permanganate oxidizable carbon; **N-NH_4 :** ammonium nitrogen; **N-NO_3:** nitrate and nitrite nitrogen; **NO_3/NH_4:** the ratio of nitrate and nitrite nitrogen to ammonium nitrogen; **AN%**: percentage of available nitrogen to total dissolved nitrogen content; **DON/TDN:** percentage of dissolved organic carbon to total dissolved nitrogen. **Bold and italic:** $p < 0.05$.

Table S4.6 *Pearson correlations matrix for the all growth parameters.*

Soil properties	$N\text{-}NH_4$	$N\text{-}NO_3$	NO_3/NH_4	DON	TDN	AN%	DON%	TC	DOC	POXC	DOC/DON
$N\text{-}NO_3$	.649**										
NO_3/NH_4	.474**	.966**									
DON	-0.209	-0.266	-0.250								
TDN	.781**	.807**	.710**	0.236							
AN%	.843**	.896**	.813**	-.499**	.716**						
DON%	-.844**	-.901**	-.819**	.488**	-.725**	-.998**					
TC	-.462**	-.436**	-.417**	.721**	-0.132	-.654**	.658**				
DOC	-.464**	-.438**	-.419**	.721**	-0.135	-.656**	.660**	1.000**			
POXC	0.073	0.111	0.132	.526**	.370*	-0.031	0.028	0.199	0.205		
DOC/DON	-.383*	-0.263	-0.252	-0.268	-.483**	-0.273	0.294	.472**	.473**	-.371*	
TC/TN	-.826**	-.819**	-.752**	0.277	-.771**	-.901**	.911**	.727**	.729**	-0.139	.658**

Note. **. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Table S4.7 *Statistical summary of Redundancy analysis.*

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.2687	0.2161	0.0313	0.0077
Explained variation (cumulative)	26.87	48.48	51.6	52.37
Pseudo-canonical correlation	0.773	0.587	0.3628	0.5917
Explained fitted variation (cumulative)	95.46	98.6	99.66	99.78

Permutation Test Results:
On First Axis: pseudo-F = 1.2, P = 0.002
On All Axes: pseudo-F = 3.6, P = 0.002

5. Effects of microplastics on common bean rhizosphere microbial communities

Microplastic pollution in terrestrial ecosystem is a growing concern due to its potential influences on soil properties and crop growth. Little is known about the effect of microplastic on bacterial community structure and diversity in the rhizosphere. Here, we studied the effects of two microplastics (MPs), low density polyethylene (LDPE-MPs) and biodegradable (Bio-MPs) of poly-butylene-adipate-co-terephthalate (PBAT) mixed with polylactic acid (PLA), on rhizosphere microbial communities of Phaseolus vulgaris at contaminant doses of 0.5%, 1.0% and 2.5% (w/w, dry weight ratio between MPs and soil). Results of 16S revealed rhizosphere microbial α-diversity (Chao 1, ACE, Shannon and Simpson) was highest at 1.0% Bio-MPs and 0.5% LDPE-MPs, respectively, whereas lowest at 2.5% LDPE-MPs and Bio-MPs. This indicating 2.5% dose of MPs might pose selective effect on rhizosphere microbial community. According to β-diversity, bacteria communities in 1.0% and 2.5% Bio-MPs were distinctive from control treatment without microplastic addition, 0.5% Bio-MPs and LDPE-MPs. Bio-MPs and LDPE-MPs addition also affected rhizosphere bacterial composition. As compared to control, family Comamonadaceae exhibited higher relative abundance in all microplastic treatments, family Rhizobiaceae exhibited higher relative abundance in 2.5% LDPE-MPs, while lower relative abundance in 2.5% Bio-MPs, family Micrococcaceae exhibited higher relative abundance in 2.5% Bio-MPs and 2.5% LDPE-MPs. Our findings evidenced LDPE-MPs and Bio-MPs exerted profound effects on rhizosphere microbial community composition and diversity, these effects might have far reaching effects on soil nutrient cycling and plant health in agroecosystems. Therefore, future efforts to assess the ecological risks of microplastics in terrestrial ecosystem are urgently needed.

Based on:

Meng, F., Harkes, P., van Steenbrugge, J J.M., Yang. X., Riksen, M., Geissen, V., 2021. Effects of microplastics on common bean rhizosphere microbial communities. To be submitted.

5.1 Introduction

Microplastics (MPs) are generally defined as plastic particles smaller than 5 mm (Thompson et al., 2004) and are considered an environmental pollutant. Previous research of MPs pollution was mainly focused on marine and sediment systems (Andrady, 2011; Ivar do Sul and Costa, 2014; Koelmans et al., 2013). Realizing that about 80% of the microplastics in marine systems originate from land-based sources (Li et al., 2016b), research interest towards microplastic pollution in terrestrial ecosystems has increased. Especially studies to agroecosystems, which has been identified as a very important entry point for microplastics in soils (de Souza Machado et al., 2018a; Ng et al., 2018; Nizzetto et al., 2016). Numerous studies showed that microplastics are able to reach agricultural soils via sewage water irrigation, compost and organic fertilization (Corradini et al., 2019b; Qi et al., 2020a; Van den Berg et al., 2020; Zhu et al., 2019). Yet, plastic mulching has been identified as one of the major sources of microplastic pollution in terrestrial ecosystems (Huang et al., 2020; Steinmetz et al., 2016). A study by Zhang et al. (2020) found 107 particles·kg^{-1} of low density polyethylene microplastics (LDPE-MPs) in plastic mulching fields of northeast China. Liu et al. (2018) found microplastics of 62-78 particles·kg^{-1} in plastic mulching vegetable fields in Shanghai.

To overcome agricultural plastic pollution from mulching residues, biodegradable plastics were introduced into agricultural production. Biodegradable plastics are designed to maintain the advantages of conventional polyethylene film while at the same time could be tilled into soils and decompose into carbon dioxide, water, and microbial biomass (Bandopadhyay et al., 2018; Siwek et al., 2019). Now it turns out that, out of sight does not mean out of soil (Sintim and Flury, 2017). Recent research claimed that biodegradable materials, such as polylactide (PLA)-based films and starch-based films, are prone to break down into smaller plastic particles rather than completely biodegrade under natural field conditions, resulting in the accumulation of bio-microplastics in agricultural soils (Briassoulis, 2004; de Souza Machado et al., 2018a; Whitacre, 2014).

Microplastics in agricultural soils have been found to reduce soil bulk density and increase soil water evaporation, reduce soil aggregate stability and alter soil water repellency (de Souza Machado et al., 2018b; Lehmann et al., 2019; Qi et al., 2020b). Microplastics can also affect soil fauna activity and fitness. For instance, Kim and An (2019) found microplastics can inhibit the movement of springtails. Huerta Lwanga et al. (2016a) observed higher mortality of earthworm *Lumbricus terrestris* in litters with higher concentration of PE microplastics. Microplastics can also affect crop growth. Dong et al. (2020) found

microplastics of polystyrene (PS) and polytetrafluoroethylene (PTFE) decrease rice biomass and root activity. Wang et al. (2020a) found 10% PLA microplastics decreased maize biomass and leaf chlorophyll content. Our previous research found ≥ 1.5% Bio-based microplastics significantly reduced root and shoot biomass of common bean (Meng et al., 2021). Furthermore, microplastics can also affect soil microbial community and nutrient status. Research by Liu et al. (2017) indicated 28% polypropylene (PP) microplastics can increase the activities of fluorescein diacetate hydrolase (FDAse) and phenol oxidase, thus stimulating the decomposition of soil dissolved organic matter and enhancing the accumulation of soil N-NO_3^-. Yan et al. (2020) found 0.1% and 1.0% polyvinyl chloride (PVC) microplastics showed no significant effects on overall soil bacterial community, but significantly increased soil available P content. A study by Qi et al. (2020c) found starch-based microplastics can significantly affect rhizosphere microbial community and produce volatile compounds like dodecanal. Rhizosphere microbes are essentially for soil nutrient cycling and respond rapidly to environmental changes (Cui et al., 2018b; Fei et al., 2020; Zhu et al., 2014). However, current data of the effects of microplastics on rhizosphere communities are still scares. This greatly impedes our understanding of the influence of microplastics on the soil-plant system.

This study investigates the effects of conventional LDPE-MPs and Bio-MPs on rhizosphere microbial communities by means of high throughput sequencing. Our previous findings showed the existence of LDPE microplastics (LDPE-MPs) and biodegradable microplastics (Bio-MPs) stimulated the formation of common bean nodules for fixing the nitrogen (Meng et al., 2021). Soil available nitrogen content was not significantly affected by LDPE-MPs, while significantly reduced in 2.5% Bio-MPs. Therefore, we hypotheses that (1) the presence of microplastics affects the composition and structure of the rhizosphere microbiome and (2) these effects vary according to plastic doses and types. (3) both LDPE-MPs and Bio-MPs stimulate the growth of nitrogen fixing. These results will contribute to our understanding of the effect of microplastic on the soil microbiome and thus the functioning of agroecosystems.

5.2 Materials and Methods

5.2.1 Soils, microplastics and common bean seed

The test soil was sandy soil collected from Unifarm, Wageningen University, the Netherlands (Supplementary Table S5.1). Two types of microplastic particles (MPs) were

selected in current study: 1). low-density polyethylene (LDPE-MPs) and 2). biodegradable plastic (Bio-MPs). The parental industrial pellets of biodegradable plastic consisted of 85% poly-butylene-adipate-co-terephthalate (PBAT), 10% of polylactic acid (PLA) and 5% of calcium carbonate. For both types, the size categories of microplastics used in this experiment were 60% 250~500 mm and 40% 500~1000 mm. Additional information about the used microplastic are provided in Meng et al. (2021) and Supplementary Figure S5.1. Common bean (*Phaseolus vulgaris L.*) seeds were obtained from Unifarm, Wageningen University, the Netherlands.

5.2.2 Pot experiment design and soil sampling

The pot experiment took place from 28th, June 2019 till 18th, October 2019 in an outdoor net house (diameter 0.25 mm) at Unifarm, Wagenigen University & Research (WUR), the Netherlands. Each type of MPs was mixed into the soil at doses of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w by weight of dry soil). A control treatment (CON, pure soil) without MPs were also included. In total 11 treatments with 8 replicated for each treatment were included in this study, resulting in 88 pots (Supplementary Figure S5.2). To achieved target doses, microplastics were added to homogenized dried soil in an iron tank and thoroughly mixed. Thereafter, 6 kg soil-MPs mixture substrate was weighed into 7 L polypropylene (PP) pots (21 cm height, 16 cm bottom diameter and 21 cm top diameter). During the experiment, all pots were unified to 10% (by weight of dry sandy soil) moisture. Further details regarding the experiment and cultivation have been reported in Meng et al. (2021). Rhizosphere and bulk soil samples were collected 46 days after seeding (D46), near the end of the vegetative stage when plant roots and leaves completed the early development stage. Rhizosphere samples were collected from soils that loosely adhered to the roots by shaking the roots gently. Soil from each pot were mixed thoroughly. The soil samples were transferred in a Styrofoam box with ice and immediately stored at −80 °C refrigerator for further analysis. Each bulk soil sample was compromised by 5 soil subsamples from each pot. The soil samples were air-dried and passed through 2 mm steel sieve, stored at 4 °C.

5.2.3 DNA extraction and bioinformation analysis

Due to budget and time constrains, only three out of five microplastic doses were selected for DNA extraction and analysis. Our previous research (not published yet) observed an accumulation of soil available nitrogen at ≤ 1.0% LDPE-MPs and decrease at ≥ 1.5% LDPE-

MPs. For Bio-MPs, significant responses of root growth were observed at ≥ 0.5% Bio-MPs, significant responses of soil carbon and nitrogen cycling were observed at ≥ 1.5% and most significant at 2.5% w/w. Therefore, MPs doses of 0.5%, 1.0% and 2.5% w/w were selected for assessing the effects of LDPE-MPs and Bio-MPs on soil bacterial community.

Soil DNA was extracted from 2 grams of soil, using a lab-made protocol based on a phenol-chloroform-isoamylalcohol extraction (Harkes et al., 2019). Quality and quantity of the extracted DNA was measured with a Nanodrop and Qubit. Thereafter, DNA samples were diluted to 1 ng/µl and used as template for PCR amplification. The variable V4 region of bacterial 16S rRNA gene was utilized as target for the analyses of Illumina sequencing. A two-step PCR was performed according to (Harkes et al., 2019). First a bacterial targeted primer combination, extended with an Illumina read area and the appropriate adapter were used to produce primary amplicons (in triplicate). A second PCR was conducted on 40x diluted amplicons of PCR1 to attach the Illumina index and the Illumina sequencing adaptor. Products of PCR 1 and 2 were randomly checked on gel to ensure amplification was successful. PCR1 was performed with the adapted version of primer 515F and 806R. All PCR2 products were pooled and sent for sequencing (Bioscience, Wageningen Research, Wageningen, The Netherlands) using the Illumina MiSeq Desktop Sequencer (2*250 nt paired-end sequencing) according to the standard protocols.

5.3 Results

5.3.1 Effect of plastic residues on the level of disease suppressiveness, plant biomass and plant nutrient status

A total of 980,000 bacterial sequences were obtained after passing quality filtering. The number of sequences reads per sample ranged from 12,000 to 947,00. In total, 10,474 OTUs were detected. α-diversity was used to analyze observable bacterial community complexity in each treatment (Chao 1, ACE, Shannon and Simpson). Highest species richness (Chao 1, ACE) and diversity (Shannon, Simpson) was observed in 1.0% Bio-MPs and 0.5% LDPE-MPs, respectively, and lowest in 2.5% Bio-MPs and 2.5% LDPE-MPs. 1.0% Bio-MPs also showed the highest α-diversity among all the treatments (Figure 5.1). In addition, a principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity matrix was used for comparing bacterial communities across treatments (β diversity analysis) (Figure 5.2). The first two principal components explained 27.6% of the observed community variance. Multivariate permutational ANOVA (PERMANOVA) was used to compare the microbial community

structure. The R^2 value indicates how much of the observed variance is explained by each individual variable. MPs dose explains most of the observed shifts in bacterial community (22%), while MP type explains only 8%. The interaction between dose and type was also significant (p = 0.002) and explains another 14% of the observed variance (Table 5.1). Both weighted and unweighted UniFrac confirmed that MP dose was the most important factor, followed by the interaction of dose x type and MPs type (p ≤ 0.02) (Supplementary Table S5.2).

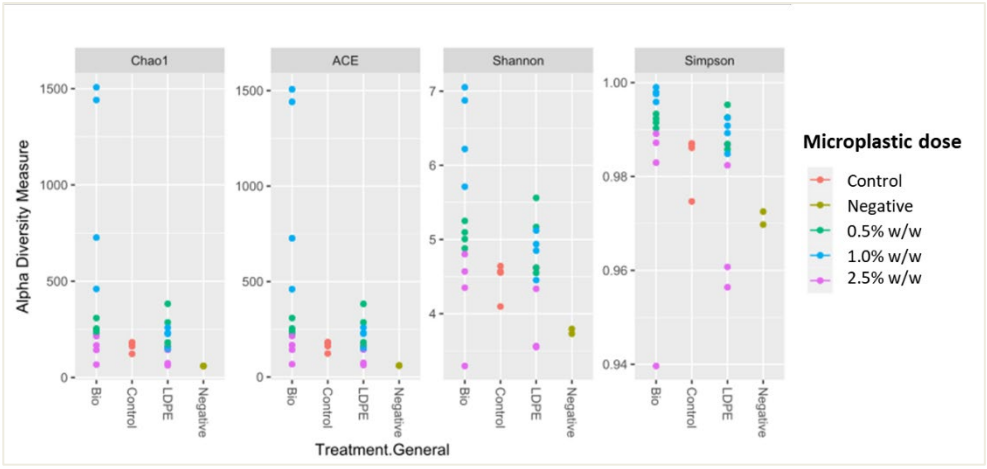


Figure 5.1 *Alpha diversity of bacterial, Chao 1, ACE, Shannon, Simpson index.*

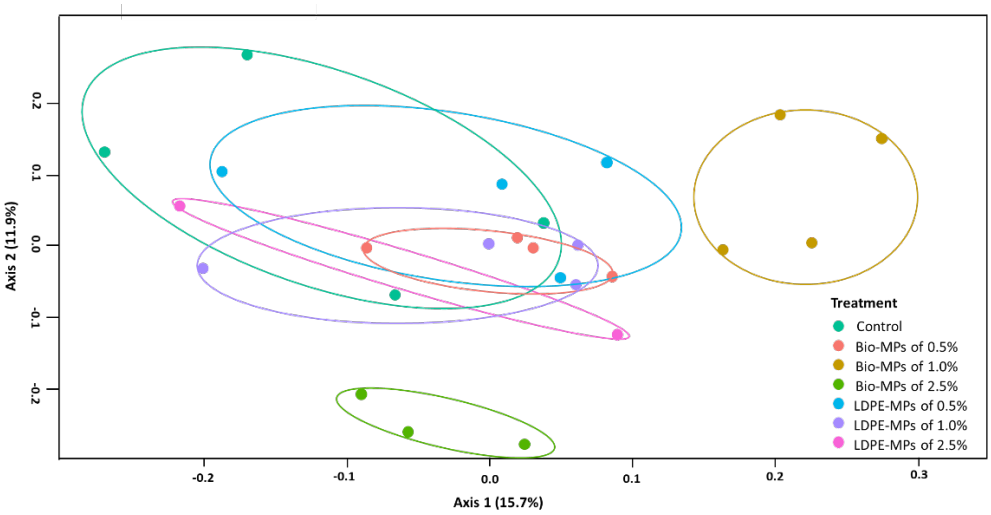


Figure 5.2 *Principal coordinate analysis of the rhizosphere microbial communities.*

5.3.2 3.2. Effects of microplastics on microbial community composition

The rhizosphere bacterial community at phylum level were dominated by *Proteobateria*, *Actinobacteria, Gemmatimonadetes and Acidobacteriota* (Supplementary Figure S5.3). The relative abundance at family level is illustrated in Figure 5.3. As compared to control, family *Comamonadaceae* was observed more abundant in all LDPE-MPs and Bio-MPs treatments. Family *Micrococcaceae* was more abundant in both 2.5% Bio-MPs and 2.5% LDPE treatments, while family *Rhizobiaceae* exhibited higher relative abundance in 2.5% LDPE-MPs while lower in 2.5% Bio-MPs than in control. Family *Xanthobacteraceae* was mostly abundant in 2.5% LDPE-MPs and leat in 1.0% Bio-MPs. Family *Sphingomonadaceae* was least abundant in 2.5% LDPE-MPs. In addition, LEfSe analysis with a LDA cutoff value of (≥ 3.5) illustrated the taxon-specific differences in

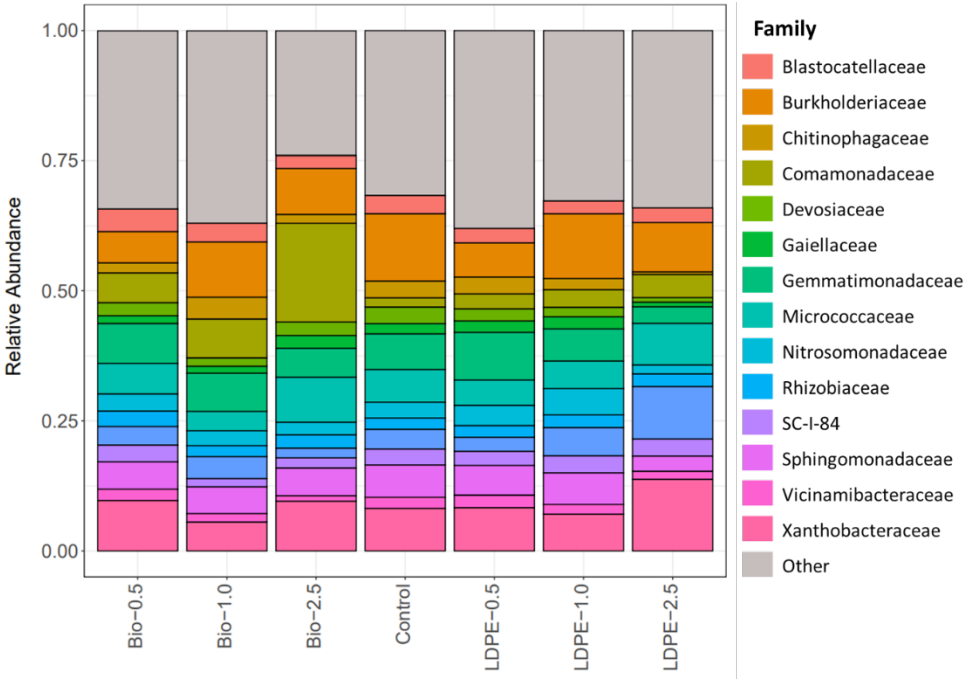
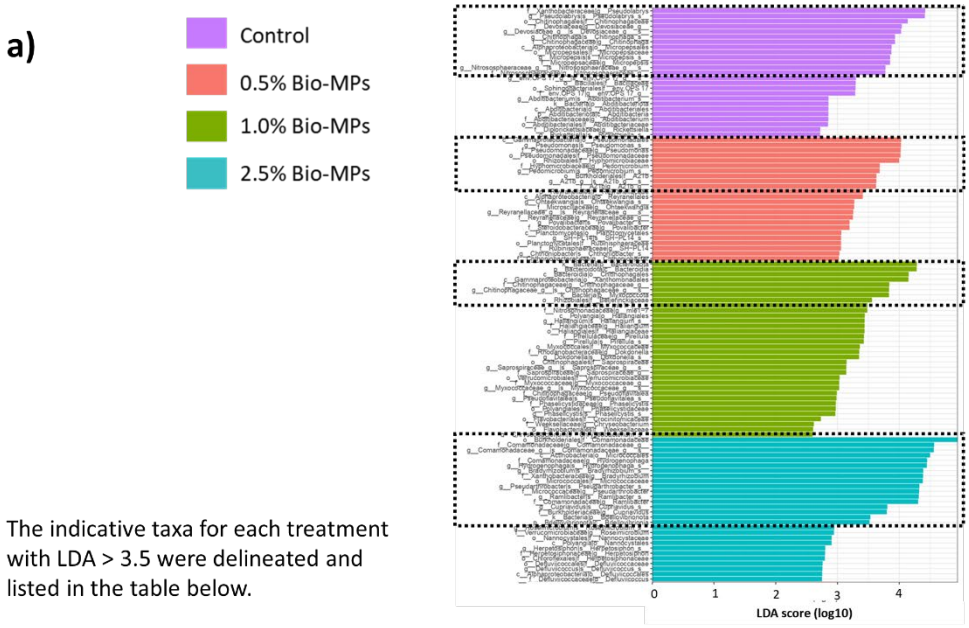


Figure 5.3 *Relative abundance of bacteria at family level in each treatment. LDPE-0.5: soil with LDPE microplastics of 0.5% w/w; LDPE-1.0: soil with LDPE microplastics of 1.0% w/w;LDPE-2.5: soil with LDPE microplastics of 2.5% w/w; Bio-0.5: soil with biodegradable microplastics of 0.5% w/w; Bio-1.0: soil with biodegradable microplastics of 1.0% w/w; Bio-2.5: soil with biodegradable microplastics of 2.5% w/w.*



The indicative taxa for each treatment with LDA > 3.5 were delineated and listed in the table below.

Treatment	*Indicative taxa (LDA > 3.5)*	
Control	f__Xanthobacteraceae	g__Pseudolabrys
	g__Pseudolabrys	s__Pseudolabrys_s__
	o__Chitinophagales	f__Chitinophagaceae
	f__Devosiaceae	g__Devosiaceae_g__
	g__Devosiaceae	s__Devosiaceae
	g__Chitinophaga	s__Chitinophaga
	f__Chitinophagaceae	g__Chitinophaga
	c__Alphaproteobacteria	o__Micropepsales
	o__Micropepsales	f__Micropepsaceae
	g__Micropepsis	s__Micropepsis
	f__Micropepsaceae	g__Micropepsis
	g__Nitrososphaeraceae	s__Nitrososphaeraceae
	f__Nitrososphaeraceae	g__Nitrososphaeraceae
0.5% Bio-MPs	c__Gammaproteobacteria	o__Pseudomonadales
	g__Pedomicrobium	s__Pedomicrobium
	f__Hyphomicrobiaceae	g__Pedomicrobium
	o__Rhizobiales	f__Hyphomicrobiaceae
	o__Pseudomonadales	f__Pseudomonadaceae
	f__Pseudomonadaceae	g__Pseudomonas
	g__Pseudomonas	s__Pseudomonas
	o__Burkholderiales	f__A21b
	g__A21b	s__A21b
	f__A21b	g__A21b

Treatment	*Indicative taxa (LDA > 3.5)*	
1.0% Bio-MPs	k__Bacteria	p__Bacteroidota
	p__Bacteroidota	c__Bacteroidia
	c__Bacteroidia	o__Chitinophagales
	c__Gammaproteobacteria	o__Xanthomonadales
	f__Chitinophagaceae	g__Chitinophagaceae
	g__Chitinophagaceae	s__Chitinophagaceae
	k__Bacteria	p__Myxococcota
	o__Rhizobiales	f__Beijerinckiaceae
2.5% Bio-MPs	o__Burkholderiales	f__Comamonadaceae
	f__Comamonadaceae	g__Comamonadaceae
	g__Comamonadaceae	s__Comamonadaceae
	c__Actinobacteria	o__Micrococcales
	f__Comamonadaceae	g__Hydrogenophaga
	g__Hydrogenophaga	s__Hydrogenophaga
	g__Bradyrhizobium	s__Bradyrhizobium
	f__Xanthobacteraceae	g__Bradyrhizobium
	o__Micrococcales	f__Micrococcaceae
	g__Pseudarthrobacter	s__Pseudarthrobacter
	f__Micrococcaceae	g__Pseudarthrobacter
	g__Ramlibacter	s__Ramlibacter
	f__Comamonadaceae	g__Ramlibacter
	g__Cupriavidus	s__Cupriavidus
	f__Burkholderiaceae	g__Cupriavi
	k__Bacteria	p__Bdellovibrionota
	p__Bdellovibrionota	c__Bdellovibrionia

Figure 5.4 *a) LEfSe analysis identifying active taxa (LDA score > 3.5 are delineated) of rhizosphere bacterial communities resulting from biodegradable microplastic treatments.*

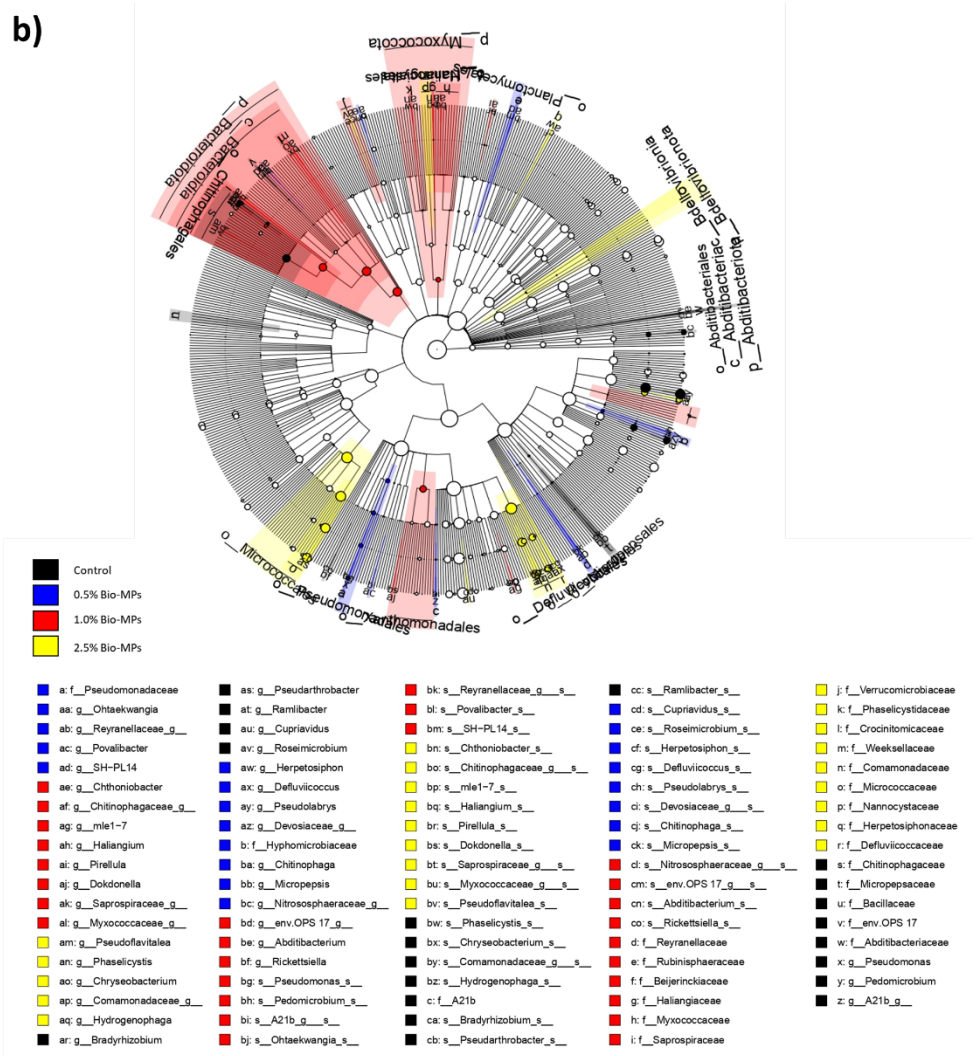
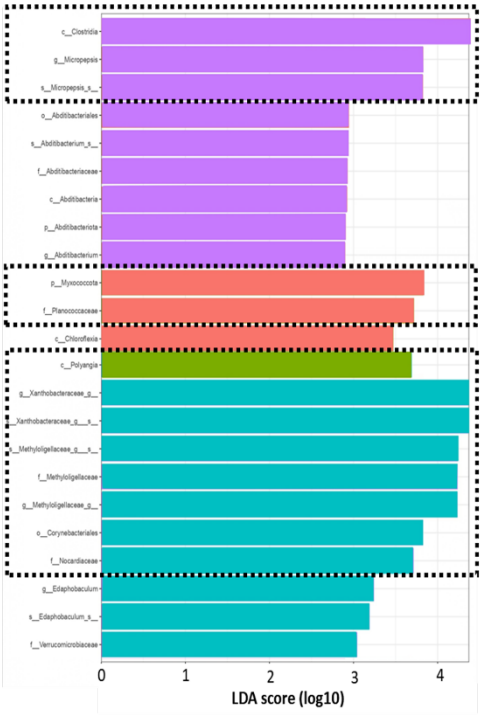
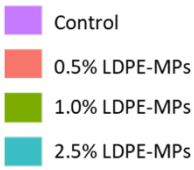


Figure 5.4 *b) Cladograms indicate the phylogenetic distribution of microbial lineages associated biodegradable microplastic treatments. Differences are represented in the color of the abundant groups.*

c)





The indicative taxa for each treatment with LDA > 3.5 were delineated and listed in the table below.

Treatment	***Indicative taxa (LDA > 3.5)***
Control	c__Clostridia g__Micropepsis s__Micropepsis
0.5% LDPE-MPs	p__Myxococcota f__Planococcaceae
1.0% LDPE-MPs	c__Polyangia
2.5% LDPE-MPs	g__Xanthobacteraceae s__Xanthobacteraceae s__Methyloligellaceae f__Methyloligellaceae g__Methyloligellaceae o__Corynebacteriales f__Nocardiaceae

Figure 5.4 *c) LEfSe analysis identifying active taxa (LDA score > 3.5 are delineated) of rhizosphere bacterial communities resulting from LDPE microplastic treatments.*

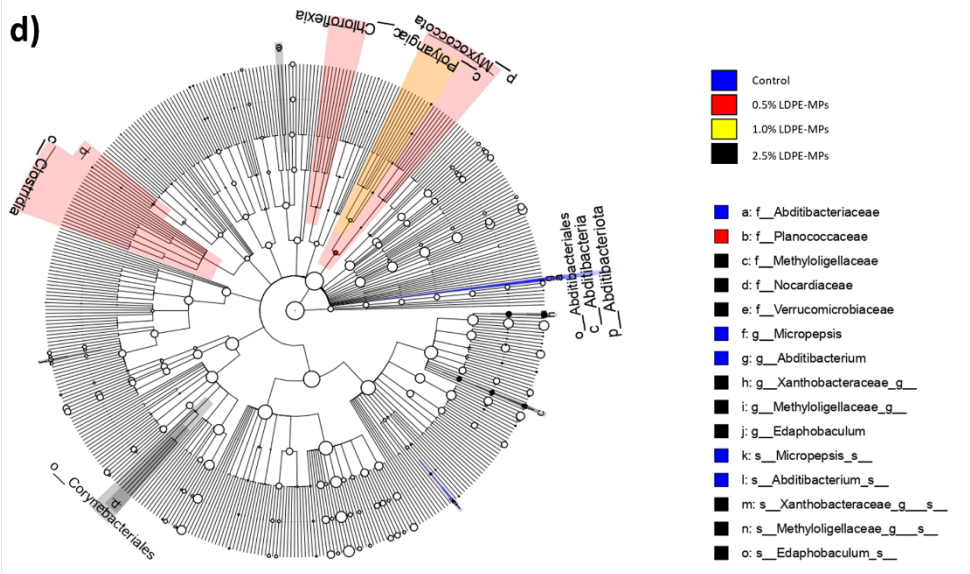


Figure 5.4 *d) Cladograms indicate the phylogenetic distribution of microbial lineages associated LDPE microplastic treatments. Differences are represented in the color of the abundant groups.*

the rhizosphere bacterial communities between control treatment and different types of microplastics, respectively. The pairwise comparison showed more bacterial taxa were detected by LEfSe as important contributors for the difference between control and Bio-MPs than between control and LDPE-MPs (Figure 5.4). Specifically, the comparison between control and Bio-MPs showed the indicative taxa for control soils were genus *Pseudolabrys, Devosiaceae, Chitinophaga, Micropepsis* and *Nitrososphaeraceae*; in 0.5% Bio-MPs, the genus *Pedomicrobium*, *Pseudomonas* and *A21b* were considered indicative; in 1.0% Bio-MPs, the families *Chitinophagaceae* and *Beijerinckiaceae* were considered indicative. Finally, in 2.5% Bio-MPs, the family *Comamonadaceae*, *Hydrogenophaga*, *Bradyrhizobium*, *Pseudarthrobacter*, *Ramlibacter* and *Cupriavidus* were considered indicative. The comparation between control and LDPE-MPs showed that the genus *Micropepsis* is an indicative taxon in control soils. In 0.5% LDPE-MPs, the family *Planococcaceae* and phylum *Myxococcota* were considered indicative; in 1.0% LDPE-MPs class *Polyangia* were considered indicative and in 2.5% LDPE-MPs families *Xanthobacteraceae*, *Nocardiaceae* and *Methyloligellaceae* were considered indicative.

Table 5.1 *Results of PERMANOVA based on Bray-Curtis dissimilarity distances. The factors microplastic dose, microplastic types, and their interaction were analyzed.*

Factors	F	R^2	p
Microplastic dose	30.504	0.22182	0.001
Microplastic type	24.175	0.0879	0.001
Dose: Type	19.926	0.1449	0.002
Residuals		0.54539	

Note. Differences are considered significant if $p < 0.05$.

5.4 Discussion

5.4.1 Microplastics changed the common bean rhizosphere bacterial community

Microplastics-induced dynamics on microbial diversity have been reported earlier and the observed shifts in microbial community composition were highly various. Zhou et al. (2021) conducted a mesocosm experiment with poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBA), they found the addition of PHBA to be increasing soil α-diversity. Wang et al. (2020a) and Yang et al. (2021) observed no significant effects of up to 10% PLA-MPs on α-

diversity of soil arbuscular mycorrhizal fungal community. The impacts on soil microbial community from PE-MPs also varied from positive (Ren et al., 2020), insignificant (Huang et al., 2019b; Wang et al., 2020a) to negative (Fei et al., 2020; Gao et al., 2021). In this study, the α-diversity of rhizosphere microbial communities was found enhanced at lower doses of MPs (0.5% LDPE-MPs and 1.0% Bio-MPs), while decreased at highest dose (2.5% LDPE-MPs and 2.5% Bio-MPs) (Figure 5.1, Figure 5.2 and Table 5.1). In addition, the PCoA (β-diversity) depicted 1.0% and 2.5% Bio-MPs treatments were clearly separated from LDPE-MPs treatments (Figure 5.2). Those results demonstrate that microplastics can alter soil rhizosphere microbial community composition and structure, but effects vary with the microplastic types and doses.

Several mechanisms might explain the increase in soil microbial α-diversity in 0.5% LDPE-MPs and 1.0% Bio-MPs and decrease α-diversity in 2.5% LDPE-MPs and 2.5% Bio-MPs. Firstly, the surface of microplastics, also known as "plastisphere" (Jiang et al., 2018; Zettler et al., 2013), can provide a novel and distinct habitats for soil microorganisms (Zhang et al., 2019d). Xie et al. (2021) stated that plastisphere of microplastics can provide an inclusive and compatible niche for a wide variety of rhizosphere microbes to colonize, resulting in more diverse microbial community. On the other hand, an excessive amount of microplastics can exhibited selective effects on the indigenous bacteria which are more compatible with microplastic surface (Qi et al., 2020c; Ren et al., 2020; Xie et al., 2021), leading to the decrease of microbial α-diversity in 2.5% dose microplastic treatments. Secondly, carbon supplied by Bio-MPs (such as PBAT, PLA and PHA) has can be utilized by microorganisms to gain biomass and energy (Urtuvia et al., 2014; Zumstein et al., 2018b). However, by gaining access to this bioavailable-C source, these microorganisms might outcompete microorganisms who are unable to metabolize this carbon source (Dini-Andreote et al., 2015; Rüthi et al., 2020), thus lowering microbial diversity at 2.5% Bio-MPs.

The PCoA showed a clear separation of 1.0% Bio-MPs and 2.5% Bio-MPs treatments, while control, 0.5% Bio-MPs and all LDPE-MPs treatments were clustered together. One explanation might be the divergence of the microbial community composition between LDPE-MPs and Bio-MPs. Bio-MPs used in current experiment contains heteroatomic biopolymers that could be biodegraded by microorganisms into CO_2, water and CH_4 (Guerrini et al., 2017; Madhavan Nampoothiri et al., 2010). LDPE is a petroleum-derived hydrocarbon that has stable C-C bones and is almost non-degradable in soil (Briassoulis et al., 2004; Steinmetz et al., 2016). Hence, LDPE-MPs and Bio-MPs present distinct physical and chemical properties which likely account for the divergence of the rhizosphere microbial community structure (Seeley et al., 2020). Finally yet importantly, the PERMANOVA results showed the dose of microplastics contributed most to the changes in

microbial community composition, followed by the interaction of type and dose of microplastics and the type of microplastics. This result emphasized that the accumulation of microplastics in soil, no matter Bio-MPs or LDPE-MP, when reaching to a certain level might finally pose environmental threats to the soil-plant system. To date, studies on the effects of differrent types and doses of microplastics on rhizosphere microbial community are still scares. In order to make stronger statements this needs to be further evaluated.

5.4.2 Microbial taxa affected by MPs dose and type

The response of bacterial relative abundance at family level varied with microplastic type and dose. The relative abundance of family *Comamonadaceae* was higher in all LDPE-MPs and Bio-MPs treatments compared to control, with Bio-MPs exerting stronger effects as compared to LDPE-MPs. *Comamonadaceae* is an aerobic family that known to harbor hydrocarbon decomposers and play a role in the decomposition of various organic compounds (Kerster et al., 2006; Nuccio et al., 2013; Willems, 2014). The higher relative abundance of *Comamonadaceae* in LDPE treatments could be a result from the increased soil porosity by the addition of the microplastics. This allows more air diffusion (Qi et al., 2020b; Zhang et al., 2019a), thus affiliating the growth of family *Comamonadaceae* in our LDPE-MPs treatments. As a main decomposer for biodegradable materials (PHBV and PLA) (Khan et al., 2002; Takahashi et al., 2011; Xu et al., 2018). *Comamonadaceae* has previous been observed thrives in starch-based plastic treated soil (Qi et al., 2020c) and PHBV-MPs treated soil (Zhou et al., 2021). As such, the higher relative abundance of *Comamonadaceae* in our Bio-MPs treatments might be attributed to the carbon substrate supplied from Bio-MPs (PLA and PBAT). Additionally, LEfSe showed the genus *Hydrogenophaga* and *Ramlibacter (Family Comamonadaceae)* to be stimulated by 2.5% Bio-MPs treatment. Previous study by Bandopadhyay et al. (2020) found the genus *Hydrogenophaga* was enriched on PLA-containing plastics. (Chen et al., 2019a) found *Ramlibacter* was positive responded to PLA-MPs. Members of *Ramlibacter* have been reported have the catabolic potential of utilizing complex organic fractions like hydroxybenzoate, 3-hydroxybenzoate, and D-melibiose as sole carbon sources (Wang et al., 2012; Yan et al., 2020). Member of *Hydrogenophaga* are characterized by the ability to oxidize hydrogen (Fagervold et al., 2014; Willems, 2014), it has also been reported prefer using carboxylic acids as growth substrate (Magic-Knezev et al., 2009). Hence, our findings, combine with these previous findings, suggesting that biodegradable materials can be used as a carbon source for rhizosphere microbial community and affect its composition.

The relative abundance of families *Rhizobiaceae* and *Xanthobacteraceae* were higher in 2.5% LDPE-MPs, while family *Rhizobiaceae* was lower in 2.5% Bio-MPs. In addition, family *Xanthobacteraceae* was observed as a biomarker in higher in 2.5% LDPE-MPs treatments (LEfSe). Both *Xanthobacteraceae* and *Rhizobiaceae* are aerobic and involved in organic matter decomposition and symbiotic nitrogen fixation process (Chen et al., 2019b; Cheng et al., 2017; Khalid et al., 2020; Oren, 2014). Hence, the explanation of higher soil porosity by the addition of LDPE-MPs may also apply for the observed increase of the relative abundance in 2.5% LDPE-MPs. To date, very few studies reported the effects of microplastics on relative abundance of family *Rhizobiaceae*. Zhu et al. (2018a) observed that 10% polystyrene nanoplastics (0.05-0.1mm) significantly decreases the relative abundance of *Rhizobiaceae* in the gut microbiome of *Enchytraeus crypticuswhich*. Similar to LDPE, polystyrene polymers have stable C-C bones and are resistant to degradation (Shen and Worrell, 2014). However, the smaller sizes However, the small particle size of the nanoplastics is likely to allow a higher higher carbon accessibility for the microorganisms in the study of Zhu et al. (2018a). Here, we also observed a lower relative abundance of *Rhizobiaceae* in 2.5% Bio-MPs. Hence, combine with their results, we speculate that the lower abundance of family *Rhizobiaceae* in 2.5% Bio-MPs might attributed to its over amount of carbon availability.

Previously, 2.5% LDPE-MPs and 2.5% Bio-MPs induced higher specific root nodules (nodules per gram dry root) compared to control treatment. We therefore hypothesized higher relative abundance of N_2 fixation bacteria in 2.5% LDPE-MPs and 2.5% Bio-MPs contained (Meng et al., 2021). Our current result seems to contradict this hypothesis at first as 2.5% Bio-MPs lowered the *Rhizobiaceae*, which harbors the nitrogen fixing genus *Rhizobium*. Considering the higher specific root nodules and lower relative abundance of *Rhizobiaceae* of 2.5% Bio-MPs treatment, it might be that others are responsible for the nodulation and N_2 fixation process in the Bio-MPs treated soil-plant system. For example, LEfSe analysis revealed the genus *Bradyrhizobium* as indicative in 2.5% Bio-MPs. *Bradyrhizobium* is one of the main nitrogen fixation genera that is capable of forming symbiotic nodules and develop in legumes plant (Avontuur et al., 2019; Ormeno-Orrillo and Martinez-Romero, 2019).

The relative abundance of the family *Micrococcaceae* was observed significantly enhanced by 2.5% LDPE-MPs and 2.5% Bio-MPs treatments. Recent study showed *Micrococcaceae* is primary decomposer of bean plant residues and positively related to soluble carbon content, as well as for cellobiose and glucose under oxic conditions (Monreal et al., 2018; Ortiz-Cornejo et al., 2017; Schellenberger et al., 2010). Our previous research found 2.5% Bio-MPs led to higher root decay of common bean (Meng et al., 2021) and higher DOC (unpublished), this might account for the increase in the relative abundance of family

Micrococcaceae in 2.5% Bio-MPs treatment. However, 2.5% LDPE-MPs showed no significant effects on neither soil DOC nor root biomass (Meng et al., 2021). Several studies showed that *Micrococcaceae* was positively related to high C:N ratio and considered as potential biomarkers of microbial nutrition limitation (Aanderud et al., 2018; Cui et al., 2018a; Huang et al., 2019a). Thus, the increased relative abundance of family *Micrococcaceae* in our study suggesting that the addition of higher amount of LDPE-MPs might have legacy effects on influencing soil C and N cycling.

5.4.3 Implication and changed rhizosphere bacterial community

According to LEfSe (Figure 5.4), the pair comparison of Bio-MPs VS Control induced more distinct taxa than LDPE-MPs VS Control, indicated Bio-MPs exerted stronger effects than LDPE-MPs on rhizosphere microbial communities. We should notice that changes in soil microbial composition may pose potential legacy effects on soil quality (Chen et al., 2019a). For example, members of the family *Comamonadaceae* can mineralize organic forms of sulfate into inorganic forms, thus inhibiting nitrification process in sediments and soils systems (Ouyang et al., 2019; Schmalenberger et al., 2008). Family *Comamonadaceae* and *Micrococcaceae* were also played crucial roles in denitrification process (Huang et al., 2014; Khan and Hiraishi, 2002; Takahashi et al., 2011). Thus, microplastic-induced changes in microbial communities might impose effects on soil nitrogen availability. In addition to influencing the growth of bacteria related to organic matter-degrading and nitrogen cycling, some of the taxa that were stimulated by microplastic pollution are also associated with pathogenic bacteria. For example, the family *Nocardiaceae* was observed as the biomarker taxa on 2.5% LDPE-MPs treatment in our study (Figure 5.4c). Previous research by Huang et al. (2019b) also observed the relative abundance of family *Nocardiaceae* enriched by LDPE-MPs. The family *Nocardiaceae* is known to harbor causal pathogens of suppurative and granulomatous diseases of humans and animals (Goodfellow, 1996; Goodfellow, 1998; Goodfellow and Maldonado, 2006). It is noted that not all members of family *Nocardiaceae* are pathogenic. However, it is now known which species is enriched by the occurrence of microplastics and microplastics may act as a vector for transporting those opportunistic pathogens, which requires a more in-depth evaluation.

LDPE-MPs exerted less significant effects on rhizosphere microbial communities than Bio-MPs. Is this implying that there being no immediate implications for microplastics to terrestrial ecosystems (van Weert et al., 2019)? We suggest the readers take this idea with a grain of salt. We only applied pure microplastics (without additives) in a pot experiment.

However, plastics entering soil often contain additives, such as plasticizers and antioxidants (Hurley et al., 2020; Yang et al., 2020). Once released into soil, those additives can pose serious threats to soil biological processes (Steinmetz et al., 2016; Wang et al., 2016). In addition, agricultural soils receive large amounts of heavy metal, pesticides, herbicides and other toxic chemicals via various routes (Beriot et al., 2021; Briassoulis et al., 2010). The interactions between different microplastics and agricultural pollutants remains largely unexplored. Therefore, broader approach will be needed to thoroughly evaluate the effects of microplastics in soil-plant systems (Khalid et al., 2020; Rillig, 2020).

5.5 Conclusion

This study shows that the impacts of LDPE-MPs and Bio-MPs (PLA+PBAT) on common bean rhizosphere bacterial community diversity vary with the polymer type and doses: α-diversity (richness and diversity) was significantly improved by 1.0% Bio-MPs and 0.5% LDPE-MPs while decreased by 2.5% Bio-MPs and 2.5% LDPE-MPs, this might be attributed to the selective effects of two types of polymers. PCoA showed rhizosphere microbial communities of 1.0% and 2.5% Bio-MPs were clearly separated from other treatments, LEfSe showed the addition of Bio-MPs induced more distinct taxa in rhizosphere than LDPE. These results indicate Bio-MPs exerted stronger effects in our soil-plant system than LDPE-MPs. The shifts of relative abundance of families *Comamonadaceae*, *Rhizobiaceae* and *Micrococcaceae* were observed varied with microplastic type and dose, these families play important roles in soil organic matter decomposition and nutrient cycling, implying that increasing contamination by microplastics in soil might has profound effects on soil nutrient cycling. PERMANOVA results revealed the dose of microplastics contributed most more to the changes in microbial community composition. This emphasized that the no matter conventional LDPE-MPs or Bio-MPs, when accumulating in soil to a certain level might finally impose threats to soil-plant systems. The outcomes of current study revealed the complexity of the interactions among soil-plant-microplastics. As such, future studies on investigating the interaction between microplastic, plant root and rhizosphere are advocated to provide better understanding of the ecological effects of microplastics.

Acknowledgements

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Supplementary Material

Table S5.1 *Detailed information about soil used in the experiment*

Soil parameters	Unit	Results
Total nitrogen (N)	$kg \cdot ha^{-1}$	3775
C/N ratio		17
Available nitrogen	$kg \cdot ha^{-1}$	45
Total sulfur (S)	$kg \cdot ha^{-1}$	995
C/S ratio		66
Available sulfur	$kg \cdot ha^{-1}$	15
Total phosphorus (P)	$kg \cdot ha^{-1}$	695
Total potassium (K)	$kg \cdot ha^{-1}$	505
Total calcium (Ca)	$kg \cdot ha^{-1}$	3840
Total magnesium (Mg)	$kg \cdot ha^{-1}$	260
pH		6
Organic carbon (SOC)	%	2
Organic matter (SOM)	%	4
Inorganic carbon	%	0.07
Carbonated lime	%	< 0.2
Clay	%	< 1
Silt	%	11
Sand	%	83

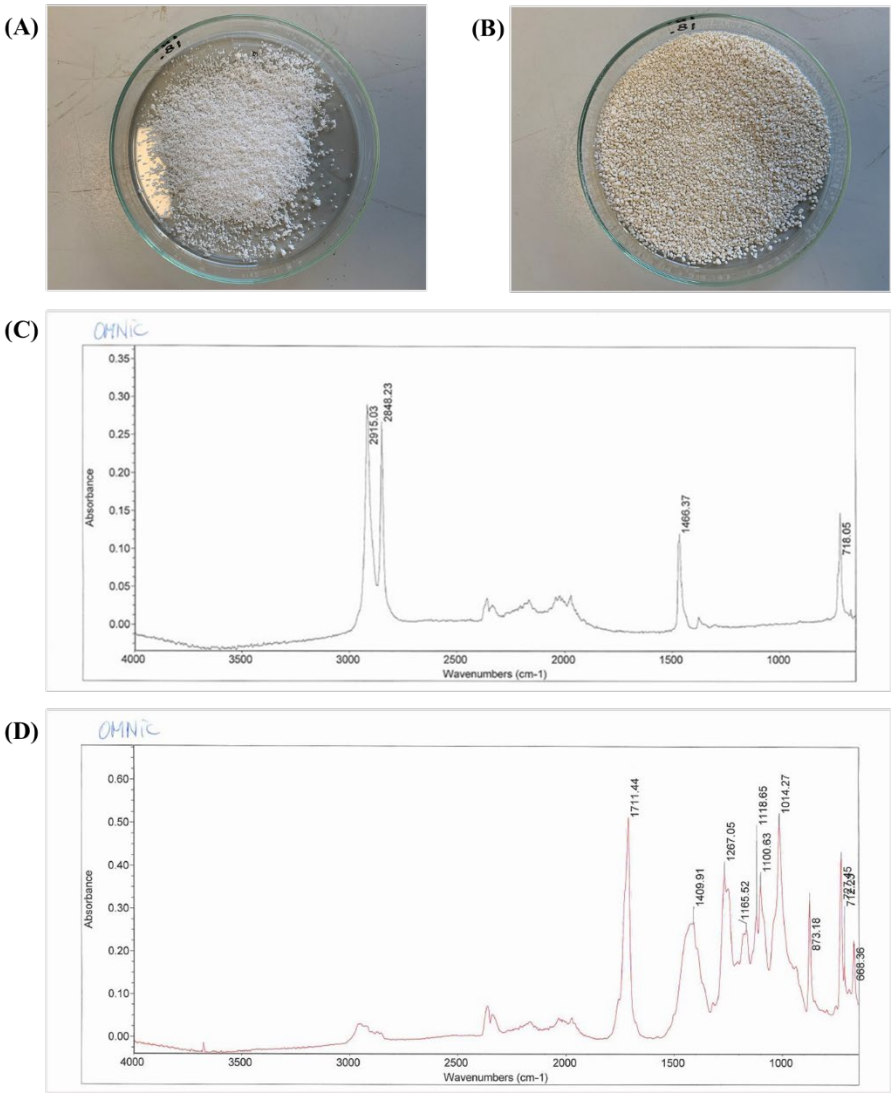

Figure S5.1 *The MPs applied in current research. (A) LDPE-MPs; (B) Bio-MPs; (C) FTIR of LDPE-MP; (D) FTIR of Bio-MPs.*

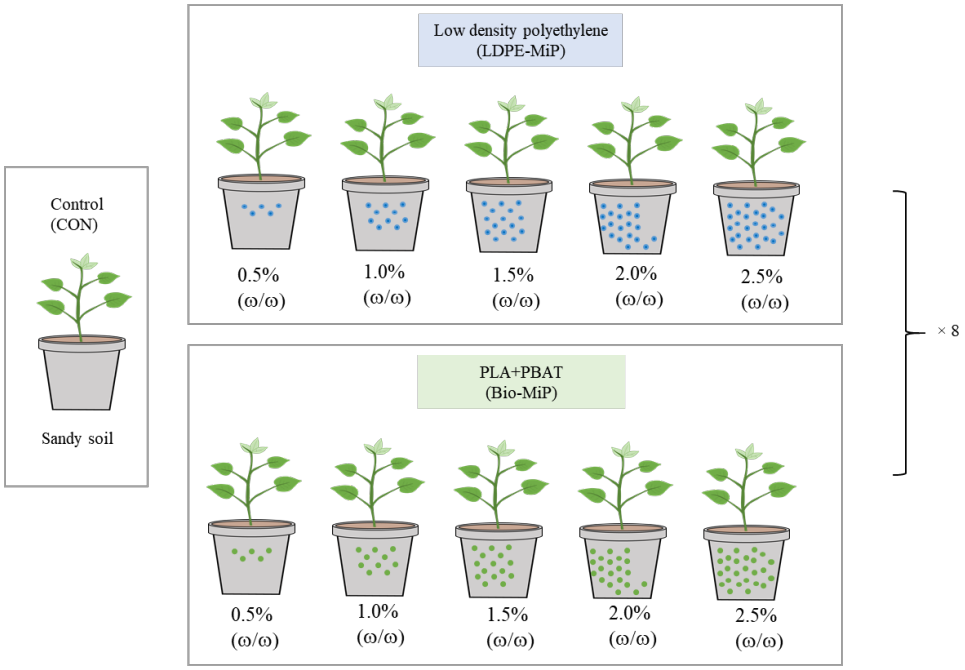

Figure S5.2 *Pot experimental design. All 11 treatments were repeated 8 times (4 replicates per harvesting moment).*

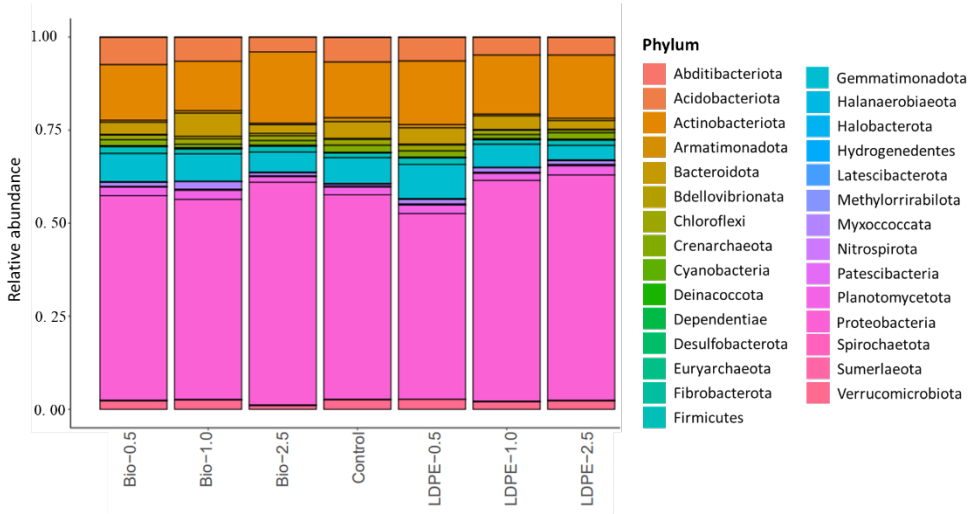

Figure S5.3 *Relative abundance of bacteria at phylum level in each treatment. LDPE-0.5: soil with LDPE microplastics of 0.5% w/w; LDPE-1.0: soil with LDPE microplastics of 1.0% w/w;LDPE-2.5: soil with LDPE microplastics of 2.5% w/w; Bio-0.5: soil with biodegradable microplastics of 0.5% w/w; Bio-1.0: soil with biodegradable microplastics of 1.0% w/w; Bio-2.5: soil with biodegradable microplastics of 2.5% w/w*.

6. Synthesis

6.1 General conclusions

The thesis provided a valuable "field-to-pot experiment" view of current plastic pollution. The outline of the findings is presented in Figure 6.1. In chapter 2, we studied accumulation and distribution of agricultural macroplastics and microplastics in the agricultural fields of two regions in Northwest China. Both selected regions had a long history (dating back to the mid-1980s) of plastic mulching application but followed two different farming systems. The first study region (S1) was characterized by small-scale farmlands with low-density machinery tillage while the second study region (S2) was characterized by large-scale farmlands and high-density machinery tillage. Plastic residues in the first 0–30 cm of soil was collected and analysed. In this chapter, macroplastics were defined as plastic particles with a size area of > 0.25 cm^2 while microplastics were defined as plastic particles that had broken down from LDPE plastic films and had a diameter of < 2 mm and a density smaller than 1 $g \cdot cm^{-3}$.

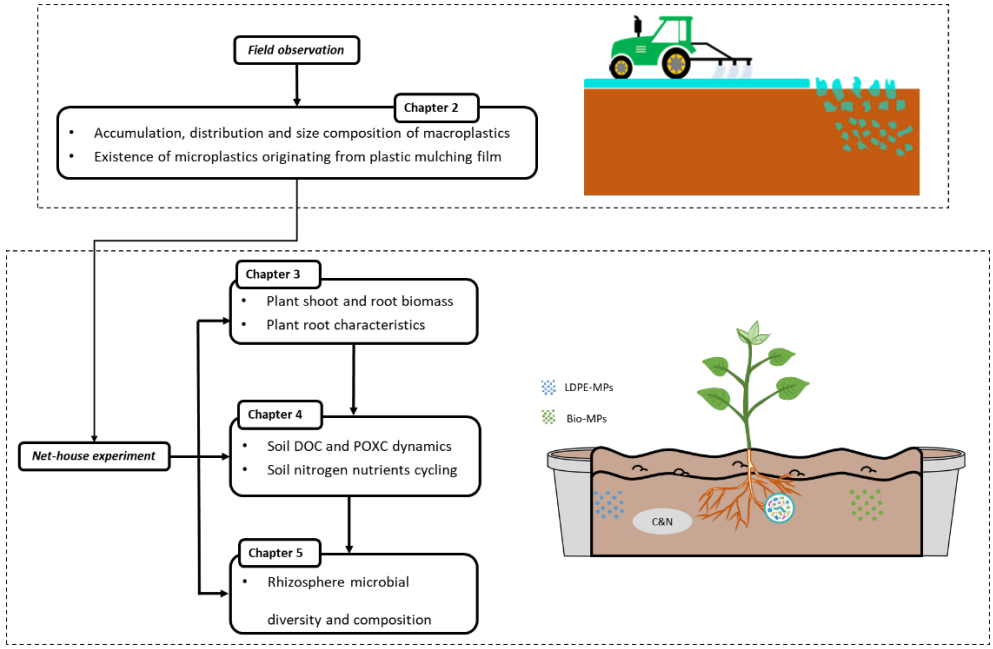

Figure 6.1 *An outline of the main findings in this thesis.*

After identifying the occurrence of LDPE microplastics (LDPE-MPs), we conducted an outdoor net-house pot experiment to investigate the effects of microplastics on the soil-plant system (Chapter 3 and 4). In the pot experiment, two types of microplastics were applied: LDPE-MPs and biodegradable microplastics (Bio-MPs). Bio-MPs used in this research consisted of 10% polylactic acid (PLA), 85% poly-butylene-adipate-co-

terephthalate (PBAT) and 5% calcium carbonate. Both LDPE-MPs and Bio-MPs contained no additives or plasticizers. These two types of microplastics were mixed into sandy soil at 5 doses (0.5%, 1.0%, 1.5%, 2.0%, 2.5% w/w dry soil weight). Common bean (*Phaseolus vulgaris L*) was selected as the model plant to assess the effects of microplastics on the soil-plant system. The wide range of microplastic concentrations was used to determine the potential effect of thresholds and amplify any subtle effects that might otherwise be ignored. In Chapter 5, a control treatment and three microplastic concentrations, 0.5%, 1.0% and 2.5% w/w, were selected to examine the effects of microplastics on rhizosphere microbial communities.

The outputs of this thesis have made fundamental contributions to understanding the growing concerns surrounding plastic pollution in agricultural fields, as well as assessing the ecological impacts of the effects of conventional LDPE-MPs and Bio-MPs the on the soil-plant system. The main findings of this PhD thesis are:

- In Chapter 2, we found farming systems could substantially affect the accumulation and distribution of agricultural plastic residues. Specifically, in S1, we found that macroplastic accumulation in fields with 6-8 years of continuous plastic mulching was significantly higher than in fields with over 30 years of intermittent plastic mulching. Macroplastics in the size category 10–50 cm^2 accounted for over 40% of the collected macroplastics, followed by 2–10 cm^2 which accounted for over 30%. Microplastics were mainly detected in fields with over 30 years of intermittent mulching. In S2, we found that the accumulation of macroplastics did not increase with increasing years of mulching. The collected macroplastics were mainly in the size category 0.25–2 cm^2 (40.6%) and 2–10 cm^2 (41.1%). Microplastics were detected in all the selected fields in S2. By comparing S1 and S2, we concluded that high-intensity machine tillage could lead to higher fragmentation of macroplastics and more severe microplastic pollution.

- In Chapter 3, we measured a suite of proxies for common bean *(Phaseolus vulgaris L.; P. vulgaris)* growth. Overall, Bio-MPs exhibited stronger effects than LDPE-MPs. LDPE-MPs showed no significant effects on shoot or root biomass, while Bio-MPs at 1.5%, 2.0% and 2.5% w/w significantly inhibited the root and shoot biomass. All the Bio-MPs showed higher specific root length and specific root nodules, ≥ 1.0% LDPE-MPs also showed higher specific root nodules. The results indicated that both LDPE-MPs and Bio-MPs were able to elicit responses in *P. vulgaris* growth, and Bio-MPs exerted stronger effects. One potential explanation might be attributed to the fact that LDPE and Bioplastic polymers varied in composition and structure. Carbon supply from Bio-MPs is more bioavailable to microorganisms while carbon from

LDPE-MPs is not. These differences might induce different effects on soil biota and consequently, nutrient availability, thus contributing to the different responses in common bean growth. Therefore, we decided to further study the dynamics of soil carbon and nitrogen cycling and rhizosphere microbial communities in the following chapters.

- In Chapter 4, the effects of LDPE-MPs and Bio-MPs on dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), available nitrogen of N-NH_4^+ and N-NO_3^-, and dissolved organic nitrogen (DON) were measured on the 46th and 105th day of plant growth. Corresponding to Chapter 3, Bio-MPs showed stronger effects on measured soil nutrient indicators than LDPE-MPs. LDPE-MPs showed minor to no significant impacts on soil available nitrogen (N-NH_4^+ and N-NO_3^-), soil DOC and DON. However, we observed that soil available nitrogen had accumulated from 0.5% to 1.0% LDPE-MPs and decreased from 1.5% to 2.5% LDPE-MPs. Bio-MPs of 2.0% and 2.5% w/w (46th day) led to significantly higher soil DOC and DON and significantly lower available nitrogen. Soil POXC was significantly lower in both LDPE-MPs and Bio-MPs on the 105th day. In addition, we also measured soil organic carbon (SOC) by using the "loss on ignition" (LOI) method, the loss masses included SOC from the soil-plant system and mass of added microplastics. The results of the SOC suggested that the treatments with Bio-MPs experienced a rapid degradation from the first to the 46th day, while LDPE-MPs did not degrade during the experiment (0 to 105th day). Considering the different compositions, we speculated that the changes in the rhizosphere microbial communities might provide insight into the mechanisms behind the microplastic effects on the soil labile organic carbon fractions and nitrogen cycling.

- In Chapter 5, we observed the effects of two types of microplastics on the diversity and composition of rhizosphere microbial communities. Due to budget and time constrains, only three out of the five microplastic doses were selected for metagenome analysis. According to the results of chapter 3 and chapter 4, microplastics doses of 0.5% LDPE-MPs lowered leaf relative chlorophyll content and 1.0% LDPE-MPs increased leaf area. ≥ 1.5% Bio-MPs lowered root biomass and nutrient availability. Hence, 0.5%, 1.0% and 2.5% were selected. Results showed that for each type of microplastic, rhizosphere microbial alpha diversity (Chao 1, ACE, Shannon and Simpson) was highest at 1.0% Bio-MPs and 0.5% LDPE-MPs and were lowest at 2.5% LDPE-MPs and Bio-MPs. This implied that higher doses of microplastics might exert selective pressure on rhizosphere microbial communities. PCoA results showed that 1.0% and 2.5% Bio-MPs led to clearly different microbial community structures as compared to the control, 0.5% Bio-MPs, and all LDPE-MPs

treatments. Relative abundances of specific bacterial taxa were also affected, i.e., as compared to the control. Family *Comamonadaceae* presented higher relative abundance in MPs treatments, especially Bio-MPs; relative abundance of Family *Rhizobiaceae* was higher for 2.5% LDPE-MPs while lower for 2.5% Bio-MPs. These bacterial taxa were closely related to soil organic matter decomposition and nitrogen dynamics. The results indicated that both LDPE-MPs and Bio-MPs could exert profound effects on the rhizosphere microbial community and these effects might have far reaching effects on soil nutrient cycling and plant health in agroecosystems.

6.2 General discussion

I hope the findings of this PhD thesis can provide a basic understanding of current concerns surrounding agricultural macro and microplastic pollution and provide insight into how plant growth, soil nutrient dynamics and the rhizosphere microbial community respond to microplastics in the soil-plant system. Accumulation of agricultural plastic waste (APW), the fate of microplastics broken down from macroplastics and any additional effects of microplastics in agricultural systems remain largely unknow and require future study. In this chapter, we synthesized and discussed the findings and shortcomings of this thesis as well as the implications associated with our findings and their contribution to the current concerns surrounding agricultural plastic pollution.

6.2.1 Factors affecting agricultural macroplastic accumulation

Removing plastic mulching films left in fields after harvest is extremely labour intensive and thus, most farmers don't do it and recycling or reusing the films proves to be more difficult than its worth. As a result, plastic mulch films have been identified as a major source of agricultural macroplastic and microplastic pollution (Blanco et al., 2018; Hurley et al., 2020; Liu et al., 2014). To date, only a few studies have focused on agricultural macroplastic accumulation. For example, one study in the Barletta-Andria-Trani Province (Italy) found that plastic mulching produced APWs at an annual rate of 627 kg·ha^{-1} (Blanco et al., 2018). He et al. (2018) conducted a field observation in continuously mulched fields in Xinjiang (China) where they found that APW accumulated at a rate of 121.85 kg·ha^{-1} (5 years of application) to 352.38 kg·ha^{-1} (19 years of application) at a soil depth of 0-40 cm, with an annual rate of 15.69 kg ha^{-1}. Over the years, scientists believed that the accumulation of AWP was solely related to years of mulching exposure (He et al., 2009), as such, the factors

affecting the distribution and accumulation of APW have received surprisingly little attention. Notwithstanding, according to our findings, there are many factors that have been overlooked. Here, we would like to discuss some obstacles that we faced during our field observation and hopefully give you some insights.

Figure 6.2 *Macroplastics in S1 (a) and S2 (b).*

Our findings in Xinjiang (S2) showed that the mass of macroplastics were 43.5 kg·ha^{-1}, 88.9 kg·ha^{-1} 80.6 kg·ha^{-1}, 148 kg·ha^{-1} and 81.1 kg·ha^{-1} for 6, 7, 8, 15- and 18-years of continuously mulched fields, respectively. Our findings did not linearly increase with increased mulching years as reported by He et al. (2018) and Yan et al. (2008), who conducted field observations in the same region. In addition, the amounts of macroplastics found in our study were much lower than in these two previous studies. Some insights into the discrepancy might be drawn from our field observation experiences. During the field observation, famers claimed that the plastic films on the soil surface, which accounted for 70-80% of the applied amount of plastic films, would normally be removed along with the cotton stalk. Hence, the intact plastic films on the surface of soils were not recorded in our study, only plastic residues that were buried into the soil profiles were collected and weighed. In the study of He et al.

(2018), researchers collected all of the residues found in the fields, including the plastic left of the surface. Additionally, in S2, which was subjected to high-intensity machinery tillage, plastic residues were heavily fragmented into very small plastic particles (Figure 6.2b). This combined with the local strong wind climate (Xiong et al., 2019), led to the unpredictable accumulation and distribution pattern of APW (Barnes et al., 2009; Blanco et al., 2018; Lanorte et al., 2017; Vox et al., 2016).

The type of farming system used substantially affects the accumulation of macroplastics in soil. Our findings in S1, exposed to a low-intensity machinery tillage farming system, showed that intermittently mulching fields allowed farmers to perform more recycling activities that reduced the amount of buried macroplastics during crop rotation intervals (3-4 years). However, due to the intensive application of plastic mulching films, fields exposed to continuous mulching accumulated significantly more macroplastics. As a result, fields exposed to 6 to 8 years of continuous mulching accumulated more plastic residues than fields exposed to 30 years of intermittent mulching. Different crop rotations also showed significant effects on the accumulation of macroplastics since different cropping procedures can affect the timing of plastic recycling. We observed that plastic residues recycled in spring were more fragmented than those collected in autumn. This is due to the fact that plastic residues recycled in spring went through “freeze and thaw” cycles, and were subjected to longer UV-solar radiation and natural weathering, all of which can accelerate the physical degradation of LDPE (Briassoulis et al., 2004; Kamal and Huang, 1992; Kasirajan and Ngouajio, 2012). Our results showed that over time, the small differences in farming activities among the farmers, such as ploughing date and crop rotations, can lead to various distribution and accumulation patterns of macroplastics. However, without a systematic monitoring system, these small differences can be easily overlooked. Considering the growing concerns surrounding agricultural plastic pollution, it is imperative to establish a more comprehensive monitoring framework.

Machinery tillage intensity also significantly affected the accumulation and distribution of agricultural macroplastics. Comparing the two study regions, plastic residues in Xinjiang (S2) were much more difficult to collect than those in Gansu (S1). The size of macroplastics in S1 were relatively bigger and the loose soil structure facilitated the plastic residue collection (Figure 6.2). In S2, the high intensity machinery tillage not only heavily fragmented the plastic mulching films and generated very small plastic particles, but it was also responsible for the compact soil structure (Figure 6.2). As a result, the plastic particles were very difficult to collect. According to our results, macroplastics measuring 0.25-2 cm^2 accounted for more than 40% of the total collected particles in S2, while in S1, only 3.55% - 4.20% of the plastics fell within this range. Climate should also be considered when studying plastic accumulation. Although S1 and S2 were both located in a temperate continental climate

zone, the climate in the two regions were not exactly same. During the field observation, we tried to minimize the effects caused by climate. To do this, only plastic particles that were mixed into soil profiles were collected and we assumed that the mixing procedure responsible for the plastic distribution in the soil profile mainly depended on the farming management system. Natural factors such as solar radiation intensity, storms, precipitation or any combination of these play an important role in LDPE degradation (Briassoulis et al., 2004; Briassoulis et al., 2015; Briassoulis et al., 2013a). However, the effects of the natural factors on accumulation and distribution patterns of macroplastics have long been ignored. Therefore, for future research, it will be important to identify the contributions that climate makes to the accumulation and distribution of macroplastics.

Last but not least, the mobility of macroplastics through the soil profiles should also be considered in any future study. During the field observation, even though farmers claimed that the ploughing depth for both regions was not deeper than 30 cm, there were still macroplastics observed deeper than the first 30 cm of soil. He et al. (2018) examined the first 0-40 cm of soil in the same region as our S2 site and their results showed that plastic particles that accumulated in the 35-40 cm soil layer had a mass < 10 mg. This finding inspired us to examine the possible mechanisms responsible for the downward mobility of macroplastics after entering the soil. Several factors might influence this mobility: the size and shape of the macroplastic, the disturbances caused by machinery tillage and rotation, precipitation, and soil microflow, or even the activity of soil microorganisms. Unfortunately, any questions remained unanswered since most studies, including ours, were mainly only focused on the accumulation of plastic residues.

Overall, the findings of Chapter 2 supported our scientific hypothesis that the farming system affects the pattern of agricultural plastic accumulation in soil. However, the findings of Chapter 2 also brought to light the need for a long-term monitoring system that can keep track of the fate of agricultural plastic residues in order to fill in the gaps in the plastic cycle.

6.2.2 Extraction and identification of microplastics from soil samples: a challenge

When our field observation began, studies on microplastic extractions were scarce and remained largely unexplored. We now realized that there were many aspects of microplastic extraction that could be improved in relation to soil sampling, microplastic extraction and identification. During the field observation, soil samples were stored in a polypropylene bag. Even though the material was very sturdy and not prone to disintegrate

during the short period of time that it took to transfer the samples from the field to the laboratory, the results in our study could have been influence by the bag during collection and transport. We applied the method that was developed by Zhang et al. (2018), but we faced many challenges when trying to get our work published. We feel that it is important to discuss these challenges.

First, fellow researchers questioned why we followed the method described in Zhang et al. (2018) instead of another well-known method that was developed for sediments (Masura et al., 2015). Although soil has similar properties to sediments, still, the extraction method used for sediments cannot be directly applied to the soil matrix. The second question that we faced concerned our plastic identification methods using a heating plate and glass slides. We placed the glass slides on the electric heating plate (130 °C) for 7 s rather than 3-4 s as Zhang et al., (2018) stated. The heating time was extended because some of the aged microplastics were resistant to melting within 5 s, which might be attributed to the changes of molecular structure of LDPE films during field exposure. It is reported that the aging of LDPE is usually coupled with an increase in carbonyl groups and crystallinity, resulting in a higher melting point (Briassoulis et al., 2004). Thirdly, using distilled water as a reagent also limited the extraction results, since only particles with < 1 $g \cdot cm^{-3}$ were able to be extracted. Nowadays, the preferred extraction solution is usually zinc chloride (ρ = 1.55 $g \cdot cm^{-3}$) or sodium iodide (ρ = 1.70 $g \cdot cm^{-3}$) (Corradini et al., 2019b; Van den Berg et al., 2020), which allows more particles to be extracted. Quantification of microplastics in the soil matrix has experienced considerable advancements since 2017. Machines such as visible to near infrared (vis-NIR) spectroradiometers and Fourier-transform infrared spectroscopy (FTIR) have been increasingly used along with a machine-learning algorithm, making considerable contributions to the identification of microplastics (Corradini et al., 2019a; Paul et al., 2019; Tofa et al., 2019). Using an apparatus like FTIR also pushed the boundary of the classification of microplastics since the characteristic peaks of certain types of plastic could be labelled. With these innovative microplastic extraction and identification methods, mounting evidence of the microplastics that have accumulated in agricultural soils has been revealed. For example, Van den Berg et al. (2020) found that a Spanish agricultural field that received sewage sludge irrigation contained a light density plastic load of 18,000 ± 15,940 $microplastics \cdot kg^{-1}$ soil and a heavy density plastic load of 32,070 ± 19,080 $microplastics \cdot kg^{-1}$ soil. Another study in the coastal plain of Hangzhou Bay in East China measured microplastics ranging from 263 $pieces \cdot kg^{-1}$ (non-mulching fields) to 571 $pieces \cdot kg^{-1}$ (mulching fields), most of the particle sizes ranged from 1 to 3 mm (Zhou et al., 2020).

Looking back, there are many aspects of the method developed by Zhang et al. (2018) can be improved. However, at the time, this method enabled us to verify our study hypothesis which posed a question concerning the existence of microplastics originating from

agricultural plastic mulching film. By using this extraction method, Chapter 2 has made valuable contributions to understanding the fate of agricultural microplastics in the plastic cycle. To date, although many efforts have been made to quantify microplastics in soil, it remains a great challenge. To overcome this challenge, transdisciplinary collaboration is urgently needed to establish systematic quantification and classification methods for microplastics in soil.

6.2.3 Effects of LDPE and biodegradable microplastics on the soil-plant system

Since 2018, mounting evidence has shown that along with plastic mulching, microplastics can be introduced to agricultural fields via multiple sources such as waste water irrigation, wind erosion, compost, etc. (Beriot et al., 2021; Huang et al., 2020; Li et al., 2020d; Piehl et al., 2018; Zhou et al., 2020). These findings further emphasize the immediate need to investigate the effects of microplastics on soil-plant systems. Based on existing findings, we conducted an outdoor net-house pot experiment in 2019. As we were conducting our pot experiments, biodegradable alternatives for conventional LDPE mulching films began to receive more and more attention (Guerrini et al., 2017; Yan et al., 2016). However, Whitacre (2014) proposed that current biodegradable materials available on the market were more prone to break down into smaller particles than actually biodegrade, which in turn would generate more bio-microplastics. The toxicity and ecological effects of these bio-microplastics in soil-plant systems remained unclear (Palsikowski et al., 2017b; Sintim and Flury, 2017). As a result, we decided to incorporate these biodegradable microplastics into our study and thus, two types of microplastics were examined. LDPE was selected due to its wide use in plastic mulching films. In terms of biodegradable materials, a biodegradable film that was based on polylactic acid (PLA) mixed with polybutylene adipate terephthalate (PBAT) was chosen from a list of various candidates. Both the LDPE-MPs and Bio-MPs chosen for this study contained no additives and plasticizers.

By referencing several previous findings (de Souza Machado et al., 2019; de Souza Machado et al., 2018b; Qi et al., 2018), a gradient of microplastic concentration doses were chosen: 0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/w (ratio of weight of microplastics to dry soil). Admittedly, some of these doses were much higher than the environmentally relevant concentrations of microplastics. However, considering the ever-increasing additions of microplastics to soil, these doses were deemed appropriate to determine the potential environmental threshold, as well as to depict the effects that triggered by the microplastics

in soil-plant systems. In order to simulate the heterogeneity of agricultural microplastics, we choose the microplastics in relative bigger size range of 250 mm -1000 mm.

According to the findings of chapters 3 to 5, our thesis provided experimental evidence that microplastics could influence biochemical processes and plant growth in the soil-plant system and that Bio-MPs exerted stronger effects than LDPE-MPs. Similar findings have also been reported in other studies. For example, a pot experiment by Wang et al. (2020a) showed that 10% PE exerted no noticeable phytotoxicity and 10% PLA decreased maize biomass and chlorophyll content in leaves. Similarly, another study by Qi et al. (2018) also showed that exposure to starch-based microplastics resulted in significantly lower shoot and root biomass in wheat, while LDPE-MPs did not. A subsequent publication by the same team indicated that the stronger negative effects could have been attributed to the fact that the starch-based microplastics substantially changed the rhizosphere microbial communities and also stimulated rhizosphere bacteria to released higher quantities of volatile compounds like dodecanal, which is harmful to plant growth (Qi et al., 2020c). Another study by Zhou et al. (2021) concluded that carbon derived from a type of Bio-MPs, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), can enhance microbial biomass growth and intensify soil nitrogen limitation. The effects of biodegradable microplastics on microbial communities have also been reported in other systems. Seeley et al. (2020) conducted a sedimentary incubation experiment which showed that PLA-based microplastics promote sedimentary nitrification and denitrification processes and lead to lower availability of N-NH_4^+ and N-NO_3^- in surface water.

So, you've gotten this far, and you may be wondering that if LDPE-MPs have no significant effects on plant growth and soil biochemical processes, do we still need to worry about microplastic pollution? The answer is yes. Indeed, the LDPE-MPs used in this thesis did not elicit significant responses in the soil-plant system. However, microplastics have existed since plastic was invented and plastic accumulation in the environment is unpredictable. Rillig et al. (2021) suggested that the target experimental concentrations should be higher than current reported levels. For example, a study from Liu et al. (2017) indicated that 28% of polypropylene (PP) microplastics can stimulate soil enzymatic activity, thus enhancing the accumulation of dissolved organic C and N while suppressing the accumulation of available nitrogen (N-NH_4^+ and N-NO_3^-). However, these positive effects are not necessarily desirable. Rillig et al. (2021) stated that "It merely means that increases in certain performance parameters can occur, but that these still represent deviations from the natural state." Another concern surrounding microplastic pollution in soils is the release of additives into the environment. In the current experiment, the LDPE-MPs and Bio-MPs applied contained no additives. However, in today's market, various additives are often mixed into plastic materials to improve their performance and prolong useful life during

manufacture (Hurley et al., 2020; Yang et al., 2020). For example, plasticizers including phthalic esters (PAEs), dipentyl phthalate (DPP), and Di-(2-ethylhexyl) adipate (DEHA) can improve the flexibility and durability of polymeric films; antioxidants including arylamines, phenolics and organophosphates are used to improve oxidation resistance (Hahladakis et al., 2018). Once released into soil, these additives can pose serious threats to soil biological processes (Steinmetz et al., 2016; Wang et al., 2016). In addition, owing to the small size and big specific area, microplastic surfaces can provide a unique niche for soil microbes. Microbes attaching to microplastic surfaces form "plastispheres" (Zettler et al., 2013). Many studies have verified that this "plastisphere" can alter soil microbial community structure and function, in turn affecting soil biogeochemical properties (Jiang et al., 2018; Xie et al., 2021; Zhou et al., 2021). Agricultural microplastics are found in many different types and sizes with many different additives (Naji et al., 2017; Shruti and Kutralam-Muniasamy, 2019; Wang et al., 2020b). These characteristics of microplastics are likely to affect soil ecological function via various mechanisms (Qi et al., 2020a). As such, the current available data about microplastics in soil-plant systems are only the tip of the iceberg, further studies looking at the effects of microplastics on terrestrial ecosystems still require enormous efforts.

It should be noted that due to the current knowledge gaps surrounding microplastics in soil-plant systems, the findings of this current thesis and the other publications mentioned should be considered species-specific effects. For example, even though in our results, as well as many other results, LDPE-MPs showed insignificant effects on plant growth and soil physiobiological processes, it doesn't mean that it poses no ecological risks to soil-plant systems. Agricultural soil is a complex organo-mineral matrix and receives large amounts of heavy metal pollution, pesticides, herbicides, and other toxic chemicals in various ways. After entering soils, these pollutants can be absorbed to microplastics and transferred to broader ecosystems, which may have far reaching effects in terms of crop production and food safety (Khalid et al., 2020; Teuten et al., 2009; Torres et al., 2021). To date, there is still very limited data available, more comprehensive investigations concerning the size and shape of microplastics and microplastic interactions with soil pollutants are needed to effectively evaluate the effects of microplastics on soils. To summarize, microplastic pollution is a multifaced threat and requires joint efforts from all disciplines to solve this problem.

6.3 Implications and recommendations

Many different elements of plasticulture, including plastic mulching and green house plastics as well as low and high tunnels, have brought numerous benefits to modern agriculture. With the increasing food demand, it is nearly inevitable that the use of plastic

materials in agricultural sectors will continue increasing. However, for every plus, there is a minus. The escalating plastic pollution caused by plasticulture has drawn global attention (Huang et al., 2020; Muise et al., 2016; Steinmetz et al., 2016). Recycling agricultural plastic mulch films has long been commonplace (Clarke, 1996; Levitan and Barros, 2003). However, farmers' attitudes towards the recovery and recycling of agricultural plastic wastes are now in limbo. On the one hand, farmers are willing to reduce agricultural plastic waste. On the other hand, the recycling and recovery of plastic residues does not generate economic profit and requires significant time and labour from farmers. In all practicality, agricultural plastic particles smaller than 10 cm^2 and the particles that are buried in the soil profiles will never be fully removed. Based on our field data and our interviews with farmers, we believe that a dedicated recycling program which can provide service and support to farmers and help them control agricultural plastic waste as well as facilitate more sustainable agricultural plastic management is urgently needed. Such a program should not only focus on the post-consumption of plastics, but also take into consideration other factors such as crop rotation and ploughing intensity during crop growth and mulching (the factors that were discussed in Chapter 2). In addition, the program should require input from policy makers and planners to develop a stronger regulation system that would protect agricultural farmland from being exposed to plastic film residues.

There have been great expectations placed on biodegradable materials to solve the plastic pollution problem by serving as promising alternatives that will provide an end-of-life option for agricultural plastic films. Unfortunately, the full degradation of these biodegradable films has only been accomplished under laboratory conditions. Under in-situ conditions, these biodegradable materials tend to breakdown rather than actually degrade, which can create more microplastics (de Souza Machado et al., 2018a; Li et al., 2014). Scientists have expressed their concerns about the effects of biodegradable plastic residues on soil ecosystems (Serrano-Ruiz et al., 2021; Sintim and Flury, 2017). The data from this thesis also confirmed that the worries caused by the use biodegradable materials are justified. Current knowledge about the ecological effects of biodegradable material on soils is scarce and remain largely unexplored. Hence, thorough investigations looking at the impacts of these biodegradable materials on agricultural environmental safety are needed before there is a universal adoption of these biodegradable materials for the global commercial market. Along with this, strict regulations on the products already on the market are needed. Recently, Pro-oxidant Additive Containing plastic materials that contain oxo and photo additives have been excluded from the list of biodegradable materials (Siwek et al., 2019), however, there are still many companies that have launched these products on the market and labelled them as "Biodegradable materials". Customers and stakeholders may release these products into the environment believing that the products are completely biodegradable. Knowing this, governments are obligated to establish a stronger

supervisory mechanism that would bring credible products to the market, thereby strengthening the protection of agricultural soils from the threats posed by non-fully biodegradable materials (Brodhagen et al., 2017).

The diverse consumption of plastic materials has led to various microplastic streams, along with agricultural microplastics, the microplastics found in terrestrial ecosystems could come from personal care products, laundry machines, tire disintegration, or the textile factory as primary and/or secondary microplastics (section 1.3) (Torres et al., 2021). From 2015, global efforts have been made to reduce plastic pollution through a variety of strategies, i.e., in order to ban the "upstream sources", United States was the first country that banned microplastics from rinse-off cosmetic products in 2015 (Kentin, 2018). The European Commission also initiated a restriction on microplastics in cosmetic products in January 2018 (Kentin and Kaarto, 2018). In 2019, many nations including China, Zimbabwe, Kenya, UK, US and EU pledged to reduce or ban single-use plastics by 2030 (Godfrey, 2019). Nowadays, many markets are embracing biodegradable materials since people assume that the use of these materials will help reduce plastic pollution. There are other alternatives for conventional plastic mulching such as straw mulching, which also contributes to soil water conservation, decreases runoff and erosion and increases crop production (Akhtar et al., 2019; Keesstra et al., 2019; Yu et al., 2018a). Moreover, considering that water shortage in arid and semi-arid regions is one of the key factors that affect agricultural plastic mulching use, a proper agricultural restructuring relating to cropping systems and water resource redistribution might also contribute to reducing agricultural plastic wastes and increasing sustainable agricultural development (Davis, 2006; Hanisch, 2015; Yu et al., 2018b). In conclusion, mitigating plastic pollution requires not only policy restrictions, but also requires interdisciplinary efforts from agricultural economists, soil scientists and policymakers. Citizens and consumers need to take responsibility for the reuse, recovery, and recycling of plastic materials wherever possible in order to attempt to reduce the amount of plastic currently used worldwide.

6.4 Research challenges and outlook

This PhD work is expected to provide more understandings of current microplastic pollution in terrestrial ecosystems. However, as research moved along and we began to learn more, more questions arose. Here are some of these questions listed as suggestions for future research to further reveal the mysteries of "plasticulture", namely:

- To thoroughly investigate the factors that affect the accumulation and distribution of agricultural plastic mulch residues. A long-term monitoring framework

concerning farming systems (i.e., mechanical tillage intensity, ploughing strategy, crop rotation, fertilization, irrigation) and climate information (i.e., solar radiation intensity, annual temperature) should be established. This would facilitate a "track and trace" system for agricultural plastic residues and future agricultural plastic management.

- To develop a clearer definition for the classification of plastic particles. Current definitions of microplastics were derived from the definitions used for marine systems, which is not always applicable to terrestrial systems. Future definitions should clearly classify macro, meso, micro and nanoplastics in terrestrial ecosystems. With clear definitions, scientists can study the evolution process of macro to nano-sized agricultural plastic waste more thoroughly.

- To improve the statistical unit used for agricultural plastic residues. For example, during the field observation, we found that macroplastic particles measuring 0.25-2 cm^2 accounted for more than 40% of the total collected particles, however, plastics from this size category accounted for less than 10% of the total mass. Small particles may pose greater threats than bigger particles. Therefore, a unit defined as the mass of macroplastic per kilogram soil ($g \cdot kg^{-1}$) or per hectare soil ($g \cdot ha^{-1}$) is not suitable for describing particles and might lead scientists to overlook their potential threats.

- To establish a systematic quantification method for agricultural macroplastic and microplastic sampling. This includes a standardized sampling strategy (tools, instrument, sampling sizes, etc.), extraction method, microplastic particle recognition and quantification model.

- To establish an effective assessment system for evaluating the safety of biodegradable materials in agricultural soils. Biodegradable plastics are more likely to degrade into microplastics. The interactions between biodegradable materials and the soil microbial community, soil organic pollutants, heavy metals and soil animals should be rigorously studied to ensure the environmental safety of biodegradable plastics.

- To study the interactive effects of microplastics and different soil pollutants on soil microbial communities and plant growth. As we discussed in the "General discussion", soils receive large amounts of agrochemicals that could be adsorbed to microplastic particles, yet little information is available and wider studies are required.

- To study the desorption of various plastic additives in soil-plant systems and their effects on soil microbial communities, as well as the dynamics of microbial communities in “microplastispheres”.

- To find alternative sustainable farming systems for “plasticulture” in semi-arid regions.

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English summary

In recent years, scientists have become increasingly concerned about microplastic pollution. Efforts to raise awareness about microplastic pollution were initiated by marine and aquatic scientists who saw increasing amounts of plastic polluting surface water. However, recently studies reveal that continental environments have received 4-23 times the amount of plastic residues than the oceans. Some scientists estimate that agricultural soil alone may store more microplastic than all the world's oceans put together. After entering soil, microplastics can affect soil physical, chemical and biological processes and be ingested by soil organisms. Plastic debris originating from mulching films has made a significant contribution to agricultural microplastic pollution. Just how much of a contribution remains unclear since comprehensive data about the accumulation of plastic residues and the occurrence of microplastics in agricultural fields are missing. The effects of LDPE and biodegradable microplastics on soil-plant systems have scarcely been addressed. Biodegradable plastic films were designed to be tilled into the soil and be biodegraded into water and carbon dioxide by soil microorganisms. However, current biodegradable materials have only been tested under laboratory conditions. Full biodegradation under in-situ conditions have rarely been observed. Instead, biodegradable materials tend to break down into smaller bio-plastic fragments and microplastics rather than fully biodegrade. Worryingly, the ecological effects of LDPE and biodegradable microplastics in soil-plant systems remains largely unknown. For this reason, this PhD thesis aims to fill some knowledge gaps concerning the effects of plastic mulching and microplastic pollution on terrestrial ecosystems. To achieve this, a field observation and a net house mesocosm experiment were carried out to investigate the growing concerns surrounding agricultural plastic waste as well as the effects of microplastics on soil-plant systems.

In **Chapter 2**, we share the results of a field observation conducted in September 2017 in North western China. We selected two regions with a long history of plastic mulching use but with two different farming systems. The first study region (S1) was characterized by small scale farmlands, crop rotation (3 harvests every 2 years) and low intensity machinery use. The second study region (S2) was characterized by large scale farmlands, single yearly harvests and high intensity machinery use. Agricultural plastic wastes were collected from the top 0-30 cm of soil. Our results indicated that in S1, fields with continuous application of plastic mulching accumulated significantly higher amounts of macroplastics than fields with intermittent application of plastic mulching. Different crop rotations affected the field preparation time, thus affecting the number of macroplastics found. From the total amount of macroplastics that were collected, macroplastics in the 10–50 cm^2 size category

accounted for over 40% of the collected macroplastics, followed by macroplastics from 2–10 cm^2 which accounted for over 30%. In S2, we found that the accumulation of macroplastics did not increase with increasing years of mulching use. The collected macroplastics were mainly from the size category 0.25–2 cm^2 (40.6%) and 2–10 cm^2 (41.1%). Comparing the two study regions, macroplastics were more fragmented in S2 than in S1. This result was in line with the occurrence of microplastics in the two study regions. In S1, microplastics were only detected in fields that were exposed to more than 30 years of mulching use, while in S2, microplastics were detected in all the selected fields. The study emphasized that farming systems play important roles in the accumulation of macroplastics and the transformation of macroplastics to microplastics.

After identifying the occurrence of LDPE microplastics in soil, we focused on the effects of microplastics in the soil-plant system. In the meantime, using biodegradable material as an alternative to traditional LDPE mulching film has drawn growing attention. Considering biodegradable materials tend to generate bio-microplastics in field conditions, but the toxicity and ecological effects of these bio-microplastics on soil-plant systems during the degradation process remains unclear. Hence, we also incorporated biodegradable microplastics into our study. In 2019, an outside net-house pot experiment was conducted for 105 days to examine the effects of microplastics on soil-plant systems. Common bean *(Phaseolus vulgaris L.; P. vulgaris)* was selected as the model plant. Two types of microplastics were used in this pot experiment: LDPE microplastics (LDPE-MPs) and biodegradable microplastics (Bio-MPs) of polybutylene adipate terephthalate mixed with polylactic acid (PBAT+PLA). The MPs used in this experiment measured 250-500μm and 500-1000 μm (weight ratio 3:2). Microplastics were mixed into soil at doses of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/w (weight of microplastic to dry soil).

In **Chapter 3,** a suite of proxies on crop growth were measured on the 46th day of growth (at the end of the vegetative stage) and the 105th day of growth (after harvest). The results showed that LDPE-MPs exerted no significant effects on shoot and root biomass as compared to the control treatment (no microplastics) and Bio-MPs at higher doses of 1.5%, 2.0% and 2.5% w/w significantly inhibited the root and shoot biomass. All Bio-MPs treatments and ≥ 1.0% LDPE-MPs showed significantly higher numbers of specific root nodules. Overall, Bio-MPs showed stronger effects on the growth of *P. vulgaris* than LDPE-MPs. The results indicated that the existence of microplastics in agricultural soils affect *P. vulgaris* growth. We speculate that the different responses of the shoots and roots of *P. vulgaris* to LDPE-MPs and Bio-MPs might be attributed to the different compositions of the two types of microplastics, thus affecting the different responses of soil nutrients and rhizosphere bacterial communities.

In **Chapter 4**, we examined the dynamics of total organic matter, soil labile organic matter fractions of soil dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), available nitrogen (AN) of N-NH_4^+ and N-NO_3^-, and dissolved organic nitrogen (DON) on the 46th day and the 105th day of plant growth. Overall, Bio-MPs exerted stronger effects on soil carbon and nitrogen cycling than LDPE-MPs. The SOC results indicated that Bio-MPs experienced a rapid biodegradation from the start of the experiment to the 46th day, while LDPE-MPs did not degrade during the whole growth period. Compared to the control treatment, LDPE-MPs exerted no significant effects on DOC, AN and DON. 2.0% and 2.5% treatments of Bio-MPs showed significantly higher DOC and DON (46th and 105th day), while ≥ 1.5% Bio-MPs showed significantly lower AN availability (46th day). In order to gain more insights into microplastic pollution in soil-plant systems, we further explore the effects of microplastics on the rhizosphere microbial community.

Chapter 5 examined the changes in rhizosphere microbial communities exposed to three selected microplastic doses, 0.5%, 1.0% and 2.5% w/w. The results showed that the *P. vulgaris* rhizosphere bacterial communities were strongly affected by different types of microplastics. For both types, 0.5% and 1.0% w/w showed higher diversity in rhizosphere microbial communities, while 2.5% w/w showed similar or lower diversity, as compared to the control. This implied that a higher dose (2.5% w/w) of microplastics might have exerted selective pressure on the rhizosphere microbial communities. The rhizosphere microbial communities in 1.0% and 2.5% w/w Bio-MPs were clearly separated from other treatments. The control treatment, 0.5% Bio-MPs and all the LDPE-MPs treatments were clustered together. The relative abundance of specific bacterial taxa (i.e. *Comamonadaceae Rhizobiaceae* and *Micrococcaceae*) that related to soil organic matter degradation and nutrient cycling were affected by the occurrence of microplastics and the effects were varied according to the types and doses of microplastic. The results indicated that both LDPE-MPs and Bio-MPs could exert profound effects on the rhizosphere microbial community. Although the complex interactions among the soil-microplastic-plant systems remained largely unexplored, this chapter provide evidence that the accumulation of microplastics in agricultural soils has the potential to affect soil ecological function and nutrient cycling.

To conclude, this thesis provided a “field to pot experiment” perspective of the escalating plastic pollution problem. The accumulation of macroplastics and the transformation from macro to micro size under different farming systems were studied. The pot experiment results not only showed that LDPE and biodegradable microplastics could substantially affect plant growth, soil carbon and nitrogen cycling, and soil rhizosphere microbial communities but also showed that these effects may have far reaching impacts for future crop production, soil health and food safety. Moreover, in **Chapter 6,** we discussed the

implications and outlook for future research based on our results. This thesis has made valuable contributions to the systematic assessment of the ecological impacts of microplastics on soil-plant systems. It also brings new insights that will help policymakers develop sustainable agricultural plastic management programs and carefully consider the use of biodegradable plastic films in agricultural fields.

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About the author

Fanrong Meng was born on January 9th, 1992 in Heilongjiang, China. He grew up in the small village Baofeng, where his attachment to the soil and agriculture was shaped. After graduate from Daqing Normal University (BSc) in 2013, he continued to study his master degree in Soil chemistry in Jilin Agricultural University (2013-2016). During his master, he worked on the effects of biochar on characteristics of soil humus and on soil organic matter turnover. This great experience prompted him to pursue a PhD on soil quality. At the beginning of 2017, he moved to the Netherlands and start his PhD on the soil microplastic pollution at the Wageningen University under the supervision of Prof. Violette Geissen. In 2018 he obtained the financial support from China Scholarship Council (CSC). Results of the PhD research project are presented in this thesis. Through his research Meng has gained experience in assessing the dynamics of soil carbon, nitrogen and rhizosphere community under microplastic pollution. Besides his work, Meng prefer to stay at home, or only travel with intimacy friends, meeting new people is a challenge, he also like to try different kind of cuisines, and local market is his favorite.

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Scientific Publications

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SENSE PhD Courses

- Environmental research in context (2017)
- Research in context activity: 'Youtube video: Response of common bean (Phaseolus vulgaris L.) growth to soil contaminated with microplastics' (2021)

Other PhD and Advanced MSc Courses

- Applied multivariate analysis data mining and chemometrics, Universitie de Liege (2016)
- Guide to writing scientific papers, Universitie de Liege (2016)
- Academic Writing and Presenting in English, CAAS-WUR program (2016)
- Efficient and Effective Academic Development CAAS-WUR program (2016)
- Introduction to R for statistical analysis, PE&RC and SENSE (2017)
- Basic Statistics, PE&RC and SENSE (2017)
- Linear Models, PE&RC and SENSE (2017)
- Data management Planning, Wageningen Graduate Schools (2017)
- Brain training, Wageningen Graduate Schools (2017)
- Reviewing a Scientific Paper, Wageningen Graduate Schools (2017)
- Risk Assessment, Leiden University, University of Groningen, University of Amsterdam, Wageningen University (2018)
- Meta-analysis, PE&RC and SENSE (2018)
- Soil Ecology, PE&RC (2019)
- Design of Experiments, PE&RC (2019)
- Scientific publishing, Wageningen Graduate Schools (2019)
- Root Ecology, PE&RC and University of Copenhagen (2020)

Management and Didactic Skills Training

- Member of the WIMEK PhD council (2020-2021)

Oral Presentations

- *Effect of plastic mulching on the accumulation and distribution of macro and micro plastic particles in the soil - A case study of two farming systems in North West China.* EGU2020, 5 May 2020, Online
- *Accumulation and distribution of macro and micro plastic particles in the soil under different farming system.* Netherlands Annual Ecology Meeting, 11-12 February 2020, Lunteren, The Netherlands

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

Colophon

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