Science of the Total Environment 900 (2023) 165179

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

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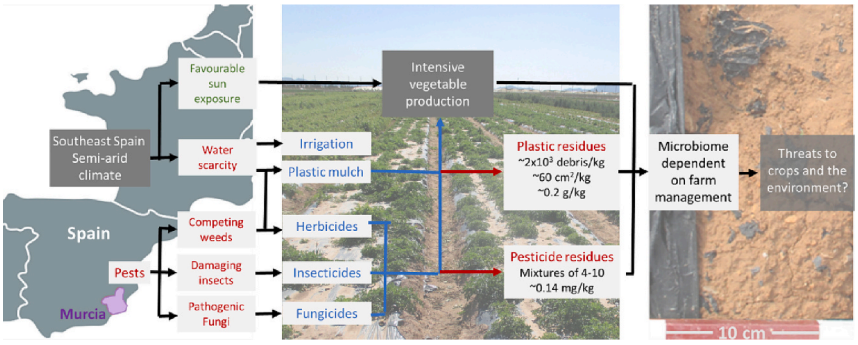
HIGHLIGHTS

- Assessment of plastic and pesticide residues and the soil microbiome from vegetable farms.
- All soil samples contained plastic residues, ~2 × 10^3 particles kg^{-1} ~60 cm^2 kg^{-1}.
- All soils under conventional farming contained >4 pesticide residues.
- Records of pesticide and plastic mulch use did not predict soil residue content.
- Plastic debris and pesticide residues contributed to soil microbiome variations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yolanda Picó

Keywords:
Agriculture contamination
Pesticides residues
Microplastic
Soil microbiome
Intensive vegetable production
Plastic mulch

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 contamination in agricultural soils. In conventional agriculture, the use of pesticides also leaves residues accumulating 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

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https://doi.org/10.1016/j.scitotenv.2023.165179
Received 7 December 2022; Received in revised form 25 June 2023; Accepted 26 June 2023
Available online 27 June 2023

soil fungal and bacterial communities. Plastic debris (>100 μm) was found in all samples with an average number of 2×10^3 particles kg^{-1} and area of 60 cm^2 kg^{-1}. We found 4–10 different pesticide residues in all conventional soils, for an average of 140 μg kg^{-1}. Overall, pesticide content was ~100 times lower in organic farms. The soil microbiomes 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 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.

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 contamination (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, 2021).

Southern Spain is one 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 (Pérez Hernández et al., 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. 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. The most commonly used plastic mulch is made of Low-Density Polyethylene (LDPE), which is resistant to weathering (Crawford and Quinn, 2017). 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 (Pérez Hernández et al., 2021). The most commonly used plastic mulch is made of Low-Density Polyethylene (LDPE), which is relatively resistant to weathering compared to other materials (Crawford and Quinn, 2017). 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 $\sim 1.7 \times 10^6$ tonnes of vegetables on $\sim 53 \times 10^3$ ha in Murcia in 2020 (Pérez Hernández et al., 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 n.d.). Both plastic mulches and pesticides leave residues in the soil.

In this study, we focused on the accumulation of plastic and pesticide residues in the soil. 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. 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 (Courtney et al., 2009). We considered that debris above 2 mm was easily identifiable visually and could be extracted by sieving. Pieces above 2 mm would also inhibit the proper identification of smaller μP under a microscope. The degradation of LDPE plastic debris is expected to ultimately produce 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 >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. For examples, a study of European farms found a maximum individual pesticide content in soil of 2.05 mg kg^{-1} (Silva et al., 2018); and a study in China estimated a maximum plastic residues content of 325 kg ha^{-1} in fields was estimated in a field where plastic mulch was applied over 30 years in China (Zhang et al., 2020). More specifically, a preliminary study in the same region reported an average of 2240 ± 980 μPs kg^{-1} for particles from 30 μm to 2 mm in agricultural soil (0–10 cm depth) (van Schothorst et al., 2021). 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 plant growth (Lozano and Rillig, 2020; Meng et al., 2021; Qi et al., 2018), 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., 2017) 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 plastic and AS content in the soils of a region representative of intensive agriculture. 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 assumed that the bacterial communities would differ on each farm and that the variation would be explained by the measured parameters.

2. Materials and methods

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 and an alkaline pH. The site has been under vegetable cultivation since the early 1990s. 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 S1). All farms used plastic mulch >5 times in the last 7 years. No farms in the same area, with similar vegetable production and without plastic mulch use could be found in the area for comparison. Three parcels of ~0.5–5 ha was 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. 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 parcel. 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). 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 S3) (MAPA, 2020). 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 S4) using the formula:

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

We present the calculations for Azoxystrobin, Oxyfluorfen and Pendimethalin as examples in Table 1.

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). 90 months is the longest record we could obtain for all the farms. All farmers declared having used only LDPE mulch for >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. To keep the interview with the farmer short, we did not ask for the exact thickness or colour of the plastic mulch used applied. Knowing the thickness of the plastic would give a better estimate of the potential input (Manzano et al., 2019).

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 <2 mm. The second aliquot was sieved at <2 mm 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

2.4. Soil physicochemical analyses

At each of the three sampling locations per parcel, the soil temperature and moisture were recorded (ECH-5TM/5 from Pessl Instruments) and the hydraulic conductivity (ks) was measured in triplicate using three mini disk infiltrometers (from METER 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 (Dekker et al., 2009).

Table 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

Table 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

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, 1986). The dry bulk density (ρb) was measured using the dry mass of the ring sample and the ring volume (Klute, 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 analyzer 2000LF (Malvern Instruments) with previous oxidation of organic matter and dispersion of clays. Soil NH_4^+ was extracted with 2 M 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 (ISO13536, 1995) using ICP-MS (Agilent 7500CE). In total, 18 soil physicochemical parameters were measured and included in the statistical analysis.

2.5. Plastic content determination

2.5.1. Macroplastic visual estimations

Macroplastic (MP) debris were visually identified from the remaining fraction of 20–50 g of soil samples after 2 mm sieving. Macroplastics were then put in a 50 mL tube with water, put in ultrasonic bath for 10 min, shaken for 30 min, and rinsed. The process was repeated until the water stayed clear. Then, Macroplastics were dried, weighed, counted and categorised according to their size: <25 mm^2, 25–400 mm^2 and >400 mm^2. An estimated area was calculated by multiplying the number of particles by the estimated size per category, 10 mm^2, 40 mm^2 and 470 mm^2, 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.

2.5.2. 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 2 mm sieved dried soil were 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.

2.5.3. 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.

2.5.4. 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 (Fig. S1) 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}.

2.5.5. 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 three 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.

2.6. 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 S4).

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 min. Then, 1 g of sodium acetate and 4 g of magnesium sulfate 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.

2.6.2. 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. 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.

2.6.3. 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.

2.6.4. 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 S4).

2.7. Microbial community assessment

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.

2.7.2. Amplification and sequencing of bacterial 16S rRNA gene

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

2.7.3. Amplification and sequencing of fungal ITS1 region

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., 2010). The PCR amplifications of ITS regions were performed at a final volume of 30 μL consisting of 0.7 μL of each primer (10 mM), 0.9 μL of 50 mM MgSO4, 0.6 μL of 10 mM 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 s), annealing at 50 °C (1 min) and extension at 72 °C (1 min) followed by a final extension of 10 min 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.

2.7.4. 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. Fungal amplicon sequences variant (ASVs) were processed with Quantitative Insights Into Microbial Ecology version 2 (QIIME2 version 19.10) software (Bolyen et al., 2019) following the protocol initially established in Comeau et al. (2017). Alpha biodiversity indexes Shannon and Simpson were calculated with the function estimate_richness().

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://g

ithub.com/NGBeriot/LDPE_Mulch_Cartagena.

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 S5. Among these, parameters leading to a significant difference between farms among the 18 soil physicochemical parameters were included in a principal component analysis (Fig. S2).

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 management type and plastic content effect for indicator taxa. A cut off value of 1.8 was applied on the LDA score (log10) to highlight the most responsive order. For the pesticides, the samples were classified between organic and conventional farms. For plastic, the samples were classified as low or high plastic content with the median plastic area found in the soil (38 $cm^2\ kg^{-1}$).

3. Results

3.1. Soil physicochemical properties

Soil physicochemical properties are used in this study to characterise the soil and explain the observed variations of soil bacterial and fungal communities (Fig. 7). All results are available in the GitHub repository and an average for the 0–10 cm soil depth for the organic (O) and conventional (C) farms of the main properties is presented in Table 3. In short, the soil pH is comprised between 7.8 and 9.1, the soil organic carbon between 0.7 % to 2.1 % and the soil nitrogen between 0.7 % to 1.9 %.

3.2. 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} (Fig. 1). Overall, the μPs represented 92 % of the total number of plastic debris found and 2.1 % of the total plastic debris area in soil. The size distribution was similar for all farms and soil depths except for the farm C2 which had more smaller particles (Fig. S3). There were no significant differences between soil depth with regards to the amount of plastic debris found (Fig. 2) or the area it covered (Fig. S4). Only two conventional farms, C2 and C3, showed significantly fewer plastic particles than the other farms (Fig. 2).

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) correlated with the measured amount of plastic debris found or the calculated area.

Table 3
Average values for the 0–10 cm soil depth for the organic (O) and conventional (C) farms of the main soil physicochemical properties explaining the variation of bacterial and fungal communities (Fig. 7).

Farm	O1	O2	O3	C1	C2	C3
Clay [%]	11	15	7	10	11	6
Sand [%]	46	39	62	57	49	74
Dry bulk density [g cm^{-3}]	1434	1403	1370	1324	1327	1425
Porosity [−]	0.45	0.43	0.45	0.50	0.48	0.47
Field capacity [−]	0.23	0.22	0.21	0.21	0.22	0.17
$WDPT_{lab}$ [s]	9.0	9.1	7.1	1.3	1.4	1.6
pH [−]	8.29	8.65	8.56	8.05	8.16	8.73
N_tot [%]	1.53	1.36	0.97	1.40	1.44	0.93
C_org [%]	1.17	1.33	0.82	1.31	1.28	1.02
NH4 [mg kg^{-1}]	1.74	2.96	0.05	10.1	9.65	7.18
NO3 [mg kg^{-1}]	35.2	14.2	104	12.6	124	172
Na [mg kg^{-1}]	459	581	182	257	392	323
CEC [cmol charge kg^{-1}]	19.2	21.1	11.0	17.6	17.7	11.6

3.3. 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} (Fig. 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 (Fig. 4). Only soil samples from Farm O1 were free of detected pesticide residues.

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 (Fig. 5, Table S6). 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 >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

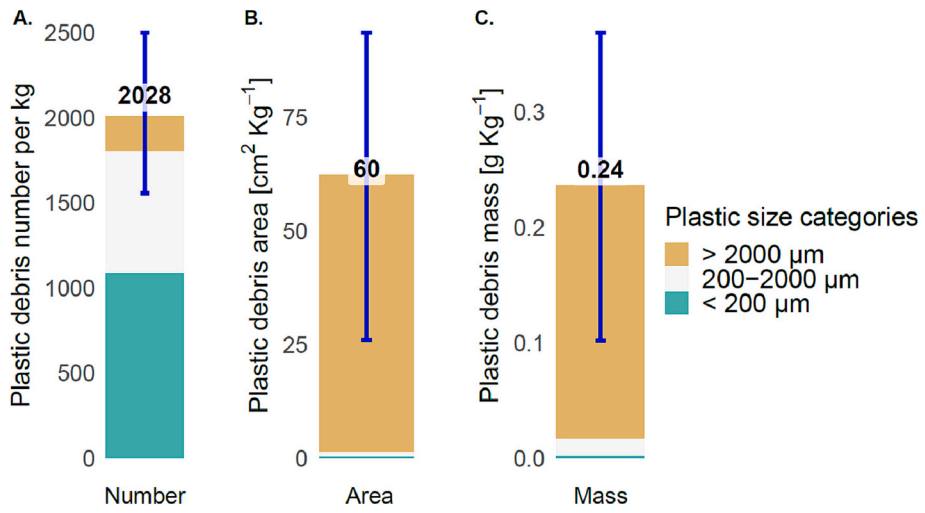

Fig. 1. Average amount of plastic debris, area and estimated mass in all the soil samples for three plastic size categories: <200 µm, 200–2000 µm and >2000 µm. The vertical blue line represents the standard deviation among all soil samples.

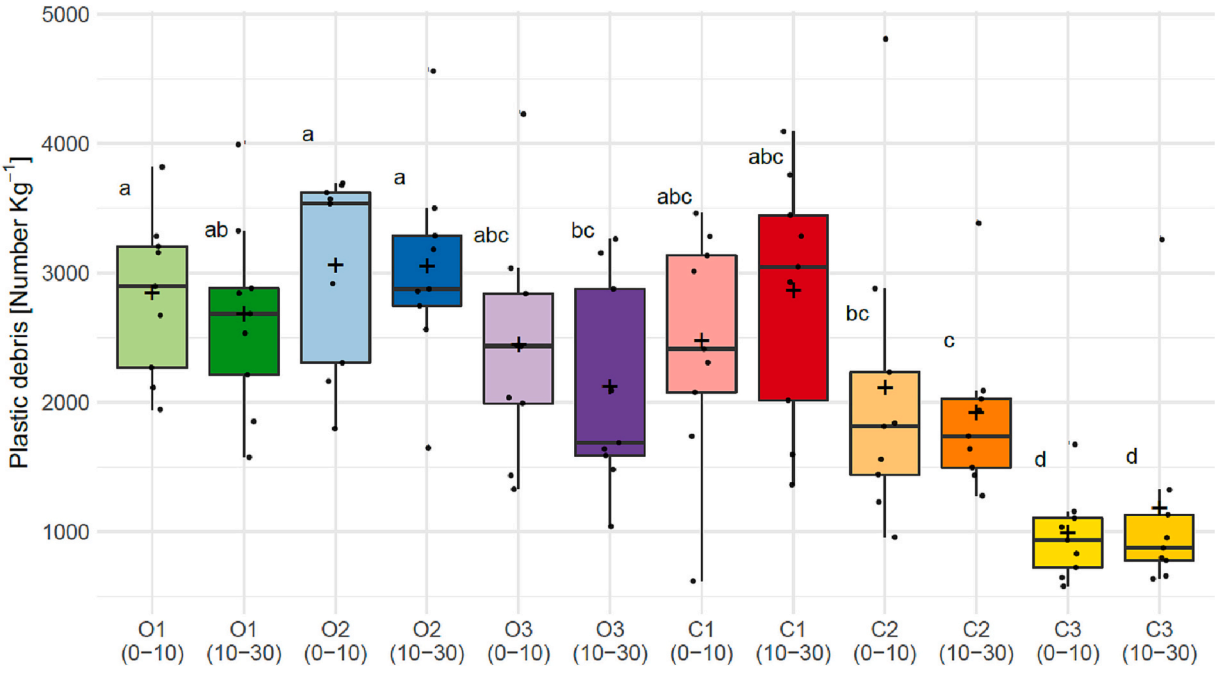

Fig. 2. Total number of plastic particles (>100 µm and <50 mm) per kg of soil in organic (O) and conventional (C) farms for both the top soil (0–10 cm) and the subsurface soil (10–30 cm). The box plots (horizontal lines) represent content for at least 25 %, 50 % and 75 % of the samples. The vertical 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 $p < 0.05$).

kg^{-1} and 0.2 mg kg^{-1} (Table S6), 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.

3.4. Microbiome analysis

A total of 14,168 bacterial sequences and 4340 fungi sequences were obtained after filtration, including 10,581 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 S7). PCoA for bacterial data showed that organic farms had the same scores in Axis 1, with differences related with Axis 2 (Fig. 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 (Fig. 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) (Fig. S5).

A first Permanova including the sample location within each farm as factors showed that the farm explained most of the variation between

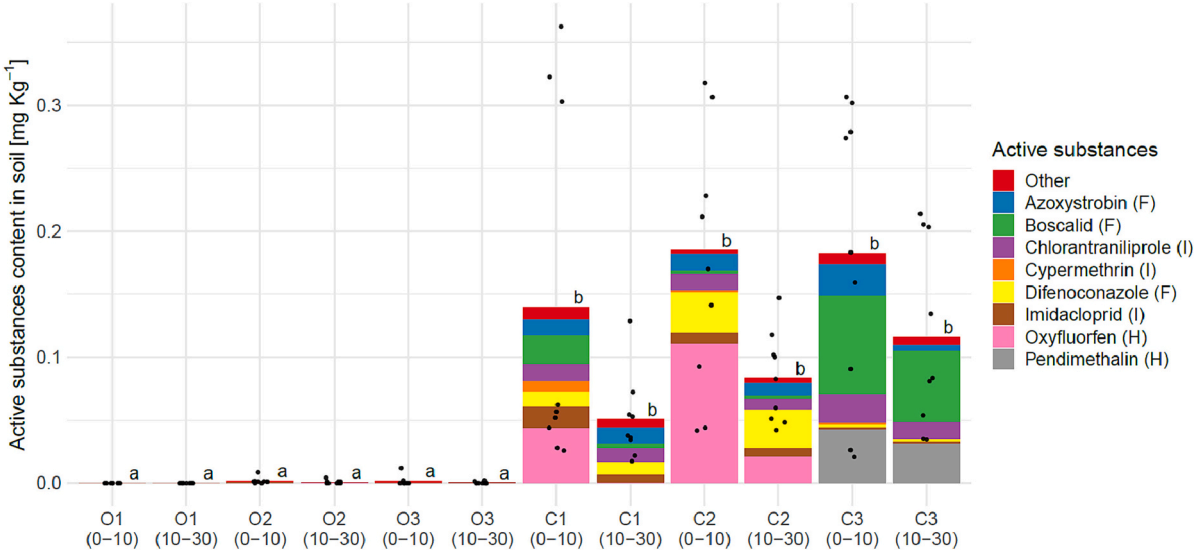

Fig. 3. Sum of active substances measured per kg of soil in organic (O) and conventional (C) farms for both the top soil (0–10 cm) and the deeper soil (10–30 cm). The eight most abundant substances are given a different colour and classified as fungicide (F), insecticide (I) or herbicide (H). Other substances 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 $p < 0.05$).

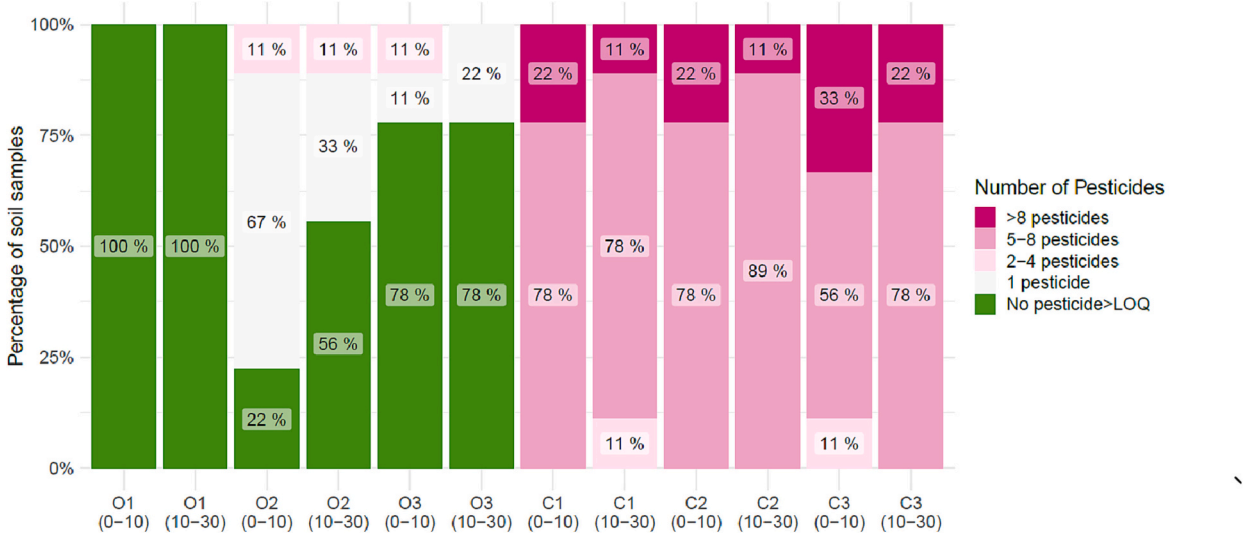

Fig. 4. Percentage of soil samples with no quantified pesticide residues, 1 pesticide residue and multiple pesticide residues in organic (O) and conventional (C) farms for both the top soil (0–10 cm) and the deeper soil (10–30 cm).

communities. A second permanova, without location factors, showed that seven parameters significantly explained the variation of both the bacterial and fungal communities: N.tot, NH4, NO3, FC, C.org, WDPT, CEC. The pH, porosity, dry bulk density, plastic area, Azoxystrobin, Chlorantraniliprole and total pesticide residue content in soil related to the bacterial communities only (Table S8). Sodium (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 (Fig. 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 (Fig. 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 (Fig. 7B). Thus, fungal communities were more strongly affected by soil physico-chemical properties than by pollutants such as pesticides and plastics, bacteria. in comparison with the bacteria.

The Linear discriminant analysis (LDA) effect size (LEFSe) showed that for pesticides (Fig. 8A and 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 (Fig. 8C and D), the bacterial order Clostridales was related to low plastic content, while Solirubrobacterales, an unknown order of S0134 terrestrial group and an unknown Acidobacteriota were related to high plastic content. For fungi, the order Agaricales and Pyxidiophorales

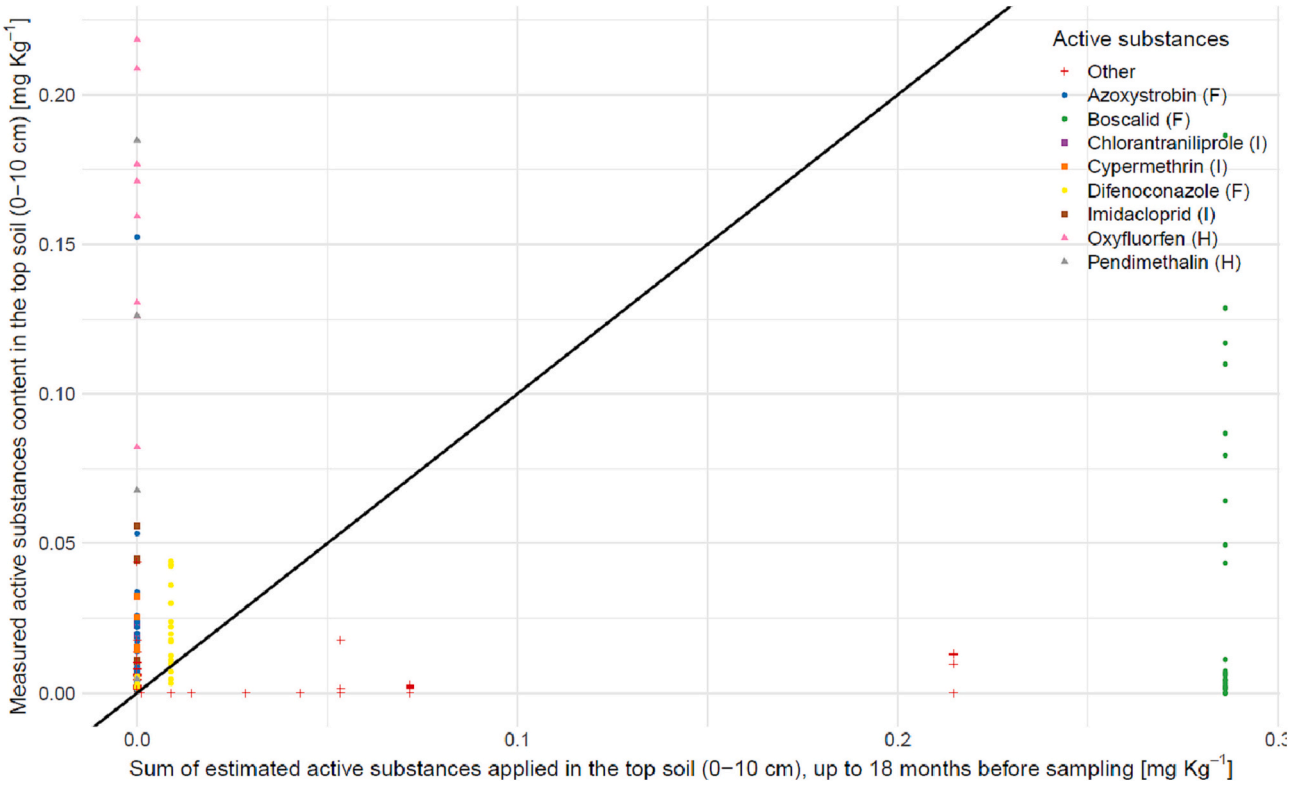

Fig. 5. Measured compared to estimated applied content of active substances in the soil. The eight most abundant substances are represented with a different colour and classified as fungicide (F, circle), insecticide (I, square) or herbicide (H, triangle). The black line represents the equality between 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 visualization and all values are presented in Table S6.

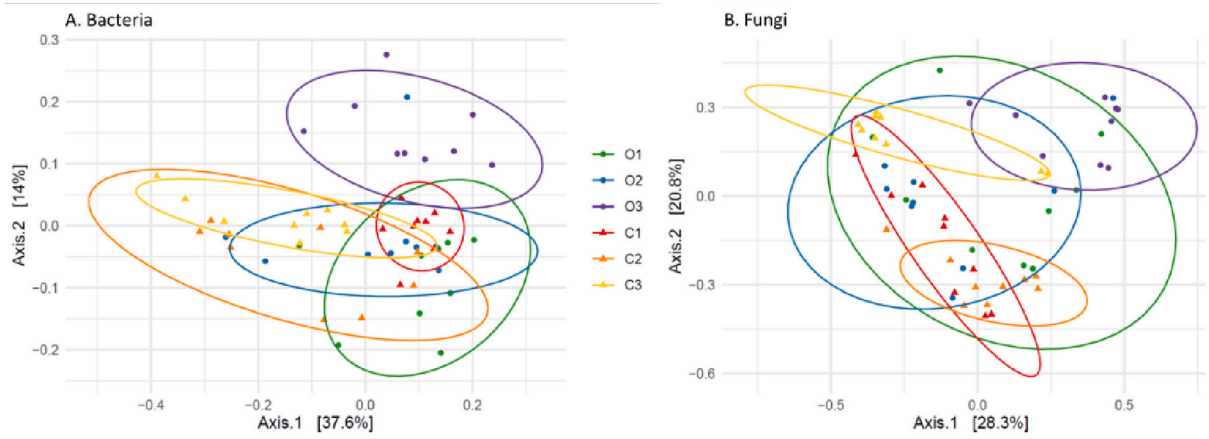

Fig. 6. Principal coordinate analysis (PCoA) ordination of the soil bacterial (A.) and fungal (B.) communities, respectively from the 16S rRNA gene and ITS sequencing. Communities are coloured by farms, from organic (O) and conventional (C) management.

were related to a high content of plastic, and no order got a LDA score above 1.8 for low plastic content.

4. Discussion

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 et al., 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 <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. Farmers put efforts to remove the plastic mulch after harvest and manually collect debris by

Fig. 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, water drop penetration time (WDPT), pH, cation exchange capacity (CEC), sodium (Na), plastic area (Area.Plastic), total pesticide active substances (AS.content), Azoxystrobin-F, Chlorantraniliprole-I and Boscalid-F.

hand. The plastic removal effort can vary per farm and per season. The residues left are typically ploughed into the soil during the preparation of the field for the next crop. The removal rate can depend on the technique used and on the plastic mulch thickness (Gómez-Águila et al., 2021; James 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

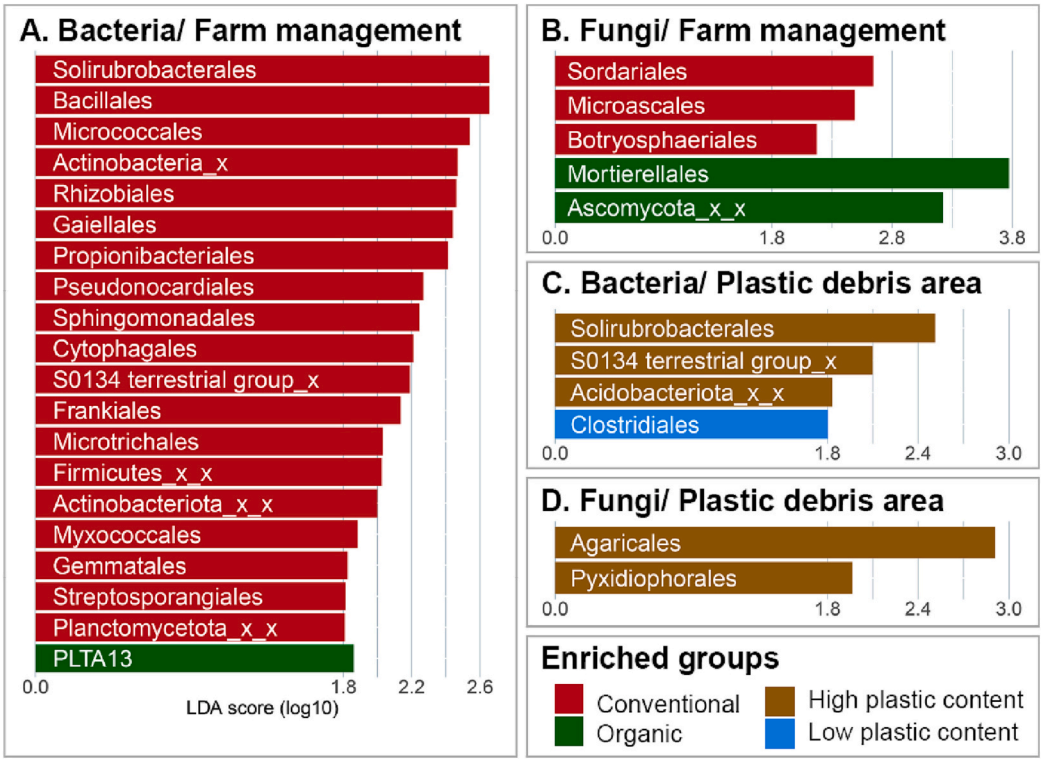

Fig. 8. LEfSe analysis (LDA score log10) of bacterial (A and C) and fungal (B and D) communities identifying order for which a major part of the population was active in soil with organic farming (green) or conventional (red) (A and B) and with a lower (<38 cm^2 kg^{-1}, blue) or higher (>38 cm^2 kg^{-1}, brown) plastic debris area (C and D). A cut off value of 1.8 was applied to the LDA score to highlight the strongest differences. If the order is unknown, it is marked with an '_x' and the class is given; if the class is unknown, it is marked with an '_x' and the phylum is given.

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.

4.2. Plastics debris analysis in soil, limitations and recommendations

4.2.1. MP: Testing more soil to recover more particles

In this study, the MP assessment was based on soil samples weighing <50 g. 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) 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. Assessing the MP area is important because we showed that MP greatly contributed to the total plastic area found 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.

4.2.2. μP: Testing more than the light density plastic and going beyond the detection size limit

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 (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.

4.2.3. 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., 2020); 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 and 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., 2020) and nm for plant roots (Chae and Youn-Joo, 2020). Therefore, providing the complete size distribution will allow the data to be reused to study other processes.

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 and 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 0-10 cm, 10-20 cm and 20-30 cm soil samples from cereal fields intensively ploughed from 0 to 10 cm 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 40 cm (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.

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., 2018 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.

4.5. Potential toxicity of pesticides and plastics

It is worth noting that >80 % of the soil from conventional agriculture contained >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 (Fred-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 Youn-Joo, 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 bioaccumulation along the food chain (Sun et al., 2022). All these interactions between pesticides and plastic should be investigated under field conditions.

4.6. Analysis of the soil microbiome

In this field assessment, we defined microbiomes with 16S rRNA gene 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 Fungal community, 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). Comparatively, Han et al., 2022 reported With regards to the increase of the relative abundances in soil of four bacterial genus (*Citrobacter*, *Castellaniella*, *Starkeya* and *Sphingomonas*) after the application of Boscalid at recommended dose (Han et al., 2022). More specifically, *Starkeya* and *Sphingomonas* showed an upward trend with the repetitive application of boscalid. This finding relates to our results as we identified Sphingomonadales as more abundant in soils under conventional management. With regards to the taxa most responsive to general 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, varying from 7.8 to 9.1 in the studied soil. 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. Rousk et al. (2010) explains it with a narrower optimal pH range for the growth of most bacteria compared to most fungi.

Plastic area residues in the soil 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., 2020a), 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, whereas Meng et al. (2023) showed a bacterial alpha diversity higher for 0.5 % (w/w) LDPE μP compared to no plastic but lower for 2.5 %. In the same study, the bacteria orders Hyphomicrobiales and Mycobacteriales were associated to 2.5 % plastic contamination. Qi et al. (2020b) only reported a higher relative abundance of the genus *Saccharibacteria* with 1 % (w/w) LDPE μP and MP. Blöcker et al. (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.

5. Conclusion

Our research has demonstrated that the use of plastic mulch in both conventional and organic vegetable production leads to plastic contamination in soils, with an average quantity of ~0.2 g kg^{-1}. The use of pesticides in the conventional farm led to mixtures AS residues in the soil (4–10 AS for an average of 140 μg kg^{-1}), with content 100 times lower in organic farms. Concerning the estimation of the measured contents of plastic and AS residues by the recorded applications of plastic mulch and pesticides in the farms, neither the number of mulch or pesticide applications in the past correlated with the measured amount of plastic debris and AS residues. Thus, no prediction could be done with the applications record in the farms. More understanding of the plastic degradation and better assessments are required to predict environmental plastic contamination from the plastic use. In addition, AS residues and soil plastic content significantly contributed to changes in soil bacterial community, highlighting the high sensitivity of bacteria to plastic and pesticide contamination. Comparatively, fungal communities were more affected by soil physicochemical properties than by pesticides or plastics, contrary to bacteria, responding only to the presence of the fungicide boscalid. Thus, more studies are required 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.

CRediT authorship contribution statement

Nicolas Beriot: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition. **Raúl Zornoza:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Esperanza Huerta Lwanga:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Paul Zomer:** Methodology, Resources, Validation, Writing – review & editing. **Benjamin van Schothorst:** Investigation, Writing – review & editing. **Onurcan Ozbolat:** Methodology, Validation, Writing – review & editing. **Eva Lloret:** Resources, Validation, Data curation, Writing – review & editing. **Raúl Ortega:** Resources, Writing – review & editing. **Isabel Miralles:** Resources, Writing – review & editing. **Paula Harkes:** Resources, Validation, Data curation, Writing – review & editing. **Joris van Steenbrugge:** Resources, Validation, Data curation, Writing – review & editing. **Violette Geissen:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All scripts and raw data tables are available on Github https://github.com/NGBeriot/LDPE_Mulch_Cartagena.

Acknowledgments

We are thankful for the contribution of the farmers from the region of

Murcia, Spain. This work was supported by the European Commission Horizon 2020 project MINAGRIS [grant agreement 101000407]. 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165179.

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