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Cesare Accinelli [a, *], Hamed K. Abbas [b], Veronica Bruno [a], Lorenzo Nissen [a], Alberto Vicari [a], Nacer Bellaloui [c], Nathan S. Little [d], W. Thomas Shier [e]

[a] *Department of Agricultural and Food Sciences, Alma Mater Studiorum - University of Bologna, Bologna 40127, Italy*

[b] *USDA-ARS, Biological Control of Pests Research Unit, Stoneville, Mississippi 38776, USA*

[c] *Crop Genetic Systems Research Unit, US Department of Agriculture, Agricultural Research Service, Stoneville, MS 38776, USA*

[d] *USDA-ARS, Southern Insect Management Research Unit, Stoneville, Mississippi 38776, USA* [e] *Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, USA*

** Corresponding author.*

E-mail address: cesare.accinelli@unibo.it (Cesare Accinelli)

ABSTRACT

An increasing number of states and municipalities are choosing to reduce plastic litter by replacing plastic items, particularly single-use ones, with same-use products manufactured from compostable plastics. This study investigated the formation and persistence of compostable film microplastic particles (CFMPs) from ultra-thin compostable carrier bags in soil under laboratory conditions, and the potential impact of CFMPs on *Aspergillus flavus* populations in the soil.

During a 12-month incubation period, compostable film samples in soils with small, medium or large populations of indigenous *A. flavus*, underwent 5.9, 9.8, and 17.1% reduction in total surface area, respectively. Despite the low levels of deterioration, the number of CFMPs released increased steadily over the incubation period, particularly fragments with size < 0.05 mm. Up to 88.4% of the released fragments had associated *A. flavus* and up to 68% of isolates from CFMPs produced aflatoxins. *A. flavus* levels associated with CFMPs increased rapidly during the initial part of the 12-month incubation period, whereas the percent aflatoxigenicity continued to increase even after *A. flavus* density leveled off later. During 12 months incubation, *A. flavus* DNA amounts recovered from CFMPs increased in soils with all levels of indigenous *A. flavus*, with the largest increases (119.1%) occurring in soil containing the lowest indigenous *A. flavus*. If confirmed in field studies, these results suggest that burying compostable film in soil, or application of compost containing CFMPs, may reduce soil quality and increase risk of adverse impacts from elevated aflatoxigenic *A. flavus* populations in soil.

Keywords

Plastic; compostable plastic; compost; microplastic; *Aspergillus flavus*; aflatoxins

1. Introduction

Petroleum-based plastic has played a crucial role in modern societies since the beginning of its large-scale production in the early 1950s. Due to its versatility and ease of processing, plastic is used for manufacturing a wide variety of products, including durable and single-use items for both domestic to industrial applications (Andrady and Neal, 2009). Nearly 40% of the global plastic production (~ 120 million tons) is represented by single-use packaging items, including carrier bags

(Kale et al., 2007; Hopewell et al., 2009; Geyer et al., 2017). Less than 30% of post-consumer plastic is recovered for reuse or recycling for various reasons, including difficulties of sorting plastic products from mixed waste, high cost of separate collection, etc. (Geyer et al., 2017). In the case of single-use plastic packaging, especially lightweight (LW; plastic bags less than 50 μm thick) and ultra-thin (UT; thickness less than 15 μm) plastic bags, percentages of recovery are even lower, with the bulk of these products being incinerated or ending up in landfills (Rivers et al., 2017). However, due to their light weight and parachute-shaped design, these plastic bags are also inadvertently dispersed in the environment. Because LW and UT plastic bags are commonly manufactured from high-density polyethylene, their persistence in the environment (e.g., soil, sea) as litter is a major environmental issue (Napper and Thomson, 2019; Accinelli et al., 2012). In addition, after long exposure to sunlight and to atmospheric agents, they become brittle and readily fragment into small-sized, persistent particles, usually referred to as microplastics (MPs) (Ryan and Moloney, 1990; Barnes et al., 2009). The term MP was originally proposed by Thompson et al. (2004) in their studies on the effects of plastic debris on marine ecology. MPs have since been found in surface water, debris and soil, as well as seawater. MPs are defined not by the type of plastic they are made of, but by particle size, so that MPs are defined as plastic particles that pass through a 5-mm sieve (Arthur et al., 2009). Two major classes of MPs have been defined: (i) primary MPs, which were plastic particles that were < 5-mm when they entered the environment, such as microfibers from clothing, microbeads added to cosmetics as exfoliants and microgranules added to detergents as foam suppressants; and (ii) secondary MPs, which are < 5-mm plastic particles generated in the environment through fragmentation of larger plastic items by natural weathering processes. An increasing volume of evidence has shown that MP particles and films can be ingested by

wildlife, thus entering the food chain (Van Franeker et al, 2011; Besseling et al., 2013; Huerta Lwanga et al., 2017). Other studies have indicated that the environmental impact and health risk of MPs is greater than originally expected due to their potential to selectively adsorb xenobiotics, and other chemical substances, especially those with low water solubility (Velzeboer et al., 2014; Hodson et al., 2017; Huerta Lwanga et al., 2016).

During the last decade, governments and other organizations worldwide have tried to reduce the use of disposable plastic bags that are usually made of high-density polyethylene in the form of thick film produced by the blown film extrusion process. In England, Ireland, Greece, and other countries, the use of plastic bags plummeted following the introduction of a charge for LW plastic bags (Xanthos and Walker, 2017). In other countries, including France, Italy, South Africa, and some states in the United States have passed laws banning LW plastic bags. Alternatives to LW plastic bags are available and allowed, including disposable paper bags, reusable tote bags and compostable LW plastic bags, which are the preferred choice for many supermarket consumers. In Italy, LW plastic supermarket bags were banned in 2011, but UT plastic bags were excluded from the ban until 2018, when the ban was extended to all but UT compostable plastic bags, resulting in the market for this category of bags increasing to approximately 500 million dollars. Single-use UT compostable plastic bags are manufactured with compostable plastic, and intended to be disposed in organic waste collectors and then composted in industrial facilities.

In the last 40 years there has been increasing interest in using compost from biological wastes in agriculture as a soil organic matter amendment. Source-separated collection and composting of organic waste have gained importance as an approach to reducing carbon footprints. However, use of different sorting procedures and composting technologies results in differences in compost quality as measured

by factors such as total organic carbon content, C/N ratios, pH values, etc. Compost quality is also affected by physical contaminant levels, including glass, metal and plastic particles, which are routinely assessed gravimetrically by sieving the compost through 4 and/or 2-mm sieve, then sorting and weighing unpassed material (Khalid et al., 2017). Although thresholds for physical contaminants larger than 4 or 2 mm have been established in various countries, the content of smaller-sized impurities, such as plastic and compostable plastic particles, is not regulated or measured. As a result, it is not known what effect application of compost from urban waste to agricultural fields will have on MP contamination of agricultural soil, which may subsequently enter surface waters (Rilling, 2012; Nizzetto et al., 2016b; Horton et al., 2017; Rilling et al., 2017; Bläsing and Amelung, 2018; Weithmann et al., 2018; Chae and Youn-Joo, 2018). Despite the growing concern about terrestrial contamination by MPs, many other aspects of the phenomenon remain unclear or unexplored, particularly for MPs generated from biodegradable and/or compostable plastic packaging products. For example, investigations conducted with film fragments from biodegradable agricultural mulching films have shown that these fragments are frequently colonized by soil-borne fungi, including the aflatoxin-producing fungus *Aspergillus flavus* and other mycotoxigenic fungi (Moore-Kucera et al., 2014).

The present study was undertaken to investigate the effect of UT compostable carrier bags in soil in a defined, laboratory-based experimental system, in particular the persistence of CFMPs in soil and their potential to alter the population size and toxigenicity of *A. flavus* in CFMP-containing soil.

2. Materials and methods

2.1. Soil selection

Three soils with different *A. flavus* indigenous population sizes were selected for this study. Soils with low, medium, and high levels of *A. flavus* propagules were collected in agricultural farms located in Northern Italy, Southern Italy and the Mississippi Delta (U.S.), respectively. Properties of the three soils are presented in Table 1. In each location, soil samples were collected from the topsoil layer (0-10 cm), passed through a 2-mm sieve and kept at 4 °C for no longer than 2 weeks.

2.2. Preparation of UT compostable plastic film samples

UT compostable bags (12-µm thin) obtained from Polycart S.p.A. (Perugia, Italy) were cut into 2.8 cm x 6.0 cm rectangles or into 2.1 mm x 2.1 mm squares. In both cases, compostable film samples were prepared to achieve single 2 mm x 2 mm exposed film surfaces. Rectangle films were placed between two polyethylene nets with mesh opening of either 2-mm or and 5-mm square, secured by hot-melt glue and then surface disinfected, as described in Accinelli et al. (2012).

Table 1. Properties of the three soils used in this study. Soil samples were collected from agricultural fields located in Southern (Low Af from Catania, Italy) and Northern Italy (Medium Af from Padova, Italy), and the Mississippi Delta (High Af from Stoneville, Mississippi, USA). Values are average of five replicates.

Natural soil *Aspergillus flavus* level[a]	Soil textural class (particle size)			pH	Organic C	*Aspergillus flavus* level
	Sand	Silt	Clay			
	(%)	(%)	(%)		(g kg^{-1})	(cfu g^{-1})[b]
Low Af	60.2	8.1	31.7	8.0	1.2	2.9
Medium Af	31.6	59.6	5.8	8.1	6.4	3.1
High Af	32.7	59.2	8.1	5.8	9.4	4.3

[a] Low natural *A. flavus* levels were found in soil from Northern Italy (Low Af); medium levels were in soil from Southern Italy (Medium Af); and high levels were in soil from the Mississippi Delta, USA (High Af).
[b] Enumeration of colony forming units (cfu) of *A. flavus* by the procedure of Accinelli et al., 2014

2.3. Soil incubation of UT compostable plastic film samples

Assembled UT rectangular films were then secured in centrifuge tubes (3.1 cm diameter x 11.5 cm long) containing 50 g of soil. Another group of soil-containing tubes were prepared with 2.1-mm square films, which were placed between two 2-mm square mesh of the same type described above. Each of these tubes contained 50 g of soil and 4 square films. In both rectangular or squared films, soil moisture of each sample type was adjusted to the field capacity and samples were incubated at 25 °C in the dark in a ventilated incubator for 12 months. Soil moisture was monitored at two-day intervals and water added as needed. At selected intervals, samples were removed and processed for film degradation evaluation and microbiological analysis. The experiment with rectangular UT films was also carried out using film samples obtained from food waste disposal bags manufactured with recycled paper (Sumus Italia s.r.l., Padova, Italy). For each film type and soil, six replicates were prepared, including tubes with no films as controls.

2.4. Measurements of film deterioration and fragments enumeration

At each sampling time, soil from tubes containing rectangular and square-shaped UT films was transferred to clean 50-mL centrifuge tubes and placed under a dissecting microscope equipped with a Nightsea Fluorescence Adapter (Electron Microscopy Sciences, Hatfield, PA). Images were analyzed using the software imageJ version 1.5oi (National Institutes of Health, Bethesda, USA) and film degradation was calculated considering the total area of visible lacerations and holes, with respect to the exposed surface area (2.0 mm^2) of single squares. In the case of the rectangular-shaped films, measurements were done considering the single 6 central 5 mm^2 grid areas.

Fragments that were released from compostable film samples in the soil were recovered by a modified density separation approach (Coppock et al., 2017). A 50-mL centrifuge tube (lower tube) was connected to another tube with the same diameter (upper tube) by means of a ball valve, mounted on a 4-mL cylinder. A volume of 52 mL of a glycerol/water mixture (7:3, v/v) and 5 g of soil were loaded into the upper tube and then mixed. After standing for 10 min in vertical position, the ball valve was closed, and floating CFMPs were removed by washing the tube two times with 7.5 mL of a 4% polyvinyl alcohol solution prepared in 1:1 water/ethanol (v/v) (PVA; molecular weight ~195,000; Sigma-Aldrich KGaA, Darmstadt, Germany). PVA dispersion was left to solidify for 2 hrs at 60 °C and particles were subsequently enumerated and measured as described above.

2.5. A. flavus isolation and aflatoxin biosynthesis

Soil from samples prepared with the single 2 mm x 2 mm square films were transferred to a metal knurled plate (6 cm wide x 25 cm long) equipped with lateral edges and mounted on an oscillator base unit. A thin (0.2 mm) circular (5-cm diameter) surface-sterilized pieces of polyvinyl chloride foil with a central 4-mm opening for securing a modified ionizer probe MJ II, which was attached to a charging generator AG35 (Haug GmbH, Leinfelden-Echterdingen, Germany), was positioned at a distance of 10 cm above the oscillating plate. Plates were oscillated for 3-min intervals and attached UT film fragments were transferred to 9-cm diameter plates containing potato dextrose agar (PDA) or modified rose Bengal agar (MDRBA). Plates were then incubated at 25 °C and 37 °C, respectively. After 2 weeks of incubation, the percentage of UT film fragments colonized by *A. flavus* was determined by visual observation using a dissecting microscope. Observations were confirmed by sequencing analysis following the procedure described in Accinelli et

al. (2009), and sequences deposited in the NCBL GenBank database (accession numbers from MN845191 to MN845210).

Thirty *A. flavus* isolates were then randomly picked from each size-class of incubated film fragments and evaluated for their aflatoxin biosynthesis capability. Isolates were grown in test tubes containing 2 mL of yeast extract sucrose broth and incubated without shaking in the dark at 30 °C. After 7 days of incubation, broth was extracted with chloroform and evaporated until dry under vacuum. Residues were dissolved in methanol/H_2O (70:30, v:v) and aflatoxin concentration determined by HPLC. Samples were loaded on a minicolumn packed with aluminum oxide (Alltech Co., Deerfield, IL), and 20 mL of the eluate injected on an HPLC system equipped with a Nova-Pak C18 column (150 mm x 3.9 mm, 4 μm) and a multi-wavelength fluorescence detector (Waters Inc., Macclesfield, UK). Chromatographic separation was performed with a mobile phase consisting of water: methanol:1-butanol (60:25:1) and a flow rate of 0.9 mL min^{-1} at 30 °C (Accinelli et al., 2016). Aflatoxins were detected at 365 nm (excitation) and 440 nm (emission). Data are expressed as total aflatoxins (aflatoxin B1, B2, G1and G2) with the quantitation limits of 0.1 ng g^{-1} of crude extracts.

Soil from incubated tubes were also used for quantitative polymerase chain reaction (qPCR) for monitoring the *A. flavus* population over incubation time, following the procedure described by Accinelli et al. (2016). Briefly, soil DNA was isolated using the PowerSoil DNA isolation kit (MoBio Laboratories), following the manufacturer's instructions. Recovered DNA was measured using a BioDrop spectrophotometer (BioDrop Ltd, Cambridge, UK). Each 25 μL of reaction contained 12.5 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.2 μM of each primer, and 40 ng of DNA. Thermocycling conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1

min at 60 °C. Reactions were performed using an Open qPCR (ChaiBio, Santa Clara, CA). A standard curve (r2 = 0.92; efficiency = 94%; slope = −0.21) was generated by plotting cycle threshold values (Ct) against logarithmic-transformed amounts of target DNA obtained from 10-fold dilutions of DNA isolated from *A. flavus* spore dispersions.

2.6. Statistical analysis

Mean values of data in single experiments were compared by Fisher's least significant difference (LSD), and P values < 0.05 were considered statistically significant.

3. Results and discussion

3.1. CFMP deterioration and fragment formation

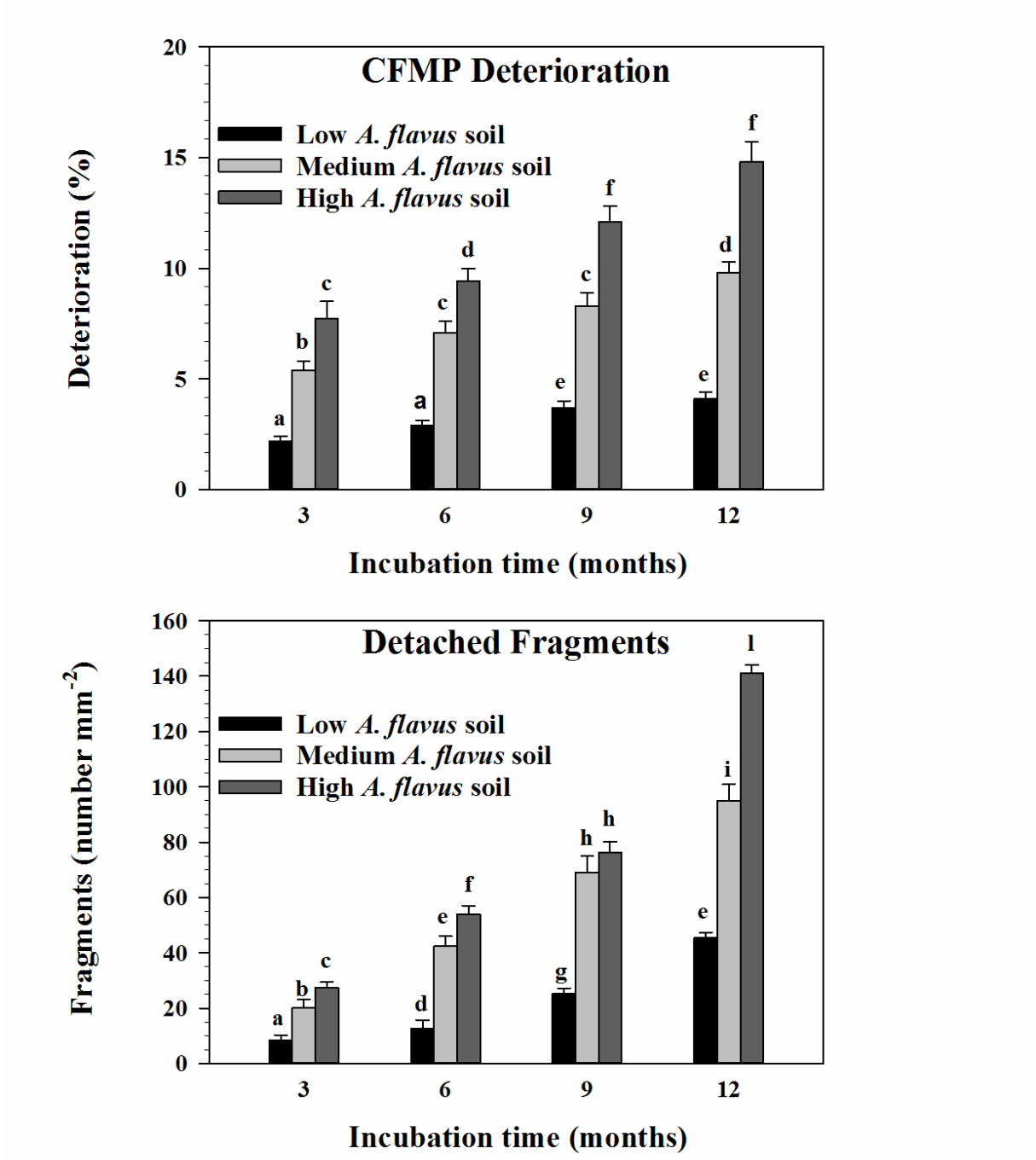
Extent of deterioration of CFMPs over the one-year period in the three selected soils is shown in Fig. 1. During the first 3 months of incubation, surface deterioration of 12-µm thin films from compostable bags expressed as surface area loss was only about 2.2% in soil with low *A. flavus* content, 5.6% in soil with medium *A. flavus* content and 7.7% in soil with high *A. flavus* content. At the end of the 12-month incubation period, film deterioration accounted for 4.1, 9.8, and 14.8% of the total surface in low, medium and high *A. flavus* containing soils, respectively. Low rates of deterioration (> 6% surface area loss) were also observed in a recent agricultural field soil burial study conducted in the northern USA for 12 months with bioplastic mulching films having a chemical composition comparable to those used in this study (Li et al, 2014). Data from the current study were generated by incubating film samples from plastic bags compostable industrially under conditions favorable to microbial growth, which are the type currently required by law for carrying loose

fruits and vegetables in supermarkets and grocery stores in Italy. According to the Italian law, allowed UT bags must satisfy the EN 13432 standard, which includes 90% mineralization within 6 months and 90% disintegration to fragment having a size less of 2 mm within 3 months under industrial composting conditions (temperature approximately of 60 °C, high humidity, etc.). Since compostable plastic fragments less than 2 mm in size are not quantified in the final product from the industrial composting process, it is not regulated by the EN 13432 or other international standards (e.g., ISO, EN, ATSM, etc.). Therefore, the application of industrially processed compost to agricultural fields is likely to contaminate the soil with these UT film particles. Considering the size of single exposed film squares (< 2 mm^2) used in this study and their slow deterioration in soil, our findings are consistent with the above assumption. Except for Italy, where plastic bags have been banned, other alternatives for customers are allowed, including paper bags, reusable plastic bags, reusable cotton net bags, etc. The experiment described here was thus repeated with sheets of the paper found in paper grocery bags. As expected, deterioration of these cellulose-based sheets was rapid, and was completed within 3 months of incubation in all of the three soils (data not shown). In the biodegradability evaluation standard test of packaging materials in soil and/or compost (i.e., ISO 17556:2012, ATSM D 5988-12), pure cellulose powder is used as reference material (e.g., positive control) for assessing the biodegradation capability of a substrate's biomass. To eliminate any possible complications caused by shape and dimension, test materials are added to the substrate as powder. Cellulose-based foils that were used for this experiment were manufactured from recycled paper items, with the additions of natural wax for preventing tear-off. Sheets have a lesser exposed surface area than powder and, in addition, a wax lining reduces the high wettability of cellulose (Sintim and Flury, 2017). Although both aspects are expected to delay

285 microbial attack, the observed rapid deterioration of cellulose sheets demonstrated
286 that the three soils were conducive to microbial activity.
287 In recent years, with the increasing number of bioplastic-based products that have
288 entered the market, studies concerning their environmental impact have received
289 growing interest. Nowadays, most of the commercialized bioplastic products are
290 single-use items (e.g., grocery bags, dishes, cutleries, etc.), which are specifically
291 designed to be disposed of in industrial composting facilities. Consequently, for
292 regulatory purposes and product certification requirements, published studies have
293 mainly focused on mineralization and/or deterioration of bioplastic under controlled
294 composting conditions (Harrison et al., 2018; Herman et al., 2011; Yagi et al., 2012;
295 Emadian et al., 2017). In contrast, information concerning the fate of these products
296 in natural environments (e.g., soil, sea water) is very limited and little information on
297 the release of microplastic particles or films into soil or other environments is
298 available in the literature (Accinelli et., 2012; Balestri et al., 2019).
299 The present study was conducted using samples of UT bioplastic bags labelled with
300 the brand name Mater-Bi®, which are of the same type of bioplastic as those
301 provided in all Italian supermarkets and grocery stores. Grocery store bags
302 manufactured with poly(butylene adipate coterephtalate) (approximately 70%),
303 thermoplastic starch (approximately 30%), and minor amounts of synthetic additives
304 have been studied (Zuo et al., 2019). Their chemical composition, in addition to the
305 white pigment that is used for coloring these bags, greatly facilitated their
306 visualization under UV light. In preliminary experiments that were carried out in this
307 laboratory following the procedure described by Ernie-Cassola et al. (2017),
308 fluorescence of film fragments was increased by staining films with Nile red dye.
309 However, these studies showed that addition of dye was not necessary to correctly

visualize film fractions (data not shown). For this reason, and to avoid possible dye effects on microbial degradation, staining of UT film was not done in this study. Enumeration of released fragments during soil incubation of CFMPs is reported in Fig. 1. As expected, the total number of detached fragments steadily increased over the incubation period. At the end of the 12-month incubation period, the number of recovered fragments was 5.5, 4.7, and 5.1 times higher than that recorded at the first sampling time (i.e., 3 months) in the low *A. flavus*, medium *A. flavus* and high *A. flavus* soils, respectively. While the percent of fragments greater than 0.07 mm in size decreased during the incubation time and those between 0.07 and 0.05 mm remained approximately constant, the percent of smaller fragments (i.e., < 0.05 mm) showed an approximately 50% increase, with no significant differences among the three soil types (Fig. 2). During the same time interval, the increase in released fragments was up to 4-fold higher than the increase in deteriorated film area.

The fate of microplastic in soil is still largely unexplored (Nizzetto et al., 2016a; Rilling et al., 2017). Studies have shown that these fragments selectively adsorb organic pollutants onto their surfaces, and also have the potential to negatively affect germination and early growth of herbaceous plants (Zuo et al., 2019; Balestri et al., 2019). The number or mass of small-sized plastic and compostable plastic film fragments (i.e., fragments with size less than 2 or 4 mm) remaining in composted products is not subject to government regulations at present. If our laboratory findings are confirmed in ongoing field studies, the observations suggest that this type of regulation should be seriously considered. Soil tillage, especially plowing, is expected to spread plastic fragments throughout the soil profile, which may increase the risk of them entering subsurface waters or exposing fragments to surface run-off and wind transportation (Rilling et al., 2017).



335

336 Figure 1. Deterioration of microplastic compostable films (CFMP) (upper) and abundance of
337 released fragments (lower) from incubated samples of soil from Northern (Low *A. flavus* soil)
338 and Southern Italy (Medium *A. flavus* soil), and the Mississippi Delta (High *A. flavus* soil).
339 Bars with same letter are not significantly different (P > 0.05).

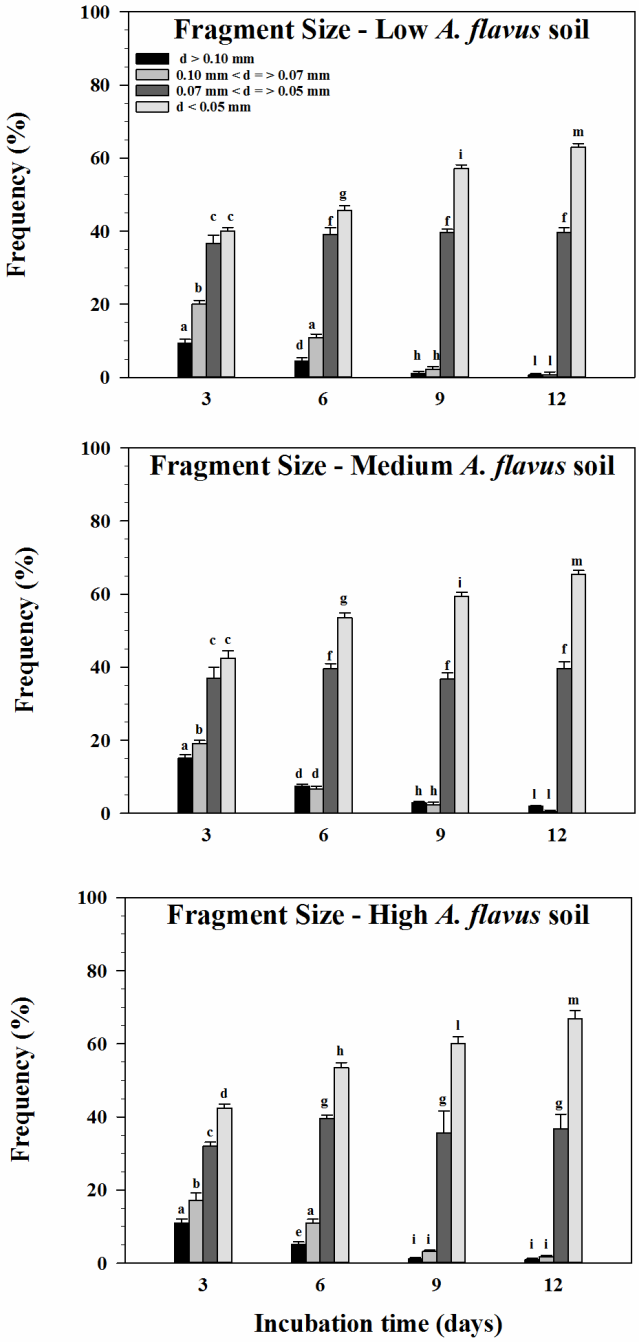


Figure 2. Relative frequency distribution of released fragments from microplastic compostable films (CFMP) that were incubated in samples of soil from Northern Italy (Low *A. flavus* soil), Southern Italy (Medium *A. flavus* soil), and the Mississippi Delta (High *A. flavus* soil). Fragments were grouped in different size classes and data expressed as percentage of fragments per surface area of CFMP. Bars with same letter are not significantly different (P > 0.05).

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

Percentages of film fragments infected by the filamentous fungus *A. flavus* over the 12-month incubation period are summarized in Table 2. During the entire incubation period, the relative frequency of contaminated CFMPs remained significantly higher in the high *A. flavus* soil than in the other two soils. These results and those in Table 1 and Table 3 are consistent with the colonization rate of CFMPs being related to the size of the *A. flavus* population in the soil. In contrast, and despite differences in the size of the indigenous *A. flavus* soil populations, the frequency of *A. flavus*-infected fragments was not significantly different ($P > 0.05$) between the low *A. flavus* soil and the medium *A. flavus* soil (Table 2). These findings are consistent with Mater-Bi® starch-based bioplastic having a substantial ability to support the growth of this fungus (Accinelli et al., 2009; Accinelli and Abbas, 2011; Moore-Kucera et al., 2014), a property which has been used to develop practical applications of it in agriculture. For instance, the size of soil *A. flavus* populations in soil can be effectively monitoring by inserting baiting rods manufactured from Mater-Bi® starch-based bioplastic into the soil (Accinelli et al., 2012). At selected sampling time, baits are removed and analyzed for the number of *A. flavus* propagules. Another technique relying on the rapid growth of this fungus on starch-based bioplastic matrices involves field application of granular or sprayable bioplastic-based formulations containing adherent spores of non-aflatoxin-producing *A. flavus* isolates. It is well established that *A. flavus* populations in soils are composed of both aflatoxin- and non-aflatoxin producing isolates (Abbas et al., 2009). After application of these bioplastic-based formulations, the fungus begins rapid growth, competing with indigenous aflatoxin-producing isolates, resulting in a reduction of risk of aflatoxin contamination of corn (Accinelli et al., 2009, 2012, 2016).

As indicated in Table 2 for the high *A. flavus* level soils, the percentage of *A. flavus* colonized fragments increased after 6 and 9 months of incubation. No further increases were observed at 12-month sampling time, when up to 75.4 % of the compostable fragments were infected by the fungus. Similar findings were observed in the low and medium *A. flavus* level soils, except that in these soils the relative increase was even more pronounced.

In the present study, *A. flavus* isolates that were recovered from detached fragments were evaluated for their aflatoxin-producing ability. As indicated in Table 3, the frequency of aflatoxigenic isolates was significantly higher after 9 and 12 moths of incubation for all soils. Data from qPCR analysis showed that the size of the soil *A. flavus* population increased during the incubation time, especially in the low level *A. flavus* soil (Table 1 and Table 2). More specifically, DNA of this fungus showed average increases of 119, 81.9, and 57.6 % during the 12-month incubation period in the low, medium and high *A. flavus* level soils, respectively, while no significant changes were observed in the control soils. These results are consistent with the occurrence and accumulation of bioplastic film fragments in soil impacting the composition of the soil microbial population. The health risk of increasing the soil *A. flavus* population has already been identified by other researchers (Moore-Kucera et al., 2014; Brodhagen et al., 2015) and this fungus is of particular concern for asthmatic and immunosuppressed individuals. In addition, the elevated *A. flavus* levels at an increased percent aflatoxigenicity are expected to substantially increase aflatoxin contamination of crops grown in the soil.

In an exhaustive review, Brodhagen et al. (2015) stressed that incorporation of bioplastic fragments into the soil is expected to increase the number of fungi, especially filamentous fungi, capable of using them as nutrient sources. Research has shown that fungi belonging to the genus *Aspergillus* are frequently detected in

starch-based bioplastic buried in soil, along with other mycotoxin-producing fungi (i.e., *Fusarium* spp., *Penicillium* spp.) (Moore-Kucera et al., 2014; Bergenholtz and Nielsen, 2002; Accinelli et al., 2009, 2012; Kim et al., 2000). Factors affecting the ratio between aflatoxigenic and non-aflatoxigenic isolates in natural environments (i.e., soil) remain unclear (Drott et al.; 2019), although it is widely believed that *A. flavus* uses aflatoxins to kill competitors, so that these mycotoxins provide a selective advantage in densely populated microbial environments. In this study the largest increases in percent aflatoxigenicity were observed when *A. flavus* populations were the highest incorporation. The physical form of CFMPs produced in soil from compostable UT bioplastic films results in a very high surface-to-mass ratio and it is expected that the large nutrient-releasing surface area would be conducive to the growth of aflatoxin-producing *A. flavus* isolates. Further studies are needed to determine if these observations can be replicated under field conditions and if the physical form and composition of these CFMPs contribute to unusually large increases in *A. flavus* levels and aflatoxigenicity.

413 Table 2. Relative frequency of *Aspergillus flavus* infection of compostable film microplastic (CFMP) fragments and percent aflatoxigenicity of *A.*

414 *flavus* isolates recovered from incubated soil samples with low, medium and high levels of indigenous *A. flavus*

415

	% *A. flavus*-infected fragments in soils with different indigenous *A. flavus* levels			% of *A. flavus* isolates producing aflatoxin		
Incubation time (days)	Low Af[b]	Medium Af	High Af	Low Af	Medium Af	High Af
3	39.2 a	41.3 a	52.5 c	27.2 a	31.0 a	32.3 a
6	47.4 b	53.4 b	67.1 e	29.0 a	29.6 a	27.3 a
9	74.0 c	75.4 c	88.4 f	44.5 b	40.2 b	46.3 b
12	75.2 d	79.1 c	86.0 f	68.0 c	61.3 c	63.2 c

416 [a] For each column, values with same letter are not significantly different form each other ($P > 0.05$).
417 [b] Low natural *A. flavus* levels were found in soil from Northern Italy (Low *A. flavus* soil); medium levels were in soil from Southern Italy (Medium
418 *A. flavus* soil); and high levels were in soil from the Mississippi Delta, USA (High *A. flavus* soil).

Table 3. Total *Aspergillus flavus* DNA recovered from incubated soil samples containing microplastic compostable films (CFMPs). Soil were collected from Northern Italy (Low *A. flavus* soil), Southern Italy (Medium *A. flavus* soil) and the Mississippi Delta (High *A. flavus* soil). Samples with no CFMPs were included (Control). For each soil, values with same letter are not significantly different ($P > 0.05$).

	Aspergillus flavus DNA (ng g^{-1})					
	Low *A. flavus* soil		Medium *A. flavus* soil		High *A. flavus* soil	
Incubation time (d)	Control	CFMPs	Control	CFMPs	Control	CFMPs
0	48.2 a	-	82.2 b	-	133.2c	-
3	44.1 a	51.0 a	78.3 b	75.7 b	104.5 c	102.3 c
6	49.1 a	69.6 b	74.6 b	82.3 b	112.1 c	112.0 c
9	51.0 a	78.7 b	79.0 b	101.4 c	134.0 c	137.0 d
12	44.3 a	111.8 c	84.1 b	137.7 d	128.0 c	161.2 e

4. Conclusions

In recent years, there has been increasing interest in using bags for groceries and other goods that are made of bioplastic materials intended for disposal under industrial composting conditions. However, when these materials are placed in soil, and presumably during industrial composting processes, variable numbers of small-sized fragments or compostable film microplastic particles (CFMPs) are generated and they unavoidably contaminate either the soil in which they were formed or soil that has been amended with industrially composted material. The results of this study show that under controlled laboratory conditions CFMPs are degraded very slowly in soil, suggesting that if similar rates of CFMP degradation occur in field soil, these small-size fragments may accumulate in the soil at higher levels than currently expected. In addition, as observed by others, we have observed that in soil under controlled laboratory conditions, naturally-present *Aspergillus flavus* fungus associated with deteriorating CFMPs, which then resulted in increases in the size and percent aflatoxigenicity of the *A. flavus* soil population.

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***Declaration of Interest Statement**

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: