Effect of Environmental Weathering on Biodegradation of Biodegradable Plastic Mulch Films under Ambient Soil and Composting Conditions

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Abstract
Plastic mulch films contribute to better crop production. Concerns for lack of sustainable disposal methods for conventional polyethylene (PE) mulch led to development of biodegradable plastic mulches (BDMs) that can be soil-incorporated or composted after use. Environmental weathering of BDMs during crop growth reduces their mechanical strength and alters the molecular structure of their polymeric components. However, the impact of weathering on BDMs’ biodegradability is not fully understood. The biodegradability of agriculturally weathered and unweathered BDMs in soil and compost was compared using standardized laboratory tests (ASTM D5988 and D5338) using four BDMs (experimental polylactic acid and polyhydroxyalkanoate-based film [PLA/PHA] and three commercially available polybutyrate [PBAT]-based BDMs). In soil, biodegradation of weathered PLA/PHA was greater than its unweathered counterpart. For PBAT-based BDMs, the extent of biodegradation varied. A decrease of the weight-averaged molecular weight (Mw) of PBAT and PLA and thermostability of PLA, PHA, PBAT, and starch components was observed during biodegradation in the soil. The proportion of the minor components PHA and starch decreased during biodegradation, indicating preferential utilization of PHA over PLA and starch over PBAT by microbes. Bacterial abundance was significantly higher than fungal abundance in soil and was more prominent in soil adjacent to weathered than unweathered BDM treatments. Under composting conditions, unweathered PBAT-enriched mulches yielded higher CO2 evolution than their weathered counterpart. Together, these results suggest that environmental weathering enhances biodegradation of BDMs and mulch’s polymeric constituents also influence the microbial degradation, more so for bacterial than fungal communities.

Keywords Biodegradable plastic mulch · Agricultural weathering · Biodegradation in soil · Composting · Polylactic acid · Polybutylene adipate terephthalate

Introduction
The use of plastic mulch film for agricultural production has led to increased yield and profitability through reduction of weeds, control of soil temperature, and prevention of evaporative loss of water. However, a major drawback of mulch films’ use is the poor environmental sustainability associated with their disposal, particularly for conventional polyethylene (PE) films that are resistive to degradation [1–3]. Recovery of mulch films from agricultural fields after crop harvest is not an economically feasible practice due to labor and disposal costs and fragmentation of plastic that results from environmental weathering, forming debris that often remains in the soil, which can reduce soil fertility, or accumulates in nearby water as litter [4–6].

Biodegradable mulches (BDMs) have been prepared as alternatives to PE mulches to address the ongoing problem of plastic waste accumulation in the environment [2, 5, 7, 8]. BDMs provide similar benefits to specialty crop production as PE mulches but are designed to be tilled into the soil where they will biodegrade [6, 9]. Due to the high cost of biodegradable polymers such as polybutylene adipate terephthalate (PBAT), BDMs are more expensive than PE...
mulches, however they do not incur extra labor costs for removal at the end of their usable life, and therefore may provide savings to farmers.

A new standard for BDMs was recently released by the European Union, EN 17033, to ensure mulch products meet performance specifications both during deployment and biodegradation [10]. EN 17033 contains requirements for inherent biodegradability in soil, chemical composition, mechanical properties, and ecotoxicity. According to EN 17033, BDMs should achieve at least 90% biodegradation under ambient soil conditions within 2 years, utilizing a standardized testing method such as ASTM D5988 [10]. Composting is considered a sustainable alternative choice for waste management disposal of BDMs [2, 11, 12]. According to ASTM D6400, a standard for compostability, a plastic can be considered “compostable” if it meets specified criteria on mineralization, deterioration and ecotoxicity compliance [13]. One criterion is 90% biodegradation in 3 months under industrial composting conditions using a standardized lab test [14]. However, the standardized tests may not accurately reflect biodegradation of BDMs due to the inability to simulate environmental conditions (e.g. pH, moisture, oxygen content, temperature, and microbial communities) and changes of inherent characteristics and properties of BDMs due to environmental weathering [7, 15–19].

The impact of environmental weathering on biodegradability is still not well understood. Upon deployment of BDMs in the field, environmental factors (e.g. solar radiation, temperature, and soil moisture) cleave polymeric constituents of the mulch films [20, 21]. Loss of BDMs’ mechanical properties, e.g., embrittlement, is attributable to photodegradation and hydrolysis [22, 23]. Both depolymerization and embrittlement can enhance biodegradation of BDMs due to the increased availability of weaker morphological regions and lower-molecular weight polymers for microbial utilization [24]. On the other hand, ultraviolet (UV) light exposure can result in crosslinking and increased gel content via a Norrish Type I reaction [22, 25, 26]. The resultant cross-linked polymeric networking may be recalcitrant to biodegradation. Particularly susceptible to photodegradation are polymers with higher aromatic content such as PBAT [18, 26, 27]. In summary, weathering plays a significant role in controlling the degree and rate of biodegradation of mulches in soil and compost, either by enhancing or inhibiting the process. One study found that simulated weathering enhanced the biodegradation of a nonwoven geotextile-based BDM composed of a PLA/PHA blend under composting conditions from 68 to 72% mineralization in 90 days [21].

In addition, the biodegradation process is not fully understood, particularly the relationship between physicochemical property changes of the polymeric material and microbial conversion of polymer carbon atoms to CO$_2$. In a previous study, the authors investigated physicochemical property changes of nonwovens prepared from neat PLA or PLA/PHA blends during biodegradation in compost-enriched soil [28]. They found that during the first stage of biodegradation (1–3 mo), the geotextiles underwent an opening up of their supramolecular structure, observed by an increase of embrittlement, with minimal molecular-level changes. During a second biodegradation stage (5–12 mo), depolymerization occurred slowly and steadily. It is anticipated that environmental weathering will significantly decrease the duration of the initial stage, perhaps minimizing the duration of a lag phase in CO$_2$ evolution, and increase the accessibility of the plastic fragments’ interior region. Hence, microbial assimilation is enhanced, particularly of local regions that are amorphous and enriched in lower-molecular weight polymers and components that are more readily assimilated, such as starch.

The objective of this study was to determine how agricultural weathering affects biodegradation of BDMs in the soil and under composting conditions. Our hypothesis was that the enhancement of biodegradation due to increased embrittlement will outweigh the decrease of microbial assimilation due to cross-link formation. We investigated four BDM films composed of PBAT, starch, PLA and PHA as their polymeric constituents, and related physicochemical property changes that occur during environmental weathering to CO$_2$ evolution to better understand the underlying mechanisms.

**Materials and Methods**

**Materials**

Four black BDMs were employed in this study: an experimental PLA/PHA film and three commercially available PBAT-based films, namely Bio360, Organix, and Naturecycle. In addition, clear and white-on-black versions of Organix were investigated, that used the same polymeric feedstock as black Organix. A cellulosic (paper) mulch (WeedGuardPlus) was employed as the positive control. Naturecycle was a mulch employed for the field and biodegradation studies; however, the change of its physicochemical properties was not monitored to the same extent as Bio360 and Organix, per the request of the manufacturer. Table 1 provides information of the mulches and their physicochemical properties before and after weathering. More detailed information, including color and apparent density, can be found elsewhere [29].

Soil (a sandy loam, Shady-Whitwell complex soil) was collected from the field site used for agricultural weathering of mulches (discussed below), at the University of Tennessee (UT) East Tennessee Research and Education Center, Knoxville, TN, USA. Soil samples were taken on May 2017 (Soil B) for biodegradability testing of black Organix and
Naturecycle and September 2018 (Soil A) for biodegradation testing of PLA/PHA and Bio360. Large particles (e.g. roots and gravel) from the soil were removed through a 2-mm sieve and manually inspected for other debris to ensure a homogenous mixture. Soil was stored in the lab at room temperature (22 °C) for at least 3 days prior to the initiation of the biodegradation experiments to allow for re-equilibration from disturbances that occurred during soil collection from the field. Soil had a C:N ratio within 9.9–10.0 w/w (Organic Production Lab, UT, Knoxville, TN, USA). No adjustments were made to control the initial soil water content since the water holding capacity of the soil was 50–70%, and the soil water content was at 86% (dry weight basis) of total water holding capacity at saturation of soil, both of which are within specifications of ASTM D5988 [30]. The soil pH was 5.43.

Table 1  Biodegradable mulch films employed in this study and their physicochemical properties before and after agricultural weathering a

| Major polymeric component; (Mulch)b | Thickness, (μm)c | Tmax, (°C)d | Gel content, (%)e | Peak load, (N)f | % Elongationf | Carbon (%g) | Manufacturer |
|-------------------------------------|-----------------|-------------|------------------|----------------|---------------|-------------|--------------|
| PLA, PHA; PLA/PHA h                  |                 |             |                  |                |               |             |              |
| Unweathered                         | 37 ± 1.4        | 309 ± 1.2   | 22 ± 0.9         | 16 ± 2.0       | 202 ± 31.0    | 47 ± 0.10   | Experimental Film |
| Weathered                           | 49 ± 3.1        | 310 ± 3.1   | 31 ± 1.8         | 13 ± 4.0       | 5 ± 1.0       | 44 ± 0.60   |              |
| PBAT; Bio360                        |                 |             |                  |                |               |             |              |
| Unweathered                         | 29 ± 1.2        | 401 ± 2.9   | 24 ± 0.3         | 11 ± 1.5       | 320 ± 104.0   | 58 ± 0.16   | Dubois Agrinovation, St. Rémy, QC, Canada |
| Weathered                           | 38 ± 2.0        | 400 ± 0.1   | 31 ± 3.7         | 6 ± 1.3        | 9 ± 4.0       | 46 ± 4.76   |              |
| PBAT; Organix AG (black)            |                 |             |                  |                |               |             |              |
| Unweathered                         | 20 ± 0.7        | 395 ± 5.6   | 31 ± 0.1         | 9 ± 0.8        | 237 ± 16.0    | 51 ± 0.12   | Organix Solutions, Maple Grove, MN, USA |
| Weathered                           | 32 ± 1.7        | 392 ± 3.5   | 33 ± 2.7         | 6 ± 0.8        | 13 ± 3.0      | 48 ± 0.73   |              |
| PBAT; Organix AG, White-on-black (WOB) |             |             |                  |                |               |             |              |
| Unweathered                         | 18 ± 1.3        | 392 ± 2.3   | 23 ± 0.1         | 9 ± 0.3        | 215 ± 10.0    | 51 ± 0.12   | Organix solutions |
| Weathered                           | NDk             | 390 ± 1.7   | 34 ± 1.3         | 7 ± 0.9        | 9 ± 2.0       | 48 ± 0.73   |              |
| PBAT; Organix AG, Clear             |                 |             |                  |                |               |             |              |
| Unweathered                         | 13 ± 1.0        | 396 ± 3.0   | 13 ± 0.1         | 12 ± 1.2       | 207 ± 3.0     | 51 ± 0.12   | Organix Solutions |
| Weathered                           | NDk             | 396 ± 1.5   | 31 ± 1.0         | 9 ± 1.1        | 8 ± 2.0       | 48 ± 0.73   |              |
| Copolyester; Naturecycle            |                 |             |                  |                |               |             |              |
| Unweathered                         | 57 ± 1.8        | NDk         | NDk              | 10 ± 1.2       | 310 ± 102     | 55 ± 0.28   | Custom Bioplastics, Burlington, WA, USA |
| Weathered                           | 55 ± 3.3        | NDk         | NDk              | 10 ± 2.0       | 8 ± 1.8       | 52 ± 0.73   |              |
| Cellulosic; WeedGuardPlus           |                 |             |                  |                |               |             |              |
| Unweathered                         | 562 ± 13        | NDk         | NDk              | 61 ± 17.0      | 7 ± 1.1       | 46 ± 0.90   | Sunshine Paper Co., Aurora, CO, USA |

aAgricultural weathering of mulches occurred in 2017 (Jun-Sept) during mulches’ use in field trials in Knoxville, TN, USA, with pepper as test crop. (For clear Organix, weathering took place during the same period in Mount Vernon, WA, USA, using sweet corn as test crop.) A description of the field trials is described in [33, 34] and environmental conditions in [29]

bPLA, PHA and PBAT refer to polylactic acid, polyhydroxyalkanoate and polybutylene adipate terephthalate, respectively

cValues reported for weathered mulches reflect treatment using artificial weathering, since adsorbed soil impacted the thickness values for weathered mulches [22]

dTemperature at which rapid and maximum thermal degradation of the major polymer (PLA for PLA/PHA and PBAT for the others) occurred (thermogravimetric analysis)

eRefers to the percent gel content of major polymeric constituents (PLA, PHA and PBAT)

f along the machine direction of the films

gValues obtained from [22]

hIts polymeric components consist of 68–71 wt % PLA and the remainder PHA; was prepared by Metabolix Inc., Cambridge, MA [22]

iMater-Bi® grade EF04P (Novamont, Novara, Italy)

jEcovio® grade M2351 (BASF, Ludwigshafen, Germany)

kNot determined (no analyses were performed)

lOnly initial (unweathered) Weedguard was employed herein
Samples of registered organic compost, made of yard and food scraps, was provided by Cedar Grove Composting (Everett, WA, USA) [31] and sieved to less than 8 mm particle size. The maturity level of compost passed the germination (Everett, WA, USA) [31] and sieved to less than 8 mm and food scraps, was provided by Cedar Grove Composting. Supplementation to the test performed by the provider of compost maturity test kit from Solvita (Mt. Vernon, ME, USA), is essential for controlling the maturation process. A compost maturity test kit from Solvita (Mt. Vernon, ME, USA) was employed to ensure the compost was at the appropriate maturity stage (CO2 level of 6, as described in the test kit instructions). The moisture content of the compost was consistently within 55-60% (gravimetric analysis), meeting the requirements of ASTM D5338 [14]; therefore, the moisture content was not further adjusted. Several properties of the compost were evaluated. Volatile solids were determined via gravimetry, compost samples were dried in the oven at 105 °C for 10 h until constant mass was achieved and after calcination at 550 °C in an electric furnace. Volatile solids were present at 36%. For pH determination, a compost sample (10 g) was mixed with deionized water (50 mL) under stirring for 30 min and allowed to settle for 1 h. pH was measured at 7.87 for the resultant supernatant. The C:N ratio, 17.7 w/w, was determined through measuring total carbon and nitrogen content on oven-dried compost samples by combustion (Flash EA 1112 NC Soil Analyzer; Thermo Fisher Scientific Inc., Waltham, MA, USA). Further information and properties of the compost are available from the provider [31].

Solvents (e.g., hydrogenated and deuterated chloroform and HCl (aq)) were HPLC grade, and chemical reagents employed for microbial analysis were of high purity (> 98%), and purchased from Fisher Scientific (Pittsburgh, PA, USA). Vermiculite (Mg_{2.18}Fe_{0.7}Al_{4.3}SiO_{10}(OH)_2 \times 4H_2O), Grade 4, mesh size 7.9 mm, was purchased from Uline (Pleasant Prairie, WI, USA). Vermiculite particles possessed an average particle size of 4.65 ± 2.39 mm (length-to-width ratio of 1.39), measured with ImageJ software [32]. Deionized water was used throughout.

### Methods

#### Environmental Weathering of BDMs

Environmental weathering took place for PLA/PHA, Bio360, and white-on-black Organix in June-Sept, 2017 during field trials conducted for green pepper production in Knoxville, TN, USA. For black Organix and Naturecycle, weathering took place in June–Sept 2016 at the same location, using pie pumpkin as test crop, while clear Organix underwent weathering in June-Sept 2018 at Mount Vernon, WA, USA, using sweet corn as test crop. Further information on the field trials and the impact of environmental weathering on BDMs' properties is given elsewhere [33, 34]. BDMs retrieved from the field were cleaned using a soft-bristle brush and stored at room temperature until the mineralization experiments were to be conducted, at which time BDMs were cut into 1 cm² pieces for the soil biodegradation study and 4 cm² pieces for investigating biodegradation under composting conditions.

#### Testing of Biodegradability of BDMs in the Soil

The testing of biodegradability of BDMs in soil was carried out according to a standardized test method, ASTM D5988 [30]. Tightly sealed 473 mL mason jars (Item # 10500, Ball® Corporation, Westminster, CO, USA) served as incubators. Jar lids were mounted with 20 mm gray butyl stoppers (Milipore Sigma, Burlington, MA, USA) to enable sampling of the air headspace for CO2 determination. For each test, BDM weight per jar was determined based on ASTM 5988 recommendation (200–1000 mg carbon/100–500 g soil). Jars contained soil (50 g) and small (1 cm²) pieces of mulch (100 mg of BDM carbon). Mulch film pieces were effectively dispersed in the soil by adding BDM pieces and soil layer-by-layer in the jars. Glass scintillation vials (20 mL) containing deionized water were also included in each jar to maintain humidification of the air headspace and therefore to minimize moisture loss of the soil. Jars were incubated at 27 °C. Soil moisture was maintained by periodically adding deionized water to the soil when the moisture loss was > 1%, as determined by weekly gravimetric analysis.

An infrared gas analyzer (LI-820, LiCor, Lincoln, NE, USA) was used to measure CO2 evolution in the headspace of the jars versus time. CO2 concentrations were calculated based on a calibration curve using gas standards with CO2 concentrations of 1000 ppm, 1 vol %, and 5% in N2 (Gasco, Oldsmar, FL, USA). Soda lime served as a zero-concentration standard. During the initial phase of the experiments, sampling was taken three times per week. Sampling frequency was reduced to once per week when the respiration from the microorganisms appeared to slow. Once per week,
the jars were opened to air to purge the jars of accumulated CO₂.

Each series of biodegradability tests involved the use of four BDM treatments (weathered and unweathered versions of two BDMs) and three controls: soil that contained unweathered WeedGuardPlus (WGP; positive control), a blank (soil only), and a technical control (air only). WGP, a paper mulch with cellulose as its major constituent, rapidly degrades in the field (< 1 year) and is biodegradable [23]. The selection of WGP as positive control rather than microcrystalline cellulose, as specified in ASTM D5988, was based on its use as a control mulching treatment in field trials and soil quality assessments that were done in parallel with the research described herein [33–36]. Each BDM treatment and control were replicated thrice. Biodegradability experiments for PLA/PHA and BioAgri were initiated on 4 Sept 2018, while experiments for black Organix and Naturecycle began on 17 May 2017.

The percentage of biodegradation was calculated according to the procedure described in ASTM D5988 [30]. The net CO₂ evolution resulting from microbial assimilation was determined by measuring the average CO₂ produced for a given mulch treatment and time, and subtracting the CO₂ measured for the controls. Biodegradation was determined by comparing the net CO₂ evolution to the theoretical maximum CO₂ that would be produced. The carbon content of BDMs, determined previously via elemental analysis [22], is given in Table 1.

For microbial abundance assessment, DNA extraction and quantitative polymerase chain reaction (qPCR) with three replications were performed. A subsample of soils from jars used for CO₂ evolution were stored in − 80 °C freezer. Genomic DNA was extracted using DNeasy PowerSoil kit and PowerLyzer 24 (Qiagen; Hilden, Germany). Concentration of extracted DNA was measured using Quant-iT™ PicoGreen™ dsDNA assay kit (Life Technologies, Eugene, OR USA; now Invitrogen, Carlsbad, CA, USA), and Synergy H1 hybrid plate reader (BioTek, Winooski, VT, USA). Extracted DNA was stored at − 20 °C. qPCR was performed in replicates using Femto Bacterial and Fungal DNA quantification kits (Zymo Research, Irvine, CA, USA) to quantify bacterial 16S rRNA and fungal internal transcriber space (ITS) region genes as a proxy for bacterial and fungal abundance, respectively. qPCR reactions were set up according to the manufacturer’s instructions and qPCR performed on a CFX Connect Real-Time PCR Detection System (Bio Rad, Hercules, CA, USA). All qPCR standard curves had an R-squared of ≥ 98%.

Chemical Changes in BDMs During Soil Biodegradation

Changes in molecular weight, polymer composition, surface chemical changes and thermal properties were measured over time during biodegradation in the soil. Nine replication jars for each of weathered and unweathered PLA/PHA and weathered and unweathered Bio360 BDMs were added to the corresponding CO₂ evolution experiments described above, and therefore underwent identical environmental conditions as those employed for CO₂ evolution. Mulch films were retrieved from one of the nine replication jars at specific times: after 3 mo and subsequently at 2-mo intervals. Only intact mulch film pieces were carefully taken out from the jar and used for analyses. Approximately 60 mg were taken for GPC (3 replications), 40 mg for NMR (2 replications) and 4 mg for TGA (2 replications).

Weight-averaged molecular weight (M_w) and polydispersity index (PDI) were determined through gel permeation chromatography (GPC) performed using an HPLC system (Shimadzu Columbia, MD, USA), equipped with a model Mark IIII evaporative light scattering detector (ELSD; WR Grace, Deerfield, IL, USA) and a 300 × 7.5 mm ID PL Gel mixed D column purchased from Agilent (Santa Clara, CA, USA). Samples (20 mg) were dissolved in 5 mL chloroform, magnetically stirred for 1 h, centrifuged at 10,000 rpm (6149 × g) for 1 min and filtered (1.0 μm and 0.2 μm). Filterate (200μL) was injected into the HPLC system, with chloroform serving as mobile phase at 0.8 mL/min with a run time of 13 min. Molecular weight was determined based on polystyrene standards. For PLA/PHA, the detector signal was much more sensitive toward PLA and therefore the measured results reflect those of the PLA component. For Bio360, since starch is insoluble in chloroform, GPC results reflect PBAT only.

1H-NMR spectra of PLA/PHA were determined using a Varian 400 MHz spectrometer (Agilent), with pulse width of 90°. BDM sample (20 mg) was dissolved in CDCl₃ containing 1% tetramethylsilane as an internal standard (800 μL; Acros, Geel, Belgium). Spectral assignments for PLA and PHA are given in our previous paper [28]. Each sample was performed with two replications. The methodology employed for determination of thermal stability through thermogravimetric analysis (TGA) and particle size analysis of BDM fragments through microscopy after 12 mo incubation in soil is described in the Supporting Information (SI).

Biodegradability of BDMs Under Industrial Composting Conditions

A laboratory scale respirometric system was constructed to measure aerobic biodegradation of BDMs based on the system described previously [21], following guidelines given
in ASTM D5338 [14]. A circuit was formed for each testing jar, consisting of the following: (1) air supply (house air removed of its CO₂ content by passage through a series of three packed 621 kPa columns [28.9 cm height × 6.6 m inner diameter] of Drierite™), (2) air humidification system, consisting of the bubbling of CO₂-free air through deionized water contained in a stoppered flask, (3) rotameter (Cole Parmer, Vernon Hills, IL, USA), to control the flow rate of moist air from the flask at 40 mL/min (4) a 1.9-L capacity composting jar (Fillmore Container, Lancaster, PA, USA), and the outlet air flow from the jar was sent to a (5) CO₂ trap, consisting of two test tubes in series that contained 60 mL of 0.024 M Ba(OH)₂ solution. The entire system, except for the Drierite™ packed columns, was retained in an incubator controlled at 58 ± 1 °C, in the dark. (Jars were equipped with connectors near the bottom for the inlet air, tightly sealed to the jars through epoxy glue, and the lids for the outlet flow.) A mesh screen was included in the interior of test jars to act as barrier to minimize mulch adhesion to jar walls and the mixture to prevent clogging of the air inflow, which occurred near the bottom of the jars. The composition of composting media followed the conditions employed in a previous study [21], consisting of compost (240 g, dry weight), vermiculite (50 g; moisture level adjusted to 55%), and BDM (9 g of 2 cm × 2 cm pieces), which were thoroughly mixed by hand to ensure a homogeneous mixture and good mulch-compost contact. BDMs were added into mature compost (noted by its dark color and less pungent odor) where majority of bacteria are no longer active because of the absence of available carbon sources [37]. The selection of mature compost is to ensure that carbon evolution will be ideally derived from carbon components of BDMs and not mainly from soil carbon. Separate batches of compost were used for the three separate experimental runs, evaluating the compostability of PLA/PHA, Bio360, and Organix plus Naturecycle. Except for Bio360, all other BDMs used a completely mature compost. Vermiculite was added to ensure aeration and retention of compost moisture.

CO₂ concentrations were determined through measuring free Ba(OH)₂ remaining in solution in the traps via titration with 0.05 N HCl solution, with phenolphthalein serving as an indicator. The net CO₂ production was calculated through subtraction of the evolved CO₂ from the controls. Percent biodegradation was calculated similarly to that described above for biodegradability in soil [14, 30].

The number of composting jars incubated simultaneously for a series of tests was within 12–18, consisting of two or four BDM treatments (e.g., weathered vs unwethered versions of one or two BDMs), a positive control (unweathered WeedGuardPlus) and a blank (compost), with three replicates per treatment. All biodegradation experiments were conducted for 90 days. Composting experiments for PLA/PHA, Bio360, and black Organix plus Naturecycle were initiated on 23 September 2018, 26 October 2017, and 17 February 2019, respectively. An experiment comparing the compostability of three varieties of environmentally-weathered Organix BDM that differed in color was initiated on 18 July 2019.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SAS (Statistical Analysis System Version 9.2 for Windows; SAS Institute, Cary, NC, USA) to calculate statistical difference on physicochemical properties of mulches upon biodegradation. Differences between treatment means were performed using Tukey’s honestly significant difference (HSD) analysis at significant difference level of α = 0.05.

For microbial abundances, a linear model was made using copy numbers as a response of mulch treatment and weathering condition. Two-way ANOVA was used to test the model, and Post-Hoc analysis included Tukey’s test and least-squared means; used to compare treatments, and effect of weathering conditions for each treatment, respectively. Gene copy statistical analysis was done in R programing language, version 3.5.3 [38].

Results and Discussion

Effect of Environmental Weathering on Physicochemical Properties of BDMs

Weathered BDMs used in field trials in 2017 (clear Organix in WA and all other BDMs in TN) were retrieved and compared with virgin BDM films to determine the effect of environmental weathering on physicochemical properties and biodegradation in soil and in compost. Weathering caused significant degradation of BDMs, as seen through the decrease of physical properties and change of chemical properties (thermal stability, polymer composition, and gel content; Table 1). The change of properties was slightly influenced by the composition of the BDMs [22, 29].

Specifically, for PLA/PHA, crosslinking, or formation of chemical bonds between PLA, PHA and the filler used (CaCO₃) via solar radiation, was evidenced by an increase of peak load and gel content, which may inhibit biodegradation. Change of physicochemical properties of PBAT-based BDMs (Bio360, Organix, Naturecycle) varied quite significantly between mulches, except for the latter, whose physicochemical properties were not extensively studied. Physicochemical properties of Bio360 changed to a greater extent (e.g. decreased peak load and increased gel content and thermal stability) than Organix (Table 1). Among the three Organix BDMs, clear Organix underwent more degradation during weathering than black and white-on-black films (e.g. FTIR spectral changes and decreased gel content and peak load), even though the
clear film, unlike the other two, was weathered in WA, a cooler and drier climate than TN. The difference is at least partially attributable to carbon black, a photostabilizer known to inhibit photodegradation for black mulch films such as the black and white-on-black Organix BDMs.

**Effect of Environmental Weathering on Biodegradation in Soil**

**CO₂ Evolution Profiles**

CO₂ evolution continuously increased during the initial phase of biodegradation, then eventually reached a plateau phase at ~100 days for PLA/PHA, Bio360 and Organix (black) and ~200 days for Naturecycle (Fig. 1). Individual CO₂ evolution profiles used to calculate % biodegradation are given in Figure S1 of the Electronic Supplementary Materials (ESM). As for WGP, the positive (cellulosic) control, CO₂ continually increased, reaching a plateau (~80%) for the first experiment (involving PLA/PHA and Bio360; 10 mo) and 55% biodegradation for the second experiment (Black Organix and Naturecycle; 11 mo) but, a plateau was not reached for the latter run. The criterion for a positive control within ASTM D5988 [30], 70% mineralization within 6 mo, was not met by WGP for either experiment. For the second experiment, only 2 replicates were used for WGP due to one replicate jar forming a crack after 4 mo. The latter experiment produced lower CO₂ values that varied substantially from the values of two other WGP replications after 4 mo. CO₂ released from the soil only remained minimal (<2% biodegradation) throughout the time-course of biodegradation (Figure S1).

CO₂ production from PLA/PHA underwent a lag phase during the initial 2–4 weeks for weathered and ~7 wk for unweathered BDMs. Weathering enhanced the biodegradation of PLA/PHA and Bio360, but not for the other two BDMs in the soil. PLA/PHA had the highest rate and extent of biodegradation among the BDMs (Fig. 1a). Weathered PLA/PHA achieved 35% biodegradation after soil-incubation (365 days), whereas unweathered PLA/PHA underwent only 16% biodegradation. Other studies using the same ASTM testing method demonstrated higher biodegradation of PLA-based plastics in soil had longer incubation period. For example, a PLA/PHA resin was able to reach...

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**Fig. 1** Cumulative mineralization of weathered and unweathered BDMs during 1 year of soil incubation, under ASTM D5988 standardized test conditions. Data for a and b were collected simultaneously; therefore, the panels share the same data for WeedGuardPlus (positive control). Similarly, since data for c and d were also obtained simultaneously, the two panels share the same WeedGuardPlus data. Data points are means and errors bars reflect standard deviation (n = 3; except for WeedGuardPlus in Figures c and d, where n = 2)
70% biodegradation in 660 days [11] whereas powder and pellets made from a PLA/starch blend attained 90% biodegradation, equal to that for cellulose, after 276 days [27]. One explanation for the different results was that the PLA/PHA mulch fragments introduced in our study (1 cm² pieces) were larger in size compared to the other studies. We did not use any methods for size reduction, such as mechanical grinding, to ensure that we would not introduce artifacts in the physicochemical properties of the mulches, since the heat of friction can increase the temperature inside of the grinding mill and melt the plastic [39, 40]. Larger particle size and surface area may be a contributing factor to a slower microbial colonization on mulch surfaces. The same study predicted that at nano size level, full biodegradation would require 15–20 days [27]. Another explanation may be that the soils used herein differ in properties from those employed in other investigations; e.g. the soil pH of 5.43, C:N ratio of 10:1) obtained for our source of soil [35] are lower than values reported in other studies [11, 27].

Among PBAT-based mulches, Bio360 underwent lower biodegradation than Organix (black) and Naturecycle. Weathered and unweathered Bio360 (Fig. 1b) reached only 19% and 12% biodegradation in 350 days, respectively. For Organix (black) and Naturecycle, unweathered mulches underwent a slightly higher biodegradation than weathered (25% cf. 20% and 22% cf. 20%, respectively) (Fig. 1c, d). A study on PBAT-based film reported that a higher adipate (A/T) ratio than terephthalate (A/T) for PBAT can give rise to greater amorphous regions available for microorganisms which enhances degradation [41]. Our results are inconsistent with the cited study [41] since a slightly higher biodegradation was achieved for Organix (black) compared to Bio360 (Fig. 1), even though the latter had a 2% higher A/T ratio (p = 0.0007, NMR analysis). The difference in the biodegradation of the three PBAT-based BDMs may be attributable to differences in minor polymeric constituents of the BDMs or the processing method employed to prepare the mulch films.

**Size Reduction of Mulch Pieces During Biodegradation**

After soil incubation (365 days), mulch film pieces were retrieved from the soil and analyzed for size and shape. The results demonstrated that mulch pieces produced from weathered BDMs had smaller particle sizes (Figure S2), consistent with our hypothesis. The average particle size diameter (dp) of mulch films recovered from soil was 300 ± 33 µm and 225 ± 20 µm for unweathered and weathered PLA/PHA, respectively, and 648 ± 119 µm and 273 ± 23 µm for unweathered and weathered Bio360, respectively (Table S1). The relatively large dp value for unweathered Bio360 compared to the other three mulch treatments is consistent with the relatively low % biodegradation achieved (Fig. 1). The particle size distribution was also narrower for weathered mulch films (Figure S3). Embrittlement due to environmental weathering promoted rapid size reduction of mulch pieces during the time course of biodegradation which results in an increase of surface area. Increased surface area (e.g. microplastics in agriculture) provides more accessibility to numerous microorganisms, which can be more heavily colonized and different in microbial community structure than the surrounding soil especially for microplastics that are kept underneath the soil for longer periods, that accelerates biodegradation [27, 42]. Further detail on the particle size analysis is given in the ESM.

**Microbial Abundances in Soils**

Total bacterial and fungal abundances were determined for soils recovered from the biodegradability experiments upon their completion. As shown in Fig. 2, bacterial abundance was significantly different among BDM treatments (p = 0.0303) for soil biodegradation using soil A (WGP A, PLA/PHA and Bio360, and bare soil control in soil A), but not for biodegradation using soil B (WGP B, Organix (black), Naturecycle, and bare soil control in soil B). Yet, for experiment B, interaction of treatment (weathered, unweathered) and BDMs yielded a significant difference for bacterial abundance (p = 0.0031). The mean bacterial abundance of soil A was higher than soil B, which may explain the higher biodegradation rate observed for Experiment A compared to Experiment B (Fig. 1). A study from the field site where our soil was taken showed that bacterial communities significantly varied in structure and activity between seasons at
The addition of mulch in both experiments resulted in an increase in both bacterial and fungal abundances compared to the no mulch added controls (Figs. 2 and 3). This is generally consistent with other studies, which have documented increases in soil microbial abundances and activities as a result of BDM incorporation [45]. Specifically, during field studies at the sites where our soils were collected, it was previously found that 2 years of BDM mulch use and incorporation resulted in significant enrichment of bacteria in the soil [43]. Other studies have also reported increases in fungal abundance as a result of incorporating BDMs into soil [23, 46, 47]. These observed increases are generally attributed to the increase in microbial biomass as part of biodegradation of the mulch.

Weathered mulches induced a significantly higher bacterial abundance compared to unweathered mulch, particularly for Naturecycle, and to a lesser extent for Bio360 and PLA/PHA. The greater bacterial abundance for weathered than for unweathered BDMs is consistent with the higher biodegradation observed for weathered PLA/PHA and Bio360 (Fig. 1), and the greater degree of particle size reduction observed for weathered BDMs. Weathering not only introduces physical changes to the mulch properties, but field deployment would also provide time for soil microbes to colonize the plastics. We know that field-weathered plastics are colonized by diverse bacterial and fungal communities, different from microbes found on virgin plastics [48]. We can presume that at least some of the microbes colonizing plastic film surfaces in the environment are doing so because of an ability to degrade the plastic. Thus, the field-weathered plastics used in this study likely started with an attached microbial community “primed” to degrade the plastic.

The opposite trend was observed for Organix (black) mulch, indicating a relatively greater bacterial abundance in treatments with unweathered BDMs than weathered mulch. This result is consistent with the earlier initiation of the plateau phase (180 days) and lower % biodegradation for weathered Organix (black) (Fig. 1c), as well with the low impact of weathering on the physicochemical changes of Organix (black) (Table 1).

Filamentous fungal communities have been reported to extensively colonize PBAT film surfaces and directly contribute to accelerated degradation in soil [41, 46, 49]. In our study, after 365 days of soil incubation, fungal abundance was higher for soil B compared to soil A (retrieved in Fall). Fungal abundances after biodegradation were significantly different among BDM treatments for both soil experiments A ($p < 0.0001$) and B ($p = 0.0009$), but not within weathering treatments (Fig. 3). There was no significant difference with regard to fungal abundance of weathered BDMs relative to their unweathered counterparts in soil biodegradation Experiment A. The BDM treatments of Experiment A had lower fungal abundance than WGP A; but, no statistically significant difference of fungal abundance existed between BDMs and the bare soil control (soil A). For Experiment B, unwithered Naturecycle and weathered Organix (black) had fungal abundance significantly similar to Soil B, but lower than the other treatments: weathered Naturecycle, unwithered Organix, and WGP ($p = 0.0235$). Uniquely observed for Organix (black), both bacterial and fungal communities were higher for soils with unweathered than weathered mulch which can be attributed to crosslinks formation during photodegradation that inhibits biodegradation [18]. In a recent study though, both photodegradation of films and appropriate use of bacterial strain (e.g. Bacillus subtilis) significantly increased the biodegradation of BDMs (PBAT and PBAT/PLA) [50]. In another study, 1 × 1 cm² pieces of PBAT-based BDM with minor biobased components (e.g. PLA, starch) were mineralized in soils, even in soils with poor organic matter content: About 40–50% biodegradation was achieved after 350 days, a value slightly higher than what we found in our study [6]. Our previous field enrichments indicate similar microbial communities found in our initial soil (lab enrichments) [43], so other factors (e.g. organic matter, soil chemistry) may have limited degradation capabilities in our study as also observed by others [44, 51].

Fig. 3  Abundance of fungi in soil after 12 mo biodegradation experiments. Note: Soil biodegradation experiment A was comprised of WeedGuardPlus (WGP) A, PLA/PHA and Bio360 and Soil A, whereas experiment B was comprised of WGP B, Organix, Naturecycle and Soil B. “Soil A” and “Soil B” refer to control treatments where plastic mulches were not included. “NA” or not applicable, refers to soil treatment, technical control, that do not contain any mulch samples
Changes of Biopolymer Chemical Properties

The polymeric composition of both weathered and unweathered PLA/P HA BDMs changed during biodegradation in soil. Results show a consistent increase of PLA content (i.e., decrease of PHA) for weathered and unweathered PLA/PHA mulch vs. time, but with the change being significantly higher for the weathered mulch (p < 0.0001; Fig. 4a). This result clearly indicates that PHA was preferentially utilized over PLA as a carbon source by soil microorganisms as a study also found out [47]. Similarly, during the time-course of biodegradation for a meltblown PLA + PHA nonwoven mulch in compost-enriched soil, %PLA also increased [16, 28]. A significant decrease of starch concentration vs. time occurred for both weathered and unweathered Bio360, indicating preferential utilization of starch over PBAT as a carbon source (Fig. 4b).

According to gel permeation chromatographic analysis of weathered and unweathered PLA/P HA and Bio360, M_w consistently decreased throughout biodegradation, but to a greater extent for PBAT in Bio360 than for PLA in PLA/P HA (p < 0.0001, Fig. 5, Table S2). M_w decreased significantly after 3 mo for both weathered and unweathered Bio360, but to a greater extent for the former (p < 0.0001). M_w reached a minimum value after 7 mo and 9 mo for unweathered and weathered Bio360, respectively (p < 0.0112; Fig. 5), and remained constant thereafter. Unlike PBAT, PLA has a high glass transition temperature, T_g (60-65 °C). The ambient and soil temperature (27 °C) was far below T_g, minimizing the contribution of thermal degradation. Yet, a decrease of M_w was
still observed, but with no significant differences in $M_w$ values for weathered PLA/PHA at a given time through 7 mo ($p = 0.0872$). $M_w$ reached minimum values for both PLA/PHA mulches at 9 mo ($p < 0.0001$). Other studies of molecular weight decrease for PLA during biodegradation used PLA sheets or films or nonwoven geotextiles of PLA blends. PLA in compost disintegrated after 5 days, causing a significant $M_w$ reduction from 151.90 kDa to 4.45 kDa, and a 50% reduction of $M_w$ after 4 weeks of burial in soil [52, 53]. A study on the biodegradation of nonwovens prepared using PLA/PHA blends in compost-enriched soil observed that $M_w$ increased during the early stage of biodegradation, but then decreased subsequently [28]. At this initial stage, microorganisms would consume the monomeric and oligomeric regions near the mulch surface [54]. The PDI of weathered Bio360 significantly decreased ($p < 0.0001$) during soil biodegradation from 3.74 to 2.43; however, no significant changes were observed for PLA/PHA ($p = 0.3944$, Table S2).

Figure 5 displays the $M_w$ relative to the rapid degradation temperature ($T_{max}$) of PLA for PLA/PHA and PBAT for Bio360 throughout biodegradation. The decrease of $M_w$ corresponds to the decrease of main heating stages of major polymeric constituents of BDMs. Heating stages of PLA and PBAT consistently shifted to lower temperatures throughout 9 mo of biodegradation in the soil, an indication of thermal stability decrease. TGA thermograms clearly illustrate the decrease of thermal stability, occurring to a greater extent for weathered mulches (Figure S4). Biodegradation in the soil led to significant decrease of $T_{max}$ for PLA and PBAT ($p < 0.0001$), to a greater extent for weathered PLA/PHA and Bio360 than unweathered mulch counterparts ($p < 0.0001$). The $T_{max}$ for PLA (PLA/PHA) and PBAT (Bio360) increased from 0 to 3 mo then decreased from 3 to 8 mo and remained constant thereafter (Fig. 5a). During the first stage of biodegradation (0-3 mo), microbes may be slowly becoming colonized (“situated” or established) on the mulch surface, especially on unweathered films not previously exposed to soil microbes. The increase of temperatures may indicate selective depolymerization of lower molecular weight polymer molecules and lower crystalline morphological regions of the plastic mulch. The rate of decrease for both temperatures after 3 mo is consistent with the rate of decrease for $M_w$ of PLA (Fig. 5a). A decrease of $T_{max}$ can reflect depolymerization ($M_w$ decrease) while an increase can indicate leaching out of lower-weight oligomers/monomers ($M_w$ increase).

**Effect of Environmental Weathering on Biodegradability Under Composting Conditions**

The time courses of biodegradation for BDMs and the cellulosic mulch (WGP; positive control) under industrial composting conditions over a 3 mo period are shown in Fig. 6. The portion of the CO$_2$ evolution attributable to the compost alone was consistently low throughout the experiment, ranging from 2 to 11% biodegradation (Figure S5). Within 10 days, 46–59 mg of CO$_2$ were produced per g of volatile solids for the plastic-free controls of the PLA/PHA, Organix and Naturecycle experiments, which is within or near the specified range of ASTM D5338, 50–150 mg/g. In contrast, the Bio360 experiment’s plastic-free control produced only 21 mg CO$_2$/g within 10 days. The level of biodegradation achieved was higher under composting conditions than in soil due to several factors that likely enhance microbial activity, such as higher moisture content and temperature. As expected, WGP, the positive control, underwent a steady increase of CO$_2$ production and achieved the highest biodegradation: 60–90% (Fig. 6). WGP met the criterion for a positive control within ASTM D5338 [14] 70% mineralization within 45 days, for the experiments involving PLA/PHA, Organix and Naturecycle, but not for Bio360 (Fig. 6).
Biodegradation of PLA/PHA under composting conditions was higher than for PBAT-based BDMs. PLA/PHA achieved 76% and 38% biodegradation for weathered and unweathered mulches (Fig. 6a). PLA films that were slightly thinner than PLA/PHA films used in our study (22–34 µm cf. 37 µm) were incorporated into compost as smaller sized fragments (1 cm²) showed higher biodegradation of about 47–68% (3 mo), values higher than biodegradation of unweathered (but not weathered) PLA/PHA mulch in our study [55]. Although both studies employed the same temperature, 58 °C, our study used a yard and food scrap-based compost whereas another biodegradation study [55] used a manure-straw-based compost, suggesting the two studies may differ in the C:N ratio, quality and characteristics of the compost [17].

PLA is susceptible to biotic and abiotic hydrolysis, both of which break down the polymer chain into lactic acid oligomers or monomers that are easily assimilated by microorganisms [55]. Biodegradation of PLA is greatly affected by temperature and the closer the environmental temperature approaches \( T_g \), the faster the biodegradation rate [2]. The environmental temperature for composting is near \( T_g \) for PLA, which may explain the higher biodegradation observed for PLA/PHA in compost than in soil. PHA, a minor polymeric component of PLA/PHA, may also have significantly influenced higher biodegradation. A study showed biodegradation of PHA occurred to the same extent as cellulose during a 660-d soil incubation period [11]. Microbial polymerases that utilize PHA can biodegrade solid PHA/PHB blends into water-soluble monomers and oligomers, utilizing carbon as nutrient source within cells [56].

In our study, a lag phase occurred for PLA/PHA during biodegradation under composting conditions that lasted for 1–2 week (Fig. 6a), a shorter time than the lag phase observed for PLA/PHA during biodegradation in the soil (~45 days; Fig. 1a) and in other studies utilizing PLA and PBAT films [55, 57]. Weathered mulches had shorter lag phase (8 days) than unweathered mulch (15 days) (Fig. 6a). However, various PLA fragment sizes (e.g. resins, 1 cm² films, 5 cm² films) during biodegradation in compost, nevertheless, affect the duration of the lag phase [58].

Unlike PLA/PHA, biodegradation of PBAT-based BDMs was relatively lower during composting and a lag phase was not observed (Fig. 6b). Biodegradation of weathered

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**Fig. 6** Cumulative biodegradation of weathered and unweathered BDMs (PLA/PHA, Bio360, Organix and Naturecycle) during 90 days of biodegradation under industrial composting conditions, per the standardized test conditions of ASTM D5338. Error bars reflect standard deviation and error bars for some data points appear smaller than markers. Data for Figures c and d were taken simultaneously; thus, data for WeedGuardPlus is identical between the two panels.
Bio360 was nearly identical to WGP; however, biodegradation of WGP (%) was lower for the experiment with Bio360 (Fig. 6b) than other experiments (Figs. 6a–d), and this may be attributed to the compost material not being at an optimal maturity index level (active compost), as it was for the other experiments. Nevertheless, weathering significantly enhanced the biodegradation of Bio360, and to a greater extent in the compost than soil. Weathered mulches can be in “primed” condition with microbial communities present in the field. In high-moisture environments (e.g. compost), microbial hydrolysis increases relative to soil conditions, resulting in depolymerization, thereby increasing the available sites for microorganisms to attack the polymer chain.

For PBAT, hydrolytic degradation of samples takes place mainly through cleavage of the adipate ester groups [17, 59]. The high susceptibility of PBAT to hydrolysis (for Bio360 and other PBAT mulches used in the study) may also explain the similar extent of biodegradation of WGP to PBAT-based BDMs in compost (Fig. 6), in contrast to the situation in soil (Fig. 1). In addition, although the % biodegradation achieved for all three weathered PBAT-based BDMs were similar (near 60% at 3 mo), the % biodegradation for Bio360 was closer to that achieved by the cellulosic control (WGP). Among PBAT-based BDMs, Bio360 underwent greater physicochemical changes due to weathering than Organix and Naturecycle. The embrittled structure of weathered PBAT-based BDMs (per low % elongation values, Table 1) enhanced the susceptibility of BDMs during biodegradation in compost. The higher A/T ratio for Bio360 compared to Organix may also play a role, as other studies also found that degradation rate of PBAT was significantly enhanced by a higher temperature of the degradation media and higher A/T ratio [59]. Higher A/T ratios likely correspond to highly amorphous regions, which are likely to serve as the sites of hydrolytic reactions [17]. The slightly higher level of aromatic constituents (i.e., lower A/T ratio) of Organix may have led to a higher degree of cross-linkage through agricultural weathering, as observed by a higher gel content for weathered Organix compared to weathered Bio360 (Table 1). The smaller difference of gel content between weathered and unwashed Organix is smaller than for Bio360 (Fig. 6). Differences in the films’ preparation, and minor and inorganic components may be factors that make Bio360 more susceptible to degradation among the PBAT-based BDMs.

Colorants, particularly carbon black, which acts as a photostabilizer, inhibit photodegradation of mulches. Three varieties of weathered Organix that differed in color (black, white-on-black and clear) were compared for their inherent biodegradability under composting conditions (Fig. 6), to determine if differences in biodegradation were related to the extent of degradation of the mulches due to weathering, or if the colorants influencing gel content formation [29] inhibited microbial assimilation. The CO₂ profile of the compost alone was low until the later phase of biodegradation, ultimately reaching ~20% at the end (Figure S6). Comparing the three mulches, the clear film underwent the slowest rate of biodegradation, followed by white-on-black. The gel content of three Organix mulches were almost identical but the clear film had a slightly lower % elongation (hence higher degree of embrittlement) than the two Organix mulches, which may explain this result. Up to ~30 days, the time course of biodegradation for both black and white-on-black Organix were nearly equal; but, white-on-black eventually slowed in its biodegradation rate relative to the other two Organix BDMs, as evidenced by a plateau in CO₂ production (Fig. 7). Carbon black as a photostabilizer may have inhibited photodegradation, leading to a lower gel content for the fully black Organix. However, the three weathered Organix mulches generally underwent similar rates of biodegradation (Fig. 7), suggesting that the photodegradation due to colorants (carbon black) did not inhibit microbial activity under the conditions employed as also found out by other studies [60, 61].

Conclusions

Biodegradation of BDMs in soil and compost depended upon the polymeric composition of mulches, soil and compost used, and the events occurring during their previous life stages. Weathering enhanced the rate of biodegradation for BDMs in soil and compost, particularly for the latter. Different starting soil samples and compost maturity in the
standardized tests resulted in different rates of biodegradation, as evidenced by differences in the mineralization of the paper mulch (WGP) positive control between experiments that shared common environmental conditions (e.g. temperature, RH) in both soil and compost.

Biodegradation of BDMs occurred from highest to lowest was in the following order: PLA/PHA > Bio360 > Naturecycle > Organix, which was independent of weathering treatment. Weathered BDMs underwent a greater extent of biodegradation than unweathered BDMs in both soil and compost, suggesting that the beneficial impact of embrittlement and depolymerization outweighed the detrimental formation of gel resulting from cross-link formation. Three ecovio®-based Organix mulches that differed in color (black, white-on black, clear) differed slightly in their compostability. Clear Organix mulch, which had greater gel content (%), also exhibited a slightly lower rate and extent of biodegradation in compost than other Organix mulches, especially as time increased. Under composting conditions, the order of biodegradation was PLA/PHA > Organix > Naturecycle > Bio360. The enhanced rate of biodegradation for weathered mulches can be attributed to depolymerization (via a Norrish Type II photodegradative chain scission reaction and hydrolysis) and embrittlement. The increased embrittlement leads to more rapid size reduction during biodegradation in soil, hence, increasing the surface area exposed to the microorganisms. Microbial abundances were significantly higher for soil that contained weathered rather than unweathered BDMs. Except for Organix, microbial abundance was influenced more by bacterial communities than fungal communities.

This study demonstrates that agricultural weathering impacted the biodegradation of BDMs, primarily enhancing the process, while at the same time biodegradation was influenced by polymeric composition of the mulches. The study provides us an information that BDMs’ biodegradation in the field after specialty crop production is possible but factors such as microbial communities and abiotic conditions (e.g. soil chemistry, C:N, pH, moisture, organic matter, others) must be known to understand the biodegradability of BDMs when left on soil or retrieved for composting after harvest.

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Author Contributions All authors contributed to the study conception and design and were involved with the retrieval of mulch specimen from our ongoing field studies. MBA performed the biodegradation experiment in compost, assisted by CDCB on biodegradation experiment in the soil. AFA performed the microscopy and microplastics assessment. MBA and LCW performed the measurements of the chemical properties of the mulches. JMD and JELG performed the microbial assessment and its statistical analysis. MBA performed other statistical analysis while MBA, DGH, and LCW analyzed other data. MBA and DGH wrote the first draft of the manuscript and all authors commented on the previous versions of the manuscript and read and approved the final manuscript.

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Data Availability The datasets generated during the current study are available at https://doi.org/10.5061/dryad.2v6wpznm.

Compliance with Ethical Standards

Conflicts of interest We have no conflicts of interest or competing interests to declare.

Consent to Participate and for Publication All authors have provided consent to participate and to pursue publication of this manuscript.

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