Field studies on the deterioration of microplastic films from ultra-thin compostable bags in soil

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ABSTRACT

In recent years, some countries have replaced single-use plastic bags with bags manufactured from compostable plastic film that can be used for collecting food wastes and composted together with the waste. Because industrial compost contains uncomposed fragments of these bags, application to field soil is a potential source of small-sized residues from these bags. This study was undertaken to examine deterioration of these compostable film microplastics (CFMPs) in field soil at three different localities in Italy. Deterioration of CFMPs did not exceed 5.7% surface area reduction during the 12-month experimental period in two sites located in Northern Italy. More deterioration was observed in the Southern site, with 7.2% surface area reduction. Deterioration was significantly increased when fields were amended with industrial compost (up to 9.6%), but not with home compost. Up to 92.9% of the recovered CFMPs were associated with the soil fungus *Aspergillus flavus*, with 20.1% to 71.2% aflatoxin-producing isolates. Application of industrial compost resulted in a significant increase in the percentage of CFMPs associated with *A. flavus*. This observation provides an argument for government regulation of accumulation of CFMPs and elevation of hazardous fungi levels in agricultural soils that receive industrial compost.

Keywords

Bioplastic; biodegradable plastic; compost; soil; *Aspergillus flavus*; aflatoxins; mycotoxin; separate collection organic waste.

1. **Introduction**

First introduced in the late 1970s, single-use plastic bags have rapidly become the preferred choice for carrying purchased items, including packaged foods, clothes, and many other consumer products. Consumption of disposable petroleum-based plastic bags steadily increased over the years, reaching a global annual consumption of over a trillion units (Harrison et al., 2018). However, due to difficulties in proper disposal and recycling, and their long persistence in the environment, these lightweight bags pose a serious environmental threat (Accinelli et al., 2012; Xanthos and Walker, 2017). As with other thermoplastic products, prolonged exposure to sunlight and other physico-chemical agents result in formation of thin plastic particles, which subsequently fragment into small-sized particles (Rhodes, 2019). As proposed by Thompson et al. (2004), plastic fragments having size less than 5 mm are defined as microplastics (MPs). MPs generated from thin and ultra-thin disposable carrier bags are then easily transported by wind from urbanized areas into natural and agricultural areas, where they can enter the food chain and adversely affect water and soil quality (Balestri et al., 2019; Chae and Youn-Joo, 2018; Huerta Lwanga et al., 2016, 2017; Nizzetto et al., 2016). Consequently, plastic waste generated from single use plastic bags has prompted much debate, forcing many governments and municipalities to adopt or promote alternatives and restrictions to their usage. Replacing petroleum-based disposable carrier bags with compostable ones has become a common option in some countries, along with use of reusable totes and paper bags and other solutions (Battista et al., 2021; Dolci et al., 2021). For example, lightweight plastic bags (thickness < 50 µm) were initially banned in Italy in 2011, and in 2018 the ban was extended to ultra-thin (UT) plastic bags (thin < 15 μm). While the former were designed for carrying purchased packaged items from supermarkets and stores, the latter were intended for carrying unpackaged fruits and vegetables. Since UT compostable bags are thus the sole bags currently permitted for carrying loose fruits and vegetables from either supermarkets or local grocery stores in Italy, their annual consumption has increased rapidly up to 300 units per capita. These single use compostable bags are disposed of after their primary use by placing in organic waste bins along with food waste and other compostable items and processed together in industrial composting facilities. The resulting compost is then applied as a soil amendment to agricultural fields for improving soil structure, organic matter content, and other soil properties, including water holding capacity, etc. (Kranz et al., 2020). However, industrial compost is highly variable in terms of quality and technical parameters. Major parameters affecting compost quality include its maturity and stability, nutrient content, pH value, C/N ratios, and levels of chemical (e.g., heavy metals, pharmaceuticals) and physical contaminants, including glass and metal particles, and plastic fragments (Khalid et al., 2017). Compost should also be free of plant pathogenic agents and/or phytotoxins to avoid any negative effects on seed germination and seedling growth (Haas et al., 2016; Luo et al., 2018). Although different standardized procedures for evaluating compost quality are available, none of them take into consideration the number of millimeter-sized fragments of materials, such as compostable plastic particles, still present in the final product of the composting process, nor do they consider the impact material of that composition will have on the soil ecosystem. One explanation for this deficiency in existing evaluation procedures is that these protocols have been specifically designed to evaluate composting processes starting from material only composed of food waste and/or other biowaste residues (i.e., yard/garden wastes), with minor amount of impurities such as inert materials (i.e., glass, metal, and plastic fragments). The adoption of single use compostable bags into standard public use has inevitably resulted in their introduction into industrial composting processes at relevant levels (approximately 5% w/w of the composting mass). This situation has created a need for a better understanding of the potential impact of the small-sized compostable plastic particles present in industrial compost on the quality and functionality of soil to which it is added (Bläsing and Amelung, 2018; Lavagnolo et al., 2020; Weithmann et al., 2018). Among the various possible effects of adding biodegradable and compostable film fragments to soil is the possibility of altering the composition of the soil microbial community in ways that could result in adverse effects such as reduced seed germination or increased root infection by soil microorganisms (i.e., damping off) (Li et al., 2021; Ruggero et al., 2019). Specifically, it has been reported (Brodhagen et al., 2015; Moore-Kucera et al., 2014) that small-sized compostable film fragments, called compostable film microplastics (CFMPs), can promote the growth of soil-inhabiting filamentous fungi, including mycotoxin-producing species. In a previous laboratory-based study, it was demonstrated that CFMPs from UT compostable bags have the potential to persist in soil and to increase the size of the *Aspergillus flavus* population (Accinelli et al., 2020). The aim of the present research was to study the deterioration of CFMPs in soil under three typical field conditions and to investigate the potential effect of adding compostable bag-derived CFMPs in home or industrial compost on the persistence and population size of *A. flavus* by comparing values in amended and non-amended soils under those field conditions.

2. Materials and methods

2.1. Field sites, management and application of UT film samples

Three experimental sites were selected for this study, two located in Northern Italy in Montagnana (MO) and in Mezzolara (ME) and one in Southern Italy in Siracusa (SI). In all locations, experiments were conducted in flat and uniform fields (15 m x 15 m) that were uncropped during the entire 12-month experimental period (from September, 2019, to September, 2020). Selected properties of the three soils are summarized in Table 1. After harvesting wheat in June at the MO and ME sites and in May at the SI site, the soil was moldboard plowed and then disked three times. During the whole experimental period, the soil was not tilled. Fields were divided in to 3 blocks (3 m x 3 m), which were separated by a 1-m wide buffer area. Home or industrial composts were applied at the rate of 10 t ha^{-1} before disking. Industrial compost was obtained from an industrial compost facility located at Voltana di Lugo, Italy and operated by Herambiente s.p.a. (Bologna, Italy). Home compost (food waste only) was obtained by local restaurants and combined to achieve comparable properties to those of the industrial compost (Table 1).

Samples of UT films (12-μm thin) were prepared as described in Accinelli et al. (2020). Briefly, rectangles (2.8 cm x 6.0 cm) obtained from Mater-Bi® compostable bags (Novamont s.p.a., Novara, Itay) were retained between two high-density polyethylene plastic nets with openings of 2 mm x 2 mm. The same approach was adopted for preparing single square UT films with exposed surface of 2 mm x 2 mm. Both sample types were surface disinfected by UV exposure for 20 min and stored in sterilized glass tubes before inserting into the soil. Rectangular and single square UT films were buried into the soil at a 5-cm depth and identified by placing hardwood plant labels. Soil and assembled films were sampled throughout the experimental period using a soil auger (10 cm diameter and 20 cm height). Assembled films were separated from soil, and the remaining soil was gently homogenized by hands, air dried for 24 hrs., and then used for plastic fragment recovering and microbiological analysis.

2.2. Deterioration and fragmentation of UT film samples

Fragmentation of rectangular UT films was evaluated following the procedure described elsewhere (Accinelli et al., 2020). Briefly, assembled films were secured inside 50-mL centrifuge tubes, vortexed at low speed for 30 s, and then photographed with a dissecting microscope equipped with a Nightsea Fluorescence Adapter (Electron Microscopy Sciences, Hatfield, PA, USA). Images were uploaded into the ImageJ software version 1.53a (National Institutes of Health, Bethesda, USA), and film deterioration was estimated by summarizing areas showing lacerations and holes present in six central areas of exposed 4-mm^2 film.

Detached fragments were recovered from soil samples by laying 10 g of air-dried soil on a pre-warmed (120 °C) metal plate covered with a thin removable nylon 6,6 foil (150 µm thick) which was fixed on the plate. The metal plate was mounted on a shaking block and shook horizontally for 6 s. After 30 s of contact time, soil samples were discharged, and nylon foils with attached CFMPs were then removed from the plate and directly analyzed by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). ATR-FTIR analyses were performed using a Cary 630 FTIR spectrometer equipped with diamond ATR (Agilent Technology, Santa Clara, CA, USA) operating at room temperature with 64 scans within the range of 4000-650 cm^{-1} at 4 cm^{-1} resolution.

2.3. Aspergillus flavus recovery from UT film fragments and percentage of aflatoxigenic isolates

Soil samples from each burial point were processed using a patented benchtop electrostatic generator machine for separating UT fragments from soil (Accinelli, 2019). Soil samples (15 g of air-dried soil) were transferred to an oscillating metal plate, and fragments were separated from the soil using an electrostatically charged sterilized plastic film mounted 15 cm above the plate. The film was then transferred to a Petri plate containing modified Rose Bengal agar and incubated at 37 °C for 5-7 days (Abbas et al., 2004). *A. flavus* isolates were randomly selected and used for assessing their capability to produce aflatoxins. Briefly, isolates were incubated at 30 °C for 7 days in test tubes containing 2 mL of yeast extract sucrose broth, which was then extracted with chloroform, dried under vacuum, and redissolved in methanol/H_2O (70:30 v/v). Total concentrations of aflatoxin B1, B2, G1 and G2 were determined by HPLC as described elsewhere (Accinelli et al., 2020). Soil used for recovering film fragments was then used for quantifying *A. flavus* DNA by qPCR. Briefly, total soil DNA was isolated using the PowerSoil Isolation kit (Qiagen Ltd., Manchester, UK) and quantified using a BioDrop spectrophotometer (BioDrop Ltd, Cambridge, UK). Each 25 μL of reaction mixture contained 12.5 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.2 μM of each primer (Accinelli et al., 2012), and 40 ng of DNA. Samples were amplified on an Open qPCR (ChaiBio, Santa Clara, CA, USA) using the following conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A standard curve (r^2 = 0.92; efficiency = 94%; slope = − 0.21) was generated by plotting cycle threshold values (Ct) against logarithmic-transformed amounts of known *A. flavus* DNA.

2.4. Statistical analysis

Data were processed by one-way analysis of variance using the software package SPSS ver. 27 (IBM Corp., Armonk, NY, USA), and statistical significance was determined by Tukey's multiple comparisons test ($p < 0.05$).

3. Results and discussion

3.1. CFMP deterioration and fragment formation

The present experiment was conducted in three different experimental fields, two located in the North and one in the South of Italy. Weather conditions during the experimental period are shown in Figure 1. During the 12-month experiment, total rainfall was 848 and 605 mm in MO and ME, Northern Italy, respectively. Lesser rainfall was recorded in the Southern Italy site, SI. This site also experienced drought during the summer season, and temperatures never fell below 4.5 °C during the whole 12-month experimental period.

Deterioration of CFMPs that were buried into field soil at the three experimental sites is shown in Figure 2 (left panel). In both Northern sites in plots not receiving compost application, CFMPs showed reduced deterioration during the fall and winter seasons, with values that did not exceed 1.5%. More deterioration of CFMPs occurred during the June and September sampling operations, with values that reached 5.4 and 5.7% at the MO and ME sites, respectively. Similar deterioration patterns were observed in samples from plots amended with home compost. In contrast, amending the soil with compost from an industrial process resulted in a greater deterioration of CFMPs ($p < 0.05$). At the end of the 12-month experiment, CFMP deterioration in industrial compost plots was 7.0 and 7.5% reduction in surface area at the MO and ME sites, respectively. Deterioration of CFMPs at the site in Southern Italy was also more intense during the spring and summer season, with a final value of 7.2% reduction in surface area. The significant stimulatory effect of industrial compost was also observed at this site, in which CFMP deterioration reached 9.6% reduction in surface area.

Results from this field study are generally consistent with those of a previous laboratory study conducted using the same type of assembly and compostable film samples (Accinelli et al., 2020). However, less deterioration was observed under field conditions than under the more favorable laboratory conditions (i.e., soil samples incubated at 25 °C with soil moisture maintained at field capacity). Soil temperature and moisture level have well-known effects on the rate and extent of microbiological processes. In addition, the presence of more recoverable *A. flavus* propagules in soil during the last sampling operation, in early September, particularly at the Southern site, along with conditions favorable for *A. flavus* growth would be expected to contribute to increased degradation of compostable bioplastic, because *A. flavus* is known to be an efficient degrader of poly(butylene adipate co-terephthalate), the major component of compostable bioplastic (Accinelli et al., 2009, 2012, 2020; Moore-Kucera et al., 2014). This explanation for increased degradation is further supported by the observation that amending with the industrial compost was associated with increased degradation. Industrial composting processes use high temperatures (approximately 55-65 °C) to reduce the number of microbes in waste, including several human and plant pathogens, but *Aspergillus* species spores are relatively heat resistant (Franceschini et al., 2016). Consequently, the final product of industrial composting processes is expected to add *A. flavus* propagules to soils amended with it. This is not expected to occur in home composting, a process in which temperatures at such elevated values are never reached (Di Piazza et al., 2020; Franceschini et al., 2016). Thus, the higher deterioration of CFMPs in plots receiving the industrial compost plots at the three sites during the second half of the experimental period may at least partly be explained by increased number of heat-resistant species and spore-formers added with the compost and increased proliferation of these microorganisms as a response to the added organic matter and higher temperatures (Abbas et al., 2004, 2009).

As stated above, the main objective of these studies was to provide data under real field conditions to confirm a previous laboratory study of the deterioration of small-sized films (< 2 mm) from compostable plastic bags. This experimental system was considered a model of what happens when CFMPs enter the soil by compost application, especially when compost is obtained from urban organic wastes (Cattle et al., 2020; Corradini et al., 2021). In the European Union and many other countries existing regulations (e.g., EN 13432, 2002) do not consider the amount of microplastic industrial composting processes have left in their final product due to the need to minimize production costs. There are a number of different reasons for this regulatory oversight, including technical difficulties in recovering and separating small plastic-like fragments in order to monitor the wastes, and the expectation at the time compostable plastic bags were approved for use that the final industrial composting product would not be free of bag fragments. Industrial composting processes are usually operated at short residential times, usually 6-12 weeks (Lavagnolo et al., 2020). Field application of industrial compost is thus a potential source of MPs, including fragments from compostable plastic bags and from any petroleum-based plastic fragments that might have contaminated the waste stream (Accinelli et al., 2020).

During the last decade, a growing number of studies have focused on MP occurrence and their effects in the marine environment. More recently, there is also an increasing interest in focusing on agricultural soil, as a sink for MP contamination (Horton et al., 2017; Rillig, 2012; Scheurer and Bigalke, 2018). However, a major obstacle in studying MP occurrence, persistence and accumulation in soil are the technical challenges in recovering small-size plastic fragments from the heterogeneous and variable soil matrix (i.e., variability in particle size, level and nature of organic components, etc.). Most of the available methods for recovering MPs from soil are based on floatation or other density separation approaches. In the typical process, soil or sediment samples are first chemically or enzymatically digested to remove organic matter, then separated in an aqueous medium, from which samples are recovered by filtration and analyzed by Fourier transform infrared (FTIR) microscopy or Raman microspectroscopy (Bläsing and Amelung, 2018; Yang et al., 2021). More recently, other alternatives have been proposed, including critical fluid extraction, use of electrostatic forces, etc. (Fuller and Gautam, 2016). However, none of these methods have been designed for recovering compostable plastic film particles from soil or for studying their fate in soil ecosystems. A proposed novel method for monitoring CFMP fate in soil was developed and shown in the present studies to be very effective and easily applied under field conditions. Basically, dried soil samples are shaken onto a nylon foil, which had been pre-warmed to a temperature that causes partial melting, creating an adhesive consistency for detached compostable film fragments. Soil particles are removed by air-flush, while CFMPs remain stuck to the foil where they can be directly processed for analysis. Results of a recovery test are summarized in Figure 3. In samples of the three soils, recovery of CFMPs with sizes ranging from 0.1 to 4.0 mm^2 was above 97%. Addition of compost at the same dosage as that of the field experiment did not significantly affect CFMP recovery. Fragments can be easily visualized and enumerated using a simple dissecting microscope. For polymer identification, fragments are then directly analyzed by ATR-FTIR with no need of further costly equipment (e.g., FTIR microscope and dedicated software applications). Before developing this solution, single fragments were analyzed using a Survey IR microspectroscopy accessory, which was equipped with a high-resolution color video camera (SRA Instruments s.p.a., Milano, Italy). The accessory was mounted on the FTIR Cary 630 spectrometer. Unfortunately, this approach did not lead to reliable and consistent results. The procedure was time-consuming and some recovered fragments, including fragments with size larger than 1 mm, were not clearly visible, and no distinguished peaks were displayed. However, all fragments were correctly visualized and analyzed using the newly developed procedure described above (Fig. 4). These findings suggest that replacing an FTIR microscope with an FTIR spectrometer equipped with a scan camera accessory is not recommended for MP analysis.

Results obtained using this novel approach are summarized in Figure 2 (right panel). The total number of detached fragments increased over the 12-month experimental period. More specifically, higher increases were observed during the spring-summer period. Approximately 3.1-, 2.5-, and 6.2-times more fragments were recovered at the end of the experiment than in the initial 3 months, at the MO, ME and SI sites, respectively. In all three sites, significantly more fragments were recovered from industrial compost plots than from unamended plots, but the increases were not observed with home compost amendment. These results are consistent with those of a previous laboratory study and they show that CFMPs were not rapidly degraded in the soil, and thus have the potential to affect soil fertility and other ecological processes, including soil organic matter evolution and turn-over, microbial processes and microbial composition. Given that a large fragment can generate multiple small fragments in the course of deterioration, the observed differences in CFMP numbers were consistent with CFMP deterioration data, in which the SI site showed the highest number of recovered fragments. The data presented here suggested the power of this novel approach for measuring deterioration and persistence of bioplastic items in soil. Monitoring MPs numbers in soil is expected to provide very useful and practical information for regulatory agencies.

3.2. Occurrence of A. flavus in compostable films and soil

The three experimental sites were also characterized by having soils with different sizes of the indigenous *A. flavus* population in addition to differences in weather conditions and soil type (Table 1; Figure 1). The level of *A. flavus* was monitored during the 12-month experimental period (Figure 5). The size of the *A. flavus* population remained relatively stable over the whole 12-month period in both non-amended and plots amended with home compost. In contrast, during the second half of the period, the size of the *A. flavus* population significantly increased ($p < 0.05$) in plots receiving the industrial compost, especially at the SI site. The results are consistent with industrial compost both adding *A. flavus* propagules and stimulating indigenous *A. flavus* proliferation with the added nutrients in the form of CFMPs, as was demonstrated in a previous laboratory-based study (Accinelli et al., 2020).

Buried CFMP fragments were recovered from soil using a technique based on electrostatic charges, incubated on a selective medium, and the percentage of *A. flavus*-infected fragments recorded. In all three sites, the percent of *A. flavus* infected fragments increased over the 12 months (Table 2). Although application of home compost stimulated CFMPs deterioration in soil (Figure 3), this soil amendment did not affect ($p > 0.05$) the percent of infected fragments. In contrast, the percent of *A. flavus*-infected fragments significantly increased ($p < 0.05$) in plots amended with industrial compost. More specifically, at the end of the experiment, the percent increase of infected fragments from these plots was 79.0, 69.3, and 92.9 % in the MO, ME, and SI sites, respectively. The SI site was selected in this study for its low level of soil *A. flavus* and hot, dry summers (Table 1; Figure 1). The results from this site confirmed that *A. flavus* is a major colonizer of poly(butylene adipate co-terephthalate)-based compostable films, especially when environmental conditions are favorable to this fungus, such as at the SI site (Accinelli et al., 2009, 2020; Accinelli and Abbas, 2011; Moore-Kucera et al., 2014). More than 24% of the *A. flavus* isolates that were recovered from CFMPs were capable of producing aflatoxins (Table 2). This percentage increased over the experimental period, reaching values of 60.1, 57.3, and 59.9% at the MO, ME, and SI site, respectively. Samples from home and industrial compost plots showed similar values, except that at the SI site. At this site, the percent of aflatoxin producing isolates reached a value of 71.2%. Although the capability of *A. flavus* isolates to produce aflatoxins has been the subject of numerous investigations, factors affecting ratios of aflatoxigenic to non-aflatoxigenic isolates have still not been clarified. Some studies indicated that aflatoxin-producing isolates have competitive advantages for colonizing nutrient-rich substrates in soil, such as plant residues (e.g., corn residues) or seeds (e.g., corn, peanut seeds, etc.) (Abbas et al., 2008; Accinelli et al., 2008, 2018). The high percentage of aflatoxigenic *A. flavus* isolates recovered from CFMPs is consistent with these observations. Aflatoxins are regulated contaminants of food and feed, and most published studies have focused on the occurrence of aflatoxigenic *A. flavus* and concentrations of aflatoxins in edible products. Only a few studies have investigated on the soil ecosystem (Accinelli et al., 2009). High levels of soil inhabiting aflatoxigenic *A. flavus* isolates are expected to lead to increased infection of crop plants where they pose a health risk in foods and feeds, and they are expected to produce carcinogenic aflatoxins in the organic matter they inhabit as a way to compete with other soil microorganisms and those aflatoxins pose a serious health risk for wildlife, particularly birds. The effect of CFMPs from added industrial compost on soil should be considered by agricultural scientists and regulatory agencies.

4. Conclusions

Field studies on the on the deterioration of small-sized fragments from compostable ultra-thin plastic bags in soil confirmed results from a previous laboratory study.

Deterioration of small compostable film microplastic fragments that were buried in field soil at three different locations proceeded very slowly during the entire experimental period of 12 months. Compostable film microplastic fragments from bags used for collecting food wastes that are composted together with the waste in standard practice and get added as an amendment to field soil persist as small-sized fragments (< 2 mm) and were found to have been extensively colonized by the fungus *A. flavus* when recovered from amended soil. Although the application of industrial compost resulted in a greater deterioration of these film fragments, it also increased the size of the soil population of *A. flavus* and the percentage of isolates capable of producing aflatoxins. Compostable film microplastic fragments added to soil with industrial compost are not currently taken into account by regulatory agencies, but the observed effects on soil *A. flavus* and its aflatoxigenicity suggest that they should be.

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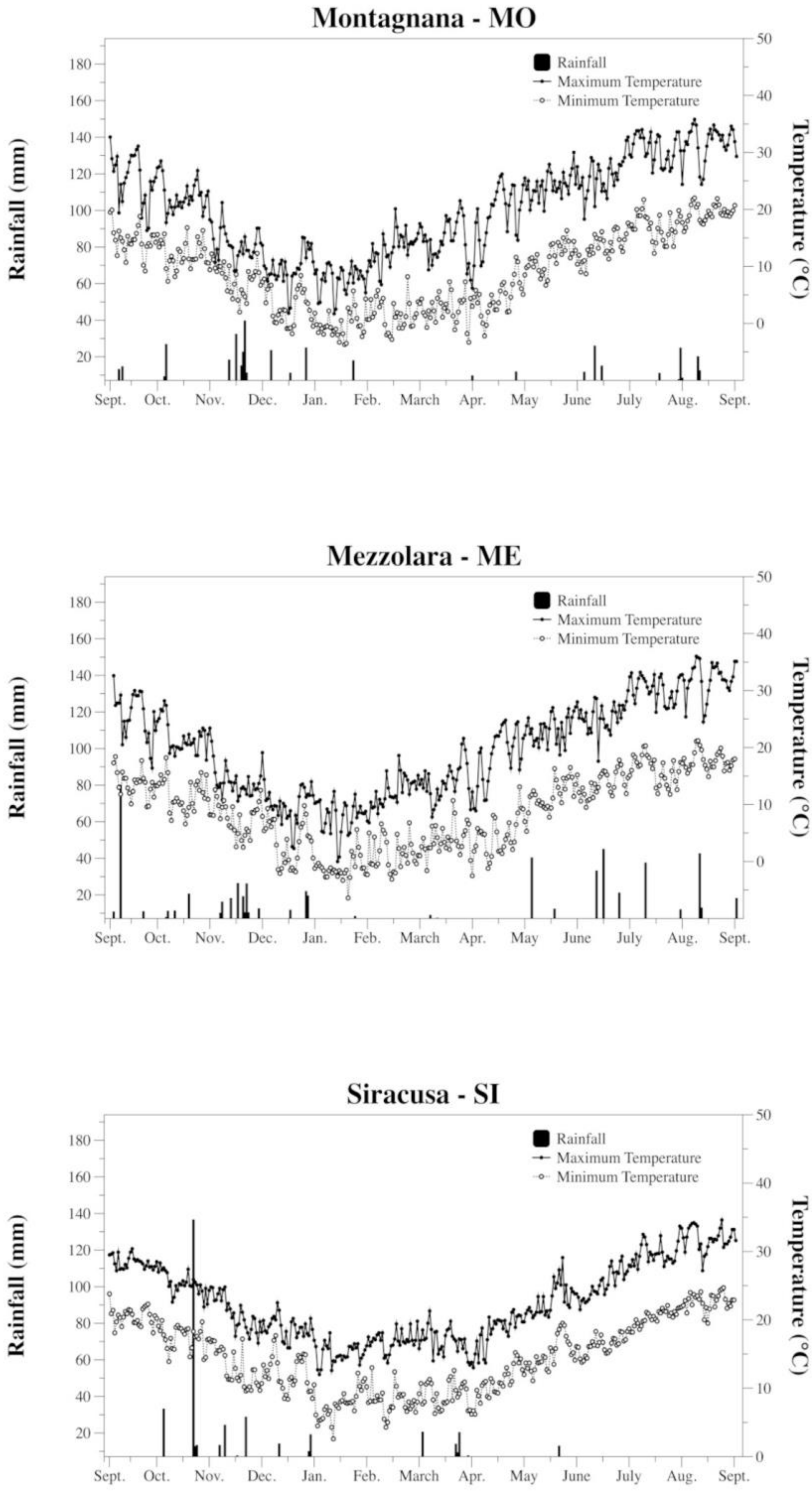

Figure 1. Meteorological data recorded at the three experimental sites (Montagnana, Mezzolara and Siracusa) from September 2019 to September 2020. Maximum and minimum daily temperatures are shown with closed and open circles, respectively. Rainfall data are shown as solid bars.

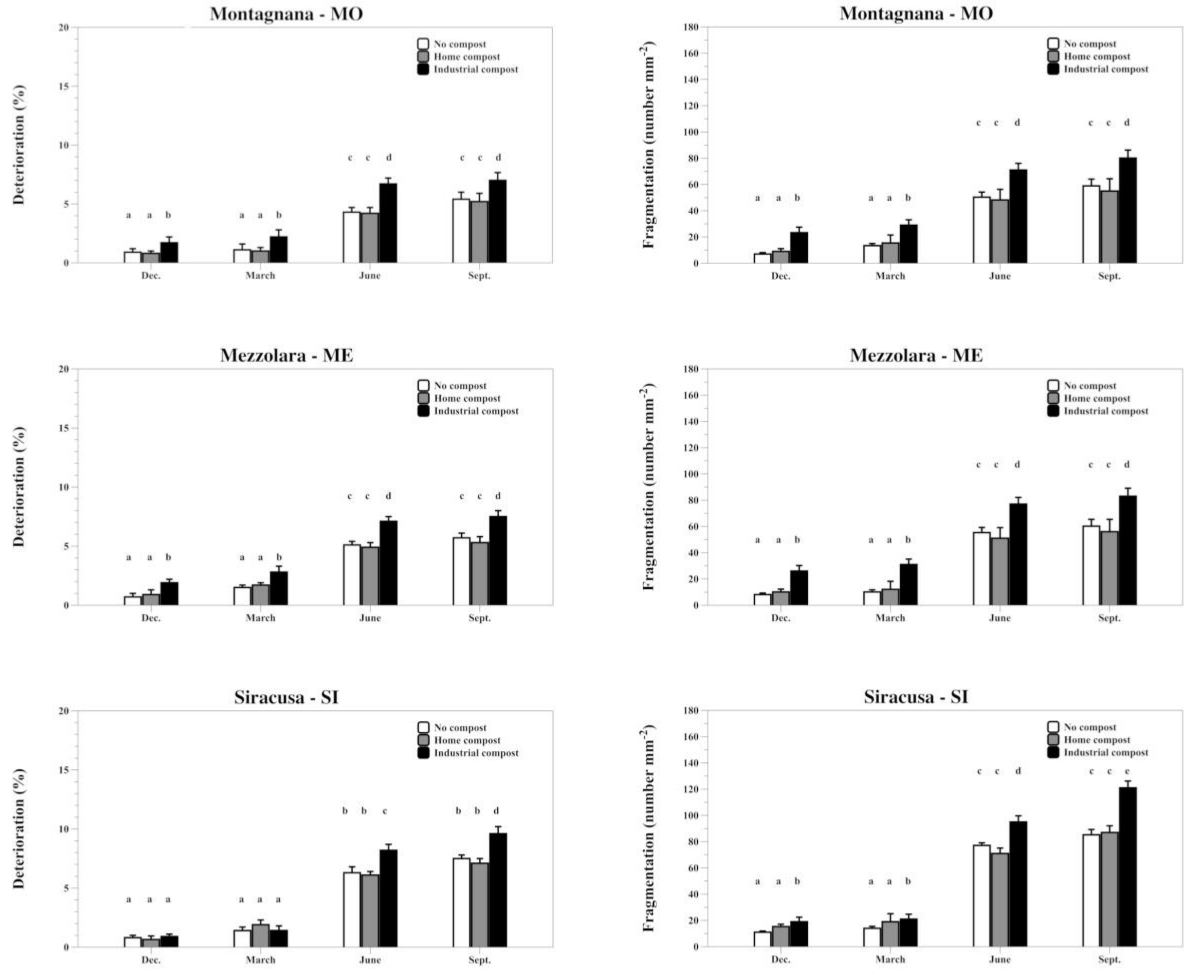

Figure 2. Deterioration and fragmentation of CFMPs in field soil during a 12-month period starting from September 2019. CFMPs were buried in experimental field plots located in three sites, two in Northern Italy (Montagnana, Mezzolara), and one in Southern Italy (Sirucusa). Deterioration, measured as % reduction in the total surface area of CFMPs, is shown in panels on the left. Fragmentation during the same experimental period, measured as number of recoverable-sized fragments detached from CFMPs, is shown in panels on the right. Field plots were non-amended or amended with home or industrial compost. Bars with same letters are not significantly different from each other ($p > 0.05$).

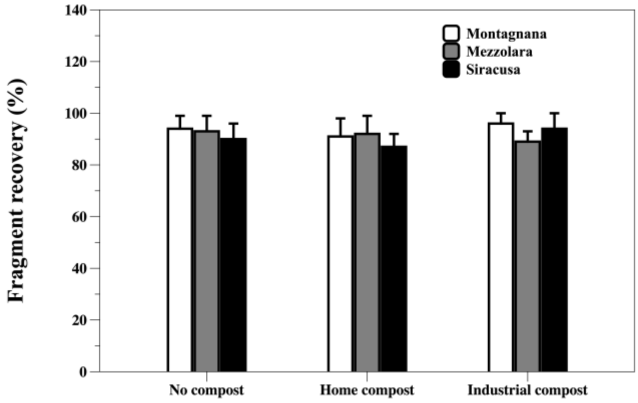

Figure 3. Results of a validation study assessing the percent recovery of small-sized fragments (0.1-4 mm^2) obtained from CFMPs. A fixed numenr of fragments were mixed with soil samples collected from non-amended and home or industrial compost-amended plots of the three experimental sites (Montagnana, Mezzolara, Siracusa). Bars are means of four replicates ± STD. Data were not significantly different ($p > 0.05$).

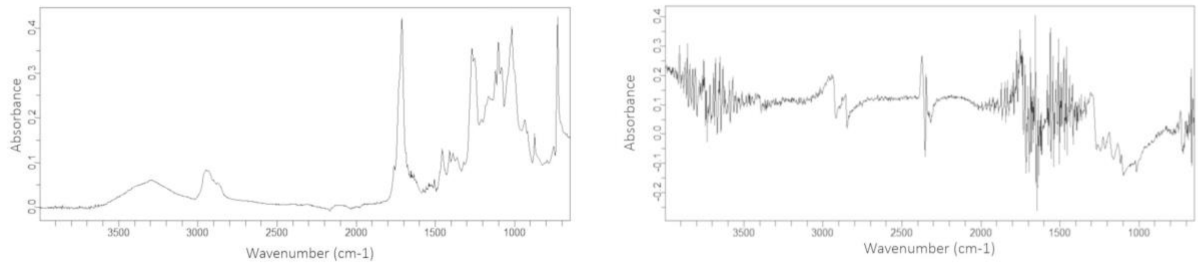

Figure 4. FTIR spectra of a compostable film microplastic sample obtained using the Agilent Diamond ATR Cary 630 module (left) and the SurveyIR infrared micro-spectroscopy accessory (right).

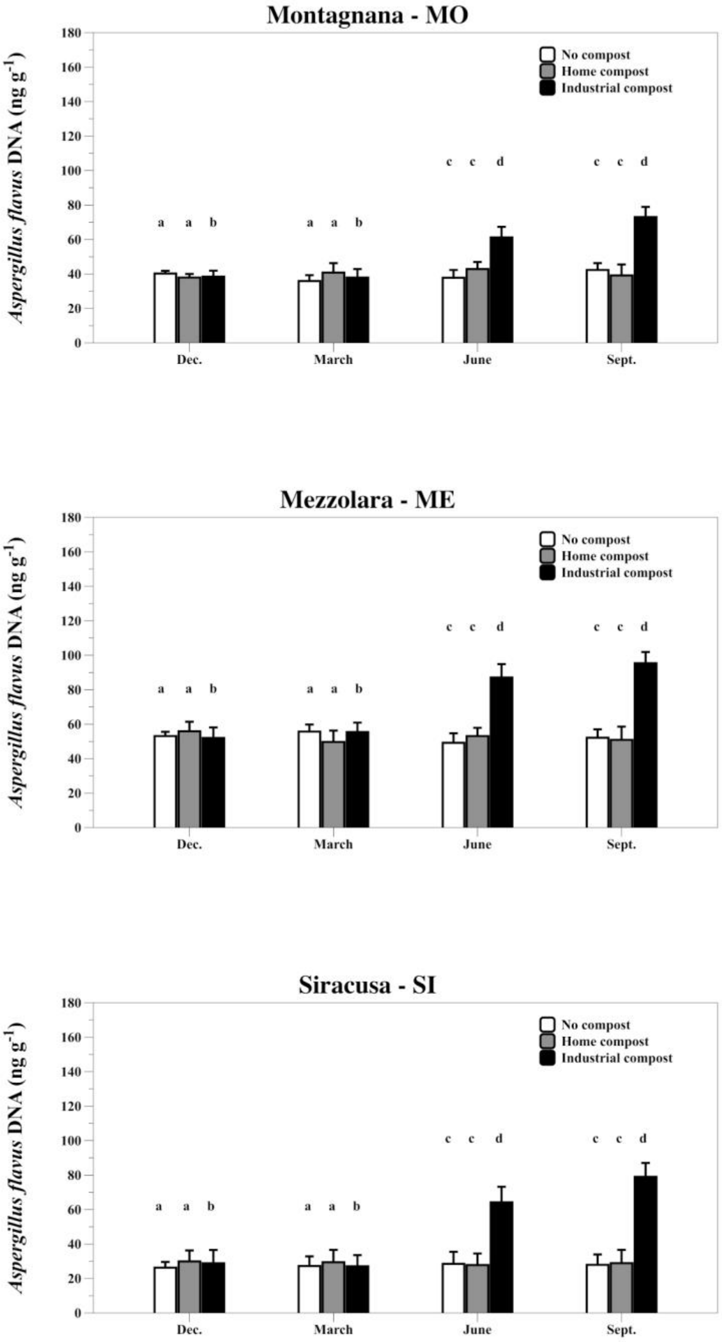

Figure 5. Quantification by qPCR of total soil *Aspergillus flavus* DNA recovered from field plots that were non amended or amended with home or industrial compost. The 12-month study was started in September 2019 and was conducted in three localities, two in Northern Italy (Montagnana, Mezzolara), and one in Southern Italy (Siracusa). Bars with same letters are not significantly different ($p > 0.05$).

Table 1. Selected properties of soils at the three experimental sites and of the home and industrial compost.

Experimental site	Soil textural class			pH	Organic Carbon	*Aspergillus flavus* level
	Sand	Silt	Clay			
	(%)	(%)	(%)		(g kg^{-1})	(cfu g^{-1})*
Montagnana	39.1	40.3	20.6	7.8	1.5	2.8
Mezzolara	36.4	45.2	18.4	8.0	1.0	3.1
Siracusa	58.3	20.6	21.1	8.2	1.3	1.1
Home compost	-	-	-	7.6	28.3	1.5
Industrial compost	-	-	-	7.9	26.5	6.1

* Enumeration of colony forming units (cfu) of *A. flavus* was by the procedure of Accinelli et al., 2009.

Table 2. Percent of detached CFMP fragments in soil infected by the fungus *Aspergillus flavus* and percent of aflatoxin-producing *A. flavus* isolates. Fragments that became detached during deterioration of CFMPs buried in soil in field plots at three localities (Montagnana, Mezzolara, Siracusa), during a 12-month experimental period starting from September 2019 were recovered from soil samples and examined for *A. flavus* culturable on modified Rose Bengal agar. Aflatoxin production was determined on selected isolates by HPLC analysis of yeast extract sucrose culture broths. Values with same letter are not significantly different ($p > 0.05$).

		% of fragments infected with *A. flavus*			% of *A. flavus* isolates producing aflatoxins		
Site	Month	Unamended	Home compost	Industrial compost	Unamended	Home compost	Industrial compost
Montagnana	March 2019	29.1 a	31.2 a	27.3 a	24.0 a	26.8 a	29.1 a
	Sept. 2020	61.3 b	58.9 b	79.0 c	60.1 b	57.7 b	63.1 b
Mezzolara	March 2019	21.0 a	28.0 a	24.3 a	20.1 a	24.4. a	27.2 a
	Sept. 2020	49.4 b	51.1 b	69.3 c	57.3 b	51.0 b	59.2 b
Siracusa	March 2019	22.1 a	25.3 a	21.2 a	25.5 a	27.0 a	29.1 a
	Sept. 2020	57.4 b	62.9 b	92.9 c	56.9 b	49.9 b	71.2 c