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## DEGRADATION OF AGRICULTURAL BIODEGRADABLE PLASTICS IN THE SOIL UNDER LABORATORY CONDITIONS

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**Abstract.** Mulches, usually consisting of polyethylene films, are used in agriculture to improve production. The main drawback of using polyethylene is its extremely high stability. Removing it from the field is usually not feasible, and so wastes remain accumulating in the field and pollute the environment. As an alternative, five potentially biodegradable plastic films for mulching (maize thermoplastic starch–copolyester, cereal flour–copolyester, polylactic acid–copolyester, polyhydroxybutyrate, and potato thermoplastic starch–copolyester) were tested to evaluate their degradation in an agricultural soil. Polyethylene film was used as control. A soil burial test was carried out during 6 months under laboratory conditions and film weight loss, chemical changes and soil microbial activity were monitored. Weight loss was fastest for the polyhydroxybutyrate film, followed by potato thermoplastic starch–copolyester and cereal flour– copolyester. Maize thermoplastic starch–copolyester and polylactic acid–copolyester required 5–6 months to disintegrate. Concomitant to the weight loss, chemical changes in the films and an increase in soil microbial activity were noticed. From the disintegration and biodegradation results of the biodegradable tested films, it is concluded that these films are an alternative for avoiding the soil pollution drawbacks of the polyethylene mulching films.

Additional keywords: biodegradation, mulch, organic farming, polymers, soil enzyme activity.  
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### Introduction

Mulching is a widely used technique that consists of covering the soil with a material. In agriculture, it improves crop productivity by enhancing crop microclimate, saving irrigation water and fertilisers and avoiding the use of herbicides (Lamont 2005; Schettini *et al.* 2007). It also reduces soil loss and erosion (Giménez-Morera *et al.* 2010) and water runoff (Moreno- Ramón *et al.* 2014). Moreover, mulches improve forest management by increasing afforestation (Jiménez *et al.* 2013; Prats *et al.* 2013). Although different materials are used for mulching (straw, crop residues, wood chips, paper, stones, etc.), in intensive agriculture, most mulches are composed of polyethylene (PE), a highly durable, resistant and cheap plastic produced from oil. The massive use of PE constitutes an environmental and economic problem; agricultural plastic wastes abandoned or burnt in rural areas may cause severe damage to the environment (Scarascia-Mugnozza *et al.* 2008).

Recently, biodegradable plastics have arisen as an alternative to replace this conventional PE mulch and reduce waste pollution (Martín-Closas and Pelacho 2011). Biodegradable materials can be degraded in the field through the joint action of two mechanisms: photodegradation, due to the exposure of films to solar radiation; and biodegradation, the degradation process resulting from the action of soil microorganisms. It is acknowledged that soil biodiversity supplies many ecosystem services essential to humans and the environment (Brevik *et al.* 2015); among others, soil microorganisms may be instrumental in decontaminating the environment. As the most promising feedstock for manufacturing plastic mulches intended to biodegrade in the field after harvest, attention has focused on thermoplastic starch (TPS) (Bastioli 2005), polylactic acid (PLA) (Tokiwa and Calabria 2006), polyhydroxyalkanoates (PHAs) (Harding *et al.* 2007), poly(butylene adipate-co-terephthalate) (PBAT) and polybutylene succinate (PBS) (Tserki *et al.* 2006). These polymers have drawn recognition because their blends exhibit suitable features for mulching and they are obtained from low-cost agricultural resources, such as potato, maize, wheat, rice or sugarcane. Recently, plant and microbiological biotechnology have played an important role in the development and improvement of several of these polymers, as is the case for PHAs and PLA (Akaraonye *et al.* 2010; Madhavan Nampoothiri *et al.* 2010). The mechanical properties of the mulches, stability on water contact or water vapour permeability, have been improved by making blends with specific additives or aliphatic polyesters derived from dicarboxylic acids and glycols. These mixtures have resulted in plastic mulches with enhanced properties and biodegradability (Gross and Kalra 2002; Feuilloley *et al.* 2005).

The development of biodegradable mulches that retain properties similar to those of PE for the

cultivation period and subsequently degrade *in situ* is necessary to provide benefits both to farmers and to the environment. Therefore, there is a need to identify suitable methods for testing the biodegradability of these plastics.

Several standardised methods to monitor biodegradation of plastics under different environmental conditions are available for field and laboratory evaluation. Among them, the indoor soil burial test, which determines the material weight loss with time, is the most widely used (Chandra and Rustgi 1998). Compared with an equivalent test done outdoors in the field, its main advantages are simplicity, reproducibility and reliability (Degli Innocenti 2005). In addition, it enables further investigation of the changes the sample undergoes during the testing period, for example, chemical changes in polymer chains (by means of Fourier transform infrared (FTIR) spectroscopy), biological erosion of the mulch surface (by means of electron microscopy), or altered physical and mechanical properties associated with the degradation. In addition, the indoor soil burial test allows the isolation and identification of bacteria involved in the degradation process (Kumari *et al.* 2009). Other existing methods frequently measure the dynamics of the gaseous phase of the plastic–soil system, specifically, the biochemical oxygen demand (BOD) or/and carbon dioxide production (Barragán *et al.* 2012; ISO 2012). However, they usually require the assembling of complex and expensive testing equipment.

Most published reports focus on evaluating biodegradability of only one or a few polymers or polymer blends of known composition; they are usually carried out only with the raw granulate, not with the final commercial film, which incorporates other components (e.g. additives). Furthermore, most studies are carried out in compost media and seldom in soil (Degli Innocenti 2005).

The present paper investigates, under laboratory conditions, the degradation of five biodegradable, agricultural mulch plastics of different composition, in a soil from an organic farming field. Additionally, it examines the effects of the degradation of the plastics on soil microbial activity.

## Materials and methods

### *Test materials*

Five biodegradable, agricultural mulch films of different basic composition were selected to cover the most representative plastic mulch materials on the market: Mater-Bi (MB), thermoplastic starch–vegetable oil–copolyester; Biofilm (BF), cereal flour–copolyester; Bio-Flex (BFx), PLA–copolyester. Some promising experimental materials were also included: Bioplast (BP), thermoplastic potato starch–copolyester; Mirel (MI), polyhydroxybutyrate (PHB). Manufacturers are listed in Table 1. A conventional mulching film made of black, lineal, low-density PE was used as negative control. Before starting the test, the average thickness of the films was obtained by measuring 20 random samples per film with a micrometer (ND 287; Heidenhain, Hamburg, Germany) (Table 1); other main features of the films are also provided.

### *Soil sampling*

Soil was collected from a vegetable experimental field under organic farming management at Lleida, Spain. Soil was sieved with a 2-mm mesh screen to remove inert objects, rocks and plant materials. To retain the native soil microbial activity, soil samples were incubated for 1 week in an environmental chamber at 25°C. Physico-chemical features of the soil at sampling in February are presented (Table 2).

### *Soil burial test*

Glass jars were filled with 400 g of conditioned soil. A jar with soil only and another one with sterile soil served as biotic and abiotic controls, respectively. Three pieces 7 cm by 7 cm were cut from each film, weighed and clamped by non-degradable plastic frameworks, then introduced into the jars. Distilled water was added to the jars for adjusting the water soil content up to 50% of water-holding capacity. This was checked and corrected weekly. Immediately, the glass jars were taken to an environmental chamber at 25°C in the dark. Periodically from the beginning of the test up to 6 months, the frameworks with the samples were visually inspected during the soil burial test. They were retrieved approximately every 2 weeks (10 measurements) from the jars and their individual weights recorded. For this, the soil adhering to the films was removed by immersing the frameworks in a 0.02% NaN<sub>3</sub> (Panreac, Barcelona, Spain) solution at 25°C; after 24 h, they were rinsed with distilled water and dried for 24 h in an air-circulating chamber at 25°C. The weight of each frame was registered using an analytical scale of ±0.1 mg. Weight loss of the material was calculated as the decrease, in percentage, from the initial sample weight. Results obtained were expressed as weight-loss percentage, which was calculated as follows:

$$\% \text{ Weight loss} = \frac{W_0 - W_i}{W_0} \times 100 \quad (1)$$

where  $W_0$  is the weight of the framework with the sample before the test and  $W_i$  is the weight registered for the framework with the sample at the generic moment 'i' during the test.

#### *Chemical changes*

Chemical changes in the films during the soil burial test were evaluated by means of FTIR spectroscopy after weighing. Data were taken with a Nicolet Magna 560 FTIR spectrophotometer (Thermo Fisher, Waltham, MA, USA), as an average of 32 scans with  $4\text{ cm}^{-1}$  resolution in the range  $4000\text{--}650\text{ cm}^{-1}$ . The carbonyl index was calculated as the ratio of the optical density of the absorption band between  $1840$  and  $1640\text{ cm}^{-1}$  ( $\text{C}=\text{O}$  carbonyl peak) and that between  $1480$  and  $1350\text{ cm}^{-1}$  ( $\text{C}-\text{H}$  bending peak). For every material, indices of hydroxyl groups ( $\text{OH}$ ) and of crystallinity were determined. The hydroxyl index was measured as the ratio of the  $3450\text{--}3300\text{ cm}^{-1}$  band ( $\text{OH}$  groups) absorbance to the  $1480\text{--}1350\text{ cm}^{-1}$  band ( $\text{C}-\text{H}$  bending peak) absorbance. Crystallinity index was determined as ratio of the  $1150\text{--}1065\text{ cm}^{-1}$  ( $\text{C}-\text{O}-\text{C}$  symmetric) absorbance to the  $1028\text{--}875\text{ cm}^{-1}$  band (skeletal; amorphous vibration) absorbance.

#### *Soil microbial activity*

Soil microbial activity during the biodegradation of the mulch films was estimated by the fluorescein diacetate (FDA) hydrolysis test method according to Adam and Duncan (2001). Soil samples in the jars were taken regularly, starting 10 days from the initiation of the experiment, every 20 days. Soil (0.7 g) was weighed in a 15- mL test tube to which 5 mL of 60 mM potassium phosphate buffer pH 7.6 ( $\text{K}_2\text{HPO}_4\text{--K}_2\text{H}_2\text{PO}_4$ ) was added. Then, 0.07 mL of a  $1\text{ g L}^{-1}$  stock solution of FDA ( $3^0_6^0$ -diacetyl-flourescein) (Acros Organics, Geel, Belgium) was added to start the reaction. The tubes were shaken for 3 min and then placed in an incubator at  $30^\circ\text{C}$  for 20 min at 80 rpm. Aliquots (5 mL) of a chloroform: methanol solution (2:1) (Panreac) were added to the tubes, which were shaken again for 3 min. The tubes were then centrifuged at 3000 rpm for 3 min. Supernatants were filtered (Whatman No. 1) and placed in new 15-mL tubes. Optical density of the eluates was measured at 490 nm in a UV/VIS spectrophotometer Helios Gamma (Thermo Fisher). Fluorescein concentration was calculated by a calibration graph obtained by using standard solutions of fluorescein disodium salt (Acros Organics) dissolved in 60 mM potassium phosphate buffer (pH 7.6). For this assay, autoclaved soil was used to assess the abiotic effects on the mulches during the experiment. Two repetitions per sample and date were analysed.

## Results and discussion

#### *Soil burial test*

From 28 days onward, several physical changes in the films were noticed, including cracks and holes (Fig. 1). Among the five polymers tested, MI showed the earliest fragmentation under soil burial conditions, followed by BP and BF at 38 days, and then MB and BFx, which started disintegration at day 67.

All plastic mulches decreased their weight over the 6-month period of burial in soil (Fig. 2), thereby showing that the films underwent degradation. In the absence of UV radiation, biodegradation is very likely the main degrading process. The degradation was dependent on the material. The film that degraded fastest was MI, which despite being the thickest material (Table 1), was totally degraded after 116 days (Fig. 2). Thus, the MI chemical composition appears as the most sensitive to degradation.

These results agree with those of Wen and Lu (2012), who found that  $>50\%$  of PHB film weight was lost after 60 days in

a garden soil at room temperature. Rudnik and Briassoulis (2011) reported a fast disintegration of the PHB-based film MI; after 2 months of burial in a clay-loam soil in the field, they recovered only 1–2% of the film surface area. Rudnik and Briassoulis also found a faster degradation for MI than for a PLA film. Similarly, in the present study, a PLA-based film, BFx, degraded much slower than MI (Figs 1 and 2). One of the key attributes of MI (PHB) copolymers is their ability to biodegrade in soil at ambient temperature (Krishnaswani *et al.* 2008). The high degradability of MI in soil is not surprising; it is well known that soil microorganisms produce extracellular PHB depolymerases that degrade PHB copolymers. The resulting compounds are assimilated by the microorganisms as nutrients (Ha and Cho 2002).

All films except MI showed a similar weight loss at the beginning of the test, up to 40 days of incubation. From then on, BP started a quicker degradation than the other films (BF, MB and BFx), which evolved similarly until day 100 of the test. Maximum weight loss, total degradation, was

achieved at day 158 for BP and BF, whereas MB and BFx were close to total degradation only at day 186 (Fig. 2). Although BP, MB and BF can be considered similar biodegradable matrices (potato or maize TPS and cereal flour), they are probably blended with different amounts and types of copolyesters. This may be responsible for the variations in weight-loss dynamics among the three materials during their degradation processes. It is likely that the additives and the presence of vegetable oils in the MB blend with starch accounts for the slower degradation of this film. The presence of amylose–lipid complexes has a negative effect on the enzyme digestibility of starch (Vázquez *et al.* 2011), which may be the case for MB.

Degradation studies conducted by Mostafa *et al.* (2010) showed that MB strips buried in a loamy soil at 25°C degraded up to 80% in 5 months. The soil burial test used in the present study provides 85% MB weight loss in the same timeframe. The soil characteristics and incubation environment were very similar in both studies; therefore, the slightly higher degradation obtained here may be explained by the greater MB surface contact with the clay loam soil compared with loamy soil. There may have been some other differences between the studies; those authors do not specify the film thickness, an essential film property for the degradation time span of the material (Rudnik and Briassoulis 2011).

The products MB and BFx degraded similarly at the end of the experiment (day 186) (Fig. 2). However, BFx degraded considerably slower than MB up to day 116. Several studies (Ho and Pometto 1999; Ho *et al.* 1999; Kaplan 1998) have concluded that the slow degradation of PLA-based materials in soil is due to the usually low soil temperature and little PLA hydrolysis in the soil environment. Rudnik and Briassoulis (2011) also found a slow degradation of PLA films in a field soil and argued that the prevailing soil field temperatures (16–21°C) were below the minimum threshold temperatures for PLA degradation (>30°C). In our study at 25°C, the PLA-based material disintegrated, probably because the well-watered clay loam soil in the jars allowed a close film–soil contact and maintained a humid environment favouring hydrolysis. Additionally, relatively high organic matter content of the soil favours biodegradation processes by soil microorganisms. Mostafa *et al.* (2010) also found less degradation for BFx (70%) than for MB (80%) after 5 months of soil burial. Soil degradation of PLA- and MB-based films can vary sharply depending on the biodegradable copolyester blends and additives. Poly-butylene adipate/terephthalate (Ecoflex) is a copolyester commonly used for blending with some biopolymers (e.g. PLA, MB) (Martín-Closas and Pelacho 2011). The concentration of this copolyester in the blend has an effect on degradation. According to Mostafa *et al.* (2010), degradation in the soil is slower for Ecoflex alone than for MB but quicker than for BFx.

As expected, no visual changes were identified for PE, which did not show significant degradation, and an insignificant 0.8% weight loss was recorded.

#### *Chemical changes (FTIR analysis)*

Seven regions, together with their corresponding positions, were identified in the FTIR spectra of the materials tested (Table 3). During the soil burial test, no new peaks arose in the FTIR spectra of the biodegradable films (Fig. 3), as Rudnik and Briassoulis (2011) also report for MI and a PLA-based film. However, after 15 days of exposure, significant changes were found for all materials. Two modifications were seen throughout the study: the decrease in the absorbance of carbonyl ( $\text{-C=O}$ , 1840–1640  $\text{cm}^{-1}$ ) and hydroxyl ( $\text{-OH}$ , 3450–3300  $\text{cm}^{-1}$ ) bands. Carbonyl groups are part of the polyester structure, specifically of the ester bonds present in all tested biodegradable materials. Hydroxyl bonds belong to hydroxyl endings of starch (MB, BP, BF), dihydric alcohols (e.g. ethylene glycol) and  $\text{-OH}$  of 4-hydroxyalkanoic acid (MI). Thus, these results suggest that both carbonyl and hydroxyl groups were the main molecular bonds involved in the degradation arising from microorganism activity. BFx was the only film in which  $\text{-OH}$  bands were not detected, suggesting that at least to some extent, its slower degradation could be associated to lacking of hydroxyl ‘reactive bonds’ within its structure.

The MB, BF and BP spectra also showed absorbance decreases within the 1450–1050  $\text{cm}^{-1}$  range (Fig. 3), revealing changes in the vibrations of  $\text{-C-O}$ , and  $\text{-CH}_2$  groups and the presence of some additives in the mulches. Similarly, changes in the 1150–1065  $\text{cm}^{-1}$  range (Fig. 3) were detected for MI, which are characteristic of the asymmetric and symmetric stretching vibrations of the  $\text{C-O-C}$  group (Xu *et al.* 2002). On the other hand, in the BFx spectra, absorbance decreased from day 67 in the bands 3000–2840  $\text{cm}^{-1}$  and 1480–1350  $\text{cm}^{-1}$  (Fig. 3). According to Gonçalves *et al.* (2010), these bands can be assigned to  $\text{-CH}$  stretching ( $\text{-CH}_3(\text{Asym})$ ,  $\text{-CH}_3(\text{Sym})$  and  $\text{-CH}$  modes) and to  $\text{-CH}_3$  bending modes, respectively. All of these changes in the spectra represent secondary signals of the degradation of the carbonyl groups of the polymers.

#### *Carbonyl and hydroxyl index*

Structural changes of the films were detected by FTIR, which also allowed us to obtain quantitative information regarding carbonyl (-C=O) and hydroxyl (-OH) group modifications. However, due to the unavailability of detailed information about the chemical structure of the commercial and experimental films tested, the reference bands remain unknown. Therefore, because the 1480–1350  $\text{cm}^{-1}$  band assigned to the -CH<sub>3</sub> bending vibration was not altered by the degradation process, we used it as reference. Carbonyl index values (Table 4) allow the clustering of the materials into two groups. For the first group, MB and BF, carbonyl index continuously decreased along the incubation time (from 11.3 to 2.4 and from 6.7 to 1.9, respectively). For the second group, MI, BP and BFx, after an initial increase up to day 50 (MI, BP) or 116 (BFx), it suddenly decreased (from 3.7 to 1.1, 10.7 to 3 and 2.7 to 1, respectively). In the second group, the increase suggests that the -OH groups were susceptible to oxidation, which could produce additional -C=O (e.g. ketones, aldehydes, etc.). The consequent decrease in carbonyl index indicates that most of the -OH groups had already been degraded, prompting the degradation of compounds with -C=O groups.

For all materials, the -OH index decreased with incubation time (Table 5). MB, MI and BP showed greater decreases (from 2 to 0.7, 3 to 0.9 and 1.6 to 0.9, respectively), whereas for BF, the decrease was lower (from 1.8 to 1). This confirms that -OH groups were more susceptible to both chemical degradation and to microbial access than -C=O groups. Altogether, the dynamics of both indices are indicative of the bio/degradation extent of the films (Picuno 2014).

#### *Crystallinity index*

Biopolymer degradation is known usually to proceed in a selective manner, with the amorphous regions of the films being degraded before the crystalline ones (Tserki *et al.* 2006). Thus, the degree of crystallinity may vary throughout the degradation process. We used FTIR for quantitatively assessing crystallinity (Fakirov and Bhattacharyya 2007). However, it should be considered that the crystallinity index obtained (Table 6) is not the absolute degree of the polymer crystallinity. In the present study, the crystallinity index was calculated as the absorbance ratio of 1150–1065  $\text{cm}^{-1}$  (identified as C-O-C(Sym)) to 1028–875  $\text{cm}^{-1}$  (skeletal) band, the latter found to be constant for all materials tested.

From the start of the burial test to the end of the experiment, MB, BF and BP increased their crystallinity index (Table 6). This is most likely associated with the preferential degradation of the amorphous phase by the microorganisms, which led to the corresponding relative increase of the material crystallinity phase (Tserki *et al.* 2006). For BFx, crystallinity did not increase (Table 6), thus supporting the results of Rudnik and Briassoulis (2011), which show that the degradation (biodegradation) during soil burial is not pronounced for PLA-based films. For MI film, although the enzymatic degradation commences in the amorphous region of the polymer, in later stages, both amorphous and crystalline phases are degraded without preference (Djonlagic and Nikolic 2011). The slight but unexpected decrease in crystallinity index obtained may indicate a very advanced stage of degradation, where both amorphous and crystalline phase are very much degraded.

#### *Soil microbial activity*

The hydrolysis of FDA in the soil is considered a convenient index for determining microbial activity (Schnurer and Rosswall 1982). It can be used as an indicator of the soil microbial activity along with the biodegradation of the soil organic compounds such as biodegradable plastics. During the biodegradation process, different enzymes released by microorganisms to the soil environment may hydrolyse FDA. For all materials, FDA hydrolytic soil activity increased during the biodegradation of the biodegradable plastics (Fig. 4). Microbial activity increased most for BF and MI, up to 90 mg FDA  $\text{g}^{-1}$  dry soil (Fig. 4a). MB, BP and BFx followed a similar trend, reaching 70–80 mg FDA  $\text{g}^{-1}$  dry soil (Fig. 4b). Although this demonstrates that all biodegradable materials increased soil microbial activity, the minor microbial activity increase in non-degradable PE film is indicative of a priming effect, which arises by introducing an innocuous substance into most biological environments. In addition, some additives used in the PE film may suffer some biodegradation. Soil without material and abiotic control jars provided the lowest FDA hydrolytic soil activity values (Fig. 4). Thus, the soil microbial activity of the biodegradable mulch films tested in this study provides evidence that the constituents of the materials (raw materials and additives) are not toxic to the soil environment; on the contrary, to some extent they stimulate soil microbial activity.

Soil microbial activity proceeded concurrently to the increasing disintegration (Fig. 1), weight loss (Fig. 2) and chemical changes of the films (Fig. 3). All of these results support the degradation of the material through a biodegradation process. As shown for MI (Fig. 4), although the disintegration in the soil burial test ended at day 95 (Fig. 1), soil microbial activity continued to the end of the

experiment. The same was for BP and BF after they fully disintegrated at day 116. Thus, the biodegradation process of the film particles continues in the soil after fragmentation. The results suggest that the FDA assay, in combination with the soil burial test, provides a simple and reliable method for monitoring the biodegradation processes and the putative ecotoxic effects of bioplastics in soil. To our knowledge, this is the first report of FDA methodology being used for evaluating the biodegradation of plastics for agriculture.

### Conclusions

The plastic materials tested disintegrate in the soil at different times depending on composition. The disintegration timeframe varied from 116 (MI) to 160 (BP and BF) days or even to >180 days (MB and BFx). FTIR analysis identified several chemical changes during disintegration. The carbonyl and hydroxyl groups were the main bonds targeted by microorganisms during the degradation. Changes in the crystallinity index suggest a preferential degradation of the amorphous phase of the polymer matrices. During and after the disintegration period, soil microbial activity increased for all materials, demonstrating that the biodegradation process continues after disintegration of the films, without ecotoxicological effects on soil microorganisms. FDA hydrolysis was demonstrated to be a reliable and practical tool to monitor biodegradation and ecotoxicity on soil microbial activity.

We conclude that the commercial and experimental tested films are adequate for mulching in organic farming systems. In this context, they contribute to reducing the environmental impact of agriculture, especially providing an alternative to PE film. The tested films degraded in soil under laboratory conditions in a timeframe compatible with the most crops cultivated with mulching; further research is needed to provide more knowledge on the time course for the degradation of biodegradable materials under field conditions.

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Table 1. Product name, composition, manufacturer and thickness of the selected plastic mulching films  
Comm., Commercial material; expt, experimental material

Product name	Main composition	Manufacturer	Thickness (mm)
Biofilm (BF), comm.	Cereal flour, copolyester	Limagrain, Saint-Beauzire, France	20.8
Bio-Flex (BFx), comm.	Polylactic acid, copolyester	FKuR Kunststoff, Willich, Germany	14.2
Bioplast (BP), expt	Potato thermoplastic starch, copolyester	Sphere Group Spain, Zaragoza, Spain	11.6
Mater-Bi (MB), comm.	Maize thermoplastic starch, copolyester, vegetable oils	Novamont, Novara, Italy	14.7
Mirel (MI), expt	Polyhydroxybutyrate (PHB)	Metabolix, Cambridge, MA, USA	44.6
Polyethylene (PE), comm.	Lineal, low density polyethylene	Solplast SA, Murcia, Spain	15.1

Table 2. Soil physico-chemical properties

Moisture 1058C	1.90%
pH	8.1
Wilting point	10%
Field capacity	26%
Organic matter	3.13%
Total carbonates	29%
Total nitrogen concentration	0.084 g kg <sup>-1</sup>
Assimilable phosphorus concentration	0.030 g kg <sup>-1</sup>
Exchangeable potassium concentration	0.394 g kg <sup>-1</sup>
Ca	7.144 g kg <sup>-1</sup>
Mg	0.266 g kg <sup>-1</sup>
Na	0.044 g kg <sup>-1</sup>
Soil textural class	Clay-loam <sup>-1</sup>
Electric conductivity	0.5 dS m <sup>-1</sup>

Table 3. Main bands detected in the Fourier transform infrared (FTIR) spectra for the Mirel, Bioplast, Biofilm, Mater-Bi, Bio-Flex and polyethylene films

Band	Peak position (cm <sup>-1</sup> )
-OH hydroxyl stretch	3450–3300
-CH <sub>2</sub> stretch alkyl	3000–2840
-C=O carbonyl	1840–1640
-CH <sub>2</sub> - bending	1480–1350
-C-O-C- symmetric	1150–1065
-C-O- stretch	1070–1000
Skeletal (amorphous vibration)	1028–875

Table 4. Carbonyl index for the biodegradable mulch films tested during 158 days of burial test biodegradation in the soil  
MB, Mater-Bi; BF, Biofilm; BFx, Bio-Flex; MI, Mirel; BP, Bioplast

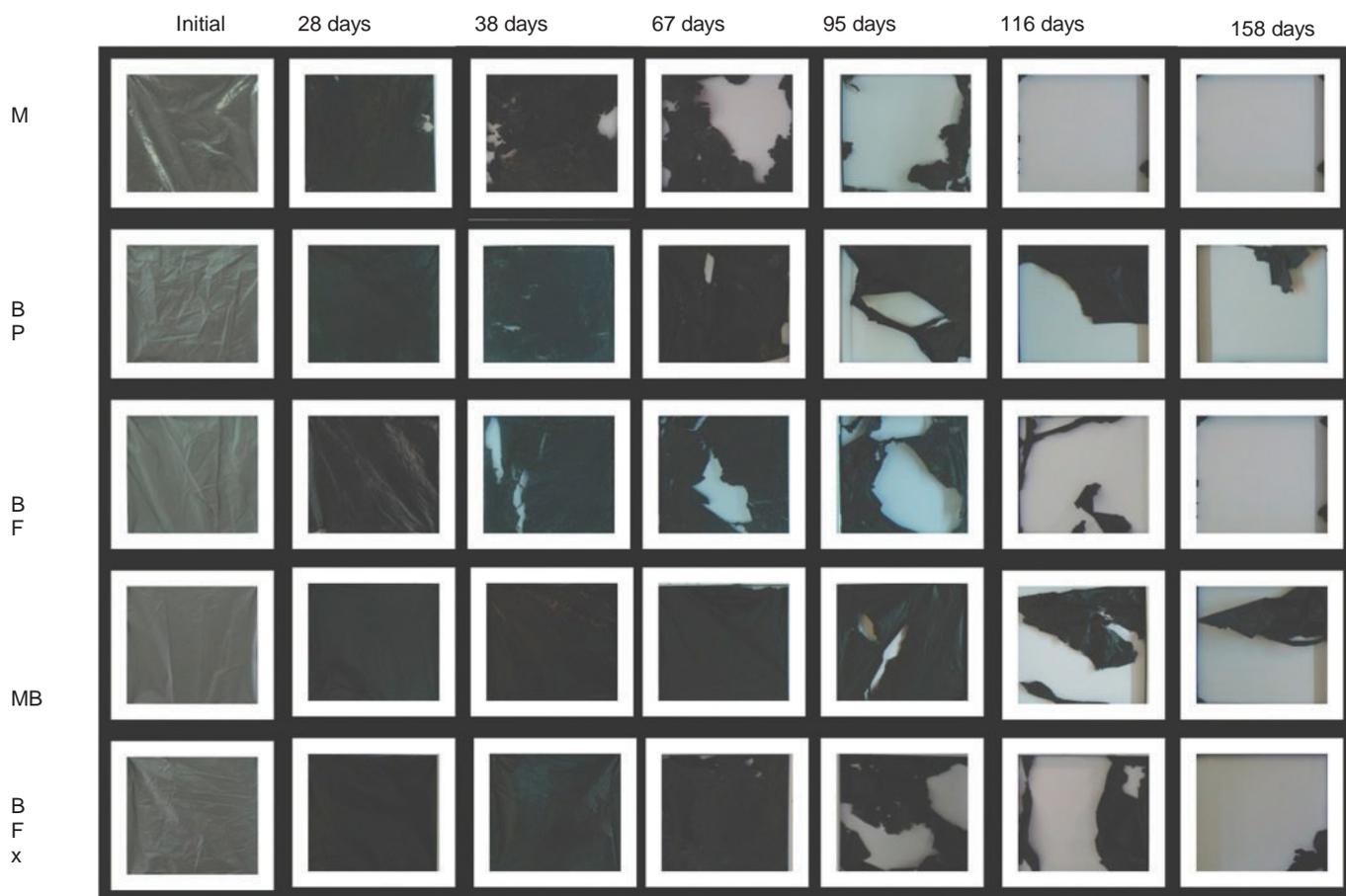
Test days	MB	BF	BFx	MI	BP
0	11.3	6.7	2.2	3.0	10.3
15	9.5	6.7	2.3	3.2	10.5
28	9.4	6.7	2.4	3.3	10.5
38	9.1	4.3	2.5	3.4	10.5
50	9.1	3.5	2.5	3.7	10.7
67	9.0	3.1	2.6	1.9	4.8
79	9.0	3.1	2.6	1.2	4.3
95	8.6	3.1	2.7	1.1	4.0
116	8.2	1.9	2.7	–	3.0
158	2.4	–	1.0	–	–

Table 5. Hydroxyl index for the biodegradable mulch films tested during 158 days of burial test biodegradation in the soil  
MB, Mater-Bi; BF, Biofilm; BFx, Bio-Flex; MI, Mirel; BP, Bioplast

Test days	MB	BF	BFx	MI	BP
0	2.0	1.8	–	3.0	1.6
15	1.1	1.0	–	1.5	0.9
28	1.1	0.9	–	1.2	0.9
38	1.0	1.0	–	1.2	0.9
50	1.0	1.2	–	1.0	0.9
67	0.9	1.1	–	0.9	0.9
79	0.9	1.1	–	1.0	0.9
95	0.8	1.0	–	0.9	0.9
116	0.8	1.0	–	–	0.9
158	0.7	–	–	–	–

Table 6. Crystallinity index for the biodegradable mulch films tested at the initial and final times of the 158 days of soil burial test  
(based on FTIR transmission spectra)  
MB, Mater-Bi; BF, Biofilm; BFx, Bio-Flex; MI, Mirel; BP, Bioplast

Sample	Initial	Final
MB	1.4	2.9 (158)
BF	1.4	1.6 (116)
BFx	2.7	2.7 (158)
MI	1.2	1.0 (95)
BP	1.2	1.8 (116)



**Fig. 1.** Physical changes of Mirel (MI), Bioplast (BP), Biofilm (BF), Mater-Bi (MB) and Bio-Flex (BFx) films at different biodegradation stages (28, 38, 67, 95, 116 and 158 days) in soil, under laboratory conditions.

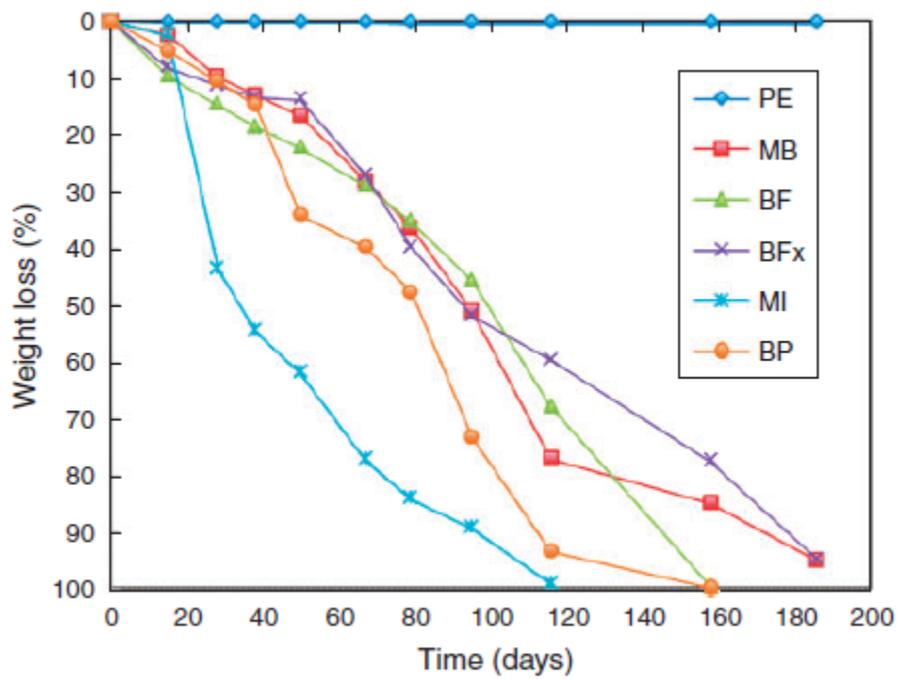
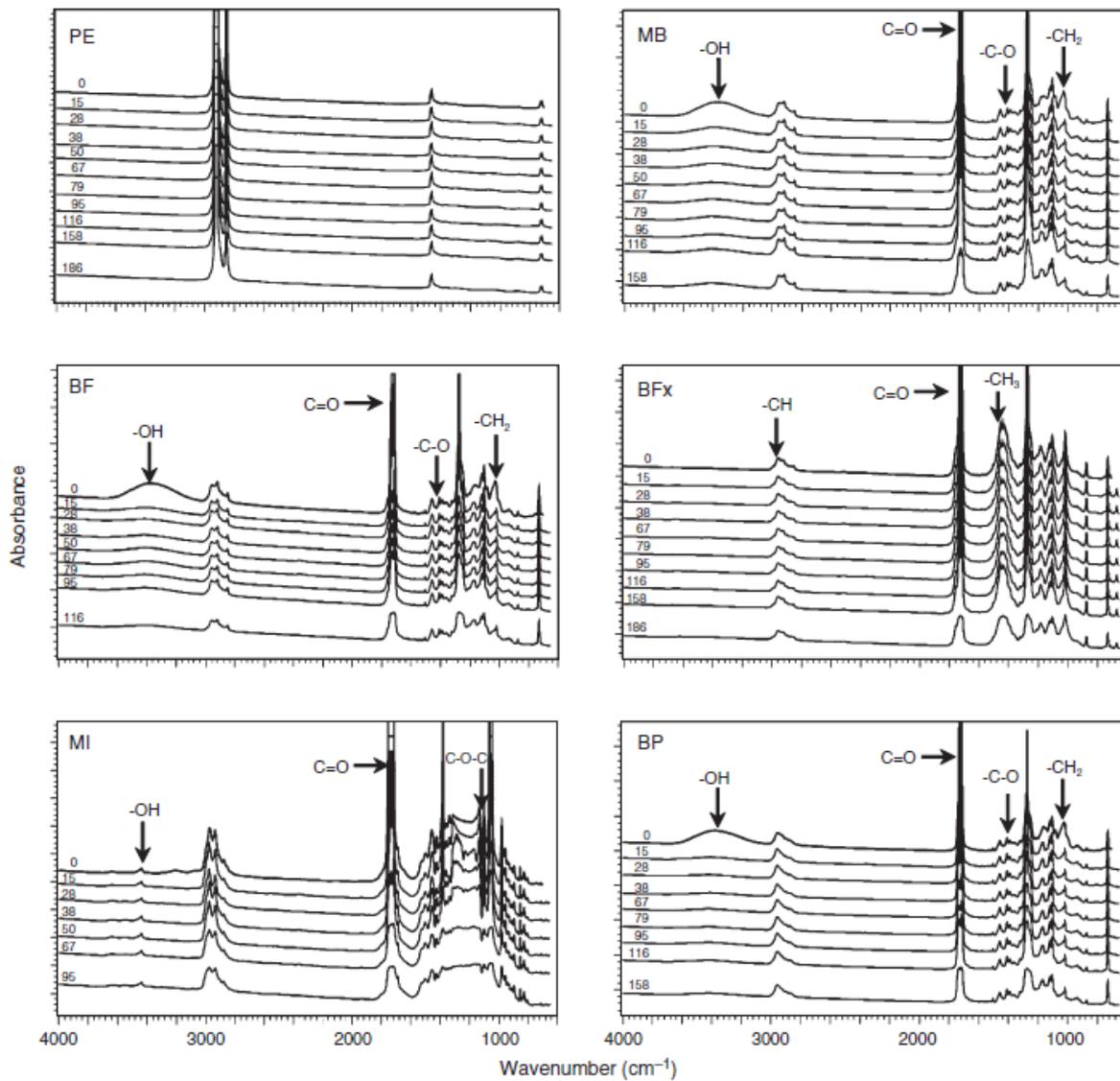
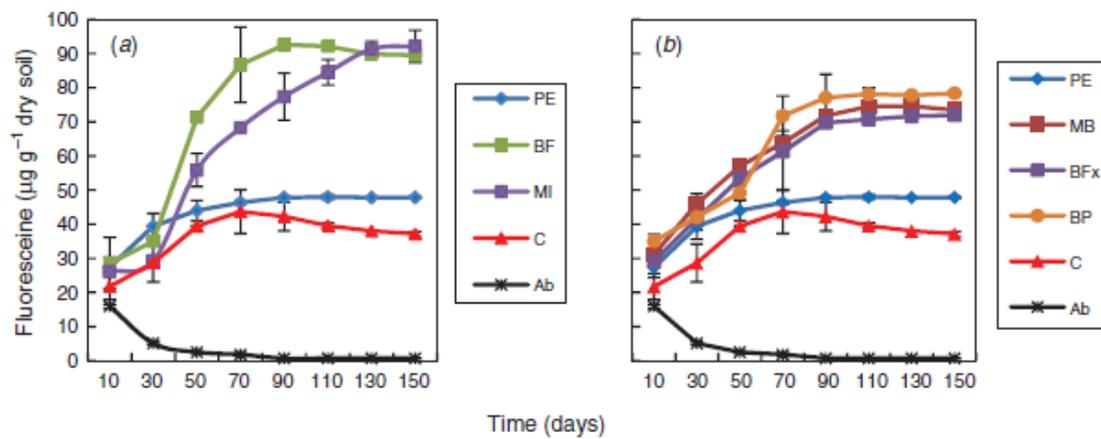


Fig. 2. Weight loss of the biodegradable mulch films Mirel (MI), Bioplast (BP), Biofilm (BF), Mater-Bi (MB), Bio-Flex (BFX) and polyethylene (PE) along 6 months of burial test in soil.



**Fig. 3.** Fourier transform infrared (FTIR) spectra of Mater-Bi (MB), Biofilm (BF), Bio-Flex (BFx), Mirel (MI), Bioplast (BP) and polyethylene (PE) films during the soil burial test.



**Fig. 4.** Evolution of soil microbial activity during degradation of the biodegradable plastics compared with polyethylene (PE), control soil (C), and abiotic soil control (Ab): (a) Biofilm (BF) and Mirel (MI); (b) Mater-Bi (MB), Bio-Flex (BFx) and Bioplast (BP). Bars indicate standard deviation.