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Plastics in Agricultural Production: Impacts, Lifecycles and LONg-term Sustainability

Summary description of PAPILLONS field-scale experiments

For stakeholders' knowledge and comments

HORIZON 2020 of European Union

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Summary

This document is intended to openly disclose relevant information with the stakeholders of the PAPILLONS project with respect to the large-scale pan-European experiment that will be carried out between 2022 and 2023. The aim of this experiment is to study the potential effects of mulching film residues on soil ecosystems and their agricultural production. By presenting the experimental design in advance, PAPILLONS offers stakeholders the possibility of commenting and providing feedback and ideas prior to execution. These will be considered if they contribute to add quality, meaningfulness and objectivity to the experiment, and if their implementation is technically and economically feasible.

The main objective of the experiment is to address the following question:

Do legacy micro- and nanoplastic fragments accumulated in soil from deteriorated or partially degraded mulching films have an effect on farmed soil ecosystems and on soil fertility?

The experiment will be simultaneously and consistently conducted in three European countries: Spain, Germany and Finland. The same two microplastic materials will be separately tested at all sites (polyethylene (PE) mulching film fragments and biodegradable (Mater-bi- based) mulching film fragments), using soil cultivated with the same crop, and applying the local farming practices. Fragments will be obtained through cryomicronization of new films or from pellets made of recycled commercial films. The selection of the materials is performed by qualified experts with agronomy, polymer science and engineering background on the base of the material likelihood to represent a source of plastic fragments to soil. The ecotoxicology team involved with the assessment of effects and in running the experiment was not directly involved in this choice.

The experiment will be conducted in five fully randomized blocks that include one control and two concentration levels per each microplastic type. These concentration levels represent a worst-case scenario (75 g of plastic fragments per square meter of soil, equivalent to 0.05% of plastic in the soil ploughing layer) and an intermediate exposure scenario with (15 g per square meter, or 0.01%) for each type of material. The worst-case scenario is defined based on earlier observations of soils contaminated with polyolefin mulching film fragments. It also roughly represents a situation in which an entire film is mixed in the form of microplastic in a unit surface of soil, which is a relevant scenario also for the biodegradable film. **We offer the stakeholders the opportunity to give feedback on the exposure scenario and the treatment levels.**

The experiment will take place over two years, but the plastics will be added to the soil only at the beginning of the first growing season. Several endpoints will be monitored during the growth and post-harvest periods to study possible mid- to long-term impacts of plastic fragments on soil ecosystem health and soil fertility. The endpoints measured will relate to a broad range of aspects such as agricultural performance, crop yields, soil properties and functioning and the health and functioning of soil invertebrates and microbiota.

Criteria for quality and objectivity are described.

Contents

Background, context and scientific question

The PAPILLONS team is committed to deliver knowledge and data on the behaviour of agricultural plastic fragments in the form of micro- and possibly nano-plastic in agricultural soils and on the potential long-term effects this pollution may have on the farm soil ecosystem. For this, PAPILLONS will conduct (among other types of experimental activity) a large field-scale experiment taking place simultaneously in 3 regions of Europe.

Our main scientific question is:

1. Do legacy micro- and nanoplastic fragments accumulated in soil from deteriorated or partially degraded mulching films have an effect on farmed soil ecosystems and on soil fertility?

The experiment will also address:

2. What is the behaviour and further fragmentation/degradation of these fragments in soils.

With the term "legacy" we refer to the presence of micro- and nanoplastics in soils from past use of mulching films. Our experiment is therefore not intended to provide an assessment of the effects of the mulching films during their use or on the specific type of agriculture treated with mulching films. It rather focuses on addressing whether soils that contain mulching film fragments from past application will be healthy and performant when cultivated in future with a generic (reference) crop.

Rationale of the scientific objective

The rationale for the scientific question is to study the potential pressure that residual fragments from agricultural plastic may have on soil ecosystems, in the mid- to long-term. This addresses the call of the EU research programme and is considered by the PAPILLONS group as an important scientific question. It acknowledges the need for focusing research on the long-term sustainability and resilience of soils that represent recipients of micro- and nanoplastic residues from the use of agricultural plastics.

The experiment

The experiment will cover different climatic and agricultural contexts. This will be achieved by running the experiment simultaneously in three field case studies located in Spain, Germany and Finland. The fields are located in experimental farm areas on fields that have no history of treatment with materials that could constitute direct sources of plastic contamination (e.g. sewage sludge or agricultural plastics).

Experiment field in Spain	Experiment field in Finland
	

Figure 1. Experiments field sites in Spain and Finland

The experimental design is equivalent in all cases. Differences will be relative only to the geoclimatic context and agricultural practices concerning the timeline of soil ploughing, seeding and harvesting, and the type of watering and use of pesticides and mineral fertilizers according to local practices. In all cases we will use barley as a reference crop. The experiment will take place over two years.

There is of course an understanding that mulching films are not used in barley cultivation. This choice is, however, pertinent and justified by the research question posed and by the need of having the same reference crop in all regions to test ability of soils containing residues of mulching fragments to dispense agricultural services, and facilitating comparability between the experiences gained from the different locations.

Each field will be divided into 25 plots, as described in Figure 2, relative to different types of treatments and divided by untreated buffers. The experiment is designed in a randomized block form. (Figure 2).

In each case study we will measure atmospheric deposition of microplastics and assess the relevance of this flux to alter the exposure scenario during the experiment.

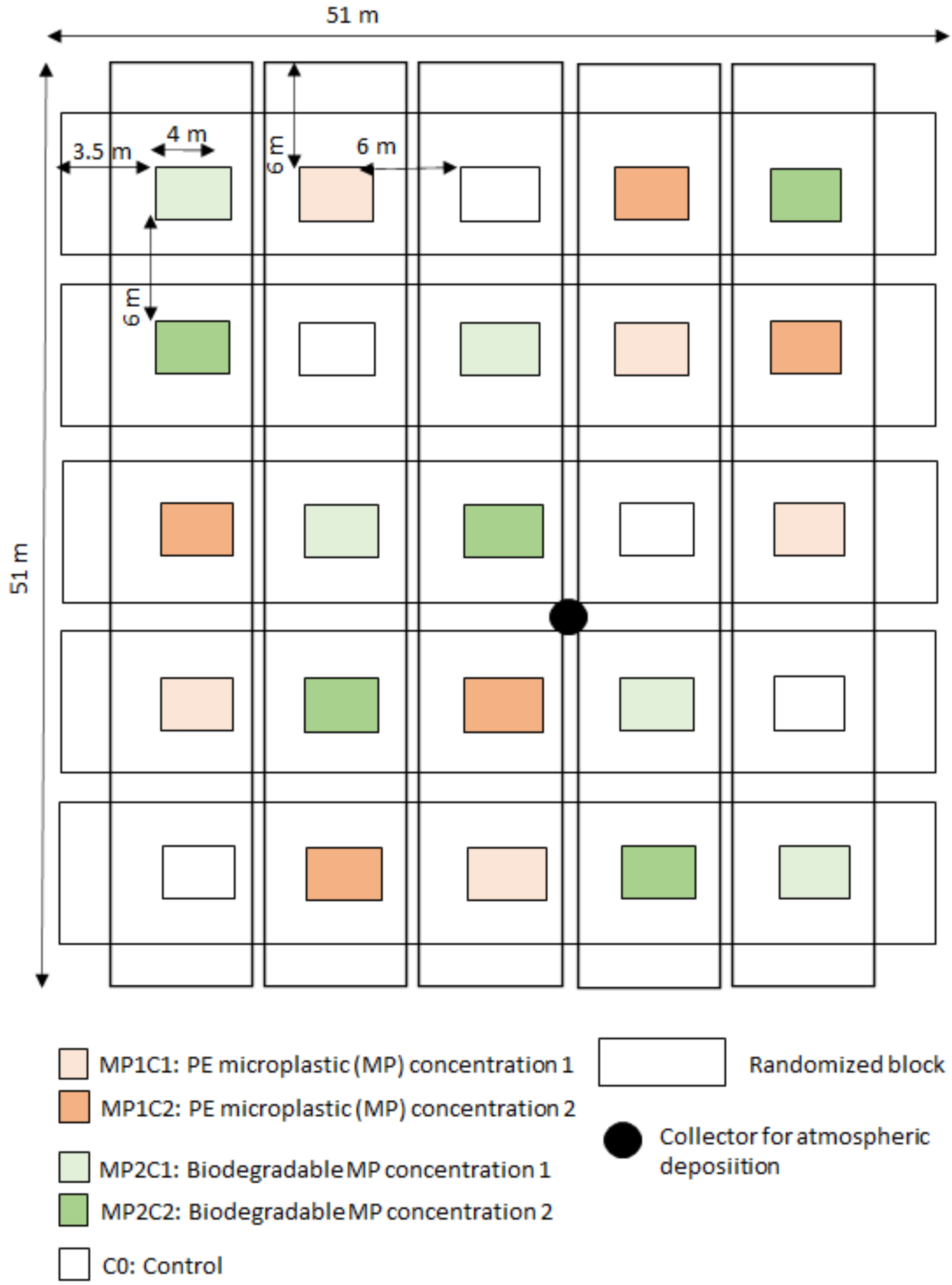

Figure 3. Randomized block scheme of the experiment (including buffer zones between plots.

Exposure scenario and tested materials

The treatments in the factorial experimental design will represent different levels of microscale fragments of two types of materials. **The fragments are obtained from artificially micronizing pellets from recycled black mulching films or directly from new black mulching films. In both cases we will use one type based on PE and one type based on Mater-bi.** The materials are obtained from commercially available films, whereby producers and retailers will retain anonymity, but that will be technically and chemically characterized by our team. The selection of the materials is performed by qualified experts with agronomy, polymer science and engineering background on the base of the material likelihood to represent a source of plastic fragments to soil. The ecotoxicology team involved with the assessment of effects and in running the experiment was not directly involved in this choice.

Micronization is by cryomilling, achieving fragments in the range of 10-1000 micrometres in their major dimension, with a prevalence of fragments between 500-600 micrometres. In any case, these materials originate from commercial products used in European agriculture. The identity of the manufacturer will not be disclosed.

The exposure levels are defined based on a literature review of all available data on contamination of soils treated with plastic mulching films (Annex 1).

Type	**N. replicates**	**Provisional levels of added plastic** (in g of fragmented mulching per square meter of soil)	**Description**
Control	5	0	No artificial addition of plastics
MP1C1-PE	5	15	Lower concentration of PE-based mulching film fragments
MP1C2 -PE	5	75	Higher concentration of PE-based mulching film fragments
MP2C1-mater-bi	5	15	Lower concentration of Mater-bi based mulching film fragments
MP2C2-mater-bi	5	75	Higher concentration of Mater-bi based mulching film fragments

The provisionally selected levels represent a “worst case scenario” and an intermediate scenario (the source of information used to define these scenarios is reported in Annex 1 at the end of this document).

We recall that the scope is to study the effect of soil contamination accumulating over time, e.g., after continuous addition of plastic to soil. The worst-case scenario corresponds to about 0.05% of plastic content in soil (by weight). These levels have been reported from monitoring of soils outside of Europe which document PE film fragments resulting from the mismanagement of mulching films.

Since the aim here is to represent a worst-case scenario – and considering that mismanagement can locally occur in Europe too – we argue that this is a reasonable higher concentration to use.

The worst-case value of 75 g of plastic per square meter of soil also roughly coincides with the addition of an entire film to soil in a microparticle form (e.g. if a hypothetical film has a thickness of 75 micrometers). It is therefore relevant also for the case of biodegradable mulching film, whereby the full mass of the film is typically left to degrade in the soil.

The low exposure scenario was selected to represent a factor of 5 lower level of fragments compared to the worst-case scenario.

We offer PAPILLONS stakeholders the possibility of commenting on or providing feedback to the selected exposure scenarios.

Experiment timeline and monitored effects

Microplastics will be added quantitatively only once (at the beginning of the experiment) to the soil surface just before mixing with a rotavator to a depth of 10 cm. It is assumed that their distribution will be pseudo-homogeneous within the plough layer in the treated plots. The same type of machinery and tools will be used in all the countries whenever possible.

Figure 3. Rotavator for mixing the ploughing layer

Figure 4. Seeder used to sow barley seeds after the mixing of the MP with the soil.

Figure 5. Combine harvester used to collect the barley crop at the end of the harvesting period in Spain.

Treated and control plots will be managed consistently based on the local farming practices for barley. At the end of the first harvesting season, soil, soil organisms and crops will be sampled from half of each plot and analysed for the following parameters only through state-of-the-art validated protocols previously utilized in ecological and ecotoxicological research, internationally (See table below for a summary). The remaining half of the plots will be sampled and analysed after 12 months from the first sampling, in order to explore potential mid/long term effects on the soil ecosystem.

Type of endpoint	**End point**	**Explanation**
Ecosystem functioning	Litter degradation	Litter degradation and hence C dynamics (C-losses and C storage).
Impacts on plants during growth season	Measures of plant characteristics at flag leaf stage	Biochemical and toxicological analyses, analysis of necrotic tissue presence and abundance, characteristics of the leaves.
Agricultural performance at harvesting)	Yield	Grain yield, harvest index and grain quality factors
The fate of microplastics and plastic additives	Changes of MP composition, morphology and distribution in soil	Distribution of microplastics and plastic additives in soils (at different depths), earthworms and earthworm faeces
Soil ecosystem in post harvesting	Effects on soil properties	Bulk density, soil moisture content, soil aggregation
	Effects on microbial community and microbial activity	DNA amplicon sequencing, microbial activity
	Effects on soil invertebrates	Earthworm and microarthropod abundance and community structure. Accumulation of microplastics in earthworm body and faeces.

Quality assurance and blindness in dataflow.

The experiment was scaled based on a statistical power pre-analysis. Experimental design is based on fully randomized blocks where each treatment type appears at least once in each block in a random location (See Figure 2). This is useful to minimize the effects of systematic errors. The effects due to variations between the different blocks should be eliminated.

In order to maximize objectivity throughout the experimental workflow, the identity of each treatment will be kept unknown to the field operator, lab analysts and data analysts until the end of the experimental work. This will be done by attributing an anonymized sample ID to each treatment immediately after the experiment has commenced and using this for each generated sample. The original identity of each plot will not be shared with operators of the sampling, laboratory analysis, data analysts and activity managers. The key for linking a random sample code to the original identity of the plots will be disclosed to the research teams only after completing the statistical analysis based on the randomized groups of treatments.

Annex 1

Sources of information used for the definition of the exposure scenario.

Note: Peer reviewed literature on the occurrence of plastic fragments in soils from the use of mulching films was considered. Note, to the date of the selection specific observation and usable data from European soils, specifically treated with mulching films were not available to the independent scientific community.

Country	Reported soil treatment information	Depth of the sampling	Reported n. of fragments per kg of soil	Reported estimated mass	Reported estimated %of plastic in soil by mass.	Reference
India	Film mulching practices for 2 years	0-10	43 MPs/kg	0.092-1.94 mg/kg	0.0000092-0.000194	(Kumar M and Sheela A, 2021)
		11-20	26 MPs/kg	0.07-1.79 mg/kg	0.000007-0.000179	
		21-30	12 MPs/kg	0.015-2.10 mg/kg	0.0000015-0.00021	
	Film mulching practices for 4 years	0-10	60 MPs/kg	0.082-3.75 mg/kg	0.0000082-0.000375	
		11-20	38 MPs/kg	0.059-2.56 mg/kg	0.0000059-0.000256	
		21-30	21 MPs/kg	0.015-2.10 mg/kg	0.000015-0.000210	
	Film mulching practices for 6 years	0-10	58 MPs/kg	0.92-3.26 mg/kg	0.000092-0.000326	
		11-20	28 MPs/kg	0.07-2.80 mg/kg	0.000007-0.00028	
		21-30	19 MPs/kg	0.015-2.10 mg/kg	0.0000015-0.00021	
	Film mulching practices for 10 years	0-10	84 MPs/kg	0.092-4.96 mg/kg	0.0000092-0.000496	
		11-20	67 MPs/kg	0.075-3.45 mg/kg	0.0000075-0.000345	
		21-30	50 MPs/kg	0.01-2.81 mg/kg	0.000001-0.000281	

	Control sample	0-10	28 MPs/ kg			
		11-20	8 MPs/kg			
		21-30	0 MPs/kg			
China	Greenhouse land, mulching film land, arable land	0-3	53.2 ± 29.7 MPs/kg			(Feng et al., 2021)
		3-6	43.9 ± 22.3 MPs/kg			
China	Mulching practice	0-20(30)	0-800 MPs/kg, mean 107 MPs/kg	0-8.5 mg/kg, mean 0.27 mg/kg	0.00085	(Zhang et al., 2020)
China	5 years of continuous mulching	0-5	61.9 ± 20.6 MPs/kg			(Huang et al., 2020)
		5-20	102.9 ± 69.4 MPs/kg			
		20-40	68.0 ± 41.4 MPs/kg			
	15 coyears of continuous mulching	0-40	308 ± 138.1 MPs/kg			
	24 coyears of continuous mulching	0-40	1075.6 ± 346.8 MPs/kg			
China		0–3	78.00±12.91 MPs/kg			(Liu et al., 2018)
		3–6	62.50±12.97 MPs/kg			
China		0-6	136.6±41.7 MPs/kg			(Liu et al., 2019)
		0-6	155±95.2 MPs/kg			
		0-6	190±31.2 MPs/kg			
		0-6	256.7±62.2 MPs/kg			
China	mulched		571 MPs/kg			(Zhou et al., 2020)
	nonmulched		263 MPs/kg			
China			320–6.9 × 10^5 MPs/kg			(Chen et al., 2020)
China			1430 to 3410 MPs/kg			(Ding et al., 2020)

China	LDPE mulching	0-10	40 ± 126 MPs/kg	0.008 ± 0.025 g/kg	0.0008-0.0025	(Zhang et al., 2018)
		10-30	100 ± 141 MPs/kg	0.368 ± 0.740 g/kg	0.0368	
		0-10	320 ± 329 MPs/kg	0.540 ± 0.603 g/kg	0.054	
		10-30	120 ± 169 MPs/kg	0.460 ± 0.735 g/kg	0.046	
		0-10	100 ± 254 MPs/kg	0.130 ± 0.307 g/kg	0.013	
		10-30	80 ± 193 MPs/kg	0.024 ± 0.051 g/kg	0.0024	

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