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Soil refinement accelerates in-field degradation rates of soil-biodegradable mulch films

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Contributions

MF, LT, MB and PD, data curation, formal analysis, and investigations. PD, MT and MG, resources; MF, LT, MB and PD, writing – original draft; LL and MT, writing – review and editing; MF and MB, methodology and visualization; PD and MT supervision.

Highlights

- Degradation rates of three biodegradable mulch films were evaluated in the open-field.
- Soil refinement accelerates the degradation of film weight (14%) and surface (17%).
- Highest degradation rates were observed for one Mater-bi-based film.
- Fastest degradation rates were observed in spring for all the tested films.
- Weight and surface area loss indicators showed positive relationship.

Abstract

Soil-biodegradable mulch films are a promising solution to replace conventional polyethylene-based mulch films, the use of which has led to negative environmental impacts. Soil-biodegradable mulch films are specifically designed to be incorporated into the soil at the end of the cropping cycle, and are expected to be biodegraded by soil microorganisms. The biodegradability of such products must be tested under laboratory-controlled conditions following international standards, although these can fail to represent real environmental conditions where mulch films are used. The objective of this study was to evaluate the effects of soil refinement on the degradation rates of three different commercial soil-biodegradable mulch films after their incorporation into the soil. The hypotheses were that: (i) soil refinement (i.e., ploughing followed by grubbing) creates more favourable conditions for film biodegradation compared to ploughing alone; and (ii) different mulch films show different degradation rates. An open-field completely randomised design was applied to test the effects of soil refinement by ploughing to 0.35 m depth without and with subsequent grubbing to 0.15 m depth twice. Three commercially available soil-biodegradable mulch films were sampled in 2020 (i.e., two Mater-bi-based, one Ecovio-based) at the end of a zucchini growing season (~3 months) when films were still lying above ground, and were later buried at 0.2 m depth inside mesh bags. Biodegradation rates of the sampled films were assessed with the indirect indicators of film weight loss and surface area loss at ~2-month intervals over 314 days. The results showed that soil refinement significantly accelerated degradation of the three tested mulch films by 14% and 17% according to the loss of weight and surface area indicators, respectively. One Mater-bi-based film showed higher degradation rates compared to the other two films. Future studies are needed to quantify the time needed for these different mulch films to be completely biodegraded. Such studies should be carried out following standards for laboratory incubation and/or in-field quantification of residual polymers in the soil over time.

Introduction

The correct use of mulch films enhances crop yields and quality through numerous positive effects, including moderation of soil temperature, and reduction of soil water evaporation and weed cover (Briassoulis and Giannoulis, 2018; Gao *et al.*, 2019). Most of the mulching films currently used worldwide are based on polyethylene and its by-products, which are relatively easy to manufacture, inexpensive, and show excellent chemical resistance, durability and flexibility (Kasirajan and Ngouajio, 2012; Briassoulis and Giannoulis, 2018). However, polyethylene-based mulch films cannot naturally decompose in the soil, which implies that they must be removed at the end of each cropping cycle. This leaves incineration or landfill as undesired disposal options for the polyethylene-based mulch films used (Sander, 2019). Unfortunately, the removal and/or recycling of used mulch films is time and manpower demanding, and the risk of plastics fragments remaining after each crop cycle is high. This creates conditions for macroplastics and microplastics pollution of the environment (Scaringelli *et al.*, 2016; Marí *et al.*, 2019; Yang *et al.*, 2020).

Recent studies have shown how medium- to long-term use of polyethylene-based mulch films can cause major side effects in terms of altered nutrient availability and microorganism communities, modified soil structure and properties, and delayed root development, while also contributing to greenhouse gas emissions (Steinmetz *et al.*, 2016; Briassoulis and Giannoulis, 2018; Gao *et al.*, 2019). To solve these issues, research over the last decade has focused on the development of mulch films that are designed to be biodegraded in the soil environment, and that have comparable properties to polyethylene-based mulch films.

Commercially available mulch films that are certified as ‘biodegradable in the soil environment’ are specifically designed to be incorporated into the soil at the end of the cropping cycle. Soil-biodegradable mulch films can be used by microorganisms as a source of energy, whereby they ultimately transform the plastic carbon into CO_2, water and new microbial biomass (Sander 2019). This should result in overall manpower and CO_2 emissions savings, and in no microplastics waste released into the ecosystem (Scaringelli *et al.*, 2016; Malinconico, 2017), which is an increasing source of concern resulting in increasing scientific interest (Huang *et al.*, 2020; Jacques and Prosser, 2021). To date, different soil-biodegradable mulch films are available on the market, while new experimental biodegradable mulch films are being tested all over the world (Martin-Closas *et al.*, 2008; Miles *et al.*, 2012; Marí *et al.*, 2019). Nevertheless, the use of soil-biodegradable mulching films is still not widespread, and the market remains dominated by polyethylene-based products (Briassoulis and Giannoulis, 2018).

Most of the soil-biodegradable mulch films available on the market are composed of polyesters that are often blended with starch and other additives (Sander, 2019). Such combinations can vary widely from one commercial product to another, and consequently different biodegradation rates might be expected across these different mulch films under the same environmental conditions (Francioni *et al.*, 2021).

The biodegradation rates of mulching films do not depend only on their chemical composition, as they are also highly driven by the actions and interactions of abiotic and biotic factors. Abiotic factors include mechanical stress from wind, rain and pollutants, UV radiation, air temperature and relative humidity. Most of these factors act when the films are lying above ground, and this whole process is known as ‘weathering’ (Wypych and Faulkner, 1999). When mulch films are finally incorporated into the soil, other abiotic factors such as soil moisture, temperature and oxygenation affect the biodegradation process that is carried out by soil microorganisms (mainly fungi and bacteria, but also actinomycetes and algae).

Three distinct phases can be identified during the biodegradation of biodegradable plastic products, including mulch films: (i) biodeterioration; (ii) biofragmentation; and (iii) mineralisation. Biodeterioration occurs when weathering together with enzymes attack the plastic surface (i.e., the mulch film), which begins to weaken, erode and fragment. Biofragmentation occurs through the constant work of the soil communities that degrade complex polymers into ever smaller oligomers. Mineralisation occurs when the oligomers are broken down into basic monomers and they can be assimilated by the soil micro-organisms and finally excreted as CO_2, water and other metabolites (Kjeldsen *et al.*, 2019).

Mineralisation under controlled laboratory conditions is also used to demonstrate the ultimate biodegradability of plastics in a soil environment (e.g., EN 17033, 2018). In these tests, the materials are incubated in real soil with controlled moisture (water holding capacity, 50% ±10%) and temperature (25 ±3 °C). To be considered 'biodegradable', the tested material must show conversion of 90% of its carbon into CO_2 (i.e., its mineralisation level) in less than 2 years. However, albeit necessary to confirm the biodegradability of materials, these tests do not consider that the soils can differ greatly and that the same soil can be subjected to different management, both in the short-term (e.g., different tillage) and in the medium- to long-term (e.g., long-term fertilisation with manure). Moreover, even the different crops and the related agronomic practices required during their growth can have key roles during both mulch film weathering (i.e., shadowing) and after their incorporation into the soil (e.g., activity of microorganisms, root exudates). These are important aspects that need to be taken into consideration, especially because soil-biodegradable mulch films are designed to be used and then buried under open-field conditions and not in the laboratory (Yang *et al.*, 2020; Francioni *et al.*, 2021; Griffin-LaHue *et al.*, 2022).

As of today, most of the scientific literature dealing with soil-biodegradable mulch films (or with the polymers that compose them) are 'chemical oriented' and focus on the production processes and mechanical properties of the products, leaving the soil environment as a secondary aspect. Such studies have been carried out mainly under laboratory-controlled conditions, while studies under open-field conditions are fewer, although increasing (Francioni et al. 2021). To date, studies that have considered soil-biodegradable mulch films under open-field conditions have mainly considered their effects on crop yield (Moreno *et al.*, 2009; Filippi *et al.*, 2011; Miles *et al.*, 2012; Marí *et al.*, 2019) the film technical characteristics (Shogren *et al.*, 2003; Scarascia-Mugnozza *et al.*, 2006; Touchaleaume *et al.*, 2018) and the microorganism communities (Sakai *et al.*, 2002; Moore-Kucera *et al.*, 2014; Zhang *et al.*, 2019).

In addition to these in-field trials, there is the need for further studies that investigate the various factors that can affect the biodegradation rates of mulch films, such as different soil tillage, which changes the physical and chemical conditions. To date, studies that have investigated the effects of different agricultural management practices on soil-biodegradable mulch films are rare, if not completely lacking (Francioni *et al.*, 2021).

Horticultural crop systems in central Italy are mainly located in plain areas of river valleys, and are characterised by soil with significant amounts of clay (ASSAM, 2005). In such cropping systems, and based on the crop rotation adopted by the farms, soil refinement can be necessary before winter, to allow for the sowing of a winter cereal (e.g., durum wheat) or for advanced preparation of the soil for a spring crop (e.g., zucchini). In this second case, the soil refinement performed at the end of the summer ensures that the actions of rain and freeze-thaw cycles result in the optimal conditions of the soil for the sowing of spring horticultural crops. Alternatively, if a non-horticultural spring crop is planned in the crop rotation of the farms (e.g., corn), soil refinement is not necessary and the soil management is limited to ploughing carried out at the end of the summer.

In this context, we hypothesise that by changing the soil physical characteristics (i.e., by grubbing), the soil refinement carried out at the end of the summer creates more favourable conditions for the biodegradation of mulch films compared to ploughing alone. Moreover, it is also possible that as different soil-biodegradable mulch films have different chemical compositions, this might result in different biodegradation rates even under the same soil conditions. This study thus had two objectives: to evaluate the effects of soil refinement on the biodegradation rates of three different commercial soil-biodegradable mulch films after their incorporation into the soil, and to evaluate the biodegradation rates of three different commercial soil-biodegradable mulch films in a traditional horticulture system in central Italy.

Materials and methods

Experimental site and soil characterisation

The experiment was carried out in the field of a private farm '*Società Agricola Fratelli Ercoli*' located in Morrovalle (MC), Marche region, Italy (43°17'12.55"N, 13°36'40.03"E), at an elevation of 64 m a.s.l.. The soil was typical of agricultural land of the mid-lower Chienti River valley. According to USDA soil taxonomy, it was classified as Inceptisol, Typic Haploxerept, fine, mixed, thermic (ASSAM 2005, Smith, 2014), and it was characterised by a clay loam texture with 43.1% sand, 23.0% silt and 33.9% clay. Its main characteristics were as follows: pH 7.33; cation exchange capacity, 5.2 meq/100 g; organic matter, 22.9 g/kg; organic C, 13.28 g/kg; C/N ratio, 17.7; total N, 0.75 g/kg; available P, 52.3 mg/kg; exchangeable K, 137 mg/kg; available Na, 118 mg/kg; exchangeable Ca, 690 mg/kg; exchangeable Mg, 108 mg/kg; total $CaCO_3$, 37 g/kg; and active $CaCO_3$, 14 g/kg.

The farm where the experiment was carried out was mainly focused on vegetable production (e.g., zucchini, lettuce, fennel), which was alternated with renewal crops (mainly grain cereals, such as corn and wheat). Before the start of the trial, the study field was cultivated with curled endive and escarole (September 2019 to January 2020) followed by fennel (February to June 2020). From July to November 2020 zucchini (*Cucurbita pepo* L. 'opera' cultivar) was cultivated using the soil-biodegradable mulch films which would later be sampled for this study. The basal fertilization was carried out before the mulch film deployment by applying 0.8 t ha^{-1} of a NPK compound fertilizer (12-12-17). During the zucchini growing season the crop was drip fertigated twice a week using variable rates of NPK-based and plant-based amino acid products.

The farm had been distributing cattle manure (~30 t ha^{-1} y^{-1}) for at least 15 years, and had been using biodegradable mulching films for 5 years, to improve thermoregulation, prevent weed growth, and obtain a clean product that was destined for widespread distribution. The study site is in the bioclimate temperate oceanic-subcontinental variant or ‘Warm temperate, summer dry, cool summer’, according to the Koppen climate classification (Kottek *et al.*, 2006), and is characterised by a mean annual precipitation of ~780 mm and a mean annual temperature of ~14.5 °C (Figure 1).

Mulch films

Three different commercial soil-biodegradable mulch films were used by the farm before the start of the trial, during the zucchini (*Cucurbita pepo* L. ‘opera’ cultivar) growing season of 2020. The commercial names of the tested films were: ‘*Ecopac Bio Black*’ (ECO; Guarniflon Spa - PATI Division, Treviso, Italy); ‘*Film Biologico per Pacciamatura di* Mater-bi’ (PAR; Manifatture Roberto Pardini & Figli, Lucca, Italy); and ‘*Film Multibio Nero*’ (EIF; Eiffel Spa, Parma, Italy). According to the suppliers, ECO and PAR were based on Mater-bi (Novamont), while EIF was based on Ecovio (BASF). All of the mulch films used were black and certified as ‘biodegradable in the soil environment’ by the manufacturers.

Mulch film management, sampling and mesh bag preparation

All of the films tested (i.e., ECO, PAR, EIF) were laid down on 5 July, 2020, and zucchini was seeded by hand in the same day. The mulch films were sampled on 2 October, 2020, a month before the zucchini were removed at the end of their cycle. Each film underwent the same weathering before being sampled, and according to the weather data supplied, they received 2239 °C cumulated temperature and 113 mm cumulated precipitation (Figure 1, ‘mulch film weathering’). Samples of mulch film were collected using a round cutter of 9-cm diameter, taken immediately to the laboratory, and gently cleaned in pure water and air dried for about 1 week. Thickness of sampled films (i.e., weathered) was measured with a ratchet thimble micrometre (Mitutoyo series 102, Kawasaki, Japan) on ten random samples for each film and equalled ~30, ~20 and ~28 µm for ECO, PAR and EIF, respectively. Subsequently, the samples were inserted inside mesh bags (12×12 cm wide; 1-mm opening), which were considered sufficient for microorganisms to penetrate after their incorporation into the soil.

Study design and management practices

The overall design of this study involved investigation of the *in-situ* degradation of these soil-biodegradable mulch films after their incorporation into soil that was subjected to two different tillage regimes: ploughing to 0.35 m depth (ploughing); and the same ploughing followed by grubbing (twice) to 0.15 m (ploughing+grubbing). Ploughing was carried out on 7th November 2020 while grubbing on 16th November 2020. The experimental design adopted was a split plot design with tillage regime as main plot (i.e., ploughing; ploughing+grubbing) and mulch film type as subplots (i.e., ECO, PAR, EIF). Within each subplot, 15 mesh bags that contained the film samples were buried, which corresponded to the three mulch films tested (i.e., ECO, PAR, EIF) and to five destructive sampling dates (T1-T5). Thus, the total number of mesh bags buried was 150 (2 tillage × 5 replications × 3 mulch film types × 5 destructive sampling dates). All of the mesh bags were buried on 19 November, 2020, at 0.20 m, laid horizontally, and distanced 0.1 m from each other. The mesh bags were sampled at about 2 month intervals, from 18 January, 2021 (T1), to 29 September, 2021 (T5).

Estimation of film biodegradation by indirect indicators

The biodegradation rates of the three mulch films tested were assessed by their ‘weight loss’ and ‘surface area loss’, which are not equivalent to their actual biodegradation, but can be considered indirect indicators of biodegradation (Rudnik and Briassoulis, 2011; Sintim *et al.*, 2020; Griffin-LaHue *et al.*, 2022). Both weight and surface area loss are suitable for open-field trials when the biodegradability of the mulch films has already been shown in the laboratory, following international standards (Francioni *et al.*, 2021).

At each sampling time (i.e., T1-T5), the samples retrieved were gently cleaned in pure water, air dried for 1 week, and weighed using an electronic balance (1 µg precision; BCA 200; Orma). The weight loss is expressed as the proportion (%) of the remaining weight, and was calculated according to

Equation (1):

$$\% \, remaining \, weight = 1 - \frac{Initial \, weight - Final \, weight}{Initial \, weight} \times 100 \qquad (1)$$

After weighing, the samples were placed in laminating pouches of 148×210 mm and were later scanned with an image scanner (XP-322; Epson) using the following settings: resolution, 300 dpi; gamma correction, default 2.2. The images obtained were saved as .jpg RGB files with a 210×297 mm format. Subsequently, each image was processed using the ImageJ software (Schneider et al. 2012) following the method described by Cowan et al. (2013), with slight modifications to adjust the selection area to the round shape of the mulch film samples. The scanned images were first transformed into only black and white pixels (command: 'Make binary'), then the 'surface area loss' (expressed as proportion [%] of the remaining area) was automatically calculated by the programme via the 'Analyze particles' function (parameters: 'Size: 0-Infinity'; 'Circularity: 0.00-1.00').

Statistical analysis

Pearson correlation coefficients were determined to explore the relationships between the 'weight loss' and 'surface area loss' estimations. Both the weight loss and surface area loss estimations were analysed according to repeated measure ANOVA using the 'mixed model procedure' with SPSS version 25.0 (SPSS Inc., IBM, Chicago, IL, USA), to determine the effects of time (date of sampling, within factor), tillage (between factor), film type (between factor) and their interactions, over the whole study period. Two-way ANOVA was carried out for each of the sampling dates to detect differences between mulch film types, tillage, and their interactions. The data were Box-Cox transformed to meet the ANOVA assumptions.

Results

Degradation rates of the mulch films

Both of the ‘weight loss’ and ‘surface area loss’ indicators showed significant and positive correlations for each of the mulch films tested and for each tillage regime (Supplementary Figure S1). The rate of degradation was initially low for all of the mulch films and for both ploughing and ploughing+grubbing (Figure 2). Subsequently, their degradation showed higher rates in the spring (from March to May), especially for the ploughing+grubbing, which then slowed down significantly in the late summer (from July to September) (Figures 2, 3).

A significant interaction of tillage and sampling date emerged, and demonstrated overall higher degradation rates for ploughing+grubbing compared to ploughing (Table 1). This was also supported by the scanned mulch film samples, which appeared more fragmented for ploughing+grubbing compared to ploughing for each sampling date (Figure 4). However, the effects of ploughing+grubbing were not significantly different from ploughing for the first two sampling dates (T1, T2), with significance seen from T3 to T5 (i.e., from 187 to 314 days after sample burying) (Supplementary Table S1). No other significant interactions were detected for either weight loss or surface area loss.

Among the mulch films, PAR showed the highest degradation rates throughout the monitoring period (Table 1), although this did not reach significance for all of the sampling dates (Supplementary Table S1). Indeed, there were no differences between the mulch films tested between T3 and T4 (i.e., from 187 to 249 days after sample burying). Later, PAR again showed the highest degradation rate, at T5 (i.e., 314 days after sample burying). No significant interactions emerged between the different mulch films and the tillage for the weight loss and surface area loss indicators (Supplementary Table S1).

Discussion

Mulch film degradation as affected by environmental factors

Despite a large number of studies that have focused on biodegradation rates of soil-biodegradable mulch films under laboratory-controlled conditions (which remain necessary to demonstrate the biodegradability of these tested materials), a growing number of studies are now beginning to focus on open-field estimations, to also include the effects of environmental factors (Francioni *et al.*, 2021). Under open-field conditions, both abiotic and biotic degradation phases are key to the fate of biodegradable mulch films (Sintim *et al.*, 2020; Griffin-LaHue *et al.*, 2022). When a crop is still in place, the mulch films are expected to moderate soil temperature, water content and weed pressure, and the actions of soil biodegraders are expected to be low because the mulch films are predominantly outside the soil. However, weathering (i.e., the actions and interactions of environmental factors, such as UV radiations, rainfall, wind) can significantly change the physicochemical properties of the mulch films and greatly influence their biodegradation following their soil burying (Scarascia-Mugnozza *et al.*, 2006; Miles *et al.*, 2012).

Previous studies that have examined the weathering effects on mulch films have shown how these decrease the molecular and carbon contents and increase the high-melting compounds (Hayes *et al.*, 2017), with decreased tensile strength (Hablot *et al.*, 2014), modified elasticity and damage caused to the mulch films (Martín-Closas *et al.*, 2016). In general, weathering modifies the mechanical properties of the mulch films, which makes them easier to degrade (Wypych and Faulkner, 1999; Hablot *et al.*, 2014; Sintim *et al.*, 2020). The loss of the mulch film mechanical properties are commonly used to test the weathering effects by measuring 'elongation at brake' and 'tensile strength' of mulch films (Martin-Closas *et al.*, 2008; Hayes *et al.*, 2017).

Recent studies have highlighted how the loss of mulch film mechanical properties is mainly determined by the composition, suggesting that warmer climates can facilitate this process (Anunciado *et al.*, 2021). Moreover, in addition to weathering, the ongoing crop can also affect mulch film degradation directly or indirectly. For example, large-canopy crops (e.g., zucchini) can shade the mulch film, thus screening or filtering the UV radiation (Hayes *et al.*, 2017). Furthermore, it has been suggested that the ongoing crop can affect the degradation of mulch films because it can press the mulch against the soil, to create a humid environment underneath the plastic, which might favour both biotic and abiotic degradation (Martín-Closas *et al.*, 2016).

In the present study, all three of the mulch films (i.e., ECO, PAR, EIF) were laid down when mean temperatures were relatively high (>25 °C) and above pluriannual means (Figure 1). Thus, it is likely that from July 2020 (i.e., when the mulch films were laid down and the zucchini was sown) to August 2020, UV radiation modified mechanical properties of the mulch films to some extent, with potentially different effects between those based on Materi-bi (i.e., ECO, PAR) and that based on Ecovio (i.e., EIF). In this study area, it is possible that first the UV radiation and then later the rainfall had key roles in early and late summer, respectively (Figure 1). Later, the crops will have come into play, depending on plant growth habit (e.g., creeping or erect) and/or canopy (i.e., height, width), as suggested by Martín-Closas *et al.* (2016). Future studies are needed to clarify these issues considering that fragmentation, tears and holes in the mulch films are important factors from the farmer perspective, because they are likely to affect production (Hayes *et al.*, 2017).

Mulch film degradation as affected by management practices

On clay-loam soil in climates characterised by a dry summer and a rainy spring, as in the present study, ploughing is largely used, and is indeed the standard practice for almost all of temperate Europe (Birkás *et al.*, 1989; Karlen *et al.*, 1991; Holland, 2004). In a cropping system that includes the use of soil-biodegradable mulch films, ploughing has a dual function: it prepares the soil for the new crop; and it buries mulch films so that they can be biotically degraded (Francioni *et al.*, 2021).

Once the mulch film is buried, its fate will be determined by abundance, composition and metabolic activity of the soil microbial communities, and also by the physiochemical characteristics of the soil (Kjeldsen *et al.*, 2019; Anunciado *et al.*, 2021). Many studies have highlighted how different tillage practices can significantly alter soil temperature (e.g., Malhi and O’Sullivan, 1990; Young and Ritz, 2000), moisture (e.g., Dick, 1992; Mohammadi *et al.*, 2011; Bienes *et al.*, 2021) and aeration (e.g., Ojeniyi, 1986; Malhi and O’Sullivan, 1990; Young and Ritz, 2000). By altering these factors, soil tillage can also influence the microbial activity that is responsible for mulch film biodegradation (Andrady, 1994; Kjeldsen *et al.*, 2019; Francioni *et al.*, 2021). Indeed, previous studies carried out in clayey soils of central Italy suggested that ploughing might enhance richness and diversity of active bacterial community (e.g., Pastorelli *et al.*, 2014). Usually, in soils such as the one in the present study, conventional ploughing to 0.3 m to 0.4 m in depth tends to decrease the bulk density and mix the soil aggregates, which creates more meso- and macro-porosities with larger cracks and voids that drain water faster and deeper than for a non-tilled soil (Malhi and O'Sullivan, 1990; Holland, 2004). This will promote soil aerobiosis, and therefore accelerate carbon turnover (Young and Ritz, 2000; Bienes *et al.*, 2021). However, despite these favourable conditions for their activity, some bacteria might leach away together with the water during rainy periods, potentially delaying the actions of biodegradation (Abu-Ashour *et al.*, 1994; Young and Ritz, 2000; Sintim *et al.*, 2020).

Together with low temperatures, this might explain the low degradation rates observed in the present study from November 2020 to February 2021 for all of the treatments (Figures 1, 3), even though the soil conditions in this period were very different between the ploughing and ploughing+grubbing plots (Figure 2). On the other hand, the grubbing disintegrated the soil clods and macro-aggregates, to create a more homogeneous and water-retentive substrate. This will have virtually maximised the mulch film–soil contact surface, which is expected to be particularly relevant for soils with significant amounts of clay, such as the soil in the present study. This would allow the soil to maintain sufficient moisture by capillarity and tension, and favour the displacement of microorganisms via the circulating solution and their adhesion together with the soil particles to the mulch films from the very first part of the year (Abu-Ashour *et al.*, 1994; Young and Ritz, 2000). Later, in May 2021, the clods formed by ploughing disappeared in all of the plots (Figure 2), and it is possible that all of the surface of the samples was in contact with soil particles. This, as well as optimal environmental conditions for soil biodegraders during spring, might explain the high degradation rates observed for all of the treatments (Figure 3), as it is well known that biodegradation of plastic materials depends on the surface exposed to biodegraders (Chinaglia *et al.*, 2018). Albeit the mesh bags were perforated, they can create a physical barrier between the film and the soil, and it is possible that they slowed down the degradation process. However, recent in-field studies have reported that mesh bags do not significantly impede mulch film degradation (e.g., Sintim *et al.*, 2020), and are thus well suited for this kind of open-field trial.

The soil in the present study had received long-term amendment with cattle manure. It is well-known that long-term management of organic amendments does affect the soil biological properties, and that animal manure inputs increase the biological activity and microbial biomass (Dick, 1992). Generally, animal manure application appears to enhance the activity and diversity of bacteria to a greater extent compared to fungi (Parham *et al.*, 2003; Rayne and Aula, 2020). However, it has been suggested that biodegradation of mulch films in soil is more dependent on fungi than bacteria, as fungi are more efficient in degrading polyesters (Yamamoto-Tamura *et al.*, 2020) and are less affected by limitations of soil nitrogen (Sander, 2019). Indeed, a greater refinement of the soil as observed for the ploughing+grubbing, on the one hand, might have slowed the infiltration of fungi between the empty spaces of the soil, while on the other hand, this might have facilitated the action of the bacteria, which need more contact and a liquid medium (Sander, 2019). Indeed, the mulch film degradation rates started to increase significantly from March 2021, to reach the highest rates between May and July 2021, when higher temperatures and lower precipitation were recorded (Figures 1, 3). This is in agreement with Parham *et al.* (2003), who reported that microbial activity and biomass (both bacterial and fungal) were directly related to seasonal temperatures. Future studies could deepen these aspects and clarify the interactions between fungi and bacteria, to emphasise also the ‘fungal highway’ concept. Indeed, bacteria are expected to exploit the hyphae as preferential pathways, which might be very significant where manure is applied. However, the actions and interactions of fungi and bacteria are expected to be highly context dependent and regulated by the soil characteristics and application rates and origins of the manure (Rayne and Aula, 2020).

The different degradation rates shown by the three mulch films tested (Figures 3, 4; Table 1) between the two types of tillage most likely depend on their different chemical compositions. This is in agreement with recent open-field studies that reported that different soil-biodegradable mulch films buried in the same soil and under the same conditions showed different degradation rates (Sintim *et* *al.*, 2020; Anunciado *et al.*, 2021). Even if mulch films are certified as soil-biodegradable (i.e., under laboratory conditions they must show biodegradation >90% in 2 years; EN 17.033), this does not guarantee that this is reflected under open-field conditions, where the biodegradation rates are indeed likely to be lower (Francioni *et al.*, 2021).

Some open-field studies have shown no evidence of ecotoxicity in the soil after incorporation of soil-biodegradable mulch films (e.g., Scarascia-Mugnozza *et al.*, 2006). However, further studies are needed over the long-term perspective to determine whether the samples tested in the present study affect crop yield and/or quality. This would be particularly relevant in the case where these films do not reach 100% biodegradation before the beginning of the new cropping cycle, which would lead to constant film accumulation over the years (Gao *et al.*, 2019; Huang *et al.*, 2020; Jacques and Prosser, 2021; Griffin-LaHue *et al.*, 2022).

Future perspectives for open-field trials

Estimation of mulch film biodegradation rates with indirect indicators are relatively easy to implement, but it is important to emphasise that these methods cannot directly demonstrate their biodegradability (Sander, 2019). Methods that measure the complete mineralisation of the carbon of the tested material into CO_2 (e.g., EN 17.033) are needed if the aim is to directly test their biodegradability. Conversely, indirect indicators (e.g., weight and surface area loss) are useful for open-field trials when the tested materials have been previously certified as biodegradable under soil conditions (Francioni *et al.*, 2021).

It is also important to note that only open-field trials can consider the real environmental conditions under which mulch films are applied, incorporated into the soil, and hopefully completely biodegraded. Moreover, open-field trials are the only option to test the performances of mulch films in terms of crop production and mulch film compliance with the farmer’s needs (e.g., ease of application with machines, life duration).

Advanced techniques such as ^{13}C tracking (e.g., Zumstein *et al.*, 2018) can be used in open-field trials to unequivocally demonstrate complete mulch film degradation, although these are costly and laborious, and they are thus not likely to be widely adopted in future studies. However, alternative and more accessible techniques have recently been proposed, which include quantification and monitoring of the residual polymers in the soil over time through Soxhlet or accelerated solvent extraction followed by nuclear magnetic resonance (Nelson *et al.*, 2019). The integration of such techniques with indirect indicators for the estimation of mulch film degradation and agronomic performances might represent the answer to the need to develop a protocol for open-field studies that is currently missing (Francioni *et al.*, 2021; Griffin-LaHue *et al.*, 2022).

Conclusions

Overall, the data from the present study indicate that soil refinement derived from ploughing followed by grubbing accelerates the degradation of the mulch films tested. The effects of the soil refinement were already visible over the short-term, which was probably due to increased soil–film contact surface. However, the highest degradation rates became evident only in spring, when the soil temperature and moisture were probably under optimal conditions for soil biodegraders.

The results obtained also show that one of the Mater-bi-based mulch films (PAR) showed higher degradation rates than the other two mulch films used (EIF, ECO; Ecovio, Mater-bi based, respectively). This could be attributable to the lower thickness of PAR (i.e., ~ 20 µm) compared to EIF and ECO (28 and 30 µm, respectively). However, it might also reflect the different potential of the studied soil for the biodegradation of one or more of the components of the films, although future studies are needed to test this hypothesis.

This study used indirect indicators for the estimation of mulch film biodegradation and future studies are needed to confirm whether and to what levels the mulch films tested are biodegraded by the soil microorganisms. Other aspects should also be investigated in the future including: (i) the effects of a long-term manure application, which might substantially modify the soil microbial diversity, abundance and activity; (ii) the direct and indirect effects of different crops on mulch film degradation during the crop growing phase; (iii) the possible interactions of mulch film biodegraders with crop residues after their incorporation into the soil; and (iv) the effects of different soil tillage regimes in different soils and across different seasons.

References

Abu-Ashour J, Joy DM, Lee H, Whiteley HR, Zelin S, 1994. Transport of Microorganisms through soil. Water, Air Soil Pollut. 75:141–58.

Andrady AL, 1994. Assessment of environmental biodegradation of synthetic polymers. J. Macromol. Sci. Part C 34:25–76.

Anunciado MB, Hayes DG, Wadsworth LC, English ME, Schaeffer SM, Sintim HY, Flury M, 2021. Impact of Agricultural Weathering on Physicochemical Properties of Biodegradable Plastic Mulch Films: Comparison of Two Diverse Climates Over Four Successive Years. J. Polym. Environ. 29:1–16. Available from: https://doi.org/10.1007/s10924-020-01853-1

ASSAM, 2005. Suoli e paesaggi delle Marche: programma interregionale Agricoltura e qualità, misura 5, carta dei suoli, scala 1:250.000 (D.G.R. n. 2805 del 18/12/2000 ASSAM. Agenzia Servizi Settore Agroalimentare delle Marche.

Bienes R, Marques MJ, Sastre B, García-Díaz A, Esparza I, Antón O, Navarrete L, Hernánz JL, Sánchez-Girón V, Sánchez Del Arco MJ, Alarcón R, 2021. Tracking changes on soil structure and organic carbon sequestration after 30 years of different tillage and management practices. Agronomy 11:291. Available from: https://doi.org/10.3390/agronomy11020291

Birkás M, Antal J, Dorogi I, 1989. Conventional and reduced tillage in Hungary - A review. Soil Tillage Res. 13:233–52.

Briassoulis D, Giannoulis A, 2018. Evaluation of the functionality of bio-based plastic mulching films. Polym. Test. 67:99–109. Available from: https://doi.org/10.1016/j.polymertesting.2018.02.019

Chinaglia S, Tosin M, Degli-Innocenti F, 2018. Biodegradation rate of biodegradable plastics at molecular level. Polym. Degrad. Stab. 147:237–44. Available from:

https://doi.org/10.1016/j.polymdegradstab.2017.12.011

Cowan JS, Inglis DA, Miles CA, 2013. Deterioration of three potentially biodegradable plastic mulches before and after soil incorporation in a broccoli field production system in Northwestern Washington. Horttechnology 23:849–58.

Dick RP, 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric. Ecosyst. Environ. 40:25–36.

Filippi F, Magnani G, Guerrini S, Ranghino F, 2011. Agronomic evaluation of green biodegradable mulch on melon crop. Ital. J. Agron. 6:111–6. Available from: https://doi.org/10.4081/ija.2011.e18

Francioni M, Kishimoto-Mo AW, Tsuboi S, Takada Hoshino Y, 2021. Evaluation of the mulch films biodegradation in soil: a methodological review. Ital. J. Agron. (AOP). https://doi.org/10.4081/ija.2021.1936

Gao H, Yan C, Liu Q, Ding W, Chen B, Li Z, 2019. Effects of plastic mulching and plastic residue on agricultural production: A meta-analysis. Sci. Total Environ. 651:484–92. Available from: https://doi.org/10.1016/j.scitotenv.2018.09.105

Griffin-LaHue D, Ghimire S, Yu Y, Scheenstra EJ, Miles CA, Flury M, 2022. In-field degradation of soil-biodegradable plastic mulch films in a Mediterranean climate. Sci Total Environ. 806:150238. Available from: 10.1016/j.scitotenv.2021.150238.

Hablot E, Dharmalingam S, Hayes DG, Wadsworth LC, Blazy C, Narayan R, 2014. Effect of Simulated Weathering on Physicochemical Properties and Inherent Biodegradation of PLA/PHA Nonwoven Mulches. J. Polym. Environ. 22:417–29. Available from: 10.1007/s10924-014-0697-0

Hayes DG, Wadsworth LC, Sintim HY, Flury M, English M, Schaeffer S, Saxton AM, 2017. Effect of diverse weathering conditions on the physicochemical properties of biodegradable plastic mulches. Polym. Test. 62:454–67. Available from: http://dx.doi.org/10.1016/j.polymertesting.2017.07.027

Holland JM, 2004. The environmental consequences of adopting conservation tillage in Europe: Reviewing the evidence. Agric. Ecosyst. Environ. 103:1–25. Available from: doi:10.1016/j.agee.2003.12.018

Huang Y, Liu Q, Jia W, Yan C, Wang J, 2020. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environ. Pollut. 260:114096. Available from: https://doi.org/10.1016/j.envpol.2020.114096

Jacques O, Prosser RS, 2021. A probabilistic risk assessment of microplastics in soil ecosystems. Sci. Total Environ. 757:143987. Available from: https://doi.org/10.1016/j.scitotenv.2020.143987

Karlen DL, Berry EC, Colvin TS, Kanwar RS, 1991. Twelve-year tillage and crop rotation effects on yields and soil chemical properties in northeast Iowa. Commun. Soil Sci. Plant Anal. 22:1985–2003. Available from: https://doi.org/10.1080/00103629109368552

Kasirajan S, Ngouajio M, 2012. Polyethylene and biodegradable mulches for agricultural applications: A review. Agron. Sustain. Dev. 32:501–29. Available from: 10.1007/s13593-011-0068-3

Kjeldsen A, Price M, Lilley C, Guzniczak E, Archer I, 2019. A Review of Standards for Biodegradable Plastics with support from. Ind. Biotechnol. Innov. Cent. IBioIC:33.

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F, 2006. Updated world map of the Köppen-Geiger climate classification. Meteorol. Zeitschrift 15:259–63. Available from: https://doi.org/10.1127/0941-2948/2006/0130

Malhi SS, O'Sullivan PA, 1990. Soil temperature, moisture and penetrometer resistance under zero and conventional tillage in central Alberta. Soil Tillage Res. 17:167–72.

Malinconico M, 2017. *Soil Degradable Bioplastics for a Sustainable Modern Agriculture* (M. Malinconico, Ed.). Springer Berlin Heidelberg, Berlin, Heidelberg. Available from: http://link.springer.com/10.1007/978-3-662-54130-2

Marí AI, Pardo G, Cirujeda A, Martínez Y, 2019. Economic evaluation of biodegradable plastic films and paper mulches used in open-air grown pepper (*Capsicum annum* L.) crop. Agronomy 9:36.

Available from: https://doi.org/10.3390/agronomy9010036

Martín-Closas L, Costa J, Cirujeda A, Aibar J, Zaragoza C, Pardo A, Suso ML, Moreno MM, Moreno C, Lahoz I, Mácua JI, Pelacho AM, 2016. Above-soil and in-soil degradation of oxo- and biodegradable mulches: A qualitative approach. Soil Res. 54:225–36. Available from: http://dx.doi.org/10.1071/SR15133

Martin-Closas L, Pelacho AM, Picuno P, Rodríguez D, 2008. Properties of new biodegradable plastics for mulching, and characterization of their degradation in the laboratory and in the field. Acta Hortic. 801:275–82. Available from: https://doi.org/10.17660/ActaHortic.2008.801.27

Miles C, Wallace R, Wszelaki A, Martin J, Cowan J, Walters T, Inglis D, 2012. Deterioration of potentially biodegradable alternatives to black plastic mulch in three tomato production regions. HortScience 47:1270–7. Available from: https://doi.org/10.21273/HORTSCI.47.9.1270

Mohammadi K, Heidari G, Khalesro S, Sohrabi Y, 2011. Soil management, microorganisms and organic matter interactions: A review. African J. Biotechnol. 10:19840–9. Available from: https://doi.org/10.5897/AJBX11.006

Moore-Kucera J, Cox SB, Peyron M, Bailes G, Kinloch K, Karich K, Miles C, Inglis DA, Brodhagen M, 2014. Native soil fungi associated with compostable plastics in three contrasting agricultural settings. Appl. Microbiol. Biotechnol. 98:6467–85. Available from: https://doi.org/10.1007/s00253-014-5711-x

Moreno MM, Moreno A, Mancebo I, 2009. Comparison of different mulch materials in a tomato (*Solanum lycopersicum* L.) crop. Spanish J. Agric. Res. 7:454–64.

Nelson TF, Remke SC, Kohler HPE, McNeill K, Sander M, 2019. Quantification of Synthetic Polyesters from Biodegradable Mulch Films in Soils. Environ. Sci. Technol. 54: 266−275. Available from: https://doi.org/10.1021/acs.est.9b05863

Ojeniyi SO, 1986. *Effect of zero-tillage and disc ploughing on soil water, soil temperature and growth and yield of maize (Zea mays L.)*. Available from: https://linkinghub.elsevier.com/retrieve/pii/0167198786900164

Pastorelli R, Vignozzi N, Landi S, Piccolo R, Orsini R, Seddaiu G, Roggero PP, Pagliai M, 2013. Consequences on macroporosity and bacterial diversity of adopting a no-tillage farming system in a clayish soil of Central Italy. Soil Biol. and Biochem. 66:78–93. Available from: https://doi.org/10.1016/j.soilbio.2013.06.015

Parham JA, Deng SP, Da HN, Sun HY, Raun WR, 2003. Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. Biol. Fertil. Soils 38:209–15. Available from: https://doi.org/10.1007/s00374-003-0657-7

Rayne N, Aula L, 2020. Livestock manure and the impacts on soil health: A review. Soil Syst. 4:1–26. Available from: https://doi.org/10.3390/soilsystems4040064

Rudnik E, Briassoulis D, 2011. Degradation behaviour of poly(lactic acid) films and fibres in soil under Mediterranean field conditions and laboratory simulations testing. Ind. Crops Prod. 33:648–58. Available from: http://dx.doi.org/10.1016/j.indcrop.2010.12.031

Sakai Y, Isokawa M, Masuda T, Yoshioka H, Hayatsu M, Hayano K, 2002. Usefulness of soil p-nitrophenyl acetate esterase activity as a tool to monitor biodegradation of polybutylene succinate (PBS) in cultivated soil. Polym. J. 34:767–74.

Sander M, 2019. Biodegradation of Polymeric Mulch Films in Agricultural Soils: Concepts, Knowledge Gaps, and Future Research Directions. Environ. Sci. Technol. 53:2304–15. http://dx.doi.org/10.1021/acs.est.8b05208

Scarascia-Mugnozza G, Schettini E, Vox G, Malinconico M, Immirzi B, Pagliara S, 2006. Mechanical properties decay and morphological behaviour of biodegradable films for agricultural mulching in real scale experiment. Polym. Degrad. Stab. 91:2801–8. Available from: https://doi.org/10.1016/j.polymdegradstab.2006.02.017

Scaringelli MA, Giannoccaro G, Prosperi M, Lopolito A, 2016. Adoption of biodegradable mulching films in agriculture: Is there a negative prejudice towards materials derived from organic wastes? Ital. J. Agron. 11:92–9. Available from: https://doi.org/10.4081/ija.2016.716

Schneider CA, Rasband WS, Eliceiri KW, 2012. NIH Image to ImageJ: 25 years of image analysis.

Nat. Methods 9:671–5.

Shogren RL, Doane WM, Garlotta D, Lawton JW, Willett JL, 2003. Biodegradation of starch/polylactic acid/poly(hydroxyester-ether) composite bars in soil. Polym. Degrad. Stab. 79:405–11.

Sintim HY, Bary AI, Hayes DG, Wadsworth LC, Anunciado MB, English ME, Bandopadhyay S, Schaeffer SM, DeBruyn JM, Miles CA, Reganold JP, Flury M, 2020. In situ degradation of biodegradable plastic mulch films in compost and agricultural soils. Sci. Total Environ. 727:138668. Available from: https://doi.org/10.1016/j.scitotenv.2020.138668

Smith DW, 2014. *Soil survey staff: keys to soil taxonomy*. 12th ed. Natural Resources Conservation Service, Washington, DC, USA.

Steinmetz Z, Wollmann C, Schaefer M, Buchmann C, David J, Tröger J, Muñoz K, Frör O, Schaumann GE, 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. 550:690–705. Available from: http://dx.doi.org/10.1016/j.scitotenv.2016.01.153

Touchaleaume F, Angellier-Coussy H, César G, Raffard G, Gontard N, Gastaldi E, 2018. How Performance and Fate of Biodegradable Mulch Films are Impacted by Field Ageing. J. Polym. Environ. 26:2588–600. Available from: http://dx.doi.org/10.1007/s10924-017-1154-7

Wypych G, Faulkner T, 1999. Basic Parameters in Weathering Studies. Weather. Plast.:1–13.

Yamamoto-Tamura K, Hoshino YT, Tsuboi S, Huang C, Kishimoto-Mo AW, Sameshima-Yamashita Y, Kitamoto H, 2020. Fungal community dynamics during degradation of poly(butylene succinate-co-adipate) film in two cultivated soils in Japan. Biosci. Biotechnol. Biochem. 84:1077–87. Available from: https://doi.org/10.1080/09168451.2020.1713718

Yang Y, Li P, Jiao J, Yang Z, Lv M, Li Y, Zhou C, Wang C, He Z, Liu Y, Song S, 2020. Renewable sourced biodegradable mulches and their environment impact. Sci. Hortic. 268:109375. Available from: https://doi.org/10.1016/j.scienta.2020.109375

Young IM, Ritz K, 2000. Tillage, habitat space and function of soil microbes. Soil Tillage Res.

53:201–13. Available from: https://doi.org/10.1016/S0167-1987(99)00106-3

Zhang M, Jia H, Weng Y, Li C, 2019. Biodegradable PLA/PBAT mulch on microbial community structure in different soils. Int. Biodeterior. Biodegrad. 145:104817. Available from: https://doi.org/10.1016/j.ibiod.2019.104817

Zumstein MT, Schintlmeister A, Nelson TF, Baumgartner R, Woebken D, Wagner M, Kohler HPE, McNeill K, Sander M, 2018. Biodegradation of synthetic polymers in soils: Tracking carbon into CO_2 and microbial biomass. Sci. Adv. 4:7 eaas9024. Available from: https://doi.org/10.1126/sciadv.aas9024

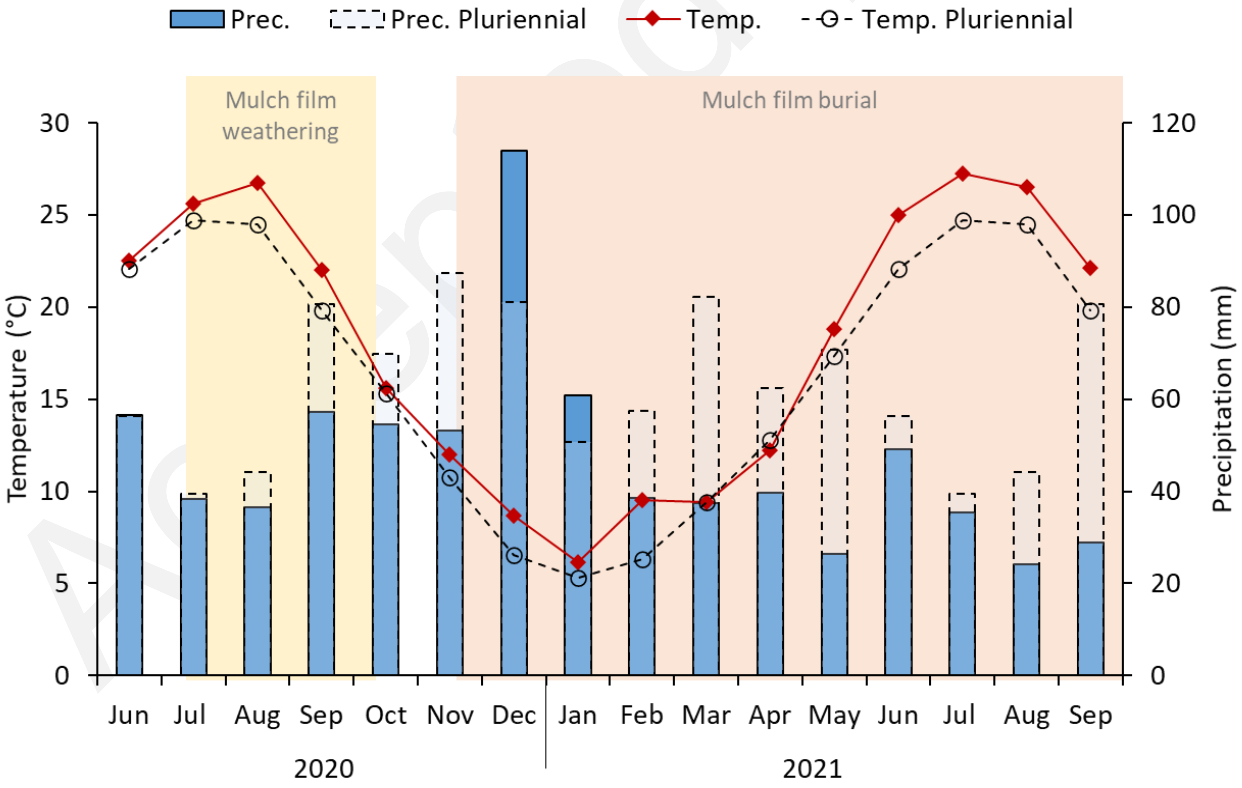

Figure 1. Monthly and plurennial (2019-2021) mean air temperatures and precipitation in the study area during the experimental period (source: *Regione Marche*, *Servizio Protezione Civile*; ASSAM). ‘Mulch film weathering’ refers to the period when the mulch films were laid down together with the crop; ‘Mulch film burial’ refers to the period when the mulch films were incorporated into the soil.

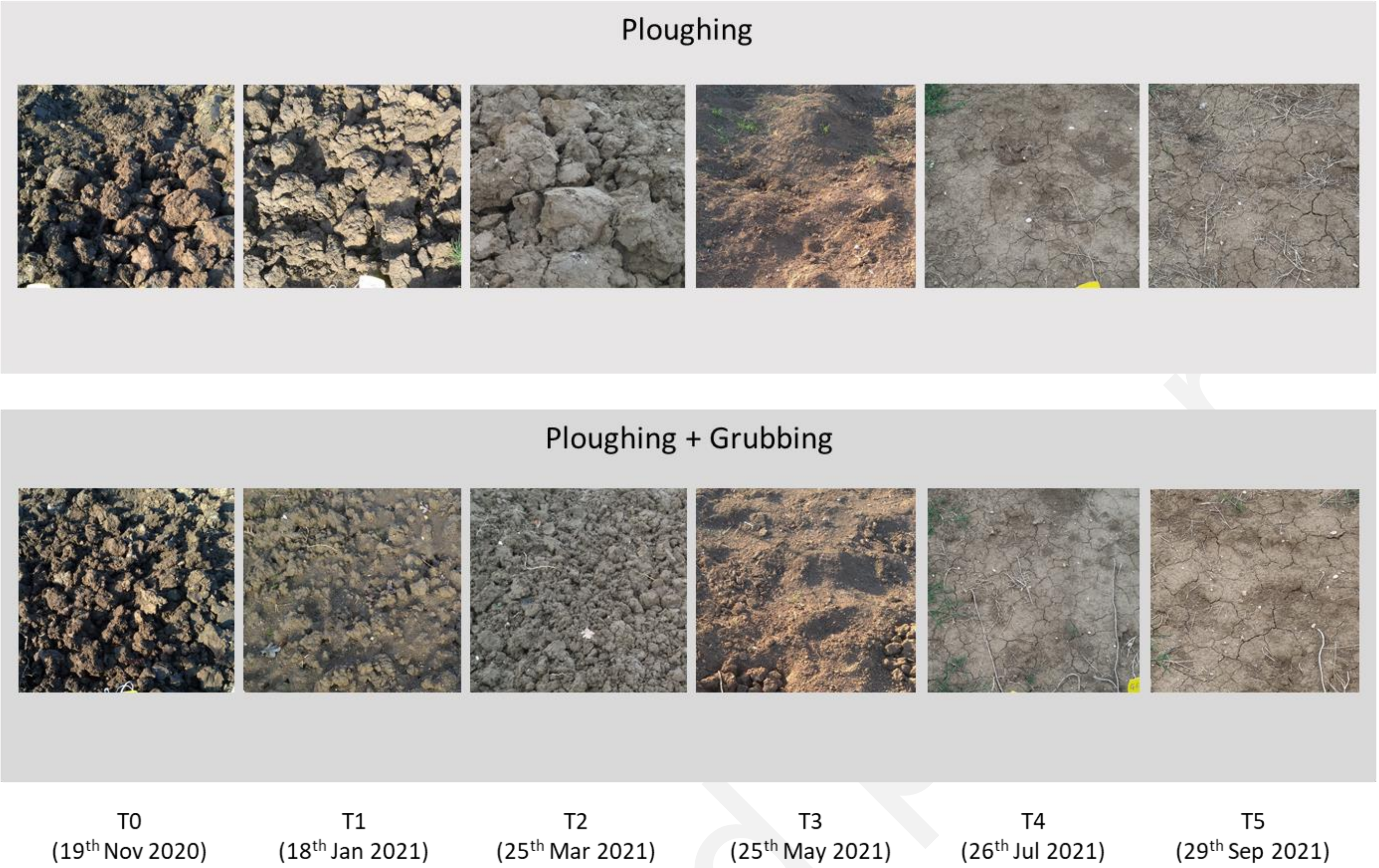

Figure 2. Aspects of the representative plots subjected to ploughing and ploughing+grubbing over time.

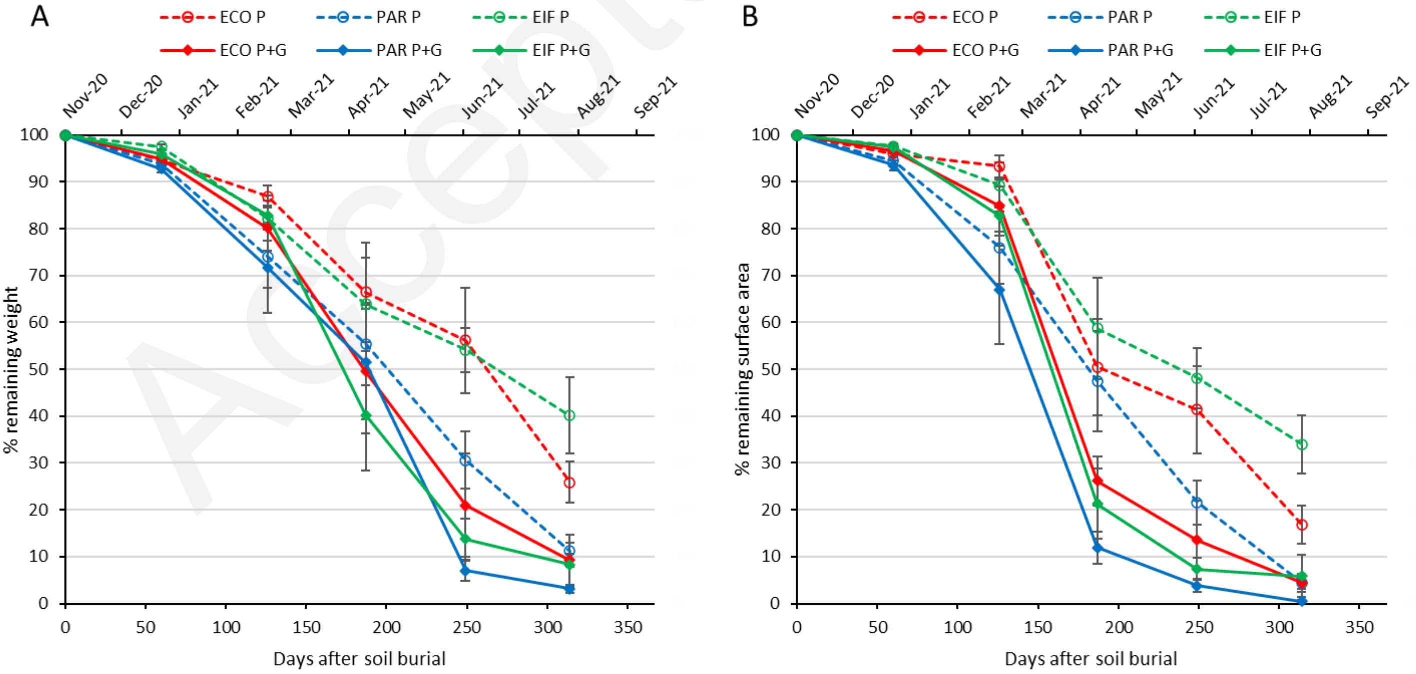

Figure 3. Weight loss (**A**) and surface area loss (**B**) for the soil tillage (P: ploughing, dashed lines; P+G: ploughing+grubbing, full lines), film type (ECO, 'Ecopac'; PAR, 'Pardini'; EIF, 'Eiffel'), and sampling dates (~2 months interval). Data are means ±standard error of the means.

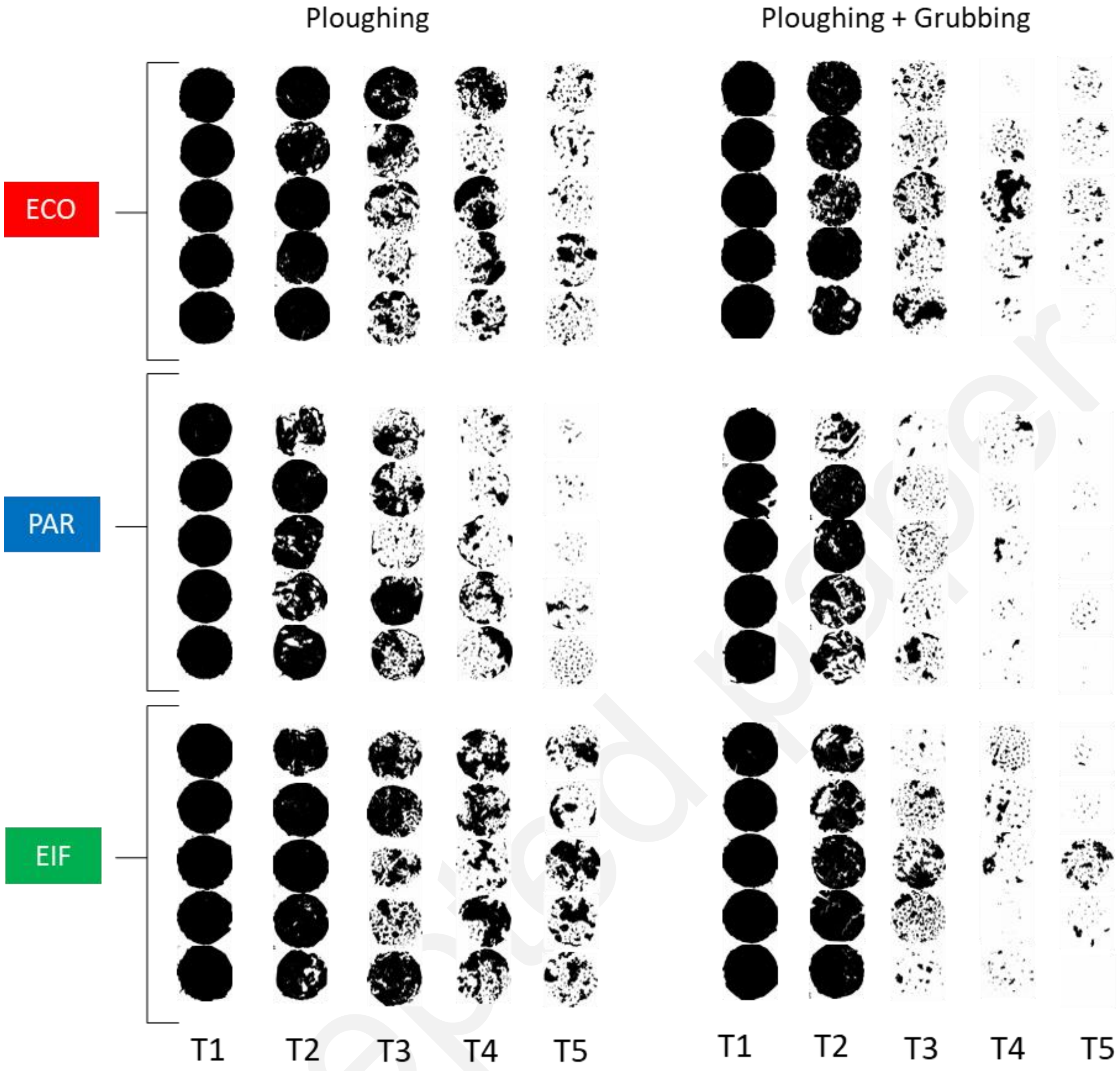

Figure 4. Aspects of each film sample after the scanning following the binary transformation, applied with ImageJ for soil tillage (ploughing; ploughing+grubbing), film type (ECO, ‘Ecopac’; PAR, ‘Pardini’; EIF, ‘Eiffel’) and sampling date (from November 2020 to September 2021, T1-T5, ~2 month intervals).

Table 1. Repeated measure ANOVA for soil tillage, film type and sampling date. The overall means report the non-transformed proportions of weight loss and surface area loss over the study period.

Variable	**Source of variation**	**df**	**F**	***P***	**Overall mean ±standard error**	
					Ploughing	**Ploughing+ grubbing**
Weight loss (% remaining)	Tillage	1	42.93	0.01	62.20 ±3.37 a	48.13 ±4.36 b
	Film	2	8.04	0.01		
	Sampling date	4	275.56	0.01		
	Tillage × Film	2	0.93	0.41		
	Tillage × Sampling date	4	13.19	0.01		
	Film × Sampling date	8	1.43	0.19		
	Film × Tillage × Sampling date	8	0.93	0.50		
Surface area loss (% remaining)	Tillage	1	43.23	0.01	57.98 ±3.85 a	41.13 ±4.64 b
	Film	2	8.21	0.01		
	Sampling date	4	279.44	0.01		
	Tillage × Film	2	0.94	0.40		
	Tillage × Sampling date	4	13.02	0.01		
	Film × Sampling date	8	8.17	0.20		
	Film × Tillage × Sampling date	8	0.92	0.50		

Supplementary Materials

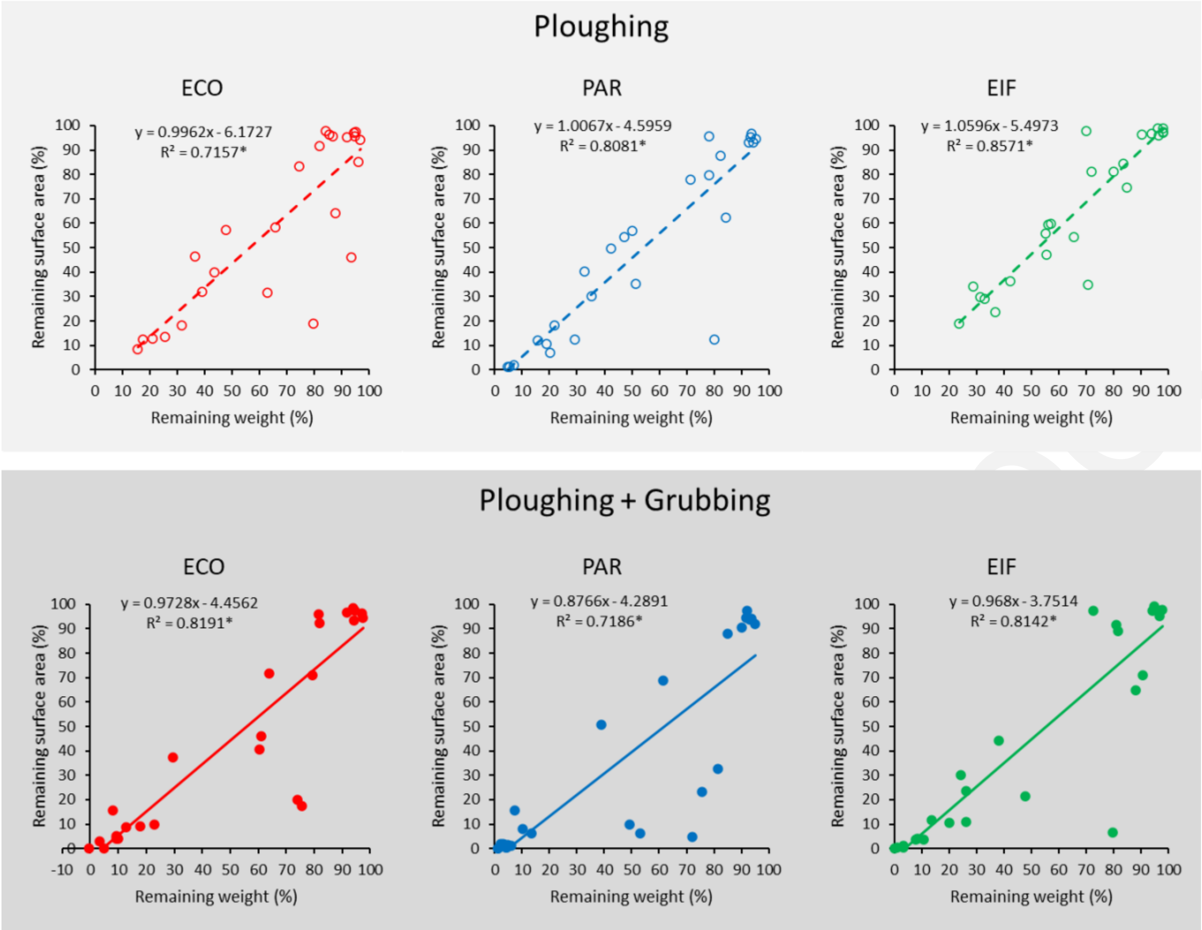

Figure S1. Relationship between the mulch film ‘remaining mass’ and ‘remaining surface area’. ECO, Ecopac; PAR, Pardini; EIF, Eiffel. *, P <0.05.

Table S1. ANOVA within sampling dates for soil tillage and mulch films and sampling dates (from November 2020 to September 2021, T1-T5, ~2 month intervals), with the overall means that report the non-transformed proportions of weight loss and surface area loss over the study period. Different letters indicate significant differences (*P* =0.05, Tukey tests).

Variable	**Source of variation**	**Sampling date**	**df**	**F**	**P**	**Ploughing**	**Ploughing + grubbing**	**Ecopac**	**Pardini**	**Eiffel**
Weight loss (% remaining)	Tillage × Film	T1	2	0.52	0.60					
		T2	2	0.01	0.99					
		T3	2	0.60	0.56					
		T4	2	0.61	0.55					
		T5	2	2.70	0.09					
	Tillage	T1	1	0.04	0.84	95.33 ±0.52	94.56 ±0.58			
		T2	1	2.12	0.16	81.06 ±3.02	78.23 ±3.69			
		T3	1	22.52	0.00	61.89 ±5.39 a	47.06 ±6.76 b			
		T4	1	36.49	0.00	46.96 ±5.22 a	13.96 ±4 b			
		T5	1	32.42	0.00	25.74 ±4.37 a	6.89 ±1.69 b			
	Film	T1	2	11.03	0.00			94.81 ±0.88 a	93.32 ±0.66 b	96.71 ±0.65 a
		T2	2	3.59	0.04			83.52 ±3.92 a	72.89 ±7.88 b	82.53 ±3.83 ab
		T3	2	1.10	0.35			57.97 ±11.95	53.43 ±10.09	52.01 ±11.67
		T4	2	2.60	0.10			38.61 ±13.32	18.82 ±7.07	33.96 ±10.42
		T5	2	10.49	0.00			17.58 ±4.97 a	7.18 ±3.03 b	24.19 ±9.76 a
Surface area loss (% remaining)	Tillage × Film	T1	2	0.52	0.60					
		T2	2	0.01	0.99					
		T3	2	0.60	0.56					
		T4	2	0.64	0.5					

				4					
	T5	2	2.83	0.08					
Tillage	T1	1	0.04	0.84	96.03 ±0.47	95.82 ±0.62			
	T2	1	2.13	0.16	86.16 ±3.51	78.18 ±4.89			
	T3	1	22.54	0.00	52.24 ±5.83 a	19.79 ±3.4 b			
	T4	1	36.86	0.00	37.02 ±4.83 a	8.26 ±2.91 b			
	T5	1	32.98	0.00	18.42 ±4.01 a	3.6 ±1.57 b			
Film	T1	2	11.09	0.00			96.27 ±0.6 a	94.04 ±0.91 b	97.47 ±0.55 a
	T2	2	3.59	0.04			89.06 ±4.48 a	71.44 ±9.48 b	86.01 ±5.52 a
	T3	2	1.10	0.35			38.31 ±9.64	29.71 ±11.25	40.02 ±12.46
	T4	2	2.69	0.09			27.48 ±10.58	12.71 ±5.33	27.73 ±10.63
	T5	2	10.96	0.00			10.64 ±4.09 a	2.46 ±1.58 b	19.93 ±8.35 a