Plastic mulch and pesticide residues in intensive agriculture Nicolas Bériot 2022

Plastic mulch and pesticide residues in intensive agriculture

Nicolas Bériot

Propositions

1. Plastic and pesticide residues are typical of the constrained environmental management in agriculture: solutions of today are the problems of tomorrow.
   (this thesis)
2. Refuse plastics or refuse pesticides are not necessarily the most environmentally sustainable option.
   (this thesis)
3. Farmers should not be held responsible for poor practices and environmental contamination if society demands cheap production regardless the pedoclimatic conditions.
4. The free market in capitalist systems inevitably leads to inequalities and therefore less effective freedom.
5. The majority judgment voting system exemplifies why better democracy needs better science.
6. Science is fiction supported by facts.
7. Cultural content is too often considered as entertainment only whereas it is a way of gaining a better understanding of oneself, others, and the world around.
8. The collaborative part in individual achievement is not sufficiently acknowledged.

Propositions belonging to the thesis, entitled
Plastic mulch and pesticide residues in intensive agriculture

Nicolas Bériot
Wageningen, 19 April 2022

Plastic mulch and pesticide residues in intensive agriculture

Nicolas Bériot

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This research was conducted under the auspices of the Research School for Socio-Economic and Natural Sciences of the Environment (SENSE)

Plastic mulch and pesticide residues in intensive agriculture

Nicolas Bériot

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 19 April 2022
at 1:30 p.m. in the Aula

Nicolas Bériot
Plastic mulch and pesticide residues in intensive agriculture,
202 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2022)
With references, with summaries in English, Spanish and French

ISBN: 978-94-6447-126-7
DOI: 10.18174/565338

Table of contents

Chapter 1

General introduction

Adapted from:

Beriot, N. 2020. Reducing the plastic footprint of agriculture. EIP-AGRI Focus Group Starting Paper.

1.1 Plastic and pesticide use in intensive agriculture

In 2020, the total production of primary crops reached 9.8 billion tonnes (FAOstats 2021) for an estimated world population of 7.8 billion people (FAO 2021a). This means that yearly ~1250 kg are produced per person. This is mostly possible thanks to intensive agriculture (Kopittke et al. 2019). Intensive agriculture aims at producing crops with the highest value and the lowest input of resources. The value of a crop is defined by the yield, the type and the quality of the crop. The resources include time, water, nutrient management, work load, machinery, fuel and crop protection managements (Kershen 2012). We want to focus on two specific inputs: the pesticide and the plastic use in agriculture.

1.1.1 Pesticide use in agriculture

Pesticides are chemicals used for controlling weeds (herbicides), pests and diseases, caused by fungi (fungicides), insects (insecticides) or others. Most of them are synthetic organic chemicals (Laws and Edward 2013) (e.g. Figure 1.1). In Europe nearly 500 different active substances are currently on the market, which combined with different additives, amounts to 2000 different pesticides (Silva et al. 2019). Inorganic compounds are also frequently used (e.g. Ag, Cu, SiO_2, TiO_2, ZnO, Al_2O_3) (Patinha et al. 2018). In the past decades an increasing number of pesticides have been introduced based on natural substances such as plant extracts (Cavoski et al. 2011), pheromones (Shani 1982) or also micro-organisms and viruses (Sun and Peng 2007).

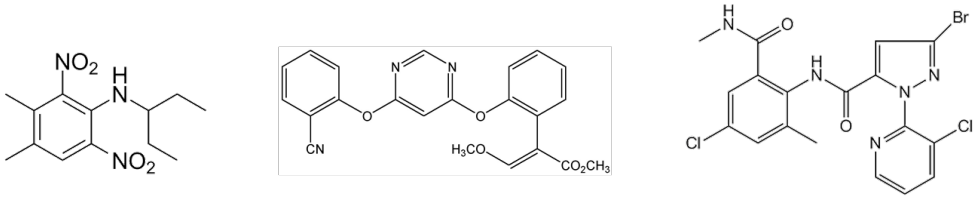


***Figure 1.1**:Chemical formulation for three examples of synthetic organic pesticides: Pendimethalin (Herbicide), Azoxystrobin (Fungicide) and Chlorantraniliprole (Insecticide).*

Commercial pesticides are composed of one or several active substances toxic for the targeted organisms (Mullin et al. 2016). They also contain additives that enhance their efficacy (*e.g.* surfactants, attractants/repellents, stabilisers), which can comprise up to 99% of the formulation, but are often not disclosed (van de Merwe et al. 2018).

The main benefit of pesticides is to improve crop productivity by decreasing plants' pests and competitors (Sharma et al. 2019). Global pesticide use reached 4.2 million tonnes and an average of 2.6 kg ha^{-1} in 2019 (FAOstats 2021). The application of pesticides depends on the recommended dose, the need of the crop and the pedoclimatic conditions (Pimentel 1996). Some pesticides are used when a pest is spotted by the farmer. Others are applied preventively. For example, pre-emergent herbicides can be applied on the bare soil before planting to prevent other plants from growing (Anthony and Isha 2020). The action of these pesticides can be extended with coating that allow a slow release in the soil (Roy et al. 2014). Some coatings can be made of plastic polymers (Mogul et al. 1996). This is only one example of plastic use in agriculture.

1.1.2 Plastic use in agriculture

What do we call plastic?

A common definition of plastic is a material which is at least partly made of an organic polymer and can be moulded into solid, non-soluble, objects (Hartmann et al. 2019). An organic polymer consists of a repetition of many monomers that contains carbon. For example, polyethylene is composed of a chain of carbons atoms whereas polylactic acid, a polymer used in biodegradable plastics, is composed of a chain of lactic acid (Figure 1.2).

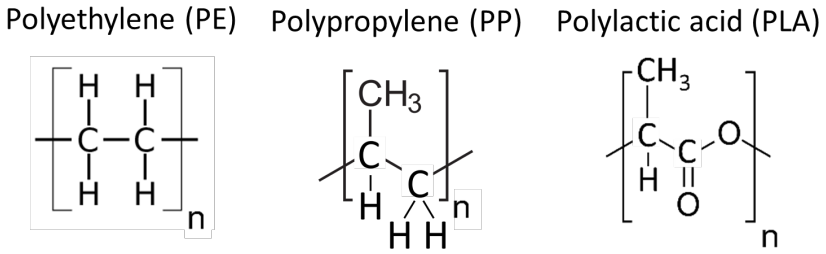


***Figure 1.2**: Chemical structure of common polymers used in agriculture: polyethylene (PE), polypropylene (PP) and polylactic acid (PLA).*

Most conventional plastics are petroleum-based, meaning that they are made from fossil resources such as natural gas, oil or coal. In Europe plastic production accounts for 4% to 6% of all the oil and gas used and agricultural plastics represents about 4% of the plastic production in Europe (PlasticsEurope 2020). Plastics can also be produced from crops in which case they are called bio-based. For example, sugar cane can be processed to produce ethylene, which can then be used to manufacture polyethylene (Liptow and Tillman 2012). Plastics can be made of a single polymer or a blend of several types, associated in different manners. The two main polymers used in agriculture are polyethylene (PE) and polypropylene (PP) (PlasticsEurope 2020). Additives are added to adjust the elasticity, the colour, the mechanical strength and the degradability of the plastic. Plastic chemical composition and manufacturing are tailored to fit the plastic's intended function.

Plastic use in agriculture

In agriculture, plastics are mostly used in the form of film as it constitutes a light, resistant, elastic, cheap and waterproof barrier (Figure 1.3) (FAO 2021b). Plastic mulch, greenhouse cover and bale wrapping (Figure 1.4) are the three main agricultural practices that use plastic films. Plastics provide other services: crop protective nets; irrigation pipes; ropes and twines, crates and containers for farming products; coating for controlled-release coatings. Some of these plastics are placed directly in contact with the soil. This is the case for mulches, irrigation pipes and controlled-release coatings. We will focus on plastic mulches as they are in direct contact with the soil and dominant in the market.

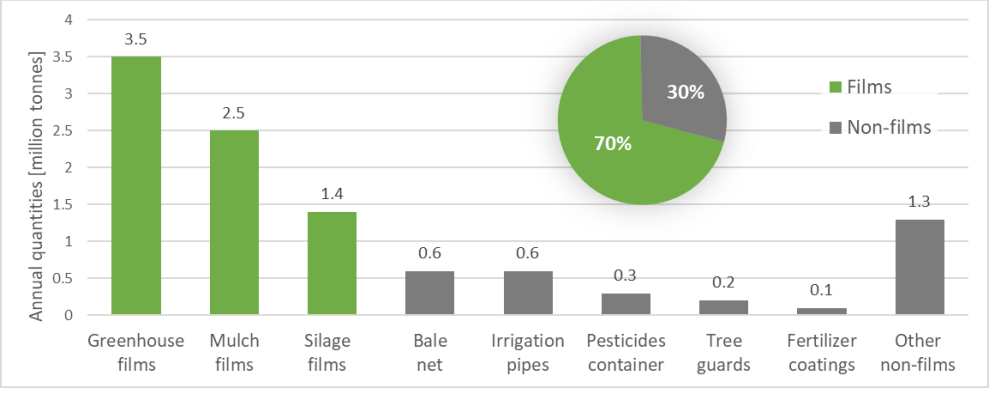


Figure 1.3*: Estimated global annual quantities of agricultural plastics for different uses add up to 10.5 million tonnes. Plastic food packaging is not included and represents an additional 37 million tonnes (Based on data from (APE-Europe 2021; FAO 2021b; Moine 2018).*

***Figure 1.4**: Bale wrapping with nets and twines (A.) and with silage film (B.) and the mainly used polymers. Adapted from (FAO 2021b).*

Plastic mulches in agriculture

An estimated 2.5 million tonnes of plastic mulch are annually used worldwide, covering about 140 000 km^2 (twice the size of the Netherlands and Belgium together) (FAO 2021b). Plastic mulch is used for one or all of the three following reasons:

- **Increasing soil temperature :** Plastic mulch was first noted for its ability to increase soil temperature in the 1950s (Kasirajan and Ngouajio 2012). Higher soil temperatures increase nutrient availability, enhance nutrient uptake by roots, increase the number and activity of soil microorganisms, speed up plant germination and growth, and can help controlling pathogens (e.g. Tuta absoluta in tomato plants (Asma and Kaouthar 2017) leading to higher and earlier yields (Jabran 2019). Therefore, plastic mulch may reduce the need for fertilization.
- **Increasing water use efficiency:** The water use efficiency is estimated by dividing the yield per ha by the total amount of water applied. Plastic mulch is a barrier that prevents water evaporation from the soil and therefore increases water availability for plants (Deng et al. 2006). Plastic mulch can also increase rainwater harvesting when associated to a ridge-furrow tillage, the ridge being mulched by plastic and plants growing in the furrows (Yang et al. 2020). An analysis of 266 studies in China, showed that plastic mulching significantly increased crop yield by 24% and water use efficiency by 28% on average (Gao et al. 2019).
- **Decreasing weed growth:** Opaque (often black) plastic mulch limits weed growth by preventing the light from reaching the soil (William James 1993). Plastic mulch can reduce weed emergence by 64% to 98% during the growth season, depending on the surface covered with plastic (Kasirajan and Ngouajio 2012). In this sense plastic mulch can contribute to reducing the use of herbicides. Nevertheless, with a clear or transparent plastic mulch, the application of a pre-emergent herbicide is needed.

Because of these benefits, plastic mulch is used under various climatic conditions, in open fields and in greenhouses, for the production of different crops, and both organic and conventional farming practices. Plastic mulch is often partially buried in the soil to prevent it from being blown away by the wind (Figure 1.5).

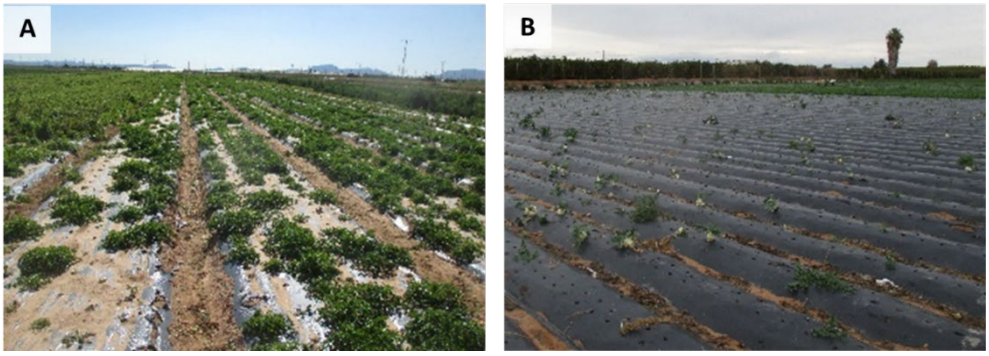


***Figure 1.5**: Fields covered with low density polyethylene (LDPE) plastic mulch before the harvest of parsnip (Panel A) and after the harvest of kohlrabi (Panel B) in Southeast Spain.*

In conclusion, we showed that plastic and pesticides are very diverse and provide many benefits to farmers. Intensive agriculture has great potential to feed the world population. However this success is not without drawbacks. Both plastic mulches and pesticides leave residues in the soil.

1.2 Plastic and pesticides, from ubiquitous use to ubiquitous contaminants in agricultural soil

1.2.1 Pesticide use and residues in soil

The persistence of pesticides in soil has been known for a long time (Carson 1962). Pesticide residue contents in soil have been documented by many studies (Sabzevari and Hofman 2022). For instance, a study of agricultural soils in Europe showed that 70% of the soils contaminated contained mixtures of residues (Vera et al. 2019). Sabzevari and Hofman (2022) analysed the data from 72 studies published in the last 50 years worldwide. They identified ubiquitous pesticides with Mevinphos, Esfenvalerate and Cyfluthrin having detection frequency of 71%, 65% and 63% worldwide. The study alarms about the high contents of pesticide residues. The maximum content reported for Diuron was 867 µg kg^{-1} which is 100 times more than its permissible concentration in soil in the Netherlands,

namely 8 µg kg^{-1} (Crommentuijn et al. 2000). Permissible concentrations are thresholds to limit the risks of detrimental effects of pesticides in the environment.
A major detrimental effect of pesticides is their toxicity for non-targeted organisms (Mahmood et al. 2016). An emblematic example is the adverse effect on beneficial insects and pollinators (Grubisic et al. 2018; Sánchez-Bayo and Wyckhuys 2019). Hallmann et al. (2017) reports the decline of 75% of the insect populations in Germany over the last 30 years and suggested pesticide use as one of the main contributing factors. Pesticides also affect the soil microbiome (Hartmann et al. 2015). Some microorganisms are negatively affected and others are able to degrade the pesticide residues (Arya et al. 2017; Kumar et al. 2018). Moreover, pesticides also contaminate water (Ochoa and Maestroni 2018) and affect aquatic organisms (Moura and Souza-Santos 2020; Spycher et al. 2018). When contaminating an organism, pesticide residues can be transferred through the food chain and affect other organisms (Baudrot et al. 2020; Kim 2020).

Some farmers decide to not use pesticides to avoid these negative consequences. They are practicing organic agriculture. Only some targeted chemicals are used under organic agriculture and their application dose and frequency are restricted (EC 2008). Farmers use other techniques such as crop diversification (Hufnagel et al. 2020), tillage (Kells and Meggitt 2018) or predatory insects (Kenis et al. 2017) to manage pests without or with fewer synthetic chemicals. The European Commission requires a conversion time of two years of organic management before certification for annual crops (EC 2008). However a field assessment of pesticide residues in organic farms suggested that two years transition time is not enough for the complete degradation of the pesticide residues present in soil (Geissen et al. 2021).

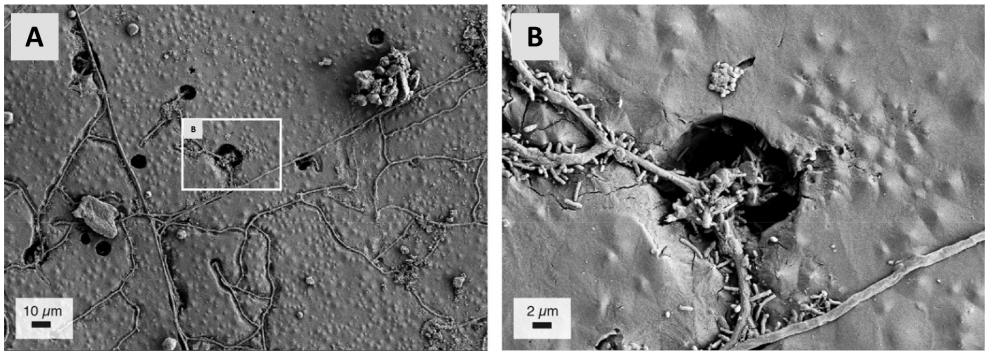
1.2.2 Plastic use and debris in soil

The general public and the scientific community first became aware of the issue of plastic debris by observing their accumulation in the oceans (Moore 1997). Since then, land has been identified as a major source and reservoir of plastic debris (Boyle and Örmeci 2020). Plastic debris is formed through the fragmentation of plastics in the environment. Plastic degradation relies on two main processes: weathering and biodegradation.

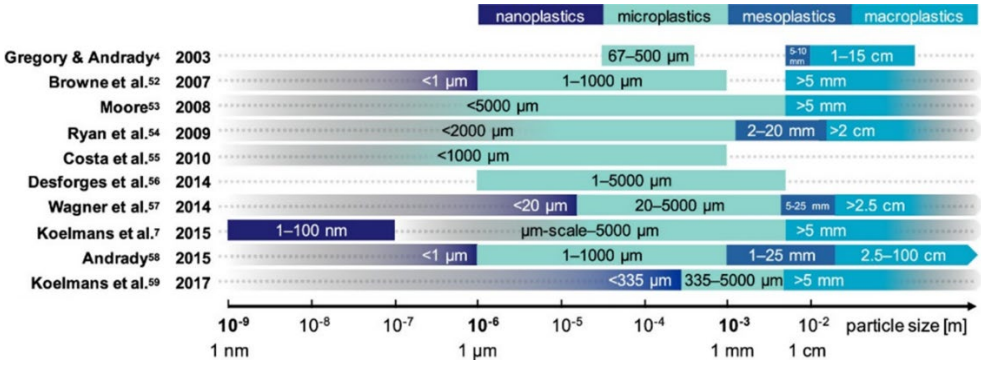
Weathering refers to abiotic reactions such as thermal degradation, photo-degradation, oxidation, hydrolysis and to mechanical degradation (e.g. wind or ploughing). Weathering plays an important role in the degradation processes, as weathered plastic will undergo faster biodegradation (Restrepo-Flórez et al. 2014). For example, photo-degradation can change the chemical structure of plastic polymers making them easier to degrade for microorganisms (Napper and Thompson 2019b).

Biodegradation of a polymer is a biological process leading to its complete or partial conversion to water, CO_2, methane and new biomass by microorganisms (mostly bacteria and fungi) (van Ginkel 2007a). The biodegradation process can be divided in three different steps (Sander 2019):

1. The organisms colonize the polymer and grow on its surface (Figure 1.6).
2. The organisms degrade the polymer. They mostly do this by secreting enzymes (e.g. hydrolases) that can depolymerise the polymer. Depolymerisation is the breaking of chemical bounds in the polymer that leads to smaller molecules. The main process of depolymerisation is the catalysis of hydrolysis with enzymes.
3. Finally, the hydrolysis products released from the polymer are used as an energy source or a carbon source for the microorganisms leading for example to emission of CO_2 or the increase of biomass.



The degradation of plastic produces smaller and smaller debris. Debris size is frequently classified into three categories: “nanoplastics < microplastics < macroplastics” (Hartmann et al. 2019). However the size of plastic debris is, in fact, a continuous variable and no defined boundaries exist for these categories (Figure 1.7). Most often the boundary between microplastics and macroplastics will be based on the identification method used, and so varies from one study to the other from 500 µm to 5 mm.



Plastic debris can also be classified based on their origin. As plastics have many functions, plastic debris have many different origins. For agricultural sources we can distinguish direct sources: the plastic is used for the beneficial action it is expected to offer; and indirect sources: the plastic is transported to the field without expecting a benefit from it.

Additionally, when debris is small, it is interesting to know if it was manufactured like this or if it was degraded. Therefore we distinguish primary microplastic from secondary microplastic which are particles coming from the fragmentation of bigger debris. Secondary microplastic have gone through degradation, which has modified their size, shape, physical and/or chemical properties.

Examples of direct sources of plastic in agriculture are plastic mulch, irrigation pipes and plastic coatings (Table 1.1). The degradation of plastic mulch and irrigation pipes into smaller debris is a direct source of secondary microplastics (Figure 1.8). The use of fertilizers coated in plastic is a direct source of primary microplastic (Heuchan et al. 2019). Organic fertilizers such as sewage sludge (Corradini et al. 2019c) and compost (Gui et al. 2021) are examples of indirect sources of microplastics. Indeed sewage sludge can contain primary microplastics from cosmetics and compost can contain secondary microplastics from the fragmentation of a wrongly sorted plastic item. When they are applied to the field for fertilization, contaminated sewage sludge and compost bring with them microplastics that are of no use to the farmers.

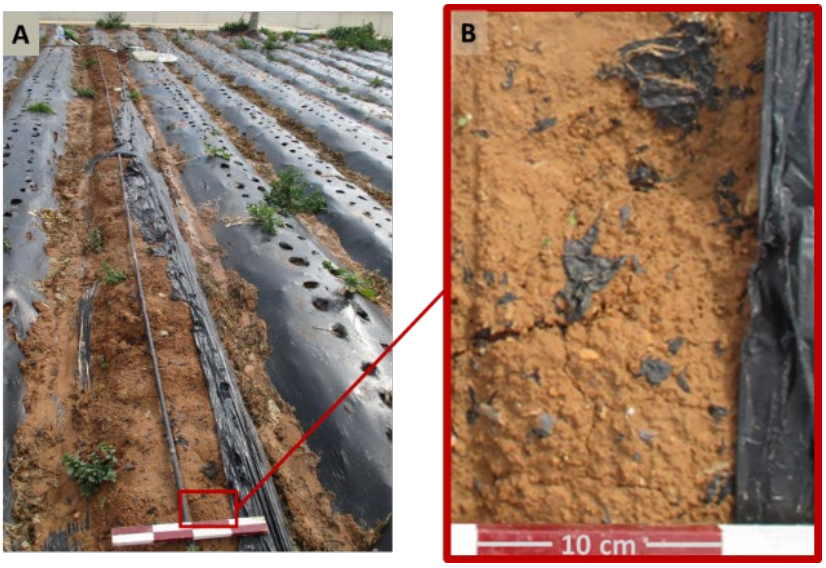


***Figure 1.8**: Light density polyethylene plastic mulch after harvest of Kohlrabi in Southeast Spain. The plastic mulch covers an irrigation pipe to improve the water use efficiency (Panel A). The sides of the mulch film are buried into the soil making complete removal impossible and leading to debris accumulation (Panel B).*

***Table 1.1**: Examples of direct and indirect sources of primary and secondary microplastics in agriculture.*

		Degradation stage of the plastic	
		Primary microplastics	**Secondary microplastics**
Origin of the plastic	**Direct source**	Microplastic coating for slow release fertilizers	Degradation of agricultural plastics in the soil, such as, twines, plastic mulch, irrigation pipes
	Indirect source	Microplastic from personal care products in sewage sludge	Tire fragment in sewage sludge, wrongly sorted plastic packaging in compost, oversight contamination

The occurrence of plastic in agricultural soils has been described in many studies (Hurley et al. 2020). For instance, it has been found in soils after plastic mulching (Meng et al 2021a), compost (van Schothorst 2021) and sewage sludge application (van den Berg et al. 2020). In China, the extensive use of low density polyethylene (LDPE) plastic mulch lead to worrying levels of residues (Liu et al. 2014a). Detrimental effects of plastic residues have been observed when the contents in soil exceeds 270 kg ha^{-1} (Gao et al. 2019). Zhang et al. (2020) estimated a 3% drop in crop yield for every additional 100 kg ha^{-1} of film residue in soil.

LDPE is the most used polymer for plastic mulches. LDPE plastic mulch has to be removed from the fields after the harvest and incomplete removal leads to plastic debris accumulation (Zacharias Steinmetz et al. 2016b). Some plastic producers have tried to improve the degradation processes of plastic mulch by adding pro-oxidant additives to LDPE in order to avoid the need for plastic mulch removal and thus, the accumulation of plastic debris (Selke et al. 2015). With these aims in mind, Pro-oxidant Additive Containing (PAC) plastic, also called “oxo-degradable” or “oxo-biodegradable”, was developed but it has ultimately proven to be poorly degraded in soils (Hogg 2016) leading to the ban of oxo-degradable plastic in June 2019 by the European Parliament (EP 2019). Another attempt to solve the problem of plastic debris accumulation from mulch has resulted in the production of biodegradable (BIO) plastic polymers. Biodegradable mulch can be made from a diversity of biobased or petroleum-based polymers or a blend of both (Sintim and Flury 2017). Biodegradable mulches are expected to degrade into water, CO_2, methane, energy and new biomass in soil with the help of microorganisms (Carol et al. 2017). These mulches were designed such that they should be degraded by 90% after two years in the soil (Standard ISO 16929). However, studies have found that biodegradable plastic mulch does not degrade as fast as expected under some field conditions and leaves residues in the soil after more than two years (ACBD 2020; Sintim et al. 2020). Beside the potential incomplete degradation, BIO plastic mulch also presents a threat to plant growth. Two studies have found a reduction of the production of wheat (Qi et al. 2018) and common beans (Meng et al. 2021b) in presence of 2% (w/w) of BIO residues added to the soil. Processes leading to the crop production decrease are not well understood yet and need more investigation.

One reason to explain the crop production decrease could be the reduction of beneficial microorganisms or the increase of plant pathogens in the presence of BIO plastics. Indeed, plastic debris can affect the soil microbiome (Lear et al. 2021) and BIO plastics have a stronger potential to do so because they are degraded by microorganisms (Sander 2019). Plastic debris could be shelters for microorganisms, with the formation of a biofilm for example (Amaral-Zettler et al. 2020). Additionally, plastics could be a source of nutrients, mostly carbon. In fact several organisms have been reported to degrade plastic (Bahl et al. 2020), even LDPE (Gajendiran et al. 2016). Finally, plastic could release chemicals that are toxic for some microorganisms. As explained previously, we have barely any information about the chemicals present in commercial plastic and they could be released due to plastic degradation in the soil (Wang et al. 2019a). In fact, we know that some additives can directly affect the soil microbiome (Kong et al. 2018).

Plastics and their additives can affect other organisms. Study of exposure of soil animals to microplastics showed that many organisms do ingest plastics. However, ecotoxicological effects at environmental concentrations are still uncertain (Chae and An 2018; Ng et al. 2018). Microplastic ingested by organisms can travel in the food chain. For example microplastic concentrations increased from soil (0.87 ± 1.9 particles g^{-1}), to earthworm casts (14.8 ± 28.8 particles g^{-1}), to chicken faeces (129.8 ± 82.3 particles g^{-1}) in home gardens in southeast Mexico (Huerta Lwanga et al. 2017b). An increase in earthworms mortality has been recorded, when exposed to 28%, 45%, and 60% of microplastics in the litter (Huerta Lwanga et al. 2016) but these plastic contents are higher than average environmental contents. Moreover, nanoplastics decreased the growth, locomotor activity, and intestinal microbiota viability of snails that were feeding on plants grown in soil with 10 mg kg^{-1} and 100 mg kg^{-1} nanoplastics added (Chae and An 2020). The effects of plastics on terrestrial organisms can also be caused by additives and other contaminants that may leak from the debris (Mai et al. 2018). Many plastic additives are suspected to be endocrine disruptors. This means that they may interfere with animal hormones and therefore impact the entire organism (Hermabessiere et al. 2017). Plastic debris is also ingested by bigger organisms (Mekuanint et al. 2017; Zhao et al. 2016) resulting in various negative effects (Prata et al. 2020). The effects include blockage of the intestinal tract, inhibition of gastric enzyme secretion, reduced feeding stimuli, decreased steroid hormone levels, delays in ovulation and even failure to reproduce (Li et al. 2016). Though it is still hard to draw definitive conclusions from the literature, early studies about plastics in soil concur with the wider base of aquatic plastic toxicology in the sense that plastics are a threat to soil biota (Helmberger et al. 2019). Toxicity of aquatic plastic debris has been better described and marine animals like sea turtles became emblems of plastic contamination in the environment (Duncan et 2019). Water and wind transportation link land and aquatic plastic contamination, making plastic debris an ubiquitous contaminant (Horton al. 2018).

1.3 Outline of this thesis

From the previous section we conclude that plastic and pesticides are widely used in agriculture for beneficial but also detrimental consequences. We showed that plastic and pesticide residues have the potential to accumulate in soil. Samples have been analysed but data about the co-occurrence of pesticides and plastics is required to evaluate the risk of combine effects on the environment. Both, plastic and pesticides are very diverse so we narrow down the investigation to synthetic organic pesticides and plastic mulch applications. Synthetic organic pesticides and plastic mulch are representative at the same time of the dominant use and the important threat to the environment. Some laboratory studies rang warning bells but more data in field conditions are required to encompass the full potential consequences. We defined six main research questions :

1. What are representative pesticide and plastic residues contents in agricultural soils?
2. Do pesticide and plastic residues alter the soil microbiome in agricultural fields?
3. What is the potential sorption of different pesticides on plastic mulches?
4. What are the effects of plastic contamination on soil properties?
5. Is plastic debris bioaccumulated in the terrestrial food chain?
6. What are the consequences of plastic and of pesticide residues for crop production?

We also understood that data relevant to field conditions is required. We selected the region of Murcia in Southeast Spain as representative to the intensive plastic mulch and pesticides application.

1.3.1 The case of vegetable intensive agriculture in Murcia, southeast Spain

Southern Spain is an area where transformative, intensive agriculture has taken off in recent decades (Caparrós-Martínez et al. 2020). For instance, in the Murcia agricultural region in southeast Spain, intensive vegetable production represents ~66% of agricultural production (Hernández and Martínez 2021). Here, intensive vegetable production benefits from warm weather and beneficial soil properties. Murcia has a mean annual temperature of 17.5˚C, mean annual precipitation of 280 mm and annual potential evapotranspiration of 1300 mm. Murcia is called “the garden of Europe” (tr. “Huerta de Europa”, EuropaPress 2017). However, the semi-arid climate makes water a limiting resource and all the vegetable production in Murcia is irrigated production (Figure 1.9) (Hernández and Martínez 2021). To improve water use efficiency, farmers frequently use plastic mulch. In fact, in Murcia, 26% of the land surface utilized for vegetable cultivation is covered with plastic mulch production (Hernández and Martínez 2021). Under conventional management, weeds are also controlled with herbicides. The intensive production is supported by the use of pesticides

in conventional farms. However, organic farms are also intensively producing vegetables with less or no use of pesticides to answer the growing European demand for organic products. Irrigation, plastic mulching and pesticide application aided in the successful production of ~1.7 $x10^6$ tonnes of vegetables on ~53 $x10^3$ ha in Murcia in 2020 production (Hernández and Martínez 2021). For example, the ~404 kt of lettuce produced in Murcia in 2020 represent ~10% of the lettuce production in the European Union (FAOstats 2021). This success story is not without drawbacks and the bill has come due. Irrigation leads to severe depletion of fresh water resources, which has long term consequences for ecology (Burgen 2021). Both plastic mulches and pesticides leave residues in the soil. Moreover, in Murcia, ~638,000 sheep were bred in 2019 for meat production (Agrarios 2019). A common practice is to bring sheep to the vegetable fields after the harvest to eat the crop residues. Few sheep farms own land and they strongly rely on crop residue grazing and fallow land grazing. Little fodder is provided. We investigated the effects of the plastic and pesticide residues on the soil microbiome, the plants and sheep grazing in the fields.

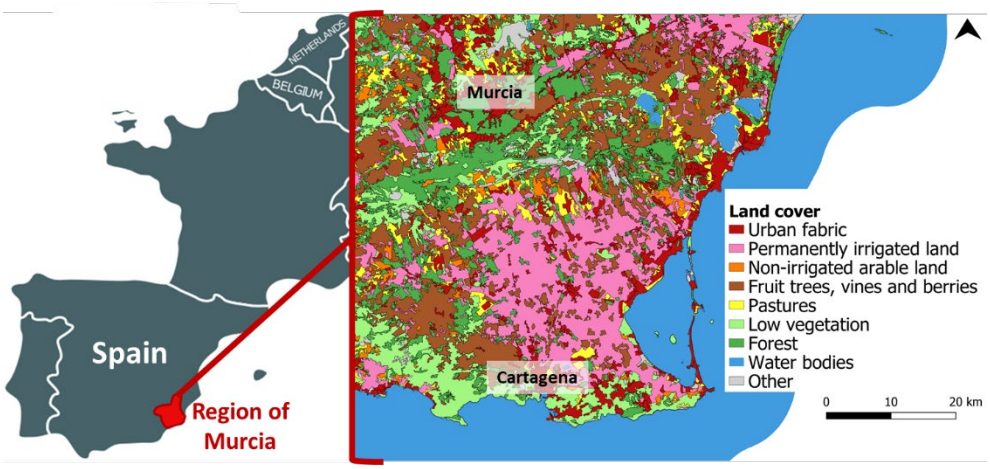


Figure 1.9 *: Land cover in the region of Murcia (2018 Corrine land Cover). The country side is dominated by permanent irrigated lands.*

1.3.2 Specific research objectives

We developed five research chapters (2,3,4,5,6) to answer the research questions within the context of intensive vegetable agriculture in Murcia (Figure 1.10).
These five research chapters are introduced and discussed together in chapter 1 and 7. More specifically:

Chapter 1 describes the issue around the use of plastic and pesticides in agriculture. We gave an overview of the current knowledge and the research gaps. We conclude by outlining the objectives of the thesis.

Chapter 2 is a field assessment of six different vegetable farms in the region of Murcia. Soils from organic and conventional farms were sampled to be representative of the diversity of pesticide and plastic mulch application. It draws a representative picture of the plastic and pesticide contamination in vegetable fields in Murcia. It also describes the effects on the soil microbiome.

Chapter 3 focuses on the potential sorption of pesticides onto plastics. We tested 38 pesticide active substances with LDPE, PAC and BIO plastic mulches in an incubation solution of 90% distilled water and 10% acetonitrile at 35 °C for 15 days in the dark.

Chapter 4 explores the effects of 0.5%, 1% and 2% (w/w) micro- and macro- debris of LDPE and BIO mulches on the soil physicochemical properties. The plastic was incubated in a sandy soil at 20°C for 30 days in a lab before analysis.

Chapter 5 is a field assessment of the ingestion of plastic debris by sheep grazing on vegetable fields. We collected sheep faeces in the field from 5 different sheep herds and analysed the light density microplastics.

Chapter 6 tests the effects of LDPE, PAC and BIO mulches residues on lettuce growth in presence of 3 pesticides. The plastic mulches were exposed to field conditions before preparing plastic debris from 50 µm to 5 mm. A sandy soil was then incubated for a year with 520 cm^2 kg^{-1} (~1g kg^{-1}) of the plastic debris, irrigation and two applications of the pesticides at the recommended dose. After a year, lettuce seedling (*Lactuca sativa*) were plantef and plant growth was assesed 3 months later.

Chapter 7 summarizes the major findings of the thesis. We discuss the results of the different chapters and compare them to the available literature. Finally, we explore solutions to avoid detrimental consequences of plastic and pesticide residues in soil.

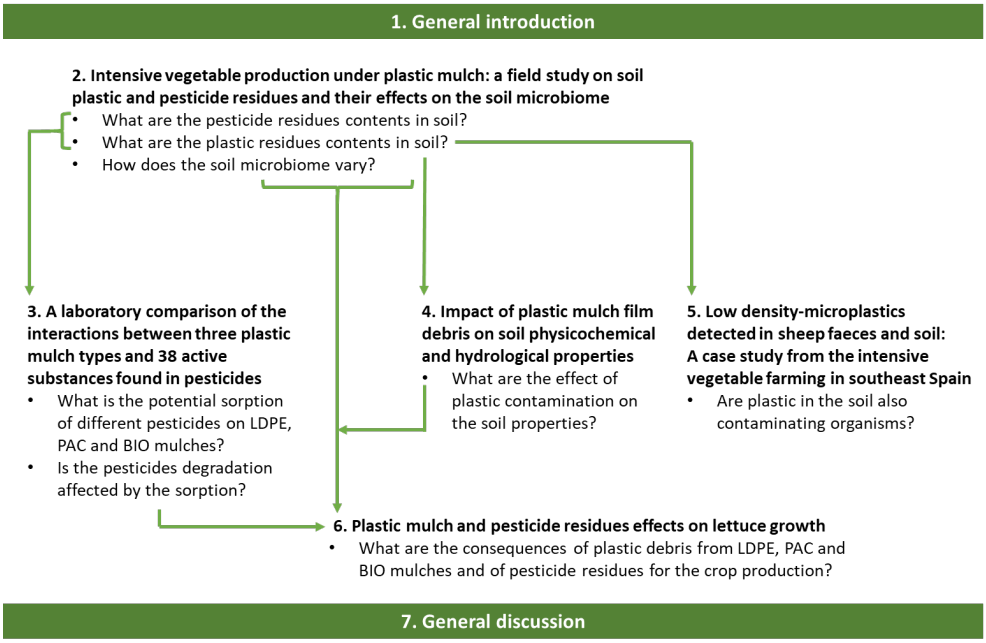


***Figure 1.10**: Schematic Outline of the thesis and main research questions answered in the chapters 2-6.*

Chapter 2

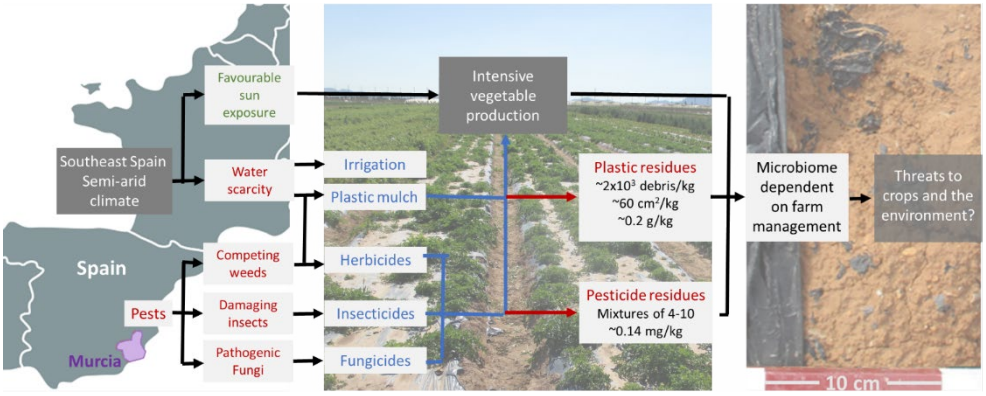
Intensive vegetable production under plastic mulch: a field study on soil plastic and pesticide residues and their effects on the soil microbiome

Based on:

Beriot, N., Zornoza, R., Huerta Lwanga, E., Zomer, P., Ozbolat, O., van Schothorst, B., Lloret, E., Ortega, R., Miralles, I., Harkes, P., van Steenbrugge, J., Geissen, V. 2022. Intensive vegetable production under plastic mulch: a field study on soil plastic and pesticide residues and their effects on the soil microbiome. Submitted to Science of the Total Environment

Abstract

Intensive agriculture relies on external inputs to reach high productivity and profitability. Plastic mulch, mainly in the form of Low-Density Polyethylene (LDPE), is widely used in agriculture to decrease evaporation, increase soil temperature and prevent weeds. The incomplete removal of LDPE mulch after use causes plastic pollution in agricultural soils. In conventional agriculture, the use of plastic mulch is combined with the use of pesticides, which also accumulate in soils. Thus, the objective of this study was to measure plastic and pesticide residues in agricultural soils and their effects on the soil microbiome. For this, we sampled soil (0-10 cm and 10-30 cm) from 18 parcels from 6 vegetable farms in SE Spain. The farms were under either organic or conventional management, where plastic mulch had been used for > 25 years. We measured the macro- and micro- light density plastic debris contents, the pesticide residue levels, and a range of physiochemical properties. We also carried out DNA sequencing on the soil fungal and bacterial communities. Plastic debris was found in all samples and 4-10 different pesticide residues were found in all conventional soils. Overall, pesticide content was ~100 times lower in organic farms, whereas no significant difference in plastic content was observed between organic and conventional farms. The measured plastic and pesticide residue contents were not correlated with the plastic mulch application records in the past 90 months nor the pesticide application records in the past 18 months. The fungal and bacterial communities were farm-specific and related to different soil physicochemical parameters and contaminants. Regarding contaminants, bacterial communities responded to the total pesticide residues, the fungicide Azoxystrobin and the insecticide Chlorantraniliprole as well as the total plastic area. The fungicide Boscalid was the only contaminant to influence the fungal community. The wide spread of plastic and pesticide residues in agricultural soil and their effects on soil microbial communities may impact crop production and other environmental services. More studies are required to evaluate the total costs of intensive agriculture.



2.1 Introduction

Intensive agriculture aims at producing crops with the highest value and the lowest input of resources. A crop's value is defined by the yield, the type and the quality of the crop. The resources include time, water, nutrients present in the soil or added, work load, machinery, fuel and crop protection managements (Kershen 2012). Although intensive agriculture has great potential to transform the lives of farmers, it is also associated with several severe drawbacks such as the dependency on mineral fertilizer and the depletion of natural resources. This kind of farming requires adequate waste management strategies to avoid environmental pollution (Egea et al. 2021) from agricultural plastic (Hurley et al. 2020) and pesticide residues (Geissen et al. 2021). In intensive agricultural systems, local circumstances play a major role in explaining the choice of certain crops and the use of resources. For instance, in arid and semiarid areas, plastic mulch is a cost-effective solution used to improve water use efficiency (Jabran 2019). Plastic mulch is applied extensively with an estimated yearly total use of 2.5 million tonnes covering about 0.14 million km^2 (more than a ¼ the size of France) (FAO 2021b).

Southern Spain is one area where transformative, intensive agriculture has taken off in recent decades (Luis Caparrós-Martínez et al. 2020). For instance, in the Murcia agricultural region in southeast Spain, intensive vegetable production represents ~66% of agricultural production (Fulgencio Pérez Hernández and Esteban Barba Martínez 2021). Here, intensive vegetable production takes advantage of warm weather and beneficial soil properties. However, the semi-arid climate makes water a limiting resource and all the vegetable production in Murcia is irrigated (Fulgencio Pérez Hernández and Esteban Barba Martínez 2021). To improve water use efficiency, farmers frequently use plastic mulch. In fact, in Murcia, 26% of the land surface utilized for vegetable cultivation is covered with plastic mulch (Fulgencio Pérez Hernández and Esteban Barba Martínez 2021). The most commonly used plastic mulch is made of Low-Density Polyethylene (LDPE), which is resistant to weathering (Crawford et al. 2017b). Apart from limiting water evaporation, plastic mulch also prevents weed growth. Under conventional management, weeds are also controlled with herbicides. Together with fungicides and insecticides, pesticides protect the crops from diseases and pests. Pesticides are composed (2% to 80%) of specific active substances (AS) mixed with other chemicals such as solvents or surfactants to improve the pesticide efficacy. Irrigation, plastic mulching and pesticide application aided in the successful production of ~1.7 $x10^6$ tonnes of vegetables on ~53 $x10^3$ ha in Murcia in 2020 (Fulgencio Pérez Hernández and Esteban Barba Martínez 2021). For example, the ~404 kt of lettuces produced in Murcia in 2020 represent ~10% of the lettuce production in the European Union (FAOstats 2021). This success story is not without drawbacks and the bill has come due. Irrigation leads to

severe depletion of fresh water resources, which has long term consequences for ecology (Burgen 2021). Both plastic mulches and pesticides leave residues in the soil.

In this study, we focused on the accumulation of plastic and pesticide residues. After being laid on the fields, plastic mulch is altered by weathering due to UV-light, heat, wind, rain, plant growth and the use of machinery. After harvest, LDPE plastic mulch needs to be manually or mechanically removed. However, the total removal of plastic mulch remains a challenge since i) part of the plastic deteriorates due to weathering and remains in the soil and ii) the edges of the mulch that are buried in the soil during crop development break off and remain in the soil during mulch removal. The resistance of the mulch will depend on the polymer properties and film thickness. For instance, Manzano et al. (2019) reported removal rates of 90% for plastic mulch thicker than 25 µm but of only 32% for LDPE mulch 20 µm thick (Manzano et al. 2019). The fragmentation of the plastic generates larger pieces of debris called macroplastics (MP) and smaller particles called microplastics (µP). We decided to use a limit of 2 mm to differentiate between MP and µP, unlike the 5 mm threshold suggested by Arthur et al. in 2008 (Arthur et al. 2009). We considered that debris above 2 mm was easily identifiable visually and could be extracted by sieving. Pieces above 2mm would also inhibit the proper identification of smaller µP under a microscope. LDPE plastic debris is expected to be ultimately degraded into CO_2 and water under aerobic conditions (Kijchavengkul et al. 2006). For example, the half-life of a 100 µm thick LDPE plastic bag buried in soil after exposure to UV and heat is estimated to range from 7 months to 32 years (Chamas et al. 2020). Pesticides also degrade in the soil but at a much faster rate than LDPE debris. In fact, the half-life of pesticides in soil ranges from less than a day for some AS, like Spirotramat ($DT50_{field}$=0.7 days), to more than 6 months for persistent AS, like Chlorantraniliprole ($DT50_{field}$=204 days) (PPDB 2019). Plastics and pesticides are inputs from the soil surface therefore, we expect to find the residues in the topsoil. Long-term accumulation would mean that deeper soil has also been contaminated by residues. Therefore, in this study, we provide an assessment of both top soil (0-10 cm) and deeper soil (10-30 cm).

The concurrent large-scale application of plastic mulch and pesticides in intensive agriculture means that these contaminants can accumulate to high concentrations, which carries consequences for the environment and provides the opportunity for these compounds to react in unexpected ways. Pesticides have been shown to have adverse effects on different taxa including beneficial insects (Sánchez-Bayo and Wyckhuys 2019), earthworms (Pelosi et al. 2021) and soil microorganisms (Wołejko et al. 2020). Plastics have been proven to not only change soil physicochemical properties and the soil microbiome, but also affect the plant community structure (Lozano and Rillig 2020), migrate to aquatic environments(Horton and Dixon 2018) and be ingested by a wide range of organisms (Guo

et al. 2020), from earthworms (Huerta Lwanga et al. 2017b) to whales (Kühn and van Franeker 2020). Plastics and pesticides can also become sorbed together (Wang et al. 2020b) which could lead to an increased transport of pesticides (Hüffer et al. 2019) and increase the toxicity of the plastics (Abdolahpur Monikh et al. 2020). Data about the co-occurrence of pesticides and plastics is needed to better predict these processes.

The presented field assessment had three specific aims. The first aim was to assess the total plastic and AS content in the soils of a region representative of intensive agriculture. We sampled 6 farms in the region of Murcia, Spain, focusing on light density plastic debris and AS that are commonly applied in the region. We included both organic and conventional farms in the assessment, to estimate the effects of management on the pesticide AS content. We sampled soil at two different depths to compare the accumulation in top soil versus deeper soil. We hypothesized that plastic contamination would be found at all farms as the use of plastic mulch and other plastic material is ubiquitous. We expected to find more pesticide AS residues at conventional farms than at organic farms. The second aim was to compare the measured contents of plastic and AS residues to the recorded applications of plastic mulch and pesticides to check if the records could predict the level of soil contamination. The final goal was to assess bacterial and fungal communities in the soil samples, aiming to link them to our other measurements. We compared the microbial communities between the different farms and we analysed the variations between them with measured soil parameters, including plastic and pesticide AS residues. We also highlighted the most responsive taxa to pesticide AS and plastic contamination. We assumed that the bacterial communities would differ on each farm and that the variation would be explained by the measured parameters.

2.2 Materials and Methods

2.2.1 Study site

The field assessment was carried out in the agricultural Region of Murcia, SE Spain. Murcia has a mean annual temperature of 17.5˚C, mean annual precipitation of 280 mm and annual potential evapotranspiration of 1300 mm. The soil is a Haplic Calcisol (loamic, hypercalcic) (WRB 2014), with loamy texture, an alkaline pH between 8.5 and 9, and 0.6% to 2.1% soil organic carbon. The site has been under vegetable cultivation since the early 1990's. Farmers in the area use drip irrigation and have adopted the use of crop rotations and multiple cropping to increase productivity. Vegetable producers in the region were interviewed to learn more about the application rates of plastic mulch and pesticides. Based on this preliminary survey, three conventional farms (C1, C2, C3) and three organic farms

(O1, O2, O3) were selected. All farms were located in an area measuring 30 km in diameter. All farms intensively produced vegetables with similar crop diversification patterns (e.g., melons, pumpkins or maize in summer and lettuces, cabbages, broccoli or celery in winter, Table S2.1). Three parcels of ~0.5-5 ha were selected on each farm to account for local management variations. A parcel defines the spatial unit over which a unique management is applied at each given time, including crop type, fertilization, plastic mulching, plant protection and irrigation.

2.2.2 Plastic mulch and pesticide application

The study sites were visited in February 2018 to collect soil samples and to carry out a detailed interview about the agricultural managements applied to each parcels. Farmers were asked about the specific commercial names of the pesticides that they used as well as the application date and rate per parcel since September 2016 (previous 18 months) (Table S2.2). Application rates were recorded in L ha^{-1} or g ha^{-1} depending on the pesticide. The application rate of each AS was calculated from the pesticide application rates and the percentage of active substances in each commercial pesticide was obtained from the Spanish agricultural department registers (Table S2.3) (MAPA 2021). The application rates were converted into maximum expected content in soil [mg kg^{-1}] assuming accumulation in the first 10 cm of soil and a soil density of 1400 kg m^{-3}. Maximum expected contents were calculated for each AS for all recorded applications during the previous 18 months. These maximum expected values were compared to the measured AS content. Application rates were also used to calculate a worst-case scenario of an application of two times the recommended dose 18 months ago. Expected contents based on this scenario were calculated with the typical DT50 in soil (Table S2.4) using the formula :

$C_{scenario} = 2x\ C_{recomended} \times 2^{-548/\ DT50}$

We present the calculations for Azoxystrobin, Oxyfluorfen and Pendimethalin as examples in Table 2.1.

Table 2.1*: Calculation of the expected content of three pesticide active substances (Azoxystrobin, Oxyfluorfen and Pendimethalin) which were not recorded as applied in the last 18 months but were detected with a content > 0.1 mg kg^{-1} in some samples. The calculations were made based on the half-life (DT50) values in soil (PPDB. (2019)), a period of 18 months and two times the recommended dose*

Active substance	**DT50 in soil [days]**	**Two times the recommended dose [mg kg^{-1}]**	**Expected content in soil after 18 months [mg kg^{-1}]**
Azoxystrobin	85	0.38	0.004
Oxyfluorfen	140	0.55	0.036
Pendimethalin	180	1.63	0.197

Farmers were also asked about the number of crops they produced in the past and the number of plastic mulch applications they carried out on each parcel of land since September 2011 (previous 90 months) (Table 2.2). 90 months is the longest record we could obtain for all the farms. All farmers declared having used only LDPE mulch for more than 25 years (the year of first plastic mulch application was not provided). Based on the records covering 90 months, plastic mulch application ranged from 1 time per year to 2.2 times per year with an average of 1.8 times per year. At each plastic mulch application, about half of the field was covered, so the plastic mulch application covered about 0.9 ha mulch ha^{-1} field yr^{-1}. This yearly average application area of plastic mulch, divided by a soil dry bulk density of 1400 kg m^{-3} and a soil depth of 0.30 m, gives an average of ~22 cm^2 kg^{-1} soil yr^{-1} and a total of ~550 cm^2 kg^{-1} of soil for the past 25 years. Multiplying by an average plastic mulch thickness of 20 µm and a density of 910 kg m^{-3} it represents ~40 mg kg^{-1} $year^{-1}$ and ~1 g kg^{-1} for the past 25 years.

Table 2.2*: Summary of agricultural practices in each of the studied farms: average number of crops produced in the last 90 months, plastic mulch application in the last 90 months and pesticides applied in the last 18 months per parcel and average estimated total pesticide active substances (AS) applied per kg of soil per parcel. O: organic management; C: conventional management*

Farm	Number of crops (90 months)	Number of plastic mulch applications (90 months)	Average number of plastic mulch use per year	Number of pesticide applications (18 months)	Calculated total AS content applied [mg kg^{-1}] (18 months)
O1	15	11	1.47	0	0
O2	8	8	1.07	0	0
O3	18	13	1.73	0	0
C1	17	17	2.26	12	0.4
C2	17	17	2.26	8	3.0
C3	17	16	2.13	10	2.6

2.2.3 Soil sampling

Soil was sampled at two depths (0-10 cm and 10-30 cm) after the winter harvest and before the soil preparation for summer crops in February 2018. A total of six soil samples were collected with a manual auger (0.7 dm^3 boring head volume) in each parcel at each depth. Samples were taken to the lab immediately and the superficial soil samples separated into two aliquots. One aliquot was air-dried for one week for physicochemical analyses and sieved at < 2mm. The second aliquot was sieved at < 2mm and stored at -20 ˚C for biological

analysis and inorganic nitrogen content. Thus, we had 18 soil samples per farm and soil depth. Five undisturbed soil samples were also collected in each parcel using metallic cylinders (5 cm diameter x 5 cm height) in the top soil (0-10 cm depth). In total, we collected 15 soil cylinders per farm to measure porosity, dry bulk density and field capacity

2.2.4 Soil physicochemical analyses

At each of the three sampling locations per parcel, the soil temperature and moisture was recorded (ECH-5TM/5 from Pessl Instruments) and the hydraulic conductivity (ks) was measured in triplicate using three mini disk infiltrometers (from METER Group)(Group 2020). The soil water repellence was assessed using the water drop penetration time (WDPT) method (Ritsema et al. 2008). It was measured twice, once in the field at each of the three sampling locations and once in the lab on the ring samples at pF 2. An arbitrary WDPT threshold of 5 s was used to distinguish between hydrophilic (wettable) and hydrophobic (water-repellent) soils (Louis W. Dekker et al. 2009b).

In the laboratory, ring samples were water saturated for 24 h and weighed. Ring samples were then placed in a sandbox to measure the field capacity (FC) (Klute and Dirksen 1986; Topp and Zebchuk 1979). The suction was gradually increased to pF 2 and the ring samples were weighed to measure the gravimetric water content. FC is defined as the gravimetric water content at pF 2. The ring samples were finally dried at 105 °C for 48 h. The dry mass was used to calculate the water content at saturation and at pF 2. The porosity (n) was estimated using the volume of water in a saturated sample divided by the total volume (Klute and Dirksen 1986). The dry bulk density (ρb) was measured using the dry mass of the ring sample and the ring volume (Klute and Dirksen 1986). Soil pH and electrical conductivity (EC) were measured in deionized water (1:5 w/w). Total carbon (C.tot), total nitrogen (N.tot) and organic carbon (C.org) were determined by an elemental CHNS-O analyzer. Particle size distribution (percentage of sand, clay and silt) was measured using a Mastersizer analyser 2000LF (Malvern Instruments) with previous oxidation of organic matter and dispersion of clays. Soil NH_4^+ was extracted with 2M KCl in a 1:10 soil:extractant ratio and measured by spectrophotometry (Kandeler and Eder 1990). Soil NO_3^- was extracted with deionized water in a 1:10 soil:extractant ratio and measured by ion chromatography (Metrohm 861). Cation exchange capacity (CEC) and exchangeable Ca, Mg, K and Na were determined using $BaCl_2$ as the exchangeable cation following the method of international standard (ISO 13536, 1995) using ICP-MS (Agilent 7500CE). In total, 18 soil physicochemical parameters were measured and included in the statistical analysis.

2.2.5 Plastic content determination

2.2.5.1 Macroplastic visual estimations

Macroplastic (MP) debris were visually identified from the remaining fraction of 20-50g of soil samples after 2 mm sieving. Macroplastics were then cleaned, weighed, counted and categorised according to their size: < 25 mm², 25-400 mm² and >400 mm². An estimated area was calculated by multiplying the number of particles by the estimated size per category, 10 mm², 40 mm² and 470 mm², respectively. This estimation was used to compare the total area occupied by the plastic mulch in the soil to the total plastic mulch application area per farm.

2.2.5.2 Microplastic Extraction with Flotation

The extraction of the light density microplastics (µP) was adapted from the method of Zhang et al. (2018). Briefly, 5 g of dried 2 mm sieved soil were was stirred into 30 mL of distilled water and centrifuged at 3000 rpm for 10 min. The supernatant was transferred onto a Whatman No. 42 filter paper (2.5 µm particle retention). Samples were refilled with distilled water, stirred again, and put in an ultrasonic bath to further break down soil aggregates. The samples were centrifuged again, and the supernatants were poured onto the same filters. The filters were then air dried for 24 h before microplastic identification and quantification were carried out. Each time that samples were analysed in the lab, a tube without soil was added as a blank to control the plastic contamination from the tube, the water and the atmosphere. A total of 5 blank samples were used in the study.

2.2.5.3 Visual Microplastic Identification

All materials present on a filter were brushed carefully onto a glass plate and gathered into the centre of the plate while trying to avoid the superposition of particles. A stereo microscope (ZEISS Stemi 508) equipped with a digital camera (Leica) was used to take a picture of the particles with ×6 magnification. The glass plate was then put onto a hot plate at 140 °C for 5 s and a second picture was taken. The plastic particles were identified among other soil particles and organic matter by looking at their shape, colour, brightness, and response to heat. Plastic fragments were outlined using Adobe Photoshop CC 2018 before further analysis of the pictures in ImageJ.

2.2.5.4 Microplastic Particle Analysis with ImageJ and mass calculation.

All pictures were analysed using the batch process of ImageJ 1.52 with a macro (Macro S.1). The pictures were first converted to 8-bit type and a threshold was applied before using the analysed particle function. The number of particles per kg was estimated on the basis of total sample dry weight. We detected particles of ~30 µm but the analysis of the size distribution (Figure S2.1) indicated a lower abundance of the µPs smaller than 100 µm. Therefore, we assumed that µPs under 100 µm were less likely to be identified and only presented µPs results of particles between 100 µm and 2 mm.
The mass of each identified particle was estimated using the approximation proposed by Simon et al. (2018). First, the mean ratio between minor and major axes of fit ellipses was calculated. Then the thickness was estimated assuming that the ratio of the thickness and the minor dimension of the particle were the same as the mean ratio between minor and major axes. The volume was calculated as the product of the area and the estimated thickness and finally, the mass was obtained by multiplying by a density of 0.920 mg mm^{-3}.

2.2.5.5 Combining micro and macro plastic results

The number, area and mass of plastic debris obtained from the MP and µP analysis were summed for each sample. The size distribution is shown using 3 categories of plastic debris: <200 µm, 200-2000 µm and >2000 µm. The total number, area and mass of plastic debris were used for further statistical analysis.

2.2.6 Pesticide application and content determination

A list of commonly used pesticides and associated active substances (AS) was prepared based on the preliminary interviews in order to set reference substances for screening. Some active substances on the list were not analysed due to logistical and financial limitations. The final list of the 38 active substances analysed, including 17 insecticides, 15 fungicides and 6 herbicides, is presented in the supplementary materials (Table S2.3).

2.2.6.1 Pesticide extraction

The extraction method was adapted from the QuEChERS approach (Anastassiades et al. 2003). A sample of soil known to be free of pesticide residues (blank soil) was added to the soil samples. For all samples, 10 g of a dry soil was spiked with 13C-caffeine (used as internal standard to assess the procedure efficiency of the LC-MS/MS), and mixed with 5 mL of MilliQ water and 10 mL of acetonitrile containing 1% acetic acid (Mol et al. 2008). The

samples were agitated end-over-end for 30 mins. Then, 1 g of sodium acetate and 4 g of magnesium sulphate were added to induce phase separation. After centrifugation, the supernatant (acetonitrile phase) was transferred to a clean tube and stored at 4°C until analysis. The pesticide quantification was adapted from the multi-residue approach described by Mol et al. (2008) and Silva et al. (2018). It combines liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis.

2.2.6.2 LC-MS/MS analysis

Thirty-eight different AS references were used to determine AS contents in soil with LC-MS/MS. Briefly, 250 µL of the extract was mixed with 250 µL of MilliQ water and filtered in a filter vial. LC-MS/MS measurements were performed on a Xevo TQ-S (tandem quadrupole mass spectrometer) system coupled with an Acquity UPLC (ultra-performance liquid chromatography) system, both from Waters (Milford, MA, USA). Mobile phases of 0.1% formic acid and 5 mM ammonium formate in water (eluent A) or in 95% methanol and 5% water (eluent B) were used. The gradient used to elute all compounds from the column is shown in Table S2.2. Each LC-MS/MS series included a calibration curve of nine levels (0, 0.125, 0.25, 0.5, 1, 2.5, 5, 10, 25 ng mL^{-1}) in a solution of acetonitrile +1% acetic acid and MilliQ water (1:1). A standard matrix was prepared from the blank matrix extract at a level of 5 ng mL^{-1} and injected after every 10 sample measurements as a reference. The software MassLynx™ (Version 4.1, Waters) was used to collect the data and integrate the peaks.

2.2.6.3 GC-MS/MS analysis

Five different AS references were used to determine AS content in soil with LC-MS/MS. Briefly, 250 µL of the extract was transferred to a vial containing 250 µL acetonitrile, 50 mg primary secondary amine (PSA) and 150 mg $MgSO_4$ (magnesium sulfate). Then, 25 µL of PCB−198 2 µg mL^{-1} was added (used as internal standard to assess the procedure efficiency of the GC-MS/MS analysis). The vial was then shaken (clean-up using dispersive SPE) and centrifuged (13,000 rpm for 5 min) then, 150 µL of the cleaned supernatant was transferred into an amber glass vial for analysis. Additional extract from the blank soil was prepared following the same steps. GC-MS/MS measurements were performed on a 7010B MS coupled to a 7890B Gas Chromatograph and a 7693 autosampler, all from Agilent Technologies. Each GC-MS/MS analysis included a calibration curve of nine fortified blanks (0, 0.125, 0.25, 0.5, 1, 2.5, 5, 10 and 25 ng mL^{-1}) prepared with the purified extracts of blank soil. Additionally, the blank soil fortified at 5 ng mL^{-1} was injected after every 10 sample measurements as a standard for 5 ng mL^{-1}. The software MassHunter QQQ™ (Agilient) was used to collect the data and integrate the peaks.

2.2.6.4 Limit of quantification

For both methods and for each compound, a limit of quantification (LOQ) was calculated according to the lowest calibration level inside the linearity range (deviation of back-calculated concentration from true concentration within ±20%) and an ion ratio within ± 30 % of the average of calibration (European Commission 2017). Only one ion transition for Spinosyn-A and Spinosyn-D were available so no ion ratio could be calculated. Because Spinosyn-A and Spinosyn-D come from the same pesticide, Spinosad, we verified that each active substance was present in a sample to validate the quantification. The active substance contents below the LOQ were considered to be zero during data processing. After carrying out calculations using the methods LC or GC, the lowest LOQ was selected for each compound (Table S2.3).

2.2.7 Microbial community assessment

2.2.7.1 DNA extraction from soil

DNA extraction from soil was carried out with the DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's instructions using 0.5 g of soil. Assignments of purity and concentration values were done using a NanoDrop™ 2000/2000c Spectrophotometer and a Qubit® 2.0 Fluorometer combined with a Qubit dsDNA HS Assay Kit, all from Thermo Fisher Scientific.

2.2.7.2 Amplification and sequencing of bacterial 16S

Amplification of bacterial 16S hypervariable regions was carried out using an Ion 16S Metagenomics Kit (ThermoFisher Scientific). The library preparation process was carried out using an Ion Xpress Plus gDNA Fragment Library Preparation Kit (ThermoFisher Scientific) combined with an Ion Xpress™ Barcode Adapters kit (ThermoFisher Scientific) in order to pool several samples for sequencing reactions. An Agilent 2100 Bioanalyzer instrument was used to evaluate concentration, purity and size distributions of the barcoded libraries for further dilutions with the suitable Agilent High Sensitivity DNA Kit. Prepared and diluted library amplicons were processed for template preparation by using Ion Sphere Particles (ISPs) via Ion OneTouch 2 System with a suitable Ion PGM Hi-Q View OT2 Kit (ThermoFisher Scientific) followed by the enrichment of ISPs using Ion OneTouch ES. The sequencing reaction was carried out using an Ion PGM System, Ion PGM Torrent Server and a suitable Ion PGM Hi-Q View Sequencing kit (Thermo Fisher Scientific) with sequencing chips, Ion 316 Chip v2 kit. All purification processes carried out between incubation and the amplification

reactions during library preparation were processed using DynaMagTM 2 magnetic racks (Thermo Fisher Scientific) and an AMPure XP Purification Kit (Beckman Coulter). Purification of ISPs after the enrichment was conducted using a DynaMagTM 2 magnetic rack and Dynabeads™ MyOne™ Streptavidin C1 Beads.

2.2.7.3 Amplification and sequencing of Fungal ITS

Fungal ITS libraries were prepared using a custom protocol based on the method constructed by Smith and Peay (2014). Amplifications of ITS regions were carried out using primer set ITS1f-ITS2 tailed with Illumina adapters. The reverse primers ITS2 were barcoded using 12-base Golay barcodes (Caporaso et al 2012). The PCR amplifications of ITS regions were performed at a final volume of 30 µL consisting of 0.7 µL of each primer (10mM), 0.9 µL of 50 mM MgSO4, 0.6 µL of 10mM dNTP and 0.12 µL of Invitrogen Platinum Taq DNA polymerase High Fidelity (Cat no: 11304-011). PCR conditions were set as follows: 3 min. initial denaturation at 95°C, 35 cycles of denaturation at 95°C (45 sec.), annealing at 50°C (1 min.) and extension at 72°C (1 min.) followed by a final extension of 10 minutes at 72°C. Amplified ITS amplicons were then purified using Apure XP beads (Beckman Coulter) following the manufacturer's instructions. Purified ITS libraries were checked for size distribution using Agilent 2100 Bioanalyzer and Bioanalyzer DNA 1000 kit (Agilent) followed by measuring concentrations via Qubit® 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific) combined with a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Prepared ITS amplicons were pooled together and sequenced on the Illumina MiSeq system.

2.2.7.4 Bioinformatics

Bacterial sequencing analysis was performed with QIIME 2 2020.6 (Bolyen et al. 2019) adapted for IonTorrent data. Raw sequence data were quality filtered using the q2-demux plugin followed by denoising with DADA2 (Callahan et al. 2016) (via q2-dada2). Taxonomy was assigned to amplicon sequence variants (ASVs) using the q2-feature-classifier (Bokulich et al. 2018) against the Greengenes 13_8 99% OTUs reference sequences (McDonald et al. 2012). Alpha biodiversity indexes Shannon and Simpson were calculated with the function estimate_richness().

2.2.8 Statistical analysis

All data analysis and visualisations were performed with R (version 3.6) and all scripts and raw data tables are available on Github https://github.com/NGBeriot/LDPE_Mulch_Cartagena (available after publication). For every soil parameter, the normal distribution was tested with the Shapiro-Wilk test. Then, the differences among the farms (and soil-depths when applicable) were tested with ANOVA followed by a pair-wise comparison with t-test in case of normal distribution and otherwise with the Kruskal-Wallis method followed by a pair-wise comparison with the Wilcoxon rank sum test. Both the t-test and Wilcoxon rank sum test were implemented with the function compare_means() and calculated p-values were adjusted with the Holm method. The results for the 18 soil physiochemical parameters are presented in Table S2.5. Among these, parameters leading to a significant difference between farms among the 18 soil physicochemical parameters were included in a principal component analysis (Figure S2.2).

The phyloseq package was used to analyse phylogenetic sequencing data (McMurdie and Holmes 2013). First, the different microbial communities were visualised with a principal coordinate analysis (PCoA) and the difference between farms was tested with the anosim test. Then, a permanova with the Adonis function and the Bray-Curtis distance was used to identify the main parameters involved in the variations between bacterial and microbial communities. We tested the 18 measured soil physicochemical parameters, the number and total area of plastic debris, the number and total content of pesticide residues and the content of the 11 most abundant pesticide residues. Then, the parameters with a significant contribution were used to visualise the bacterial and fungal variability with a canonical analysis of principal coordinates (CAP). Finally, two linear discriminant analysis (LDA) effect size (LEFSe) analysis were performed to test the pesticide and plastic content effect for indicator taxa. A cut off value was applied to highlight the most responsive order. For the pesticides, the samples were classified between organic and conventional farms and a cut off value of 1.8 was applied. For plastic, the samples were classified between the first (101 cm^2 kg^{-1}) and third quartiles (394 cm^2 kg^{-1}) of the plastic area content in soil and a cut off value of 1 was applied.

2.3 Results

2.3.1 Soil plastic content

Both MP extraction and µP extraction methods found plastic debris in all soil samples. The minimum number of observed µPs was 5 per sample (~5 g of soil), comparatively, the 5 blank samples showed 0, 2, 0, 0 and 1 µPs. The biggest plastic debris found was about 20 cm^2. The overall plastic debris content in soil, MP and µP combined, was ~2.10^3 debris kg^{-1} and ~60 cm^2 kg^{-1} soil which represent ~0.2 g kg^{-1} (Figure 2.1). Overall, the µPs represented 92% of the total plastic debris found and 2.1% of the total plastic debris area in soil (Figure 2.1). The size distribution was similar for all farms and soil depths exept for the farm C2 which had more smaller particles (Figure S2.3). There were no significant differences between soil depth with regards to the amount of plastic debris found (Figure 2.2) or the area it covered (Figure S2.4). Only two conventional farms, C2 and C3, showed significantly fewer plastic particles than the other farms (Figure 2.2).

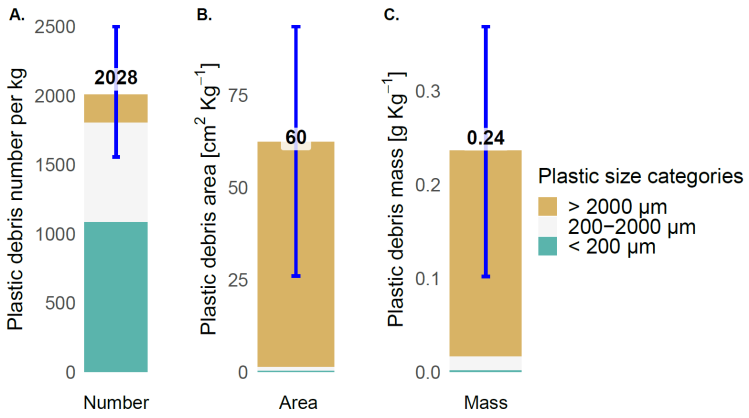


Figure 2.1*: Average amount of plastic debris, area and estimated mass in all the soil samples for three plastic size categories: <200 µm, 200-2000 µm and >2000 µm. The vertical blue line represents the standard deviation among all soil samples.*

The yearly average for plastic mulch application of ~0.9 ha ha^{-1} yr^{-1} leads to an estimated total of ~550 cm^2 kg^{-1} and ~ 1 g kg^{-1} of plastic mulch used over the past 25 years. Therefore, the measured ~60 cm^2 kg^{-1} and 0.2 g kg^{-1} represent ~10% and ~20%, respectively, of the plastic applied in the past 25 years. At the parcel level, neither the recorded number of crops nor the number of mulch applications in the past 90 months (Table 2.2) correlated with the measured amount of plastic debris found or the calculated area.

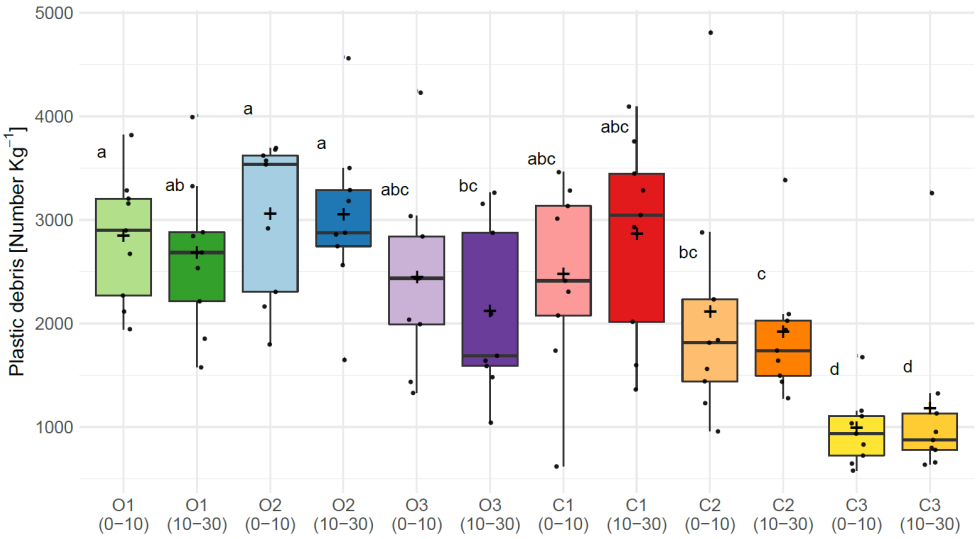


***Figure 2.2**: Total number of plastic particles (>100 µm and < 50mm) per kg of soil in organic (O) and conventional (C) farms for both the top soil (0–10 cm) and the subsurface soil (10–30 cm). The box plots (horizontal lines) represent content for at least 25%, 50% and 75% of the samples. The vertical black lines denote the minimum and maximum values, excluding outliers (1.5 IQR method). The cross represents the average content of any given sample group. The dots represent individual measurements. Soils that do not share letters are significantly different from each other (Wilcoxon test with $p < 0.05$).*

2.3.2 Pesticide application rates and soil residues

Soils from conventional farms contained > 100 times the amount of pesticide residues than organic farms, with respective averages of 140 µg kg^{-1} and 0.8 µg kg^{-1} (Figure 2.3). For all farms, higher pesticide AS content was found in the top soils than in the deeper soil, but the variation among samples does not result in a significant difference between top and deeper soils. Azoxytrobin was found in all the soils from conventional farms with a minimum of 1 µg kg^{-1}. Azoxystrobin, Imidacloprid, Chlorantraniliprole, Boscalid and Difenoconazole were found at an average of >1 µg kg^{-1} in all conventional farms. Azoxystrobin, Boscalid, Chlorantraniliprole, Cypermethrin, Difenoconazole, Imidacloprid and Oxyfluorfen all measured >100 µg kg^{-1} in some parcels. In the top soil from farms C1 and C2, the herbicide oxyfluorfen was the most abundant pesticide residue. In the subsoil from farms C1 and C2, the fungicides Azoxystrobin and Difenoconazole were dominant. In farm C3, the fungicide Boscalid was the preponderant pesticide in both the top- and subsoil. In C3, the herbicide Oxyfluorfen was not found but Pendimethalin was.

All soil samples from conventional farms contained at least 4 pesticide AS. However, soil samples from organic farms contained at most 4 pesticide AS (Figure 2.4). Only soil samples from Farm O1 were free of detected pesticide residues.

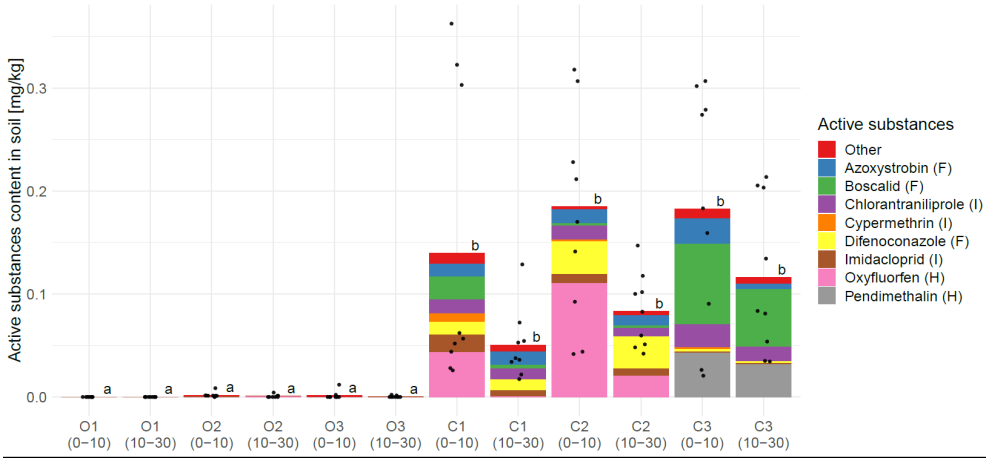


***Figure 2.3**: Sum of active substances measured per kg of soil in organic (O) and conventional (C) farms for both the top soil (0–10 cm) and the deeper soil (10–30 cm). The eight most abundant substances are given a different colour and classified as fungicide (F), insecticide (I) or herbicide (H). Other substances are summed in the same category. The dots represent individual measurements. Soils that do not share letters are significantly different from each other (Wilcoxon test with $p < 0.05$).*

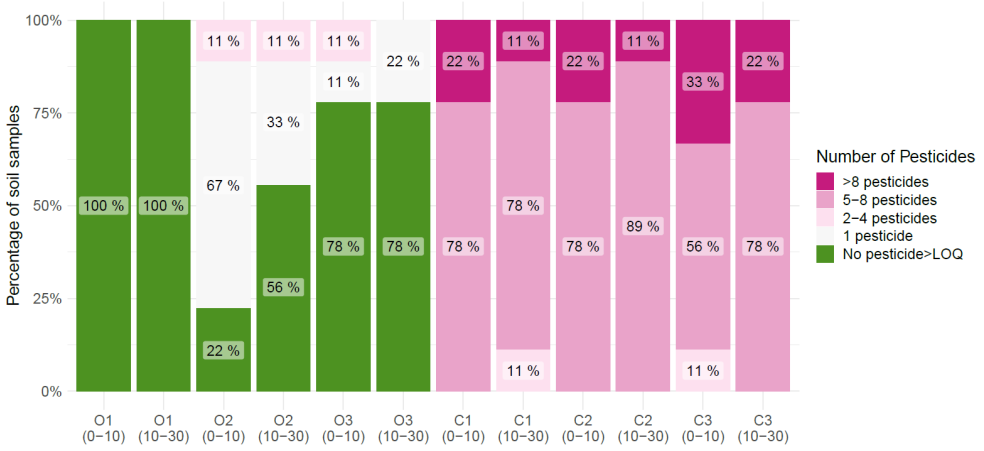


***Figure 2.4**: Percentage of soil samples with no quantified pesticide residues, 1 pesticide residue and multiple pesticide residues in organic (O) and conventional (C) farms for both the top soil (0–10 cm) and the deeper soil (10–30 cm).*

For many soil samples, the estimation of the pesticide residue applied in the past 18 months was lower than the measured pesticide residues measured in the soil (Figure 2.5, Table S2.6). This was the case for many substances that were not on the list of substances applied in the past 18 months. For example, measured contents of Azoxystrobin, Oxyfluorfen and Pendimethalin reached more than 0.1 mg kg^{-1} even though they were not registered as being applied in the past 18 months in the parcels where they were found. If we consider

the worst-case scenario of the application of two times the recommended dose and the minimum time of 18 months, we obtain expected contents in soil of 0.004 mg kg^{-1}, 0.04 mg kg^{-1} and 0.2 mg kg^{-1} (Table 2.3), respectively. These values are 2 and 1 order of magnitude below the measured contents of Azoxystrobin and Oxyfluorfen, respectively, but the same order of magnitude of Pendimethalin. So, this worst-case scenario could explain the measured pendimethalin content but not the measured Azoxystrobin or Oxyfluorfen found in soils where no applications of these compounds were recorded.

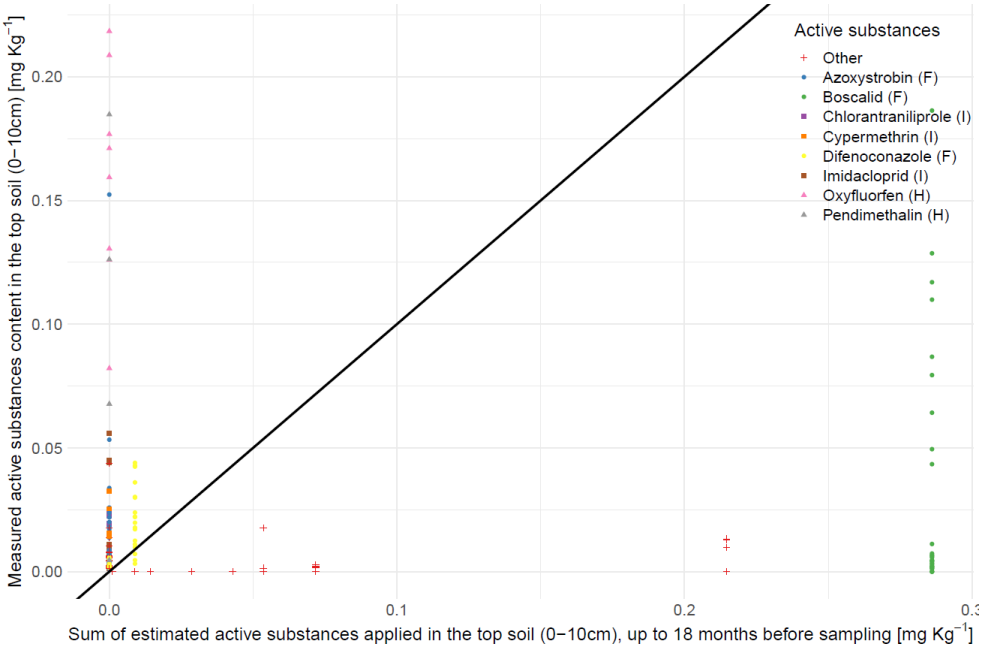


Figure 2.5*: Measured compared to estimated applied content of active substances in the soil. The eight most abundant substances are represented with a different colour and classified as fungicide (F, circle), insecticide (I, square) or herbicide (H, triangle). The black line represents the equality between measured and estimated applied content (y=x). The measured contents were expected to be below this line. The graph is centred on values <0.3 mg kg^{-1} for better visualisation and all values are presented in Table S2.6 .*

2.3.3 Microbiome Analysis

A total of 14168 bacterial sequences and 4340 fungi sequences were obtained after filtration, including 10581 and 3813 sequences with annotated phylum, respectively. The pair-wise comparisons with the Anosim test indicates that the soil bacterial and fungal communities of all farms were significantly different from each other, and only the soil fungal communities of farms O1-O2, O2-C1 and C1-C2 were not significantly different from each other (Table S2.7). PCoA for bacterial data showed that organic farms had the same

scores in Axis 1, with differences related with Axis 2 (Figure 2.6A). In this line, O3 showed positive scores along Axis 2, while O1 and O2 showed negative scores. Conventional farms showed similar scores within Axis 2, separating Axis 1 C1 from C2 and C3. PCoA for fungal data showed that O3 and C2 had the most samples with positive scores within Axis 1, while C1 and C2 showed negative scores (Figure 2.6B). Axis 2 clearly separated O3 and C3 from C1 and C2. The alpha biodiversity indexes were similar for all the farms except for farm C3 which had a higher bacterial diversity and a lower fungal diversity (Shannon and Simpson index) (Figure S2.5).

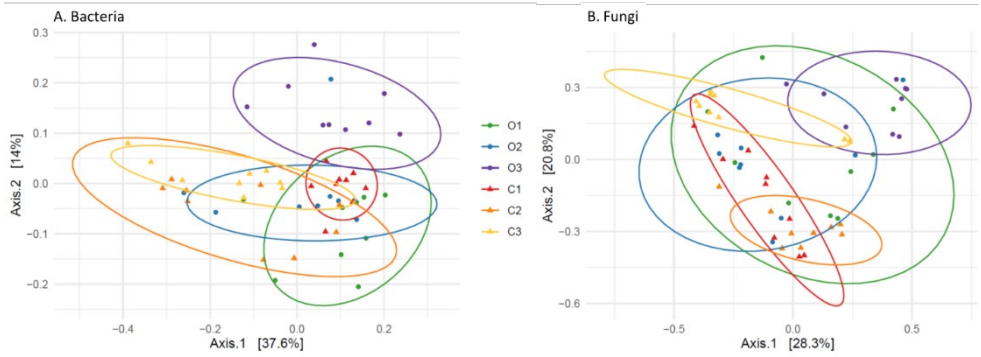


***Figure 2.6**: Principal coordinate analysis (PCoA) ordination of the soil bacterial (A.) and fungal (B.) communities*

A first permanova including the sample location as factors showed that the farm explained most of the variation between communities. A second permanova, without location factors, showed that seven parameters significantly explained the variation of both the microbial and fungal communities: N.tot, NH4, NO3, FC, C.org, WDPT, CEC. The pH, porosity, dry bulk density, total plastic area, Azoxystrobin, Chlorantraniliprole and total pesticide residue content in soil related to the microbial communities only (Table S2.8). Na and Boscalid content in soil related to the fungal communities only. These parameters were implemented in the Canonical analysis of principal coordinates (CAP) which was performed for establishing the relationship between bacterial and fungal communities as a whole (Figure 2.7). In CAP for bacterial communities, the sum of pesticide residues, Azoxystrobin and Chlorantraniliprole showed the highest significant load to explain variation in the farms under conventional management: C1, C2 and C3 (Figure 2.7A). Total N and the area of plastics were related to O1 and some C2 samples. O2 and O3 had higher pH, and O3 also with NO_3^- content. Thus, pesticide residues and plastic content significantly contributed to variations in the bacterial community. The bacterial and fungal communities on farm O3 were very different from other farms, related to higher NO3, WDPT and lower FC. Fungal

communities in conventional farms (C1, C2, C3) correlated with the Boscalid content in soil (Figure 2.7B). Thus, fungal communities were more strongly affected by soil physicochemical properties than by pollutants such as pesticides and plastics, contrary to bacteria.

***Figure 2.7**: Canonical analysis of principal coordinates (CAP) of the soil bacterial (A.) and fungal (B.) communities. The CAP shows the contribution of parameters selected to explain the variation in the communities: total nitrogen (N.tot), total carbon (C.tot), organic carbon (C.org), ammonium (NH4), nitrate (NO3), field capacity (FC), porosity, soil dry bulk density, pH, cation exchange capacity (CEC) sodium (Na) , total plastic area (Area.Plastic), total pesticide active substances (AS.content), Azoxystrobin-F, Chlorantraniliprole-I and Boscalid-F.*

The Linear discriminant analysis (LDA) effect size (LEFSe) showed that for pesticides (Figure 2.8 A. an B.) some bacterial orders were linked to a high content of pesticides (conventional), while PLTA13 (*Xanthomonadales*) was associated with low pesticide content (organic). The fungal orders Sordariales, Microascales and Botryosphaeriales were related to high pesticide content, while Mortierallales and an unknown Ascomycota was related to a low pesticide level. With regards to plastic debris (Figure 2.8 C. and D.), the bacterial order Salinisphaerales was related to low plastic content, while Subgroup 17, Pyrinomonadales, Nitrospirales and Acidobacteriae were related to high plastic content. For fungi, the order Chaetothyriales was related to a low content of plastic, and the order Kickxellales to a high content.

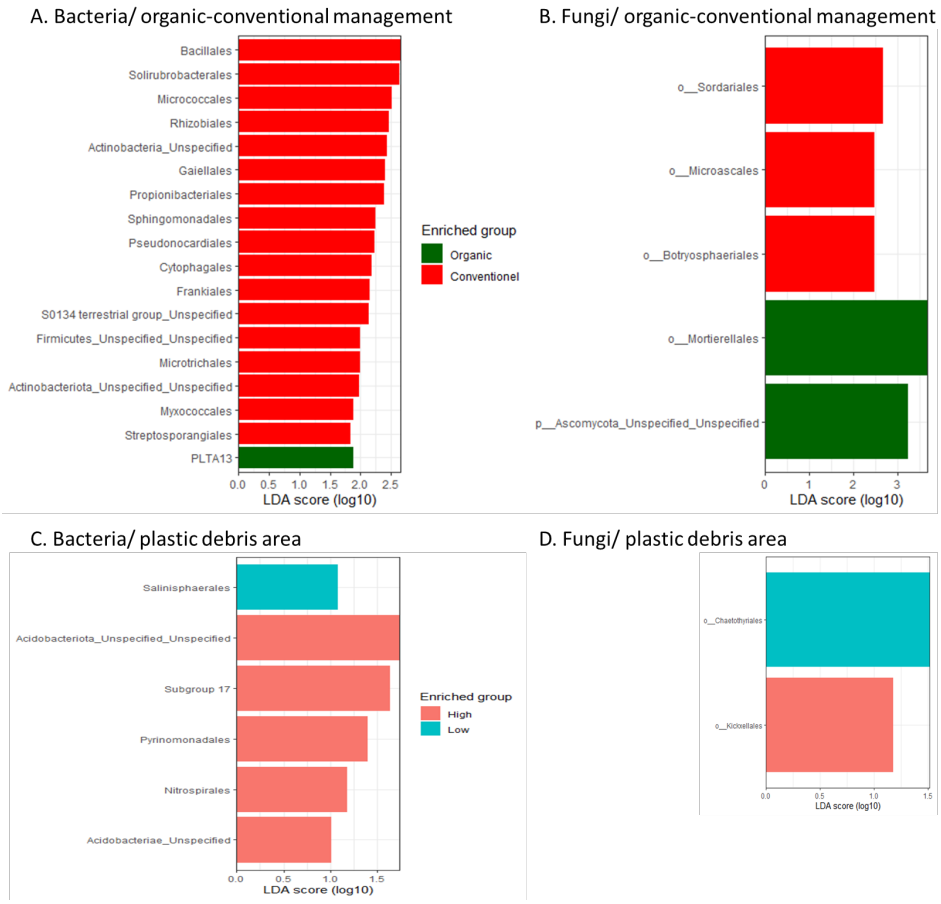


***Figure 2.8**: LEfSe analysis of bacterial (A. and C.) and fungal (B. and D.) communities identifying order for which a major part of the population was active in soil with organic farming (green) or conventional (red) (A. and B.) and with a lower (<101 cm²/kg, blue) or higher (>394 cm²/kg, pink) plastic debris area (C. and D.).*

2.4 Discussion

2.4.1 Accumulation of Plastics in Soils

This field assessment confirmed the ubiquity of microplastic contamination in intensive agriculture with all soil samples containing plastic. More specifically, this study confirmed the 2240 ± 980 µPs kg^{-1} found in a preliminary study in the same region (van Schothorst 2021). The number of plastic debris was lower for farms C2 and C3 even though the records from the previous 90 months of plastic mulch applications indicated that the same amount or even more plastic was used in the form of mulch on these farms than on the other farms. The plastic mulch application records did not correlate either with the number or the area of plastic measured in the soil. It could be because the records from the previous 90 months of applications represent less than 30% of all the plastic mulch history and the difference between parcels could have been different in the past. This fact could be related to the efficiency of mulch removal at the end of the crop cycle and the degradation rate of plastic. The plastic mulch removal rate for each parcel is missing in this study since it has not been historically monitored. The removal rate can depend on the technique used and on the plastic mulch thickness (James et al 2021; Gómez-Águila et al. 2021). Manzano et al. (2019) reported removal rates of 90% for plastic mulch thicker than 25 µm but only 32% for LDPE mulch measuring 20 µm. We are also missing other potential inputs such as the packaging of vegetables in the fields and the transport of plastic by wind and water which can bring or remove plastic debris. Overall, we estimated that the plastic area measured in the field represented ~10% of the plastic mulch applied in the past 25 years. This would mean that from all the plastic mulch applied in the past 25 years, ~10% has remained in the soil, the rest being either removed, degraded, or transported away from the field. This estimation does not consider other inputs of plastic debris in the field such as plastic packaging dropped on the field or deposition by the wind. Chen et al. (2013) gave a similar estimation when calculating a plastic mulch residual rate between 5% to 16% in Chinese provinces.

In addition to the number and the area, the average size of the plastic debris could be an indicator of the overall stage of degradation of the plastic in a soil. The degradation stage would depend on the residence time of the plastic in the soil, fragmentation reducing the size of debris over time, and the input/output balance. We can expect the input of newer/bigger debris to reduce the overall degradation stage. In other words, we expect that a more advance degradation stage would be characterized by a larger abundance of small particles. This could apply to the farm C2 which had been exposed to more years of plastic mulch applications but had a lower plastic area and a smaller average particle size than farm O2. One hypothesis could be that farm C2 has had a better plastic removal technique than

farm O2 and therefore, a more advanced degradation stage. It would be interesting to compare the plastic removal techniques and other inputs of plastic in both farms.

2.4.2 Plastics debris analysis in soil, limitations and recommendations

MP: more soil more particles

In this study, the MP assessment was based on soil samples weighing less than 50g. This small amount of soil led to very few MP being recovered from the soil thus inducing a huge variation between samples. We encourage future field assessments to measure the MP content in the soil using a quadrat sampling method as in Meng et al. (2020) (Meng et al. 2020) so that a larger proportion of the soil is sampled for MP. The MP should be cleaned, weighed and scanned to obtain a measurement of the total area that the plastic takes up in the soil. Assessing the MP area is important because we showed that MP greatly contributed to the total plastic area in the soil. The total plastic area is a determinant factor to compare plastic inputs and residues for processes such as the sorption/ desorption of contaminants or the colonization by biofilms.

µP: only the light density PE and limit of detection

In this study, the µPs assessment was adapted from the extraction and identification methods from Zhang et al. (2018). With this method, only light density plastics, less dense than the distilled water, were extracted. We expect that most of the µPs originating from the LDPE plastic mulch were extracted because LDPE has a density of ~0.91 g cm^3. Plastic packaging sometimes used on the fields is also composed of LDPE. However, other plastics such as PVC or PET are likely not to be extracted using this method. After extraction, the method relies on visual identification based on shape, colour, brightness and heat response. The visual selection presents some advantages as compared to the developing spectral technics (e.g., Raman or Fourier transform infrared) (Munno et al. 2020; Sobhani et al. 2019): it is fast, does not rely on spectra library or machine specification, adaptive to particle clustering and different shapes(Fabio Corradini et al. 2021; Weber et al. 2021). Indeed, with spectral methods some shapes like plastic fibres or particles crossing over each other are difficult to identify. Both methods have intrinsic limitations due to the plastic property tested: the response to heat with SMVS and transmission or reflection for spectral methods. Some plastic polymers do not respond to heat at the chosen temperature as some plastic polymers do not have characteristic transmission or reflection spectra at the tested wavelength. Finally, the main advantage of using spectral methods is the possibility to standardize them and to give more information about the polymer types.

Based on the analysis of the µPs size distributions, we noticed a decrease in abundance of particles smaller than 100 µm with a minimum of 30 µm. From a fragmentation point of view, we would expect to find smaller particles more abundant unless there is an important transport of small particles. Because we do not have more information about the transport of smaller particles away from the field, we decided not to include particles < 100 µm in this study. We encourage future studies to perform a more informative size distribution analysis.

Plastic debris units

In accordance with the suggestion of Horton et al. (2017), the units which are presented in this study are a unit per mass of soil, namely particles kg^{-1}, cm^2 kg^{-1} and g kg^{-1}. The amount, area and mass of the particles are representative of different processes. For example, to estimate the probability of ingestion, the number of particles would be most important (Helmberger et al. 2019); for the formation of biofilms, the total area would be the dominant factor (Sander 2019); and for input/ output balances, the mass is generally favoured (Li et al. 2020). More generally, studies should always provide the results per particle identified in order to allow comparisons with other studies. This is a requisite step for the standardization of the extraction and identification methods. For example, Harms et al. (2021) reported ~3.7 ± 11.9 debris kg^{-1} in arable lands in Germany for particles between 1-5 mm. To compare this value with our results, we need to apply the same size threshold on the raw results. We found more debris in our study with ~107 ± 113 debris kg^{-1} in the range of 1-5 mm. Moreover, many processes will be influenced by the size of the debris. For example, a study about plastic ingestion may focus on mm size debris for mammals (Mekuanint et al. 2017), µm for earthworms (Helmberger et al. 2019) and nm for plant roots (Chae and An 2020). Therefore, providing the complete size distribution will allow the data to be reused to study other processes.

2.4.3 Plastic and pesticide accumulation in different soil depths

The amounts of plastics and pesticides were not significantly different between the two soil depths. This is explained by the regular ploughing which homogenised the soil between 0-30 cm. However, the pesticides always showed greater maximums in the top soil. In both cases, the input comes from the surface, but the residence time of pesticide residues in soil is much lower than the time for plastic debris. Therefore, the pesticides recently applied on the surface contribute comparatively more to the total pesticide content than the newly applied plastic does to the total plastic content. For the plastic debris, Meng et al. (2020) gave a contrasted conclusion. They analysed MP and µP debris in 0-10cm, 10-20cm and 20-30cm soil samples from cereal fields intensively ploughed from 0-10cm and found that the amount of MP was significantly higher in top soil but found no difference for µP. For the

pesticides, various studies confirmed that there are more abundant pesticide residues in the top soil as compared to deeper soil, with more significant results for soils deeper than 40cm (Rodríguez-Cruz et al. 2006, Bhandari et al. 2020). We encourage future studies to analyse deeper soil layers to further assess the vertical transport of plastic and pesticide residues.

2.4.4 Pesticide content in soil

This field assessment confirmed the ubiquity of AS residues from pesticides in agricultural soils (Geissen et al. 2021). We found similar pesticide contents in conventional soils as those found by Silva et al. 2019 for European agricultural soils. At the same time, we found 100 times fewer AS residues in organic farms than in conventional farms. However, Geissen et al. (2021) found higher content in organic farms by including long-banned organochlorine pesticides like DDT and carrying out a wider AS screening than we did. We can conclude that the results are strongly dependent on the method and that we could have found more residues with a wider screening. However, we just focused on the pesticides applied on the farms in the last few years. Research would benefit from a longer record of pesticide applications. The application records spanning 18 months did not explain the AS measured in all samples. In fact, some samples contained AS residues from compounds that were not applied in the past 18 months. For some pesticides, such as Azoxystrobin and Oxyfluorfen, this could be explained by the fact that the compounds take longer than expected to degrade. Many factors influence pesticide degradation in soil. For example, higher clay content is expected to reduce the degradation of pesticides by increasing their sorption (Huang et al. 2015). Low soil moisture is also expected to reduce pesticide degradation (Ismail et al. 2012; Singh 2017). This could be one of the main factors explaining degradation in the semi-arid climate of Murcia because the fields are irrigated only during the crop growing period. It is worth noting that even if the high pendimethalin contents measured in some of the soils could be explained by the double-dose scenario, Kočárek et al. (2016) showed that the application of a double-dose does not necessarily lead to higher concentrations in soil. Degradation experiments carried out in field conditions are required to draw any conclusions.

2.4.5 Potential toxicity of pesticides and plastics

It is worth noting that more than 80% of the soil from conventional agriculture contained more than 4 different active substances, while plastic debris were detected in all soil samples. Therefore, we encourage ecotoxicology studies to i) study the effects of pesticides as a mixture of contaminants and ii) study the potential synergetic effects of plastics and pesticides. Indeed, previous studies have indicated that pesticides could be adsorbed on plastic debris (Beriot et al. 2020). The sorption could affect the availability of the AS in the soil, the transport of the AS in the environment and its transport in the food chain(Wang et al. 2020b).

First, AS availability in the soil water solution is a factor for the sorption onto soil mineral particles, soil organic materials and plastic debris (Sadegh-Zadeh et al. 2017). The comparative sorption coefficients in the soil are not well explored yet, however, the soil mineral particles and the soil organic materials represent a much bigger contact area than the plastic residues. Therefore, we could expect that plastic debris would have only a small or even negligible effect on the AS availability in the soil water solution(Ahmadu et al. 2020). The conclusions could be different if we take into account that some fungicides and insecticides are sprayed after the plastic mulch is laid on the soil (e.g. foliar application of Boscalid (Borrell et al. 2017)). Therefore, in this case, the plastic would have a significant sorption area with these pesticides as compared to the soil. More investigation is needed to elucidate these processes on the AS availability.

When sorbed on plastic, AS can be transported in the environment with the wind and water (Wang et al. 2020a). The particular shape and density, among other specific properties of the plastic, suggest that the transport mechanisms would be different from other soil particles or organic matter (Li et al. 2021). Therefore, the sorption of AS on plastic could affect the transport of AS in a different way than soil particles or organic matter.

Finally, the sorption of AS on plastic debris could lead to a trojan effect when the plastic debris is ingested (Beckingham and Ghosh 2017). Most available studies have focused on aquatic organisms (Kühn and van Franeker 2020; Sun et al. 2022). However, the same is expected to happen with terrestrial organisms (e.g. plants (Chae and An 2020), earthworms (Huerta Lwanga et al. 2016) or livestock (Beriot et al. 2021)). The sorption/ desorption will depend on the type of AS, plastic, organisms, etc. and needs further investigation. If we compare the ingestion of plastic to the ingestion of organic matter, both contaminated with AS from pesticides, we can expect that, in most cases, the organic matter would be more digested than LDPE debris in the organism's gut. Therefore, we could expect a faster release of the AS in the organism in the case of organic matter as compared to plastic. This would lead to a more chronical contamination and maybe more bio-accumulation along the food

chain (Sun et al. 2022). All these interactions between pesticides and plastic should be investigated under field conditions.

2.4.6 Analysis of the soil microbiome

In this field assessment, we defined microbiomes with 16S and ITS sequencing. Therefore, the results are representative of only some bacterial and fungal taxa. Moreover, only a fraction of the sequences matched identified taxa. Thus, the characterisation of the soil microbiome is a limitation of the study. The Analysis of similarities (ANOSIM) showed differences in the microbiomes associated with different farms. This was confirmed by a permanova identifying the location of the sample as the main factor explaining variation. Nevertheless, some parameters were correlated with more variation than others. This field assessment cannot conclude anything about a direct causality between a measured soil parameter and the soil microbiome, but some hypothesis can be suggested.

First, the fungal communities were more strongly affected by soil physicochemical properties than by pollutants such as pesticides and plastics, contrary to bacteria. Boscalid was the only pesticide significantly affecting the fungal community. As a broad spectrum fungicide, Boscalid is expected to affect the fungal community (Li 2021). Moreover, Boscalid was detected in many soils under conventional farms with the highest contents in soils from farm C3. We did not find studies specifically concerning Boscalid and the soil microbiome, but many studies showed that foliar fungicides were directly impacting the soil fungal communities (Santísima-Trinidad et al. 2018, Yang et al. 2011). We hypothesized that Boscalid residues, among other factors, were responsible for the lower fungal diversity in Farm C3. Bacteria were affected by the overall pesticide residues and more specifically, by the fungicide Azoxystrobin and the insecticide Chlorantraniliprole. Previous studies have shown that pesticide residues can be expected to have different effects on the bacterial and fungal communities. For example, Sahu et al. (2019) showed that the recommended dose and double the recommend dose of Chlorantraniliprole can lead to a significant decrease of heterotrophic bacteria but had no significant effects on the fungi population. In Sahu's study, all detected effects were recovered after 45 days but other field studies suggest a long-term effect of pesticides on the soil microbial community (Santísima-Trinidad et al. 2018; Sharma et al. 2019; Wołejko et al. 2020). With regards to the taxa most responsive to pesticide content, we can compare our findings with Harkes et al. (2019) who studied the different soil microbiomes from barley fields under conventional and organic farming. For the bacteria, we found in both studies that Bacillales, Micrococcales and Actinobacteria were more abundant in fields under conventional agriculture than in fields under organic agriculture. For the fungi, we found in both studies that Sordariates and Microascales were more abundant in fields under conventional agriculture than in fields under organic

agriculture. However, our analysis identified Mortierellales as being more abundant in organic agriculture, whereas Harkes et al. (2019) found it more abundant in conventional agriculture. This difference highlights the importance of pedoclimatic factors to explain the soil microbiome.

One important soil factor is pH. We observed an effect of pH on bacteria but not on fungi. This is similar to the result of Rousk et al. (2010) showing that pH strongly affected the relative abundance and diversity of bacteria but not the relative abundance and diversity of fungi.

Plastic area also affected the soil microbial community but not the fungal community. This relationship between plastic residues and soil microbes could be explained by many processes such as changes in soil moisture and temperature due to plastic mulch use (Kasirajan and Ngouajio 2012) or large debris (de Souza Machado et al. 2019; Qi et al. 2020b), plastic debris supporting biofilms (McCormick et al. 2014) and/or chemical interactions, toxic or beneficial, with the plastic additives (Kong et al. 2018). For instance, LDPE plastic can be colonised and degraded by fungal (Gajendiran et al. 2016) and bacterial (Montazer et al. 2018) communities but the extent of this process in the field and links with the soil microbial communities remain unclear. We found four incubation experiments studying the effects of LDPE debris in soil: Huang et al. (2019) reported a lower bacteria alpha diversity after incubation with 0.76% (w/w) LDPE µP (Huang et al. 2019), whereas Meng et al. (2022) showed a bacterial alpha diversity higher for 0.5% (w/w) LDPE µP compared to no plastic but lower for 2.5%. Qi et al. (2020) only reported a higher relative abundance of the genus *Saccharibacteria* with 1% (w/w) LDPE µP and MP (Qi et al. 2020b). Bloker (2020) showed a reduced microbial biomass with 1% (w/w) LDPE addition but highlighted that the two tested organic matter contents (4.0% and 2.6%) contributed to more effects than the plastic treatment. These four incubation experiments were conducted with pristine plastic debris incubated between 1 and 6 months. Therefore, we need further investigations to understand in which ways plastic debris may affect the soil microbiome.

2.5 Conclusion

Intensive agriculture successfully provides high yields of vegetables in the region of Murcia, Spain using minimum resources. However, this study reveals the un-considered costs for the environment due to an accumulation of plastic debris and pesticide residues. We show that farming practices using plastics and pesticides have effects on soil microbial communities. We conclude that more studies are required in the lab and in the field to understand the consequences of plastic and pesticide residues on the soil microbiome and ultimately, on crop production. These results are required to provide supportive information for farmers, agronomists and industry to design and apply the best agricultural managements.

Acknowledgments

We are thankful for the contribution of the farmers from the region of Murcia, Spain. We thank all the lab technicians for the technical support for the soil measurements. We would also like to thank Robin Palmer for the language editing.

Supplementary Material

Table S2.1 *: Farmers records of crops and plastic mulch applied from September 2011 to January 2018 for the three parcels of each organic farms (O1, O2, O3) and conventional farms (C1, C2, C3). Growing period are defined from the soil preparation to the crop harvest.*

			Farm O1		
Year	**growing period**		**Parcel 1**	**Parcel 2**	**Parcel 3**
2011	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2011	1	**Plastic**	PE	PE	PE
2011	2	**Crop**	Zea mays	Zea mays	Zea mays
2011	2	**Plastic**	PE	PE	PE
2012	1	**Crop**	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2012	1	**Plastic**			
2012	2	**Crop**	Cucurbita moschata	Cucurbita moschata	Cucurbita moschata
2012	2	**Plastic**	PE	PE	PE
2012	3	**Crop**			
2012	3	**Plastic**			
2013	1	**Crop**	Brassica oleracea gongyloides	Brassica oleracea gongyloides	Brassica oleracea gongyloides
2013	1	**Plastic**	PE	PE	PE
2013	2	**Crop**	Zea mays	Zea mays	Zea mays
2013	2	**Plastic**			
2014	1	**Crop**	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2014	1	**Plastic**	PE	PE	PE
2014	2	**Crop**	Cucurbita moschata	Cucurbita moschata	Cucurbita moschata
2014	2	**Plastic**			
2014	3	**Crop**			
2014	3	**Plastic**			
2015	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2015	1	**Plastic**	PE	PE	PE
2015	2	**Crop**	Zea mays	Zea mays	Zea mays
2015	2	**Plastic**	PE	PE	PE
2015	3	**Crop**			
2015	3	**Plastic**			
2016	1	**Crop**	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2016	1	**Plastic**	PE	PE	PE
2016	2	**Crop**	Cucurbita moschata	Cucurbita moschata	Cucurbita moschata
2016	2	**Plastic**	PE	PE	PE
2016	3	**Crop**			
2016	3	**Plastic**			
2017	1	**Crop**	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes
2017	1	**Plastic**	PE	PE	PE
2017	2	**Crop**	Zea mays	Zea mays	Zea mays
2017	2	**Plastic**			
2018	1	**Crop**	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2018	1	**Plastic**	PE	PE	PE

***Table S2.1 continued**: Farmers records of crops and plastic mulch applied from September 2011 to January 2018 for the three parcels of each organic farms (O1, O2, O3) and conventional farms (C1, C2, C3). Growing period are defined from the soil preparation to the crop harvest.*

			Farm O2		
Year	growing period		Parcel 1	Parcel 2	Parcel 3
2011	1	Crop	Brassica oleracea v.Italica	Brassica oleracea v.Italica	Brassica oleracea v.Italica
2011	1	Plastic	PE	PE	PE
2011	2	Crop			
2011	2	Plastic			
2012	1	Crop	Apium graveolens	Apium graveolens	Apium graveolens
2012	1	Plastic	PE	PE	PE
2012	2	Crop			
2012	2	Plastic			
2012	3	Crop			
2012	3	Plastic			
2013	1	Crop	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2013	1	Plastic	PE	PE	PE
2013	2	Crop			
2013	2	Plastic			
2014	1	Crop	Brassica oleracea v.Italica	Brassica oleracea v.Italica	Brassica oleracea v.Italica
2014	1	Plastic	PE	PE	PE
2014	2	Crop			
2014	2	Plastic			
2014	3	Crop			
2014	3	Plastic			
2015	1	Crop	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2015	1	Plastic	PE	PE	PE
2015	2	Crop			
2015	2	Plastic			
2015	3	Crop			
2015	3	Plastic			
2016	1	Crop	Apium graveolens	Apium graveolens	Apium graveolens
2016	1	Plastic	PE	PE	PE
2016	2	Crop			
2016	2	Plastic			
2016	3	Crop			
2016	3	Plastic			
2017	1	Crop	Brassica oleracea v.Italica	Brassica oleracea v.Italica	Brassica oleracea v.Italica
2017	1	Plastic	PE	PE	PE
2017	2	Crop			
2017	2	Plastic			
2018	1	Crop	Apium graveolens	Apium graveolens	Apium graveolens
2018	1	Plastic	PE	PE	PE

***Table S2.1 continued**: Farmers records of crops and plastic mulch applied from September 2011 to January 2018 for the three parcels of each organic farms (O1, O2, O3) and conventional farms (C1, C2, C3). Growing period are defined from the soil preparation to the crop harvest.*

			Farm O3		
Year	growing period		Parcel 1	Parcel 2	Parcel 3
2011	1	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2011	1	**Plastic**	PE	PE	PE
2011	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2011	2	**Plastic**			
2012	1	**Crop**	Cynara scolymus	Cynara scolymus	Cynara scolymus
2012	1	**Plastic**			
2012	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2012	2	**Plastic**	PE	PE	PE
2012	3	**Crop**			
2012	3	**Plastic**			
2013	1	**Crop**	Cynara scolymus	Cynara scolymus	Cynara scolymus
2013	1	**Plastic**			
2013	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2013	2	**Plastic**	PE	PE	PE
2014	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2014	1	**Plastic**	PE	PE	PE
2014	2	**Crop**	Cucurbita moschata	Cucurbita moschata	Cucurbita moschata
2014	2	**Plastic**	PE	PE	PE
2014	3	**Crop**	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes
2014	3	**Plastic**	PE	PE	PE
2015	1	**Crop**	Cucurbita moschata	Cucurbita moschata	Cucurbita moschata
2015	1	**Plastic**	PE	PE	PE
2015	2	**Crop**	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes
2015	2	**Plastic**	PE	PE	PE
2015	3	**Crop**	Zea mays	Zea mays	Zea mays
2015	3	**Plastic**			
2016	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2016	1	**Plastic**	PE	PE	PE
2016	2	**Crop**	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes	Brassica oleracea v.gongylodes
2016	2	**Plastic**	PE	PE	PE
2016	3	**Crop**	Zea mays	Zea mays	Zea mays
2016	3	**Plastic**			
2017	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2017	1	**Plastic**	PE	PE	PE
2017	2	**Crop**	Cucurbita moschata	Cucurbita moschata	Cucurbita moschata
2017	2	**Plastic**	PE	PE	PE
2018	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2018	1	**Plastic**	PE	PE	PE

***Table S2.1 continued**: Farmers records of crops and plastic mulch applied from September 2011 to January 2018 for the three parcels of each organic farms (O1, O2, O3) and conventional farms (C1, C2, C3). Growing period are defined from the soil preparation to the crop harvest.*

			Farm C1		
Year	growing period		Parcel 1	Parcel 2	Parcel 3
2011	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2011	1	**Plastic**	PE	PE	PE
2011	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2011	2	**Plastic**	PE	PE	PE
2012	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2012	1	**Plastic**	PE	PE	PE
2012	2	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2012	2	**Plastic**	PE	PE	PE
2012	3	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2012	3	**Plastic**	PE	PE	PE
2013	1	**Crop**	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2013	1	**Plastic**	PE	PE	PE
2013	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2013	2	**Plastic**	PE	PE	PE
2014	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2014	1	**Plastic**	PE	PE	PE
2014	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2014	2	**Plastic**	PE	PE	PE
2014	3	**Crop**			
2014	3	**Plastic**			
2015	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2015	1	**Plastic**	PE	PE	PE
2015	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2015	2	**Plastic**	PE	PE	PE
2015	3	**Crop**			
2015	3	**Plastic**			
2016	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2016	1	**Plastic**	PE	PE	PE
2016	2	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2016	2	**Plastic**	PE	PE	PE
2016	3	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2016	3	**Plastic**	PE	PE	PE
2017	1	**Crop**	Brassica oleracea v.Italica	Brassica oleracea v.Italica	Brassica oleracea v.Italica
2017	1	**Plastic**	PE	PE	PE
2017	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2017	2	**Plastic**	PE	PE	PE
2018	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2018	1	**Plastic**	PE	PE	PE

***Table S2.1 continued**: Farmers records of crops and plastic mulch applied from September 2011 to January 2018 for the three parcels of each organic farms (O1, O2, O3) and conventional farms (C1, C2, C3). Growing period are defined from the soil preparation to the crop harvest.*

			Farm C2		
Year	growing period		Parcel 1	Parcel 2	Parcel 3
2011	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2011	1	**Plastic**	PE	PE	PE
2011	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2011	2	**Plastic**	PE	PE	PE
2012	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2012	1	**Plastic**	PE	PE	PE
2012	2	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2012	2	**Plastic**	PE	PE	PE
2012	3	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2012	3	**Plastic**	PE	PE	PE
2013	1	**Crop**	Foeniculum vulgare	Foeniculum vulgare	Foeniculum vulgare
2013	1	**Plastic**	PE	PE	PE
2013	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2013	2	**Plastic**	PE	PE	PE
2014	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2014	1	**Plastic**	PE	PE	PE
2014	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2014	2	**Plastic**	PE	PE	PE
2014	3	**Crop**			
2014	3	**Plastic**			
2015	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2015	1	**Plastic**	PE	PE	PE
2015	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2015	2	**Plastic**	PE	PE	PE
2015	3	**Crop**			
2015	3	**Plastic**			
2016	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2016	1	**Plastic**	PE	PE	PE
2016	2	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2016	2	**Plastic**	PE	PE	PE
2016	3	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2016	3	**Plastic**	PE	PE	PE
2017	1	**Crop**	Brassica oleracea v.Italica	Brassica oleracea v.Italica	Brassica oleracea v.Italica
2017	1	**Plastic**	PE	PE	PE
2017	2	**Crop**	Cucumis melo	Cucumis melo	Cucumis melo
2017	2	**Plastic**	PE	PE	PE
2018	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2018	1	**Plastic**	PE	PE	PE

***Table S2.1 continued**: Farmers records of crops and plastic mulch applied from September 2011 to January 2018 for the three parcels of each organic farms (O1, O2, O3) and conventional farms (C1, C2, C3). Growing period are defined from the soil preparation to the crop harvest.*

			Farm C3		
Year	growing period		Parcel 1	Parcel 2	Parcel 3
2011	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2011	1	**Plastic**	PE	PE	PE
2011	2	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2011	2	**Plastic**	PE	PE	PE
2012	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2012	1	**Plastic**	PE	PE	PE
2012	2	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2012	2	**Plastic**	PE	PE	PE
2012	3	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2012	3	**Plastic**	PE	PE	PE
2013	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2013	1	**Plastic**	PE	PE	PE
2013	2	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2013	2	**Plastic**	PE	PE	PE
2014	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2014	1	**Plastic**	PE	PE	PE
2014	2	**Crop**	Brassica oleracea v.Italica	Brassica oleracea v.Italica	Brassica oleracea v.Italica
2014	2	**Plastic**	PE	PE	PE
2014	3	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2014	3	**Plastic**			
2015	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2015	1	**Plastic**	PE	PE	PE
2015	2	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2015	2	**Plastic**	PE	PE	PE
2015	3	**Crop**			
2015	3	**Plastic**			
2016	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2016	1	**Plastic**	PE	PE	PE
2016	2	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2016	2	**Plastic**	PE	PE	PE
2016	3	**Crop**			
2016	3	**Plastic**			
2017	1	**Crop**	Apium graveolens	Apium graveolens	Apium graveolens
2017	1	**Plastic**	PE	PE	PE
2017	2	**Crop**	Citrullus lanatus	Citrullus lanatus	Citrullus lanatus
2017	2	**Plastic**	PE	PE	PE
2018	1	**Crop**	Lactuca sativa	Lactuca sativa	Lactuca sativa
2018	1	**Plastic**	PE	PE	PE

***Table S2.2**: Farmers records of commercial pesticides applied and the dose of application from September 2016 to January 2018. All pesticides were applied at 1000 L/ ha.*

Farm	Parcel	Crop	Commercial name	Applied date	Applied dose	Pesticide ID
C1	311	Lettuce	Kerb flo	11/11/2016	2.5 cc/L	17845
C1	311	Lettuce	Cabrio duo	14/12/2016	2.5 cc/L	25408
C1	311	Lettuce	Enervin duo	14/12/2016	2.5 cc/L	25626
C1	311	Water melon	Cidely top	2/5/2017	0.1 cc/L	ES-00056
C1	311	Water melon	Cidely top	2/5/2018	0.1 cc/L	ES-00056
C1	311	Lettuce	Switch	24/11/2016	0.6 g/L	21714
C1	311	Lettuce	Cabrio duo	30/12/2016	2.5 cc/L	25408
C1	311	Lettuce	Stomp aqua	31/10/2016	2.5 cc/L	25580
C1	311	Lettuce	Karate zeon	4/1/2017	0.2 g/L	22398
C1	311	Lettuce	Revus	4/1/2017	0.6 g/L	25186
C1	311	Lettuce	Signum	4/1/2017	1.5 cc/L	23977
C1	311	Water melon	Vertimec	4/4/2018	1 cc/L	16784
C1	311	Water melon	Altacor 35 wg	4/6/2018	100 g/L	25296
C1	311	Water melon	Plenum 25 wp	4/6/2018	0.5 g/L	25047
C1	311	Water melon	Vivando	4/6/2018	0.2 g/L	24143
C1	312	Lettuce	Kerb flo	11/11/2016	2.5 cc/L	17845
C1	312	Lettuce	Cabrio duo	14/12/2016	2.5 cc/L	25408
C1	312	Lettuce	Enervin duo	14/12/2016	2.5 cc/L	25626
C1	312	Water melon	Cidely top	2/5/2017	0.1 cc/L	ES-00056
C1	312	Water melon	Cidely top	2/5/2018	0.1 cc/L	ES-00056
C1	312	Lettuce	Switch	24/11/2016	0.6 g/L	21714
C1	312	Lettuce	Cabrio duo	30/12/2016	2.5 cc/L	25408
C1	312	Lettuce	Stomp aqua	31/10/2016	2.5 cc/L	25580
C1	312	Lettuce	Karate zeon	4/1/2017	0.2 g/L	22398
C1	312	Lettuce	Revus	4/1/2017	0.6 g/L	25186
C1	312	Lettuce	Signum	4/1/2017	1.5 cc/L	23977
C1	312	Water melon	Vertimec	4/4/2018	1 cc/L	16784
C1	312	Water melon	Altacor 35 wg	4/6/2018	100 g/L	25296
C1	312	Water melon	Plenum 25 wp	4/6/2018	0.5 g/L	22021
C1	312	Water melon	Vivando	4/6/2018	0.2 g/L	24144
C1	313	Lettuce	Kerb flo	11/11/2016	2.5 cc/L	17845
C1	313	Lettuce	Cabrio duo	14/12/2016	2.5 cc/L	25408
C1	313	Lettuce	Enervin duo	14/12/2016	2.5 cc/L	25626
C1	313	Water melon	Cidely top	2/5/2017	0.1 cc/L	ES-00056
C1	313	Water melon	Cidely top	2/5/2018	0.1 cc/L	ES-00056
C1	313	Lettuce	Switch	24/11/2016	0.6 g/L	21714
C1	313	Lettuce	Cabrio duo	30/12/2016	2.5 cc/L	25408
C1	313	Lettuce	Stomp aqua	31/10/2016	2.5 cc/L	25580
C1	313	Lettuce	Karate zeon	4/1/2017	0.2 g/L	22398
C1	313	Lettuce	Revus	4/1/2017	0.6 g/L	25186
C1	313	Lettuce	Signum	4/1/2017	1.5 cc/L	23977
C1	313	Water melon	Vertimec	4/4/2018	1 cc/L	16784
C1	313	Water melon	Altacor 35 wg	4/6/2018	100 g/L	25296
C1	313	Water melon	Plenum 25 wp	4/6/2018	0.5 g/L	22021
C1	313	Water melon	Vivando	4/6/2018	0.2 g/L	24145

***Table S2.2 continued**: Farmers records of commercial pesticides applied and the dose of application from September 2016 to January 2018. All pesticides were applied at 1000 L/ ha.*

Farm	Parcel	Crop	Commercial name	Applied date	Applied dose	Pesticide ID
C2	321	Brocoli	Karate zeon	1/12/2017	0.2 g/L	22398
C2	321	Brocoli	Signum	1/12/2017	1.5 cc/L	23977
C2	321	Brocoli	Stomp aqua	18/9/2017	2.5 cc/L	25580
C2	321	Water melon	Cidely top	23/5/2017	0.1 cc/L	ES-00056
C2	321	Water melon	Teppeki	23/5/2017	10 g/L	24526
C2	321	Water melon	Altacor 35 wg	4/6/2017	100 g/L	25296
C2	322	Brocoli	Karate zeon	1/12/2017	0.2 g/L	22398
C2	322	Brocoli	Signum	1/12/2017	1.5 cc/L	23977
C2	322	Brocoli	Stomp aqua	18/9/2017	2.5 cc/L	25580
C2	322	Water melon	Cidely top	23/5/2017	0.1 cc/L	ES-00056
C2	322	Water melon	Teppeki	23/5/2017	10 g/L	24526
C2	322	Water melon	Altacor 35 wg	4/6/2017	100 g/L	25296
C2	323	Brocoli	Karate zeon	1/12/2017	0.2 g/L	22398
C2	323	Brocoli	Signum	1/12/2017	1.5 cc/L	23977
C2	323	Brocoli	Stomp aqua	18/9/2017	2.5 cc/L	25580
C2	323	Water melon	Cidely top	23/5/2017	0.1 cc/L	ES-00056
C2	323	Water melon	Teppeki	23/5/2017	10 g/L	24526
C2	323	Water melon	Altacor 35 wg	4/6/2017	100 g/L	25296
C3	331	Escarole	Movento 150	10/2/2018	0.4 cc/L	25295
C3	331	Sweet corn	Coragen 20 sc	11/5/2017	0.15 cc/L	25341
C3	331	Sweet corn	Karate zeon	11/5/2017	0.2 g/L	22398
C3	331	Sweet corn	Laudis wg	11/5/2017	0.5 cc/L	ES-00231
C3	331	Sweet corn	Laudis wg	13/3/2018	0.5 cc/L	ES-00231
C3	331	Escarole	Steward	14/2/2017	0.125 cc/L	22693
C3	331	Sweet corn	Decis evo	16/6/2017	0.5 cc/L	25838
C3	331	Escarole	Switch	2/3/2017	0.6 g/L	21714
C3	331	Escarole	Altacor 35 wg	21/11/2018	100 g/L	25296
C3	331	Escarole	Switch	21/11/2018	0.6 g/L	21714
C3	331	Escarole	Karate zeon	21/12/2018	0.2 g/L	22398
C3	331	Escarole	Altacor 35 wg	3/3/2017	100 g/L	25296
C3	331	Escarole	Karate zeon	3/3/2017	0.2 g/L	22398
C3	331	Escarole	Signum	3/3/2017	1.5 cc/L	23977
C3	331	Escarole	Signum	4/2/2019	1.5 cc/L	23977
C3	331	Escarole	Switch	4/2/2019	0.6 g/L	21714
C3	331	Sweet corn	Steward	4/5/2017	0.125 cc/L	22693
C3	331	Sweet corn	Decis evo	8/5/2018	0.5 cc/L	25838
C3	331	Escarole	Scatto	9/11/2018	0.5 g/L	ES-00012
C3	331	Escarole	Steward	9/11/2018	0.125 cc/L	22693
C3	332	Escarole	Movento 150	10/2/2018	0.4 cc/L	25296

***Table S2.2 continued**: Farmers records of commercial pesticides applied and the dose of application from September 2016 to January 2018. All pesticides were applied at 1000 L/ ha.*

Farm	Parcel	Crop	Commercial name	Applied date	Applied dose	Pesticide ID
C3	332	Sweet corn	Coragen 20 sc	11/5/2017	0.15 cc/L	25341
C3	332	Sweet corn	Karate zeon	11/5/2017	0.2 g/L	22398
C3	332	Sweet corn	Laudis wg	11/5/2017	0.5 cc/L	ES-00231
C3	332	Sweet corn	Laudis wg	13/3/2018	0.5 cc/L	ES-00231
C3	332	Escarole	Steward	14/2/2017	0.125 cc/L	22693
C3	332	Sweet corn	Decis evo	16/6/2017	0.5 cc/L	25838
C3	332	Escarole	Switch	2/3/2017	0.6 g/L	21714
C3	332	Escarole	Altacor 35 wg	21/11/2018	100 g/L	25296
C3	332	Escarole	Switch	21/11/2018	0.6 g/L	21714
C3	332	Escarole	Karate zeon	21/12/2018	0.2 g/L	22398
C3	332	Escarole	Altacor 35 wg	3/3/2017	100 g/L	25296
C3	332	Escarole	Karate zeon	3/3/2017	0.2 g/L	22398
C3	332	Escarole	Signum	3/3/2017	1.5 cc/L	23977
C3	332	Escarole	Signum	4/2/2019	1.5 cc/L	23977
C3	332	Escarole	Switch	4/2/2019	0.6 g/L	21714
C3	332	Sweet corn	Steward	4/5/2017	0.125 cc/L	22693
C3	332	Sweet corn	Decis evo	8/5/2018	0.5 cc/L	25838
C3	332	Escarole	Scatto	9/11/2018	0.5 g/L	ES-00012
C3	332	Escarole	Steward	9/11/2018	0.125 cc/L	22693
C3	333	Escarole	Movento 150	10/2/2018	0.4 cc/L	25297
C3	333	Sweet corn	Coragen 20 sc	11/5/2017	0.15 cc/L	25341
C3	333	Sweet corn	Karate zeon	11/5/2017	0.2 g/L	22398
C3	333	Sweet corn	Laudis wg	11/5/2017	0.5 cc/L	ES-00231
C3	333	Sweet corn	Laudis wg	13/3/2018	0.5 cc/L	ES-00231
C3	333	Escarole	Steward	14/2/2017	0.125 cc/L	22693
C3	333	Sweet corn	Decis evo	16/6/2017	0.5 cc/L	25838
C3	333	Escarole	Switch	2/3/2017	0.6 g/L	21714
C3	333	Escarole	Altacor 35 wg	21/11/2018	100 g/L	25296
C3	333	Escarole	Switch	21/11/2018	0.6 g/L	21714
C3	333	Escarole	Karate zeon	21/12/2018	0.2 g/L	22398
C3	333	Escarole	Altacor 35 wg	3/3/2017	100 g/L	25296
C3	333	Escarole	Karate zeon	3/3/2017	0.2 g/L	22398
C3	333	Escarole	Signum	3/3/2017	1.5 cc/L	23977
C3	333	Escarole	Signum	4/2/2019	1.5 cc/L	23977
C3	333	Escarole	Switch	4/2/2019	0.6 g/L	21714
C3	333	Sweet corn	Steward	4/5/2017	0.125 cc/L	22693
C3	333	Sweet corn	Decis evo	8/5/2018	0.5 cc/L	25838
C3	333	Escarole	Scatto	9/11/2018	0.5 g/L	ES-00012
C3	333	Escarole	Steward	9/11/2018	0.125 cc/L	22693

Table S2.3: *Pesticides applied in the farms commercial names, composition in active substances and recommended dose based on the registered pesticides at the Spanish Minister of Agriculture.*

Commercial name	Pesticide ID	Active substances composition	Recommended dose
Altacor 35 wg	25296	Clorantraniliprol 35 %	100 - 115 g/L
Cabrio duo	25408	Dimetomorf 7,2% + Piraclostrobin 4% [ec] p/v	2 - 2.5 L/ha
Cidely top	ES-00056	Difenoconazol 12,5% + Ciflufenamid 1,5% [dc] p/v	0.10%
Coragen 20 sc	25341	Clorantraniliprol 20% [sc] p/v	0.1 - 0.15 L/ha
Decis evo	25838	Deltametrina 2,5 %	0.3 - 0.5 L/ha
Enervin duo	25626	Ametoctradin 30% + Dimetomorf 22,5%	0.1 - 0.25 L/HL
Karate zeon	22398	Lambda-Cihalotrin 10 %	0.1 - 0.2 g/L
Kerb flo	17845	Propizamida 40% [sc] p/v	1.75 -3.75 L/ha
Laudis wg	ES-00231	Tembotriona 20% [wg] p/p	0.3 -0.5 kg/ha
Movento 150	25295	Spirotetramat 15% [od]	0.1 -0.5 L/ha
Plenum 25 wp	25047	Pimetrocina 70 % [wp] p/p	40 - 120 g/HL
Revus	25186	Mandipropamid 25% [sc] p/v	0.4 -0.6 %
Revus	25186	Mandipropamid 25% [sc] p/v	0.4 -0.6 %
Scatto	ES-00012	Deltametrina 2,5 %	0.3 - 0.5 g/L
Signum	23977	Piraclostrobin 6,7% + Boscalid 26,7% [wg] p/p	1 -1.5 Kg/ha
Steward	22693	Indoxacarb 30% [wg] p/p	125 g/ha
Stomp aqua	25580	Pendimetalina 45,5% [cs] p/v	2-3 L/ha
Switch	21714	Ciprodinil 37,5% + Fludioxonil 25% [wg] p/p	0.5 -0.6 Kg/ha
Teppeki	24526	Flonicamid 50% [wg] p/p	10 g/HL
Vertimec	16784	Abamectina 1,8%	0.08 -0.1 %
Vivando	24145	Metrafenona 50% [sc] p/v	0.03%

***Table S2.4**: Type (either fungicide, herbicide or insecticide), LOQ associated to the method used and properties for the 38 active substances tested. The molar mass, DT50 in soil and octanol-water partition coefficient at pH 7, 20 °C (log P) were obtained from the Pesticide Properties DataBase (PPDB, 2019).*

Active substance	LOD [ng.g^{-1}]	Method	Type	Molar mass [g.mol^{-1}]	DT50 in soil [days]	log.P [-]
Ametoctradin	11	LC	Fungicide	275.39	1.8	4.4
Azadirachtin	11	LC	Insecticide	720.721	8	1.09
Azoxystrobin	1.1	LC	Fungicide	403.4	84.5	2.5
Boscalid	1.1	LC	Fungicide	403.4	84.5	2.96
Chlorantraniliprole	1.1	LC	Insecticide	343.21	204	2.86
Chlorimuron.ethyl	1.1	LC	Herbicide	414.82	40	0.11
Chlorothalonil	22	GC	Fungicide	265.91	3.53	2.94
Chlorpyrifos	4.4	LC	Insecticide	350.58	386	4.7
Cyflufenamid	1.1	LC	Fungicide	412.36	25.3	4.7
Cyfluthrin	11	LC	Insecticide	434.29	51	6
Cymoxanil	4.4	LC	Fungicide	198.18	1.4	0.67
Cypermethrin	11	LC	Insecticide	416.3	70	5.55
Deltamethrin	11	LC	Insecticide	505.2	26	4.6
Difenoconazole	1.1	LC	Fungicide	406.26	130	4.36
Dimethomorph	1.1	LC	Fungicide	387.86	56.7	2.68
Emamectin	1.1	LC	Insecticide	886.133	46	5
Etoxazole	4.4	LC	Insecticide	359.42	19.3	5.52
Fenhexamid	2.2	LC	Fungicide	302.2	0.43	3.51
Flonicamid	1.1	LC	Insecticide	229.16	1.1	-0.24
Fluazinam	11	LC	Fungicide	465.14	72.5	4.03
Flufenoxuron	1.1	LC	Insecticide	488.77	72.5	5.11
Fluopicolide	1.1	LC	Fungicide	383.58	138.8	2.9
Folpet	4.4	GC	Fungicide	296.56	4.7	3.02
Imidacloprid	2.2	LC	Insecticide	255.66	187	0.57
Indoxacarb	1.1	LC	Insecticide	527.83	113.2	4.65
Kresoxim.methyl	11	LC	Fungicide	313.35	0.87	3.4
Lambda.Cyhalothrin	4.4	LC	Insecticide	449.85	175	5.5
Linuron	11	LC	Herbicide	249.09	57.6	3
Metalaxyl	11	LC	Fungicide	279.33	36	1.75
Metrafenone	4.4	LC	Fungicide	409.3	62	4.3
Metribuzin	2.2	LC	Herbicide	214.29	11.5	1.65
Oxyfluorfen	2.2	LC	Herbicide	361.7	138	4.86
Pendimethalin	4.4	LC	Herbicide	281.31	182.3	5.4
Pirimicarb	1.1	LC	Insecticide	238.39	86	1.7
Propamocarb	2.2	LC	Fungicide	188.3	14	0.84
Propyzamide	4.4	LC	Herbicide	256.13	50.5	3.27
Pyraclostrobin	2.2	LC	Fungicide	387.8	62	3.99
Spinosyn.A	1.1	LC	Insecticide	731.968	13	4
Spinosyn.D	1.1	LC	Insecticide	745.995	13	4
Spirotetramat	1.1	LC	Insecticide	373.48	0.19	2.51
Thiacloprid	1.1	LC	Insecticide	252.72	1.3	1.26

***Table S2.5**: Pair-wise comparisons of the six different farms (O1, O2, O3, C1, C2, C3) for the 18 measured soil physicochemical parameters. When the data was normally distributed, an Anova followed by a pair-wise comparison with T-test was performed. When the data was not normally distributed, a Kruskal-Wallis test followed by a pair-wise comparison with the Wilcoxon rank sum test was performed. p-values are adjusted with the Holm's methods. We only show p-value > 0.05*

Parameter	Method		grp1	grp2	p-value
Total carbon [%]	T-test		O1	O2	0.04
Total carbon [%]	T-test		O1	O3	0.04
Total carbon [%]	T-test		O1	C1	1.4E-05
Total carbon [%]	T-test		O1	C2	0.016
Total carbon [%]	T-test		O3	C1	0.00026
Total carbon [%]	T-test		C1	C2	0.035
Total carbon [%]	T-test		C1	C3	0.027
Total nitrogen [%]	T-test		O1	O3	7.40E-07
Total nitrogen [%]	T-test		O1	C3	1.20E-05
Total nitrogen [%]	T-test		O2	O3	0.001
Total nitrogen [%]	T-test		O2	C3	0.0011
Total nitrogen [%]	T-test		O3	C1	0.0084
Total nitrogen [%]	T-test		O3	C2	2.00E-04
Total nitrogen [%]	T-test		C1	C3	0.0054
Total nitrogen [%]	T-test		C2	C3	2.00E-04
Total organic carbon [%]	Wilcoxon		O1	O3	0.00062
Total organic carbon [%]	Wilcoxon		O2	O3	0.00062
Total organic carbon [%]	Wilcoxon		O3	C1	0.0035
Total organic carbon [%]	Wilcoxon		O3	C2	0.0011
Field Capacity [w/w]	Wilcoxon		O1	O3	0.0052
Field Capacity [w/w]	Wilcoxon		O1	C3	0.0052
Field Capacity [w/w]	Wilcoxon		O2	C3	0.0052
Field Capacity [w/w]	Wilcoxon		O3	C3	0.0052
Field Capacity [w/w]	Wilcoxon		C1	C3	0.0052
Field Capacity [w/w]	Wilcoxon		C2	C3	0.0052
Ph [-]	T-test		O1	O2	0.0015
Ph [-]	T-test		O1	O3	0.0046
Ph [-]	T-test		O1	C1	0.0083
Ph [-]	T-test		O1	C3	0.0095
Ph [-]	T-test		O2	C1	1.30E-06
Ph [-]	T-test		O2	C2	0.00039
Ph [-]	T-test		O3	C1	1.20E-07
Ph [-]	T-test		O3	C2	0.0015
Ph [-]	T-test		C1	C3	0.00062
Ph [-]	T-test		C2	C3	0.002
EC [µS/cm]	Wilcoxon		O1	C1	0.041
EC [µS/cm]	Wilcoxon		O1	C3	0.041

***Table S2.5 continued**: Pair-wise comparisons of the six different farms (O1, O2, O3, C1, C2, C3) for the 18 measured soil physicochemical parameters. When the data was normally distributed, an Anova followed by a pair-wise comparison with T-test was performed. When the data was not normally distributed, a Kruskal-Wallis test followed by a pair-wise comparison with the Wilcoxon rank sum test was performed. p-values are adjusted with the Holm's methods. We only show p-value > 0.05*

Parameter	Method	grp1	grp2	p-value
CEC [cmol charge/ kg soil]	Wilcoxon	O1	O3	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	O1	C3	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	O2	O3	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	O2	C1	0.0086
CEC [cmol charge/ kg soil]	Wilcoxon	O2	C2	0.017
CEC [cmol charge/ kg soil]	Wilcoxon	O2	C3	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	O3	C1	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	O3	C2	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	C1	C3	0.00062
CEC [cmol charge/ kg soil]	Wilcoxon	C2	C3	0.00062
NO3 [mg/ kg soil]	Wilcoxon	O1	C3	0.015
NO3 [mg/ kg soil]	Wilcoxon	O2	O3	0.012
NO3 [mg/ kg soil]	Wilcoxon	O2	C3	0.005
NO3 [mg/ kg soil]	Wilcoxon	O3	C1	0.011
NO3 [mg/ kg soil]	Wilcoxon	C1	C3	0.0048
NH4 [mg/ kg soil]	T-test	O1	C1	6.70E-08
NH4 [mg/ kg soil]	T-test	O1	C2	1.40E-05
NH4 [mg/ kg soil]	T-test	O1	C3	5.80E-06
NH4 [mg/ kg soil]	T-test	O2	O3	0.03
NH4 [mg/ kg soil]	T-test	O2	C1	2.60E-07
NH4 [mg/ kg soil]	T-test	O2	C2	0.00011
NH4 [mg/ kg soil]	T-test	O2	C3	3.60E-06
NH4 [mg/ kg soil]	T-test	O3	C1	6.80E-07
NH4 [mg/ kg soil]	T-test	O3	C2	3.60E-06
NH4 [mg/ kg soil]	T-test	O3	C3	3.20E-05
NH4 [mg/ kg soil]	T-test	C1	C3	0.0025
Na [mg/ kg soil]	Wilcoxon	O1	O3	0.00062
Na [mg/ kg soil]	Wilcoxon	O1	C1	0.033
Na [mg/ kg soil]	Wilcoxon	O2	O3	0.00062
Na [mg/ kg soil]	Wilcoxon	O2	C1	0.00062
Ca [mg/ kg soil]	Wilcoxon	O1	O2	0.0055
Ca [mg/ kg soil]	Wilcoxon	O1	O3	0.00062
Ca [mg/ kg soil]	Wilcoxon	O1	C3	0.00062
Ca [mg/ kg soil]	Wilcoxon	O2	O3	0.00062
Ca [mg/ kg soil]	Wilcoxon	O2	C3	0.00062
Ca [mg/ kg soil]	Wilcoxon	O3	C1	0.00062
Ca [mg/ kg soil]	Wilcoxon	O3	C2	0.00062
Ca [mg/ kg soil]	Wilcoxon	C1	C3	0.00062
Ca [mg/ kg soil]	Wilcoxon	C2	C3	0.00062
K [mg/ kg soil]	Wilcoxon	O1	C2	0.011
K [mg/ kg soil]	Wilcoxon	O2	O3	0.011
K [mg/ kg soil]	Wilcoxon	O2	C2	0.0012

***Table S2.5 continued**: Pair-wise comparisons of the six different farms (O1, O2, O3, C1, C2, C3) for the 18 measured soil physicochemical parameters. When the data was normally distributed, an Anova followed by a pair-wise comparison with T-test was performed. When the data was not normally distributed, a Kruskal-Wallis test followed by a pair-wise comparison with the Wilcoxon rank sum test was performed. p-values are adjusted with the Holm's methods. We only show p-value > 0.05*

Parameter	Method	grp1	grp2	p-value
WDPT [s]	Wilcoxon	O1	C1	0.0052
WDPT [s]	Wilcoxon	O1	C2	0.0052
WDPT [s]	Wilcoxon	O1	C3	0.0052
WDPT [s]	Wilcoxon	O2	O3	0.0052
WDPT [s]	Wilcoxon	O2	C1	0.0052
WDPT [s]	Wilcoxon	O2	C2	0.0052
WDPT [s]	Wilcoxon	O2	C3	0.0052
WDPT [s]	Wilcoxon	O3	C1	0.0052
WDPT [s]	Wilcoxon	O3	C2	0.0052
WDPT [s]	Wilcoxon	O3	C3	0.0052
CLAY [%]	T-test	O1	O2	0.012
CLAY [%]	T-test	O1	O3	0.00021
CLAY [%]	T-test	O1	C3	0.0088
CLAY [%]	T-test	O2	O3	1.40E-05
CLAY [%]	T-test	O2	C1	0.0022
CLAY [%]	T-test	O2	C2	0.012
CLAY [%]	T-test	O2	C3	8.20E-05
CLAY [%]	T-test	O3	C1	0.014
CLAY [%]	T-test	O3	C2	0.00023
CLAY [%]	T-test	C1	C3	0.045
CLAY [%]	T-test	C2	C3	0.0088
Dry bulk density [kg/m3]	Wilcoxon	O1	C1	0.0052
Dry bulk density [kg/m3]	Wilcoxon	O1	C2	0.0052
Dry bulk density [kg/m3]	Wilcoxon	C1	C3	0.0052
Dry bulk density [kg/m3]	Wilcoxon	C2	C3	0.0052
Mg [mg/ kg soil]	Wilcoxon	O1	O2	0.039
Mg [mg/ kg soil]	Wilcoxon	O1	O3	0.00062
Mg [mg/ kg soil]	Wilcoxon	O1	C1	0.0035
Mg [mg/ kg soil]	Wilcoxon	O1	C2	0.024
Mg [mg/ kg soil]	Wilcoxon	O1	C3	0.00062
Mg [mg/ kg soil]	Wilcoxon	O2	O3	0.00062
Mg [mg/ kg soil]	Wilcoxon	O2	C3	0.00062
Mg [mg/ kg soil]	Wilcoxon	O3	C1	0.00062
Mg [mg/ kg soil]	Wilcoxon	O3	C2	0.00062
Mg [mg/ kg soil]	Wilcoxon	C1	C3	0.00062
Mg [mg/ kg soil]	Wilcoxon	C2	C3	0.00062
Hydraulic conductivity [cm/s]	Wilcoxon	O1	C1	0.041
Hydraulic conductivity [cm/s]	Wilcoxon	O2	C1	0.041
Porosity [-]	Wilcoxon	O1	C1	0.0052
Porosity [-]	Wilcoxon	O2	C1	0.0052
Porosity [-]	Wilcoxon	O3	C1	0.0052
Porosity [-]	Wilcoxon	C1	C3	0.0052

Table S2.6 *Total applied active substances (AS) dose, expected maximum AS content in soil (E), measured AS content in soil (M) and difference E-M. For better visualisation we classified the results E-M as >=0 or <0. Measured AS content in soil (M) should theoretically not be higher than the expected maximum AS content in soil (E): E-M is expected to be >=0*

Farm	Parcel	Active substance	Number of times applied	Total application dose	Application dose units	Content expected in soil (E) [mg/kg]	Content measured in soil (M) [mg/kg]	E-M
C1	311	Cypermethrin		Not recorded as applied			< LOQ	>=0
C1	311	Oxyfluorfen		Not recorded as applied			< LOQ	>=0
C1	311	Boscalid	1	0.4005	L/ha	0.29	0.0069	>=0
C1	311	Pendimethalin	1	1.1375	L/ha	0.81	< LOQ	>=0
C1	311	Other	13	3.5195	-	2.51	0.0071	>=0
C1	312	Boscalid	1	0.4005	L/ha	0.29	0.0599	>=0
C1	312	Pendimethalin	1	1.1375	L/ha	0.81	< LOQ	>=0
C1	312	Other	13	3.5195	-	2.51	0.0127	>=0
C1	313	Cypermethrin		Not recorded as applied			< LOQ	>=0
C1	313	Oxyfluorfen		Not recorded as applied			< LOQ	>=0
C1	313	Boscalid	1	0.4005	L/ha	0.29	0.0015	>=0
C1	313	Difenoconazole	1	0.0125	L/ha	0.01	0.0059	>=0
C1	313	Pendimethalin	1	1.1375	L/ha	0.81	< LOQ	>=0
C1	313	Other	13	3.5195	-	2.51	0.0093	>=0
C2	321	Cypermethrin		Not recorded as applied			< LOQ	>=0
C2	321	Oxyfluorfen		Not recorded as applied			< LOQ	>=0
C2	321	Boscalid	1	0.4005	L/ha	0.29	0.0045	>=0
C2	321	Chlorantraniliprole	1	35	kg/ha	25.00	0.0327	>=0
C2	321	Pendimethalin	1	1.1375	L/ha	0.81	< LOQ	>=0
C2	321	Other	4	5.122	-	3.66	0.0040	>=0
C2	322	Boscalid	1	0.4005	L/ha	0.29	0.0030	>=0
C2	322	Chlorantraniliprole	1	35	kg/ha	25.00	0.0028	>=0
C2	322	Pendimethalin	1	1.1375	L/ha	0.81	0.0000	>=0
C2	322	Other	4	5.122	-	3.66	0.0032	>=0
C2	323	Cypermethrin		Not recorded as applied			< LOQ	>=0
C2	323	Boscalid	1	0.4005	L/ha	0.29	< LOQ	>=0
C2	323	Chlorantraniliprole	1	35	kg/ha	25.00	0.0046	>=0
C2	323	Pendimethalin	1	1.1375	L/ha	0.81	< LOQ	>=0
C2	323	Other	4	5.122	-	3.66	0.0000	>=0
C3	331	Cypermethrin		Not recorded as applied			< LOQ	>=0
C3	331	Imidacloprid		Not recorded as applied			< LOQ	>=0
C3	331	Oxyfluorfen		Not recorded as applied			< LOQ	>=0
C3	331	Pendimethalin		Not recorded as applied			< LOQ	>=0
C3	331	Boscalid	1	0.4005	L/ha	0.29	0.1416	>=0
C3	331	Chlorantraniliprole	2	35.03	kg/ha	25.02	0.0347	>=0
C3	331	Other	10	0.763	-	0.55	0.0108	>=0
C3	332	Cypermethrin		Not recorded as applied			< LOQ	>=0
C3	332	Oxyfluorfen		Not recorded as applied			< LOQ	>=0
C3	332	Boscalid	1	0.4005	L/ha	0.29	0.0869	>=0
C3	332	Chlorantraniliprole	2	35.03	kg/ha	25.02	0.0164	>=0
C3	332	Other	10	0.763	-	0.55	0.0198	>=0

Table S2.6 continued *Total applied active substances (AS) dose, expected maximum AS content in soil (E), measured AS content in soil (M) and difference E-M. For better visualisation we classified the results E-M as >=0 or <0. Measured AS content in soil (M) should theoretically not be higher than the expected maximum AS content in soil (E): E-M is expected to be >=0*

Farm	Parcel	Active substance	Number of times applied	Total application dose	Application dose units	Conten expected in soil (E) [mg/kg]	Content measured in soil (M) [mg/kg]	E-M
C3	333	Oxyfluorfen		Not recorded as applied			< LOQ	>=0
C3	333	Boscalid	1	0.4005	L/ha	0.29	0.0058	>=0
C3	333	Chlorantraniliprole	2	35.03	kg/ha	25.02	0.0174	>=0
C3	333	Other	10	0.763	-	0.55	0.0096	>=0
C1	311	Azoxystrobin		Not recorded as applied			0.002	<0
C1	311	Chlorantraniliprole		Not recorded as applied			0.020	<0
C1	311	Imidacloprid		Not recorded as applied			0.000	<0
C1	311	Difenoconazole	1	0.0125	L/ha	0.009	0.010	<0
C1	312	Azoxystrobin		Not recorded as applied			0.032	<0
C1	312	Chlorantraniliprole		Not recorded as applied			0.001	<0
C1	312	Cypermethrin		Not recorded as applied			0.024	<0
C1	312	Imidacloprid		Not recorded as applied			0.048	<0
C1	312	Oxyfluorfen		Not recorded as applied			0.130	<0
C1	312	Difenoconazole	1	0.0125	L/ha	0.009	0.021	<0
C1	313	Azoxystrobin		Not recorded as applied			0.004	<0
C1	313	Chlorantraniliprole		Not recorded as applied			0.019	<0
C1	313	Imidacloprid		Not recorded as applied			0.003	<0
C2	321	Azoxystrobin		Not recorded as applied			0.010	<0
C2	321	Imidacloprid		Not recorded as applied			0.002	<0
C2	321	Difenoconazole	1	0.0125	L/ha	0.009	0.022	<0
C2	322	Azoxystrobin		Not recorded as applied			0.023	<0
C2	322	Cypermethrin		Not recorded as applied			0.005	<0
C2	322	Imidacloprid		Not recorded as applied			0.016	<0
C2	322	Oxyfluorfen		Not recorded as applied			0.331	<0
C2	322	Difenoconazole	1	0.0125	L/ha	0.009	0.043	<0
C2	323	Azoxystrobin		Not recorded as applied			0.008	<0
C2	323	Imidacloprid		Not recorded as applied			0.009	<0
C2	323	Oxyfluorfen		Not recorded as applied			0.152	<0
C2	323	Difenoconazole	1	0.0125	L/ha	0.009	0.030	<0
C3	331	Azoxystrobin		Not recorded as applied			0.065	<0
C3	331	Difenoconazole		Not recorded as applied			0.002	<0
C3	332	Azoxystrobin		Not recorded as applied			0.005	<0
C3	332	Difenoconazole		Not recorded as applied			0.005	<0
C3	332	Imidacloprid		Not recorded as applied			0.002	<0
C3	332	Pendimethalin		Not recorded as applied			0.104	<0
C3	333	Azoxystrobin		Not recorded as applied			0.005	<0
C3	333	Cypermethrin		Not recorded as applied			0.005	<0
C3	333	Difenoconazole		Not recorded as applied			0.001	<0
C3	333	Imidacloprid		Not recorded as applied			0.001	<0
C3	333	Pendimethalin		Not recorded as applied			0.024	<0

***Table S2.7**: Pair-wise comparison of the bacterial and fungal communities in each farm with the Anosim test. P-values <0.05 are highlighted.*

Farms	Bacteria	Fungi
O1-O2	0.0016	0.1492
O1-O3	0.0001	0.0019
O1-C1	0.0002	0.009
O1-C2	0.0054	0.0028
O1-C3	0.0003	0.003
O2-C3	0.0004	0.0024
O2-C1	0.0002	0.0853
O2-C2	0.004	0.0054
O2-C3	0.0023	0.003
O3-C1	0.0001	0.0002
O3-C2	0.0002	0.0001
O3-C3	0.0004	0.0002
C1-C2	0.0004	0.1272
C1-C3	0.0001	0.0024
C2-C3	0.0366	0.0002

***Table S2.8**: Permanova analysis of the parameters contribution to the bacteria land fungal communities variations. Only parameters with a p.value > 0.05 are shown*

A. Bacteria

Parameter	p.value
NH4	0.001
N.tot	0.003
C.org	0.004
Azoxystrobin (F)	0.004
pH	0.006
CEC	0.006
Porosity	0.007
WDPT	0.009
FC	0.013
NO3	0.014
pb	0.015
AS.content	0.015
Plastic.Area	0.027
Chlorantraniliprole (I)	0.037

B. Fungi

Parameter	p.value
N.tot	0.001
FC	0.001
NH4	0.001
C.org	0.002
Boscalid (F)	0.02
CEC	0.021
WDPT	0.021
C.tot	0.029
Na	0.032
NO3	0.046

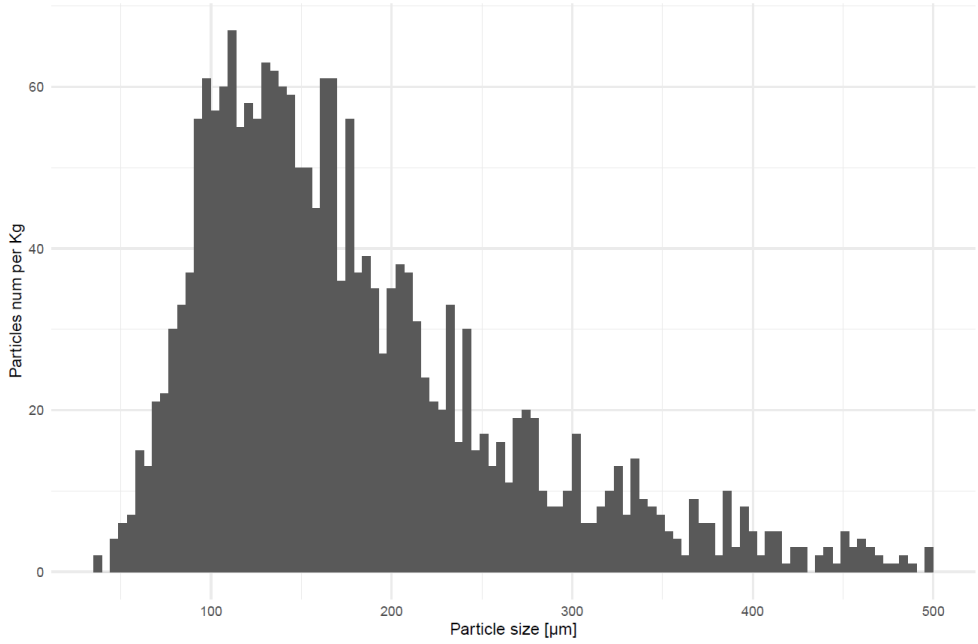


***Figure S2.1**: Plastic size distribution. Only particles< 500 µm are showed for better visualisation. Based on this result we applied a threshold of 100 µm for the rest of the analysis.*

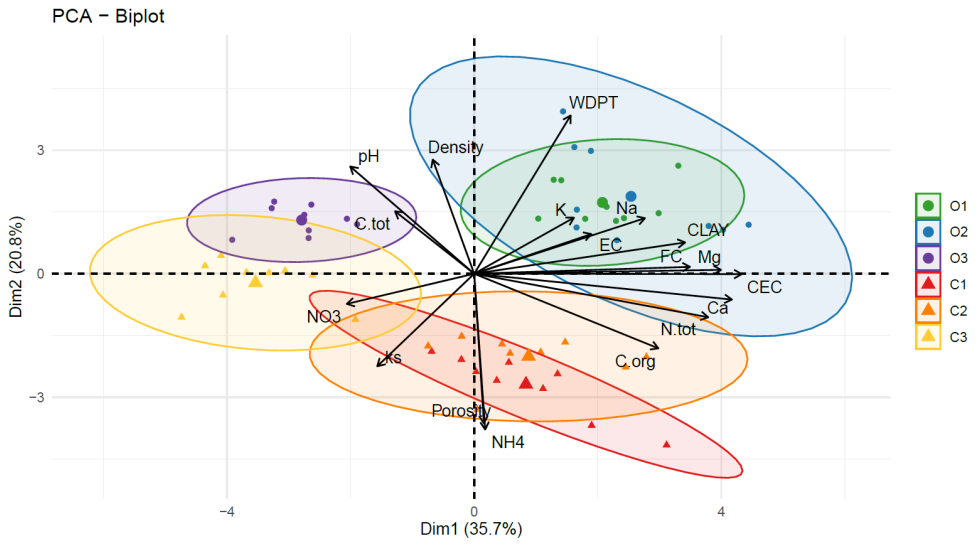


***Figure S2.2**: Principal component analysis for the soil physicochemical parameters*

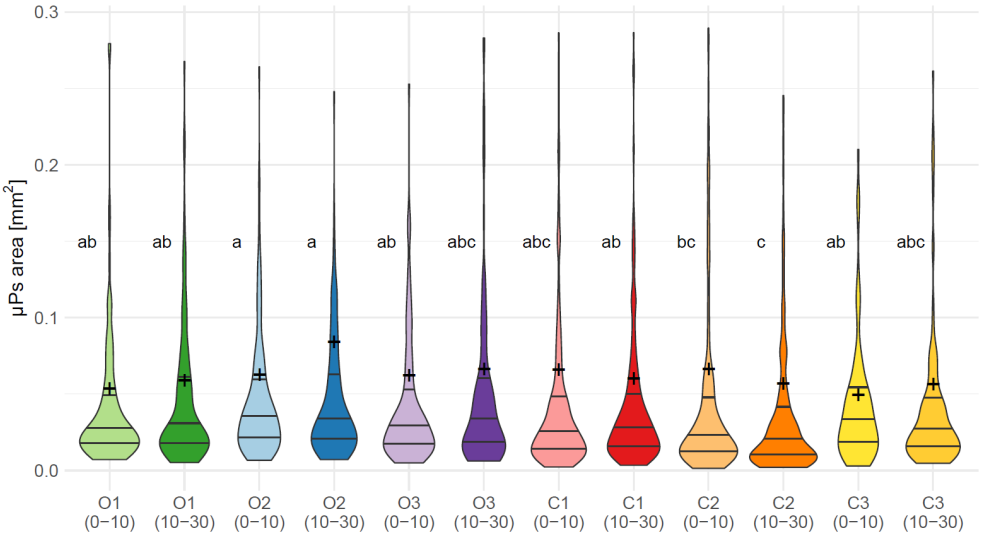


***Figure S2.3**: Microplastic particles' area distribution for all the farms for both the top soil (0–10 cm) and the deeper depth (10–30 cm). Only particles < 0.3 mm² are plotted for a better visualization. The cross indicates the mean particle area and horizontal black lines indicate respectively area for 25%, 50%, and 75% of the samples. Soils that do not share letters are significantly different from each other (Kolmogorov-Smirnov test with p < 0.05).*

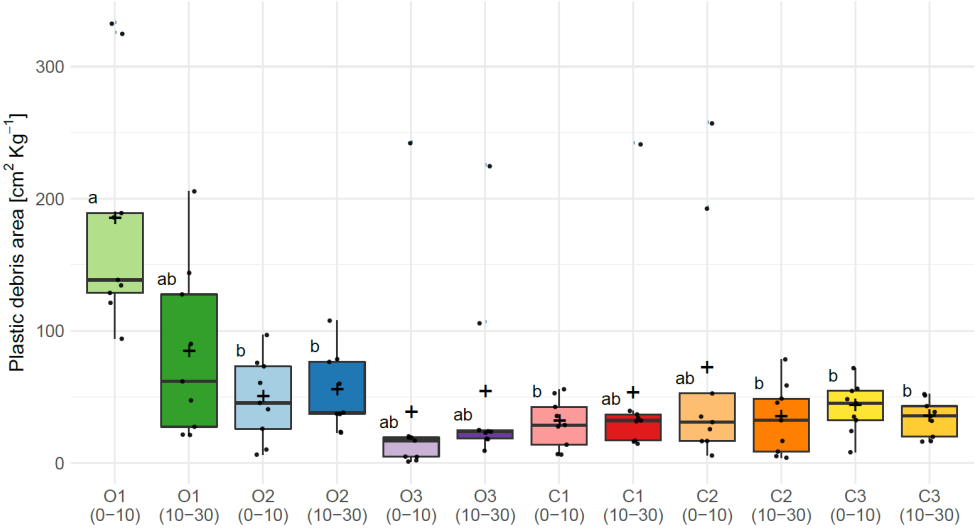


***Figure S2.4**: Total area of plastic particles (>100 µm and < 50mm) per kg of soil in organic (O) and conventional (C) farms10 for both the top soil (0–10 cm) and the subsurface soil (10–30 cm). The box plot (horizontal lines) represents content for at least 25%, 50% and 75% of the samples. The vertical black line ends represent the minimum and maximum values. The cross represents the average content of any given*

sample group. The dots represent individual measurements. Soils that do not share letters are significantly different from each other (Wilcoxon test with p < 0.05).

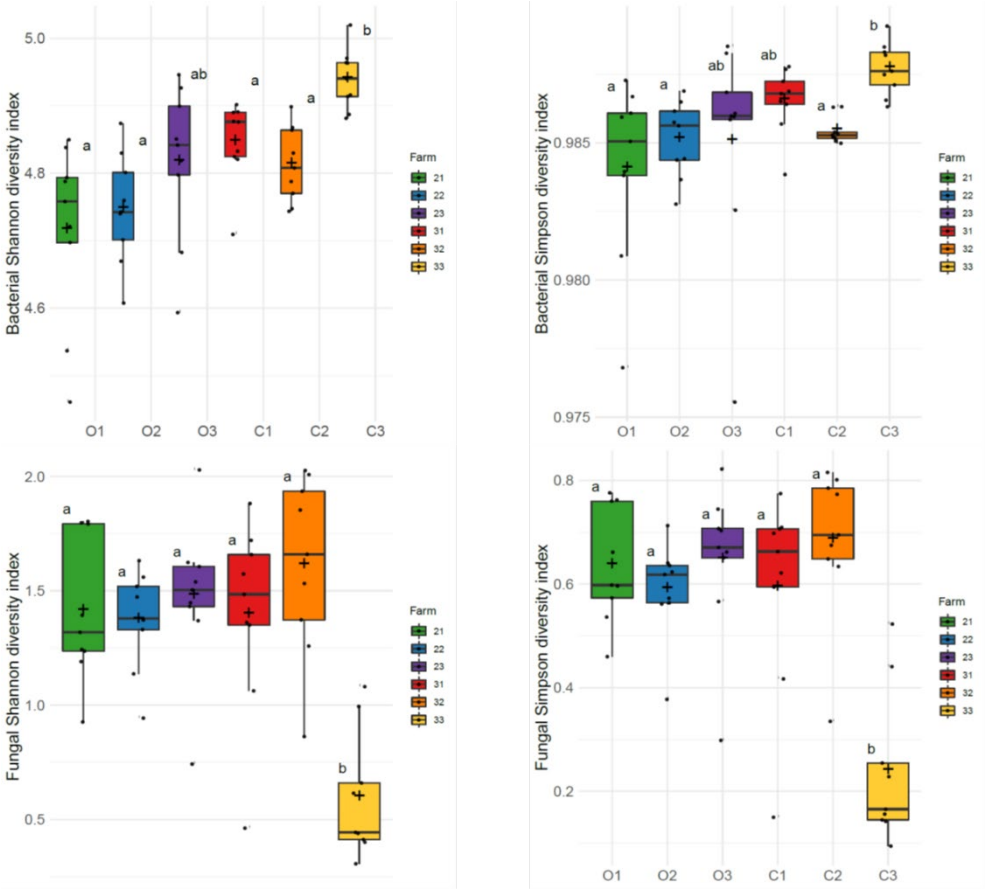


***Figure S2.5**: Bacterial (top) and fungal (bottom) alpha diversity Shannon (left) and Simpson (right) indexes for each organic (O1, O2, O3) and conventional (C1, C2, C3) farms. The box plot (horizontal lines) represents content for at least 25%, 50% and 75% of the samples. The vertical black line denotes the minimum and maximum values, excluding outliers (1.5 IQR method). The cross represents the average content of any given sample group. The dots represent individual measurements. Soils that do not share letters are significantly different from each other (Wilcoxon test with p < 0.05).*

Macro S2.1: Macro implemented in ImageJ 1.52 in order to analyse the plastic particles from a batch of preliminary processed pictures with Adobe Photoshop CC 2018

```
run("8-bit");
run("Set Scale...", "distance=0 known=0 unit=pixel");
setAutoThreshold("Default");
setThreshold(0, 245);
setOption("BlackBackground", false);
run("Convert to Mask");
run("Fill Holes");
run("Set Measurements...", "area centroid perimeter fit shape feret's display
redirect=None decimal=3");
run("Analyze Particles...", "size=4-Infinity pixel display summarize");
close();
```

Chapter 3

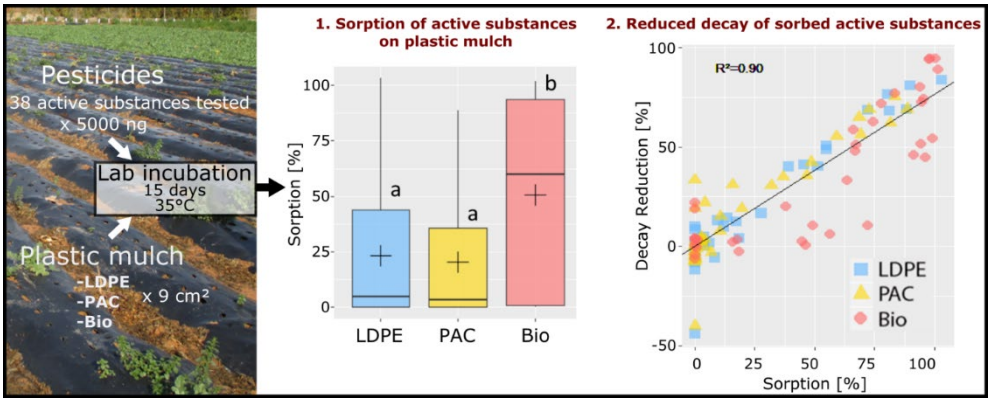
A laboratory comparison of the interactions between three plastic mulch types and 38 active substances found in pesticides

Based on:

Beriot, N., Zomer, P., Zornoza, R., Geissen, V. 2020. A laboratory comparison of the interactions between three plastic mulch types and 38 active substances found in pesticides. PeerJ 8:e9876

Abstract

In semi-arid regions, the use of plastic mulch and pesticides in conventional agriculture is nearly ubiquitous. Although the sorption of pesticides on Low Density Polyethylene (LDPE) has been previously studied, no data are available for other plastics such as Pro-oxidant Additive Containing (PAC) plastics or "biodegradable" (BIO) plastics. The aim of this research was to measure the sorption pattern of active substances from pesticides on LDPE, PAC and BIO plastic mulches and to compare the decay of the active substances in the presence and absence of plastic debris. For this purpose, 38 active substances from 17 insecticides, 15 fungicides and 6 herbicides commonly applied with plastic mulching in South-east Spain were incubated with a 3×3 cm² piece of plastic mulch (LDPE, PAC and BIO). The incubation was done in a solution of 10% acetonitrile and 90% distilled water at 35°C for 15 days in the dark. The QuEChERS (Quick Easy Cheap Effective Rugged Safe) approach was adapted to extract the pesticides. The sorption behavior depended on both the pesticide and the plastic mulch type. On average, the sorption percentage was ~23% on LDPE and PAC and ~50% on BIO. The decay of active substances in the presence of plastic was ~30% lesser than the decay of active substances in solution alone. This study is the first attempt at assessing the behavior of a diversity of plastic mulches and pesticides to further define research needs.



3.1 Introduction

The use of plastic mulching has become a well-established technique to increase the profitability of many crops (Kasirajan and Ngouajio 2012). The European Commission estimated in 2016 that 100 000 tons of plastic mulch is used per year in the European Union (EuropeanCommission 2016). Plastic mulch is generally used for one or all of the following three reasons: decreasing evaporation, decreasing weed competition or increasing soil temperature (Zacharias Steinmetz et al. 2016b). After crop harvest, some farmers try to remove the plastic mulch but debris is left in the soil. Other farmers simply incorporate the plastic into the soil (Kasirajan and Ngouajio 2012). Once the plastic is in the environment, the low degradation rate of plastic debris facilitates its accumulation (Rillig 2012).

The plastic mulch degradation process can be explained by looking at three main underlying factors: abiotic conditions, microbial requirements, and properties of the plastic mulch material (Hayes et al. 2012). The most common plastic used for mulching is Low Density Polyethylene (LDPE) (Kasirajan and Ngouajio 2012). LDPE is a fully saturated polymer of hydrocarbons which makes it highly resistant (Crawford et al. 2017b). Consequently, LDPE mulch needs to be removed after harvest and LDPE debris accumulates in the environment. Some plastic producers have tried to improve the degradation processes of plastic to avoid plastic mulch removal and plastic debris accumulation. Pro-oxidant Additive Containing (PAC) plastics are polymers, mainly LDPE, which contain a pro-oxidant additive that is used to enhance oxidation and photo-degradation (Selke et al. 2015). In the presence of light and under aerobic conditions, PAC plastics degrade quickly into small pieces. Small fragmented debris is more likely to be further degraded by microorganisms (Ahmed et al. 2018). PAC plastics are also known as “oxo-degradable” or “oxo-biodegradable” (Hogg 2016). However, when incorporated into the soil, the degradation process is minimized due to the absence of UV-light (Hogg 2016) and PAC debris may accumulate. Over the last few years, new mulching films that can be degraded by microorganisms in the soil have been developed (Hayes et al. 2017; Sintim and Flury 2017). They are usually sold as “biodegradable” (BIO) mulch (Oever et al. 2017). Biodegradable mulch can be made of a diversity of polymers (Kijchavengkul and Auras 2008) either biobased, synthetic or a blend of both. Biodegradation of polymeric mulch films relies on three fundamental steps: the colonization of the polymer surfaces by soil microorganisms, the enzymatic depolymerization of the polymer by extracellular hydrolases secreted by the colonizing microorganisms and the microbial utilization of the hydrolysis products that are released from the polymer (Sander 2019). Therefore, a larger contact area helps colonization and polymers containing functional groups that can be enzymatically hydrolyzed increase the degradation rate. About 3 000 tons of biodegradable plastic mulch are used each year in the European Union (EuropeanCommission 2016). In order to properly manufacture plastic mulches, additives

such as nucleating agents, plasticizers, performance additives, and lubricants are required (Briassoulis 2004; Hayes et al. 2012; Shen et al. 2010). It is important to note that manufacturers do not normally share the chemical structures of the raw materials or the additives that are used in plastic production in order to protect their products from being duplicated.

In arid and semi-arid areas, where water deficits are common, the use of plastic mulching for irrigated crops is widespread. It is a technically and economically feasible strategy used to prevent evaporation and reduce water consumption. This is the case in regions such as the Loess plateau in China (R. Jiang et al. 2016) and in the Murcia Region of South-eastern Spain (van der Meulen et al. 2006). In addition to plastic mulch, pesticides are used in conventional agriculture to control weeds, insects and fungi (Oever et al. 2017). The synergetic effect of plastic debris and pesticide residues on degradation and on the terrestrial environment is not sufficiently understood. Nerin et al. (1996) and Sharom and Solomon (1981) studied the sorption rates of nine different active substances in pesticides on LDPE and found a sorption rate between 20% and 100% after 15 days at 35°C. Adsorbed active substances are less likely to be degraded (Kasirajan and Ngouajio 2012) and may be released when ingested by an organism (Teuten et al. 2007). Furthermore, microplastics may be carriers for pesticide residues when transported through the terrestrial environment. The modification of the degradation patterns of active substances might affect the soil organism community due to the toxicity of the active substances. Moreover, the microbial activity plays a major role in BIO plastic degradation. Therefore, adsorption of active substances could potentially decrease plastic debris degradation.

Previous studies determined the occurrence of adsorbed organic contaminants (Polycyclic aromatic hydrocarbons, organochlorinates) on different plastic polymers (Crawford et al. 2017a; Hirai et al. 2011; Mato et al. 2001) and have characterized the sorption of different organic contaminants on plastics (Lee et al. 2014; Liu et al. 2019; Mato et al. 2001; Ramos et al. 2015; Seidensticker et al. 2018; Teuten et al. 2007). Most studies were focused on the aquatic environment and coastal areas but Hüffer and al. (2019) showed that the sorption on polyethylene microplastics influenced the transport of hydrophobic organic pollutants in soils (Seidensticker et al. 2018). More data are required to assess the sorption of commonly used pesticides on LDPE and on new types of plastic.

As a preliminary investigation to address these data gaps, the sorption of 38 active substances from 17 insecticides, 15 fungicides and 6 herbicides commonly used along with plastic mulching in South-eastern Spain, were tested on three types of plastic mulch: LDPE, PAC and BIO. The objectives of this research were to measure the sorption of a mixture of 38 active substances on plastic mulch and to compare the decay of adsorbed and non-

adsorbed active substances. We hypothesized that sorption rates would be different for each specific active substance and each specific plastic. For example, the BIO mulch was believed to be the most prone to sorb active substances (Boivin et al. 2005; Crawford et al. 2017b). Furthermore, we hypothesized that sorption would reduce the degradation of active substances (Nerín et al. 1996; Ramos et al. 2015).

3.2 Materials and Methods

A laboratory single point sorption experiment was set up to test the sorption of a mixture of active substances on plastic mulches. Previously, eight vegetable farmers in the region of Murcia (Southeast Spain) were interviewed to discover which types of pesticides and plastic mulches were commonly used in the research area. All interviewed farmers used either LDPE, PAC or BIO plastic mulch in their crop production. All farmers used similar vegetable rotations and the type of plastic mulch used was not linked to the type of crop. We were able to assemble a full list of the active substances in the pesticides that were used by the farmers. Some active substances on the list were not analysed due to logistical and financial limitations. The final list of 38 active substances from 17 insecticides, 15 fungicides and 6 herbicides is presented in supplementary (Table S3.3).

Three plastic mulches: LDPE, PAC and BIO, were incubated with or without active substances. Additionally, one control treatment containing a mixture of the active substances without plastic was also tested. Therefore, in total, seven treatments were set up in glass tubes (Table 3.1). All treatments were carried out in duplicate. Each tube contained 5 mL of a solution (either with or without the mixture of active substances) and a piece of 3x3 cm^2 plastic mulch, depending on the treatment (Figure 3.1). Therefore, we can distinguish two phases: the plastic mulch and the incubation solution.

***Table 3.1**: Type of plastic mulch and incubation solution for the seven treatments.*

Treatment	Plastic mulch	Incubation solution (10% acetonitrile and 90% H_2O)
LDPE+P	LDPE	Active substances mixture
PAC+P	PAC	Active substances mixture
BIO+P	BIO	Active substances mixture
LDPE+W	LDPE	No-active substances
PAC+W	PAC	No-active substances
BIO+W	BIO	No-active substances
P	-	Active substances mixture

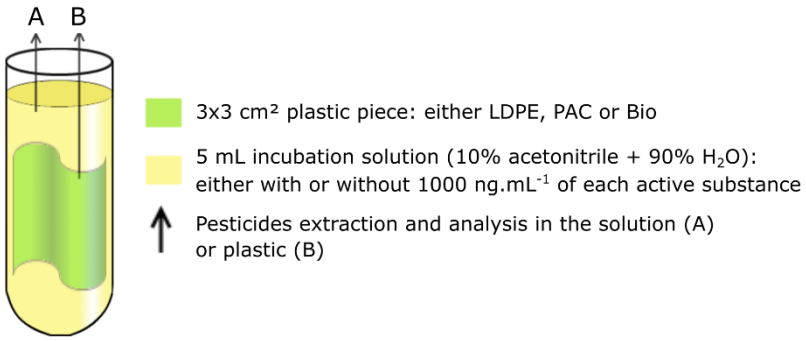


***Figure 3.1**: Glass tube set up drawing.*

LDPE, Low Density Polyethylene mulch; PAC, Pro-oxidant Additive Containing mulch; BIO, Biodegradable mulch. Pesticides content was analysed in the solution (A) and in the plastic (B). All experiments were performed in duplicate. Glass tubes were incubated at 35 °C for 15 days.

3.2.1 Plastic mulch types used in the experiment

Samples of unused plastic mulch were collected from farmers' warehouses located in the region of Murcia for each of the mulches: LDPE, PAC and BIO. All three types of mulches were black. The detailed composition of the plastic was not given by the producers since it was protected by intellectual property regulations. In addition to the main polymer, all plastics contained additives used to control the color, elasticity and resistance of the mulch (Crawford et al. 2017b; Sintim and Flury 2017). The LDPE mulch came from Reyenvas (Spain). LDPE plastic mulch is designed to be resistant and removed after the harvest. The PAC mulch (commercial name "actiblack") came from Trioplast SMS SAS (France). PAC mulch is made of LDPE with the addition of a pro-oxidant additive that increases its decay such that farmers usually incorporate it into the soil after harvest instead of removing it. Finally, BIO mulch (commercial name "Sotrafilm Black Biodegradable") was bought from Sotrafa (Spain). The available information states that it is a "biopolymer film made with biodegradable and renewable raw materials and particular carbon black content to get an optimum opacity for mulching use" (Sotrafa 2018). The compliance with the biodegradable plastic mulch norms EN 17033:2018 (CEN 2018) or ISO 17556:2019 (ISO 2019) was not specified. The composition of the biodegradable mulch was investigated using the Varian 1000 FTIR (Fourier transform infrared) spectrometer from the Aquatic Ecology and Water Quality Management group of Wageningen University. Eight scans were performed for the background and the samples. The spectrometer produced spectra ranging from 3750 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}. The comparison of the spectra with polymer libraries (HR Hummel Polymer and Additives, HR Spectra Polymers and Plasticizers by ATR, HR Sprouse Polymers by Transmission) gave high percentages of match for Polyester

terephthalic acid (78% match), Polybutylene terephthalate (72% match) and polyethylene terephthalate (65.6% match) (Figure S3.1, Table S3.1). Therefore, the biodegradable plastic mulch may have been composed of Polybutylene terephthalate, polyethylene terephthalate or other similar copolyester of terephthalic acid. It is most likely that the main polymer was Polybutylene adipate terephthalate as it is a copolyester of terephthalic acid commonly used for its biodegradability properties (Weng et al. 2013).

The pieces of plastic mulch that were collected were manually cut into 3×3 cm^2 pieces before incubation. The 3×3 cm^2 pieces of mulch were manipulated so that they fit into the glass tubes but they were not folded (Figure 3.1). Plastic pieces were fully immersed in the solution so that the incubation solution was in contact with the whole surface of the piece of plastic.

3.2.2 Incubation solution and incubation conditions

For each of the 38 active substances (Table S3.3), the Pesticide Properties Database (BPDB 2018; PPDB 2018) was used to get the molar mass, the aqueous hydrolysis half-life time at 20°C and pH 7 (DT50; indicator of degradation in water), the solubility in water at 20 °C and the octanol-water partition coefficient at pH 7, 20°C (log P). The octanol-water partition coefficient (log P) was used as a measure of the active substances hydrophobicity, which plays a key role in sorption (Leo et al. 1971). A concentration of 1000 $ng.mL^{-1}$ of each active substance was mixed in a solution of 10% acetonitrile and 90% distilled water so that there was 5000 ng of each active substance in the final volume of 5 mL. The concentration was the same as in Nerin et al (1996) and the mass of pesticides available per area of plastic was similar. Acetonitrile in the incubation solution may have helped the dissolution of hydrophobic active substances since the solubility in water for some of the substances was low (Table S3.3). The initial presence of active substances in the plastic was assessed using the same incubation solution (90% distilled water + 10% acetonitrile) without active substances applied (Treatments LDPE+W, PAC+W, BIO+W).

All glass tubes were incubated at 35°C for 15 days in a laboratory oven. The temperature was representative of the temperature under the plastic mulch in semi-arid regions (Nerín et al. 1996). Tubes were kept in the dark, without additional stirring or oxygenation during the 15 days. A period of 15 days was enough time to reach the sorption equilibrium, as reported on LDPE films by Nerin et al (1996) and allowed us to study the degradation of the substances.

3.2.3 Active substance extraction and determination

After incubation, the plastic pieces were carefully washed with distilled water, cut into 5×5 mm² pieces and transferred to a 50 mL tube for active substance extraction. The extraction method was adapted from Nerin et al (1996) and the QuEChERS approach (Anastassiades et al. (2003). Nerin et al (1996) showed with a similar extraction method that a single extraction was sufficient for a quantification. The analytical method was similar to the one described in Mol et al. (2008) and Silva et al. (2018). Plastic tubes and plastic vials were used for extraction and quantification procedures. Given the concentration of acetonitrile and the short time of extraction, we do not expect significant losses of active substances based on the quality assessment method of Mol et al. (2008) and Silva et al. (2018). All plastic samples were spiked with 13C-caffeine (used as internal standard to assess the procedure efficiency), and mixed with 5 mL of distilled water and 10 mL of acetonitrile containing 1% acetic acid (Mol et al. 2008). Tubes were exposed to an ultrasonic bath for one hour and agitated end-over-end for another hour. Then, 1 g of sodium acetate and 4 g of magnesium sulphate were added to induce phase separation. After centrifugation, 250 µL of the supernatant (acetonitrile phase) was collected, mixed with 250 µL of distilled water and filtered in a filter vial for analysis. The incubation solution was taken from the test tube and diluted 40 times in a solution of acetonitrile +1% acetic acid and distilled water (1:1).

The active substance content was analysed with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (Silva et al. 2018a) with mobile phases of 0.1% formic acid and 5 mM ammonium formate in water (eluent A) or in 95% methanol, 5% water (eluent B). The gradient used to elute all compounds from the column is shown in (Table S3.2). LC-MS/MS measurements were performed on a Xevo TQ-S (tandem quadrupole mass spectrometer) system coupled with an Acquity UPLC (ultra-performance liquid chromatography) system, both from Waters (Milford, MA, USA). Each LC-MS/MS analysis included a calibration curve of nine fortified blanks (0, 0.125, 0.25, 0.5, 1, 2.5, 5, 10, 25 $ng.mL^{-1}$) in a solution of acetonitrile +1% acetic acid and distilled water (1:1). The extraction procedure performed on the plastic not incubated with pesticides provided a matrix extract. A standard in matrix was prepared from the matrix extract fortified at 5 $ng.mL^{-1}$ and injected after every 10 sample measurements. The software MassLynx™ (Version 4.1, Waters) was used to collect the data and integrate the peaks. A limit of quantification (LOQ) was calculated for each compound according to the lowest calibration level inside the linearity range (deviation of back-calculated concentration from true concentration within ±20%) and an ion ratio within ±30% of the average of calibration (EuropeanCommission 2017) (Table S3.4). The active substance contents below the LOQ were considered to be zero during data processing.

3.2.4 Data processing

Data calculation and plotting were done in R version 3.4.2. Calculations were performed using the mean of the duplicate treatments. The percentage of sorption was calculated for each tube containing the plastic mulch and the active substances mixture as the ratio between the mass of active substances detected in the plastic extract and the mass of the active substances added. The sorption calculation did not take into account the possible decay of active substances in the solution or the incomplete extraction of sorbed substances. As a consequence, calculated sorption percentages may have been lower than the real sorption of active substances on plastic mulches. The Shapiro-Wilks test was performed at $p < 0.05$ to check the normality of the percentage of sorption with the function shapiro.test of R. Then percentages of sorption were compared among the different plastic types using a one-way non-parametric ANOVA (Kruskal-Wallis comparison at $p < 0.05$) with functions kruskal.test and dunnTest in R. Given p-values were adjusted using the Benjamini-Hochberg method.

The recovery ratio (sum of the mass of active substances measured in all compartments divided by the mass of active substances added) was calculated for each treatment where active substances were added. The difference between the mass recovered and the mass added was considered the mass of active substances decayed during the incubation. The decay calculated for active substances without plastic, minus the decay calculated in the presence of plastic, gave an estimation of the decay reduction in the case of active substance sorption.
The lm function of R calculated the linear coefficients, the coefficient of determination (R^2) and the p-value of linear regressions between percentage of sorption and log P, as well as between decay reduction and percentage of sorption.

3.3 Results

3.3.1 Active substance sorption on plastic mulch

The mean sorption rate of all active substances on each type of plastic are shown in (Figure 3.2). The measured sorption rates did not follow a normal distribution (Shapiro-Wilks test, $p < 0.001$). LDPE and PAC plastics showed no significant differences for sorption of active substances ($p > 0.05$), with an average of ~23%. BIO mulch showed a significantly higher sorption rate than LDPE and PAC mulches ($p<0.05$) with an average value of ~50%. In fact, 20 out of the 38 tested compounds showed a sorption rate > 50% on BIO, whereas only 9 and 7 had a sorption rate > 50% on LDPE and PAC mulches, respectively.

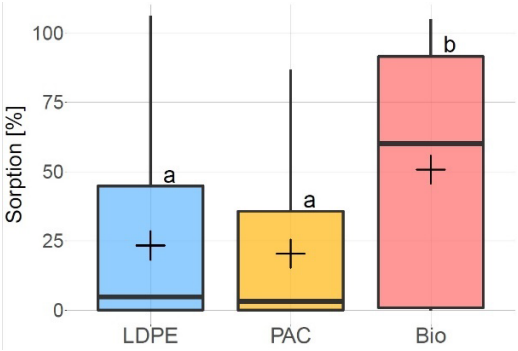


***Figure 3.2**: Sorption (%) on each type of plastic: LDPE (blue), PAC (orange) and Biodegradable (red).*

The box plot (horizontal lines) represents sorption for at least 25%, 50% and 75% of the active substances. The vertical black line ends represent the minimum and maximum values. The cross is the mean sorption for all active substances. Different letters indicate significant differences among plastic types after a Kruskal–Wallis comparison at $p < 0.05$.

Sorption rates (%) of each active substance to the different plastic types (treatments LDPE+P, PAC+P and BIO+P) are presented in (Figure 3.3) and in (Table S3.4). We observed that 14 compounds (37%) had a sorption rate > 10% on all plastic mulches. Ten compounds had a sorption rate <1% on all plastics. Three compounds, Chlorpyrifos, Oxyfluorfen and Pendimethalin, had a sorption rate > 80% on all plastics.

Active substances with higher log P values tended to show higher sorption rates. In fact, the sorption rate was positively correlated with log P (R^2 = 0.96, $p<0.001$) (Figure 3.4). Nevertheless, it is worth noting that active substances with the same log P and the same plastic type could have had significantly different sorption rates (e.g., Figure 3.3; log P(Chlorpyrifos) = log P(Cyflufenamid) = 4.7 whereas mean sorption on LDPE was 88% for Chlorpyrifos and 45% for Cyflufenamid).

For the plastic mulch with no added active substances (LDPE+W, PAC+W and BIO+W), no active substances were found in the PAC or BIO extracts but low levels (maximum: 16 ng; average: 7.7 ng) were found in the samples with LDPE in one duplicate but not in the other duplicate. These very low levels (compared to the 5000 ng of active substance added) are likely to have come from contamination in the liquid chromatography column.

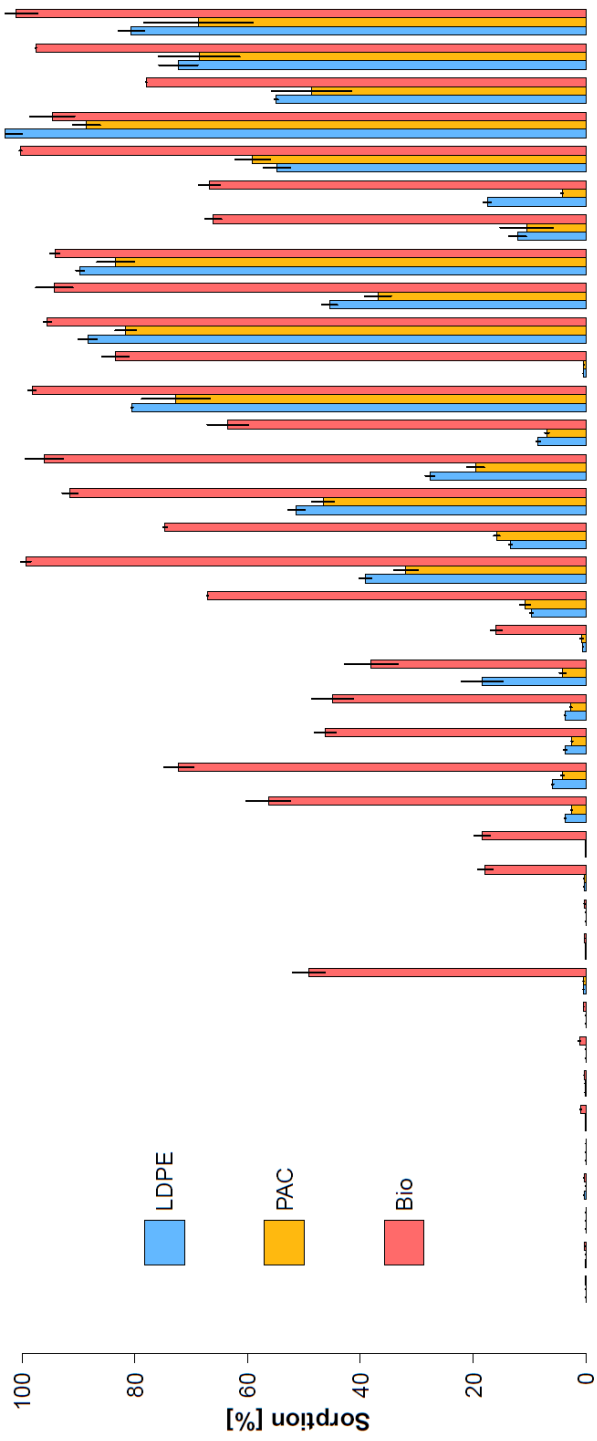
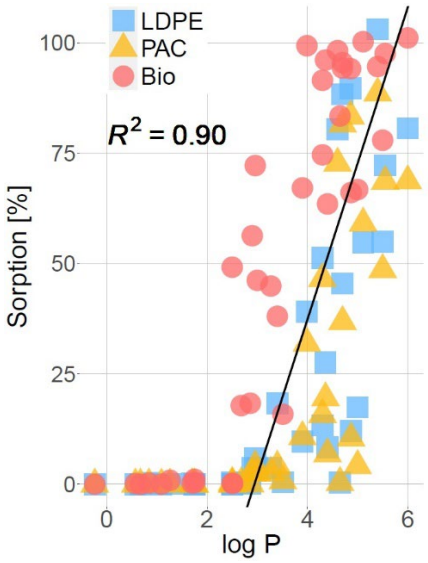


***Figure 3.3**: Mean sorption rate (%) for each active substance on LDPE (blue), PAC (orange) and Biodegradable (red) plastic mulch. Vertical black lines represent the measurement ranges (min and max). Active substances are ordered according to increasing log P (octanol-water partition coefficient).*



For active substance samples where no plastic was added, half of the compounds had a recovery ratio < 50% and 29 compounds (75%) had a recovery ratio < 90% (Figure S3.2). It is worth noting that without plastic, active substances with higher log P tended to have lower recovery. Moreover, active substances with a shorter DT50 in water tended to have a lower recovery ratio; meaning that lower recovery is likely to be explained by higher decay during incubation. In the next section, we assumed that the missing percentage (1-recovery) was due to the degradation of the active substances during incubation.

3.3.2 Decay reduction due to sorption of active substances

The sorption of active substances significantly reduced their decay in comparison to the active substances without plastic mulch (Figure 3.5). The estimated decay for active substances with sorption > 80% was ~70% lower than the decay without plastics. We measured a decay reduction of ~27% for LDPE and PAC and ~37% for BIO for active substances with a sorption > 0.01%. The decay reduction showed a significant linear relationship with the percentage of sorption (R^2 = 0.90, $p<0.001$). The greater sorption on BIO directly reflected on a greater decay reduction for active substances.

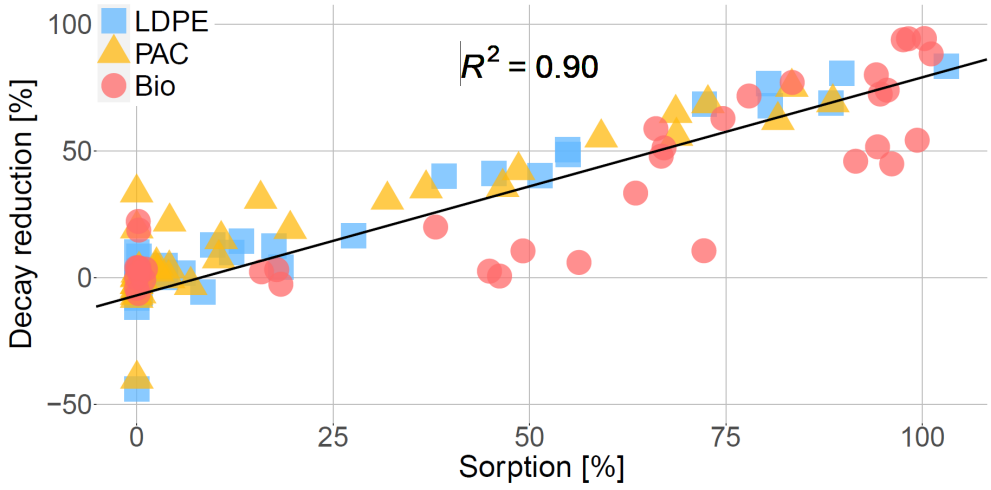


***Figure 3.5**: Decay reduction (%) for active substances sorbed on plastics related to the sorption (%) of active substances for the three types of plastic, LDPE (blue square), PAC (orange triangle) and Biodegradable (red circle).The decay reduction (%) is the difference between the decay of an active substance in presence of plastic and the decay of the same active substances without plastic. The black line is the regression calculated for sorption >0: y = 0.86x − 7; R2 = 0.90; p < 0.001.*

3.4 Discussion

We did a single point sorption experiment. Based on previous studies, 15 days of sorption at 35°C were enough to reach steady states (Nerín et al. 1996; Sharom and Solomon 1981). Kinetic sorption experiments would be needed to calculate sorption coefficients. The active substance extraction procedure was partly based on Nerín et al. (1996) and was not tested again. In case of a low extraction rate, we underestimated the sorption on plastic and over-estimated the decay of active substance incubated with plastic. We don't expect the extraction rate to perform differently for LDPE, PAC or BIO. Therefore, a low extraction rate would not change our conclusion.

The BIO mulch may have contained polybutylene adipate terephthalate and BIO mulch had a higher sorption than LDPE and PAC mulches. Higher sorption may be related to the chemical properties of the polymer used or to the specific surface area of the mulch (Aslam et al. 2013). Polybutylene adipate terephthalate may have a better affinity with the active substances than the LDPE because of aromatic-interactions and potential hydrogen bonds when the polymer is altered (Palsikowski et al. 2018). Aged polybutylene adipate terephthalate is able to form hydrogen bonds with organic chemicals (Weng et al. 2013). It is possible that the 15 day incubation at 35°C caused an alteration in the BIO mulch and

increased the formation of hydrogen bonds between the BIO mulch and the active substances. Additionally, biodegradable mulches tend to be made with smaller polymer fiber diameters that increase its specific surface area (Chinaglia et al. 2018; Hayes et al. 2012) and its biodegradation (Brodhagen et al. 2015; Chinaglia et al. 2018). A greater specific surface area would also increase the sorption (Liu et al. 2019).

Our study showed that for 20 compounds sorption on BIO mulch was higher than 50%. Sorbed active substances are likely to alter the degradation of the BIO mulch by affecting the soil microbiome (Oyeleke and Oyewole 2019). According to the International Organization for Standardization (ISO) 17556, biodegradable mulch should reach at least 90% biodegradation in the soil within two years (Carol et al. 2017). A field study showed that after 397 days in the soil, three different kinds of biodegradable mulches (Crown 1, BioAgri and SB-PLA-11) had various deterioration rates (100%, 65% and very little deterioration, respectively) (Jeremy et al. 2013). Plastic degradation studies should take into account that pesticides are likely to be sorbed on plastic and may reduce its biodegradation. On the other hand, the efficiency of pesticides in the soil (i.e. herbicides, fungicides) depends on their availability. Therefore, plastic mulch may decrease the efficiency of pesticides by decreasing their release into the soil when plastic mulch debris accumulates and the pesticides are sprayed on the soil. The aging of plastics (Liu et al. 2019) and the pesticide sorption in soils contaminated with plastics (Hüffer et al. 2019), for different soil types and organic matter contents, are factors that need to be studied to understand the interactions between plastic debris and pesticide residues.

LDPE and PAC mulches have similar sorption and their composition only differs in the additives that are added to them. Thus, the additives present in PAC mulch do not seem to change the sorption property of the original LDPE polymer. The sorption was much higher for the BIO mulch. As a consequence, knowing the exact chemical formulation of the polymers used to make plastic mulches and the specific surface area of the mulch are essential in understanding the mechanisms of the sorption of pesticides on plastic. Better, cheaper and faster analysis of plastic composition (Fabio Corradini et al. 2019b; Mintenig et al. 2017) or regulations forcing producers to share the chemical formulation of polymers could help filling this knowledge gap.

The sorption on plastic varied for all active substances, being higher for those with higher log P. In fact, log P, as a measure of hydrophobicity (Leo et al. 1971), plays a key role in sorption processes (Aslam et al. 2013). Nevertheless, the log P did not predict the sorption for all active substances so mechanisms other than hydrophobicity must play a role (L. Guo et al. 2000). Some active substances may have had an impact on the sorption of some other ones, likely due to chemical interactions between them. Interactions between active

substances might have changed the active substance degradation in solution as well as in the plastic matrix. These results highlight the need for more detailed studies to understand the mechanisms of pesticide sorption on plastic.

The highest degradation rates were obtained for active substances with low stability to hydrolysis and low stability in aqueous solution (aqueous hydrolysis DT50 (days) at 20oC, pH 7 and degradation in water DT50 (days) (PPDB 2018)). We can assume that the pesticide degradation in the glass tube was mainly due to hydrolysis (Fenner et al. 2013). Volatilization in the gaseous phase in the tube or incomplete solubilization could have played a role in the estimation of the decay. However, neither the decay reduction nor the sorption was correlated with the solubility, meaning that hydrolysis was the most likely process leading to the degradation. The decay of active substances from pesticides was reduced by sorption. It is commonly accepted that sorption limits pesticide degradation by reducing its partitioning into the liquid phase (Guerin and Boyd 1997; O'Loughlin et al. 2000). Additionally, soil microorganisms degrade preferably or exclusively chemicals that are present in the soil solution (Boivin, et al. 2005a). Thus, sorbed active substances would undergo less degradation by microorganisms (Liang et al. 2011). Since plastic debris could be transported by wind and water (Liu et al. 2014a), pesticide transport (Teuten et al. 2007) and degradation models (Silva et al. 2018b) should take into account the sorption of pesticides on plastic (Villeneuve et al. 1988).

The applied experimental design was based on Nerin et al. (1996) to reveal a potential of commonly used active substances from pesticides to be sorbed and protected from decay on conventional (LDPE) and new (PAC and BIO) plastic mulches. The sorption condition applied here does not reflect real conditions in fields. The 38 active substances were applied together at the same concentration, in the same solution. We can assume then that most hydrophobic active substances may have reduced the sorption of the rest of the substances due to the competitive sorption among all active substances. Competitive sorption would occur to a negligible extent in the field because fewer active substances would be applied simultaneously and the use of pesticides would be spread over the whole growing period resulting in seasonal variations. Moreover, the applied concentration of 1000 $ng.mL^{-1}$ exceeded the solubility in water for some compounds. A non-dissolved fraction of the active substances may had formed a stock within the liquid medium. The incubation was done in glass tubes, in the dark, at 35°C without temperature variation, stirring or oxygenation. In the field, active substances could undergo sorption on soil particles, degradation by light and by microorganisms or volatilization. Additionally, the presence of 10% acetonitrile in the incubation solution could have reduced the sorption of most hydrophobic contaminants in plastics and polymers (Teuten et al. 2007). Sorption percentages on plastic may be higher without acetonitrile and with less competitive sorption. However, sorption percentages

could be reduced by additional degradation processes (Fenner et al. 2013; Kumar et al. 2018), volatilization and sorption to soil particles (Boivin et al. 2005). Finally, plastic degradation may change the chemical properties of the polymer or the specific surface area of the mulch (Hayes et al. 2017; Liu et al. 2019) and change the sorption of active substances (Aslam et al. 2013). Despite these last issues comparing the conditions of this experiment with actual conditions in the field, our study highlights the need for further research on plastic mulch-pesticide systems since there is a real interaction between both components, which could negatively affect pesticides and plastic degradation in the field. Thus, it is essential to address these topics under field conditions (Yang et al. 2018) taking into account new and aged plastics (Liu et al. 2019).

The sorption of active substances on plastic may change the toxicity of both the pesticides and the plastic. In fact, if plastic debris is ingested by organisms (Colabuono et al. 2010; Huerta Lwanga et al. 2017a) then the sorbed active substances could potentially be desorbed in organisms (Teuten et al. 2007). On the other hand, the sorption to plastic may reduce the bioavailability of active substances, especially reducing the peak concentration after pesticides application. The reduced exposure of soil organisms could be beneficial for the ecosystem. Contaminated plastics may as well release active substances in the soil solution and contribute to plastic toxicity (Machado et al. 2018; Qi et al. 2018). In a similar way, active substances sorbed on plastic may decrease the plastic's degradation by soil organisms because of the toxicity of the active substances. Active substance sorption is of particular concern when it comes to the degradation of biodegradable mulch since the degradation of biodegradable mulch relies heavily on the activity of microorganisms in the soil. As a consequence, further studies are needed to assess the degradation of plastic debris, especially biodegradable plastics in soils where pesticides are sprayed.

3.5 Conclusions

This study reveals that sorption of active substances on plastic depends on both the chemical structure of the active substance and the type of plastic mulch. Sorption was higher for active substances with higher log P (octanol-water partition coefficient) and although it was similar between LDPE and PAC plastics, it was significantly higher on BIO mulch. Moreover, sorption of active substances on plastic reduced the decay of active substances. Therefore, the sorption of active substances can change the eco-toxicity and decay of both the active substances and the plastic debris. The sorption can also affect the transport pattern of active substances, especially when biodegradable plastic is used. More research is needed to evaluate the dynamics and consequences of the sorption of active substances from pesticides on plastic mulches in environmental conditions. With more research, scientists can propose guidelines for the use of plastic mulches in agro-ecosystems in order to avoid soil and water pollution.

Acknowledgements

We are thankful for the contribution of the farmers from the region of Murcia, Spain. We would like to thank Klaas Oostindie for his graphic support and Robin Palmer for the language editing. We are very grateful to Frits Gillissen from the Aquatic Ecology and Water Quality Management group of Wageningen University & Research for the FTIR analysis of the plastic mulch. We would also like to thank Esperanza Huerta Lwanga for her invaluable comments and revisions.

Supplementary Material

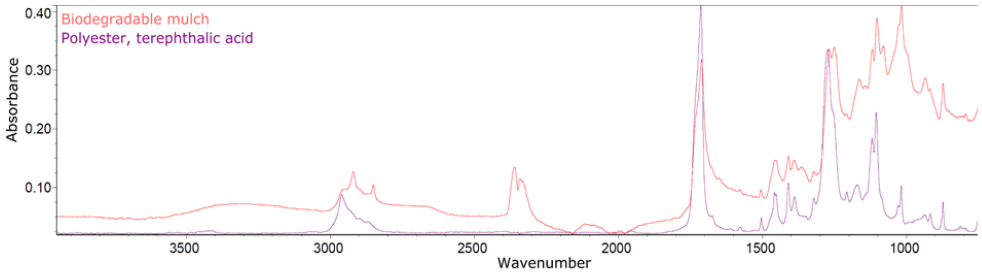


***Figure S3.1**: FTIR Spectra from the biodegradable mulch and the best match from HR Hummel Polymer and Additives library: Polyester, terephthalic acid.*

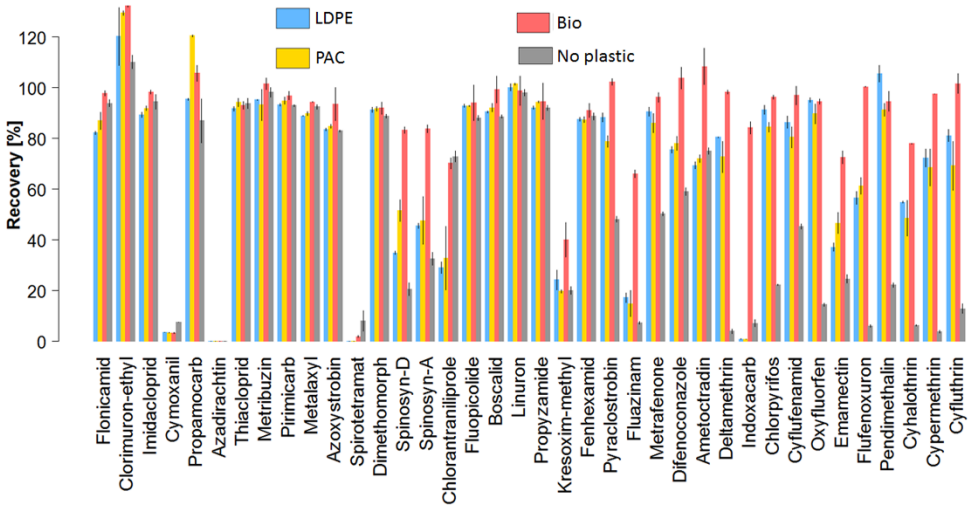


***Figure S3.2**: Recovery rate [%] for each active substance with LDPE (blue), PAC (orange) and Biodegradable (red) plastic mulch and without plastic mulch (grey).*

Black lines at the top of each column represent the measurement ranges (min and max). Active substances are ordered according to increasing log P (octanol-water partition coefficient) from left to right.

***Table S3.1**: Ten compounds that best match the biodegradable mulch spectra.*

The comparisons were made with the references spectra of the high resolution libraries HR Hummel Polymer and Additives, HR Spectra Polymers and Plasticizers by ATR and HR Sprouse Polymers by Transmission.

Match [%]	Compound Name	Library Name
78.32	Polyester, terephthalic acid	HR Hummel Polymer and Additives
72.77	Poly(butylene terephthalate)	HR Specta Polymers and Plasticizers by ATR - corrected
72.29	Polyester, tere- & isophthalic acids	HR Hummel Polymer and Additives
71.3	Poly(1,4-butylene terephthalate)	HR Hummel Polymer and Additives
70.7	Poly(1,4-butylene terephthalate)	HR Hummel Polymer and Additives
70.52	Poly(butylene terephthalate)	HR Spectra Polymers and Plasticizers by ATR
68.37	Polyester, tere- & isophthalic acids	HR Hummel Polymer and Additives
68.11	Polyester, tere- & isophthalic acids	HR Hummel Polymer and Additives
65.71	Poly(1,4-butylene terephthalate)	HR Sprouse Polymers by Transmission
65.6	Poly(ethylene terephthalate)	HR Specta Polymers and Plasticizers by ATR - corrected

***Table S3.2**: Elution gradient used for the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of the active substances.*

A mobile phases of 0.1% formic acid and 0.5% ammonium formate in water (eluent A) or in 95% methanol, 5% water (eluent B) was used for the LC-MS/MS analysis.

Time [min]	Eluent A [%]	Eluent B [%]
0	100	0
2.5	55	45
8	0	100
11	0	100
12	100	0
14	100	0

***Table S3.3**: Category of pest controlled (either fungicide, herbicide or insecticide), and properties for the 38 active substances tested.The molar mass, solubility in water at 20 °C, aqueous hydrolysis half-life time at 20 °C and pH 7 (DT50 in water) and octanol-water partition coefficient at pH 7, 20 °C (log P) were obtained from the Pesticide Properties DataBase (PPDB, 2018).*

Compound	Category	Molar mass [g/mol]	Solubility [mg/L]	DT50 in water [days]	log P[-]
Ametoctradin	fungicide	275.39	0.15	1.8	4.4
Azadirachtin	insecticide	720.721	260	8	1.09
Azoxystrobin	fungicide	403.4	6.7	84.5	2.5
Boscalid	fungicide	343.21	4.6	484.4	2.96
Chlorantraniliprole	insecticide	483.15	3446	597	2.86
Chlorpyrifos	insecticide	350.58	1.05	386	4.7
Clorimuron-ethyl	herbicide	414.82	1200	40	2.5
Cyflufenamid	fungicide	412.36	0.52	210	4.7
Cyfluthrin	insecticide	434.29	0.0066	51	6
Lambda-cyhalothrin	insecticide	449.85	0.005	57	5.5
Cymoxanil	fungicide	198.18	780	1.4	0.67
Cypermethrin	insecticide	416.3	0.004	70	5.55
Deltamethrin	insecticide	505.2	0.0002	26	4.6
Difenoconazole	fungicide	406.26	15	130	4.36
Dimethomorph	fungicide	387.86	28.95	56.7	2.68
Emamectin	insecticide	886.133	24	-	5
Fenhexamid	fungicide	302.2	24	0.43	3.51
Flonicamid	insecticide	229.16	5200	1.1	-0.24
Fluazinam	fungicide	465.14	0.135	124	4.87
Flufenoxuron	insecticide	488.77	0.0043	72.5	5.11
Fluopicolide	fungicide	383.58	2.8	271	2.9
Imidacloprid	insecticide	255.66	610	187	0.57
Indoxacarb	insecticide	527.83	0.2	113.2	4.65
Kresoxim-methyl	fungicide	313.35	2	0.87	3.4
Linuron	herbicide	249.09	63.8	57.6	3
Metalaxyl	fungicide	279.33	8400	36	1.75
Metrafenone	fungicide	409.3	0.492	250.6	4.3
Metribuzin	herbicide	214.29	10700	7.1	1.75
Oxyfluorfen	herbicide	361.7	0.116	138	4.86
Pendimethalin	herbicide	281.31	0.33	182.3	5.4
Pirimicarb	insecticide	238.39	3100	86	1.7
Propamocarb	fungicide	188.3	900000	14	0.84
Propyzamide	herbicide	256.13	9	50.5	3.27
Pyraclostrobin	fungicide	387.8	1.9	62	3.99
Spinosyn-A	insecticide	731.98	14.5	24.3	3.9
Spinosyn-D	insecticide	745.98	0.76	45.2	4.3
Spirotetramat	insecticide	373.48	29.9	0.19	2.51
Thiacloprid	insecticide	252.72	184	1.3	1.26

***Table S3.4**: Limit of quantification (LOQ), sorption on the three plastic mulches, decay in presence of a plastic mulch piece and decay in the solution alone of all active substances, for both duplicates (min;max).*

LDPE: Low Density Polyethylene mulch; PAC: Pro-oxidant Additive Containing mulch; BIO: Biodegradable mulch.

Active substance	LOQ [ng/mL]	Sorption on LDPE [%]	Sorption on PAC [%]	Sorption on BIO [%]	Decay with LDPE [%]	Decay with PAC [%]	Decay with BIO [%]	Decay without plastic [%]
Ametoctradin	2.5	8; 8.8	6.5; 7.2	59.9; 67.1	29.2; 32	26.4; 29.5	-15.6; -1.2	23.7; 26.2
Azadirachtin	2.5	Below LOQ	Below LOQ	Below LOQ	100; 100	100; 100	100; 100	100; 100
Azoxystrobin	0.125	0.4; 0.4	0.4; 0.4	46.3; 52	16; 17	14.4; 15.9	0.1; 12.9	16.6; 17.4
Boscalid	0.125	5.7; 6	3.9; 4.4	69.6; 74.8	9.1; 10	6.2; 9.5	-4.5; 6	10.8; 12
Chlorantraniliprole	0.125	0.1; 0.1	0.02; 0.04	17; 19.8	68.7; 73.3	54.6; 79.6	27.6; 31.9	25; 29.2
Chlorpyrifos	1	86.8; 90	79.8; 83.5	94.9; 96.2	7; 10.3	13.7; 17.4	2.9; 4.4	77.3; 77.9
Clorimuron-ethyl	0.125	0.01; 0.02	0.02; 0.02	0.2; 0.2	-31; -8.9	-30; -28.6	-33; -31.7	-12.9; -7.5
Cyflufenamid	0.125	44.1; 46.9	34.5; 39.1	91; 97.6	11.2; 16	15.3; 23.8	-0.6; 6.7	53.6; 55.7
Cyfluthrin	2.5	78.4; 82.9	59.1; 78.3	97.2; 105	17.1; 21.6	21.7; 40.9	-5; 2.8	85.3; 89
Lembda-cyhalothrin	2.5	54.6; 55.2	41.6; 55.7	77.9; 78	44.8; 45.4	44.4; 58.5	22; 22.1	93.5; 93.9
Cymoxanil	1	Below LOQ	Below LOQ	Below LOQ	96.4; 96.5	96.5; 96.8	96.5; 96.9	92.5; 92.5
Cypermethrin	2.5	68.8; 75.7	61.4; 75.8	97.5; 97.6	24.3; 31.2	24.2; 38.6	2.4; 2.5	95.8; 96.7
Deltamethrin	2.5	80.4; 80.6	66.6; 78.8	97.6; 98.9	19.4; 19.6	21.2; 33.4	1.1; 2.4	95.1; 96.9
Difenoconazole	0.125	26.8; 28.4	18; 21.1	92.8; 99.4	23.2; 25.7	19.1; 24.7	-8.1; 0.4	39.6; 42.5
Dimethomorph	0.125	0.3; 0.4	0.3; 0.3	16.5; 19.1	7.7; 9.6	7.4; 9.1	5.8; 10.4	10.6; 11.9
Emamectin	0.125	16.8; 18.1	4.1; 4.3	64.9; 68.6	61.2; 64.5	49.2; 57.5	24.9; 30	73.8; 76.9
Fenhexamid	0.5	0.5; 0.5	0.5; 1	14.9; 16.9	11.6; 13.1	11.6; 13.6	6.3; 11.8	9.9; 12.7
Flonicamid	0.125	Below LOQ	Below LOQ	0.01; 0.02	17.2; 18.3	9.7; 16.4	1.2; 3.2	4.7; 7.5
Fluazinam	5	10.6; 13.5	5.8; 15.2	64.7; 67.5	81; 84.6	80.1; 90.3	32.5; 35.4	92.3; 93.1
Flufenoxuron	0.125	52.5; 57.2	56; 62.2	100; 100.5	41; 45.9	35.5; 42	-0.6; -0.2	93.4; 94.5
Fluopicolide	0.125	3.5; 3.7	2.4; 2.7	52.4; 60.2	6.5; 7.8	6.9; 7.6	-1.1; 13	10.8; 13.1
Imidacloprid	0.125	Below LOQ	Below LOQ	0.2; 0.2	9.7; 11.7	7.1; 9	1; 2.4	2.6; 8.4
Indoxacarb	0.125	0.4; 0.4	0.4; 0.4	81.1; 85.8	99.1; 99.2	99.2; 99.2	13.3; 18.3	91.4; 94.3
Kresoxim-methyl	5	14.8; 22	3.5; 4.7	33.3; 42.8	71.9; 79.8	79.6; 81.1	53.3; 66.7	78.4; 81.5
Linuron	2.5	3.4; 3.8	2.3; 2.6	44.3; 48.1	-1.5; 1.2	-1.9; -1.1	-4.5; 7	0.6; 3.1
Metalaxyl	2.5	Below LOQ	Below LOQ	0.4; 0.4	11; 11.2	9.4; 11	5.5; 5.8	6.8; 8.3
Metrafenone	0.125	49.8; 52.8	44.6; 48.5	90.2; 92.9	7.8; 11.3	10.3; 17.8	1.9; 5.7	48.9; 50.6
Metribuzin	2.5	Below LOQ	Below LOQ	0.9; 1.3	4.6; 4.8	0.6; 13	-3.8; 0.6	-0.1; 3.7
Oxyfluorfen	0.25	89; 90.4	80.1; 86.7	93.3; 95	4.1; 5.6	6.5; 14.1	4.6; 6.4	84.9; 86.1
Pendimethalin	1	100; 106	86.2; 91.1	90.7; 98.6	-8.9; -2.4	6.2; 11.2	1.4; 9.3	76.8; 78.7
Pirimicarb	0.125	0.1; 0.1	0.1; 0.1	0.3; 0.3	6.1; 7.2	3.7; 6.5	1.5; 4.8	6.7; 7.4
Propamocarb	0.25	0.3; 0.3	Below LOQ	0.3; 0.3	4.1; 4.9	-20.8; -20	-8.7; -2.7	4.4; 21.6
Propyzamide	2.5	3.6; 3.8	2.5; 2.8	41.2; 48.6	7.3; 8.6	5.2; 6	-1.7; 12.5	7; 8.9
Pyraclostrobin	0.125	38; 40.2	29.9; 34	98.5; 100	10; 13.5	18.9; 23.4	-3.7; -1	50.7; 53.1
Spinosyn-A	0.125	9.4; 9.9	9.8; 11.7	67; 67.2	53.5; 55.4	43.1; 61.6	14.7; 17.8	65; 69.9
Spinosyn-D	0.125	13.1; 13.7	15.3; 16.3	74.3; 75	64.3; 65.8	44.1; 52.8	15.4; 17.9	77; 81.9
Spirotetramat	0.125	Below LOQ	Below LOQ	0.1; 0.3	100; 100	100; 100	97.8; 98.6	87.9; 95.9
Thiacloprid	0.125	0.05; 0.05	0.03; 0.03	0.8; 1	7.4; 9.1	4.2; 7.4	5.5; 8.5	4.2; 8.2

Chapter 4

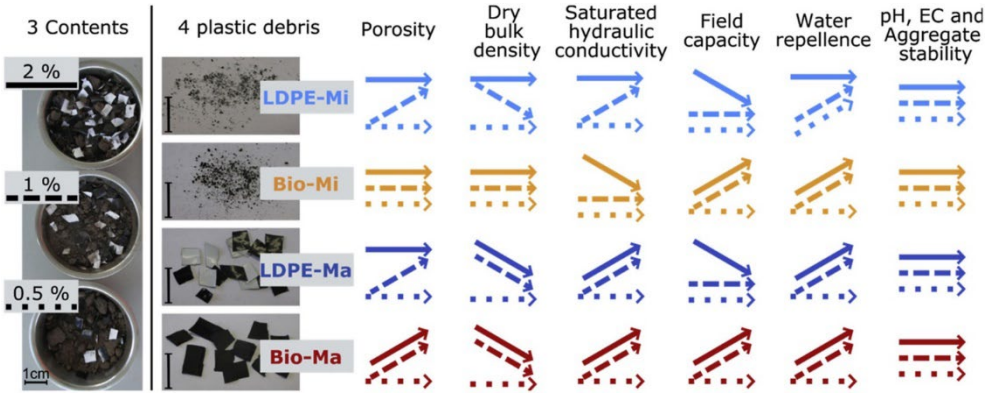
Impact of plastic mulch film debris on soil physicochemical and hydrological properties

Based on:

Qi, Y.*, Beriot, N.*, Gort, G., Huerta Lwanga, E., Gooren, H., Yang, X., Geissen, V. 2020. Impact of plastic mulch film debris on soil physicochemical and hydrological properties. Environmental Pollution, vol. 266, 115097.

Abstract

The plastic mulch films used in agriculture are considered to be a major source of the plastic residues found in soil. Mulching with low-density polyethylene (LDPE) is widely practiced and the resulting macro- and microscopic plastic residues in agricultural soil have aroused concerns for years. Over the past decades, a variety of biodegradable (BIO) plastics have been developed in the hope of reducing plastic contamination of the terrestrial ecosystem. However, the impact of these BIO plastics in agroecosystems have not been sufficiently studied. Therefore, we investigated the impact of macro (around 5 mm) and micro (< 1 mm) sized plastic debris from LDPE and one type of starch-based BIO mulch film on soil physicochemical and hydrological properties. We used environmentally relevant concentrations of plastics, ranging from 0 to 2% (w/w), identified by field studies and literature review. We studied the effects of the plastic residue on a sandy soil for one month in a laboratory experiment. The bulk density, porosity, saturated hydraulic conductivity, field capacity and soil water repellency were altered significantly in the presence of the four kinds of plastic debris, while pH, electrical conductivity and aggregate stability were not substantially affected. Overall, our research provides clear experimental evidence that microplastics affect soil properties. The type, size and content of plastic debris as well as the interactions between these three factors played complex roles in the variations of the measured soil parameters. Living in a plastic era, it is crucial to conduct further interdisciplinary studies in order to have a comprehensive understanding of plastic debris in soil and agroecosystems.



4.1 Introduction

In recent years, researchers have seen soil as a major sink for microplastics (µPs, particles with diameter < 5 mm), which is a threat to sustainable agriculture and food security (de Souza Machado et al. 2018a; Ng et al. 2018; Nizzetto et al. 2016; Rillig 2012; Rillig et al. 2017a; Rillig et al. 2019; Rochman 2018). Subsequent studies have filled certain knowledge gaps with regards to µPs in terrestrial ecosystems, particularly in agricultural soil. For instance, the effects of µPs on soil biota have been studied (Cao et al. 2017; Huerta Lwanga et al. 2016; Zhu et al. 2018c), as well as their effects on multiple trophic levels (Huerta Lwanga et al. 2017b; Zhu et al. 2018b), underground transport (Huerta Lwanga et al. 2017a; Maass et al. 2017; Yu et al. 2019), and their interactions with other soil pollutants (Hodson et al. 2017; Rodríguez-Seijo et al. 2019; Yang et al. 2018; Yang et al. 2019). Although these studies have answered many questions, the most fundamental questions concerning µPs in soil have gone unanswered. Several major problems remain unresolved: no sufficient methods to quantify diverse µPs (Blasing and Amelung 2018; Corradini et al. 2019a; Fuller and Gautam 2016; Schwaferts et al. 2019; Shan et al. 2018; Zhang et al. 2018a), very limited field surveys measuring the status of µPs in the soil (Huang et al. 2020; Liu et al. 2018; Scheurer and Bigalke 2018; Zhang and Liu 2018; Zhou et al. 2018; Zhou et al. 2020), and lack of information concerning the impacts of µPs on soil physical, chemical and biological properties (de Souza Machado et al. 2018b; Liu et al. 2017; Qi et al. 2020a). Moreover, recent studies have shown that µPs affected soil structure, hydraulic conductivity, water holding capacity, etc. (de Souza Machado et al. 2018b; Zhang et al. 2019). Therefore, it is crucial to study the impacts of µPs on soil physicochemical and hydrological properties to gain a better understanding of this emerging contaminant in soil and the agroecosystems.

As one of the main sources contributing to µPs in agricultural soil, plastic mulching practices play a crucial role in modern agriculture (Gao et al. 2019; Z. Steinmetz et al. 2016a). The use of plastic mulch film (PMF) to increase water use efficiency has been going on for years and thus it is relevant to study the effects of residual PMF on parameters related to soil water holding capacity. The prevailing use of plastics in agronomy started in the early 1950s (Espi 2006). Since then, PMF has brought multiple benefits to agriculture such as instantly improving the quality and quantity of the harvests (Steinmetz et al., 2016). After decades of application, residual PMF fragments have accumulated in the soil and have had detrimental effects on soil quality and crop yield (Liu et al. 2014b; Yan et al. 2014). With the highest amount of PMF usage in the world, China was the first to notice the plastic residue pollution in agricultural soil and has conducted many studies since the 1980s (Dong et al. 2015; Xiang et al. 1992; Xu 1985; Zhao et al. 1998). In recent years, plastic residue in the soil has aroused intensifying concerns that the macroscopic plastic debris will eventually fragment into µPs

(Barnes et al. 2009). From previous studies about residual PMF, researchers raised universal concerns about its long-term effects on farmland (Gao et al. 2019).

Due to the increasing global concern surrounding plastic pollution, a huge variety of biodegradable plastic mulch film (BIO PMF) was designed as a promising substitute for polyethylene films (Brodhagen et al. 2017; Kasirajan and Ngouajio 2012). In 2016, the European commission estimated that among the 100,000 tonnes of PMF applied in Europe, 3,000 tonnes were BIO PMF (European Commission 2016). BIO plastics are made of polymers and additives that should degrade into carbon dioxide and methane or form new biomass (van Ginkel 2007b). According to current standards (e.g. ISO 17556 and EN 13432), BIO plastic should reach at least 90% biodegradation in the soil within two years (Carol Miles et al. 2017). However, studies warned that some polymers used in these films may not be biodegradable in soil conditions (Brodhagen et al. 2017; Thompson et al. 2019). The application of BIO PMF in agriculture has aroused fierce debate (Bandopadhyay et al. 2018; Sintim and Flury 2017) and sparked controversies surrounding the fact that BIO plastics are not only used within agroecosystems (Haider et al. 2019; Ren 2003). Furthermore, only scant studies have been performed to investigate the function and disintegration of BIO PMF (Anzalone et al. 2010; Kapanen et al. 2008; Li et al. 2014; Miles et al. 2012; Moreno et al. 2017). Therefore, both fundamental and in-depth studies examining BIO PMF are urgently needed to ensure their safe and sustainable application in agroecosystems.

In this study, we conducted mesocosm experiments in the laboratory using both low-density polyethylene (LDPE) and BIO PMF. The LDPE and BIO PMF were made into macro- and micro- sized debris to investigate the impacts of the plastic debris on soil physical, hydrological and chemical properties with a plastic content gradient (0, 0.5%, 1% and 2% w/w). LDPE was chosen since it is the most common mulch material and BIO PMF was chosen because it has become increasingly popular in agricultural applications (Kasirajan and Ngouajio 2012; Steinmetz et al. 2016a). We hypothesized that (i) tested soil parameters would have predictable responses to the presence of plastic debris, e.g. a decrease of bulk density, increase of porosity, increase of water flow, increase of water repellence, and (ii) different types, sizes and content of the plastic debris may have distinct effects on soil physicochemical and hydrological properties.

4.2 Materials and methods

4.2.1 Experimental setup

The mesocosm experiments were performed at 20°C and 35% humidity in the laboratory of the Soil Physics and Land Management Group, Wageningen University & Research (WUR). Our test soil was a sandy soil (4% Organic matter, pH = 6) with 87% sand, 12% silt and 1% clay. It was collected from farmland at Unifarm, WUR and has been used for our previous studies (Qi et al. 2018; Qi et al. 2020a). More information about the soil properties can be found in Table S4.1.

LDPE and BIO PMF were bought from the plastic mulch producer. The company states that the BIO PMF is produced from a formulated compound consisting mainly of polybutylene adipate terephthalate, starch and about 5% polylactic acid, blended with a black carbon masterbatch using a copolyester as a carrier resin. The presence of polybutylene adipate terephthalate and starch was confirmed by Fourier transform infrared spectroscopy and Differential scanning calorimetry. Macro- and micro- sized debris from LDPE and BIO PMF were prepared as described in a previous study (Qi et al. 2018). Macro-sized pieces were made by cutting PMF into 5×5 mm² squares by hand and the micro-sized powder was made by freeze grinding the plastic with liquid nitrogen. The powder consisted 25% of particles between 50 and 250 µm, 62.5% of particles between 250 and 500 µm and 12.5% of particles between 500 and 1000 µm. The effects of two types and two sizes of plastic debris (i.e. LDPE-Mi, BIO-Mi, LDPE-Ma, BIO-Ma) were each tested in the experimental soil at three concentrations: 0.5%, 1% and 2% of soil dry weight (Table 4.1). This concentration gradient is environmentally relevant and was chosen based on previous studies (de Souza Machado et al. 2018b; Qi et al. 2020a). Soil without additional plastic was used as the Control.

In total, 13 treatments were tested and each treatment was replicated in three mesocosms. The three contents were always tested together with the Control treatment, during three different months making three incomplete blocks due to logistic reasons (Table 4.1).

The plastic debris was mixed with 2 mm sieved dry soil and water was added to reach a soil gravimetric water content of 20%. Four kg of the mixture was then manually packed into each plastic pot (4 L, 16.5 cm high) with a wooden pressing tool (Figure S4.1). The compaction consisted of a define pattern of 10 hits repeated every kg of soil added. Each pot was covered loosely with a plastic lid and stored at 20°C for 30 days. Every week, the mesocosms were weighed and watered to compensate for evaporation (about 10 g per week).

Table 4.1 *Treatment settings for the mesocosm experiments*

Block	Treatment	Plastic type	Plastic size	Plastic content (w/w)
1st	Control	-	-	0.0%
	LDPE-Mi_0.5	LDPE	Micro	0.5%
	BIO-Mi_0.5	BIO	Micro	0.5%
	LDPE-Ma_0.5	LDPE	Macro	0.5%
	BIO-Ma_0.5	BIO	Macro	0.5%
2nd	Control	-	-	0.0%
	LDPE-Mi_1	LDPE	Micro	1.0%
	BIO-Mi_1	BIO	Micro	1.0%
	LDPE-Ma_1	LDPE	Macro	1.0%
	BIO-Ma_1	BIO	Macro	1.0%
3rd	Control	-	-	0.0%
	LDPE-Mi_2	LDPE	Micro	2.0%
	BIO-Mi_2	BIO	Micro	2.0%
	LDPE-Ma_2	LDPE	Macro	2.0%
	BIO-Ma_2	BIO	Macro	2.0%

At the end of the experiment, four ring samples (5 cm diameter) were taken at the 0-5 cm depth and four others at the 7-12 cm depth. All the ring samples were analysed for porosity, dry bulk density (ρ_b), saturated hydraulic conductivity (ks), field capacity (FC) and water drop penetration time (WDPT). The pH, electrical conductivity (EC) and aggregate stability index (ASI) were measured from 2 mm sieved, air dried soil samples at both soil depths (two samples at 0-5 cm and two others at 7-12 cm) for each pot.

4.2.2 Measurements of soil parameters

After sampling, ring samples were water saturated for 24 h and weighed. The ks was then measured on saturated ring samples using the flow induction with constant head method (Klute and Dirksen 1986), described in (Figure S4.2). Ring samples were then placed in a sandbox to measure the FC (Klute 1986; Topp and Zebchuk 1979), described in (Figure S4.3). The suction was gradually increased to pF 2 and the ring samples were weighed to measure the gravimetric water content. FC is defined as the gravimetric water content at pF 2. Soil water repellency was assessed on the ring samples at pF 2 using the WDPT method (Ritsema et al. 2008). An arbitrary WDPT threshold of 5 seconds was used to distinguish between hydrophilic (wettable) and hydrophobic (water-repellent) soils (Louis W Dekker et al. 2009a). The ring samples were finally dried at 105 °C for 48 h. The dry mass was used to calculate the water content at saturation and at pF 2. The porosity was estimated using the volume of water in a saturated sample divided by the total volume (Klute and Dinauer 1986). The ρ_b was measured using the dry mass of the sample and the ring volume (Klute and Dinauer 1986).

pH (H_2O) and EC were measured in a suspension (1:5) of 5 g of 2 mm sieved dry soil in 25 ml demineralized water with a SenTix meter and a conductivity cell TetraCon 325, separately (Čapka et al. 2009). ASI was determined using an Eijkelkamp wet sieving apparatus with 4 g of 2 mm sieved soil and NaOH 2 g/L as a dispersing solution (Almajmaie et al. 2017; Kemper and Rosenau 1986).

4.2.3 Statistical analysis

The results of each parameter were analysed using a linear mixed effect model (Eq.S4.1) implemented in SAS® 9.4 (Littell et al. 2006). Measured variables (i.e. porosity, ρ_b, ks, FC, WDPT, pH, EC and ASI) were modelled while taking into account the content, type and size of the plastic debris applied to the soil and the soil depth of the sample. Random terms were included to correct for temporal (Block) and positional effects (Pot and Pot-Depth combination).

After fitting the mixed models, the distribution of standardized residuals was checked for approximate normality. Residuals for all parameters, except ks, loosely followed a normal distribution. The residuals for $\log_{10}$(ks) followed a normal distribution, so $\log_{10}$(ks) was used for the analysis of ks. For all parameters, the soil depth factor was relatively unimportant (Table 4.3). Therefore, we decided to present the results averaged over both soil depths. The contribution of the main effects of each factor and each factor's interaction with the fitted model was quantified, using F-values and p-values (Table 4.3). The variance components for the random terms (i.e. Block, Block×Pot, Block×Pot×Depth and Residual) were calculated. The random terms contributing to the total variance of the individual observation are shown in (Table S4.2). Means and standard errors of means were estimated for all the parameters (Table S4.3). Estimated means and standard errors of means were plotted in R version 3.4.2 (Team 2013). For convenience, the model was reparametrized, aggregating factors Type, Size and Content into one single factor Comb (Eq.S4.2) with 13 levels (1 control and 12 factor level combinations). This reparametrized model allowed for simple comparisons of treatments with the Control treatment, as well as other pairwise comparisons, using t-tests (Table 4.2).

In addition, a principal component analysis was performed for the parameters with the most effects (porosity, ρb, ks, FC and WDPT) and the correlations between porosity, ρb and ks was further explored with linear regressions. Two equations were tested to fit the porosity and ρb data. These analyses are presented in supplementary materials. The raw data, the outcomes of the model and the R script used for the plots and calculation are available on the GitHub page (https://github.com/NGBeriot/Plastic_mulch-soil_properties).

4.3 Results

4.3.1 Soil structure parameters: porosity, dry bulk density (ρ_b) and aggregate stability index (ASI)

The estimated mean porosity for the Control was 0.43 ± 0.02 (Table S4.3). Porosity of the Control was not significantly different for plastic treatments with 0.5% content (Figure 4.1A). Size-wise comparisons for treatments with BIO plastics at both 1% and 2% showed that the macro-sized pieces had higher porosity than micro-sized particles (Table 4.2). Type-wise comparisons showed that LDPE-Ma_2 had lower porosity than BIO-Ma_2. Content-wise comparisons for LDPE-Ma showed that porosity for 1% was higher than the Control, 0.5% and 2% contents. For LDPE-Mi, the porosity for 1% was higher than the Control and 0.5% content but not different from the 2% content. For BIO-Ma, the porosity at 1% and 2% were not significantly different but they were both higher than the Control.

ρ_b of the Control was not significantly different from any of the plastic treatment with 0.5% content (Figure 4.1B). ρ_b decreased with increasing 1% and 2% plastic content for all plastic debris except BIO-Mi. Size-wise comparisons showed that for LDPE_1% and LDPE_2%, the macro-sized debris had lower ρ_b than the micro-sized ones (Table 4.2). Type-wise comparisons showed that for 2% content, LDPE had lower ρ_b than BIO for both macro- and micro- sizes. Content-wise comparisons showed that the addition of LDPE-Ma significantly decreased ρ_b as the increase of content went from 0.5% to 2%.

The estimated mean value of ASI over all the treatments ranged from 0.48 ± 0.045 to 0.68 ± 0.045, with the Control being 0.56 ± 0.045 (Table S4.3). BIO-Mi_0.5 showed significantly higher ASI compared to BIO-Ma_0.5 and no other significant differences in ASI were observed among the treatments (Table 4.2).

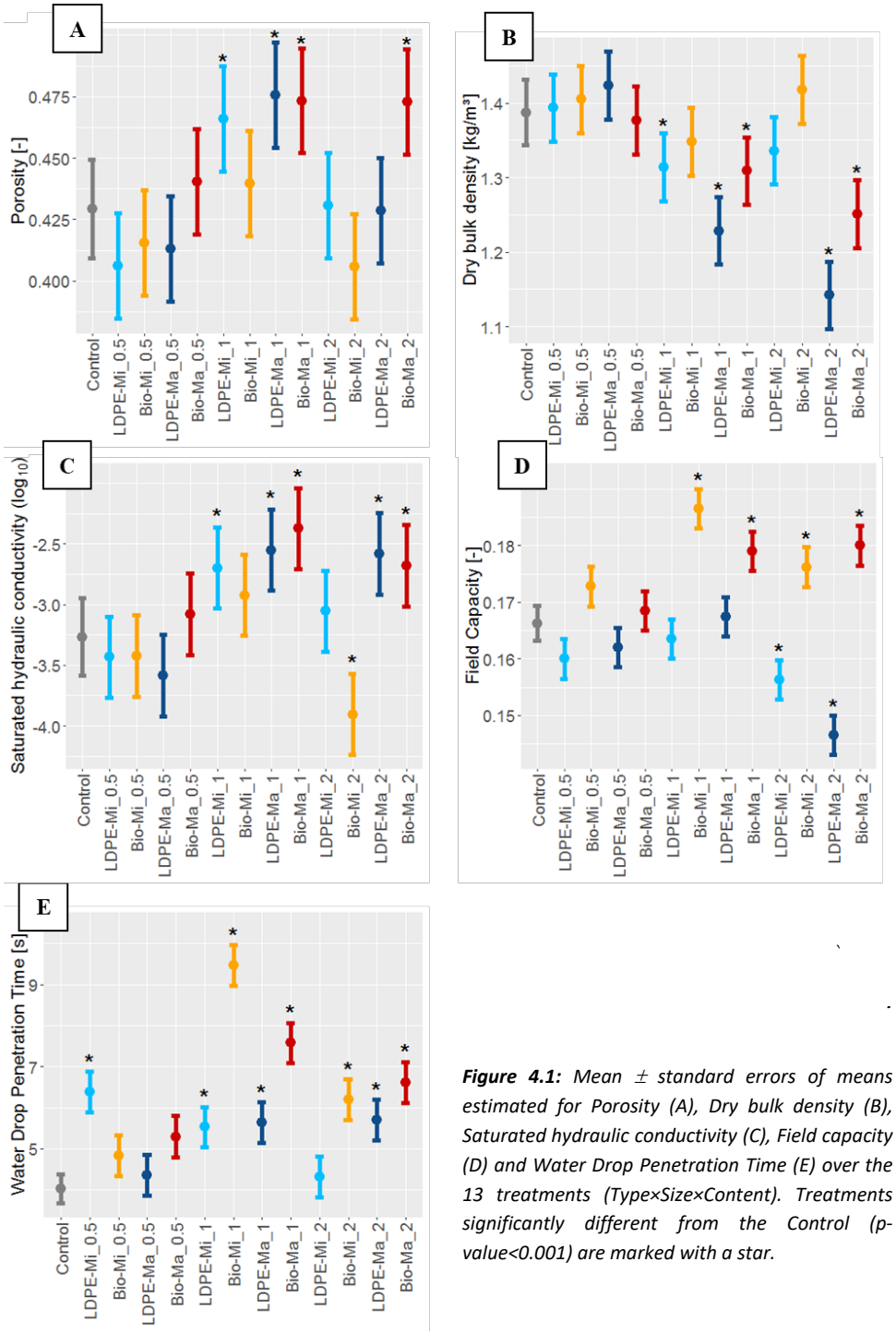


Figure 4.1: *Mean ± standard errors of means estimated for Porosity (A), Dry bulk density (B), Saturated hydraulic conductivity (C), Field capacity (D) and Water Drop Penetration Time (E) over the 13 treatments (Type×Size×Content). Treatments significantly different from the Control (p-value<0.001) are marked with a star.*

Table 4.2 *Estimates of differences between treatments associated with p-value < 0.001.*

	Porosity [-]	ρ_b [kg/m³]	$\log_{10}$(ks) [-]	FC [-]	WDPT [s]	pH [-]	EC [µS/cm]	ASI [-]
Comparison size-wise (Mi-Ma) ; same type, same content								
LDPE-Mi_0.5 - LDPE-Ma_0.5	.	.	.	.	2.0	.	.	.
LDPE-Mi_1 - LDPE-Ma_1	.	0.09	.	.	.	.	.	.
LDPE-Mi_2 - LDPE-Ma_2	.	0.19	-0.47	0.01	.	.	.	.
BIO-Mi_0.5 - BIO-Ma_0.5	.	.	.	.	.	.	.	0.16
BIO-Mi_1 - BIO-Ma_1	-0.034	.	-0.55	0.007	1.9	-0.10	.	.
BIO-Mi_2 - BIO-Ma_2	-0.067	0.17	-1.23	.	.	.	.	.
Comparison type-wise (LDPE-BIO) ; same size, same content								
LDPE-Mi_0.5 - BIO-Mi_0.5	.	.	.	-0.01	1.5	.	.	.
LDPE-Mi_1 - BIO-Mi_1	.	.	.	-0.02	-3.9	.	.	.
LDPE-Mi_2 - BIO-Mi_2	.	-0.08	0.85	-0.02	-1.9	.	.	.
LDPE-Ma_0.5 - BIO-Ma_0.5	.	.	-0.51	.	.	.	.	.
LDPE-Ma_1 - BIO-Ma_1	.	-0.08	.	-0.01	-1.9	.	.	.
LDPE-Ma_2 - BIO-Ma_2	-0.044	-0.11	.	-0.03	.	.	.	.
Comparison content-wise (0.5-1, 0.5-2, 1-2) ; same type, same size								
LDPE-Mi_0.5 - LDPE-Mi_1	-0.060	0.08	-0.73	.	.	.	.	.
BIO-Mi_0.5 - BIO-Mi_1	.	.	.	-0.01	-4.6	.	.	.
LDPE-Ma_0.5 - LDPE-Ma_1	-0.062	0.20	-1.03	.	.	.	.	.
BIO-Ma_0.5 - BIO-Ma_1	.	.	-0.70	-0.01	-2.3	.	.	.
LDPE-Mi_0.5 - LDPE-Mi_2	.	.	.	.	.	.	.	.
BIO-Mi_0.5 - BIO-Mi_2	.	.	.	.	.	.	.	.
LDPE-Ma_0.5 - LDPE-Ma_2	.	0.28	-1.01	0.015	.	.	.	.
BIO-Ma_0.5 - BIO-Ma_2	.	0.13	.	-0.01	.	.	.	.
LDPE-Mi_1 - LDPE-Mi_2	.	.	.	.	.	.	.	.
BIO-Mi_1 - BIO-Mi_2	.	.	0.98	0.01	3.3	.	.	.
LDPE-Ma_1 - LDPE-Ma_2	0.047	0.09	.	0.021	.	.	.	.
BIO-Ma_1 - BIO-Ma_2	.	.	.	.	.	.	.	.

Cells are empty (.) if the p-value > 0.001. All estimated differences and associated p-value were provided in (Table S4.4).
ρb: dry bulk density ; ks: saturated hydraulic conductivity ; FC: field capacity ; WDPT: water drop penetration time ; EC: electrical conductivity ; ASI: aggregates stability index.

4.3.2 Water infiltration parameter: saturated hydraulic conductivity (ks)

ks of the Control was not significantly different from any of the plastic treatments with 0.5% content (Figure 4.1C). Size-wise comparisons showed that for BIO_1% and BIO_2%, the macro- sized debris had higher ks than the micro-sized ones (Table 4.2). Type-wise comparison showed that treatments LDPE-Ma_0.5 had lower ks than BIO-Ma_0.5, but LDPE-Mi_2 had higher ks than BIO-Mi_2. Content-wise comparisons showed that the increase from 0.5% to 1% of plastic debris increased ks, but not all the differences were statistically significant. There was no further increase of ks with the increase from 1% to 2% plastic debris.

4.3.3 Soil water retention parameter: field capacity (FC)

FC of the Control was not significantly different from any plastic treatments with 0.5% content (Figure 4.1D). However, BIO_1% and BIO_2% of both macro- and micro- sizes had higher FC than the Control and LDPE_2% had lower FC than the Control. Size-wise comparisons showed that for BIO_1% and LDPE_2%, the macro-sized had lower FC than micro-sized ones (Table 4.2). Type-wise comparisons showed that the treatments with LDPE macro- and micro- sizes had lower FC as compared to BIO. Content-wise comparisons showed that the FC of BIO-Mi at 1% was higher than the Control, 0.5% and 2%.

4.3.4 Soil water repellency parameter: Water Drop Penetration Time (WDPT)

The WDPT was higher for all of the treatments with plastic residues as compared to the Control (Figure 4.1E). Size-wise comparisons for LDPE_0.5% and BIO_1% showed that WDPT was lower for the macro-sized plastics than for the micro-sized plastics (Table 4.2). Type-wise comparisons showed that most of the treatments with LDPE had lower WDPT as compared to the treatments with BIO. Content-wise comparisons showed that the WDPT for BIO-Mi at 1% was higher than the Control, 0.5% and 2% contents. The WDPT for LDPE-Mi decreased with increasing content from 0.5% to 2%, but the differences were not statistically significant. All treatments, except for the Control, BIO-Mi_0.5, LDPE-Ma_0.5 and LDPE-Mi_2, were above the 5 s threshold defining water repellent soils.

4.3.5 Soil chemical properties: pH and electrical conductivity (EC)

The estimated mean value of pH over all of the treatments ranged from 6.28 $\pm$ 0.052 to 6.42 $\pm$ 0.052, with the Control at 6.33 ± 0.052 (Table S4.3). The estimated mean value of EC over all the treatments ranged from 431 $\pm$ 65 to 532 $\pm$ 65, with the Control at 492 ± 65 (Table S4.3). We did not observe important variation of pH and EC caused by the addition of the plastic debris (Table 4.2).

4.3.6 Main factors and interactions

The main factor affecting porosity, ρb and $\log_{10}$(ks) was the size of the plastic whereas it was the type of the plastic for FC, WDPT and pH (Table 4.3). Both the type and the size of the plastic had important impacts on ρb.

Table 4.3 *Tests of Fixed Effects for the four factors and the factor interactions F-value (p-value).*

Factor and Interaction	DF num, DF den	Porosity	ρb	log_{10}ks	FC	WDPT	pH	EC	ASI
Content	2, 32	**11.33** **(0.0002)**	**26.13** **(<.0001)**	**15.67** **(<.0001)**	**12.1** **(0.0001)**	8.3 (0.0017)	0.48 (0.62)	0.56 (0.57)	0.55 (0.65)
Type	1, 31	1.51 (0.22)	**51.42** **(<.0001)**	2.42 (0.12)	**506.95** **(<.0001)**	**64.65** **(<.0001)**	**18.59** **(0.0002)**	3.56 (0.063)	0.19 (0.66)
Size	1, 31	**40.38** **(<.0001)**	**164.62** **(<.0001)**	**71.4** **(<.0001)**	6.09 (0.019)	2.33 (0.13)	2.63 (0.12)	0.27 (0.60)	5.32 (0.027)
Depth	1, 31	0.17 (0.68)	1.77 (0.19)	4.63 (0.038)	6.72 (0.014)	11.16 (0.0021)	4.9 (0.034)	1.35 (0.25)	0.77 (0.39)
Content × Type	2, 31	7.04 (0.0030)	**27.89** **(<.0001)**	**17.5** **(<.0001)**	**38.44** **(<.0001)**	**31.28** **(<.0001)**	1.69 (0.20)	0.41 (0.66)	1.11 (0.34)
Content × Size	2, 31	1.76 (0.19)	**72.08** **(<.0001)**	**18.82** **(<.0001)**	0.49 (0.62)	**12.06** **(0.0001)**	**10.03** **(0.0004)**	2.51 (0.089)	1.47 (0.24)
Type × Size	1, 31	**25.26** **(<.0001)**	0.17 (0.69)	**29.07** **(<.0001)**	0.75 (0.39)	0.23 (0.63)	6.66 (0.015)	0.0 (0.98)	6.15 (0.018)
Content × Type × Size	2, 31	4.8 (0.015)	6.55 (0.0042)	1.05 (0.36)	**22.84** **(<.0001)**	**16.3** **(<.0001)**	1.45 (0.24)	0.93 (0.39)	2.42 (0.10)
Depth × Content	2, 33	3.46 (0.043)	2.55 (0.0.93)	1.94 (0.16)	3.83 (0.032)	1.1 (0.34)	3.53 (0.041)	1.07 (0.35)	6.49 (0.0042)
Depth × Type	1, 33	0.83 (0.37)	0.66 (0.42)	1.81 (0.19)	4.19 (0.049)	0.52 (0.48)	12.79 (0.0011)	1.74 (0.19)	1.47 (0.23)
Depth × Size	1, 33	2.1 (0.16)	**13.7** **(0.0008)**	8.92 (0.0053)	**13.06** **(0.001)**	6.89 (0.013)	0.07 (0.80)	0.02 (0.89)	0.19 (0.67)
Depth × Content × Type	2, 33	2.45 (0.10)	3.75 (0.034)	5.13 (0.012)	6.57 (0.004)	1.55 (0.23)	4.29 (0.022)	1.49 (0.23)	1.35 (0.27)
Depth × Content × Size	2, 33	1.45 (0.25)	0.45 (0.64)	1.12 (0.34)	2.32 (0.11)	0.1 (0.90)	1.11 (0.34)	0.94 (0.40)	1.21 (0.31)
Depth × Type × Size	1, 31	4.6 (0.040)	0.27 (0.60)	0.47 (0.50)	5.31 (0.028)	0.12 (0.73)	10.27 (0.003)	1.17 (0.28)	0.63 (0.43)
Depth × Content × Type × Size	2, 33	0.12 (0.88)	2.2 (0.13)	3.31 (0.049)	1.48 (0.24)	3.63 (0.038)	4.19 (0.0239)	1.6 (0.21)	1.73 (0.19)

DF num and DF den are the degrees of freedom for numerator and denominator for the F-tests, respectively. ρb: dry bulk density ; ks: saturated hydraulic conductivity ; FC: field capacity ; WDPT: water drop penetration time ; EC: electrical conductivity ; ASI: aggregates stability index. Bold values have $p<0.001$. Underlined values are the highest per parameter when $p<0.001$.

The type of plastic itself did not affect the porosity and the $\log_{10}$(ks) very much but the Type×Size interaction was responsible for a lot of variation. The content of the plastic played a major role in the porosity, ρb, $\log_{10}$(ks), FC and WDPT and always interacted with the Type factor. For each of these five parameters, more than one factor interaction had a significant impact and the 3-factor interaction Content×Type×Size was significant except for $\log_{10}$(ks). The soil depth and its interactions with other factors was relatively unimportant. Overall, the studied factor had small effects on EC and ASI.

To further explore the correlation between parameters which were mostly affected by the main factors and their interactions, the principal component analysis for the parameters porosity, ρb, ks, FC and WDPT were conducted as additional information. The first, second and third principal components explained 89% of the variance (Table S4.5). The first and second principal components showed that porosity and ks were likely to be positively correlated, while both were likely to be negatively correlated to ρb (Figure S4.4A). The correlations porosity/ρb, porosity/ks and ρb/ks had a coefficient of determination of 0.33, 0.54 and 0.65, respectively (Figure S4.5). The correlation fit the data except for the treatment LDPE-Ma_2 which had values below the regression lines porosity/ρb and ρb/ks. Additionally, the equations Eq.S4.6 and Eq.S4.7 showed that the plastic content plays a minor role in explaining the correlation between porosity and ρb.

4.4 Discussion

The present study provides clear experimental evidence that incorporating PMF residues into the tested sandy soil aroused multiple effects on studied soil properties (Figure 4.1). Differences were observed for physicochemical and hydrological parameters, when compared to the treatments with plastic additions and/or with the Control (Table 4.2). The size, type and content of plastic debris presented idiosyncratic effects on tested soil parameters. These soil parameters are closely related with soil type and we only used one sandy soil in this study. Nevertheless, our research aligns with previous studies, suggesting that further research is urgently needed to develop a comprehensive understanding of plastic pollution in agroecosystems.

4.4.1 Effects of the size, type and content of plastic debris on soil and agroecosystems

So far, only a few studies have been carried out that examine the effects of plastic residues on soil properties and the research was either focused on macro- or micro-sized debris (de Souza Machado et al. 2018b; Dong et al. 2015; Jiang et al. 2017). In this study, both macro- and micro-sized plastic residues had significant impacts on studied soil parameters. Significant differences between the Control and treatments were observed more frequently in treatments with macro-sized debris. In the research of de Souza Machado et al. (2018b), researchers found that µPs affected the ρ_b, water holding capacity, hydraulic conductivity and water stable aggregates. Dong et al. (2015) found that plastic film residues (0 - 100 cm^2) affected soil moisture content, porosity, pH, organic matter and worsened soil quality. Jiang et al. (2017) demonstrated that residual PMF fragments changed soil properties, e.g. soil water content, ρ_b, ks and porosity, and altered soil water distribution involved with plant roots. Since different conditions were used for these studies, it is hardly feasible to directly compare the results.

In a previous study, using the same kind of soil and plastic materials, the addition of 1% plastic residues had significantly negative effects on crop growth and micro-sized plastic residues showed more negative effects than macro-sized residues (Qi et al. 2018). In the current experiment, with the same plastic type and content, treatments with micro-sized residues showed significantly lower porosity and ks, and higher ρ_b, FC and WDPT, in some cases. Although the changes, even if statistically significant, were relatively small, we hypothesized that the changes in these soil properties brought about by the addition of plastic residues may negatively affect soil quality and plant growth. In the long run, plastic debris could be eventually degraded into micro- and nanoplastics due to various biotic and abiotic stressors (Barnes et al. 2009; B. Singh and Sharma 2008). Studies about plastic debris in different sizes are needed to assess the long term effects of microplastics in soil.
In this study, we found that BIO and LDPE plastic debris showed significantly different effects on soil properties even with the same size and content. Correspondingly, with the same soil and plastic materials (at content 1%), Qi et al. (2018) concluded that BIO plastic debris had stronger negative effects on crop yield and growth than LDPE. Hence, the negative effects on plant growth could be partly explained by the effects of plastic debris on soil properties. Regarding different types of plastic debris, de Souza Machado et al. (2018b) tested four different types of µPs and found that polyester fibres showed the most noticeable impacts on the soil biophysical environment as compared with polyacrylic fibres, polyamide beads and polyethylene fragments. Unfortunately, previous studies of BIO mulch films mainly focused on their performance in agriculture (Anzalone et al. 2010; Kapanen et al. 2008; Miles et al. 2012) or their degradation and deterioration patterns (Li et al. 2014;

Moreno et al. 2017). Li et al. (2014a) buried two starch-based mulches, one polylactic acid mulch and one cellulose-based mulch, in a field for 18 months and suggested that the BIO mulch films had minor effects on the soil quality during the evaluation period. As Sintim and Flury discussed (2017), although BIO mulch films may be encouraging substitutes for traditional polyethylene films, in-depth and comprehensive studies, focussing on the potential release of micro- and nanoplastics during degradation processes among others, should be conducted before they are widely utilized. Overall, BIO plastics should not be considered as the panacea for plastic pollution in agroecosystems without in-depth research.

In our experiment, we set the same gradient for the four kinds of plastic debris tested, i.e. 0.5%, 1%, 2% and the Control at 0%. It is difficult to concisely summarize the content-wise effects of different plastic debris on various soil parameters since quite a few low-content plastic debris showed stronger effects than high-content debris even if the plastic sizes and types were all the same. Similarly, de Souza Machado et al. (2018b) added a series of concentrations for different μPs ranging from 0.05% to 2.00% to the soil and they found the apparent nonmonotonic dose responses of soil biophysical proxies. Although de Souza Machado et al. (2018b) suggested that it was unrealistic to assess this nonmonotonicity based on current experimental data, they intensively discussed the potential interactions among plastic particles and natural matter in the heterogeneous terrestrial ecosystem. The addition of plastic debris in the soil would affect multiple soil processes and the interactions between plastic particles and natural matter were unpredictable (de Souza Machado et al. 2018b).

Regrettably, to the best of our knowledge, there are no experiments that have been carried out on the effects of the μPs content gradients on crop growth so we could not estimate the dose responses of crops to μPs in the soil. Nevertheless, there are quite a few studies that have been conducted in China on the impacts of macroplastic residue gradients (from 0 to 1440 kg hm^{-2}) on crop growth and soil quality (Huang et al. 2019; Nan et al. 1996; Zhang et al. 2014; Zhao et al. 1998). For instance, Zhao et al. (1998) found monotonic responses of crop yield, ρ_b and porosity to the gradient of residual PMF weight (0, 37.5, 75, 150, 225, 300, 375 and 450 kg hm^{-2}). While Huang et al. (2019) also observed glaring adverse effects of plastic residues on the growth and yield of potato, they did not find any linear correlation between the yield and the residual amount of PMF (0, 90, 180, 360 and 720 kg hm^{-2}). Regardless, considering the undeniable nonmonotonicity in the responses of the soil matrix to plastic debris, further studies using a series of gradients are urgently needed to elucidate the mechanisms and dose responses.

4.4.2 Limitations and wider implications for ecological assessment of plastic debris in soil

We have asserted that the types, sizes and contents of the plastic debris has had distinct effects on selected soil properties in our study and interactions mattered in some cases. Parameters of soil structure, water infiltration, water retention and soil water repellency all responded vigorously during the experimental period, but not many effects were measured in the soil chemical parameters, i.e. pH and EC. On one hand, one month might be too short for plastic debris to initiate chemical alterations in the soil. On the other hand, other soil chemical parameters may react more swiftly than pH and EC. It is difficult to explain the variations of parameters in the presence of plastic debris in the soil. For instance, the effects of plastic debris on porosity and ρb cannot be explained by the lower density of plastic compared to the soil particles using Eq.S4.6 and Eq.S4.7. In this study, only the effective porosity was measured using the saturation method and only a sandy soil was tested. Therefore, more tests using different soil textures are required to understand how plastic debris may affect the soil's physical and hydrological parameters.

We did not expect the plastic to undergo significant degradation during this one-month experiment. PMF was designed to keep its integrity over the crop growing season (> 1 month) and exposure to UV irradiation from the sun is a significant factor in plastic degradation (Napper and Thompson 2019a). The properties of plastic will change during degradation and therefore, we could expect they may have different interactions with the soil. Further studies should take into account the degradation of plastics in long-term experiments and aging plastic debris could be used to compare with virgin debris.

With regards to soil properties, a soil's biological trait is a vital aspect, along with soil physical and chemical parameters (Bünemann et al. 2018). With the same plastic materials and soil, Qi et al. (2020a) found that the rhizosphere bacterial communities were significantly affected by the presence of BIO PMF residues. When Li et al. (2014a) investigated the effects of mulch film residues on soil quality, they calculated the soil quality index based on microbial biomass carbon, β-glucosidase, EC, total organic carbon and pH, so that the alterations of soil quality among treatments could be clearly presented by numerical comparisons. While scientists try to obtain an overall soil quality index for comparisons, as Bünemann et al. (2018) critically reviewed, an assessment framework based on a logical-sieve method would be useful for the assessment of targeted soil threats. Hence, establishing an assessment framework which can be applied universally for plastic debris in soil would be profoundly pragmatic for further studies.

4.5 Conclusion

Overall, we saw that both LDPE and BIO PMF debris in either macro- or micro- sizes had noticeable effects on soil physicochemical and hydrological parameters and these properties of tested sandy soil nonmonotonically responded to residual amounts of PMFs. For instance, the presence of LDPE debris decreased field capacity, while BIO plastic debris increased it. Macro-sized plastic debris presented more differences between the Control, compared to micro-sized ones. Special attention should be paid to the fundamental properties of soil in order to gain a comprehensive understanding of the potential effects of plastic residues on soils. Concerning their conspicuous mischief and long-term existence, we eagerly call on further interdisciplinary studies for various types, sizes and contents of plastic debris in soil and agroecosystems.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (41877072), the Natural Science Basic Research Plan in Shaanxi Province of China (2019JQ-639) and the EU Horizon 2020 project (ISQAPER: 635750). We are thankful for the financial support from the China Scholarship Council (CSC: 201604910510). We acknowledge Taede Stoker from Unifarm of Wageningen University & Research for providing the soil, the pots and helpful information. Many thanks to Klaas Oostindie and Piet Peters from the SLM group (WUR) for their expertise in water repellency and soil aggregate stability, respectively. Thanks to Henny Gertsen for helping prepare the plastic materials. We acknowledge Maarten van der Zee from the Agrotechnology & Food Science group of WUR for his expertise about plastics. We are very grateful to Robin Palmer for the language editing.

Supplementary Material

S4.1 Experimental set up

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

soil parameters	unit	results
Total nitrogen	kg/ha	3775
C/N ratio		17
Available nitrogen	kg/ha	45
Total sulfur	kg/ha	995
C/S ratio		66
Available sulfur	kg/ha	15
Total phosphorus	kg/ha	695
Total potassium	kg/ha	505
Total calcium	kg/ha	3840
Total Magnesium	kg/ha	260
pH		6
Organic carbon	%	2
Organic matter	%	4
Inorganic carbon	%	0.07
Carbonated lime	%	<0.2
clay	%	1
silt	%	12
sand	%	87

Figure S4.1: *Pot and wooden pressing tool.*

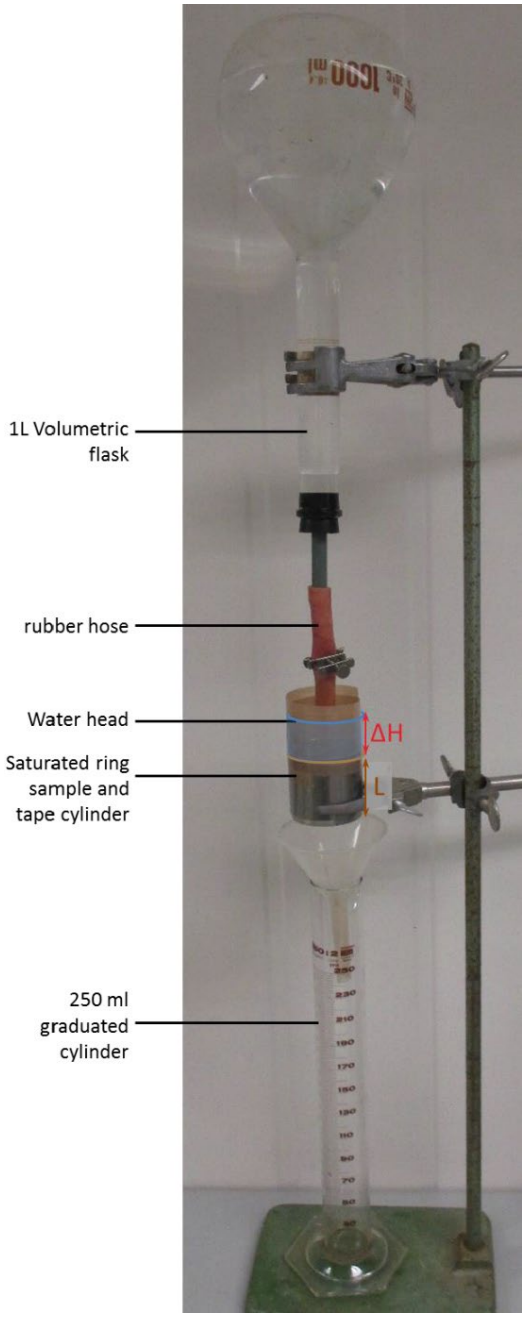


Figure S4.2: *Experimental set-up for measuring the saturated hydraulic conductivity with free outflow. The inverted flask with the tube reaching down to within the taped rim (brown) serves as a Mariotte flask that Maintains a constant water level above the sample (grey cylinder).*

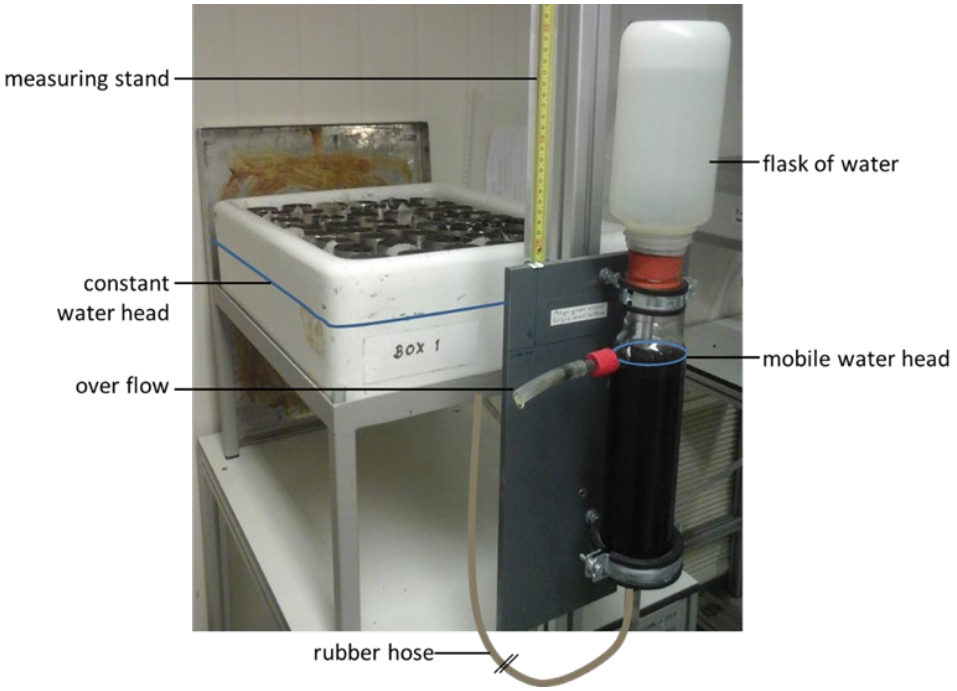


Figure S4.3: *Experimental set-up for measuring the field capacity.*

S4.2 Linear mixed effect model

Eq.S4.1: Factorial mixed effect model

$$
\begin{aligned}
Y = {} & Content + Type + Size + Depth \\
& + Content \times Type + Content \times Size + Content \times Depth \\
& + Type \times Size + Type \times Layer + Size \times Depth \\
& + Content \times Type \times Size + Content \times Type \times Depth \\
& + Content \times Size \times Depth + Type \times Size \times Depth \\
& + Content \times Type \times Size \times Depth \\
& + Random + \varepsilon
\end{aligned}
\qquad \text{S4.1}
$$

$$Random = Block + Block \times Pot + Block \times Pot \times Depth$$

In this model, Y represents the measured variable (i.e. porosity, dry bulk density, saturated hydraulic conductivity, water retention, pH, electrical conductivity, and Aggregation Stability Index). Content, Type, and Size are fixed factors for the content, type and size,

respectively, of the plastic debris added to the soil and Depth represents the position of the sample, either top depth (0-5 cm) or bottom depth (7-12 cm). Random terms were included to correct for temporal (Block) and positional effects (Pot and Pot-Depth combination). Block refers to an (incomplete) block of the experiment during a specific month, during which the Control treatment and one of the three levels of the content factor was investigated. Block×Pot refers to the set of observations stemming from one individual pot (eight observations for ring samples, and four observations for air dried soil samples). Block×Pot×Depth combination refers to the set of observations stemming from one part (top or bottom) of a pot (four observations for ring samples, two for air dried soil samples). The resulting analysis is a type of split-plot analysis with factors Content, Type and Size assigned to pots (forming the "main plots"), making pots the experimental units for these factors. Factor Depth has two distinct values within each pot, making the pot-depth combinations the experimental unit (forming the "subplots"). The individual sample (ring or air dried sample) form the measurement units at the lowest level of the hierarchy.

Factors Content, Type and Size were aggregated into a single treatment factor leading to the following model:

Eq.S4.2: Reparametrized Mixed model with aggregated treatment factor.

$$Y = Comb + Depth + Comb \times Depth + Random + \varepsilon \quad \text{Eq. S4.2}$$

$$Random = Block + Block \times Pot + Block \times Pot \times Depth$$

Comb represents the combination of factors Type, Size and Content into one single factor. There are two Type levels (LDPE and BIO), two Size levels (Ma and Mi), three Content levels (0.5%, 1%, 2%) and one control so *Comb* has 13 levels in total.

Table S4.2 *Variance component percentage of each random term for all parameters. Bold values are the highest per parameter.*

	Porosity	ρb	log10(ks)	FC	pH	EC	ASI	WDPT
Block	75%	83%	82%	67%	73%	59%	6%	17%
Block*Pot	5%	2%	3%	10%	1%	0%	5%	2%
Block*Pot*Depth	1%	3%	4%	3%	13%	25%	16%	6%
Residual	19%	11%	11%	20%	13%	16%	73%	75%

Table S4.3 *Estimated Mean and Standard Error of the estimated mean (SE) for all the parameters.*

	Porosity [-]	ρb [g/cm³]	log10(ks) [-]	FC [-]	ASI [-]	WDPT [s]	pH [-]	EC [µS/cm]
Ck	0.43	1.39	-3.27	0.166	0.56	4.0	6.33	492
LDPE-Mi_0.5	0.41	1.39	-3.43	0.160	0.59	6.4	6.32	462
LDPE-Ma_0.5	0.41	1.42	-3.59	0.162	0.59	4.4	6.35	466
BIO-Mi_0.5	0.42	1.40	-3.42	0.173	0.65	4.8	6.38	445
BIO-Ma_0.5	0.44	1.38	-3.08	0.169	0.48	5.3	6.42	463
LDPE-Mi_1	0.47	1.31	-2.70	0.164	0.60	5.5	6.32	532
LDPE-Ma_1	0.48	1.23	-2.55	0.167	0.64	5.6	6.35	524
BIO-Mi_1	0.44	1.35	-2.92	0.187	0.68	9.5	6.32	517
BIO-Ma_1	0.47	1.31	-2.37	0.179	0.62	7.6	6.41	448
LDPE-Mi_2	0.43	1.34	-3.05	0.156	0.61	4.3	6.36	490
LDPE-Ma_2	0.43	1.14	-2.58	0.147	0.56	5.7	6.28	519
BIO-Mi_2	0.41	1.42	-3.91	0.176	0.61	6.2	6.36	431
BIO-Ma_2	0.47	1.25	-2.68	0.180	0.59	6.6	6.35	505
SE	0.021	0.046	0.34	0.0035	0.045	0.50	0.052	65

Table S4.4 *Estimates of differences between treatments (p-value) for porosity, dry bulk density (ρb), saturated hydraulic conductivity (ks), field capacity (FC) in table A. and water drop penetration time (WDPT), electrical conductivity (EC), aggregates stability index (ASI) in table B. The values associated with p-value < 0.001 are in darker color.*

A.	Porosity [-]	ρ_b [kg/m³]	$\log_{10}$(ks) [-]	FC [-]
Comparison with the control				
LDPE-Mi_0.5 - Control	-0.023(0.015)	0.006(0.709)	-0.17(0.198)	-0.006(0.003)
BIO-Mi_0.5 - Control	-0.014(0.136)	0.017(0.268)	-0.16(0.226)	0.007(0.002)
LDPE-Ma_0.5 - Control	-0.016(0.083)	0.036(0.026)	-0.32(0.017)	-0.004(0.037)
BIO-Ma_0.5 - Control	0.011(0.238)	-0.01(0.503)	0.19(0.141)	0.002(0.257)
LDPE-Mi_1 - Control	0.037(0.0001)	-0.074(<.0001)	0.57(<.0001)	-0.003(0.138)
BIO-Mi_1 - Control	0.01(0.236)	-0.039(0.01)	0.35(0.006)	0.02(<.0001)
LDPE-Ma_1 - Control	0.046(<.0001)	-0.159(<.0001)	0.72(<.0001)	0.001(0.536)
BIO-Ma_1 - Control	0.044(<.0001)	-0.079(<.0001)	0.89(<.0001)	0.013(<.0001)
LDPE-Mi_2 - Control	0.001(0.891)	-0.051(0.002)	0.21(0.099)	-0.01(<.0001)
BIO-Mi_2 - Control	-0.024(0.014)	0.03(0.06)	-0.64(<.0001)	0.01(<.0001)
LDPE-Ma_2 - Control	-0.001(0.939)	-0.246(<.0001)	0.69(<.0001)	-0.02(<.0001)
BIO-Ma_2 - Control	0.043(<.0001)	-0.137(<.0001)	0.59(<.0001)	0.014(<.0001)
Comparison size-wise (Mi-Ma); same type, same content				
LDPE-Mi_0.5 - LDPE-Ma_0.5	-0.007(0.441)	-0.03(0.057)	0.15(0.231)	-0.002(0.313)
LDPE-Mi_1 - LDPE-Ma_1	-0.01(0.297)	0.085(<.0001)	-0.15(0.248)	-0.004(0.051)
LDPE-Mi_2 - LDPE-Ma_2	0.002(0.831)	0.194(<.0001)	-0.47(0.0007)	0.01(<.0001)
BIO-Mi_0.5 - BIO-Ma_0.5	-0.025(0.01)	0.028(0.08)	-0.35(0.01)	0.004(0.038)
BIO-Mi_1 - BIO-Ma_1	-0.034(0.0007)	0.039(0.016)	-0.55(0.0001)	0.007(0.0006)
BIO-Mi_2 - BIO-Ma_2	-0.067(<.0001)	0.167(<.0001)	-1.23(<.0001)	-0.004(0.063)
Comparison type-wise (LDPE-BIO); same size, same content				
LDPE-Mi_0.5 - BIO-Mi_0.5	-0.009(0.306)	-0.012(0.457)	-0.01(0.938)	-0.013(<.0001)
LDPE-Mi_1 - BIO-Mi_1	0.026(0.006)	-0.034(0.033)	0.22(0.084)	-0.023(<.0001)
LDPE-Mi_2 - BIO-Mi_2	0.025(0.01)	-0.081(<.0001)	0.85(<.0001)	-0.02(<.0001)
LDPE-Ma_0.5 - BIO-Ma_0.5	-0.027(0.005)	0.047(0.005)	-0.51(0.0003)	-0.007(0.002)
LDPE-Ma_1 - BIO-Ma_1	0.002(0.801)	-0.08(<.0001)	-0.18(0.165)	-0.012(<.0001)
LDPE-Ma_2 - BIO-Ma_2	-0.044(<.0001)	-0.109(<.0001)	0.1(0.438)	-0.034(<.0001)
Comparison content-wise (0.5-1, 0.5-2, 1-2); same type, same size				
LDPE-Mi_0.5 - LDPE-Mi_1	-0.06(<.0001)	0.079(0.0007)	-0.73(0.0002)	-0.004(0.205)
BIO-Mi_0.5 - BIO-Mi_1	-0.024(0.061)	0.057(0.011)	-0.5(0.007)	-0.014(<.0001)
LDPE-Ma_0.5 - LDPE-Ma_1	-0.062(<.0001)	0.195(<.0001)	-1.03(<.0001)	-0.005(0.053)
BIO-Ma_0.5 - BIO-Ma_1	-0.033(0.012)	0.068(0.003)	-0.7(0.0003)	-0.01(0.0005)
LDPE-Mi_0.5 - LDPE-Mi_2	-0.025(0.066)	0.057(0.014)	-0.38(0.042)	0.004(0.196)
BIO-Mi_0.5 - BIO-Mi_2	0.01(0.456)	-0.013(0.565)	0.48(0.011)	-0.003(0.225)
LDPE-Ma_0.5 - LDPE-Ma_2	-0.016(0.236)	0.282(<.0001)	-1.01(<.0001)	0.015(<.0001)
BIO-Ma_0.5 - BIO-Ma_2	-0.033(0.017)	0.126(<.0001)	-0.4(0.032)	-0.011(0.0003)
LDPE-Mi_1 - LDPE-Mi_2	0.035(0.008)	-0.022(0.298)	0.36(0.047)	0.007(0.012)
BIO-Mi_1 - BIO-Mi_2	0.034(0.011)	-0.07(0.002)	0.98(<.0001)	0.01(0.0006)
LDPE-Ma_1 - LDPE-Ma_2	0.047(0.0007)	0.087(0.0003)	0.03(0.867)	0.021(<.0001)
BIO-Ma_1 - BIO-Ma_2	0(0.97)	0.058(0.01)	0.31(0.086)	-0.001(0.713)

ρb: dry bulk density; ks: saturated hydraulic conductivity; FC: field capacity

B.	WDPT [s]	pH [-]	EC [µS/cm]	ASI [-]
Comparison with the control				
LDPE-Mi_0.5 - Control	2.36(<.0001)	-0.009(0.723)	-30.28(0.459)	0.031(0.528)
BIO-Mi_0.5 - Control	0.82(0.064)	0.059(0.026)	-47.53(0.246)	0.088(0.08)
LDPE-Ma_0.5 - Control	0.34(0.427)	0.02(0.425)	-25.62(0.531)	0.037(0.445)
BIO-Ma_0.5 - Control	1.28(0.005)	0.092(0.0009)	-29.53(0.47)	-0.075(0.132)
LDPE-Mi_1 - Control	1.51(0.0006)	-0.001(0.963)	39.46(0.301)	0.04(0.367)
BIO-Mi_1 - Control	5.45(<.0001)	-0.009(0.715)	24.55(0.519)	0.124(0.01)
LDPE-Ma_1 - Control	1.62(0.0003)	0.027(0.251)	32.38(0.395)	0.083(0.071)
BIO-Ma_1 - Control	3.55(<.0001)	0.089(0.0006)	-43.62(0.253)	0.067(0.139)
LDPE-Mi_2 - Control	0.29(0.5)	0.039(0.128)	-1.95(0.962)	0.048(0.326)
BIO-Mi_2 - Control	2.17(<.0001)	0.032(0.216)	-61.2(0.137)	0.057(0.247)
LDPE-Ma_2 - Control	1.67(0.0004)	-0.048(0.066)	27.13(0.507)	0.008(0.874)
BIO-Ma_2 - Control	2.6(<.0001)	0.028(0.267)	13.38(0.743)	0.03(0.532)
Comparison size-wise (Mi-Ma); same type, same content				
LDPE-Mi_0.5 - LDPE-Ma_0.5	2.01(<.0001)	-0.029(0.248)	-4.67(0.908)	-0.006(0.878)
LDPE-Mi_1 - LDPE-Ma_1	-0.11(0.797)	-0.028(0.262)	7.08(0.86)	-0.043(0.317)
LDPE-Mi_2 - LDPE-Ma_2	-1.38(0.002)	0.087(0.001)	-29.08(0.47)	0.04(0.345)
BIO-Mi_0.5 - BIO-Ma_0.5	-0.46(0.271)	-0.033(0.188)	-18(0.654)	0.163(0.0005)
BIO-Mi_1 - BIO-Ma_1	1.89(<.0001)	-0.098(0.0004)	68.17(0.093)	0.057(0.187)
BIO-Mi_2 - BIO-Ma_2	-0.43(0.306)	0.003(0.894)	-74.58(0.067)	0.027(0.531)
Comparison type-wise (LDPE-BIO); same size, same content				
LDPE-Mi_0.5 - BIO-Mi_0.5	1.54(0.0007)	-0.068(0.011)	17.25(0.668)	-0.058(0.18)
LDPE-Mi_1 - BIO-Mi_1	-3.94(<.0001)	0.008(0.764)	14.92(0.711)	-0.083(0.056)
LDPE-Mi_2 - BIO-Mi_2	-1.88(<.0001)	0.008(0.764)	59.25(0.144)	-0.009(0.833)
LDPE-Ma_0.5 - BIO-Ma_0.5	-0.93(0.03)	-0.072(0.007)	3.92(0.922)	0.112(0.012)
LDPE-Ma_1 - BIO-Ma_1	-1.94(<.0001)	-0.062(0.018)	76(0.062)	0.016(0.708)
LDPE-Ma_2 - BIO-Ma_2	-0.92(0.032)	-0.076(0.005)	13.75(0.732)	-0.023(0.595)
Comparison content-wise (0.5-1, 0.5-2, 1-2); same type, same size				
LDPE-Mi_0.5 - LDPE-Mi_1	0.85(0.162)	-0.008(0.82)	-69.75(0.216)	-0.01(0.889)
BIO-Mi_0.5 - BIO-Mi_1	-4.63(<.0001)	0.067(0.059)	-72.08(0.201)	-0.036(0.614)
LDPE-Ma_0.5 - LDPE-Ma_1	-1.27(0.039)	-0.007(0.839)	-58(0.303)	-0.046(0.517)
BIO-Ma_0.5 - BIO-Ma_1	-2.28(0.0005)	0.003(0.931)	14.09(0.802)	-0.142(0.066)
LDPE-Mi_0.5 - LDPE-Mi_2	2.07(0.002)	-0.048(0.186)	-28.33(0.626)	-0.017(0.808)
BIO-Mi_0.5 - BIO-Mi_2	-1.35(0.035)	0.027(0.452)	13.67(0.814)	0.031(0.662)
LDPE-Ma_0.5 - LDPE-Ma_2	-1.33(0.038)	0.068(0.065)	-52.75(0.365)	0.029(0.681)
BIO-Ma_0.5 - BIO-Ma_2	-1.32(0.039)	0.064(0.082)	-42.92(0.461)	-0.105(0.165)
LDPE-Mi_1 - LDPE-Mi_2	1.22(0.048)	-0.04(0.25)	41.41(0.461)	-0.007(0.915)
BIO-Mi_1 - BIO-Mi_2	3.28(<.0001)	-0.04(0.25)	85.75(0.129)	0.067(0.352)
LDPE-Ma_1 - LDPE-Ma_2	-0.06(0.922)	0.075(0.036)	5.25(0.925)	0.075(0.298)
BIO-Ma_1 - BIO-Ma_2	0.96(0.116)	0.061(0.086)	-57(0.311)	0.037(0.602)

WDPT: water drop penetration time; EC: electrical conductivity; ASI: aggregates stability index.

S4.3 Principal component analysis and linear regression analysis

A principal component analysis was performed for the parameters with the most effects (porosity, ρb, ks, FC and WDPT) with the function prcomp() and plotted in R version 3.5.0.

Table S4.5 *Importance of components and Eigen vector from the principal component analysis for the parameters porosity, dry bulk density, saturated hydraulic conductivity, field capacity and water drop penetration time.*

	PC1	PC2	PC3	PC4	PC5
Importance of components					
Standard deviation	1.56	1.13	0.84	0.61	0.45
Proportion of Variance	0.49	0.26	0.14	0.07	0.04
Cumulative Proportion	0.49	0.75	0.89	0.96	1.00
Eigen vectors					
Porosity	-0.5025	-0.1969	0.4645	-0.7013	-0.0338
Dry bulk density	0.5812	0.0452	0.2173	-0.3196	0.7147
Saturated Hydraulic conductivity	-0.5711	-0.1812	0.0212	0.4420	0.6670
Field capacity	0.2794	-0.6257	0.5909	0.3765	-0.1989
Water drop penetration time	0.0738	-0.7313	-0.6225	-0.2625	0.0582

Figure S4.4: *Diagrams for the first 3 components of the principal component analysis for the parameters porosity, dry bulk density, saturated hydraulic conductivity, field capacity and water drop penetration time. A: components 1 and 2, B components 1 and 3, C: components 2 and 3.*

The correlations between porosity, ρb and ks was further explored with linear regressions. The linear correlation equations and coefficient of determination (Figure S4.5) were calculated with the function lm() in R. Equations Eq.S4.4 and Eq.S4.5 were tested to fit the porosity and ρb data. Eq.S4.4 is derived from Eq.S4.5, taking into account that the plastic particles incorporated with the soil particles have a lower density than the soil particles. The normalised root-mean-square deviation for the EqS4.1, EqS4.2 and the linear regression equation between porosity and ρb were respectively 5.83%, 5.09% and 5.25%
Supporting equations:

Eq.S4.1: Porosity.

$$\text{porosity} = \frac{V_w(sat)}{V_T} = \frac{m_{sat} - m_d}{\rho_w . V_T} \quad \text{S4.3}$$

Eq.S4.2: Dry bulk density.

$$\rho_b = m_d / V_T \quad \text{S4.4}$$

Eq.S4.3: Plastic content.

$$\theta_P = \frac{mass\ (plastic)}{\text{mass (sandy soil)}} = \frac{m_P}{m_S} = \frac{\rho_P V_P}{\rho_S V_S} \quad \text{S4.5}$$

Eq.S4.4: Relationship between dry bulk density and porosity considering soil particles only.

$$\rho_b = (1 - porosity)\rho_S \quad \text{S4.6}$$

Eq.S4.5: Relationship between dry bulk density and porosity considering soil particles and plastic.

$$\rho_b = (1 - porosity)\frac{m_P + m_S}{V_P + V_S} = (1 - porosity)\frac{m_S(\theta_P + 1)}{V_S\left(\frac{\theta_P \rho_S}{\rho_p} + 1\right)} = (1 - porosity)\frac{\rho_S \rho_p (\theta_P + 1)}{\theta_P \rho_S + \rho_p} <=> \boxed{\rho_b = (1 - porosty)(\theta_P + 1)\frac{\rho_P \rho_S}{\theta_P \rho_S + \rho_P}} \quad \text{S4.7}$$

m_{sat}: mass of saturated ring samples
$V_w(sat)$: volume of water in saturated sample
m_d: mass of dry ring samples
ρ_w: density of water
V_T: volume of ring
m_S : mass of sandy soil
V_S : volume of sandy soil particles
ρ_S : density of sandy soil particles
θ_P: Plastic content
m_P : mass of plastic
V_P : volume of plastic
ρ_P : density of plastic

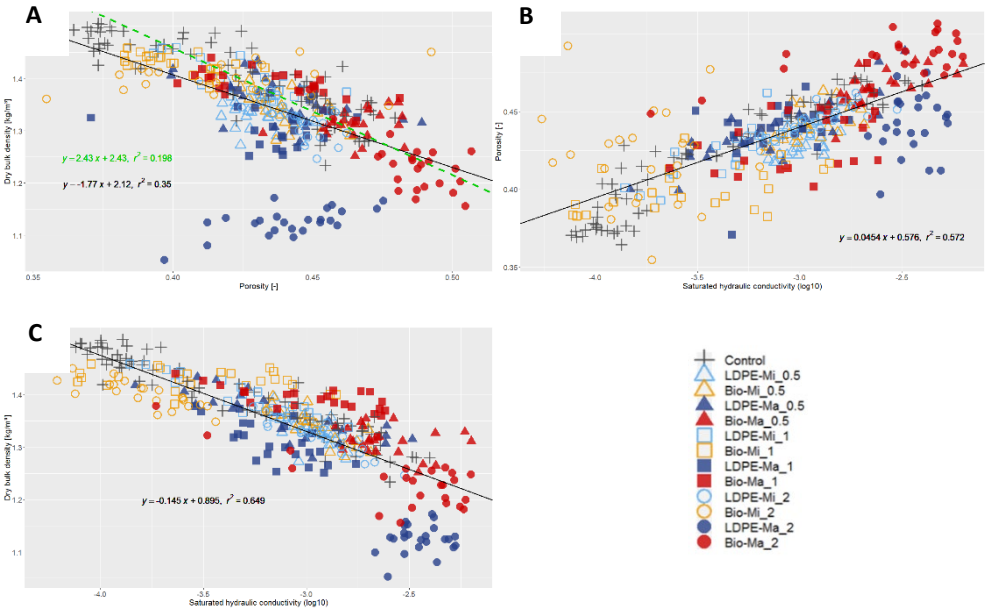


***Figure S4.5**: Linear regression and coefficient of determination between porosity and dry bulk density (A), porosity and saturated hydraulic conductivity (B) and dry bulk density and saturated hydraulic conductivity (C).*

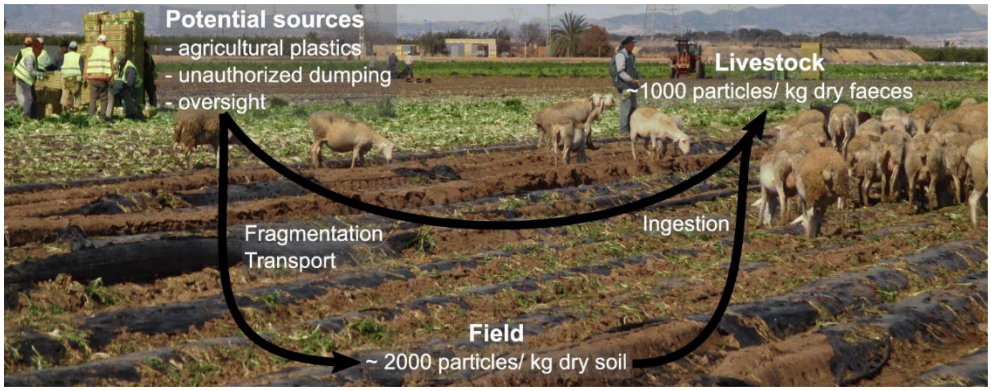
Chapter 5

Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain

Based on:
Beriot, N., Peek, J., Zornoza, R., Geissen, V., Huerta Lwanga, E. 2021. Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain https://doi.org/10.1016/j.scitotenv.2020.142653

Abstract

One of the main sources of plastic pollution in agricultural fields is the plastic mulch used by farmers to improve crop production. The plastic mulch is often not removed completely from the fields after harvest. Over time, the plastic mulch that is left of the fields is broken down into smaller particles which are dispersed by the wind or runoff. In the Region of Murcia in Spain, plastic mulch is heavily used for intensive vegetable farming. After harvest, sheep are released into the fields to graze on the vegetable residues. The objective of the study was to assess the plastic contamination in agricultural soil in Spain and the ingestion of plastic by sheep. Therefore, three research questions were established: i) What is the plastic content in agricultural soils where plastic mulch is commonly used? ii) Do livestock ingest the microplastics found in the soils? iii) How much plastic could be transported by the livestock? To answer these questions, we sampled top soils (0-10 cm) from 6 vegetable fields and collected sheep faeces from 5 different herds. The microplastic content was measured using density separation and visual identification. We found ~2 x10^3 particles·kg^{-1} in the soil and ~10^3 particles·kg^{-1} in the faeces. The data show that plastic particles were present in the soil and that livestock ingested them. After ingesting plastic from one field, the sheep can become a source of microplastic contamination as they graze on other farms or grasslands. The potential transport of microplastics due to a herd of 1000 sheep was estimated to be ~10^6 particles·ha^{-1}·y^{-1}. Further studies should focus on: assessing how much of the plastic found in faeces comes directly from plastic mulching, estimating the plastic degradation in the guts of sheep and understanding the potential effects of these plastic residues on the health of livestock.



5.1 Introduction

Microplastics have been detected in many matrices and in all different environments: from oceans to remote mountains, from earthworms to wild birds (Wu et al. 2019). Microplastics are also present in food (Prata et al. 2020) and human stools (Schwabl et al. 2019). Plastic can be transported from one environment to another by wind and water (Horton and Dixon 2018) and by other organisms. Microplastics entering the food chain in aquatic environments have been extensively studied (Wang et al. 2019b). However, only a few studies have described the transfer of microplastics via terrestrial organisms. Earthworms were the first soil-dwelling organisms to be studied for ingesting microplastics (Huerta Lwanga et al. 2017a; Rillig et al. 2017b). Huerta Lwanga et al. (2017b) demonstrated that microplastics could be transferred from the soil (0.87 ± 1.9 particles$\cdot g^{-1}$) to earthworms (14.8 ± 28.8 particles$\cdot g^{-1}$) and chickens in traditional Mayan home gardens in Southeast Mexico. Chae et al (2020) demonstrated the transfer of nanoplastic particles from the soil (10-100 mg$\cdot kg^{-1}$) to leaves of mug beans and snails (few particles, number not reported). Microplastics are identified in many agricultural fields (Yang et al. 2020) but no studies assessed the ingestion of microplastics by grazing livestock.

Microplastics can enter agricultural fields via many ways: primary use of plastic in the fields, amendment with products contaminated with microplastics or transport from other environments (Xu et al. 2020). On one hand, plastic provides many services in agriculture and is extensively used in modern managements (Liu et al. 2014a). For example plastic mulch is used for increasing soil temperature, controlling weeds, and improving water use efficiency while plastic nets are used for crop protection and plastic pipes provide irrigation. These plastics undergo degradation in the field. The degradation depends on abiotic reactions such as thermal degradation, photo-degradation, oxidation, hydrolysis and mechanical degradations (e.g. wind or ploughing) (Crawford et al. 2017b). Due to the degradation of agricultural plastics, fragments of plastic may accumulate in the soil. On the other hand microplastics may enter agricultural fields through the application of biofertilizers contaminated with plastics. Indeed sewage sludge (Fabio Corradini et al. 2019c) and composts (Weithmann et al. 2018) may contain high contents of microplastics. Finally microplastics can be transported by the wind (Zhang et al. 2020b) and flooding waters (Bläsing and Amelung 2018) in and out of the soil system.

In the region of Murcia in south-eastern Spain, plastic mulch has been used to prevent evaporation in vegetable fields for more than 20 years. Although this semiarid region suffers from severe lack of water owing to a semiarid Mediterranean climate, it is nonetheless an intensively irrigated horticultural area, commonly known as the “European garden” (tr. “Huerta de Europa” (EuropaPress 2017)). On average, two crops per year are grown in each

field. Plastic mulch is used in the summer and sometimes in the winter. Due to the intensive use of plastic mulch and its incomplete removal after harvesting, many agricultural fields are contaminated with plastic debris. Moreover, some plastic bags and plastic films (used for silage or packaging) can be lost in the fields, for example, due to oversight during vegetable wrapping at mobile harvesting stations. After harvest, a common practice is to bring sheep to eat the crop residues. In Murcia, ~638 000 sheep were bred in 2019 for meat production (Agrarios 2019). Most of the sheep farming relies heavily on crop residue grazing and fallow land grazing and little fodder is provided. Few sheep farms own land and grow fodder to feed the herd that stays in the sheepfold. The food residence time in a sheep's digestive system is about 35h (Huston et al. 1986). Therefore, we can expect that plastic particles ingested in one field are transferred to another field when the sheep defecate. This transport is particularly relevant when a herd first grazes an agricultural field contaminated with plastic and then moves to fallow land or a natural area.

The objective of the study was to assess the microplastic pollution in an agricultural area owing to intensive management practices. We focused on the light density microplastic contamination in the lithosphere and the biosphere with two representative samples: vegetable agricultural soil samples and faeces of sheep grazing in fields where plastic mulch was used. Therefore, three research questions were stablished: i) What is the light density microplastic content in farm soils where plastic mulch is commonly applied? ii) Do livestock ingest microplastic? iii) How much plastic is transported by the livestock? We hypothesized that the soil would be contaminated with plastic particles coming from mulch debris and the sheep would then ingest the plastic and excrete microplastic particles in their faeces, thus contributing to the dispersion of plastic debris in the environment.

5.2. Materials and methods

5.2.1 Study site

The study was carried out in the countryside of the Murcia region (SE Spain, Figure 5.1). Soil samples were collected from 6 vegetable fields where light density polyethylene plastic mulch was applied at least once per year over the last 10 years. All the fields had similar crop rotation history which included melons in summer and lettuces, broccoli and celery in winter, among others. Some farms used plastic much twice a year to cover soil during the winter and summer crop cycles. Five different herds of sheep were selected in the same region for faecal collection. There were approximately 1000 sheep per herd. The first four herds (A, B, C, D, Figure 5.1) grazed crop residues at surrounding vegetable farms, grass in fallow lands and ate additional feed at their sheepfolds. The herds A, B, C, D visited several

vegetable farms and fallow lands at the vicinity of their sheepfold, depending on the available crop residues and grass. Most cattle breeders in the region do not owe land for agriculture, so they depend on neighbour farmers so their cattle can graze on the vegetable residues left on the farms after harvest. The fifth herd (E) stayed at the sheepfold all year and grazed in the fields of the sheepfold daily. The herd E was fed with crop residues brought to the farm and locally produced corn, hay and silage. The shepherds were interviewed about the potential consumption of plastic by the sheep.

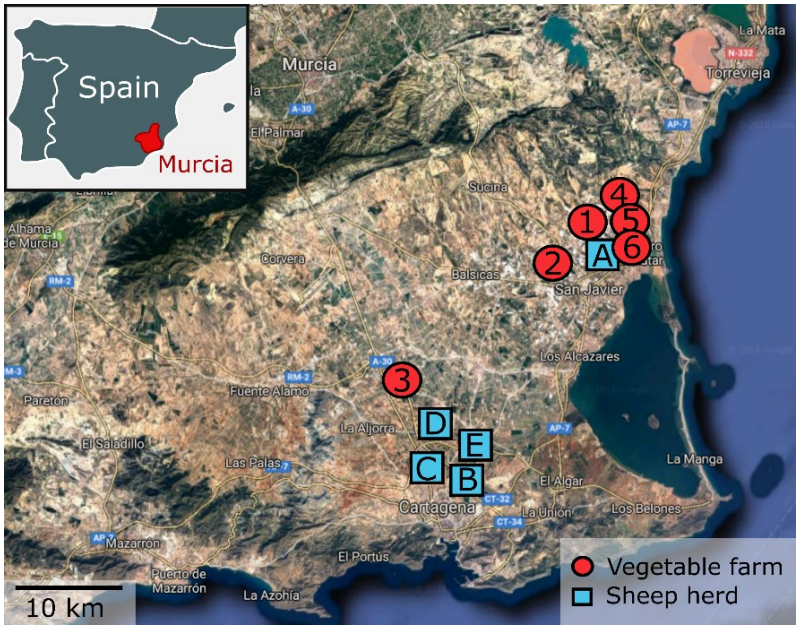


Figure 5.1*: Approximate locations of the 6 vegetable fields (in red circles: 1-6) and the 5 sheep herds (in blue squares: A-E) in the region of Murcia (SE Spain, in order to maintain the anonymity of the owners the sites are presented approximately).*

5.2.2 Sample collection

Soil sampling was carried out after the winter harvest and before the soil preparation for the summer crop cycle in 2018. Three parcels of ~0.5 ha were selected from each vegetable farm and 3 sampling points were uniformly dispersed in each sampling parcel. Soil from the top 0-10 cm was collected for each sampling point. Fresh sheep faeces were randomly collected from each herd in the field (herd A, B, C, D) or in the sheepfold (herd E). We sampled 8-18 faecal samples for each herd.

5.2.3 Microplastic extraction

Samples were air dried and analysed to assess the presence of light density microplastics. The procedure for light density microplastic extraction and identification was adapted from Zhang et al. (2018) and Corradini et al. (2019). Briefly, 5 g of dried sample were placed into a 50 ml tube with 30 mL of distilled water and shacked at 150 rpm for 30 min in a platform shaker. Tubes were then centrifuged at 3000 RPM for 10 minutes. The supernatant was transferred onto a Whatman No. 42 filter paper. Tubes were refiled with distilled water, shacked again and put in an ultrasonic bath for 10 min to further break down soil aggregates. The samples were centrifuged again, and the supernatants were poured onto the same filters. The filters were then air dried for 24 h before microplastic identification and quantification were carried out. Additionally, six blanks were performed without samples along the analysis (every ~20 samples) to detect an eventual contamination during the extraction and identification procedure.

5.2.4 Microplastic identification

All materials present on a filter were brushed carefully onto a glass plate and gathered in the centre of the plate while trying to avoid superposition of particles. A stereo microscope (ZEISS Stemi 508) equipped with a digital camera (CMEX-18 PRO) was used to take a picture of the particles. Pictures were taken with x6 magnification. The glass plate was then put onto a hot plate at 130°C for 10s and a second picture was taken. The plastic particles were then identified among other soil particles and organic matter by looking at their shape, colour, brightness and response to heat (Zhang et al 2018).

5.2.5 Calculations

The number of plastic particles per sample was presented in terms of number of microplastics per kilogram of dry matter (particles.kg^{-1}) and displayed with the function *ggplot* of R version 3.6.1 for the two different matrices and for the different fields and herds. The order of magnitude for microplastics was estimated as the smallest power of 10 used to represent that number. We performed a Shapiro-Wilk's test for the soil and faeces samples independently with the R function *shapiro.test.*

The number of microplastics potentially transported by a herd of 1000 sheep grazing in 1 ha of land in one day (MP_{herd} in particles·ha^{-1}d^{-1}) was calculated using Eq.5.1:

Eq.5.1: $$MP_{herd} = MP_{Faeces} \times Faeces_{animal} \times Defecation_{Field} \times Animal_{herd} \times Herd_{Field}$$

Where MP_{Faeces} is the number of microplastics per kg of fresh faeces (particles·kg^{-1}), $Faeces_{animal}$ is the faecal production per sheep per day (~1 kg per sheep and per day according to Ogejo et al. (2010)), $Defecation_{Field}$ is the sheep defecation percentage that occurs in the field (~36% of 1 kg per sheep, according to Taylor et al. (1987)), $Animal_{herd}$ is the number of sheep per herd (~1000 per herd; (B. Agudo 2010)) observed an optimal production for a herd size between 1200-2000 heads) and $Herd_{Field}$ is the surface grazed by the herd in one day (~1 ha for an herd of ~1000 sheep; (Rakkar and Blanco-Canqui 2018)). Eq.5.1 is suitable for other grazing animals with adapted values. The number of microplastics potentially transported (MP_{herd}) was extrapolated to a year by considering that each field was grazed two times (winter crop and summer crop) per year.

5.3 Results and discussion

5.3.1 Microplastics in agricultural soils

Microplastics were found in all soil samples (Figure 5.2). The distribution of microplastics in the dry soil followed a normal distribution (p-value = 0.33). The average content of microplastics in dry soil was 2116± 1024 particles·kg^{-1}. The amount of microplastics found in these soils is comparable to the amount of microplastics found in other agricultural soils. Van den Berg et al. (2020) reported an average of light density microplastic content of 2130 ± 950 particles·kg^{-1} in agricultural fields used to grow cereals in Eastern Spain. Zhang and Liu (2018) reported higher microplastic contents, ranging from 7100 particles·kg^{-1} to 42,960 particles·kg^{-1}, in arable soils near the Chai river valley (southwestern China). There is not much information concerning the effect of plastics on the soil function and the processes remain unclear so it is difficult to foresee the consequences of this plastic pollution on soil quality and long-term land productivity. For example, researchers have reported that plastic debris can be detrimental to plant growth (Qi et al. 2018) and can alter soil physical properties , (de Souza Machado et al. 2018b; de Souza Machado et al. 2019; Qi et al. 2020a). Hence, further studies are encouraged to understand the long-term impacts of this pollution.

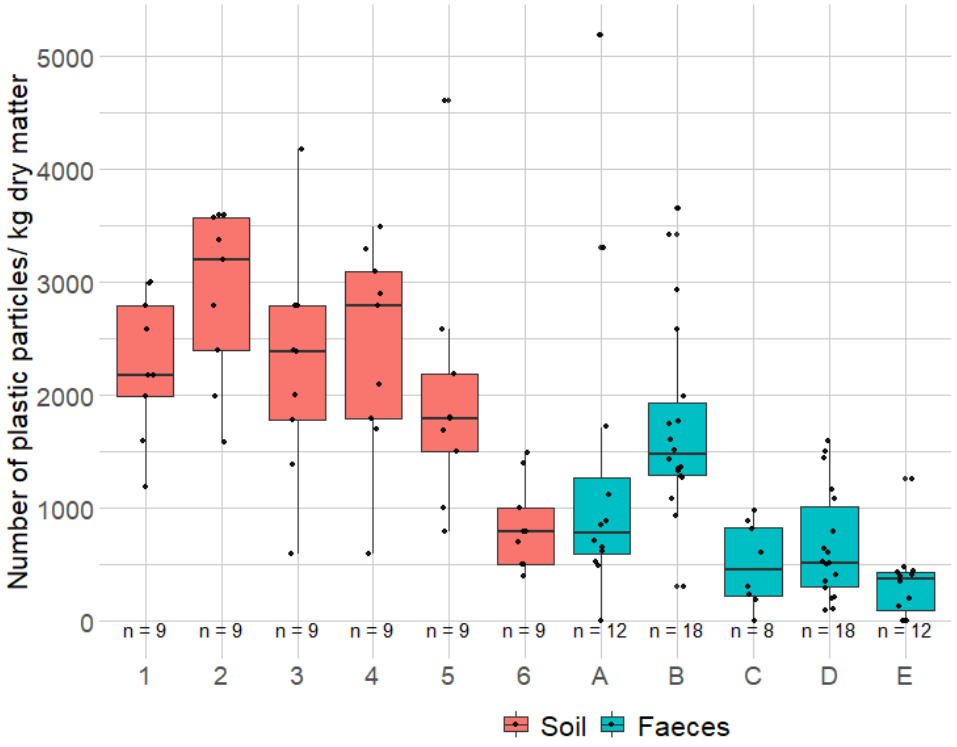


***Figure 5.2**: Microplastic content in agricultural soil (in red: 1-6) and in sheep faeces (in blue: A-E) per field and herd. The dots represent individual measurements (n=number of replicates).*

5.3.2 Microplastic in livestock faeces

This study provides the first assessment of microplastics in livestock faeces (Figure 5.2). Microplastics were found in all the herds. The distribution of microplastics in dry faeces did not follow a normal distribution (p-value < 0.001). The average content of microplastics in dry faeces was 997 ± 971 particles·kg^{-1}. We showed that plastics were ingested by sheep grazing on fields where plastic mulch was applied (Figure 5.2, herds A, B, C, D) as well as by sheep fed at the sheepfold (Figure 5.2, herd E). Shepherds of herds A, B, C and D said that sheep ingested pieces of plastic when they grazed on the vegetable fields. They reported cases of sheep eating macroplastic debris (often plastic mulch or plastic bags) stuck in the vegetation. In fact, we observed a sheep eating a plastic bag while sampling the faeces. The shepherd of herd E, whose sheep did not directly graze on vegetable fields, reported that macroplastic debris was present in the crop residues, in the corn and in the silage that were brought to the animals. He also mentioned that the wind carries macroplastic films (plastic bags, plastic mulch debris, silage sheets debris) to the field where the herd is kept. Sheep

ingesting plastic materials have been described in other studies (Ngoshe 2012; Otsyina et al. 2018) and macroplastics have been found in the digestive systems of the animals (Mekuanint et al. 2017; Ngoshe 2012; Omidi et al. 2012). These studies focus on livestock in developing country and identify the free roaming of the animals in the suburbs of cities as the main explanation for plastic ingestion. In our study we observed plastic contamination in the context of intensive agriculture, in a rural area. Calcium and phosphorous deficiency and poor nutritional supplementation are predisposing factors for plastic ingestion (Priyanka and Dey 2018). Overall, we cannot identify a unique source for the microplastics found in the sheep faeces. It is evident that one clear source of microplastic exposure for animals grazing in vegetable fields is the plastic mulch debris left on the fields (herds A, B, C, D). Nevertheless, results from herd E shows that indirect pathways also exist, for example macroplastics transported by the wind to the sheepfold or plastic debris present in the feed. On one hand, microplastics measured in the sheep faeces could have originated from microplastics in the soil or the feed. On the other hand, the microplastics could have also come from ingested macroplastics broken down in the stomachs of the animals. Assessments of the plastic content in the feed and in the environments where the sheep are kept are required to fully understand the contamination pathways.

There was a high variation of microplastic content between faecal samples, from 0 to more than 5000 particles·kg^{-1}. The variation could have come from the uncertainty of the measure caused by a variable amount of organic matter in the faeces or to other factors such as the age of the sheep. In fact, when it comes to sheep accumulating plastic debris, we can expect that the older the sheep is, the more plastic would be found in the faeces. To test this, a better extraction method, with efficient removal of organic matter and a more detailed sampling, classifying the faeces according to the age of the sheep, would be needed.

There are very few assessments of microplastic content in terrestrial animals. Huerta Lwanga et al. (2017b) measured the content of microplastics in the soil (0.87 ± 1.9 particles.g^{-1}), in the earthworms (14.8 ± 28.8 particles·g^{-1}), and in the chickens (129.8 ± 82.3 particles·g^{-1} in the faeces and 10.2 ± 13.8 particles·g^{-1} in the gizzard) in traditional Mayan home gardens (Southeast Mexico). The macroplastic content found in the chickens was 45.82 ± 42.6 of debris per gizzard and 11 ± 15.3 of debris per chicken crop (Huerta Lwanga et al. 2017b). Zhao et al (2016) identified similar plastic content in 17 wild birds around Shanghai with an average of 22.8 ± 33.4 particles per bird. Recently, (Yan et al. 2020) detected nylon fibres and polyethylene terephthalate particles of size limit of ~1 μm in five out of ten chicken faeces from a farm in Nanjing, China but don't provide quantification. These three studies showed that microplastics can reach terrestrial birds through the food chain, in farms, gardens and in the wild. With our study, we have shown that this concern must be extended to livestock, especially grazing animals.

5.3.3 Transport of microplastics

Sheep from the fields A, B, C and D grazed on crop residues and on fallow lands. By moving from one field to another and to fallow lands sheep can contribute to the transport of microplastics. The calculated transport is an estimation of how many plastic particles are excreted per day by a herd of 1000 sheep grazing on one ha. We did not measure the fragmentation rate and residence time of the plastic particles in the digestive systems of the sheep therefore, we could not estimate the ingestion rate based on the plastic content in faeces. We measured a plastic content of ~500 particles·kg^{-1} fresh faeces and we could estimate that an average herd of 1000 animals would transport ~180 000 particles·$d^{-1}ha^{-1}$. If we assume that one field is grazed two times per year, the flux is ~0.36 x10^6 particles·$ha^{-1}y^{-1}$. We can compare this value with two major fluxes of microplastics: sludge application and atmospheric deposition. Van den Berg et al (2020) reported a sludge application of ~20 t·y^{-1} on cereal agricultural fields in Eastern Spain with a sludge containing ~5000 particles·kg^{-1}. Therefore, sludge application is an input of ~10^8 particles·$ha^{-1}y^{-1}$. Zhang et al (2020) reviewed different studies of atmospheric microplastic transportation and in 17 studies, researchers reported microplastic deposition rates ranging from 0 - 11130 particles·$m^{-2}d^{-1}$. These studies used different extraction and identification procedures for microplastics, thus making a comparison of the studies complicated. We arbitrarily chose the value of ~60 particles·$m^{-2}d^{-1}$ reported in the Pyrenees mountains by Allen et al. because it was the study that was geographically the closest to our case study. We calculated a flux of ~2x10^8 particles·$ha^{-1}y^{-1}$. Therefore, sludge application and atmospheric deposition both represent a microplastic flux two orders of magnitude higher than the transport of microplastics in sheep faeces. Quantifying the flux of microplastics is important to be able to predict the amount of microplastics in the environment and determine effective actions to protect ecosystems. More studies, using a standardised plastic identification protocol, are needed to compare the different fluxes of microplastics.

5.3.4 Potential effects of plastic ingestion on livestock

Until now, only a few studies have been designed to assess the adverse health effects of plastic ingestion on livestock. Among potential effects, ingested macroplastics can cause indigestion, ruminal impaction, recurrent ruminal tympany and intestinal obstruction (Kühn and van Franeker 2020; Mekuanint et al. 2017; Priyanka and Dey 2018). The fragmentation of the debris in the digestive system could increase the number of small particles, which are then more likely to be absorbed. Plastic debris may also loosely sorb toxic chemicals during degradation (Hüffer et al. 2019) such as the heavy metals and plasticizers used in manufacturing or other contaminants. Plastics sorb many organic pollutants including

pesticides (Liu et al. 2019). These chemicals can then contaminate other tissues. For example, (Mahadappa et al. 2020) observed increased level of heavy metals in rumen fluid as well as in blood, liver, kidney and muscle of buffaloes that ingested macroplastics. Prata et al. (2020) listed oxidative stress, inflammation, translocation and cancer as potential adverse human health effects caused by microplastics. Additionally, the plastic debris could possibly modify the gut microbiome and alter digestive functions (Fackelmann and Sommer 2019). Studies investigating the consequences of plastic ingestion are urgently needed.

5.3.5 Limitations of the plastic extraction and identification method

The extraction method successfully identified plastic particles and gave an order of magnitude to the content of plastic in soil and in sheep faeces. The method was fast and inexpensive to implement for both soil and faecal samples. Blanks showed no contamination of the samples during processing. The potential fragmentation of plastic debris during the extraction procedure was not assessed since it was not expected, as reported in previous studies (Corradini et al. 2019c). Zhang et al. (2018) reported a recovery percentage of >80% for microplastic identification in soil samples. However, for some faecal samples, the high content of organic matter may have hidden some plastic particles and made the recovery of all the plastic particles impossible (Figure 5.3).

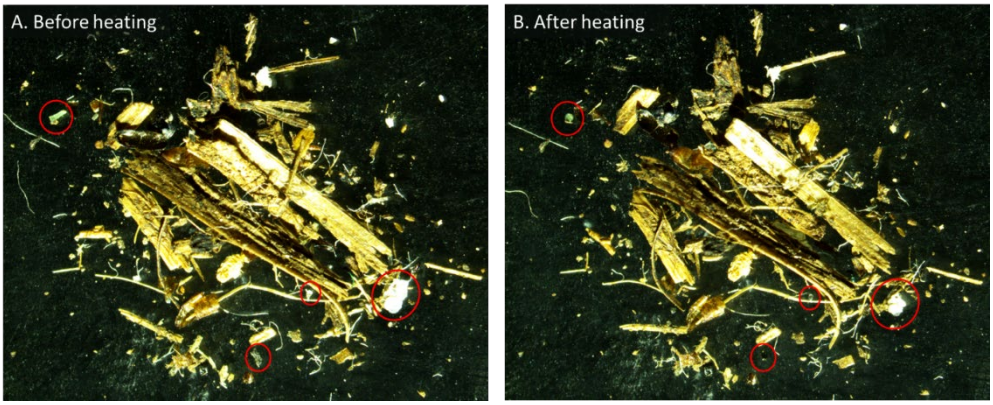


Figure 5.3*: Picture of the microplastics and organic material extracted from a faecal sample, before (A) and after (B) heating. Four plastic particles are identified with a red circle. The high amount of organic matter present in the picture does not allow for a complete assessment of the sample.*

Overall, we can expect the number of particles in faecal samples to be under-estimated because of the superposition. Pre-treatments to decrease or remove the organic matter are needed to better quantify the plastic particles. A diversity of methods have been tested to extract microplastic from complex environmental samples (soil or faeces) and rely on the diversity of plastic properties (Möller et al. 2020). The density separation methods, similar to the one we used, require an additional step to limit the organic material fraction to an appropriate level. Acidic and Alkaline Digestion, Oxidisation with Hydrogen Peroxide or Enzymatic Digestion can be used and combined to reduce the organic material fraction. For example, Yan et al. (2020), obtained more than 90% recovery for polystyrene, polyethylene and polyvinyl chloride microplastics extracted from human and chicken faeces with a density separation with ethyl alcohol and the use Fenton's reagent and nitric acid. Other methods such as the magnetic (Grbic et al. 2019), electrochemistry (Davies and Crooks 2020) and oil extraction (Scopetani et al. 2020) seems to better separate certain plastic types from organic materials. All these methods have to be adapted to the matrix and plastic type analysed and require internal recovery tests to ensure the proper extraction of the plastic materials.

The visual identification has been validated in numerous studies (Möller et al. 2020). However it does not differentiate the type of plastic and is less suitable for particles with a diameter smaller than 50 µm (Zhang et al. 2018). Spectral methods (e.g. Raman or Fourier transform infrared) are used to identify several types of plastic by comparison with a spectral library (Corradini et al. 2019b; Munno et al. 2020; Sobhani et al. 2019). Raman microspectroscopy allows microplastic identification down to a pixel resolution of 500 nm and could be improved up to 100 nm with silver colloid for surface-enhanced Raman spectroscopy (Lv et al. 2020) while micro-FTIR spectroscopy identification of particles ranges from 10 to 500 µm (Möller et al. 2020). The identification of nanoplastics remains a challenge and new methods have to be validated in environmental samples.

5.4 Conclusions

This study reported the first measured content of light density microplastics in sheep faeces (~10^3 particles·kg^{-1}). We demonstrated that livestock could ingest micro- and/or macro-plastic debris from their environment and from their feed. The light density microplastic content in the vegetables fields was ~2 x10^3 particles·kg^{-1}. We identified agricultural plastics as one but not the unique source of plastic contamination. This preliminary study emphasizes the need for standardized methods for measuring plastic content, assessments of the effects of plastic debris ingested by livestock, identification of the source of plastics ingested by livestock and proposals for alternatives to the plastics used in agriculture. The plastic contamination, from intensive agriculture management and other sources, is a threat to the fauna and humans. With the intensification of agriculture we can expect an increase of plastic use leading to an increase plastic contamination and exposure. A paradigm shift is needed in the current crop production to reverse this trend.

Acknowledgement

This work was supported by the European Commission Horizon 2020 project Diverfarming [grant agreement 728003]. We are thankful for the contribution of the farmers and sheepherders from the region of Murcia, Spain. We would also like to thank Robin Palmer for the language editing.

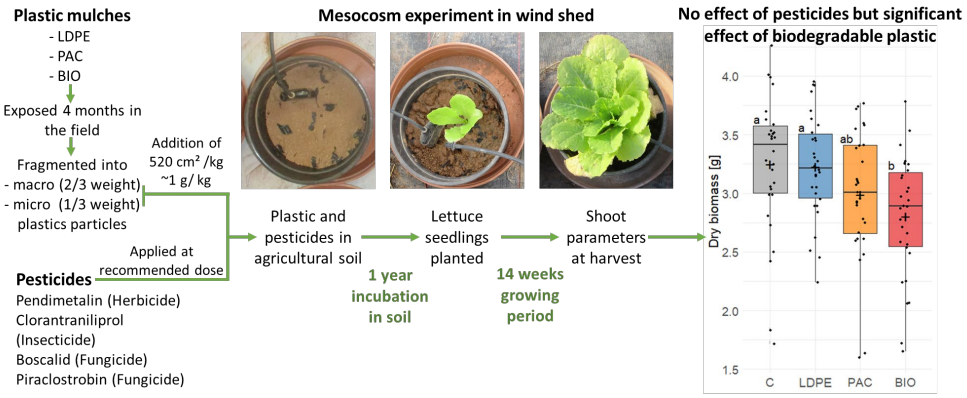
Chapter 6

Plastic mulch and pesticide residues effects on the lettuce growth

Based on:
Beriot, N., Zornoza, R., Huerta Lwanga, E., Geissen, V. 2022. Plastic mulch and pesticide residues effects on lettuce growth. To be submitted

Abstract

Plastic mulch and pesticide residues are ubiquitous contaminants in agricultural soils. In the field, pesticide residues remain in the soil for months and can accumulate over time. However, most studies investigating the effects of these contaminants use pristine chemicals and test them in lab conditions. We tested 3 plastic mulches and 3 pesticides commonly used by farmers. Low-density polyethylene (LDPE), Pro-oxidant Additive Containing plastic (PAC) and biodegradable plastic (BIO) mulches were laid in a field for 4 months, shredded into micro- and macro-sized plastic pieces and then added to a mesocosm soil experiment along with the pesticides. Plastics and pesticides were left in the mesocosm to incubate for a year in field conditions before lettuce seedlings, *Lactuca sativa*, were planted. After a 14 week growing period, we measured the basal diameter, number of leaves, leaf area, fresh shoot biomass, dry shoot biomass and shoot water content. We observed decreased leaf area, fresh shoot biomass and dry shoot biomass in plants growing in soil where BIO plastic was present as compared to the control treatments. These results follow previous studies and call for a more detailed testing procedure before BIO mulches are approved for agricultural use and made available on the market.



6.1 Introduction

The use of plastic mulch has steadily increased over the last 30 years (Liu et al. 2014a), reaching an estimated worldwide use of 2.5 million tonnes in 2021 (FAO 2021b). Plastic mulch is used to reduce water evaporation, control weeds and increase soil temperature (Espí et al. 2006). With the growing use of plastic mulch comes an increased concern about the plastic mulch debris often left in fields after harvest (Zacharias Steinmetz et al. 2016b). The most common plastic used for mulching is made of low-density polyethylene (LDPE), a fully saturated polymer of hydrocarbons resistant to weathering (Kasirajan and Ngouajio 2012). Some plastic producers have tried to improve the degradation processes of plastic mulch by adding pro-oxidant additives to LDPE in order to avoid the need for plastic mulch removal and thus, the accumulation of plastic debris (Selke et al. 2015). With these aims in mind, Pro-oxidant Additive Containing (PAC) plastic, also called "oxo-degradable" or "oxo-biodegradable", was developed but it has ultimately proven to be poorly degraded in soils (Hogg 2016). Another attempt to solve the problem of plastic debris accumulation from mulch use has resulted in the creation of biodegradable (BIO) plastic polymers. Biodegradable mulch can be made from a diversity of biobased or petroleum-based polymers or a blend of both (Sintim and Flury 2017). Biodegradable mulches are expected to degrade into water, CO_2, methane, energy and new biomass in soil with the help of microorganisms (Carol et al. 2017). These mulches were designed such that they should be degraded by 90% after two years in the soil (Standard ISO 16929). However, studies have found that biodegradable plastic mulch doesn't degrade as fast as expected under some field conditions and leaves residues in the soil after more than two years (ACBD 2020; Sintim et al. 2020).

The plastic residues left in the fields can have negative impacts on crop production. For example, in a meta-analysis carried out on farms using plastic mulch in China, Gao et al. (2019) showed a decrease in yield with an increase in the amount of plastic residue when plastic content was >240 kg ha^{-1} (~0.2 g kg^{-1} for the first 10cm). Zhang et al. (2020a) estimated a 3% drop in crop yield for every additional 100 kg ha^{-1} of film residue in soil. On a smaller scale, plastic debris is proven to affect soil physicochemical properties (de Souza Machado et al. 2019; Qi et al. 2020a) and the soil microbiome (Kim et al. 2020; Qi et al. 2020b) as well as enter the root system (Giorgetti et al. 2020). Plastic debris also interacts with other contaminants in soil such as heavy metals (Fen Wang et al. 2018) and pesticide residues (Beriot et al. 2021). In fact, pesticide residues are a ubiquitous soil contaminant in agriculture (Geissen et al. 2021) because the use of pesticides provide many services to the farmers such as control of weeds, pathogenic fungi and invading insects (Sabzevari and Hofman 2022).

Both plastic (Beriot et al. 2021) and pesticide (Geissen et al. 2021) residues are present in intensively farmed vegetable fields in Southern Spain. In the semi-arid climate, vegetable production is irrigated and plastic much is used to improve water use efficiency. For example, in the region of Murcia (Southeast Spain), intensive vegetable production represents ~66% of agricultural production. All vegetable fields are irrigated and 26% of them are covered with plastic mulch (Fulgencio Pérez Hernández and Esteban Barba Martínez 2021). Irrigation, plastic mulching and pesticide application have led to the successful production of ~404 kt of lettuce in Murcia in 2020, representing ~10% of lettuce production in the European Union (FAOstats 2021). Therefore, we chose the area of Murcia to investigate the combined effects of plastic use and pesticide residues on crop production. We bought three different plastic mulches, LDPE, PAC and BIO from a farmer's warehouse in Murcia. We selected 3 pesticides that are commonly applied in the region: the herbicide Sigmum (Pendimetalin 33%), the insecticide Altacore (Clorantraniliprol 35%) and the fungicide Bluss (Boscalid 26.7% and Piraclostrobin 6.7%). We incubated an agricultural soil with fragments (50 µm to 5mm) of plastic mulch and the separate three pesticides as well as a mixture of the three for a year. We then planted lettuce seedlings, *Lactuca sativa*, and compared the growth of the plants after 3 months.

6.2 Materials and methods

6.2.1 Soil, plastic and pesticide incubation

The soil was collected from an organic agricultural field. It was Haplic Calcisol (loamic, hypercalcic), with a loamy texture, an alkaline pH of 8.5, and 1.3% soil organic carbon. It was air-dried and sieved to 2mm.

Three commercially available black plastic mulches were purchased in Murcia : LDPE, PAC and BIO mulch. The full chemical composition of the mulches was not available as it is protected by private ownership. We investigated the composition of the mulches using a Varian 1000 FTIR (Fourier transform infrared) spectrometer. Eight scans were performed for the background and the samples. The spectrometer produced spectra ranging from 3750 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}. The spectra were compared with the polymer libraries HR Hummel Polymer and Additives and the HR Spectra Polymers and Plasticizers. Spectra of both LPDE and PAC matched the polyethylene reference spectra (> 90% match). Spectra of the BIO mulch obtained best match for Polyester terephthalic acid (78% match) and Polybutylene terephthalate (72% match) (Figure S6.1). It is most likely that the main polymer in BIO was Polybutylene adipate terephthalate as it is a copolyester of terephthalic acid commonly used for its biodegradable properties (Weng et al. 2013) but not present in the spectra library.

All 3 mulches were placed on a field in Murcia from May to September 2019 to mimic the normal exposure of the mulches during a growing season. In September, the mulches were collected, cleaned and dried. One section of the mulches was manually cut into ~5mm debris (MP). Another section was folded into layers, melted, manually cut and freeze ground into a fine powder (µP). The powder was sieved into different size fractions and the size distribution (w/w) was set at: 25% [50-250] µm, 62.5% [250-500], and 12.5% [500-1000] µm. The final plastic content added to the soil was composed of 2/3 MP and 1/3 µP (Table1).
Plastic mulch is used by farmers to cover the soil, therefore, we decided to apply the 3 plastic mulches to the same size areas. The application rate of 520 $cm^2\ kg^{-1}$ was chosen. It represents 7.5 years of use with 2 applications per year, each covering half of the field (William James 1993) and accounting for the accumulation in the first 10cm of soil with a 1400 kg m^{-3} density. 520 $cm^2\ kg^{-1}$ is also relevant to the plastic content measured in the soil (Zhang et al. 2020a). The different thicknesses and densities of the three plastic mulches lead to a different mass input into the soil (Table 6.1).

Table 6.1*: Characteristics of the low density polyethylene (LDPE), pro-oxidant additive containing (PAC) and biodegradable (BIO) commercial plastic mulches and contents of macroplastics (MP) and microplastics (µP) added.*

Plastic	Thickness [µm]	Density [g cm^{-3}]	Total Plastic [$cm^2\ kg^{-1}$ soil]	Plastic content [g kg^{-1} soil]	MP content [g kg^{-1} soil]	µP content [g kg^{-1} soil]
LDPE	22.5	0.960	520	1.2	0.8	0.4
PAC	15	0.960	520	0.75	0.5	0.25
BIO	12	1.400	520	0.88	0.59	0.29

Three commercially available pesticides were selected among commonly applied pesticides in vegetable agriculture in the region of Murcia (Geissen et al. 2021). We selected pesticides with high recovery rates when using the LC-MS method and pesticides with low degradation rates (Beriot et al. 2020). The herbicide Sigmum, the insecticide Altacore and the Herbicide Bluss were selected and applied every 6 months (M0, M6, M12) following the recommended dose (Table 6.2). The recommended dose was converted from L ha^{-1} or kg ha^{-1} to µL kg^{-1} and µg kg^{-1} by considering a soil depth of 10 cm and a soil density of 1400 kg m^{-3}.

***Table 6.2**: Characteristics of the commercial pesticides, the contents added to the soil and the associated active substances*

Commercial name	Type	Recommended dose	Applied dose	Active substances	DT50 [days]	log P
Sigmum	Herbicide	4-6 L ha^{-1}	3.6 µL kg^{-1}	Pendimetalin 33%	182.3	5.4
Altacore	Insecticide	85-115 g ha^{-1}	71 µg kg^{-1}	Clorantraniliprol 35%	246	2.86
Bluss	Fungicide	0.4-1.5 kg ha^{-1}	712 µg kg^{-1}	Boscalid 26.7%	84.5	2.96
				Piraclostrobin 6.7%	62	3.99

In October 2019, we prepared 6 replicates of the total 4x5 treatments (Table 6.3). The plastic debris were mixed in the soil, water was added to reach half the field capacity and 1kg of the soil mixture was added per mesocosm. Each mesocosm had a volume of 1020 cm^3 and height of 9 cm. Pesticides were sprayed on the soil surface. The mesocosms were randomly placed in an open-air wind shed in four lines (Figure S6.2). Mesocosms were irrigated with a dripper system to recreate field conditions. Three humidity and temperature sensors (Decagon 5TM) connected to a data logger (Zentra ZL6) were used to continuously measure the soil moisture and adapt the irrigation to keep the soil moisture at half the field capacity. This first incubation step lasted for one year.

***Table 6.3**: Summary and abbreviation of the 20 treatments applied*

	No pesticide	Herbicide	Insecticide	Fungicide	Mixture
No plastic	C_C	C_H	C_I	C_F	C_M
LDPE	LDPE_C	LDPE_H	LDPE_I	LDPE_F	LDPE_M
PAC	PAC_C	PAC_H	PAC_I	PAC_F	PAC_M
BIO	BIO_C	BIO_H	BIO_I	BIO_F	BIO_M

6.2.2 Lettuce growth

In October 2020, lettuce seedlings, *Lactuca sativa*, were planted in the mesocosms. The growing period lasted 14 weeks. Ammonium nitrate (16.9% nitrate N, 17.6% ammonium N) was added to the irrigation water from week 3 to week 12 and a total of 0.5 g of nitrogen was added to each mesocosm following the fertilization plan found in the annex (Table S6.1). Plant growth was assessed at harvest by measuring the number of leaves, the leaf area, fresh and dry shoot biomass and the stem diameter. First, the shoot was cut. The basal stem diameter was measured with a caliper. All leaves were removed from the stem, cleaned and scanned with a leaf scanner. All leaves and the stem were weighed together, dried in an oven at 50°C for two days and weighed again. We calculated the water content in the shoot as the difference between the fresh and dry shoot biomass divided by the fresh shoot biomass (Garrido et al. 2014).

6.2.3 Statistical analysis

All data analysis and visualisations were performed with R (version 3.6) and all scripts and raw data tables are available on Github https://github.com/NGBeriot/Lettuce (publicly available after publication). First outliers were removed using the Tukey's method. Then, the ANOVA hypothesis of the homogeneity of variances and the normality of the residuals were checked with the Levene's test and the Shapiro-Wilk's test (Table S6.2 and Q-Q plots Figure S6.2). The normality of the data was also checked. Each parameter was tested with a two-way ANOVA for the plastic and pesticide factors and the interactions of both. When the two-way ANOVA indicated a significant difference for a parameter and a factor level, a pair-wise comparison was performed to identify which treatments were significantly different to each other. For not normally distributed data, the results of the two-way ANOVA were confirmed with the Kruskal-Wallis method, the pair-wise comparison was performed with the Wilcoxon's test corrected with Holm's method and data was plotted in boxplot with ggplot(). For normally distributed data, the pair-wise comparison was performed with the Student's t-test corrected with Holm's method and the mean and standard deviation were plotted with ggplot().

6.3 Results

At week 11, one plant belonging to the treatment PAC_I died. It was considered an outlier and removed from the analysis. The average lettuce weighed 19.4 g and had 17 leaves for a total leaf area of 435 mm^2 (Table 6.4). All the parameters validated the assumption of homogeneity of variances and normality of the residuals for the two-way ANOVA and none of them followed a normal distribution (Table S6.2).

***Table 6.4**: Summary results for the plant parameters at harvest.*

Parameter	Mean	Standard deviation	min	max
Basal diameter [mm]	13	0.92	11	15
Number of leaves [-]	17	2.05	12	22
Leaf area [mm^2]	435	69	257	560
Fresh shoot biomass [g]	19	2.91	11	24
Dry shoot biomass [g]	3.1	0.55	1.6	4.3
Shoot water content [%]	84	0.96	80	87

The two-way ANOVA showed that the plastic factor had a significant effect on all the six measured plant parameters (two-way ANOVA p-value < 0.05 and confirmed with the Kruskal-Wallis method p-value < 0.05) except on the basal diameter. On the contrary, pesticide treatments had no effect on any of the five measured plant parameters. The two-way ANOVA indicated significant effects from the interaction plastic:pesticide for the leaf area (p-value = 0.025), but no differences were found with the pair-wise comparison of the interactions. All the

The number of leaves of the lettuce plants growing in PAC was significantly lower than the control but not lower than LDPE or BIO treatments (Figure S6.4 A). The leaf area had significantly lower values in BIO as compared to the control, and LDPE and PAC had no significant differences with the control or BIO (Figure 6.1A). The dry shoot biomass showed significantly lower values from BIO compared to the control and LDPE, and no significant differences between the control, LDPE and PAC were observed (Figure 6.1B). It was the opposite for the shoot water content, with significantly higher values from BIO compared to the control and LDPE, and no significant differences observed between the control, LDPE and PAC (Figure 6.1C). The fresh biomass was significantly lower for BIO compared to LDPE, but BIO and LDPE were both not significantly different from the control or PAC (Figure S6.4 B).

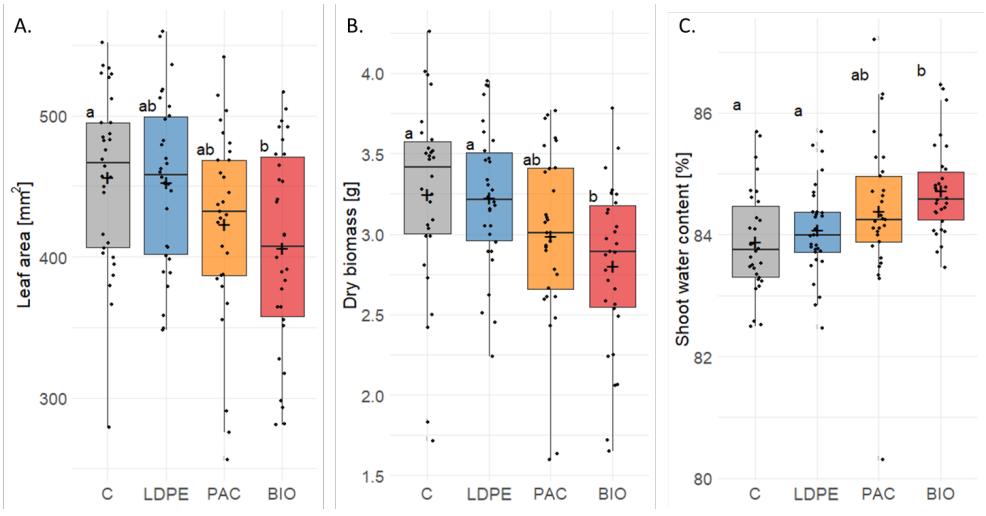


***Figure 6.1. Leaf area (A.), dry shoot biomass (B.) and shoot water content (C.)**, for each plastic treatment : no-plastic (C), low density polyethylene (LDPE), pro-oxidant additive containing (PAC) and biodegradable (BIO) plastic. The box plot (horizontal lines) represents content for at least 25%, 50% and 75% of the samples. The vertical black lines represent the minimum and maximum values, excluding outliers (Tukey's method). The cross represents the average content of any given sample group. The dots represent individual measurements. Groups that do not share letters are significantly different from each other (Wilcoxon test with $p < 0.05$).*

6.4 Discussion

The plant growth (leaf area, shoot fresh biomass and shoot dry biomass) was lower for plants growing in soil with BIO plastics. This is similar to two studies investigating the effects of LDPE and BIO plastic mulch debris on plant growth. Qi et al. (2018) compared the growth of wheat (*Triticum aestivum*) with 1% (w/w) MP and µP of LDPE and BIO added to a sandy soil in four different treatments. Wheat was cultivated in a growth chamber with controlled temperature, light and moisture. After 2 months (61 days), they reported a lower shoot biomass for plants growing in soil with µP and MP BIO debris. This difference was not significant after 4 months (139 days) but plants growing in soil with µP and MP BIO debris produced a lower fruit biomass than the control. The leaf area and leaf number was also lower for plants exposed to BIO µP and MP debris but these parameters were not measured after four months. Meng et al. (2020) compared the growth of the common bean (*Phaseolus vulgaris L.*) with 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (w/w) µP of LDPE and BIO added to a sandy soil. Common beans were cultivated in an outdoor net house in the summer in the Netherlands. 46 days after seeding, the plants growing in soils with more than 1% (w/w) plastics produced less shoot biomass than the control. Reduction of the leaf area and the fruit biomass were observed for BIO µP content ≥ 2% (w/w).

From this comparison, we point out that we found effects for contents lower than the one used in Meng et al. (2020). We applied a mixture of µP and MP while Meng et al. (2020) applied only µP < 1000 µm but results from Qi et al. (2018) seem to indicate that the debris size played a minor role regarding the impacts on the plants. The main differences we can point out are the soil pH, the crops, the climate conditions and the pre-treatment of the plastic. We exposed the plastic mulch to weathering in the field before application and left it in the soil for one year before planting the lettuce, whereas Qi et al. (2018) and Meng et al. (2020) used pristine materials. The aging of the plastic will lead to the leakage of some plastic components (Kim et al. 2020), the formation of biofilms (Amaral-Zettler et al. 2020) and the change in some polymers (Chamas et al. 2020). We can hypothesize that the aging of the plastics can lead to effects at smaller concentrations. In all three studies, a BIO plastic was tested as well as 85% PBAT industrial pellets, 10% PLA and 5% calcium carbonate in Meng et al. (2020), and a commercial mulch containing PBAT and polyscharides in Qi et al. (2018). The formulation of additives in these plastics is unknown. Therefore, no general conclusions about all biodegradable plastic, specific polymers or additives can be drawn. Nevertheless, these results call for extended ecotoxicological testing of biodegradable plastic before approval in the market.

The reduced crop growth witnessed in this study could have been the result of many processes or combinations of processes. The comparison of shoot water content indicates

that the plants growing in soil with BIO residues were not dehydrated as compared to the control. Thus, the hypothesis stating that osmotic stress caused by the BIO residues was to blame for the results could be ruled out. The other hypotheses remain possible: nutrient decrease due to the degradation of the BIO plastic, the release of toxic chemicals, a change in the microbial community increasing pathogens or decreasing beneficial organisms and/or the direct effects of the plastic on the plant via the roots. Further studies are required to test this hypothesis and understand the impacts of BIO plastics in the soil. Collaboration with industries to make more suitable plastic mulches for farmers requires the publication of the chemical composition of all the constituents of the plastic mulches.

6.5 Conclusions

We presented the first assessment of the effects of three different plastic mulches, LDPE, PAC and BIO, aged in the field and incubated in the soil for a year in the presence of commonly used pesticides on the growth of lettuce plants. We observed a reduction in plant growth (leaf area, shoot fresh biomass and shoot dry biomass) in soil incubated with BIO plastics, as compared to the control, LDPE and PAC. These results follow previous studies and call for a more detailed testing process before BIO mulches are approved for agriculture and made available on the market. These ecotoxicological tests should be performed in field conditions. Collaboration with industries to make more suitable plastic mulches for farmers necessitates the publication of the chemical composition of all the constituents of the plastic mulches.

Acknowledgment

We are thankful to the team of the experimental farm and the team of the Research Group Soil - Water - Plant of the Polytechnic university of Cartagena for their technical support. We would also like to thank Robin Palmer for the language editing.

Supplementary Material

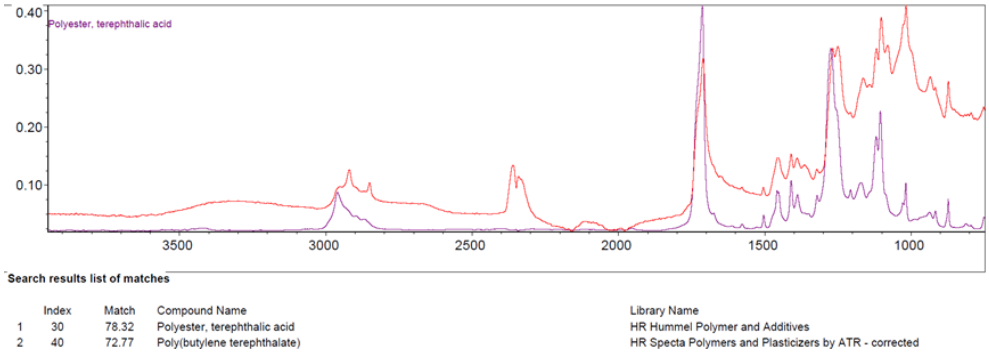


***Figure S6.1**: FTIR Spectra from the biodegradable mulch and the two best match from HR Hummel Polymer and Additives library: Polyester terephthalic acid and Poly(butylene terephthalate).*

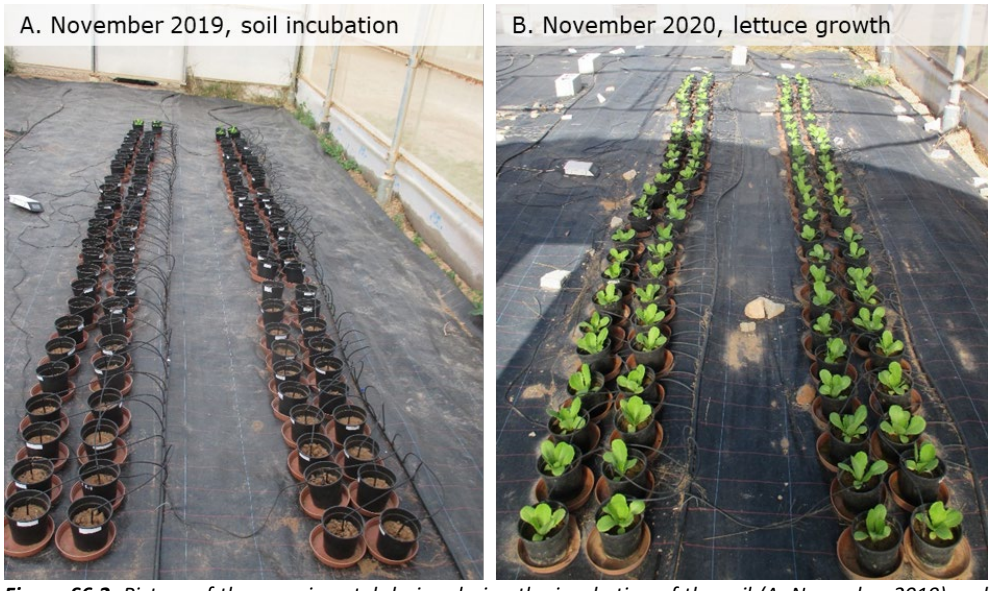


***Figure S6.2**: Picture of the experimental design during the incubation of the soil (A. November 2019) and during the lettuce growth (B. November 2020)*

***Table S6.1**: Fertilization plan for the Ammonium nitrate (16.9% nitrate N, 17.6% ammonium N) added in the irrigation water.*

Weeks	Date	Total N added in the experiment [g]	Total N added per mesocosm [g]
S3-S4	19 Oct - 2 Nov	5.2	0.04
S5-S7	2 Nov - 23 Nov	15.9	0.13
S8-S9	23 Nov - 9 Dec	13.1	0.11
S10-S12	9 Dec - 30 Dec	26.4	0.22
	Total	**60.6**	**0.51**

***Table S6.2** : P.values for the Levene's test of the homogeneity of variances, Shapiro-Wilk's test of the normality of the residuals, Shapiro-Wilk's test of the normality of the data.*

Parameters	Levene's test of the homogeneity of variances	Shapiro-Wilk's test of the normality of the residuals	Shapiro-Wilk's test of the normality of the data
Basal diameter	0.92	0.65	0.0082
Number of leaves	0.16	0.21	0.0008
Leaf area	0.90	0.68	0.0002
Fresh shoot biomass	0.86	0.09	0.008
Dry shoot biomass	0.46	0.07	0.0095
Shoot water content	0.15	0.10	0.0010

***Table S6.3**: Results of the two-way anova for the six measured plant parameters at the harvest (Basal diameter, Number of leaves, Leaf area, Fresh shoot biomass, Dry shoot biomass and Shoot water content) and the two factors (Plastic and Pesticide).Parameters and factor with p.values>0.05 are highlighted.*

Parameter	Factor	Df	Sum Sq	F value	Pr(>F)
Basal diameter	Plastic	3	1.45	0.64	0.59
Basal diameter	Pesticide	4	4.98	1.65	0.17
Basal diameter	Plastic:Pesticide	12	17.75	1.96	0.056
Basal diameter	Residuals	99	74.78		
Number of leaves	**Plastic**	3	31.6	3.15	0.029
Number of leaves	Pesticide	4	8.8	0.66	0.62
Number of leaves	Plastic:Pesticide	12	60.1	1.50	0.14
Number of leaves	Residuals	96	320.7		
Leaf area	**Plastic**	3	52703	4.39	0.006
Leaf area	Pesticide	4	6982	0.44	0.78
Leaf area	**Plastic:Pesticide**	12	99706	2.08	0.025
Leaf area	Residuals	99	395816		
Fresh shoot biomass	**Plastic**	3	82	4.20	0.008
Fresh shoot biomass	Pesticide	4	12	0.46	0.76
Fresh shoot biomass	Plastic:Pesticide	12	148	1.89	0.055
Fresh shoot biomass	Residuals	97	632		
Dry shoot biomass	**Plastic**	3	3.05	4.55	0.005
Dry shoot biomass	Pesticide	4	0.50	0.56	0.69
Dry shoot biomass	Plastic:Pesticide	12	4.49	1.68	0.08
Dry shoot biomass	Residuals	96	21.42		
Shoot water content	**Plastic**	3	1.01	3.98	0.010
Shoot water content	Pesticide	4	0.14	0.41	0.80
Shoot water content	Plastic:Pesticide	12	1.06	1.05	0.41
Shoot water content	Residuals	92	7.79		

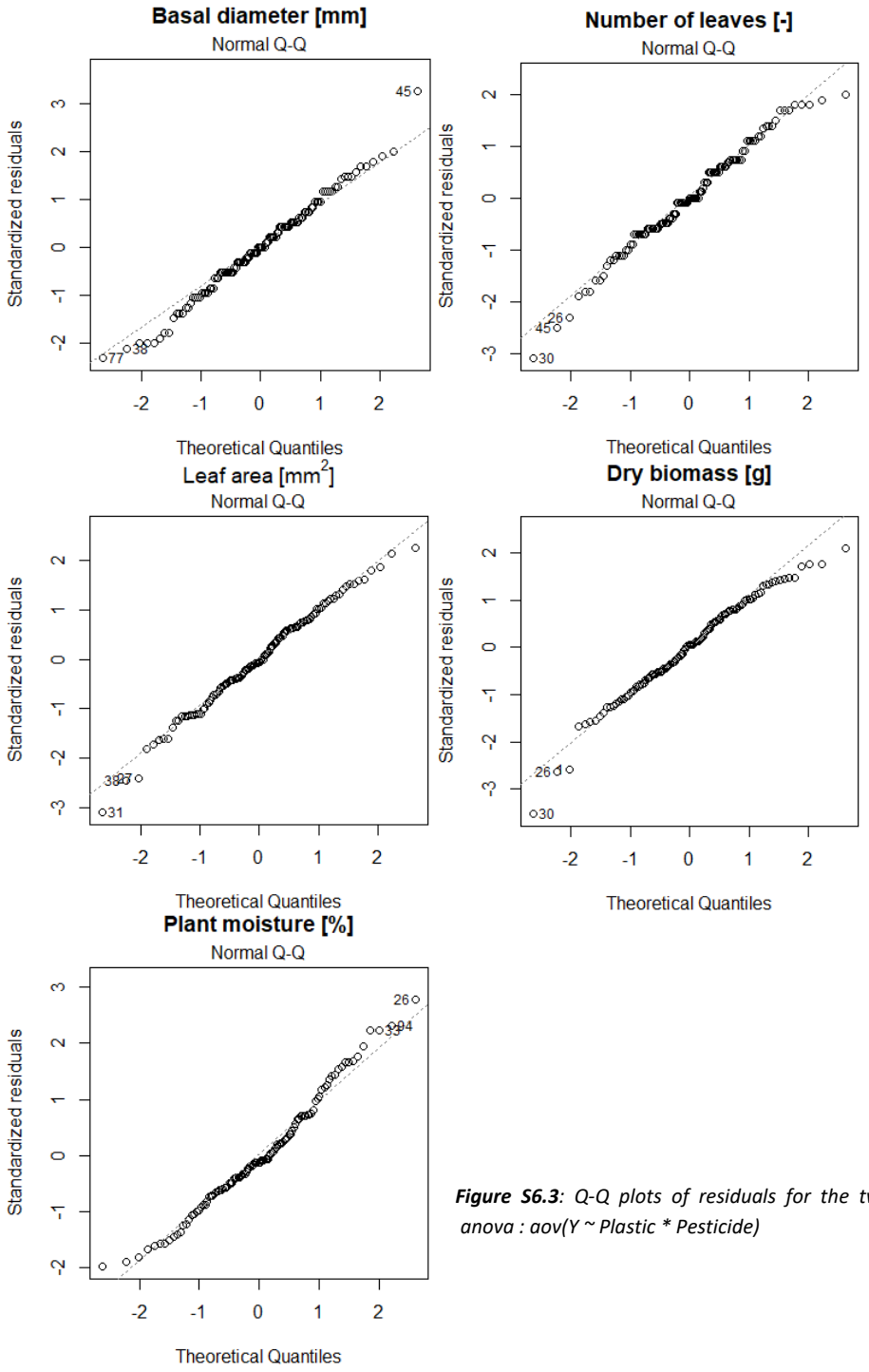


***Figure S6.3**: Q-Q plots of residuals for the two-way anova : aov(Y ~ Plastic * Pesticide)*

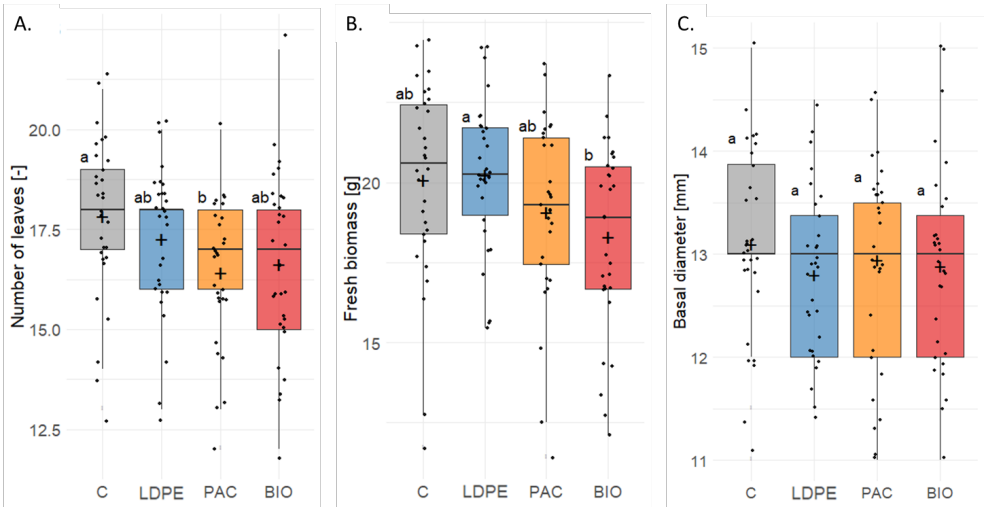


***Figure S6.4**: Number of leaves (A.), fresh shoot biomass (B.) and basal diameter (C.), for each plastic treatment : no-plastic (C), low density polyethylene (LDPE), pro-oxidant additive containing (PAC) and biodegradable (BIO) plastic. The box plot (horizontal lines) represents content for at least 25%, 50% and 75% of the samples. The vertical black line ends represent the minimum and maximum values, excluding outliers (1.5 IQR method). The cross represents the average content of any given sample group. The dots represent individual measurements. Groups that do not share letters are significantly different from each other (Wilcoxon test with $p < 0.05$).*

Chapter 7

General discussion

7.1 Overview of the thesis findings :

This PhD thesis describes the risks behind the intensive use of plastic mulch and pesticides in agriculture. We went through the example of intensive vegetable production in Southeast Spain. Along the way we explored the plastic and pesticide contamination levels, their interactions and various impacts they cause to the environment. A summary of the main findings per chapter can be find below (Figure 7.1). After the summary, we discuss the main findings as a whole, in the light of the scientific literature. We conclude the journey by reflecting on the possible actions to control the plastic and pesticide residues contamination in soil.

Chapter 1: In introduction we presented the diverse benefices of pesticide and plastic use in agriculture. The many services they provide explain why they are use abundantly worldwide. However both plastic and pesticide residues have the potential to accumulate in the soil. For plastic we made the difference between direct (e.g. plastic mulch, plastic coated fertilizers) and indirect sources (e.g. sewage sludge, compost). We also differentiated primary (~pristine) and secondary (~degraded) microplastics. We explained the degradation processes of plastic debris into microplastics and ultimately into water and CO_2. We narrowed down the focus to plastic mulch as it is used abundantly worldwide and is recognize as an important source of plastic debris. We introduced three different plastic types: Low density Polyethylene (LDPE), Pro-oxidant Additive Containing (PAC) and biodegradable (BIO) mulches. Finally, we presented the threats posed by plastic and pesticide residues and the need for more research. We elaborated six main questions that are addressed in the following five research chapters.

Chapter 2: The objective of this field assessment was to measure plastic and pesticide residues in agricultural soils and their effects on the soil microbiome. For this, we sampled soil (0-10 cm and 10-20 cm) from 18 parcels from 6 vegetable farms in Southeast Spain. The farms were under either organic or conventional management, where plastic mulch had been used for > 25 years. We measured the macro and micro light density plastic debris content, the pesticide residue levels, and a range of physicochemical properties. We also carried out DNA sequencing on the soil fungal and bacterial communities. Plastic debris was found in all samples and 4-10 different pesticide residues were also found in all conventional soils. Overall, pesticide content was ~100 times lower on organic farms, whereas no significant difference in plastic content was observed between organic and conventional farms. The fungal and bacterial communities were farm-specific and related to different soil physicochemical parameters and contaminants. Regarding contaminants, bacterial communities responded to the total pesticide residues, the fungicide Azoxystrobin and the insecticide Chlorantraniliprole as well as the total plastic area. The fungicide Boscalid was

the only contaminant to influence the fungal community in our results. We proved that plastic and pesticide residues were present together in the soil so we wondered to which extend there could be sorption of pesticides on plastic debris.

Chapter 3: The aim of this research was to measure the sorption pattern of active substances from pesticides on LDPE, PAC and BIO plastic mulches and to compare the decay of the active substances in the presence and absence of plastic debris. For this purpose, 38 active substances from 17 insecticides, 15 fungicides and six herbicides commonly applied with plastic mulching in South-East Spain were incubated with a 3 × 3 cm^2 piece of plastic mulch (LDPE, PAC and BIO). The incubation was done in a solution of 10% acetonitrile and 90% distilled water at 35 °C for 15 days in the dark. The sorption behaviour depended on both the pesticide and the plastic type. On average, the sorption percentage was ~23% on LDPE and PAC and ~50% on BIO. The decay of active substances in the presence of plastic was ~30% lesser than the decay of active substances in solution alone.

Chapter 4: We investigated whether the plastic debris, as found in the soil in chapter 2, could modify the soil properties. We tested the impact of macro (around 5 mm) and micro (< 1 mm) sized plastic debris from LDPE and one type of starch-based BIO mulch film on soil physicochemical and hydrological properties. The bulk density, porosity, saturated hydraulic conductivity, field capacity and soil water repellency were altered significantly in the presence of the four kinds of plastic debris, while pH, electrical conductivity and aggregate stability were not substantially affected. The type, size and content of plastic debris as well as the interactions between these three factors played complex roles in the variations of the measured soil parameters.

Chapter 5: We expanded the focus of our work from the soil to living being walking on it by investigating the question: Do sheep ingest the plastic debris when they are grazing in contaminated fields? To give an answer, we collected sheep faeces from five different herds and analysed the microplastic content. We found ~10^3 particles·kg^{-1} in the faeces. The data show that livestock ingested plastic, in the form of microplastics and/or macroplastics. Further studies should focus on: assessing how much of the plastic found in faeces comes directly from plastic mulching, estimating the plastic degradation in the guts of sheep and understanding the potential effects of these plastic residues on the health of livestock.

Chapter 6: In a final mesocosm experiment, we replicated field conditions to test if plastic mulch and pesticide residues could affect the plant production. We tested three plastic mulches and three pesticides commonly used by farmers. Low density polyethylene (LDPE), Pro-oxidant Additive Containing (PAC) and biodegradable (BIO) mulches were laid in a field for four months in summer, shredded into micro- and macro- plastics and added to the soil

with pesticides. Plastic and pesticides were left in the mesocosm to incubate for a year in quasi-field conditions before lettuces seedlings, *Lactuca sativa*, were planted. After 14 weeks growing periode, we measured the basal diameter, number of leaves, leaf area, fresh shoot biomass and dry shoot biomass. We observed a decreased leaf area, fresh shoot biomass and dry shoot biomass in plants growing in soil where BIO plastic was present compared to the control. These results add up to previous studies in a call for more detailed test before approval of BIO mulches on the market.

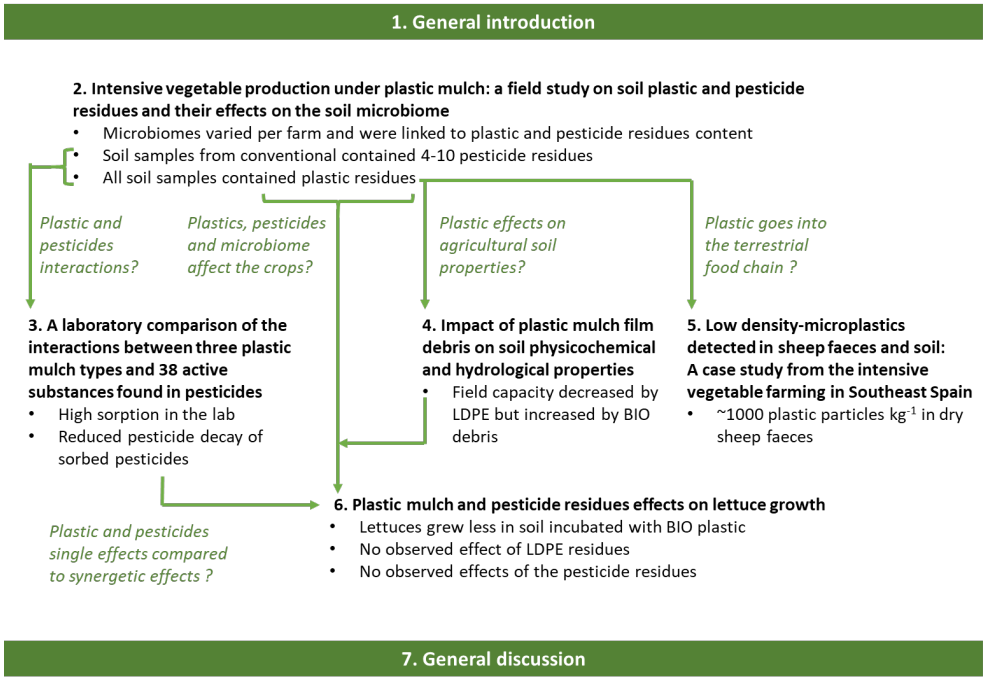


***Figure 7.1**: Schematic Outline of the thesis with logical connections and main results per chapter.*

7.2 Plastic and pesticide residues: ubiquitous contaminants in agricultural soils (Chapters 1, 2, 3, 5)

Pesticides and plastic use in agriculture expanded in the 40's and in the 70's respectively (Kasirajan and Ngouajio 2012; Whitaker 1974). But it is only 20 years later, in the 60's and 90's, that the scientific community became widely aware of the contamination and its potential detrimental consequences for the environment (Carson 1962; Moore 1997). In a sense we could say that science is moving steadily forward but it is moving slowly and behind the facts. Since the first pesticide and plastic introduction, over decades there were no wide scale monitoring programs installed to identify residues in the environment. Only recently field assessments have been conducted (Meng et al. 2020; Silva et al. 2018a) and the results of this thesis add to the consensus: plastic and pesticide residues are everywhere in agricultural soils.

In this thesis we showed that 100% of the agricultural soils we analysed contained plastic particles and 100% of the soils under conventional agriculture contained pesticide residues (**Chapter 2**). Concerning plastic residues, similar results are reported in studies sampling fields under LDPE plastic mulch (Huang et al. 2020b; Meng et al. 2020). Plastic mulch leads to both macro- and micro- residues (Steinmetz et al. 2016b). As mentioned in introduction (**Chapter 1**) Sewage sludge (Corradini et al. 2019c; van den Berg et al. 2020) or compost (Benjamin van Schothorst 2021; Sintim et al. 2020) application may also lead to plastic accumulation in soils. Comparing the different studies does not identify a practice which contributes significantly more to plastic contamination (Hurley et al. 2020). Discriminating the amount of plastic deriving from each of these agricultural practice remains a challenge.

For conventional LDPE plastic mulch, the plastic contamination depends on the frequency of applications, on the type of plastic mulch applied and on the type of method used to remove the mulch. We estimated that ~10% of the plastic mulch applied remained in the soils of the farms we visited (**Chapter 2**). The residual rate could be comprised between 5% and 55% in the extreme scenarios according to Chen et al. (2013). Plastic residual rates are higher for more resistant and thicker mulches as they are less likely to break down during removal (Manzano et al. 2019).

Beside agricultural practices, plastic debris can come from various sources and diffuse into the environment **(Chapter 1).** In **chapter 5** we gave an example of plastic ingested either as macro- or micro- debris, by sheep grazing in the field. Plastic ingestion has been observed in other organisms, from earthworms (Huerta Lwanga et al. 2017a; Yu et al. 2020) to birds (Huerta Lwanga et al. 2017b; Shiye Zhao et al. 2016) and cattle (Mekuanint et al. 2017). On a larger scale, plastic debris in soil is also transported by the run-off water (Bläsing and

Amelung 2018), ends up in rivers (Ding et al. 2019) and in the ocean (Abbing 2019) and is deposited back in fields during floods (Gündoğdu et al. 2018). The atmospheric transport is also a major pathways (Zhang et al. 2020b). Wind can transport both macro- and micro-plastics (Hurley et al. 2020). The transport is dependent on the shape and the density of the plastic, light density microplastics being the ones likely to go the furthest (Zhang et al. 2020b). Comparatively macroplastics are less likely to be transported in the atmosphere or to be ingested or inhaled by organisms.

Diffuse contamination is equally relevant for pesticides as they can be also transported by the water (Ochoa and Maestroni 2018; Sarraute et al. 2019) and the wind (Bidleman 1999; Galon et al. 2021). In **Chapter 3** we discuss the sorption of pesticides on plastic. Therefore pesticides can follow the plastic particles as they travel in the water, the wind or organisms (Hüffer et al. 2019; Li et al. 2021).

The sorption of pesticide onto plastic debris also affected the degradation of pesticides (**Chapter 3**). This is important because it could increase the persistence of pesticides in the environment (Li et al. 2021). Moreover, most conventional plastics are quite resistant to weathering and stay in the environment for years (Bahl et al. 2020); this is a lot of time to travel and carry contaminants. The sorbed contaminants can be released in the environment, in the organism who ingested the plastic (Abdolahpur Monikh et al. 2020) and/or during the degradation of the plastic (Chubarenko et al. 2018). We also showed that the sorption was higher on biodegradable plastics (**Chapter 3**). We wonder if the sorbed contaminants will affect the colonizing microbiome (Amaral-Zettler et al. 2020) and ultimately affect the degradation of the biodegradable plastics (Sander 2019).

In conclusion, we show that plastic and pesticide residues are abundant in agricultural soils, microplastics and pesticides may theoretically be everywhere because of their transport and together they might stick around for a while. We will discuss later what are and what could be the consequences for the environment but first let’s discuss the methods used to detect them in soil.

7.3 Uncertainties shrouding pesticides and plastic content assessment in soil (Chapters 2, 5, 6)

We just said that microplastics and pesticide residues are pretty much everywhere, but they are not easy to spot. If you would make a picture, you would not see them. However it is not necessarily their size but their diversity which makes them difficult to detect. We cannot emphasis too much on the fact that plastics and pesticides are diverse and complex contaminants. Commercial plastics can be composed of a blend of different polymers and contain several additives (Murphy 2001). Similarly, commercial pesticides are composed of active substances in a solution of diverse solvents and additives (Mesnage and Antoniou 2018). A noteworthy difference is that for pesticides solvents and additives toxicity is considered negligeable compared to the active substances; whereas for plastics, additives are proven to be toxic, even perhaps more toxic than the main polymer (Lear et al. 2021). The detection of plastic additives would rely on a targeted screening of expected chemicals, as we proceed for pesticide active substances (Anastassiades et al. 2003). However additives are the secret garden of industries making the targeted screening for additives very difficult. If we leave out additives in order to simplify, we can apply different technics to assess the plastic and pesticide contamination. For plastic polymers we can measure the number of particles, the contact surface, the mass of debris, the type of polymer for all these debris and their degradation over time. For pesticides, we can measure the content of active substances, the content of known metabolites, and their degradation over time. However, we don't measure all these parameters in an assessment, so we don't get the full picture. Missing information is like looking at the flatness of the floor and imagining that the earth is flat. From an uncomplete picture we need to be careful about the conclusions. For example, in chapter 2 we measured only active substances from pesticides that famers declared to apply. We concluded that the content in organic farms was 100 times less than in conventional farm. However, by including also persistent pesticides that are long-banned, like DDT, Geissen et al. (2021) found a lower difference between organic and conventional farms. **Chapter 2 and 5** give another illustration of this as we only measured light density plastics and did not report the type of polymer. We assumed that the debris were LDPE as the fields were frequently covered with LDPE mulch. However, we did not check and left-out other potential sources such as atmospheric deposition or dumping. We might underestimate the total number of plastics by not including denser plastic types. So, we must keep in mind that we do not have the whole picture.

So, what can we do to have a better vision? For pesticides we can add metabolites to the targeted screening. For plastics we can use different detection methods and even combine them. **In chapter 2 and 5** we used the extraction method of Zhang et al (2018) which was developed for the extraction of LDPE and consisted in a stereomicroscope visual selection

(SMVS). The microplastics are visually identified based on their shape, colour, brightness, and response to heat (Möller et al. 2020). SMVS has the advantage to be relatively fast and cheap but relies on the observer judgement and attention which can vary from users. Spectral methods (e.g. Raman or Fourier transform infrared) are used to identify several types of plastic by comparing the acquired spectra with a spectral library (Munno et al. 2020; Sobhani et al. 2019). Spectral methods provide a 2D picture of the sample with number, area and best polymer type matched in the library for each particle (Fabio Corradini et al. 2021). Other methods do not directly report the size or number of particles but analyse the mass of all plastic present in the sample. It is the case for the thermal extraction desorption gas chromatography mass spectrometry (TED GC-MS) (Dümichen et al. 2017) and the pyrolysis–gas chromatography-mass spectrometry (Py-GC–MS) (Cai et al. 2021). Basically, plastic particles are burned and the molecules emitted are analysed by gas chromatography. It is a sensitive and well-established method for the characterization and mass-quantification of many polymer types and their organic additives.

Each method relies on a different property of the plastic and therefore is likely to underestimate the total plastic content. Additionally, each method is suitable for a certain particle size. For instance, with SMVS, smaller particles are more difficult to identify than bigger ones simply because they are more difficult to spot. It is another factor suggesting an underestimation of the total plastic number. Generally the smaller the plastic gets the more difficult it is to analyse. The identification of nanoplastics remains a challenge and new methods have to be validated in environmental samples. Raman spectroscopy and pyrolysis–gas chromatography/mass spectrometry (py-GC–MS) are the most promising methods (Cai et al. 2021). Additionally, the sample pre-processing and the plastic extraction method are also major factors for the microplastic analysis (Li et al. 2020). In our case we used an extraction by floatation in distilled water, excluding all plastic denser than water. The high amounts of organic matter in faeces also limited the plastic identification (**Chapter 5**). Because of this restriction, we only provided a number of plastic particles. To summarize, the diversity of plastics, the diversity of matrices (e.g. soils, composts, faeces) and the diversity of variables (number, area, mass, polymer type) mean that there is not a unique method able to answer all questions.

A combination of different methods could provide a better description of the environmental samples. For example, to compare the quantity of plastic left after one year incubation of LDPE, PAC and BIO in soil (**Chapter 6**), the best option could be to use a thermal desorption method to estimate the mass balance and to combine it with the SMVS method to investigate the evolution of the plastic size distribution. The spectral methods will be less interesting because we already know the type of polymer applied. On the other hand, a

spectral method could be helpful to identify the type of plastic in the field assessments **chapter 2 and 5** to have a better idea of the source of identified particles.

In conclusion, we recommend adapting the assessment to the specific interest of the study. For examples, in case of estimating the probability of ingestion the number of debris will be most important (Helmberger et al. 2019); for the formation of biofilms, the total area will be the dominant factor (Sander 2019); and for input/output balances the mass is generally favoured (Li et al. 2020). Moreover, many processes will be influenced by the size of the debris. For example a study about plastic ingestion may focus on mm size debris for mammals (Mekuanint et al. 2017), µm for earthworms (Helmberger et al. 2019) and nm for plant roots (Chae and An 2020). Therefore we need to harmonize the methods used to answer the same questions in the same matrices but the most important step is to define the success and limits of each methods. In order to make comparisons, detailed results for each sample analysed with a particular method needs to be available. Now that we have a better idea of what we are measuring, what consequences can we expect from all these contaminants?

7.4 Effects of pesticides and plastic residues on the environment

Plastic and pesticide residues can affect the environment in many ways. In the different chapters we covered consequences for the soil physicochemical parameters (**Chapter 4**), for the soil microbiome (**Chapter 2**), for the plants (**Chapter 6**) and for the mammals living on the land (**Chapter 5**). So, we will go step by step but first we want to discuss which contamination level are relevant to test effects.

7.4.1 Current environmental concentrations and future scenarios (Chapter 3, 4 and 6)

To have an idea of which contamination level to test, we should look at the current environmental concentrations and future scenarios. For plastic, a maximum residues content of 325 kg ha^{-1} was estimated in a field where plastic mulch was applied over 30 years in China (Huang et al. 2020). Considering accumulation in the first 10 cm of soil and a soil density of 1400 kg m^{-3} it represents about ~0.3 g kg^{-1}. In our study we measured ~0.2 g kg^{-1} (**Chapter 2**). These values were given for conventional plastic which accumulated in the past 30 years. For biodegradable plastic we would expect a shorter life span. However if we consider mulches, most conventional plastics are removed after the harvest whereas biodegradable plastic are left in the soil (Manzano et al. 2019). So, if biodegradable plastics do not degrade as well as expected, residues would accumulate quickly in the soil and might

reach levels of conventional plastics. So, we suggest that conventional and biodegradable plastics should be studied in equal contamination levels. To study the effect of a contaminant, it can be interesting to study high contents to observe a clear effect and to account for future contamination levels. Moreover, studying different levels may allow to establish a threshold until which consequences are acceptable. Therefore, we consider relevant to discuss effects of plastic contamination up to two orders of magnitude more than the currently observed maximum, namely 20 g kg^{-1} or 2% (w/w).

Pesticides do accumulate in soil but degrade faster than conventional plastics. In **chapter 3** we tested the sorption of pesticides of plastic in a concentration of 1 mg L^{-1}. It is above the maximum we would find in the soil. This experiment was not designed to be representative of field conditions or to assess on effect of the contaminants on an organism or a soil properties. It was designed to compare the relative sorption of pesticides onto different plastic mulches. Our results encouraged to further explore the sorption and desorption processes of pesticides on plastic in the environment, using environmentally relevant concentrations.

7.4.2 Consequences for the soil physicochemical properties (Chapter 4)

In **chapter 4** we tested macro- and micro- plastic particles from LDPE and BIO mulches addition (0.5%, 1% and 2% w/w) to a sandy soil. We reported a decreased field capacity with LDPE debris and an increase with BIO debris suggesting a strong effect of the plastic type on the water retention. No effects were observed for the soil pH, electrical conductivity and aggregate stability. In different conditions, de Souza Machado et al. (2019) observed a reduction of aggregate stability in the presence of polyamide beads (diameter of 15-20 µm) and polyester fibers (~5000 µm in length and ~8 µm in diameter). In another study he also described that polyester and polyacrylic fibers reduced aggregate stability (de Souza Machado et al. 2018b). He suggested that the fiber shape, as an elastic and linear particle creates tension inside the aggregates (Figure 7.2). Apart from confirming the effect of plastic on the soil properties, these results highlight the fact that different results can be expected from different plastic particle polymer, shape and size. Additionally, most studies emphasis that aging of the plastic in the soil will likely modify the interaction with soil particles; and of course, soil parameters are closely related with soil type and texture (Yang et al, 2020). Therefore, further research is needed to focus on different soil types, plastic shape and types.

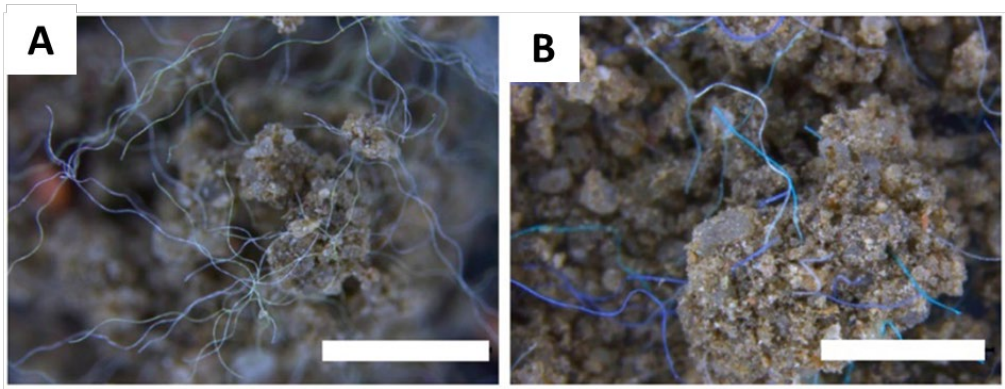


***Figure 7.2** : Polyester fibres (A) and polyacrylic fibres (B) incorporated into soil aggregates. The white bar in each panel represents 1 mm size. Adapted from de Souza Machado et al. (2018b)*

7.4.3 Consequences for the soil microbiome (Chapter 2)

In **chapter 2** we observed that bacterial communities were correlated with the total plastic area in soil, the total pesticide residues in soil and more specifically the Azoxystrobin and Chlorantraniliprole contents. The fungi communities were correlated with the Boscalid content. The interaction of plastics (Lear et al. 2021) and pesticides (M. Hartmann et al. 2015) with the soil microbiome have been observed in many studies. Meng et al. (2021) and Qi et al. (2020a) both observed a modified soil bacterial community after introduction of plastic mulch debris. There is different hypothesis which could explain the effect. First, we showed that plastic may alter the soil physical properties (**Chapter 4**) which could then modify the soil microbiome. For example, if biodegradable plastic would increase the soil water retention as reported in **chapter 4**, organisms adapted to higher water content would thrive. Another hypothesis is that plastic could serve as a shelter for microorganisms, with the formation of a biofilm for example (Amaral-Zettler et al. 2020). Additionally, plastics could be a source of nutrient, mostly carbon. In fact several organisms have been reported to degrade plastic (Bahl et al. 2020), even the LDPE (Gajendiran et al. 2016). Finally, plastic could release chemicals toxic for some microorganisms. As explained previously we have barely no information about the chemicals present in the commercial plastic and they could be released due to plastic degradation in the soil (Wang et al. 2019a). In fact, we know that some plastic additives can directly affect the soil microbiome (Kong et al. 2018). Similarly for the pesticides, the active substances could be toxic for some organisms and degraded by others (Wołejko et al. 2020). However potential combined effects of pesticides and plastic residues together remain under studied. The synergetic effects are difficult to predict. We can expect that the pesticides sorbed on plastic will alter the microbiome community

colonizing the plastic. Would sorbed pesticides reduce the biodegradation of plastic? This question needs to be answered if biodegradable plastic mulches are used widely in fields where pesticides are used. To give a first insight, it will be interesting to assess the response of the soil microbiome to the plastics and pesticides tested in the **chapter 6.** For now, we only analysed the plant response which brings us to the next paragraph.

7.4.4 Consequences for the plants (Chapter 6)

In **chapter 6** we observed a reduction of the plant production (leaf area, shoot fresh biomass and shoot dry biomass) in soil incubated with BIO plastics, compared to the control, LDPE and PAC. We did not observe any effect of the pesticides treatments. Qi et al. (2018) and Meng et al. (2021b) also observed a significant effect of BIO plastic but not of LDPE on wheat and common bean respectively. The impact of BIO could come from effects discussed earlier such as change in the soil physical property or change in the microbiome. Indeed we wonder if beneficial or pathogenic organisms have been supressed or enhanced by the BIO plastic. Another theory could be a nutrient competition between the organisms degrading the BIO plastic and the plant. For instance Meng et al. (2021a) reported in a comparable set up, less nitrate but more organic nitrogen in soils with 2% BIO plastic. This effect would be very dependent on the fertilization scheme applied and the degradation of the plastic therefore more studies are required. Moreover, we should also consider a direct effect of the plastic on the plant. It could be a mechanical effect. For example Bosker et al. (2019) reported that nanoplastics blocked the pores of cress seed capsules and reduced the germination rate. Nanoplastics could also enter the roots potentially leading to oxidative stress in the plant (Giorgetti et al. 2020). As they are more easily degraded we could expect the BIO plastics to generate more nanoplastics, explaining why they have more impact on the plant growth. The size distribution of the plastic in the mesocosm after one year remains to be measured. Finally we can imagine that chemicals leaked from the BIO plastic for un-known consequences for the plant. All these hypotheses remain unverified and could be combined to explain detrimental effects of plastic residues on the plant. If plastic ends up in plants we need to look at the plastic ingested by herbivores.

7.4.5 Consequences for the mammals living on the land (Chapter 5)

In chapter 5 we reported the presence of microplastic in sheep faeces. We suggested a link between the soil contamination and the ingestion of plastic by the sheep. We encourage further study to get a closer look at the sources of the contamination and effects on the animal. It would be important to include the study of pesticides in the assessment as we showed the ubiquitous contamination of pesticides in the conventional fields (**Chapter 2**). Pesticides sorbed on plastic could leak in the animal guts. If we extend our conclusions for the soil microbiome, we could expect a modification of the gut microbiome because of the plastic and pesticide residues. Pesticides effect on the gut microbiome have been described in many animals (Syromyatnikov et al. 2020; Utembe and Kamng'ona 2021). For plastics, modifications of the gut microbiome were observed in worms (Lou et al. 2021; Zhu et al. 2018a) and marine animals (Fackelmann and Sommer 2019). Many interrogations subsist about the effects of plastic and pesticide residues in organisms. Chronic exposure and long term consequences are particularly challenging to assess. Evidences we have are already enough to arouse concerns. So what should we do about plastics and pesticide contamination?

7.5 Plastic mulch and pesticide use in agriculture, a paradigm to be shifted?

7.5.1 Reducing the use of plastic mulch and pesticides

Reducing is the first step of the famous three 'Rs' sustainable waste management approach : 'Reduce, Reuse, Recycle' (Abdul-Rahman and Wright 2014). For pesticides, it seems to be the path taken by the European Commission with the Farm to Fork Strategy and the Zero Pollution Strategy (EC 2020b, 2020a). The aim is to reduce pesticide use by 50% by 2030 in Europe. The suggested alternative is organic farming as they also aim for having at least 25% of EU agricultural land under organic management by 2030. Alternatives to pesticides will be needed. For herbicides, we may wonder if the restriction of pesticides use will lead to more application of plastic mulch. Indeed plastic mulch is also used to reduces the growth of weeds. In **chapter 2** we did not conclude if organic farms used more plastic mulch or had more plastic residues in their soils because we did not sample enough farms.

Pesticides ban or not, data shows that the plastic mulch use is growing year by year (PlasticsEurope 2020). Indeed plastic mulch as many advantages that make it difficult to replace (Beriot 2020). In order to maintain the same crop productions it appears easier to re-design the plastic applied instead of reducing the use.

7.5.2 Better designed plastic mulches and pesticides

Re-designed is an 'R' often added to the three 'Rs' approach and provides more perspectives. For plastic mulch there are two competing ways for a better design : make the plastic mulch stronger to facilitate its recovery after the harvest or make it biodegradable to get rid of all residues without removal. The biodegradable approach comes with the issues we mentioned previously : the mulch needs to degrade in due time and without detrimental effects for the crop and the environment. The best way to ensure a mulch validates these two criteria would be to test it in field conditions where it is intended to be applied. Indeed the degradation will depend on the pedoclimatic conditions (Ahmed et al. 2018). If the mulch degrades too fast it won't fulfil its function. If it degrades too slow then we are back to the problem of accumulation and dispersion of debris. So more extensive tests are required before welcoming a new material in the field.

The approach of using stronger plastic presents different challenges. First farmers need good methods to recover the mulch. Specific tools have been designed to take out the plastic mulch and avoid collecting soil and plant residues with it (Benoit James and Vesna Miličić 2021). Then the plastic needs to be stored and collected in a proper way. For instance, the retrieving efforts will be for nothing if the plastic is blown away by the wind. Finally it needs to be collected and recycled. These last two steps bring the question of who should be responsible for the costs and why virgin plastic materials are cheaper than recycled materials, but this goes beyond the scope of this thesis. To summarize, plastic mulch should be (re)design to be either cleanly biodegraded in the soil or to be easily recovered and recycled.

Similar issues apply to pesticides. Should farmer use less toxic pesticides but with the risk they need bigger quantities to manage the pests? Should pesticides be more degradable or protect the field for a longer time? Should pesticides be more specific with the risk farmers need to use a mixture of them to deal with different pests? No easy answers can be given and there is certainly no 'one-fits-all' approach.

It is nevertheless interesting to point out that pesticide and plastic mulch applications are artificial products to answer pests and resource scarcity. Other managements are based on natural processes such as agroforestry (Dollinger and Jose 2018), beneficial insects as pest control (Kenis et al. 2017), crop diversifications (Hufnagel et al. 2020) or conservation agriculture (Knapp and van der Heijden 2018). For example, in conservation agriculture, soil compactions can be solved with earthworms instead of tractors (Kumar et al. 2020) Nevertheless, comparing different managements is complicated. It requires the collaboration of different fields of science such as contamination assessment, ecotoxicological tests and socioeconomical analysis. To conclude we could say that agriculture is built on two pillars: natural processes and artificial inputs. Science carries both to help farmers getting the most of their fields.

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Summary

In the 1960s, the agricultural "Green Revolution" brough new technologies and heavy mechanisation to the fields. These technologies brought rapid answers to increase the crop yields and improve food security. Among these technologies we found pesticide and plastic mulch applications. Since then the scientific community and the general public became worried of the long term consequences of these short term solutions. Indeed plastic mulch and pesticides leave debris which accumulate in the soil and are potentially harmful to divers organisms. With this PhD thesis we try to encompass the threat posed by plastic mulch and pesticide residues in agriculture. The research was organised in seven different chapters described below.

Chapter 1: In introduction we presented the diverse benefices of pesticide and plastic use in agriculture. The many services they provide explained why they are use abundantly worldwide. However both plastic and pesticides have the potential to accumulate in the soil. For plastic we made the difference between direct (e.g. plastic mulch, plastic coated fertilizers) and indirect sources (e.g. sewage sludge, compost). We explained the degradation processes of plastic debris into microplastics and ultimately into water and CO_2. We differentiated primary (~pristine) and secondary (~degraded) microplastics. We narrowed down the focus to plastic mulch as it is used abundantly worldwide and is recognize as an important source of plastic debris. We presented three different plastic types: Low density Polyethylene (LDPE), Pro-oxidant Additive Containing (PAC) and biodegradable (BIO) mulches. Finally, we presented the threats posed by plastic and pesticide residues and the need for more research. We elaborated six main questions that are addressed in the following five research chapters.

Chapter 2: The objective of this field assessment was to measure plastic and pesticide residues in agricultural soils and their effects on the soil microbiome. For this, we sampled soil (0-10 cm and 10-20 cm) from 18 parcels from 6 vegetable farms in Southeast Spain. The farms were under either organic or conventional management, where plastic mulch had been used for > 25 years. We measured the macro and micro light density plastic debris content, the pesticide residue levels, and a range of physiochemical properties. We also carried out DNA sequencing on the soil fungal and bacterial communities. Plastic debris was found in all samples and 4-10 different pesticide residues were also found in all conventional soils. Overall, pesticide content was ~100 times lower in organic farms, whereas no significant difference in plastic content was observed between organic and conventional farms. The fungal and bacterial communities were farm-specific and related to different soil physicochemical parameters and contaminants. Regarding contaminants, bacterial communities responded to the total pesticide residues, the fungicide Azoxystrobin and the

insecticide Chlorantraniliprole as well as the total plastic area. The fungicide Boscalid was the only contaminant to influence the fungal community. We proved that plastic and pesticide residues were present together in the soil so we wondered to which extend there could be sorption of pesticides on plastic debris.

Chapter 3: The aim of this research was to measure the sorption pattern of active substances from pesticides on LDPE, PAC and BIO plastic mulches and to compare the decay of the active substances in the presence and absence of plastic debris. For this purpose, 38 active substances from 17 insecticides, 15 fungicides and six herbicides commonly applied with plastic mulching in South-east Spain were incubated with a 3 × 3 cm2 piece of plastic mulch (LDPE, PAC and BIO). The incubation was done in a solution of 10% acetonitrile and 90% distilled water at 35 °C for 15 days in the dark. The sorption behaviour depended on both the pesticide and the plastic mulch type. On average, the sorption percentage was ~23% on LDPE and PAC and ~50% on BIO. The decay of active substances in the presence of plastic was ~30% lesser than the decay of active substances in solution alone.

Chapter 4: We investigated whether the plastic debris found in the soil in chapter 2 could modify the soil physicochemical properties. We tested the impact of macro (around 5 mm) and micro (< 1 mm) sized plastic debris from LDPE and one type of starch-based BIO mulch film on soil physicochemical and hydrological properties. The bulk density, porosity, saturated hydraulic conductivity, field capacity and soil water repellency were altered significantly in the presence of the four kinds of plastic debris, while pH, electrical conductivity and aggregate stability were not substantially affected. The type, size and content of plastic debris as well as the interactions between these three factors played complex roles in the variations of the measured soil parameters.

Chapter 5: We expanded the focus of our work from the soil to living being walking on it by investigating the question: Do sheep ingest the plastic debris when they are grazing in contaminated fields? To give an answer, we collected sheep faeces from 5 different herds and analysed the light density microplastic content. We found ~10^3 particles·kg^{-1} in the faeces. The data showed that livestock ingested plastic, in the form of microplastics and/or macroplastics. Further studies should focus on: assessing how much of the plastic found in faeces is coming directly from plastic mulching, estimating the plastic degradation in the guts of sheep and understanding the potential effects of these plastic residues on the health of livestock.

Chapter 6: In a final mesocosm experiment, we replicated field conditions to test if plastic mulch and pesticide residues could affect the plant production. We tested three plastic mulches and three pesticides commonly used by farmers. Low density polyethylene (LDPE),

Pro-oxidant Additive Containing (PAC) and biodegradable (BIO) mulches were laid in a field for four months, shredded into micro- and macro- plastics and added to the soil with pesticides. Plastic and pesticides were left in the mesocosm to incubate for a year in field condition before lettuces seedlings, *Lactuca sativa*, were planted. After 14 weeks growing periode, we measured the basal diameter, number of leaves, leaf area, fresh shoot biomass, dry shoot biomass and shoot water content. We observed a decreased leaf area, fresh shoot biomass and dry shoot biomass in plants growing in soil where BIO plastic was present compared to the control. These results add up to previous studies in a call for more detailed test before approval of BIO mulches for agriculture on the market.

Chapter 7: The main findings of the thesis were summarized in the last chapter. We compared our results with existing literature and discussed the links between the chapters. We explored the limitations of current plastic and pesticide residues detection. More specifically, the detection of small plastic debris, microplastics and nanoplastics, in soil remains a challenge and a diversity of methods are being developed. We suggested that the diversity of methods is required to describe the diversity of plastic types, sizes and shapes and the methods need to be adapted to the specific objective of the study. We also discussed the current levels of plastic and pesticide residues in soil and which concentrations should be used to assess their potential impacts. Finally we explored different options to control the impacts of plastic and pesticide residues in agriculture. No 'one-fits-all' approach can be suggested as agricultural systems are very diverse and face many challenges. Nevertheless best practices are recommended to limit the accumulation of residues in the environment: use stronger plastic mulch for a more efficient removal and recycling or use biodegradable plastic tested in field conditions; be mindful of the climate conditions when applying pesticides and take into account their specific residence time in soil. We conclude that plastic and pesticides use are artificial inputs which affect natural processes in some detrimental manner while many natural processes are beneficial to crop production. We need more investigation and initiatives to tailor agricultural management to their specific conditions and challenges.

Resumen

En la década de 1960, la "Revolución Verde" de la agricultura trajo nuevas tecnologías y una gran mecanización en los campos. Tecnologías como la aplicación de pesticidas y la instalación del acolchado plástico, aportaron respuestas rápidas para aumentar los rendimientos de los cultivos y mejorar la seguridad alimentaria. Desde entonces, la comunidad científica y la sociedad en general se preocuparon por las consecuencias de estas soluciones a corto plazo. De hecho, el acolchado plástico y el uso de pesticidas dejan residuos que se acumulan en el suelo y son potencialmente peligrosos para algunos organismos. Con esta tesis doctoral tratamos de describir el riesgo de la acumulación de residuos de plástico y del uso de pesticidas en agricultura. Este trabajo está organizado en siete capítulos que se describen a continuación.

Capítulo 1: En la introducción, presentamos varios beneficios del uso de pesticidas y plásticos en agricultura. Los muchos servicios que ofrecen explican por qué se usan abundantemente en todo el mundo. Sin embargo, tanto el plástico como los pesticidas tienen el potencial de acumularse en el suelo. Con respecto al plástico, hicimos la diferencia entre fuentes directas (p. ej., acolchado plástico, fertilizantes recubiertos con plástico) e indirectas (p. ej., lodos de depuradora, compost). Explicamos los procesos de degradación de los residuos plásticos en microplásticos y, finalmente, en agua y CO_2. Diferenciamos entre microplásticos primarios (~prístinos) y microplásticos secundarios (~degradados). Nos centramos en el uso del acolchado plástico, debido principalmente al uso abundante de estos en todo el mundo, y que se reconozca como una fuente importante de desechos plásticos. Presentamos tres tipos de plástico diferentes: Polietileno de baja densidad (LDPE), acolchados que contienen aditivos prooxidantes (PAC) y biodegradables (BIO). Finalmente, presentamos las amenazas que representan los residuos de plástico y pesticidas, y la necesidad de más investigación. Elaboramos seis preguntas principales que se abordan en los siguientes cinco capítulos de investigación.

Capítulo 2: El objetivo de esta evaluación de campo fue medir los residuos de plásticos y pesticidas en los suelos agrícolas y sus efectos en el microbioma del suelo. Para eso, tomamos muestras de suelo (0-10 cm y 10-20 cm) de 18 parcelas de 6 huertas del sureste de España. Las fincas estaban bajo manejo orgánico o convencional, donde se había usado acolchado plástico por más de 25 años. Medimos el contenido de desechos plásticos de densidad micro y macro, los niveles de residuos de pesticidas y una variedad de propiedades fisicoquímicas. También llevamos a cabo la secuenciación del ADN de las comunidades fúngicas y bacterianas del suelo. Se encontraron residuos plásticos en todas las muestras y se encontraron de 4 a 10 residuos de diferentes pesticidas en todos los suelos bajo manejo convencional. En general, el contenido de pesticidas fue ~100 veces menor en manejo

orgánico, mientras que no se observaron diferencias significativas en el contenido de plástico entre manejos. Las comunidades fúngicas y bacterianas eran específicas de cada finca y estaban relacionadas con diferentes parámetros fisicoquímicos y el contenido de contaminantes en el suelo. En cuanto a los contaminantes, las comunidades bacterianas respondieron al total de residuos de pesticidas, al fungicida Azoxystrobin y al insecticida Clorantraniliprol, así como al total del área de plástico. El fungicida Boscalid fue el único contaminante que influyó en la comunidad fúngica. Demostramos que los residuos de plástico y pesticidas estaban presentes en el suelo, por lo que nos preguntamos hasta qué punto podría haber absorción de pesticidas en los residuos plásticos.

Capítulo 3: El objetivo de esta investigación fue medir la sorción de las sustancias activas de los pesticidas en acolchados de LDPE, PAC y BIO plástico, y comparar la descomposición de las sustancias activas en presencia y ausencia de desechos plásticos. Para eso, se incubaron 38 sustancias activas de 17 insecticidas, 15 fungicidas y 6 herbicidas comúnmente aplicados con acolchado plástico en el sureste de España con una pieza de acolchado de 3 × 3 cm^2 (LDPE, PAC y BIO). La incubación se realizó en una solución del 10% de acetonitrilo y 90% de agua destilada a 35 °C durante 15 días en oscuridad. La sorción dependió tanto del pesticida como del tipo de acolchado plástico. En promedio, el porcentaje de sorción fue de ~23% en LDPE y PAC, y ~50% en BIO. La descomposición de las sustancias activas en presencia de plástico fue ~30% menor que la descomposición de las sustancias activas en solución sola.

Capítulo 4: Investigamos si los residuos plásticos encontrados en el suelo en el capítulo 2 podrían modificar las propiedades fisicoquímicas del suelo. Probamos el impacto de residuos plásticos de tamaño macro (~5 mm) y micro (< 1 mm) de LDPE y un tipo de acolchado BIO sobre las propiedades fisicoquímicas e hidrológicas del suelo. La densidad aparente, la porosidad, la conductividad hidráulica saturada, la capacidad de campo y la repelencia al agua se alteraron significativamente en presencia de los cuatro tipos de residuos plásticos, mientras el pH, la conductividad eléctrica y la estabilidad de los agregados no se vieron afectados sustancialmente. El tipo, tamaño y contenido de los residuos plásticos, así como las interacciones entre estos tres factores, resultaron en efectos complejos sobre las variaciones de los parámetros del suelo.

Capítulo 5: Ampliamos el enfoque de nuestro trabajo desde el suelo hasta los seres vivos que caminan con la pregunta: ¿Las ovejas ingieren los desechos plásticos cuando pastan en campos contaminados? Para dar una respuesta, recolectamos heces de ovejas de 5 rebaños diferentes y analizamos el contenido de microplásticos bajo densidad. Encontramos ~10^3 partículas·kg^{-1} en las heces. Los datos mostraron que el ganado ingirió plástico, en forma de microplásticos y/o macroplásticos. Estudios adicionales deberían centrarse en: evaluar la cantidad de plástico en las heces que venga directamente del acolchado plástico, estimar la

degradación del plástico en las entrañas de las ovejas y comprender los efectos potenciales de estos residuos plásticos en la salud del ganado.

Capítulo 6: En un experimento final, replicamos las condiciones de campo en un mesocosmos para probar si el acolchado plástico y los residuos de pesticidas podrían afectar la producción de la planta. Probamos tres acolchados plásticos y tres pesticidas comúnmente utilizados por los agricultores. Se colocaron acolchados de polietileno de baja densidad (LDPE), que contienen aditivos prooxidantes (PAC) y biodegradables (BIO) en un campo durante cuatro meses, se fragmentaron en micro y macroplásticos, y se agregaron al suelo con pesticidas. El plástico y los pesticidas se incubaron en el mesocosmos durante un año en condiciones de campo antes de plantar plántulas de lechuga, *Lactuca sativa*. Después de un período de crecimiento de 14 semanas, medimos el diámetro basal, el número de hojas, el área foliar, la biomasa de brotes frescos, la biomasa de brotes secos y el contenido de agua de los brotes. Observamos una disminución del área foliar, la biomasa de brotes frescos y la biomasa de brotes secos en plantas que crecían en suelo donde había plástico BIO en comparación con el control. Estos resultados se suman a estudios previos en los que se pone de manifiesto la necesidad de realizar pruebas más detalladas antes de la aprobación institucional del uso de acolchados BIO en agricultura.

Capítulo 7: Los principales resultados de la tesis se resumieron en el último capítulo. Comparamos nuestros resultados con la literatura existente y discutimos los vínculos entre los capítulos. Exploramos las limitaciones de la detección actual de residuos de plásticos y pesticidas. Más específicamente, la detección de pequeños residuos plásticos, micro- y nano-plásticos en el suelo sigue siendo un desafío. Se están desarrollando una diversidad de métodos. Sugerimos que la diversidad de métodos es requerida para describir la diversidad de tipos, tamaños y formas de plástico y que los métodos deben ser adaptados al objetivo específico del estudio. También discutimos los niveles actuales de residuos de plástico y pesticidas en el suelo y qué concentraciones deberían usarse para evaluar sus impactos potenciales. Finalmente, exploramos diferentes opciones para controlar los impactos de los residuos de plásticos y pesticidas en la agricultura. No se puede sugerir una solución única, ya que los sistemas agrícolas son muy diversos y enfrentan muchos desafíos. No obstante, se recomienda usar mejores prácticas para limitar la acumulación de residuos en el medio ambiente: utilizar acolchados plásticos más fuertes para una eliminación y reciclaje más eficientes o utilizar plásticos biodegradables probados en condiciones de campo. Además, es necesario tener en cuenta las condiciones climáticas al aplicar pesticidas y el tiempo de residencia de los residuos en el suelo. Concluimos que el uso de plásticos y pesticidas son insumos artificiales que afectan procesos naturales, muchos de los cuales son beneficiosos para la producción de cultivos. Necesitamos más investigación e iniciativas para adaptar la gestión agrícola a sus condiciones y a desafíos específicos.

Résumé

Dans les années 1960, la « Révolution Verte » apporte de nouvelles en agriculture. Ces technologies, comme par exemple les pesticides et le paillis plastique, offrent des réponses rapides pour augmenter les rendements des cultures et améliorer la sécurité alimentaire. Depuis lors, la communauté scientifique et le grand public se sont inquiétés des conséquences à long terme. En effet, les paillis plastiques et les pesticides laissent des débris qui s'accumulent dans le sol et sont potentiellement nocifs pour divers organismes. Avec ce manuscrit, nous décrivons dans sept chapitres, la menace posée par les résidus de paillis plastique et de pesticides dans les sols.

Chapitre 1: En introduction, nous présentons les divers bénéfices de l'utilisation des pesticides et du plastique en agriculture. Les nombreux services qu'ils fournissent expliquent pourquoi ils sont abondamment utilisés dans le monde entier. Cependant, le plastique et les pesticides ont le potentiel de s'accumuler dans le sol. Pour le plastique, nous distinguons les sources directes (ex. le paillis plastique, les engrais enrobés de plastique) et les sources indirectes (ex. les boues d'épuration, le compost). Nous expliquons les processus de dégradation des débris plastiques en microplastiques et finalement en eau et CO_2. Nous distinguons également microplastiques primaires (manufacturés comme microplastiques) et secondaires (issues de dégradation). Par la suite nous nous concentrons sur le paillis plastique car il est utilisé abondamment dans le monde et est reconnu comme une source importante de débris. Nous présentons trois types de films plastiques différents: le polyéthylène basse densité (LDPE), les plastiques contenant des additifs pro-oxydants (PAC) et les plastiques biodégradables (BIO). Enfin, nous présentons les menaces posées par les résidus de plastique et de pesticides et la nécessité de nouvelles recherches. Nous avons élaboré six questions principales qui sont abordées dans les cinq chapitres suivants.

Chapitre 2: L'objectif de cette étude de terrain est de mesurer les résidus de plastique et de pesticides dans les sols agricoles et leurs effets sur le microbiome du sol. Pour cela, nous avons échantillonné le sol (0-10 cm et 10-20 cm) de 18 parcelles dans six fermes maraîchères du sud-est de l'Espagne. Trois fermes étaient en agriculture conventionnelle et trois étaient en agriculture biologique. Toutes les fermes utilisaient le paillis plastique depuis plus de 25 ans. Nous avons mesuré la quantité de macro- et micro- débris plastiques de faible densité, la concentration de résidus de pesticides ainsi que plusieurs propriétés physicochimiques du sol. Nous avons également réalisé un séquençage d'ADN pour les communautés fongiques et bactériennes du sol. Des débris de plastique ont été trouvés dans tous les échantillons et 4 à 10 résidus de pesticides différents ont également été trouvés dans tous les sols en agriculture conventionnelle. Dans l'ensemble, la teneur en pesticides était environ 100 fois plus faible dans les fermes biologiques, alors qu'aucune

différence significative dans la teneur en plastique n'a été observée entre les fermes biologiques et conventionnelles. Les communautés fongiques et bactériennes étaient spécifiques de chaque ferme et liées aux paramètres physicochimiques du sol et la teneur en contaminants. Concernant les contaminants, les différences entre communautés bactériennes étaient corrélés à la surface totale de plastique ainsi qu'aux résidus totaux de pesticides et plus spécifiquement au fongicide Azoxystrobine et à l'insecticide Chlorantraniliprole. Le fongicide Boscalid était le seul contaminant à influencer la communauté fongique. Nous avons prouvé que des résidus de plastique et de pesticides étaient présents dans le sol et nous nous sommes demandé dans quelle mesure ils pouvaient y avoir sorption de pesticides sur des débris de plastique.

Chapitre 3: Le but de cette recherche était de mesurer la sorption des substances actives de pesticides sur les paillis plastiques LDPE, PAC et BIO et de comparer la décomposition des substances actives en présence et en l'absence de débris plastiques. À cette fin, 38 substances actives de 17 insecticides, 15 fongicides et six herbicides couramment appliqués dans le sud-est de l'Espagne ont été incubées avec un morceau de paillis plastique de 3 × 3 cm^2 (LDPE, PAC et BIO). L'incubation a été réalisée dans une solution de 10% d'acétonitrile et 90% d'eau distillée à 35°C pendant 15 jours dans l'obscurité. Le processus de sorption dépendait à la fois du type de pesticide et du type de paillis plastique. En moyenne, le pourcentage de sorption était d'environ 23 % sur LDPE et PAC et d'environ 50 % sur BIO. La décomposition des substances actives en présence de plastique était d'environ 30 % inférieure à la décomposition des substances actives en solution seule.

Chapitre 4: Nous avons testé l'impact de débris de taille macro (environ 5 mm) et micro (< 1 mm) provenant de LDPE et d'un type de film biodégradable sur les propriétés physicochimiques et hydrologiques du sol. La densité apparente, la porosité, la conductivité hydraulique à saturation, la capacité au champ et l'hydrophobicité du sol ont été modifiés en présence des quatre types de débris plastiques, tandis que le pH, la conductivité électrique et la stabilité des agrégats n'ont pas été sensiblement affectés. Le type, la taille et la teneur en débris plastiques ainsi que les interactions entre ces trois facteurs ont joué des rôles complexes dans les variations des paramètres mesurés.

Chapitre 5: Nous avons élargi notre étude du sol aux animaux marchant dessus: les moutons ingèrent-ils les débris de plastique lorsqu'ils paissent dans des champs contaminés? Pour y répondre, nous avons collecté les fèces de moutons de 5 troupeaux différents et analysé la teneur en microplastiques. Nous avons trouvé ~10^3 particules·kg^{-1} dans les fèces. Les données ont montré que le bétail ingérait du plastique, sous forme de microplastiques et/ou de macroplastiques. D'autres études devraient se concentrer sur : l'évaluation des différentes sources des plastiques trouvés dans les matières fécales, l'estimation de la

dégradation du plastique dans les intestins des animaux et la compréhension des effets potentiels de ces résidus plastiques sur la santé du bétail.

Chapitre 6: Dans une dernière expérience, nous avons testé si le paillis plastique et les résidus de pesticides pouvaient affecter la production végétale. Nous avons testé trois paillis plastiques et trois pesticides couramment utilisés par les agriculteurs. Des paillis de LDPE, PAC et BIO ont été étendus dans un champ pendant quatre mois d'été, réduis en micro- et macro-plastiques et ajoutés au sol avec des pesticides (Pendimetalin, Clorantraniliprol, Boscalid, Pyraclostrobine et mixte des quatre). Le plastique et les pesticides ont été incubés dans le mésocosme pendant un an dans des conditions de terrain avant que des laitues, *Lactuca sativa*, ne soient plantés. Après une période de croissance de 14 semaines, nous avons mesuré le diamètre basal, le nombre de feuilles, la surface foliaire, la biomasse fraîche, la biomasse sèche et la teneur en eau des tiges et feuilles. Nous avons observé une diminution de la surface foliaire, de la biomasse fraîche et de la biomasse sèche chez les plantes poussant dans le sol avec le plastique BIO par rapport au témoin. Ces résultats s'ajoutent aux études précédentes dans un appel à des tests plus détaillés avant l'approbation sur le marché de nouveaux paillis BIO pour l'agriculture.

Chapitre 7: Les principales conclusions de la thèse sont résumées dans le dernier chapitre. Nous comparons nos résultats avec la littérature existante et discutons des liens entre les chapitres. Nous explorons les limites de la détection des résidus de plastique et de pesticides. Plus précisément, la détection de petits débris plastiques, microplastiques et nanoplastiques, dans le sol reste un défi et de multiples méthodes sont en cours de développement. Nous suggérons que la diversité des méthodes est nécessaire pour décrire la diversité des types, tailles et formes de plastique et que les méthodes doivent être adaptées à l'objectif spécifique de l'étude. Nous discutons également des niveaux actuels de résidus de plastique et de pesticides dans le sol et des concentrations à utiliser pour évaluer leurs impacts potentiels. Enfin, nous explorons différentes options pour contrôler les impacts des résidus de plastique et de pesticides dans l'agriculture. Aucune approche unique ne peut être suggérée car les systèmes agricoles sont très divers et font face à de nombreux défis. Néanmoins, certaines bonnes pratiques sont recommandées pour limiter l'accumulation de résidus dans l'environnement : utiliser un paillis de plastique plus résistant pour une élimination et un recyclage plus efficaces ou utiliser du plastique biodégradable testé et validé dans des conditions de terrain ; être attentif aux conditions climatiques lors de l'application des pesticides et tenir compte de leur temps de dégradation spécifique dans le sol. Nous concluons que l'utilisation du plastique et des pesticides sont des intrants artificiels pouvant affecter les processus naturels, et à terme la production agricole, de manière préjudiciable. Plus de recherches et d'innovations sont nécessaires pour adapter la réponse technique aux défis agricoles.

Acknowledgements

Writing the acknowledgments is a good time to think back to the good moments and challenges of the past years. All happened thanks to very special people. I would like to thank them all personally but I am sure I will be missing some. So the minimum I can do is to thank you, the reader, at least for your interest in these lines.

First and foremost, I would like to thank my fantastic supervision team Violette, Raúl and Esperanza. Your great support from the first day until the last made this thesis possible. Violette, thank you for your open mind, open on science; you have been an insatiable source of inspiration, and on people; if you had not trust a young master student from an unknown French university I would not be here today. Raúl, thank you very much for being always so kind and supportive. Advice, technical support, energy, motivation, fun, you gave it all and more! Esperanza, thank you for always being available and welcoming to new challenges. Talking with you always made the pressure cool down and solutions come up.

The PhD journey actually started with the MOOC soil4life and three very kind coordinators Coen, Michel and Jerry. Thank you for disseminating soil science with passion and for having been so helpful. Coming to work in a foreign research group would not have been that smooth without Marnella. Thank you so much for always being thoughtful and solving any issue. I was welcomed by very inspiring PhD students at SLM, which motivated me to start the PhD. I have very special thanks for Yueling. I discovered so much with you, a lot of science of course, and also another way of thinking the world in an unpredicted funny way. Thank you Carlos for showing me around and for the very nice moments in the office and outside. Fabio, it was always a pleasure to work with you, no matter the struggles of the plastic analysis. I admire the optimism with which you solve any problem. Thank you very much Vera for your help and support in the PhD journey. From the beginning you have been and still are a lighthouse in SLM. I try to do my best to follow your example. With Lingtong, Ricardo, Jan and Joao you also initiated the board game sessions which are always great moments. Meng, you showed me that having a field study abroad and combining it with lab experiments was possible. I always feel very comfortable and safe with you around, thanks a lot for your kindness!

Many more colleagues provided me with support. Thank you Xiaomei for all the energy and motivation you bring in the SLM corridor. I want to give a big thanks to the microplastic team Meng, Hui, Ines, Foskea, Davi, Nuno, Suzy, Loes, Svenja! It is great to work with you and to support each other. I am very grateful to Meindert for the support, the good advice in all circumstances and the fun we have in the office. I am very grateful to Paula for all your help with the microbiome analysis and for the good atmosphere you bring to SLM. Of course

my stay in SLM would not have been the same without all the amazing SLM people, the table tennis games, the lunches and drinks. Thank you Demie, Karrar, Dennis, Pavan, Yan, Nikola, Hao, Pop, Rozita, Miao, Jantiene, Klaas, Rianne, Róger, Isabel, Kaveh, Belyse, Coleen, Corjan, Mara, Jinfeng, Darrell, Marta, and thank you all!

After SLM I got the chance to meet the team of GARSA in Cartagena. I was warmly welcomed and I found a quite unique, motivating and friendly atmosphere, my gratitude to all of you! Thank you Virginia for making every part of science, field work, lab measurements, writing and going to conferences, a fun and memorable experience. Thank you Juan for the precious help in the field and the fun game organization! Thank you Amalia, Jacinto, Perla, Onurcan, Eva, Angelica, Oumaima, Mariano, Maria, Melisa, Enrique, Jose, Silvia for your support, and all the time together, the field work, the drinks and the amazing Christmas dinners!

I also wish to express my special gratitude to my colleagues without whom this work would not have been possible. I cannot be too grateful to Benjamin who made possible the sampling campaign in a single month, with intense working days and no less intense and enjoyable discussions. I am glad we continue to have dinners with you and Anna! Thank you to Paul and Onurcan for your expertise, being patient with me and bringing my laboratory skills up to the task. Thank you Gerrit and Eva for your support with the analysis. A big thanks to Harm, Piet, Hennie and Rima for their technical support in all circumstances. My gratitude goes to Abdel, Pablo and Susana for their support in the lettuce experiment. I would like to thank as well Gilson and Genesys for their help in the lab and the nice discussions.

Besides the work for the PhD I had the opportunity to join other projects. My gratitude goes to the students and the co-supervisors I had the chance to work with. Thank you Julia for sharing your motivation and enthusiasm in research and thank you Nathalie for initiating the project and introducing me into the world of protists. Thank you Joost for hard work and dedication. Thank you Carlotta for your inspiring determination and open mind. The months in Cartagena were intense in work, sport and enriching discussions and I enjoyed every bit of it! My gratitude also goes to all the experts of the EIP focus groups about soil contamination and about agricultural plastics. I learned a lot thanks to you. I give a special thanks to Liisa, Alexander and Magdalena for giving me this great opportunity and the good nice time together. Many thanks also to Edisa, Raul, Violetta, Erqin and Jen. I am proud we managed to finish the opinion paper, four years after it started on the beach in Schiermonnikoog.

The work would not have been possible without the great external support I got. I want to thank all my amazing housemates for the great time we had living together. I got to live in different houses and it was always a unique and enriching experience. My deepest gratitude

to the friends with whom I lived during the 2020 lockdown, Tanvi, Michael, Niamh and Kavya. You transformed the stressful period into a joyful mix of science, games and food in a matrix of fun and kindness. I also want to thank all the people in Centauri, Acro, Discachos and WAF. Playing with you was a great way to get energy for the rest of the week. I am able to work abroad thanks to the support and motivation I receive from France. It starts of course with my friends in Berry. I would also like to thank my very first science comrades Jeremy, Nicolas, Erwan and the very special supervisors who gave us their trust and time, Mme Marcelet, Mr Beauvais, Mr Pellerin and Didier. You showed me that research was a lot of fun! Thank you Jeanne for accompanying me with joy and enthusiasm in soil science and the PhD journey. Thank you Sandie and Nicolas for sharing with fun and wisdom your vision of the world. My gratitude also goes to Mathilde for always supporting me. Thank you Hanfang, our regular epistolary support means a lot to me. Thank you Aaron for all the picnics and great time we shared. Thank you to Coralie, Marthe, Marin, Tom and Quentin for the very valuable time we spent and will spend together, with a cup of tea and a slice of bread or on the steep slopes of a mountain.

Finally, I would like to thank my family for the great support I can get in all circumstances and at all times. You always make me feel confident and safe. Thank you for taking such great care of me even when I get into challenging situations. You make everything possible. I love you.

About the author

Nicolas Bériot was born on the 21st of March 1994 in Bourges (France). He lived in Baugy until 2012 when he moved to Rouen to follow two years of intensive preparatory courses about mathematics, physics, chemistry and biology.

After failing the national competitive exams to enter veterinary schools, he joined the ENSEGID engineering schools of environment in Bordeaux. There he developed a keen interest for field work. In 2015 he went to Tübingen (Germany) for a Erasmus exchange with the Msc Applied & Environmental Geoscience. The next year he spent five months in Ecuador for an internship about preventing soil erosion with reforestation of the dry tropical forest. In April 2017 he joined the SLM group in Wageningen for a six months MSc thesis about the effects of plastic debris on soil properties.

After graduating from the ENSEGID in November 2017, he started a joined PhD program between WUR and UPCT in Cartagena (Spain). During the PhD he started studying soil physicochemical properties in field samples and headed towards soil microbiology analysis in the lab. He tested different microplastic assessment methods and participated in implementing them in the SLM lab. In 2019 he got the opportunity to be part of the experts panel for the EIP-focus group about agricultural soil contamination. The next year, this successful experience led him to become coordinating expert of the EIP-focus group about reducing the plastic footprint of agriculture. In 2020, he also collaborated writing a H2020 application for the MINAGRIS project to study the effects of plastics in Agriculture in Europe. The project got accepted and Nicolas joined the coordination team as of April 2021.

Besides science, Nicolas is interesting in meeting new people; travels, food and board games being perfect occasions. He enjoys a wide range of outdoor activities. In Spain he discovered Ultimate and now believes that flying discs are making the world a better place.

List of publications

Lwanga, E. H., **Beriot, N.**, F. Corradini, V. Silva, X. Yang, J. Baartman, M. Rezaei, L. van Schaik, M. Riksen and V. Geissen (2022). "Review of microplastic sources, transport pathways and correlations with other soil stressors: a journey from agricultural sites into the environment." Chemical and Biological Technologies in Agriculture **9**(1): 20.

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- Environmental research in context (2018)
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- Principles of Ecological And Evolutionary Genomics (2021)

Other PhD and Advanced MSc Courses

- Plant Nutrients in Terrestrial Ecosystems - acquisition and turnover, University of Copenhagen (2018)
- Frontiers in Microbial Ecology, RSEE, PE&RC & SENSE (2018)
- Design of Experiments, PE&RC (2018)
- Scientific writing, Wageningen Graduate Schools (2018)
- Soil Ecology, PE&RC, WIMEK and RSEE (2019)
- Root Ecology, PE&RC (2020)
- Supervising BSc & MSc thesis students, Wageningen Graduate Schools (2021)
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- Machine Learning for researchers, Polytechnical University of Cartagena (2021)

Management and Didactic Skills Training

- Co-organisation of workshop on micro-plastic detection in soil, Wageningen Soil Conference (2019)
- Supervising two MSc students with thesis (2019-2020)
- Supervising BSc student with thesis (2020)

Oral Presentations

- *Plastic mulch in agriculture: the case of low density polyethylene and its interactions with pesticides and soil microbiota*. Netherlands Annual Ecology Meeting. 11-12 February 2020, Lunteren, The Netherlands
- *Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain*. European Geosciences Union General assembly, 19-30/04, 2021, Online
- *Acolchado plástico en agricultura: acumulación de restos de plástico y de residuos de pesticidas en el suelo y efectos en la comunidad microbiana*. Simposio Nacional sobre Control de la Degradación y Recuperación de Suelos, 24-25 May 2021, Online
- Plastic mulch debris: transfer to grazing animals and interaction with pesticides. Eurosoil, 23-27 August 2021, Online

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

Colophon

The research described in this thesis was financially supported by the EU Horizon 2020 project Diverfarming [grant agreement 728003].

Cover in reference of the Discworld novels by Terry Pratchett, design by Nicolas Bériot, illustration by Camille Jacquet (*ccamillejacquet.wixsite.com*, @ccamille.j)

Printed by Digiforce (*proefschriftmaken.nl*) on FSC-certified paper