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

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

3  
4  
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21  
22  
23 **ABSTRACT**

24 In recent years, some countries have replaced single-use plastic bags with bags  
25 manufactured from compostable plastic film that can be used for collecting food  
26 wastes and composted together with the waste. Because industrial compost contains  
27 uncomposed fragments of these bags, application to field soil is a potential source of  
28 small-sized residues from these bags. This study was undertaken to examine  
29 deterioration of these compostable film microplastics (CFMPs) in field soil at three  
30 different localities in Italy. Deterioration of CFMPs did not exceed 5.7% surface area  
31 reduction during the 12-month experimental period in two sites located in Northern  
32 Italy. More deterioration was observed in the Southern site, with 7.2% surface area  
33 reduction. Deterioration was significantly increased when fields were amended with  
34 industrial compost (up to 9.6%), but not with home compost. Up to 92.9% of the

35 recovered CFMPs were associated with the soil fungus *Aspergillus flavus*, with 20.1%  
36 to 71.2% aflatoxin-producing isolates. Application of industrial compost resulted in a  
37 significant increase in the percentage of CFMPs associated with *A. flavus*. This  
38 observation provides an argument for government regulation of accumulation of  
39 CFMPs and elevation of hazardous fungi levels in agricultural soils that receive  
40 industrial compost.

41

42 *Keywords*

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

45

## 46 1. Introduction

47 First introduced in the late 1970s, single-use plastic bags have rapidly become the  
48 preferred choice for carrying purchased items, including packaged foods, clothes, and  
49 many other consumer products. Consumption of disposable petroleum-based plastic  
50 bags steadily increased over the years, reaching a global annual consumption of over  
51 a trillion units (Harrison et al., 2018). However, due to difficulties in proper disposal  
52 and recycling, and their long persistence in the environment, these lightweight bags  
53 pose a serious environmental threat (Accinelli et al., 2012; Xanthos and Walker, 2017).  
54 As with other thermoplastic products, prolonged exposure to sunlight and other  
55 physico-chemical agents result in formation of thin plastic particles, which  
56 subsequently fragment into small-sized particles (Rhodes, 2019). As proposed by  
57 Thompson et al. (2004), plastic fragments having size less than 5 mm are defined as  
58 microplastics (MPs). MPs generated from thin and ultra-thin disposable carrier bags  
59 are then easily transported by wind from urbanized areas into natural and agricultural  
60 areas, where they can enter the food chain and adversely affect water and soil quality  
61 (Balestri et al., 2019; Chae and Youn-Joo, 2018; Huerta Lwanga et al., 2016, 2017;  
62 Nizzetto et al., 2016). Consequently, plastic waste generated from single use plastic  
63 bags has prompted much debate, forcing many governments and municipalities to  
64 adopt or promote alternatives and restrictions to their usage. Replacing petroleum-  
65 based disposable carrier bags with compostable ones has become a common option in  
66 some countries, along with use of reusable totes and paper bags and other solutions  
67 (Battista et al., 2021; Dolci et al., 2021). For example, lightweight plastic bags  
68 (thickness < 50  $\mu\text{m}$ ) were initially banned in Italy in 2011, and in 2018 the ban was

69 extended to ultra-thin (UT) plastic bags (thin < 15  $\mu\text{m}$ ). While the former were  
70 designed for carrying purchased packaged items from supermarkets and stores, the  
71 latter were intended for carrying unpackaged fruits and vegetables. Since UT  
72 compostable bags are thus the sole bags currently permitted for carrying loose fruits  
73 and vegetables from either supermarkets or local grocery stores in Italy, their annual  
74 consumption has increased rapidly up to 300 units per capita. These single use  
75 compostable bags are disposed of after their primary use by placing in organic waste  
76 bins along with food waste and other compostable items and processed together in  
77 industrial composting facilities. The resulting compost is then applied as a soil  
78 amendment to agricultural fields for improving soil structure, organic matter content,  
79 and other soil properties, including water holding capacity, etc. (Kranz et al., 2020).  
80 However, industrial compost is highly variable in terms of quality and technical  
81 parameters. Major parameters affecting compost quality include its maturity and  
82 stability, nutrient content, pH value, C/N ratios, and levels of chemical (e.g., heavy  
83 metals, pharmaceuticals) and physical contaminants, including glass and metal  
84 particles, and plastic fragments (Khalid et al., 2017). Compost should also be free of  
85 plant pathogenic agents and/or phytotoxins to avoid any negative effects on seed  
86 germination and seedling growth (Haas et al., 2016; Luo et al., 2018). Although  
87 different standardized procedures for evaluating compost quality are available, none  
88 of them take into consideration the number of millimeter-sized fragments of materials,  
89 such as compostable plastic particles, still present in the final product of the  
90 composting process, nor do they consider the impact material of that composition will  
91 have on the soil ecosystem. One explanation for this deficiency in existing evaluation  
92 procedures is that these protocols have been specifically designed to evaluate  
93 composting processes starting from material only composed of food waste and/or other  
94 biowaste residues (i.e., yard/garden wastes), with minor amount of impurities such as  
95 inert materials (i.e., glass, metal, and plastic fragments). The adoption of single use  
96 compostable bags into standard public use has inevitably resulted in their introduction  
97 into industrial composting processes at relevant levels (approximately 5% w/w of the  
98 composting mass). This situation has created a need for a better understanding of the  
99 potential impact of the small-sized compostable plastic particles present in industrial  
100 compost on the quality and functionality of soil to which it is added (Bläsing and  
101 Amelung, 2018; Lavagnolo et al., 2020; Weithmann et al., 2018). Among the various  
102 possible effects of adding biodegradable and compostable film fragments to soil is the

103 possibility of altering the composition of the soil microbial community in ways that  
104 could result in adverse effects such as reduced seed germination or increased root  
105 infection by soil microorganisms (i.e., damping off) (Li et al., 2021; Ruggero et al.,  
106 2019). Specifically, it has been reported (Brodhagen et al., 2015; Moore-Kucera et al.,  
107 2014) that small-sized compostable film fragments, called compostable film  
108 microplastics (CFMPs), can promote the growth of soil-inhabiting filamentous fungi,  
109 including mycotoxin-producing species. In a previous laboratory-based study, it was  
110 demonstrated that CFMPs from UT compostable bags have the potential to persist in  
111 soil and to increase the size of the *Aspergillus flavus* population (Accinelli et al., 2020).  
112 The aim of the present research was to study the deterioration of CFMPs in soil under  
113 three typical field conditions and to investigate the potential effect of adding  
114 compostable bag-derived CFMPs in home or industrial compost on the persistence and  
115 population size of *A. flavus* by comparing values in amended and non-amended soils  
116 under those field conditions.

117

## 118 **2. Materials and methods**

### 119 *2.1. Field sites, management and application of UT film samples*

120 Three experimental sites were selected for this study, two located in Northern Italy in  
121 Montagnana (MO) and in Mezzolara (ME) and one in Southern Italy in Siracusa (SI).  
122 In all locations, experiments were conducted in flat and uniform fields (15 m x 15 m)  
123 that were uncropped during the entire 12-month experimental period (from September,  
124 2019, to September, 2020). Selected properties of the three soils are summarized in  
125 Table 1. After harvesting wheat in June at the MO and ME sites and in May at the SI  
126 site, the soil was moldboard plowed and then disked three times. During the whole  
127 experimental period, the soil was not tilled. Fields were divided in to 3 blocks (3 m x  
128 3 m), which were separated by a 1-m wide buffer area. Home or industrial composts  
129 were applied at the rate of 10 t ha<sup>-1</sup> before disking. Industrial compost was obtained  
130 from an industrial compost facility located at Voltana di Lugo, Italy and operated by  
131 Herambiente s.p.a. (Bologna, Italy). Home compost (food waste only) was obtained  
132 by local restaurants and combined to achieve comparable properties to those of the  
133 industrial compost (Table 1).

134 Samples of UT films (12-µm thin) were prepared as described in Accinelli et al.  
135 (2020). Briefly, rectangles (2.8 cm x 6.0 cm) obtained from Mater-Bi® compostable  
136 bags (Novamont s.p.a., Novara, Italy) were retained between two high-density

137 polyethylene plastic nets with openings of 2 mm x 2 mm. The same approach was  
138 adopted for preparing single square UT films with exposed surface of 2 mm x 2 mm.  
139 Both sample types were surface disinfected by UV exposure for 20 min and stored in  
140 sterilized glass tubes before inserting into the soil. Rectangular and single square UT  
141 films were buried into the soil at a 5-cm depth and identified by placing hardwood  
142 plant labels. Soil and assembled films were sampled throughout the experimental  
143 period using a soil auger (10 cm diameter and 20 cm height). Assembled films were  
144 separated from soil, and the remaining soil was gently homogenized by hands, air dried  
145 for 24 hrs., and then used for plastic fragment recovering and microbiological analysis.  
146

## 147 *2.2. Deterioration and fragmentation of UT film samples*

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

156 Detached fragments were recovered from soil samples by laying 10 g of air-dried soil  
157 on a pre-warmed (120 °C) metal plate covered with a thin removable nylon 6,6 foil  
158 (150 µm thick) which was fixed on the plate. The metal plate was mounted on a  
159 shaking block and shook horizontally for 6 s. After 30 s of contact time, soil samples  
160 were discharged, and nylon foils with attached CFMPs were then removed from the  
161 plate and directly analyzed by attenuated total reflection Fourier transform infrared  
162 spectroscopy (ATR-FTIR). ATR-FTIR analyses were performed using a Cary 630  
163 FTIR spectrometer equipped with diamond ATR (Agilent Technology, Santa Clara,  
164 CA, USA) operating at room temperature with 64 scans within the range of 4000-650  
165 cm<sup>-1</sup> at 4 cm<sup>-1</sup> resolution.  
166

## 167 *2.3. Aspergillus flavus recovery from UT film fragments and percentage of* 168 *aflatoxigenic isolates*

169 Soil samples from each burial point were processed using a patented benchtop  
170 electrostatic generator machine for separating UT fragments from soil (Accinelli,

171 2019). Soil samples (15 g of air-dried soil) were transferred to an oscillating metal  
172 plate, and fragments were separated from the soil using an electrostatically charged  
173 sterilized plastic film mounted 15 cm above the plate. The film was then transferred to  
174 a Petri plate containing modified Rose Bengal agar and incubated at 37 °C for 5-7 days  
175 (Abbas et al., 2004). *A. flavus* isolates were randomly selected and used for assessing  
176 their capability to produce aflatoxins. Briefly, isolates were incubated at 30 °C for 7  
177 days in test tubes containing 2 mL of yeast extract sucrose broth, which was then  
178 extracted with chloroform, dried under vacuum, and redissolved in methanol/H<sub>2</sub>O  
179 (70:30 v/v). Total concentrations of aflatoxin B1, B2, G1 and G2 were determined by  
180 HPLC as described elsewhere (Accinelli et al., 2020). Soil used for recovering film  
181 fragments was then used for quantifying *A. flavus* DNA by qPCR. Briefly, total soil  
182 DNA was isolated using the PowerSoil Isolation kit (Qiagen Ltd., Manchester, UK)  
183 and quantified using a BioDrop spectrophotometer (BioDrop Ltd, Cambridge, UK).  
184 Each 25 µL of reaction mixture contained 12.5 µL of 2× TaqMan Universal PCR  
185 Master Mix (Applied Biosystems, Foster City, CA, USA), 0.2 µM of each primer  
186 (Accinelli et al., 2012), and 40 ng of DNA. Samples were amplified on an Open qPCR  
187 (ChaiBio, Santa Clara, CA, USA) using the following conditions: 2 min at 50 °C, 10  
188 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A standard curve ( $r^2 =$   
189 0.92; efficiency = 94%; slope = - 0.21) was generated by plotting cycle threshold  
190 values (Ct) against logarithmic-transformed amounts of known *A. flavus* DNA.

191

#### 192 2.4. Statistical analysis

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

196

### 197 3. Results and discussion

#### 198 3.1. CFMP deterioration and fragment formation

199 The present experiment was conducted in three different experimental fields, two  
200 located in the North and one in the South of Italy. Weather conditions during the  
201 experimental period are shown in Figure 1. During the 12-month experiment, total  
202 rainfall was 848 and 605 mm in MO and ME, Northern Italy, respectively. Lesser  
203 rainfall was recorded in the Southern Italy site, SI. This site also experienced drought

204 during the summer season, and temperatures never fell below 4.5 °C during the whole  
205 12-month experimental period.

206 Deterioration of CFMPs that were buried into field soil at the three experimental sites  
207 is shown in Figure 2 (left panel). In both Northern sites in plots not receiving compost  
208 application, CFMPs showed reduced deterioration during the fall and winter seasons,  
209 with values that did not exceed 1.5%. More deterioration of CFMPs occurred during  
210 the June and September sampling operations, with values that reached 5.4 and 5.7% at  
211 the MO and ME sites, respectively. Similar deterioration patterns were observed in  
212 samples from plots amended with home compost. In contrast, amending the soil with  
213 compost from an industrial process resulted in a greater deterioration of CFMPs ( $p <$   
214 0.05). At the end of the 12-month experiment, CFMP deterioration in industrial  
215 compost plots was 7.0 and 7.5% reduction in surface area at the MO and ME sites,  
216 respectively. Deterioration of CFMPs at the site in Southern Italy was also more  
217 intense during the spring and summer season, with a final value of 7.2% reduction in  
218 surface area. The significant stimulatory effect of industrial compost was also observed  
219 at this site, in which CFMP deterioration reached 9.6% reduction in surface area.

220 Results from this field study are generally consistent with those of a previous  
221 laboratory study conducted using the same type of assembly and compostable film  
222 samples (Accinelli et al., 2020). However, less deterioration was observed under field  
223 conditions than under the more favorable laboratory conditions (i.e., soil samples  
224 incubated at 25 °C with soil moisture maintained at field capacity). Soil temperature  
225 and moisture level have well-known effects on the rate and extent of microbiological  
226 processes. In addition, the presence of more recoverable *A. flavus* propagules in soil  
227 during the last sampling operation, in early September, particularly at the Southern  
228 site, along with conditions favorable for *A. flavus* growth would be expected to  
229 contribute to increased degradation of compostable bioplastic, because *A. flavus* is  
230 known to be an efficient degrader of poly(butylene adipate co-terephthalate), the major  
231 component of compostable bioplastic (Accinelli et al., 2009, 2012, 2020; Moore-  
232 Kucera et al., 2014). This explanation for increased degradation is further supported  
233 by the observation that amending with the industrial compost was associated with  
234 increased degradation. Industrial composting processes use high temperatures  
235 (approximately 55-65 °C) to reduce the number of microbes in waste, including several  
236 human and plant pathogens, but *Aspergillus* species spores are relatively heat resistant  
237 (Franceschini et al., 2016). Consequently, the final product of industrial composting

238 processes is expected to add *A. flavus* propagules to soils amended with it. This is not  
239 expected to occur in home composting, a process in which temperatures at such  
240 elevated values are never reached (Di Piazza et al., 2020; Franceschini et al., 2016).  
241 Thus, the higher deterioration of CFMPs in plots receiving the industrial compost plots  
242 at the three sites during the second half of the experimental period may at least partly  
243 be explained by increased number of heat-resistant species and spore-formers added  
244 with the compost and increased proliferation of these microorganisms as a response to  
245 the added organic matter and higher temperatures (Abbas et al., 2004, 2009).

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

263 During the last decade, a growing number of studies have focused on MP occurrence  
264 and their effects in the marine environment. More recently, there is also an increasing  
265 interest in focusing on agricultural soil, as a sink for MP contamination (Horton et al.,  
266 2017; Rillig, 2012; Scheurer and Bigalke, 2018). However, a major obstacle in  
267 studying MP occurrence, persistence and accumulation in soil are the technical  
268 challenges in recovering small-size plastic fragments from the heterogeneous and  
269 variable soil matrix (i.e., variability in particle size, level and nature of organic  
270 components, etc.). Most of the available methods for recovering MPs from soil are  
271 based on floatation or other density separation approaches. In the typical process, soil

272 or sediment samples are first chemically or enzymatically digested to remove organic  
273 matter, then separated in an aqueous medium, from which samples are recovered by  
274 filtration and analyzed by Fourier transform infrared (FTIR) microscopy or Raman  
275 microspectroscopy (Bläsing and Amelung, 2018; Yang et al., 2021). More recently,  
276 other alternatives have been proposed, including critical fluid extraction, use of  
277 electrostatic forces, etc. (Fuller and Gautam, 2016). However, none of these methods  
278 have been designed for recovering compostable plastic film particles from soil or for  
279 studying their fate in soil ecosystems. A proposed novel method for monitoring CFMP  
280 fate in soil was developed and shown in the present studies to be very effective and  
281 easily applied under field conditions. Basically, dried soil samples are shaken onto a  
282 nylon foil, which had been pre-warmed to a temperature that causes partial melting,  
283 creating an adhesive consistency for detached compostable film fragments. Soil  
284 particles are removed by air-flush, while CFMPs remain stuck to the foil where they  
285 can be directly processed for analysis. Results of a recovery test are summarized in  
286 Figure 3. In samples of the three soils, recovery of CFMPs with sizes ranging from 0.1  
287 to 4.0 mm<sup>2</sup> was above 97%. Addition of compost at the same dosage as that of the field  
288 experiment did not significantly affect CFMP recovery. Fragments can be easily  
289 visualized and enumerated using a simple dissecting microscope. For polymer  
290 identification, fragments are then directly analyzed by ATR-FTIR with no need of  
291 further costly equipment (e.g., FTIR microscope and dedicated software applications).  
292 Before developing this solution, single fragments were analyzed using a Survey IR  
293 microspectroscopy accessory, which was equipped with a high-resolution color video  
294 camera (SRA Instruments s.p.a., Milano, Italy). The accessory was mounted on the  
295 FTIR Cary 630 spectrometer. Unfortunately, this approach did not lead to reliable and  
296 consistent results. The procedure was time-consuming and some recovered fragments,  
297 including fragments with size larger than 1 mm, were not clearly visible, and no  
298 distinguished peaks were displayed. However, all fragments were correctly visualized  
299 and analyzed using the newly developed procedure described above (Fig. 4). These  
300 findings suggest that replacing an FTIR microscope with an FTIR spectrometer  
301 equipped with a scan camera accessory is not recommended for MP analysis.  
302 Results obtained using this novel approach are summarized in Figure 2 (right panel).  
303 The total number of detached fragments increased over the 12-month experimental  
304 period. More specifically, higher increases were observed during the spring-summer  
305 period. Approximately 3.1-, 2.5-, and 6.2-times more fragments were recovered at the

306 end of the experiment than in the initial 3 months, at the MO, ME and SI sites,  
307 respectively. In all three sites, significantly more fragments were recovered from  
308 industrial compost plots than from unamended plots, but the increases were not  
309 observed with home compost amendment. These results are consistent with those of a  
310 previous laboratory study and they show that CFMPs were not rapidly degraded in the  
311 soil, and thus have the potential to affect soil fertility and other ecological processes,  
312 including soil organic matter evolution and turn-over, microbial processes and  
313 microbial composition. Given that a large fragment can generate multiple small  
314 fragments in the course of deterioration, the observed differences in CFMP numbers  
315 were consistent with CFMP deterioration data, in which the SI site showed the highest  
316 number of recovered fragments. The data presented here suggested the power of this  
317 novel approach for measuring deterioration and persistence of bioplastic items in soil.  
318 Monitoring MPs numbers in soil is expected to provide very useful and practical  
319 information for regulatory agencies.

320

### 321 *3.2. Occurrence of A. flavus in compostable films and soil*

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

333 Buried CFMP fragments were recovered from soil using a technique based on  
334 electrostatic charges, incubated on a selective medium, and the percentage of *A. flavus*-  
335 infected fragments recorded. In all three sites, the percent of *A. flavus* infected  
336 fragments increased over the 12 months (Table 2). Although application of home  
337 compost stimulated CFMPs deterioration in soil (Figure 3), this soil amendment did  
338 not affect ( $p > 0.05$ ) the percent of infected fragments. In contrast, the percent of *A.*  
339 *flavus*-infected fragments significantly increased ( $p < 0.05$ ) in plots amended with

340 industrial compost. More specifically, at the end of the experiment, the percent  
341 increase of infected fragments from these plots was 79.0, 69.3, and 92.9 % in the MO,  
342 ME, and SI sites, respectively. The SI site was selected in this study for its low level  
343 of soil *A. flavus* and hot, dry summers (Table 1; Figure 1). The results from this site  
344 confirmed that *A. flavus* is a major colonizer of poly(butylene adipate co-  
345 terephthalate)-based compostable films, especially when environmental conditions are  
346 favorable to this fungus, such as at the SI site (Accinelli et al., 2009, 2020; Accinelli  
347 and Abbas, 2011; Moore-Kucera et al., 2014). More than 24% of the *A. flavus* isolates  
348 that were recovered from CFMPs were capable of producing aflatoxins (Table 2). This  
349 percentage increased over the experimental period, reaching values of 60.1, 57.3, and  
350 59.9% at the MO, ME, and SI site, respectively. Samples from home and industrial  
351 compost plots showed similar values, except that at the SI site. At this site, the percent  
352 of aflatoxin producing isolates reached a value of 71.2%. Although the capability of  
353 *A. flavus* isolates to produce aflatoxins has been the subject of numerous  
354 investigations, factors affecting ratios of aflatoxigenic to non-aflatoxigenic isolates  
355 have still not been clarified. Some studies indicated that aflatoxin-producing isolates  
356 have competitive advantages for colonizing nutrient-rich substrates in soil, such as  
357 plant residues (e.g., corn residues) or seeds (e.g., corn, peanut seeds, etc.) (Abbas et  
358 al., 2008; Accinelli et al., 2008, 2018). The high percentage of aflatoxigenic *A. flavus*  
359 isolates recovered from CFMPs is consistent with these observations. Aflatoxins are  
360 regulated contaminants of food and feed, and most published studies have focused on  
361 the occurrence of aflatoxigenic *A. flavus* and concentrations of aflatoxins in edible  
362 products. Only a few studies have investigated on the soil ecosystem (Accinelli et al.,  
363 2009). High levels of soil inhabiting aflatoxigenic *A. flavus* isolates are expected to  
364 lead to increased infection of crop plants where they pose a health risk in foods and  
365 feeds, and they are expected to produce carcinogenic aflatoxins in the organic matter  
366 they inhabit as a way to compete with other soil microorganisms and those aflatoxins  
367 pose a serious health risk for wildlife, particularly birds. The effect of CFMPs from  
368 added industrial compost on soil should be considered by agricultural scientists and  
369 regulatory agencies.

370

#### 371 **4. Conclusions**

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

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

386

## 387 **References**

388

389 Abbas, H.K., Accinelli, C., Zablutowicz, R.M., Abel, C.A., Bruns, H.A., Dong, Y.,  
390 Shier, W.T., 2008. Dynamics of mycotoxin and *Aspergillus flavus* levels in aging Bt  
391 and non-Bt corn residues under Mississippi no-till conditions. J. Agric. Food Chem.  
392 56, 7578-7585. doi: 10.1021/jf801771a.

393

394 Abbas, H.K., Wilkinson, J.R., Zablutowicz, R.M., Accinelli, C., Abel, C.A., Bruns,  
395 H.A., Weaver, M.A., 2009. Ecology of *Aspergillus flavus*, regulation of aflatoxin  
396 production and management strategies to reduce aflatoxin contamination of corn.  
397 Toxin Rev. 28, 142-153. <https://doi.org/10.1080/15569540903081590>.

398

399 Abbas, H.K., Zablutowicz, R.M., Weaver, M.A., Horn, B.W., Xie, W., Shier, W.T.,  
400 2004. Comparison of cultural and analytical methods for determination of aflatoxin  
401 production by Mississippi Delta *Aspergillus* isolates. Can. J. Microbiol. 50, 193-199.  
402 doi: 10.1139/w04-006.

403

404 Accinelli, C., Abbas, H.K., 2011. New perspectives in the use of bioplastic materials  
405 in the biocontrol of *Aspergillus flavus* in corn. Toxin Rev. 30, 71-78. doi:  
406 10.3109/15569543.2011.591517.

407

408 Accinelli, C., Abbas, H.K., Zablotowicz, R.M., Wilkinson, J.R., 2008. *Aspergillus*  
409 *flavus* aflatoxin occurrence and expression of aflatoxin biosynthesis genes in soil. Can.  
410 J. Microbiol. 54, 371-379. <https://doi.org/10.1139/W08-018>.  
411

412 Accinelli, C., Abbas, H.M., Little, N.S., Kotowicz, J.K., Shier, W.T., 2018. Biological  
413 control of aflatoxin production in corn using non-aflatoxigenic *Aspergillus flavus*  
414 administered as a bioplastic-based seed coating. Crop Prot. 107, 87-92.  
415 <https://doi.org/10.1016/j.cropro.2018.02.004>.  
416

417 Accinelli, C., 2019. Method to evaluate the dispersion of particles. Patent number  
418 WO/2019/073382.  
419

420 Accinelli, C., Saccà, M.L., Abbas, H.K., Zablotowicz, R.M., Wilkinson, J.R., 2009.  
421 Use of a granular bioplastic formulation for carrying conidia of a non-aflatoxigenic  
422 strain of *Aspergillus flavus*. Bioresource Technol. 100, 3997-4004. doi:  
423 10.1016/j.biortech.2009.03.010.  
424

425 Accinelli, C., Saccà, M.L., Mencarelli, M., Vicari, A., 2012. Deterioration of bioplastic  
426 carrier bags in the environment and assessment of a new recycling alternative.  
427 Chemosphere 89, 136-143. doi: 10.1016/j.chemosphere.2012.05.028.  
428

429 Accinelli, C., Abbas, H.K., Bruno, V., Nissen, L., Vicari, A., Bellaloui, N., Little, N.S.,  
430 Shier, W.T., 2020. Persistence in soil of microplastic films from ultra-thin compostable  
431 plastic bags and implications on soil *Aspergillus flavus* population. Waste Manag. 113,  
432 312-318. doi.org/10.1016/j.wasman.2020.06.011.  
433

434 Balestri, E., Menicagli, V., Ligorini, V., Fulignati, S., Raspolli Galletti, A.M.,  
435 Lardicci, C., 2019. Phytotoxicity assessment of conventional and biodegradable plastic  
436 bags using seed germination test. Ecological Indicators 102, 569-580. doi:  
437 10.1016/j.ecolind.2019.03.005.  
438

439 Battista, F., Frison, N., Bolzonella, D., 2021. Can bioplastics be treated in conventional  
440 anaerobic digesters for food waste treatment? Environ. Technol. Inno. 22, 101393.  
441 doi.org/10.1016/j.eti.2021.101393.

442

443 Bläsing, M., Amelung, W., 2018. Plastics in soil: analytical methods and possible  
444 sources. *Sci. Total Environ.* 612, 422-435. doi: 10.1016/j.scitotenv.2017.08.086.

445

446 Brodhagen, M., Peyron, M., Miles, C., Inglis, D.A., 2015. Biodegradable plastic  
447 agricultural mulches and key features of microbial degradation. *Appl. Microbiol.*  
448 *Biotechnol.* 99, 1039-1056. doi: 10.1007/s00253-014-6267-5.

449

450 Cattle, S.R., Robinson, C., Whatmuff, M., 2020. The character and distribution of  
451 physical contaminants found in soil previously treated with mixed waste organic  
452 outputs and garden waste compost. *Waste Manag.* 101, 94-105. doi:  
453 10.1016/j.wasman.2019.09.043.

454

455 Chae, Y., Youn-Joo, A., 2018. Current research trends on plastic pollution and  
456 ecological impacts on the soil ecosystem: a review. *Environ. Pollut.* 240, 387-395. doi:  
457 10.1016/j.envpol.2018.05.008.

458

459 Corradini, F., Casado, F., Leiva, V., Huerta Lwanga, E., Geissen, V., 2021.  
460 Microplastics occurrence and frequency in soils under different land uses on a regional  
461 scale. *Sci. Total Environ.* 752, 141917. doi: 10.1016/j.scitotenv.2020.141917.

462

463 Di Piazza, S., Houbraken, J., Meijer, M., Cecchi, G., Kraak, B., Rosa, E., Zotti, M.,  
464 2020. Thermotolerant and thermophilic mycobiota in different steps of compost  
465 maturation. *Microorganisms* 8, 880. doi.org/10.3390/microorganisms8060880.

466

467 Dolci, G., Catenacci, A., Malpei, F., Grosso, M., 2021. Effect of paper vs. bioplastic  
468 bags on food waste collection and processing. *Waste and Biomass Valor.* In press. doi:  
469 10.1007/s12649-021-01448-4.

470

471 EN 13432, 2002. European committee for standardization, packaging requirements  
472 for packaging recoverable through composting and biodegradation test scheme and  
473 evaluation criteria for the final acceptance of packaging. European Committee for  
474 Standardization, Belgium.

475

476 Franceschini, S., Chitarra, W., Pugliese, M., Gisi, U., Garibaldi, A., Gullino, M.L.,  
477 2016. Quantification of *Aspergillus fumigatus* and enteric bacteria in European  
478 compost and biochar. *Compost Sci. Util.* 24, 20-29.  
479 doi.org/10.1080/1065657X.2015.1046612.

480

481 Fuller, S., Gautam, A., 2016. Procedure for measuring microplastics using pressurized  
482 fluid extraction. *Environ. Sci. Technol.* 50, 5774-5780.  
483 doi.org/10.1021/acs.est.6b00816.

484

485 Haas, D., Lesch, S., Buzina, W., Galler, H., Gutschi, A.M., Habib, J., Pfeifer, B.,  
486 Luxner, J., Reinthaler, F.F., 2016. Culturable fungi in potting soils and compost. *Med.*  
487 *Mycol.* 54, 825-834. doi.org/10.1093/mmy/myw047.

488

489 Harrison, J.P., Boardman, C., O'Callaghan, K., Delort, A.-M., Song, J., 2018.  
490 Biodegradability standards for carrier bags and plastic films in aquatic environments:  
491 a critical review. *Royal Soc. Open Sci.* 5, 71792. doi: 10.1098/rsos.171792.

492

493 Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017.  
494 Microplastics in freshwater and terrestrial environments: evaluating the current  
495 understanding to identify the knowledge gaps and future research priorities. *Sci. Tot.*  
496 *Environ.* 586, 127-141. doi: 10.1016/j.scitotenv.2017.01.190.

497

498 Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M.,  
499 Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial  
500 ecosystem: implications for *Lumbricus terrestris* (*Oligochaeta*, *Lumbricidae*).  
501 *Environ. Sci. Technol.* 50, 2685-2691. doi: 10.1021/acs.est.5b05478

502

503 Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J.A., Sanchez del Cid, L.,  
504 Chi, C., Escalona Segura, G., Gertsen, H., Salánki, T., van der Ploeg, M., Koelmans,  
505 A.A., Geissen, V., 2017. Field evidence for transfer of plastic debris along a terrestrial  
506 food chain. *Sci. Rep.* 7, 14071. doi: 10.1038/s41598-017-14588-2.

507

508 Khalid, A., Soudi, B., Boukhari, S., Perissol, C., Roussos, S., Thami Alami, I., 2017.  
509 Composting parameters and compost quality: a literature review. *Organic Agric.* 8, 1-  
510 18. doi: 10.1007/s13165-017-0180-z.

511

512 Kranz, C.N., McLaughlin, R.A., Johnson, A., Miller, G., Heitman, J.L., 2020. The  
513 effects of compost incorporation on soil physical properties in urban soils - a concise  
514 review. *J. Environ. Manag.* 26, 110209. doi.org/10.1016/j.jenvman.2020.110209.

515

516 Lavagnolo, M.C., Ruggero, F., Pivato, A., Boaretti, C., Chiumenti, A., 2020.  
517 Composting of starch-based bioplastic bags: small scale test of degradation and size  
518 reduction trend. *Detritus* 12, 57-65. doi: 10.31025/2611-4135/2020.14008.

519

520 Li, B., Huang, S., Wang, H., Liu, M., Xue, S., Tang, D., Cheng, W., Fan, T., Yang, X.,  
521 2021. Effects of plastic particles on germination and growth of soybean (*Glycine max*):  
522 a pot experiment under field condition. *Environ. Pollut.* 272, 116418.  
523 doi.org/10.1016/j.envpol.2020.116418.

524

525 Luo, Y., Liang, J., Zeng, G., Chen, M., Mo, D., Li, G., Zhang, D., 2018. Seed  
526 germination test for toxicity evaluation of compost: its roles, problems and prospects.  
527 *Waste Manag.* 71, 109-114. doi.org/10.1016/j.wasman.2017.09.023.

528

529 Moore-Kucera, J., Cox, S.B., Peyron, M., Bailes, G., Kinloch, K., Karich, K., Miles,  
530 C., Inglis, D.A., Brodhagen, M., 2014. Native soil fungi associated with compostable  
531 plastics in three contrasting agricultural settings. *Appl. Microbiol. Biotechnol.* 98,  
532 6467-6485. doi: 10.1007/s00253-014-5711-x.

533

534 Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A  
535 theoretical assessment of microplastic transport in river catchments and their retention  
536 by soils and river sediments. *Environ. Sci. Process. Impacts* 18, 1050-1059. doi:  
537 10.1039/c6em00206d.

538

539 Rhodes, C.J., 2019. Solving the plastic problem: from cradle to grave, to reincarnation.  
540 *Sci. Prog.* 102, 218-248. doi.org/10.1177/0036850419867204.

541

542 Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? *Environ. Sci.*  
543 *Technol.* 46, 6453-6454. doi.org/10.1021/es302011r.

544

545 Ruggero, F., Gori, R., Lubello, C., 2019. Methodologies to assess biodegradation of  
546 bioplastics during aerobic composting and anaerobic digestion: a review. *Waste*  
547 *Manag. Res.* 37, 959-975. doi.org/10.1177/0734242X19854127.

548

549 Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss floodplain soils. *Environ. Sci.*  
550 *Technol.* 52, 3591-3598. doi: 10.1021/acs.est.7b06003.

551

552 Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G.,  
553 McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? *Sci.* 304, 838.  
554 doi: 10.1126/science.1094559.

555

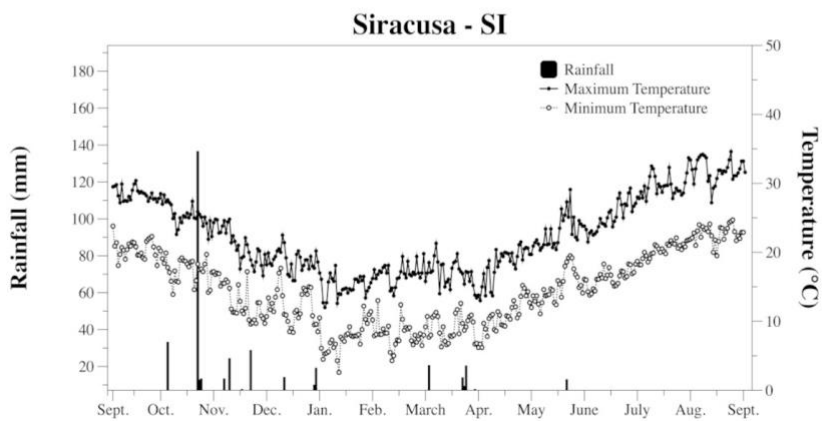
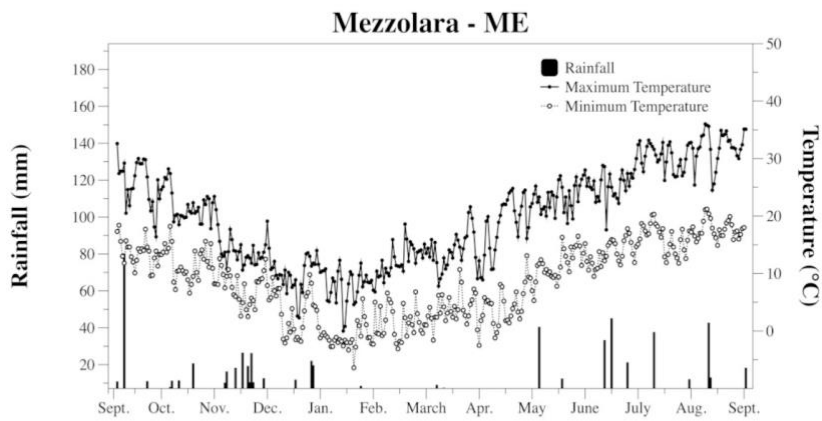
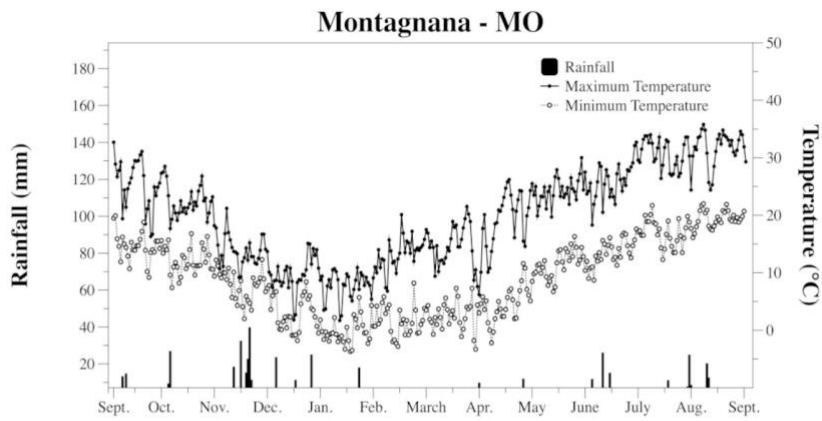
556 Weithmann, N., Möller, J.N., Löder, M.G.J., Piehl, S., Laforsch, C., Freitag, R., 2018.  
557 Organic fertilizer as a vehicle for the entry of microplastic into the environment. *Sci.*  
558 *Adv.* 4, eaap8060. doi: 10.1126/sciadv.aap8060.

559

560 Xanthos, D., Walker, T.R., 2017. International policies to reduce plastic marine  
561 pollution from single-use plastics (plastic bags and microbeads): a review. *Mar. Pollut.*  
562 *Bull.* 118, 17-26. doi.org/10.1016/j.marpolbul.2017.02.048.

563

564 Yang, L., Zhang, Y., Kang, S., Wang, Z., Wu, C., 2021. Microplastics in soil: a review  
565 on methods, occurrence, sources, and potential risk. *Sci. Total Environ.* 780, 146546.  
566 doi: 10.1016/j.scitotenv.2021.146546.



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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.

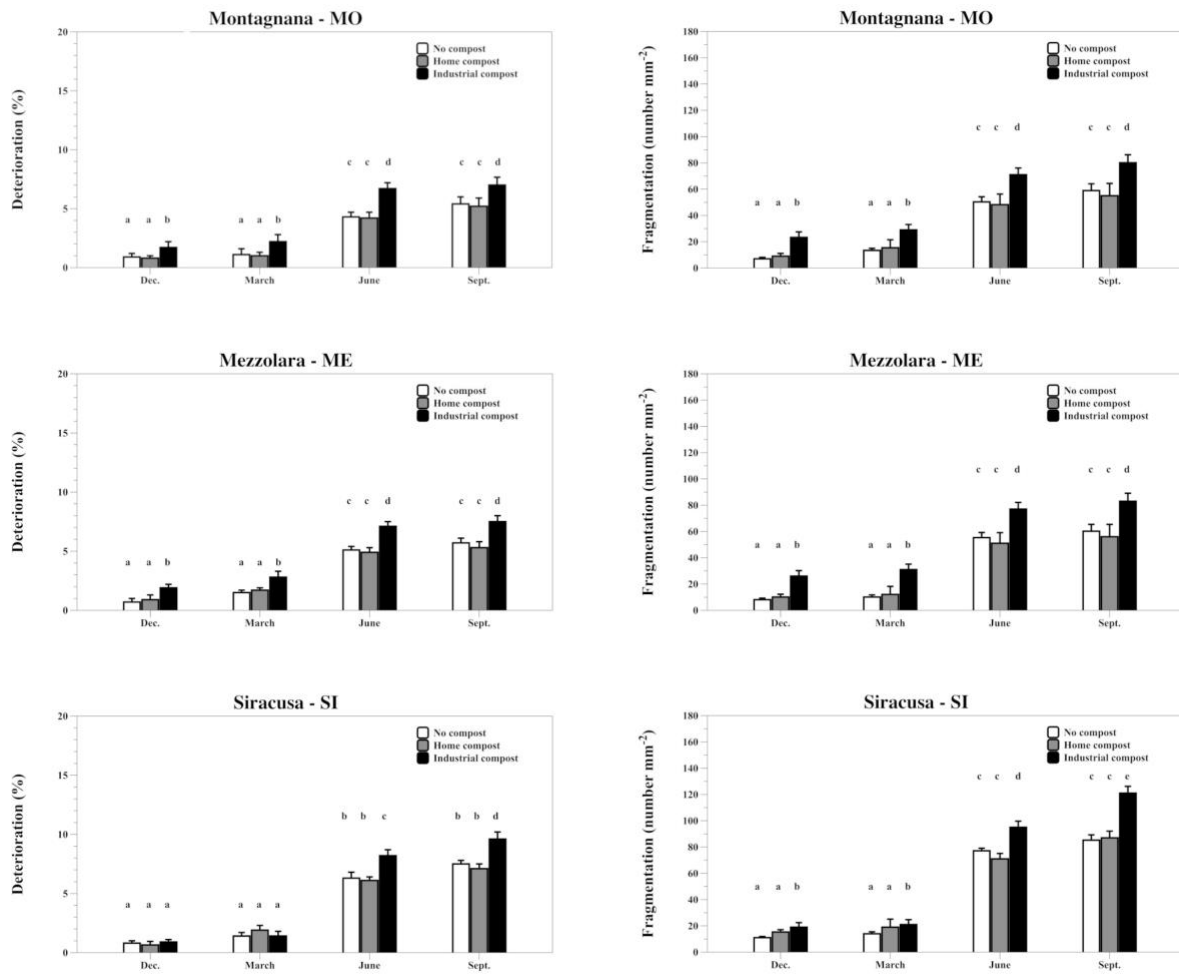


Figure 2. Deterioration and fragmentation of CFMPs in field soil during a 12-month period starting from September 2019. CFMPs were buried in experimental field plots located in three sites, two in Northern Italy (Montagnana, Mezzolara), and one in Southern Italy (Sirucusa). Deterioration, measured as % 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 ( $p > 0.05$ ).

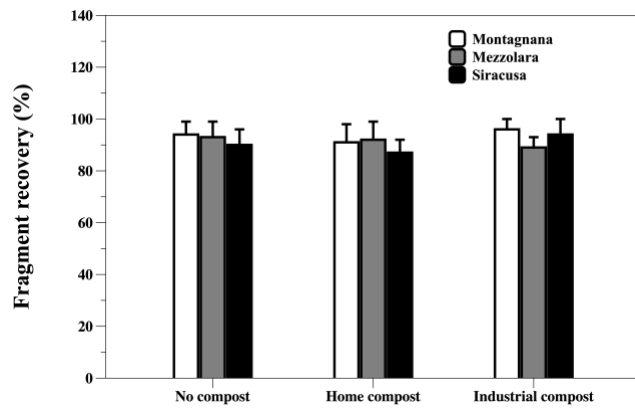


Figure 3. Results of a validation study assessing the percent recovery of small-sized fragments (0.1-4 mm<sup>2</sup>) obtained from CFMPs. A fixed number of fragments were mixed with soil samples collected from non-amended and home or industrial compost-amended plots of the three experimental sites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

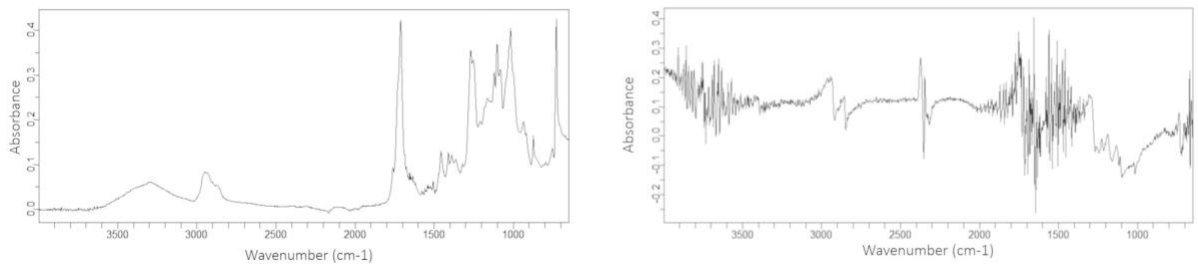


Figure 4. FTIR spectra of a compostable film microplastic sample obtained using the Agilent Diamond ATR Cary 630 module (left) and the SurveyIR infrared micro-spectroscopy accessory (right).

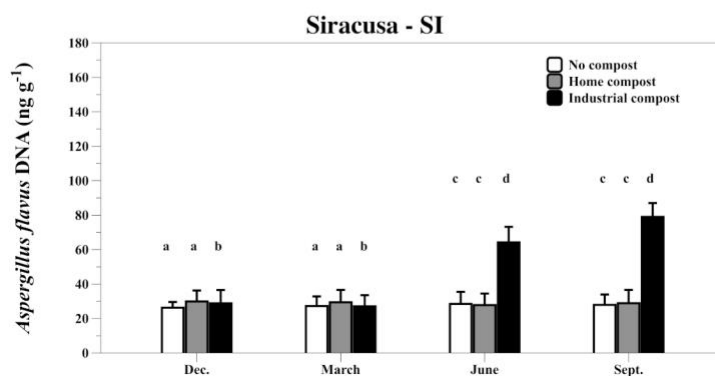
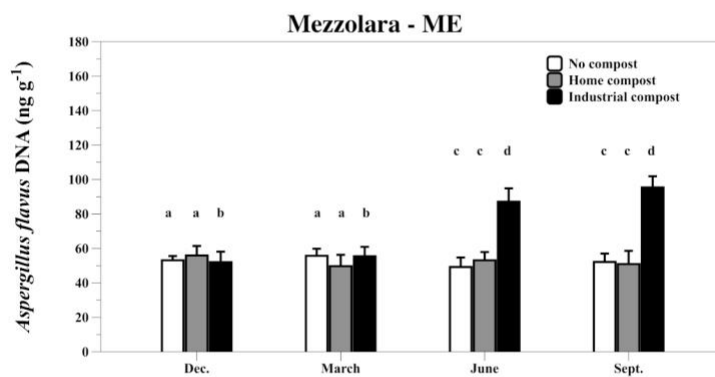
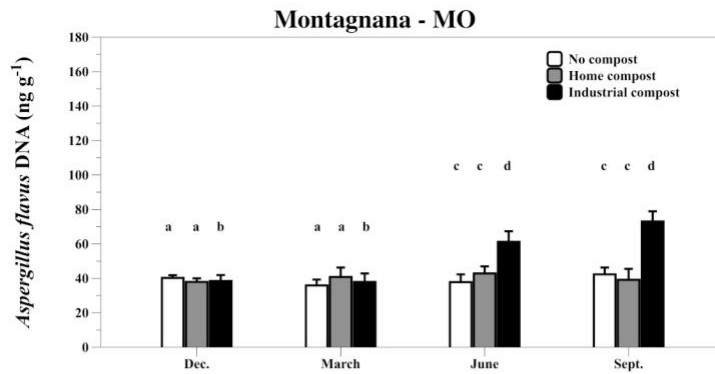


Figure 5. Quantification by qPCR of total soil *Aspergillus flavus* DNA recovered from field plots that were non amended or amended with home or industrial compost. The 12-month study was started in September 2019 and was conducted in three localities, two in Northern Italy (Montagnana, Mezzolara), and 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	<i>Aspergillus flavus</i> level
	Sand	Silt	Clay			
	(%)	(%)	(%)		(g kg <sup>-1</sup> )	(cfu g <sup>-1</sup> )*
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 ( $p > 0.05$ ).

Site	Month	% of fragments infected with <i>A. flavus</i>			% of <i>A. flavus</i> isolates producing aflatoxins		
		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