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CHANGES IN PHYSICOCHEMICAL PROPERTIES OF BIODEGRADABLE MULCH DUE TO WEATHERING AND AGEING AND THEIR INFLUENCE ON BIODEGRADATION

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I am submitting herewith a dissertation written by Marife Betinol Anunciado entitled "CHANGES IN PHYSICOCHEMICAL PROPERTIES OF BIODEGRADABLE MULCH DUE TO WEATHERING AND AGEING AND THEIR INFLUENCE ON BIODEGRADATION." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Douglas G. Hayes, Jennifer DeBruyn, Major Professor

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CHANGES IN PHYSICOCHEMICAL PROPERTIES OF BIODEGRADABLE MULCH DUE TO WEATHERING AND AGEING AND THEIR INFLUENCE ON BIODEGRADATION

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Marife Betinol Anunciado

May 2020

DEDICATION

This dissertation is dedicated:

To my colleagues, Sangeeta, Eunkyeong, Shalini, Mohsin, Beant, Surendra & Shikha, memories of laughter and fun has helped me surpass the hardships while pursuing the degree;

To friends who extended their guidance and warm welcoming hearts, namely, Ngo's, Bercero's, Basa's, Abrasaldo's, Bedayo's, Inso's, Causin's, Patrick's, Anderson's, Bonane's, Ate Clar, Christine Balong and Pyang;

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Lastly, to GOD, who is my All and made me know joy in life.

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I am grateful working with Dr. Larry Wadsworth, who helped me get acquainted into mechanical testing of plastic materials.

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ABSTRACT

Plastic mulches are thin films used to cover the soil surface for an improved agricultural production of vegetables and specialty crops. Disposal of conventional polyethylene (PE) mulches, commonly used worldwide, is an ongoing problem due to poor biodegradability and costly, laborious retrieval. As a sustainable alternative, biodegradable plastic mulches (BDMs) were developed. Unlike PE mulches, BDMs can be plowed into the soil or retrieved and composted after vegetable harvest. The impact of each preceding life-stage on BDM biodegradation, however, is not well understood.

The overall objective of my PhD dissertation study is to understand how biodegradation of BDMs is influenced by processes along BDMs' life stages (i.e., storage, degradation above-soil, during their service life). Mulches retrieved from two field studies (TN, WA) were analyzed to address three main objectives: (i) evaluate change of mechanical and chemical properties of BDMs due to agricultural weathering in two locations with diverse climates during four cropping seasons (2015-2018); (ii) evaluate the effect of agricultural weathering on biodegradation of BDMs under ambient soil and composting conditions via standardized laboratory tests; and (iii) determine the effect of mulches' ageing during long-term (three-year) storage on their structural integrity.

Results showed significant decrease of mechanical properties of BDMs due to agricultural weathering as influenced by environmental factors that did not differ significantly between years. Changes in chemical properties were observed, the extent of which was not significantly different between two field locations. There was a temporal variability on physicochemical properties of BDMs throughout the four successive cropping seasons, which were attributed to some extreme weather events experienced at both field sites. Results showed that weathering significantly

enhanced the extent of biodegradation of BDMs, especially during composting and to a lesser extent in soil. The enhanced rate of biodegradation for weathered mulches can be attributed to depolymerization and embrittlement that occurred during weathering in the field. The idea of storing BDMs (>1 year, recommended by manufacturers), for possible future-use, is discouraged as our study found that BDMs started to lose their mechanical functionality and chemical properties after 1 year even when kept under ideal environmentally controlled storage conditions.

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1.1. PLASTIC MULCH FILMS

1.1.1. Agricultural benefits of plastic mulch films

Plastics are increasingly produced for a variety of products due to low-cost and durability (Shimao, 2011; Quecholac-Piña et al., 2016). In agriculture, plastics are utilized but is not limited to trays, pots, mulch films and other materials for transplanting crops (Lamont, 2001). Plastics are made up of long-chain polymer molecules that contain a large number of structural units joined by the same type of linkage (Scott, 2002), which can be designed to possess stability and durability for molding into various shapes (Bregg, 2006). Plasticulture started with polyethylene materials used as greenhouse film (Kasirajan & Ngouajio, 2012). Agricultural plastics are also utilized to control unintentional leaching of pesticides and fertilizers and seed coatings (Chandra & Rustgi, 1998).

Plastic mulches contributes as the largest source of agricultural plastics used today. Mulching is a technique that uses plastic film and laid into the soil surface for crop production (Kasirajan & Ngouajio, 2012). Mulching can also employ geotextiles or solid agricultural residues such as straw or sawdust (Tachibana et al., 2009). Mulch is a thin sheet of extruded plastic, which may contain black or white coloring, or is transparent, and is accompanied by use of drip irrigation (Miles et al., 2012). Other possible colors include green, red, yellow, silver, blue, gray, and orange for, for controlling insects and diseases (Mitchell et al., 2004). Green mulches are also desired by farmers to provide a more aesthetic visual appearance. A more detailed discussion of mulch color is given elsewhere in this proposal in the colorants under preparation of mulch films (Section 2.2). Mulch films are usually available as 0.09 to 1.32 m-wide rolls and up to 1800 m long. Films ranging in thickness from 0.02 to 1.5 mm are used in many different climates, soils, and seasons (Kasirajan & Ngouajio, 2012).

Early use of plastic mulches occurred in colder regions of upper Midwest USA, mainly black films for crop protection from the cold. Today, mulch films benefit growers by making farming activities efficient (Table 1.1), thereby increasing crop yield and production. Growth of crops is improved due to soil moisture conservation and weed reduction (Chandra & Rustgi, 1998).

Table 1.1. Advantages of mulch films' application for specialty crop production.

Location of study	**Test crop**	**Observed benefits**	**Reference**
Japan	Mandarin orange	Disease and insect pests reduction	Tachibana et al., 2009
UK	Melon	Soil moisture conservation; weed management; enhanced crop quality	Chandra & Rustgi, 1998
Iran	Tomato	Disease and insect pests (pest) reduction; enhanced crop quality; increased yield	Rajablariani et al., 2012
Libya	Cucumber	Increased yield; more efficient water and fertilizer use; soil moisture conservation	El-Shaikh and Fouda, 2008

Temperature fluctuations in the soil (20-30 cm depth) can be minimized by mulch; less variable temperatures favor root development, promoting faster crop establishment and earlier harvest. When soil temperature is controlled, microbial activity improves and nutrient absorption by plants is enhanced. Cooling the soil through use of co-extruded white and black mulch can be used in warmer climates to improve microbial activity. Protecting damages from abiotic agents (e.g. water, wind and hail) can be avoided through mulch use. Mulches reduce contact between the crops and soil, thereby protecting crops from diseases carried by soil-borne pests and insects (Tachibana et al., 2009; Bilck et al., 2010).

1.1.2. Environmental impacts of end-of-life use

The disposal of conventional polyethylene (PE) films is recognized to be a major problem because of the material's recalcitrance to biodegradation. PE is not microbially utilized, independent of form (e.g. powder, pellets, film); thus it does not readily enter degradation cycles in the biosphere. Thus, films accumulate in the field after agricultural use if not manually retrieved (Shimao, 2001). Mulch film removal includes disking, physical handling, transport and storage of plastic mulch; but these methods do not guarantee complete removal in the field. When disintegrated into relatively small pieces, a frequent event due to embrittlement of the films due to agricultural weathering, residual film fragments can accumulate in the soil that will negatively impact root growth and crop development (Kasirajan & Ngouajio, 2012).

When plastic wastes accumulate, pollution with long-term effects makes field management not only expensive but complicated (Zafar et al., 2013). Due to the absence of sustainable disposal options (as a result of conventional plastics' resistance to microbial attack and the absence of microorganisms with the capability to degrade them), about 92% of conventional plastics are incinerated (Shah et al., 2008). Both landfill disposal and incineration, however, do not eliminate plastics' impact on the environment and thus residual plastic waste becomes a significant source of pollution (Sakamoto, 2012). In some cases when plastic mulches are burned, air quality is affected by the impact of particulates (Narayan, 2017). Pollutants can be adsorbed to plastics from contact with soil, water and air which disrupts natural food webs and endanger wildlife (Gewert et al., 2015) Krueger et al., 2015).

In terrestrial and marine environments, plastics wastes serve as the highest source of pollutants (Krueger et al., 2015). Fragmented PE mulch films are dispersed widely by the wind and animals, and may find their way into ocean and other pristine environments, where they can have a serious impact on environment (Narayan, 2017). Plastics account for 60% of ocean debris; upon ultraviolet (UV) light exposure and physical stress, plastic debris undergoes size reduction over time (Gewert et al., 2015). Contamination by microplastics and nanoplastics (< 5 mm and <100 nm, respectively) has been observed for the marine environment on sediments and animal samples (Welden & Cowie, 2017).

1.1.3. Alternative Mulch Feedstocks to conventional Polyethylene

PE is an inert, hydrophobic synthetic polymer materials comprised of a long (high molecular weight) hydrocarbon chain backbone (Figure 1.1).

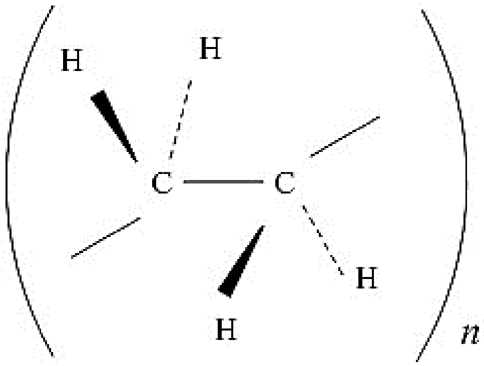

Figure 1.1. Chemical structure of polyethylene

Its hydrophobicity and high molecular weight make it an important engineering material (Shah et al., 2008). Widespread use of PE is due to easy preparation, high durability and flexibility and low cost. Low-density PE (LDPE) resins are used for conventional plastic mulches due to its resistance to puncture with other remarkable properties, while high-density PE (HDPE) resins are good as vapor barrier (Kasirajan & Ngouajio, 2012). Mulch films are prepared from LDPE resins because of its greater proportion of crystalline regions compared to HDPE, providing better stress and crack resistance (Gabriel, 2009).

1.1.4. Biodegradable Plastic Mulches: A Sustainable Option to PE Mulch

The undesirable end-of-life options for non-biodegradable polymeric materials is a growing concern and has promoted ongoing research activity to develop new biodegradable plastics (Shah et al., 2008). Laborious and costly retrieval of PE mulches inhibits the economic sustainability of using plastic mulches; hence, biodegradable plastic mulches (BDMs) were produced as an alternative to conventional PE mulches. For instance, BDM removal is much less expensive than for PE mulches, since BDMs are designed to be plowed directly onto the soil immediately after the harvest, where they are anticipated to undergo full biodegradation within two years (Maréchal, 2003)(EN17033). PE, however, is still extensively used due to its cheaper costs than BDMs (Velandia et al., 2020). Use of BDMs can potentially avoid soil pollution drawbacks and avoid damage to soils and provide the same benefits to specialty crop production as conventional PE mulch (Barragan et al., 2016, Maréchal, 2003). The increasing production of biodegradable polymers has also raised an increasing interest in their environmental safety over the long-term (Sforzini et al., 2016).

Paper mulches, unlike PE mulches, degrades faster down naturally after their incorporation in the soil and varies relative to soil properties and environmental condition (e.g. soil moisture, temperature, pH). Degradation of paper mulch is highly dependent upon paper quality and its porous characteristics makes degradation to occur faster in the field. Above-ground, degradation of paper mulches is also affected by other environmental (e.g. sunlight, wind) (Haapala et al., 2014).

In two different crop seasons with relatively different average temperatures (2011, 2012), crops (*Cucumis sativus L.*) harvested from soil with mulch films was higher than bare ground. Paper mulch in comparison to a plastic film have similar crop yield (Haapala et al., 2015). In one study, paper mulches were coated using a biodegradable polymerized vegetable oil to enhance durability and performance. Zinc oxide coating on paper mulches delayed visible degradation (e.g. formation of holes and tears) and functioned effectively during tree growth (Shogren & Rousseau, 2005). In warmer climates, paper mulches perform better than PE and BDMs by effectively suppressing the growth of weeds, specifically purple and yellow nutsedge (Moore & Wszelaki, 2019).

Sprayable mulches based from polysaccharide formulations were developed to be applied using an alternate approach to machine-laying: spraying, to be applied into the soil in a form of water solutions. As polysaccharides interact with water, they form hydrogels when sprayed into the soil (Santagata et al., 2017). Sprayable biodegradable coatings represent a safer alternative than those petroleum-derived products as soil mulch and needs less labor demand on manual operation. However, biodegradable coatings need improvement to be robust for large-scale operations. Future studies should also aim to extend mulch life and create films of greater roll width (Vox et al., 2013).

In a European Commission project on "biodegradable coverages for sustainable agriculture" or BIO.CO.AGRI, alginic acid, derived from intercellular walls of brown seaweeds, was used as a sprayable mulch. An experimental chitosan-based spray solution is now widely used. Chitosan is derived from chitin, a major component of supporting structure of crustaceans, insect exoskeletons and some algal and fungal cell walls. Biodegradable pots were developed from sodium alginate using tomato and hemp fibers as reinforcing material, which allowed for root development to enhance water and nutrient uptake. The insulating and drainage qualities of the composites are direct benefits which will further improve plant nutrition and health (Santagata et al., 2017). The color of sprayable mulch can positively affect crop yield. For an eggplant (*Solanum melongena L.*) planting bed that was treated with sprayable synthetic latex film total fruit weight and number of eggplants grown on silver-painted beds was significantly greater than other treatments. Blue and white-colored mulches provided increased numbers of fruits and total fruit yields of eggplant (Mahmoudpour & Stapleton, 1997).

Oxo- and photo-degradable mulches have additives that aid in accelerating oxidation, thereby enhancing the fragmentation of plastics when exposed to light, heat or O_2 (CEN, 2006; Kasirajan & Ngouajio, 2012). Production of oxo-degradable mulches is increasing, partially due to misconception that they biodegrade in the soil. Currently, many organizations worldwide are supporting the ban or restricted use of oxo-degradables, particularly in Europe (European Bioplastics, 2018).

1.2. DEGRADATION OF PLASTICS IN THE ENVIRONMENT

1.2.1. Hydrolytic Degradation

Water acts as a nucleophilic agent to hydrolyze ester linkages, which will reduce polyester chain length, ultimately producing oligomers of effective from microbial assimilation. The pH is also an important factor for hydrolysis, since catalysis can either occur in an acidic or basic condition (Gewert et al., 2015; Kijchavengkul et al., 2010). When polymers degrade, especially those that contain both hydrophobic and hydrophilic segments, polymer morphology changes. Synthetic polymers that have short monomeric units are also highly crystalline, making their hydrolysable bonds inaccessible to enzymes (Chandra & Rustgi, 1998). When the rate of hydrolysis on a plastic surface and water is greater than diffusion rate of water molecules through the polymeric material, the diffusion step is rate-limiting *(erosion*). Bulk erosion occurs when the surface reaction is rate-limiting (Tsuji, 2010). As shown in Figure 1.2 A, degradation of polymer starts in the outer layer causing surface erosion polymer layers and erosion and disappearance of polymer at the outer region (dark-colored polymer in figure becomes smaller upon erosion) (Siepmann & Göpferich, 2001).

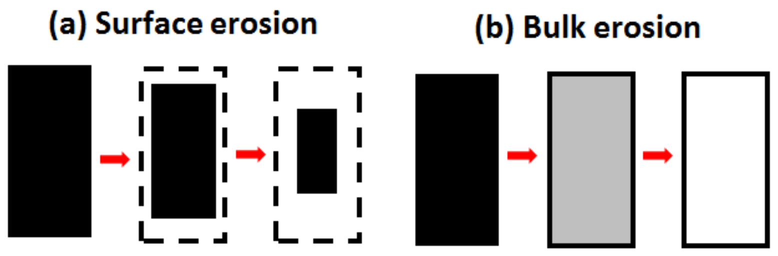

Figure 1.2. Hydrolytic degradation mechanisms of bulky PLA materials.

Figure 1.2b depicts bulk erosion, a mechanism that frequently describes PLA hydrolysis. During bulk erosion, polymers degrade slowly and uniformly throughout the volume of polymeric material (dark-colored polymer of figure becomes gray in color and does not decrease in size). When water diffuses through the polymeric material in the hydrolasable regions, hydrolysis cleaves the polymer out and reduces chain length (Kijchavengkul et al., 2009).

Under acidic and basic conditions, poly (3-hydroxybutyrate) can be hydrolyzed via random scission of the ester linkages throughout the polymer matrix, resulting in molecular weight reduction (Bregg, 2006). The mechanical properties of polybutylene adipate terephthalate (PBAT) were significantly degraded (e.g., decrease of tensile strength and elongation at break) after 30-day exposure at 50°C and 90% RH conditions. After exposure to high heat and humid conditions, a polybutylene succinate (PBS)/PBAT blend changed fracture mode from ductile to brittle, attributable to hydrolysis of PBS (Muthuraj et al., 2015). Photodegradation (Norrish Type II reaction, described below) and hydrolysis occurred concurrently chain scission on nonwoven geotextiles: spunbond (SB) PLA and meltblown (MB)-processed PLA/polyhydroxyalkanoate (PHA), as indicated by ATR-FTIR results (Hablot et al., 2014). MB PLA nonwovens were processed at higher temperatures forms small fibers relative to SB PLA. For this reason, MB PLA have low melt viscosity with lower tensile strength than SB PLA nonwovens (Dharmalingam et al., 2015).

1.2.2. Thermal Degradation

Upon heating, polymers undergoes physical and chemical changes. Changes that occurs depend on the nature and properties of the material. Thermosets, which are infusible and insoluble upon formation, do not undergo simple phase change. When heated, the chemically cross-linked molecular network of thermoset materials sets rather than melts when temperature is lower than their melting point (Gabriel, 2009). Thermoplastics, on the other hand, due to small content of cross-links, become soft through heating, not irreversibly, provided that the minimum thermal decomposition temperature is not exceeded (Beyler & Hirschler, 2001).

A polymer material that is highly crystalline has an ordered arrangement of its constituents. The glassy state of a material changing to a flexible state, particularly when heated, is a process known as the glass transition, with the temperature interval between the two states defined as glass transition temperature (T_g) (Ubbelohde, 1978). As a polymeric material is heated above $T_{g,}$ it changes from a glassy to rubbery characteristics (Kijchavengkul et al., 2008). Molecular deterioration more likely occurs on polymers at temperatures above T_g and cause changes on the polymer properties. As a result, the molecular weight changes due to redistribution of monomers, the extent of which depends on the polymer's inherent chemical properties. This further reduces ductility and causes embrittlement, chalking, color changes and finally cracking (Restrepo-Flórez et al., 2014). Chain scission can occur and be random-chain (chain scission sites randomly located), end-chain (successive removal of monomer units), chain-stripped (cleaved backbone) and cross-linked (bond creation) (Beyler & Hirschler, 2001).

The thermal degradation mechanism of PE during environmental weathering is a complex process that results in free radical species that undergo chain scission along two different degradation pathways, producing propene and 1-hexene as products. Chain scission of PE initiates on weak link sites (Peterson et al., 2001). For a PE/starch blend, infrared radiation (760-2500 cm^{-1}) accelerates thermal oxidation (Kijchavengkul et al., 2008). PLA's rate of hydrolysis for the thermal degradation of its ester linkages increased with increasing temperature and % relative humidity (Copinet et al., 2004). The lower resistance of PHA to thermal degradation resulted to chain scission of ester regions which was enhanced during prolong heating time at a temperature higher than its melting point (Rudnik, 2008; Bregg, 2006).

1.2.3. Photodegradation and Radiation Degradation

Due to the ability of polymers to absorb the UV component of solar radiation, electrons are activated to higher energy states, causing oxidation and cleavage. The UV-B (295–315 nm) and UV-A (315-400 nm) spectral regions are responsible for direct photodegradation. In the visible light spectral regions (400-760 nm), polymeric degradation is accelerated through heating (Kijchavengkul et al., 2008). Exposure of films to radiation can affect their susceptibility to degradation, either through scission of polymer chains (Norrish type I) or polymer network formation when cross-links occur (Norrish type II; Figure 1.3). Slower rates of degradation are encountered in cross-linked portions of the polymeric material (Chandra & Rustgi, 1998). Norrish type I or II mechanisms generate ketone compounds and terminal double bonds, respectively (Figure 1.3).

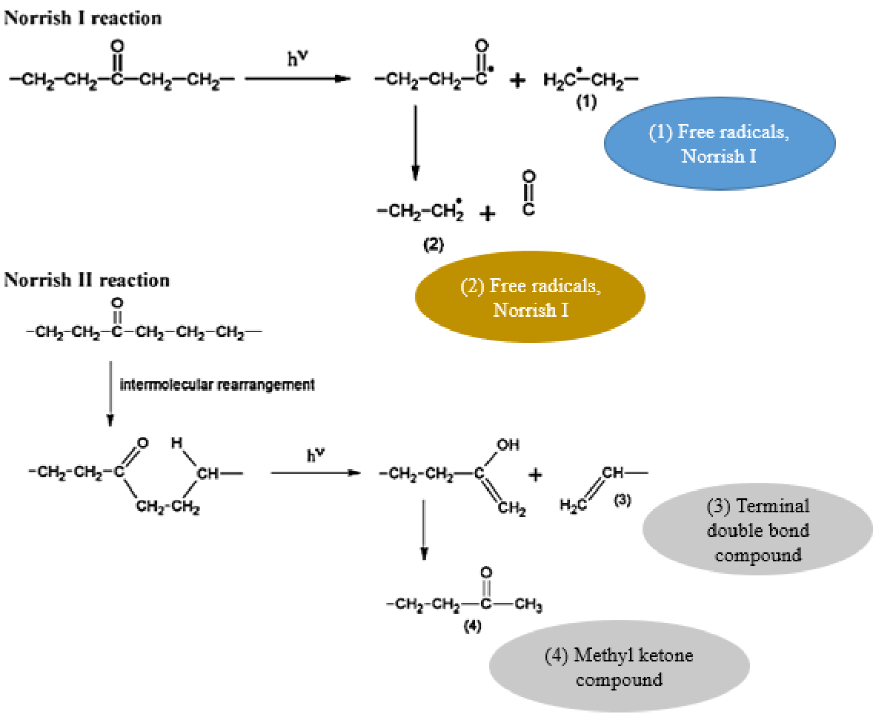

Figure 1.3. Chemical reactions during photodegradation of PBAT. Source: Kijchavengkul, 2010.

In Norrish Type I photodegradation, free radicals recombined and generate crosslinks structures (Kasirajan & Ngouajio, 2012). In the Norrish Type I reaction, homolysis of aldehydes and ketones to free radicals occur. In the Norrish Type II reaction, specifically for PBAT (Figure 1.3), the susceptible ester linkage are abstracted by a free radical, causing chain scission (Kijchavengkul et al., 2008). During UV photo-degradation of PBAT films, embrittlement and reduction of tensile strength and percent elongation were observed, which suggest the occurrence of chain scission (Kijchavengkul et al., 2008).

1.2.4. Oxodegradation

A readily biodegradable material added to the soil can be transformed through reduction-oxidation (REDOX) reactions, which involves transfer of electron pairs from one chemical species to another during aerobic respiration ($O_2 \rightarrow H_2O$) and methanogenesis ($CO_2 \rightarrow CH_4$), respectively. Oxodegradation of a polymer refers to oxidation (loss of electrons), forming smaller molecular weight fragments with an enhancement of functional groups that enhance reactivity. Oxodegradation increases the polymeric material's hydrophilicity, allowing it to increase water adsorption interactions with microorganisms. Pro-oxidants are used to initiate changes in the polymer structure (Arráez et al., 2018).

The thermal decomposition of polymers at times may proceed by oxidative processes that can be accelerated in many polymers by oxidants such as atmospheric oxygen. For PE, contact with O_2 is important in determining decomposition rates and mechanism (Beyler & Hirschler, 2001). PE, which is not inherently photodegradable in its neat form, can have enhanced photo- and chemical degradation when blended with pro-oxidants (Shah et al., 2008). Photooxidation rearranges the polymeric chains, leading to crosslinking and formation of insoluble gel (Stloukal et al., 2012)

1.2.5. Microbial Biodegradation

Biodegradation of BDMs in soil can be physically enhanced by UV, temperature, mechanical forces, and/or chemical agents prior to plastic's incorporation into the soil (Peterson et al., 2001). Microbial degradation starts with release of enzymes that cleave the polymers' chemical bonds, producing oligomers or monomers. Two categories of enzymes actively involved in biodegradation are extra- and intercellular enzymes. During surface erosion, microorganisms consume biodegradable polymers through enzymatic reactions on the polymer material's surface, causing a slow reduction in molecular weight that initiates early in the time course of biodegradation. Bacteria require substrates to be assimilated into the cellular membrane to be further degraded by intercellular enzymes (Kijchavengkul & Auras, 2012). Depolymerization through enzymatic degradation starts with hydrolysis. Hydrolases bind to the polymer substrate then subsequently catalyze a hydrolytic cleavage. Through hydrolysis, the cleaved portion of the polymer becomes a source of carbon for microbial utilization (Shah et al., 2008). Microbial attack releases exo-enzymes that breaks the complex structure of polymers, turning it into smaller molecules to allow their passage through the semi-permeable outer membranes for their utilization as carbon and energy sources. Extracellular enzymes differ between microorganism species and have uniquely structured active sites that selectively interact towards certain polymers (Kijchavengkul & Auras, 2012). A portion of the carbon in plastic is used for the assimilation and builds up new biomass (Starnecker & Menner, 1996). Plastic fragments rich in aromatic-blocks and hydrophobic regions, however, may persist in the soil or the environment where they were incorporated.

Chemical and physical factors, previously discussed in *degradation* section above, and biotic agents are involved in bond scission and transformations of polymers, leading to biodegradation of polymers (Figure 1.4) (Kijchavengkul et al., 2008).

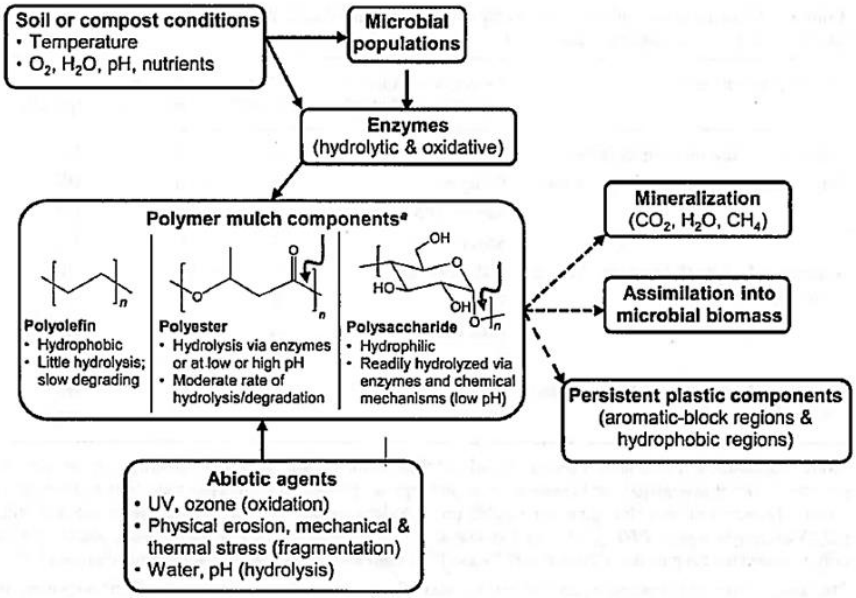

Figure 1.4. Process of biodegradation. Source: Brodhagen et al., 2015.

Microorganisms act on the films and produces extracellular enzymes that degrades the polymer, a process that could be slow or moderate depending on hydrophobicity or hydrophilicity of mulch component (Chandra & Rustgi, 1998). However, biodegradation also depends on the inherent characteristics and properties of the polymers. The hydrolysable regions of a polymer (e.g. esters), increases flexibility of the polymer structure and can increase biodegradation due to hydrolysis (Kijchavengkul et al., 2008). As molecular weight increases, chemical biodegradability tends to decrease, due in part to the inability of large molecules to enter microbial cells (Hatzinger, 2002). Lower molecular weight molecules (monomers, dimers and oligomers), on the other hand, are much more easily degraded and mineralized (Kijchavengkul & Auras, 2012). In some instances, bacteria can produce extracellular enzymes to initiate an attack on a large molecule, breaking into oligomers to be metabolized further. Higher degree of molecular branching also reduces chemical degradability through steric interference with the active site of degradative enzymes in microorganisms (Hatzinger, 2002).

Biodegradation differs between environments, with compost being a more bioactive environment than soil, water and landfill environments, the latter three providing slower and more variable biodegradation (Rujnic-Sokele & Pilipovic, 2017). Degradation in the soil depends not only on microbial activity but abiotic factors and polymer's chemical properties (Rudnik, 2008). In high-moisture environments, biodegradation occurs; but, abiotic degradation through hydrolysis increases and results in chain scission, thereby increasing the available sites for microorganisms to attack the polymer chain (Kijchavengkul et al., 2010). Full biodegradation of PHA into CO_2 and H_2O under aerobic conditions and CO_2 and CH_4 under anaerobic conditions can be catalyzed by a wide variety of microorganisms (Rudnik, 2008b).

1.3. OBJECTIVES

As introduced in this chapter, BDMs are an emerging alternative to conventional PE plastic mulches for crop production systems; but, polymeric materials degrade at different rates and to different extents, which are affected by a wide range of factors of environmental conditions, polymer and material characteristics and action of microorganisms. Compared to conventional PE mulch, which does not biodegrade and can serve as an environmental hazard to organisms in soil ecosystems and watersheds (unless retrieved, requiring expensive labor costs), use of biodegradable plastic mulch (BDM) provides less expensive and more environmentally sustainable end-of-life options. BDMs are designed to be plowed and till into the soil and left to biodegrade or retrieved to be composted. Limited studies on BDMs' long-term impact in the soil, however, is one significant challenge that should be addressed. Hence, assessing the biodegradation of mulch films used in the study will provide additional information of BDMs potential over PE mulches.

The overall objective of this dissertation research is to understand the role of both abiotic and biotic environmental factors and underlying mechanisms affecting degradation of mulch films throughout their life cycle 1) during storage; 2) field use; and 3) end-of-life (biodegradation after BDM incorporation into the soil or retrieval followed by composting). Monitoring the changes in mulch film properties during field-use is important as they can indicate underlying mechanisms for deterioration as influenced by environmental weathering factors and can influence biodegradability.

1.4. ORGANIZATION OF THE STUDY

This dissertation is divided into the following chapters:

Chapter 1: Introduction and Objectives

Chapter 2: Literature Review

Chapter 3: Effect of Agricultural Weathering on the Degradation of Biodegradable Mulch in Two Diverse Climates During Four Successive Years

Chapter 4: Effect of Environmental Weathering on Microbial Utilization of Biodegradable Mulches Under Ambient Soil and Composting Conditions

Chapter 5: Effect of Ageing on the Structural Integrity of Biodegradable Mulches

Chapter 6: Conclusions and Recommendations.

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2. LITERATURE REVIEW

2.1 BIOBASED VS. FOSSIL FUEL-DERIVED PLASTICS

This chapter encompasses a literature review of the processing of biodegradable plastics and methods that asses polymer degradation. Polymer feedstocks, blends, and other minor constituents (e.g. additives) are discussed in detail. Biodegradation, methods of biodegradation, standards relating to biodegradable plastics are also discussed. Physical, mechanical, and chemical assessment of polymer degradation are explained in detail.

Petroleum products (e.g. oil, coal and natural gas) are widely used because of their desirable characteristics (e.g. resistance to water and microorganisms) and cheaper costs (Kasirajan & Ngouajio, 2012). Biobased and fossil fuel-derived materials undergo carbon sequestration. The inherent advantage of biobased plastics are their reduction of an environmental footprint and independence from fossil fuel resources (Kijchavengkul & Auras, 2008). Thus, the need for biodegradable polymers increased as environmental safety about conventional plastics grows (Nanda et al., 2006).

2.1.1. Definition of 'biobased'

Biobased, a term that refers to feedstock source, identifies a material derived from a specific resource (e.g. photosynthetically derived or renewable) (Table 2.1). The most common biobased feedstocks used for biodegradable plastic mulches (BDM) are starch, lactic acid (derived from fermentation of corn and serving as the feedstock for PLA) and polyhydroxyalkanoate (Miles et al., 2017). Starch and cellulose recovered from harvested biomass will be broken down into simple sugars that are used as monomers in the preparation of biobased plastics (Kijchavengkul et al., 2008).

Table 2.1. Definition of terms frequently used that relates to biodegradation of plastics

Term	Definition	Reference
Biobased	"Commercial or industrial products composed in whole or in significant part of biological products or renewable domestic agricultural materials or forestry materials"	USDA, 2015
Biobased plastics	"Polymeric materials obtained through chemical or biological synthesis from raw materials which may or may not be biodegradable"	Haapala et al., 2014
Biodegradable plastic	"Degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae"	ISO
Composting	"Process where biodegradable materials are decomposed and transformed into a humus-like substance called compost, CO_2, water and minerals by microorganisms through a controlled biological process"	ASTM International
Compostable plastic	"Plastic that undergoes degradation by biological processes during composting to yield CO_2, H_2O, inorganic compounds and biomass at a rate consistent with other known compostable materials and leaves no visually distinguishable or toxic residues"	ASTM D6400-04
Degradation	"Irreversible process leading to a significant change of the structure of the material, typically characterized by loss of properties"	ASTM International; International Organization for Standardization
Renewable raw materials	"Plant, animal and microbial biomass, including delivered through food chains, whose primary production is based on photosynthesis and which are provided for material and energy uses of all kinds outside food and feed"	ETDS, 2010

Biobased polymers can be partially sourced from corn and potato starch, fat and oil through biological synthesis and other biobased feedstocks (Sakamoto, 2012). Adipic acid of polybutylene adipate terephthalate (PBAT), for instance, is traditionally 100% fossil-based but can also be substituted by fully biobased azelaic acid (-OOC(CH2)7COO-), derived from fatty acids of crops rich in oil content (Bastioli & Capuzzi, 2011). Biodegradable plastic mulch can only be listed and certified for use in organic agriculture when it fulfills the criteria of 100% biobased content as assessed through ASTM D6866, unless it is fully retrieved from the soil after harvest. (OMRI, 2015). There is no biodegradable mulch available in U.S. approved for organic production due to e inability to meet the100% biobased requirement (Miles et al., 2017).

2.1.2. Effect on climate change: biobased vs. fossil-fuel derived

Fossil fuels are resources that are likely to undergo depletion in the future. Greenhouse gases released from fossil fuels' utilization contribute to global warming among other environmental impacts (e.g. acidification, ozone depletion, eutrophication, depletion of natural resources and ecotoxicity) assessed throughout a products' Life Cycle Impact Analysis (LCIA) (TRACI, 2007; Shah et al., 2008). When evaluating the entire life cycle of plastics, for conventional plastics (e.g. polystyrene), CO_2 released to the environment is higher than what is contributed by biodegradable plastics such as poly(3-hydroxybutyrate-co-4-hydroxybutyrate (PHB) (biologically synthesized, microorganisms-processed) and polybutylene succinate/carbonate (PBSC; petroleum-based and chemically synthesized) (Sakamoto, 2012). Biobased plastics offer a great potential to minimize atmospheric greenhouse gas levels than fossil-fuel derived materials because of their carbon neutrality, that do not contribute to increased net atmospheric concentration of CO_2 (Gomez & Michel, 2013).

2.2. PREPARATION OF MULCH FILMS

2.2.1 Polymeric Feedstocks

2.2.1.1. Low-density polyethylene (for conventional plastic mulches)

PE is produced via polymerization of ethylene monomers as a feedstock, which is typically sourced from petroleum products (e.g. coal, natural gas); but recently, bio-derived (e.g., from bioethanol) PE has been developed (Huang, 2010). PE resins in pellet form are heated and processed into flexible sheets of films (*Kasirajan & Ngouajio, 2012).* The greater proportion of crystals of high-density PE (HDPE) over low-density PE (LDPE) results in greater density and greater strength but less flexibility for the former. PE, being a thermoplastic material, can melt through application of a sufficient amount of heat, with its typical melting point temperature at 120--180°C for HDPE and 105--115°C for LDPE (Gabriel, 2009).

2.2.1.2. Biodegradable polymers

Biodegradable polymers are receiving increased attention due to increasing concerns for pollution to the environment (Marechal, 2003). However, at present, only a few have a major market share compared to traditionally used conventional plastics because of their high (Vroman & Tighzert, 2009). Most BDMs are mainly composed of polyesters, consists of aliphatic or into an aromatic monomeric unit bonded via ester linkages compound. Many esters (Figure 2.1) occur in nature and enzymes that readily degrade them, hydrolases (e.g., esterases, lipases, phospholipases and cutinases), are ubiquitous in living organisms (Shimao, 2001).

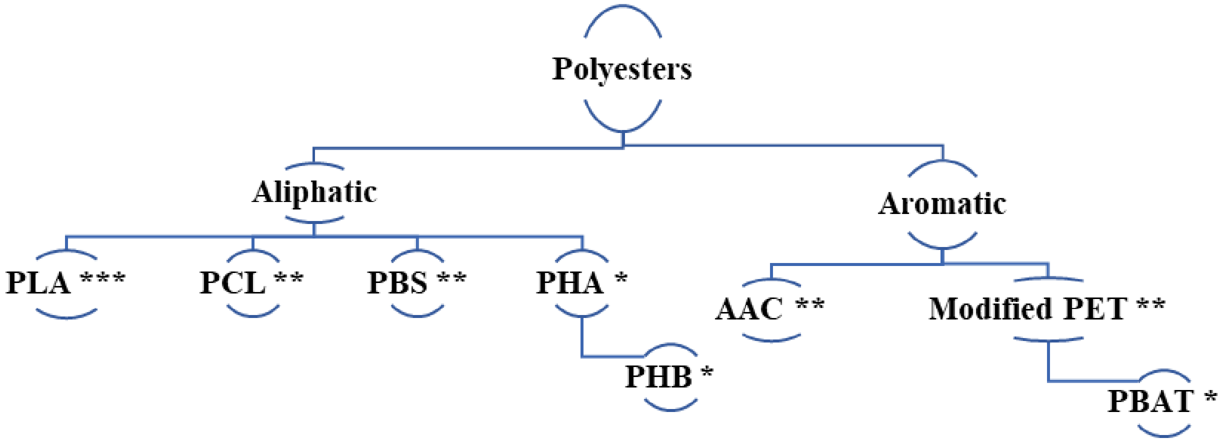

Figure 2.1. Two major families of biodegradable polyesters. Source: Kasirajan and Ngouajio, 2012

* Naturally produced renewable ** Synthetic non-renewable *** Synthetic renewable

PLA polylactic acid PCL polycaprolactone PBS polybutylene succinate

PHA polyhydroxyalkanoate PHB polyhydroxybutyrate AAC aliphatic–aromatic co-polyester

PET polyethylene terephthalate PBAT polybutylene adipate/terephthalate

Polybutylene adipate terephthalate (PBAT) is a copolymer of butylene adipate (BA) and butylene terephthalate (BT) processed through melt polycondensation of 1,4-butanediol, dimethylterephthalate and adipic acid (Figure 2.2) (Jiang et al., 2009).

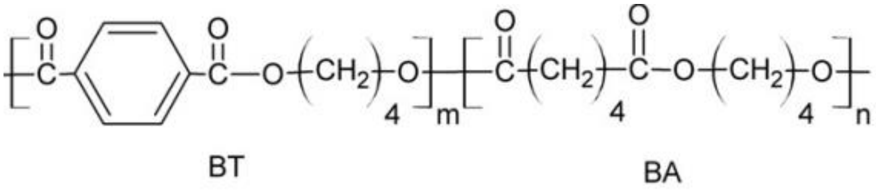

Figure 2.2. Chemical structure of poly (butylene adipate-co-terephthalate)

Today, PBAT is derived mainly from petroleum. However, Novamont (Novara, Italy) has recently introduced biobased components into its PBAT-based products. A new Novamont facility is producing 1,4-butanediol (bio-butanediol) via fermentation of sugars by an engineered *E. Coli* type bacterium as an initial step. Adipic acid is now partially replaced with azelaic acid, a diacid derived from safflower oil (Novamont S.p.A., 2016). Due to PBAT's biodegradability and mechanical properties likely similar to PE, PBAT has the potential ability to increase its commercial use (Kijchavengkul et al., 2008).

PBAT is biodegradable due to hydrolysis of ester groups in its soft aliphatic sections, whereas terephthalate regions (being hard and aromatic) contributes to its stability and mechanical properties (e.g., high elongation, up to 700%) (Coltelli et al., 2008; Shah et al., 2008; Kijchavengkul et al., 2010). For PBAT, the adipate and terephthalate mass fractions (f_{BA} and f_{BT}, respectively) are readily determined through ^{1}H NMR spectroscopy by integrating the area of signals at 2.29 ppm (eOCOC**H**2e) and 8.06 ppm (aromatic Hs) (Herrera et al., 2002). To observe the impact of diacid and α,ω-diol chain length, a recent study demonstrated that the biodegradation rate decreased with diacid and diol chain length (adipic acid (C_6) > azelaic acid (C_9) > succinic acid (C_4) and ethylene glycol (C_2) > 1,4-butanediol (C_4) (Steinbüchel, 2001).

In general, although PBAT mulch undergoes greater degradation via agricultural weathering, it performs as well as conventional PE mulch for specialty crop production. A film prepared from a cassava starch/PBAT blend underwent a reduction of mechanical properties, 8 wk after being laid on the ground for a strawberry production farm. Yet, it provided efficient mulching as crop yield and quality were relatively similar to crop yield of PE mulch (Bilck et al., 2010). Mulch films prepared from PBAT-based polymer blends of PLA and starch lost physical (tensile properties) and functional properties (water permeability) in five months after vine planting but still had comparable agronomic performance to PE mulch (Touchaleaume et al., 2016). All the films in the cited study biodegraded in soils even though the soils had poor organic matter content and microbial biomass (Bilck et al., 2010; Touchaleaume et al., 2016).

Polyhydroxyalkanoates (PHAs) are polyesters formed from hydroxy fatty acids (Figure 2.3) (Copinet et al., 2004).

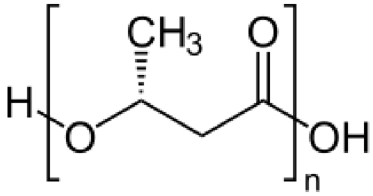

Figure 2.3. Chemical structure of poly3-hydroxybutyrate, a common PHA

PHAs' monomers can contain chain length that is short (C_3- C_5), medium (C_6- C_{14}), and long (>C_{14}) PHA (Rudnik, 2008). Polyhydroxybutyrate [PHB, particularly poly(3-*R*-hydrdoxybutyrate), i.e., P3HB], a commonly encountered PHA in nature, is highly crystalline (> 50%) with a high melting temperature, 180°C (Vroman & Tighzert, 2009). PHA, synthesized by bacteria, can accumulate at a higher dry weight during nutrient stress (e.g. N, P. Mg, or S deficiency). For the first step, a fermentation vessel is inoculated with microorganisms (Rudnik, 2008). Bacteria are then fed with nutrients to generate intercellular PHA as metabolites (Kasirajan & Ngouajio, 2012). Pellets are then blended and processed into products or packages (Rudnik, 2008; Kasirajan & Ngouajio, 2012).

PHA in its neat form has poor mechanical properties (e.g., high crystallinity) that discourage its direct use as a BDM feedstock (Zhang & Thomas, 2011). Therefore, PHA is often blended with other polymers to improve mechanical properties (discussed below). PHAs can have hard crystalline to elastic characteristics. The inherent chemical properties of the hydroxy-acid monomeric units control properties such as crystallinity and melting point temperature (Khanna and Srivastava, 2005). P3HB was discovered in 1920 as a granular component in bacterial cells that can grow in size under a wide variety of environments (Lenz & Marchessault, 2005). In natural environments, depolymerases can biodegrade P3HB and solid PHBs into water-soluble monomers and oligomers, which are then utilized as nutrient source within cells (Bregg, 2006).

Polylactic acid (PLA) is a linear aliphatic thermoplastic polyester (Figure 2.4) prepared from lactic acid (2-hydroxy propionic acid, LA), the most widely occurring carboxylic acid in nature (Narayanan et al., 2004).

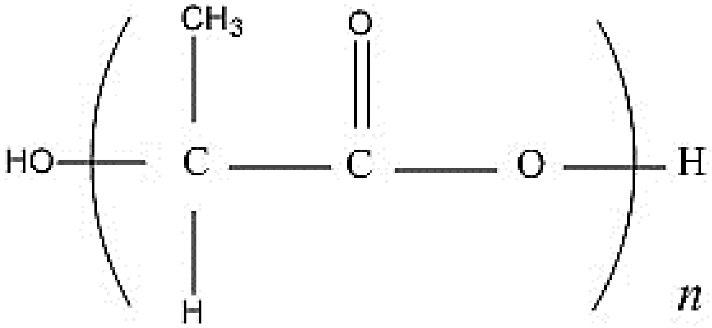

Figure 2.4. Chemical structure of polylactic acid.

LA can be derived from renewable resources that contain starch, such as maize, wheat, sugar beets, or agricultural waste. *L*- and *D*-LA are among the simplest chiral molecules in organic chemistry. While the L- form commonly occurs in nature, the D- form is produced via racemization of L-PLA. Dextrose, i.e., glucose, formed from saccharification of cellulose or starch from biomass source, is fermented, forming lactic acid, which is subsequently polymerized (Kijchavengkul et al., 2008).

LA-based polymers are prepared chemically by polycondensation of LA or ring-opening polymerization of lactide catalyzed by transition metals (e.g. Al, Pb, Zn) (Rudnik, 2008).

Polycondensation produces polyester and water as a co-products, with side-reactions being absent (Figure 2.5).

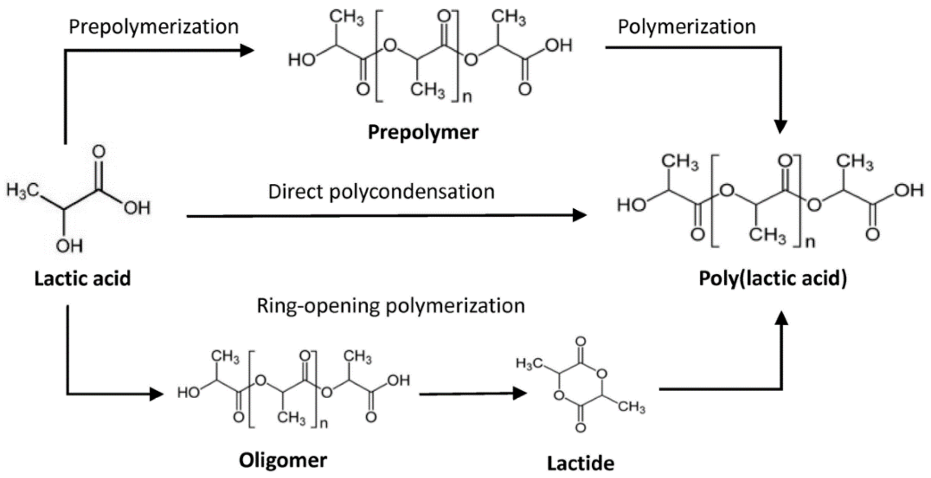

Figure 2.5. Routes of poly (lactic acid) (PLA) synthesis from lactic acid. Source: Hu et al., 2016

Polycondensation and ring-opening polymerization yield PLA with a degree of polymerization of 1.6 x $10^4$and 2.0-6.8 x 10^4, respectively (Rudnik, 2008). PLA that contains 87.5% **L**-LA and 12.5% D-LA is completely amorphous, while polymers with 92% **L**-LA (8% D-LA) are crystalline (Vert et al., 2006).

PLA undergoes degradation either through abiotic(?) hydrolysis or direct action of microorganisms. PLA ester bonds can undergo hydrolytic degradation through abiotic chain scission, resulting in water-soluble oligomers and lactic acid (Kijchavengkul et al., 2009). Through microbial action, depolymerization of PLA (also via hydrolysis) can result in monomers that are metabolized into CO_2, H_2O and biomass (Kasirajan & Ngouajio, 2012). Biodegradation of PLA is greatly affected by temperature. The closer the environmental temperature is to T_g (60-65 °C), or above it, the faster biodegradation occurs (Rujnić-Sokele & Pilipović, 2017. PLA has a lower carbon footprint than conventional polymers derived from petroleum sources of about 50-70% (Vinka et al., 2003).

Poly(Ɛ-caprolactone) (PCL) is a linear polyester manufactured by ring-opening polymerization ofε-caprolactone (Figure 2.6) derived chemically from crude petroleum (Rudnik, 2008).

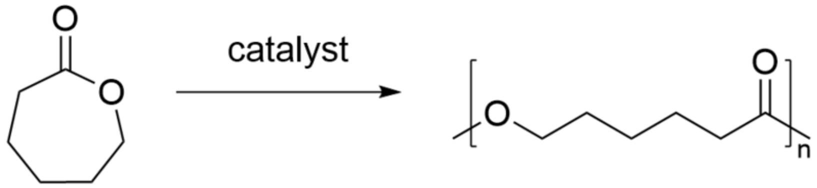

Figure 2.6. Chemical synthesis of poly(caprolactone) via ring-opening polymerization.

PCL has a low tensile strength (23 MPa) but has a high elongation (> 700%) (Vroman & Tighzert, 2009). PCL is recognized as a biodegradable aliphatic polyester that possesses good resistance to water, oil, and chlorine (Rudnik, 2008). Microorganisms present in the environment can degrade PCL (Shimao, 2001; Vroman & Tighzert, 2009). The main applications of PCL are films, adhesives, and synthetic wound dressings (Rudnik, 2008).

Polybutylene Succinate (PBS) is a biodegradable synthetic aliphatic polyester which is synthesized chemically by the polycondensation of 1,4-butanediol with succinic acid (Figure 2.7).

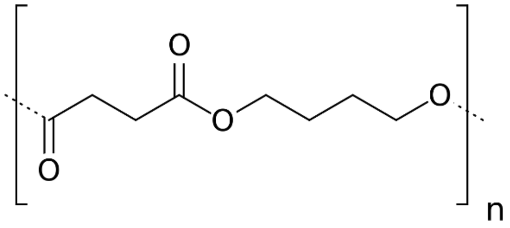

Figure 2.7. Chemical structure of poly(butylene succinate).

The excellent mechanical property of PBS makes it ideal for a wide range of applications. PBS being "hydro-biodegradable," can easily biodegrade through hydrolysis where abiotic hydrolysis initiates biodegradation (Shah et al., 2008). PBS possesses a highest tensile strength than copolymers polybutylene succinate-co-adipate (PBSA) (Vroman & Tighzert, 2009).

Polybutylene Succinat-co-adipate (PBSA) a random copolymer that contains adipic acid, succinic acid and 1,4-butanediol (Figure 2.8), is often blended to make its use economical (Shah et al., 2008). PBSA has molecular weight ranging from 10-1000 kDa (Vroman & Tighzert, 2009).

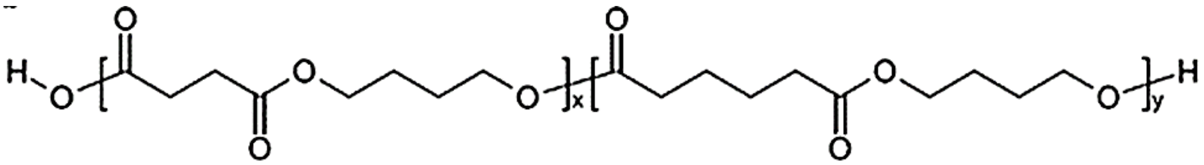

Figure 2.8. Chemical structure of poly(butylene succinate co-adipate).

Thermoplastic Starch (TPS). Starch, an abundant subfamily of polysaccharides that can be extracted from renewable materials, is composed of glucose monomers, with -OH group and -C-O-C bonds susceptible to depolymerization (Rudnik, 2008). Starch has amylose component which is linear and generates amorphous regions and amylopectin component which has a more complex structure, in a branched form (Figure 2.9).

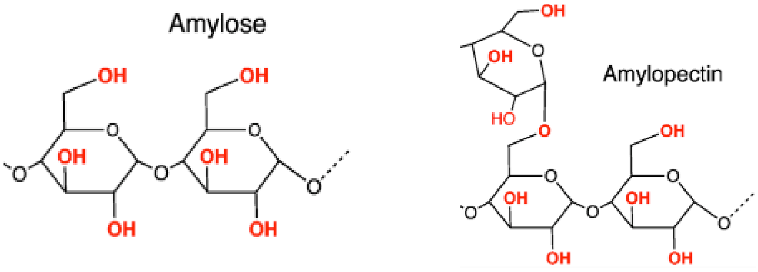

Figure 2.9. Chemical structure of amylose and amylopectin.

Under temperature stress, starch's glycosidic bonds start breaking (150°C) and eventually breaks above 250°C, granules collapse (Vroman & Tighzert, 2009). The lack of moldability and thermoplasticity of starch makes it ineffective for use as an efficient mulch material, unless it is blended (Tachibana et al., 2009). Film production using starch occurs through a gelatinization process. Techniques used in food processing are also similarly utilized during starch processing to produce pastes. Thermoplastic starch (TPS) is produced from starch and a plasticizer through an extrusion process (Rudnik, 2008).

2.2.1.3. Polymeric blends

Blends are formed to modify biodegradability, crystallinity, molecular weight and mechanical properties of homopolymers in neat form. Commonly employed polymers utilized in blends for preparing biodegradable materials include starch, PCL, PLA, PVA, PBA, PBSA, PBAT, and other synthetic polyesters (Rudnik, 2008). The rate of degradation is dependent upon the inherent chemical properties of the readily accessible biodegradable polymer component. For example, for PBSA-starch films that differed in their starch content percentages (5-30%), the rate of biodegradation in soil increased significantly with an increase of starch content (Ratto et al., 1999). Starch / PLA blends are promising candidates for biodegradable feedstocks because both are relatively inexpensive and biodegradable. Various compatibilizers or coupling agents (e.g. soybean oil) are used to improve miscibility of PLA and starch (Muller et al., 2017). The function of compatibilizers and cross-linking agents can be enhanced by controlling their distribution as well as developing a reactive blending technique (Yu et al., 2010).

Plastic films made from PLA via extrusion easily become melted at low heat temperature and are highly permeable to water and other fluids. These two major deficiencies limit use of PLA as the sole polymer for preparing mulches (Dorgan et al., 2006). Molecular parameters controlled in the extrusion of PLA include branching, D-isomer content and MW distribution. Residual metals used as additives for PLA decrease stability (Sn < Zn < Al < Fe iron salts), promoting rapid loss of molecular weight during thermal processing (Rudnik, 2008).

2.2.2 Colorants

Mulch of different colors may also differ on their optical properties and modification of UV light reaching the soil surface, thereby affecting microclimate around a crop (Table 2.2) (Kasirajan & Ngouajio, 2012).

Table 2.2. Effect of plastic mulch color on light and weed control. Source: Maughan and Frost, 2016

Color	Soil Temperature (2-14 ")	Light Reflectivity	Light Absorptivity	Light Transmission	Weed Suppression
Black	Increases (3 to 5 °F)	Low	High	Low	Excellent
Clear	Increases (6 to 14 °F)	Low	Low	Very High	Poor
While/silver	Increases (-2 to 0.7 °F)	High	Low	Low	Excellent
Infrared transmitting (IRT)	Increases (3 to 5 °F)	Low	High	High	Excellent

Blue and brown (as well as black) coloring enhance Infrared transmission, which warms the soil without losing the ability to prevent weed (Maughan & Frost, 2016; Lamont, 1999). Black mulches are typically used to increase soil temperature and reduce weed growth compared to bare soil. White and coextruded white-on-black (WOB) mulches (with white surface facing upwards towards the sun) can lower surface soil temperature because they reflect radiation (Lamont, 2001). Clear plastics are employed for soil solarization; but, these plastics do not control weeds and require other weed management practices such as fumigation and herbicide application (Mitchell et al., 2004).

Carbon black and titanium dioxide (TiO_2) are the most commonly used colorants (EPA, 2012). Carbon black, employed as colorant for black films, also stabilizes the films to mitigate photodegradation; in contrast, TiO_2 produces white color and catalyzes photodegradation, particularly Norrish type I reactions (Kijchavengkul et al., 2008). Carbon black production occurs during combustion of petroleum products (e.g. hydrocarbons) (1320-1540°C), where black particles (10-500 nm diameter) are collected after the process (EPA, 2012). Recently, biobased powder from poplar was used to produce biobased carbon black (Snowdon et al., 2014). Widespread use of nanoscale titanium dioxide (nTiO_2) has raised concerns on its toxicity to the ecosystem, not only to marine organisms but also to humans (Wang et al., 2016; (Srivastava et al., 2012). Talc and calcium carbonate ($CaCO_3$) are used as (white) color additives and fillers to PHA to improve properties (Sherman, 2008).

2.2.3 Other minor constituents

Polymers are blended with other chemicals “additives” to improve properties (Mormile et al., 2017).

Anti-bacterial additives resist biofouling by microorganisms and protect polymeric materials from bacterial growth (Murphy, 2001). Zinc pyrithione (ZnPT) and silver nanoparticle (AgNano) additives inhibit and eliminate bacterial growth (e.g. *Escherichia coli*, *Staphylococcus aureus*) on thermoplastic elastomers (Pittol et al., 2017). On the other hand, ***degradation additives*** are used to encourage a polymeric material to break down for the material to be less harmful to the environment (Murphy, 2002). Two classes of additives marketed now are sold as masterbatch concentrates ‘oxo’ and ‘organic’. Oxo-additives are used to promote chain scission of the polymer. Organic additives initiate or promote microbial attack, triggering microbial action to begin (Narayan, 2010). Pro-oxidants are also added to polyolefins to make them less resistant to hydrolysis, therefore facilitating oxidation and biodegradation (Vroman & Tighzert, 2009; Mohee et al., 2008). ***Antioxidants*** are added to prevent oxidation that occurs when mulch materials are subjected to external energy sources, to reduce the loss of mechanical strength and thermal stability (Ram, 1997). ***Fillers*** also improve mechanical properties (e.g. tear and puncture resistance) of mulch material (Callister & Rethwisch, 2015). ***Lubricants*** are added to modify viscosity of a mulch material, for better flexibility and to reduce friction so that polymers can easily slide along film layers (Murphy, 2001; Soroka, 2002). ***Nucleating or clarifying agents*** are added to modify crystallinity of mulch material and improve stiffness and tensile strength (Murphy, 2001). When crystallinity is increased, small-sized crystals are formed (Soroka, 2002; Pfister & Labowsky, 2003). ***Plasticizers*** are added to mulch material to reduce the formation of fractures and rigid structures, transforming a brittle polymer into a more flexible material (e.g., lower T_g and higher elongation) (Pfister & Labowsky, 2003). For PLA/starch blends, glycerol, sorbitol and triethyl citrate are used as plasticizers to reduce brittleness, while glycerol/ethanol solution is used in other materials to achieve maximum burst strength and elongation (Shah et al., 2008; Jiang et al., 2015). Exposure of the plasticizer di (2-ethylhexyl phthalate) (DEHP) to small children is linked to health hazards (e.g. hepatotoxicity, carcinogenicity and neurotoxicity) (Ghosh, 2017). Plasticizers such as DEHP, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), dioctyl phthalate (DOP), and di-n-hexyl phthalate (DHEXP) are prohibited in manufacturing childcare products in both the US and EU, whereas di-n-octyl phthalate (DNOP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) are currently banned on an interim basis in the US (Erickson, 2015). ***Stabilizers*** prevent photodegradation by mitigating UV light radiation contact to the bulk polymer. Absorbance depends upon thickness, color, and amount and type of stabilizers used. Stabilizers are categorized as UV light absorber/quenchers (e.g. carbon black, ZnO, TiO_2, MgO, $CaCO_3$, $BaSO_4$, Fe_2O_3), hydroperoxide decomposers, and scavengers of free radicals and singlet oxygen, ($^1O_2$) (Yousif & Haddad, 2013).

2.2.4 Mulch film processing

Prior to mulch films production, a “masterbatch” that contains polymeric feedstock, colorant, plasticizers, binder/filler (e.g. nanoclays) and other minor components is prepared. Additives and other components (e.g., peroxide, nanoclay) are used in the masterbatch preparation, dilution, and extrusion process steps to produce the mulch film (Kijchavengkul et al., 2008). Manufacturing of plastic mulches starts with extrusion process. During extrusion of films, polymer pellets are melted until they become pliable. Melted resins are extruded through a plastic tube, where air is introduced, before resins are processed to a flattened sheet. Pellets with colored pigment can be added before extrusion if needed (Tri-Cor, 2015). For BDMs, a manufacturer (“converter”) combines biodegradable polymer feedstock (e.g. Mater-Bi®) with the masterbatch. The mixture is fed to an extruder and a plastic film is produced. Production of PE-based mulch film uses LDPE extracted and refined from crude oil (Figure 2.10).

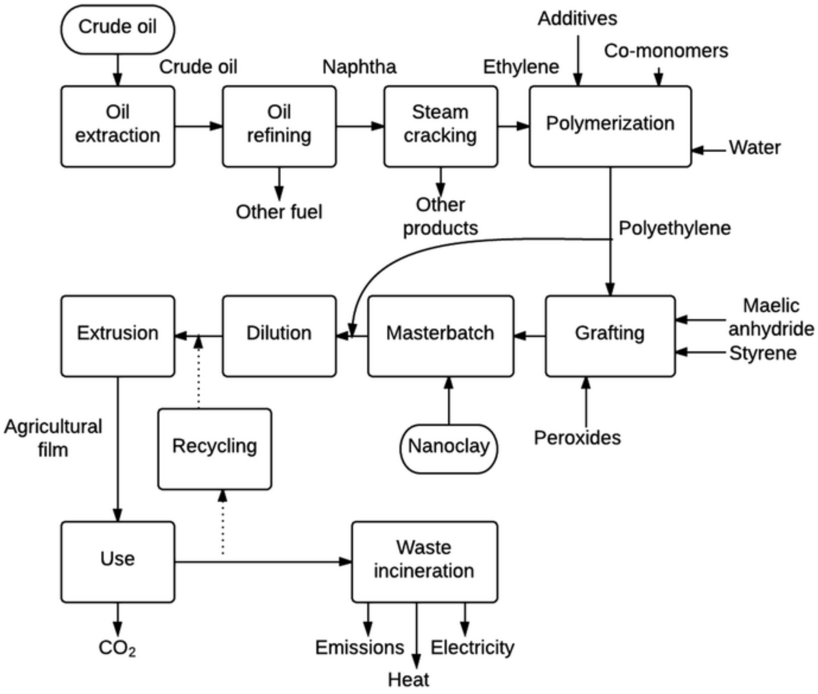

Figure 2.10. Pathway of conventional PE mulch film production from raw material acquisition, polymerization, manufacturing, consumer utilization and consumer end-use. Source: Kijchavengkul et al., 2008.

2.3. DEFINITIONS AND STANDARDS RELATING TO BIODEGRADABLE PLASTICS

2.3.1 Biodegradation

Biodegradation transforms organic substances into smaller metabolites (CO_2, H_2O, and NH_3) by microbial decomposition (OECD, 2002). Biodegradation is divided into two major categories dependent upon oxygen (O_2) availability, aerobic and anaerobic processes. For aerobic biodegradation, microorganisms cleave polymers' intramolecular bonds using oxygen during metabolism, producing CO_2 and H_2O. For anaerobic processes that occur in an oxygen-deficient environment, methane (CH_4) and H_2O are released (Kijchavengkul & Auras, 2012).

Soil respiration is the measurement of CO_2 production by organisms in the soil (USDA, 2001). Microbial activity increases with increasing temperature and moisture (until the approach of water saturation is attained) (Kijchavengkul et al., 2008). Microorganisms are essential for bioremediation of environmental plastics (Krueger et al., 2015).

Biodegradation through ***composting*** transforms biodegradable materials into compost, CO_2, water, and minerals in a controlled biological process (Kijchavengkul et al., 2008). For agricultural plastics, the increasing pressure on landfills for poorly biodegradable plastic wastes can be reduced through utilization of BDMs aided by composting (Pfister & Labowsky, 2003). Composting is a preferred disposal and treatment strategy for biodegradable plastics (Starnecker & Menner, 1996; Korner et al., 2005). Three sources of wastes can be used as compost media: manure, yard, and food. Manure waste, nitrogen-enriched, can be mixed with materials rich in carbon to maintain an adequate C:N ratio. Yard waste consists of vegetative waste from landscape areas (e.g. lawns and gardens). Food waste can be taken through post-consumer or from a food preparation process (pre-consumer) (Kijchavengkul et al., 2008). PBAT had higher and faster biodegradation in using manure than other types of compost (e.g. yard, food) due to rich nitrogen concentration coming from fecal and urinal excretion of livestock, reflecting the Influence of the C:N ratio (Kijchavengkul et al., 2010). The important parameters of composting (Table 2.3) should be regulated by aeration, free space, and agitation (Rudnik, 2008).

Table 2.3. Optimal conditions for aerobic composting. Source: Cooperband, 2002.

Conditions	Acceptable	Ideal
C:N ratio (w/w)	20-40:1	25 – 35:1
Moisture content	40 – 65%	45 – 60 %
Available oxygen concentration	> 5%	> 10%
pH	5.5 – 9.0	6.5 – 8.0
Temperature	43 – 66°C	54 - 60°C

Biodegradation is faster in compost than in soil for two reasons: higher temperature (60°C for industrial composting) and the differences between microbial communities. Biodegradation in compost also depends on factors such as polymer composition and quality of compost. The impact of biodegradation environment is particularly significant for PLA because the polymer's T_g is high, 50--60°C (Rujnić-Sokele & Pilipović, 2017). In the mesophilic stage of a natural composting process (20°C – 45°C), PLA is mainly recalcitrant to mineralization but biodegrades in the thermophilic phase (45°C – 75°C). The biodegradation rate of PHB was also higher under composting (46^{o}C) relative to biodegradation in soil. The effect of thermal ageing (192, 425, 600 h) at three temperatures (100, 120, 140°C) on the biodegradation of PHB in a compost showed an increased biodegradability only at 120 and 140°C for all thermal ageing treatments (Rudnik, 2008).

Composting can be performed through industrial composting or through home or backyard composting. Industrial composting is a thermophilic (50°C – 60°C) process to convert organic materials into CO_2, H_2O, and biomass under a hot and moist environment (45-55% relative humidity) (Greene, 2014). Industrial composting methods can be categorized into three basic types. *In-vessel systems* contain composted organic material inside a container where conditions are controlled (e.g. aeration). The in-vessel biodegradability system has three essential components: (1) air supply; (2) bioreactor' and (3) an instrument that records O_2 uptake or CO_2 release. In an *aerated static pile system*, compostable materials are formed into large piles and aerated without turning the pile over. The *windrow system* involves formation of elongated piles of compostable materials that are turned over on regular basis (Kijchavengkul et al., 2010). The methods of biodegradation measurement are shown in Table 2.4 (Kijchavengkul & Auras, 2012).

Table 2.4. Methods for measurement of biodegradation. Source: Kijchavengkul & Auras, 2012.

Methods	CO_2 Measurement system	Analysis
"Cumulative Measurement Respirometric (CMR)"	'Exhaust CO_2 is trapped in a basic solution'	Titration of free base at predetermined times
"Gravimetric Measurement Respirometric (GMR)"	'Exhaust CO_2 is captured in CO_2 absorption columns'	Mass balance based on column weight increase
"Direct Measurement Respirometric (DMR)"	'Gas chromatography or in-line infrared gas analyzer'	Measurement at predetermined times

The temperature for home or backyard composting is maintained below 30°C, producing a mesophilic condition (Greene, 2014). Two types of backyard composting, cold and hot, are possible options using compost bins. Cold/slow composting involves piling of grass clippings on the ground or in a bin, and is not managed, but can require years for waste to fully decompose. Hot composting, on the other hand requires more labor to form optimal environmental conditions that can produce compost feasible in a few weeks (NRCS, 1998).

2.3.2 Standards for Testing Biodegradability of Plastics

Biodegradable plastic can only be declared biodegradable when composting requirements are met (ASTM D6400). The key requirements include mineralization, disintegration, and safe disposal in the environment (eco-toxicity compliance). The ASTM D-6400-04 sets requirements for plastic products to be labelled accordingly (e.g. degradable, compostable and biodegradable). The standards utilize standardized tests (ASTM, ISO/DIS EN) operated under specific environmental conditions (Table 2.5) (Rudnik, 2008).

Table 2.5. Standard test methods on biodegradation.

Standardized	Description
Biodegradation in soil	
ASTM D5988	"Aerobic biodegradation in soil of plastic materials"
Composting	
ASTM D6003	"Weight loss determination of plastic materials in a simulated municipal solid waste (MSW) under aerobic compost environment"
ASTM D6340	"Aerobic Biodegradation of radiolabeled plastics in an aqueous or compost environment"
ASTM D5388	"Aerobic biodegradation of plastic materials or residual plastic materials under controlled composting conditions"
ISO 14855	"Determination of the ultimate aerobic biodegradability of materials under controlled composting conditions"
Other environments	
ISO 14852	"Ultimate aerobic biodegradability of plastic materials in an aqueous medium – method by analysis of evolved carbon dioxide"
EN ISO 14851	"Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – method by measuring the oxygen demand in a closed respirometer"
ASTM D5210	"Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge"

Table 2.5 continued.

ASTM D6691	“Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in marine environment by a defined microbial consortium”
ASTM D5526	“Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials under Controlled landfill conditions”
ASTM D5511	“Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials under high solids anaerobic-digestion conditions”

A new standard, European Standard EN 17033: Plastics-Biodegradable mulch films for use in agriculture and horticulture, was released that regulates requirements of a BDM film. The standard regulates several criteria for BDMs, such as 1) chemical composition, 2) biodegradation in soil ($\geq$ 90% conversion of organic carbon into CO_2 within 2 years), 3) ecotoxicity, and 4) selected physical characteristics.

ISO 17088, a standard for compostable plastics specifies test methods and requirements on compostable products designed to convert the plastics into CO_2 and water. The standard addresses biodegradation, disintegration, ecotoxicity, and quality of biomass (Figure 2.11). EN 13432 is another standard on compostability but under an anaerobic treatment (Rujnić-Sokele & Pilipović, 2017).

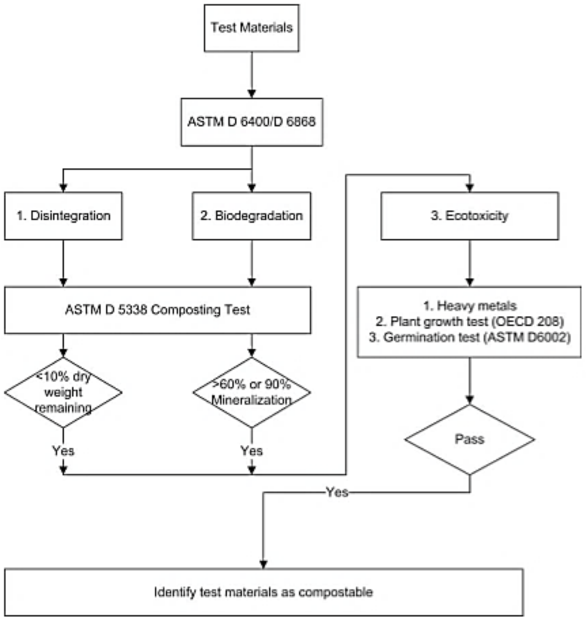

Figure 2.11. Three criteria of compostable materials based on ASTM D 6400/ D 6868 standards.

2.3.3 Ecotoxicity Tests on Compost

Ecotoxicity tests assess water pollution and contamination in soils. Inorganic materials released from plastic products' degradation may reduce soil productivity over time; hence, ecotoxicity studies are essential. Comprehensive toxicity tests can be utilized for plant, animal and perhaps microorganisms in the future. Methods of evaluating ecotoxicity includes use of plants (e.g. monocots, dicots), soil (e.g. earthworm) and aquatic fauna (e.g. daphnia), algae, and luminescent microorganisms (Kyrikou & Briassoulis, 2007; Rudnik, 2008). Degradation of biodegradable plastics used for agricultural applications occurs in the soil and due to agricultural plastics' increased usage worldwide, environmental safety also becomes an increasing concern (Sforzini et al., 2016). Ecotoxicity testing can be based on standardized tests on phytotoxicity (e.g. cress test (1STA), oats and lentils test (ISO 11269-2)) and immobilization/survival of organisms (e.g. DIN V 54900-3, ON S 2200 and ON S 2023, ISO 11268-1) (Innocenti, 2003).

In an applied ecotoxicological test, a Mater-Bi® film was intentionally left in the field after use to study degradation effects to the soil quality. Mater-Bi® underwent degradation under controlled conditions. Biodegradable mulch film produced no negative impact on organisms present in the soil, including bacteria, protozoans, green algae, sorghum and garden cress and invertebrates through acute and chronic endpoints assessment (Sforzini et al., 2016).

2.4. ASSESSMENT OF POLYMER DEGRADATION

Polymer degradation (defined in Table 1.1.) encompasses a series of physical and chemical processes. Thus, requiring a variety of techniques to characterize the long-term change in polymer properties. Several of the techniques may give similar results; but, often they provide complementary information (Karlsson & Albertsson, 2002). Changes of polymer properties can be categorized as 1) physical or 2) chemical change as assessed by the following methods which can indicate early stages of the polymer degradation process as well as the latter stages of biodegradation (Kyrikou & Briassoulis, 2007).

2.4.1. Visual degradation

Plastic mulch degradation can be indicated through visual changes such as surface roughening, holes or cracks, fragmentation, decrease of 2-dimensional surface area, decrease of thickness and changes in color (Shah et al., 2008). Changes of optical characteristics (e.g. crazing, delamination) are considered physical alterations (Kijchavengkul et al., 2008). Other methods that determine mulch fragmentation and disintegration in the soil are presented in Table 2.6.

Table 2.6. Methods used in assessment of mulch degradation on-field after agricultural-use based on visual observations of the mulch surface.

Method	Technique description	References
Qualitative Scale Visual assessment	Above-soil degradation Soil cover (%) 1 - 0% cover 9 - 100% cover In-soil degradation Dematerialization (%) 1 - 100% disintegration; 9 - Intact mulch	Martin-Closas, 2016
Percentage of Mulch Area Remaining (PMAR)	Determination of mulch area loss of a photographed mulch samples using an equation: $PMAR = \frac{original\ area - area\ after\ burial}{original\ area} \times 100$	Li et al., 2014
Percent Visual Deterioration (PVD)	Percent of soil exposed within the evaluation section 0% = intact sample 100% = completely degraded sample (not visible)	Cowan et al., 2013

2.4.2. Mechanical Properties

Mechanical testing generally determine the degree of degradation through loss of stress-strain properties (Karlsson & Albertsson, 2002). Loss of physical or mechanical strength can be measured directly through physical strength testing and viscoelastic behavior of polymers or indirectly through microscopic imaging (Hayes et al. 2015). Tensile strength measures the mechanical integrity of a material. Elongation at break, also known as fracture strain, measures the (%) increase of the plastic's length when the material is stretched during the tensile strength test at the instance of breakage, i.e., the ratio of the length after breakage to its initial length (Mormile et al., 2017).

Prior to mechanical tests, it is critical to perform thorough cleaning of retrieved mulch films from the field to remove adsorbed soil and plant debris without damaging the mulch samples to avoid artifacts for the values obtained from the tests. In previous research, mulch samples taken from field were carefully brushed with a soft bristle brush and successively with a cosmetic brush (Hayes et al., 2017). Tests that determine mass loss to assess biodegradation is commonly used; yet, the fragility of the plastic film samples can be problematic for this assessment (Shah et al., 2008).

2.4.3. Change of color

Mulch films change color during exposure to sun and other factors such as partial contact with the soil. Colorimetry, a simple method using commonly employed instrumentation, is often used to assess color changes. Colorimetry measures three parameters: *L* (lightness), *a* (redness & greenness), and *b* (blueness and yellowness) during the measurement process. Delta E (Equation 2.1) calculates the total color difference, employing a sum-of-squares analysis of the changes for *L, a,* and *b*.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \qquad \text{Equation 2.1.}$$

L refers to lightness (0 = black, 100 = white); *a* refers to redness or greenness (-*a* = green; +*a* = red); and *b* refers to blueness or yellowness of a material (-*b* = blue, +*b* = yellow). The lightening of black films (i.e., undergo a decrease of *L*) can be due to photodegradative bleaching of the carbon black pigment.

2.4.4. Surface morphology

Optical light microscopy and scanning electron microscope (SEM) can provide information on plastic degradation mechanism. SEM micrographs can detect deterioration of plastics for both crystalline and amorphous regions (Kijchavengkul et al., 2008). When microorganisms attach and form biofilms on polymer surfaces, matrix formation in a form of a clear halo around the colony indicates organisms' ability to initially depolymerize the polymer (Shah et al., 2008).

2.4.5. Surface Chemistry - Attenuated Total Reflectance (ATR)-FTIR Spectrometry Attenuated Total Reflectance

Comparison of spectroscopic data can be used to determine the extent of degradation of polymers (Shah et al., 2008). When chemical bond scission or cross-link formation occurs, chemical transformation can occur on the polymeric material's surface (Kijchavengkul et al., 2008). Analysis of Fourier transform infrared spectroscopy (FTIR) absorbance spectra (4000-600 cm^{-1}) can determine changes in chemical bonding during weathering and biodegradation. FTIR determines changes of absorbance at specific energies (i.e., frequencies, or wavenumbers) that correspond to specific chemical bonds as a result of degradation (Shah et al., 2008). FTIR spectroscopy involves sampling of a small portion of the overall surface area of a polymeric material, and therefore does not necessarily consider the highly heterogeneous nature of polymer surface chemistry and degradation processes (Karlsson & Albertsson, 2002).

2.4.6. Molecular weight (MW)

Molecular weight (MW) changes can illuminate the degradation mode, such as random chain scission, where MW underwent slower change, or chain end degradation, which leads to rapid change of MW (Karlsson & Albertsson, 2002). Number-averaged MW (M_n, Equation 2.2), weight-averaged MW (M_w, Equation 2.3) and polydispersity index (PDI, Equation 2.4) can be determined through fractionation methods such as sedimentation rate by ultracentrifugation (UC), field flow fractionation (FFF) and gel permeation (size exclusion) chromatography (GPC/SEC). Non-fractionation methods measure single molar mass averages such as static/dynamic light scattering (SLS, DLS) and small- angle scattering (SAS) to determine MW and nuclear magnetic resonance (NMR) spectroscopy to determine M_n (Molecular Weight, 2017). PDI describes the distribution. With PDI near to 1, polymer chains have the same MW. An increasing PDI value indicates a broader distribution of MWs in the polymer molecules (Karlsson & Albertsson, 2002).

$$Mn = \frac{\sum M_i n_i}{\sum n_i}$$ Equation 2.2.

$$Mn = \frac{\sum M_i w_i}{\sum w_i}$$ Equation 2.3.

$$PDI = \frac{M_w}{M_n}$$ Equation 2.4.

GPC is a fractionation technique where a suitable solvent is used to dissolve the polymers and separates polymers based on size. Size separation occurs when polymers goes through a passage inside a column packed with gel beads such as polystyrene (Gel-Permeation Chromatography, 2007). Polymers with low MW (< 1000 Dalton, Da), i.e. small molecules, are retained and penetrate through the pores of the gel beads inside the column during the process whereas large molecules are not retained and elute more quickly. Detectors (e.g. differential refractometry) reflect polymer concentration and lead to generation of a chromatogram: signal vs. time (OECD, 1996).

2.4.7. Change of polymeric composition

Nuclear magnetic resonance (NMR). Nuclear magnetic resonance (NMR) spectroscopy is a method that determines atomic and molecular level structure that uses a non-invasive probe to identify compounds. NMR takes advantage of the magnetic properties of the material's nuclei that are influenced not only by chemical environment but also by the physical alterations with its environment (Nanny et al., 1997).

Thermal properties. The thermodynamic property enthalpy equals the overall internal energy plus the product of pressure and volume product of a system. Melt temperature (T_m) is the temperature at which the material being processed undergoes melting or becomes a softened plastic during a heating cycle. T_m is an important indicator of the state of the material and the process (Encyclopedic Dictionary of Polymers, 2007). Polymer crystallization is a phase transition process going from disorder isotropic melt to the order semi-crystalline state as temperature is increased, and the temperature where transition occurs is crystallization temperature (T_c) (Müller et al., 2016).

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) measures the thermal property or heat flux variation of a sample according to time and temperature as the temperature is changed. The instrumentation is comprised of an insulated thermal conductive chamber coupled with detectors that measures heat flux between the sample and sample vessel (Le Parlouër, 2013). Heating stages are recorded and the difference on temperature of a sample relative to the reference will determine crystallinity of a material during melting (Karlsson &

Albertsson, 2002). For example, using films with PBAT component, crystallinity increased with time during biodegradation, indicating that biodegradation occurred selectively in amorphous regions (Kijchavengkul et al., 2010).

Thermogravimetric Analysis (TGA). Thermogravimetric analysis (TGA) measures the mass change of a sample mass using a thermogravimetric analyzer or thermobalance in an enclosed system as temperature is increased. A thermobalance consists a microbalance, a program for temperature that allows weighing of sample as it is heated, capturing the change of mass over time (Brown, 2001). A differential thermogravimetry (DTG) refers to the mass loss per unit time vs. temperature which automatically display and indicate onset and maximum temperature of degradation process (Beyler & Hirschler, 2001). Two different types of transformations in TGA can occur either through mass loss (e.g., via dehydration, dehydroxylation, evaporation, decomposition, desorption, pyrolysis) or mass gain (e.g., via adsorption, hydration, reaction). In TGA-MS, the balance-s headspace is connected online with a mass spectroscopy detector to determine the molecular weight and amount of volatile product emitted by the sample (Le Parlouër, 2013).

2.5. CONCLUSIONS

In this chapter, a literature review on biobased and fossil-fuel derived plastics, biodegradable polymer (as feedstocks), masterbatch and other minor constituents used for production of PE and BDMs were discussed. Definitions, standards, and testing methods on biodegradation (e.g. soil, composting condition) important for this dissertation, were also discussed. Assessment of polymer degradation was covered in detail, which is essential to the conduct of research project, particularly to address the research gaps listed below:

- BDMs undergo degradation during field-use; yet, few studies have investigated long-term duration studies. Often overlooked, the potential negative impact of weathering on BDMs (e.g. crosslinking) may inhibit and slow further biodegradation of mulches in the soil;
- BDMs are designed to biodegrade after field-use; yet, few studies have studied the impact of weathering on biodegradation of BDM and how these changes differ between soil and composting conditions;
- Prior to field-use, BDMs are susceptible to degradation during storage; yet, no research project has assessed mechanical functionality and chemical properties of BDM and how it influence agronomical performance of BDMs during field-use.

This literature review will provide a framework for the next chapters of this dissertation.

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ASTM D6474 – 12 Standard Test Method for Determining Molecular Weight Distribution and Molecular Weight Averages of Polyolefins by High Temperature Gel Permeation Chromatography (GPC)

ASTM E1131 – 08 (Reapproved 2014) Standard Test Method for Compositional Analysis by Thermogravimetry

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3. EFFECT OF AGRICULTURAL WEATHERING ON THE DEGRADATION OF BIODEGRADABLE MULCH IN TWO DIVERSE CLIMATES DURING FOUR SUCCESSIVE YEARS

A version of this chapter was submitted to the Journal of Polymers and the Environment and is titled as follows:

Marife Anunciado, Douglas G. Hayes, Larry C. Wadsworth, Marie E. English, Sean M. Schaeffer, Henry Y. Sintim and Markus Flury. Impact of Agricultural Weathering on Physicochemical Properties of Biodegradable Plastic Mulch Films: Comparison of Two Diverse Climates Over Four Successive Years

In the study, I and Larry Wadsworth were responsible for analyzing physicochemical data. I also interpreted the results and prepared the manuscript. Marie English collected the environmental data, as well as Henry Sintim who assisted in solar radiation and degree days calculations. Sean Schaeffer and Markus Flury provided technical inputs and Douglas Hayes one of my major advisors, conceived, directed the research and edited the manuscript, and serves as its corresponding author.

3.1. ABSTRACT

Biodegradable plastic mulches (BDMs) are utilized all over the world to improve crop production. Unlike conventional polyethylene (PE) mulches, BDMs can be tilled in the soil after cropping season, where they undergo biodegradation, thereby leading to minimal environmental impact. Agricultural weathering impacts both the performance of BDMs during crop production as a barrier to weeds and biodegradability of BDMs in the soil. To better understand the relative importance of climatic factors, the change of physicochemical properties (color, mechanical strength, surface characteristics and morphology, thermal properties, molecular weight and composition of polymeric constituents) of BDMs during field trials for vegetable production at two diverse climates (Knoxville, TN and Mount Vernon, WA across four successive years, 2015-2018) were evaluated. Mulch treatments consisted of two commercially available BDMs composed primarily of polybutylene co-adipate- co-terephthalate (PBAT), an experimental BDM prepared from a polylactic acid/polyhydroxybutyrate blend and a conventional PE mulch.

Results showed that trends of degradation of BDMs across years did not vary appreciably, despite extreme weather events (heat and drought, heavy rainfall) experienced at both field sites. BDMs underwent greater changes on physicochemical properties in TN than in WA, attributable to higher amount of rainfall, and soil and ambient air temperature.

3.2. INTRODUCTION

Plastic mulch films are agricultural technical tools used for production of vegetables and other specialty crops. The microclimate for the soil underneath the film is controlled, leading to reduced weeds, increased crop yield and effective use of agricultural inputs (Hayes et al., 2019; Steinmetz et al., 2016). The use of conventional mulches has a major drawback: the environmentally sustainable disposal or reuse of the plastics, especially polyethylene (PE) mulches, which are recalcitrant to biodegradation and are stockpiled on farms after their use due to the low availability of recycling programs (Chiellini & Solaro, 1997; Kasirajan & Ngouajio, 2012; Kyrikou & Briassoulis, 2007). PE mulches become embrittled during their service life due to agricultural (environmental) weathering, producing fragments that readily become dispersed in the environment that can ultimately form microplastics in soils (Rochman, 2018; Zhang et al., 2018). Terrestrial microplastics are a major concern due to their reported harm to earthworms, collendola and microorganisms in the soil, and the potential ability to form nanoplastics that can potentially adsorb pesticides and other nonpolar toxicants and enter the food chain (Bouwmeester et al., 2015; Huerta Lwanga et al., 2017; Ng et al., 2018; Zhu et al., 2018). Biodegradable mulches (BDMs) serve as an alternative to PE mulch, providing the same benefits toward sustainable specialty crop production but possessing a significantly higher inherent biodegradability (Hayes et al., 2019; Kapanen et al., 2008; Miles et al., 2017; Rujnić-Sokele & Pilipović, 2017). BDMs can be inexpensively plowed into the soil after their service life, in contrast to laborious and expensive manual removal of PE mulch by hand (Chiellini & Solaro, 1997).

Weathering can impact the performance of BDMs above and below the soil. During the useful-life stage of BDMs in cultivation of vegetables, environmental factors degrade the polymeric material, causing mechanical weakening and changes of physicochemical properties (Hablot et al., 2014; Kijchavengkul et al., 2008a). BDMs' perceived "unpredictable breakdown" during deployment in the field is attributable to limited utilization of BDMs compared to PE in the farms (Li et al., 2014; Moreno et al., 2017). Factors that influence agricultural weathering include 1) abiotic environmental factors (e.g. solar radiation, air and soil temperature (T), physical and mechanical erosion, 2) soil conditions (e.g. O_2 availability , moisture, nutrients, pH, temperature), 3) soil microbial communities, 4) conditions of use (e.g. crop type and procedure for mulch laying) and 5) inherent properties of the BDMs' polymeric constituents (Chiellini & Solaro, 1997; Copinet et al., 2004; Emadian et al., 2017; Li et al., 2014). Sunlight, particularly its ultraviolet (UV) spectral component, imparts chemical degradation of BDMs through photochemical reactions that either inhibit or enhance biodegradation in the soil (e.g., crosslink formation and depolymerization, respectively) (Copinet et al., 2004; Hablot et al., 2014; Hayes et al., 2017; Kijchavengkul et al., 2008a; Urtuvia et al., 2014). A canopy of leaves and vines that forms upon maturity of crops reduces the amount of solar radiation reaching the top surface of mulch films, thereby reducing the solar degradation of mulches (Hayes et al., 2017; Moreno et al., 2017). Soil moisture and elevated soil temperature can enhance hydrolysis, leading to depolymerization and therefore loss of mechanical strength (Andrady, 2015; Brodhagen et al., 2015; Kijchavengkul et al., 2008b; Lendlein & Sisson, 2011; Rudnik, 2008). When crop grows and canopy forms, the crops entire system can cause pressure on the mulch against the soil, or may also trap heat from the sunlight and build a humid environment under plastic mulches, which may favor degradation (Devetter et al., 2017; Martín-Closas et al., 2016). In most cases, extreme weather conditions (e.g. catastrophic rainfall, high wind turbulence, high impact flow of rainwater) can trigger mechanical stress, leading to puncture, cracks, holes, tears, and rips on the already embrittled mulch films. Localized water pools can form on mulches when soil surface is uneven causing further degradation of mulch films occurs (Briassoulis, 2007; Hablot et al., 2014; Krueger et al., 2015). Regarding below-soil effects, embrittlement and depolymerization of BDMs increases the biodegradation rate of BDMs in soil or compost. However, studies conducted in the lab are performed in short-term duration and do not reflect the actual, long-term agricultural application of BDMs in the field (Briassoulis, 2007; Hablot et al., 2014). Often misunderstood and overlooked, weathering can also pose a potential negative impact on biodegradable mulches, photodegradation causing chemical oxidation can lead to crosslinking, which may inhibit and slow further biodegradation of mulches in the soil.

3.3. OBJECTIVES

The objective of this study is to better understand the effect of environmental factors (e.g. solar radiation, temperature, and soil moisture) on degradation. A long-term field study was conducted across four years (2015-2018) at two diverse geographical conditions possessing different soil types (Knoxville, TN and Mount Vernon, WA, USA, representing hot-humid and mild-humid summer climates, respectively), to evaluate specialty crop production, weed suppression and soil health (Ghimire et al., 2018; Hayes et al., 2019; Moore & Wszelaki, 2019; Sintim et al., 2019). The four-year field study provided a unique opportunity to more deeply understand the effect of agricultural weathering on changes of physicochemical properties of BDMs. Results from 2015 were recently published (Hayes et al., 2017); this work is therefore an expansion of the cited study, probing additional years and two additional mulch products. Environmental conditions, such as solar radiation, temperature, and humidity, will vary between geographical regions and across years. Changes of physicochemical properties due to agricultural weathering were hypothesized to be more pronounced in TN than WA due to increased exposure to solar radiation, and other abiotic factors (e.g. soil moisture, soil temperature) to a lesser extent.

3.4. EXPERIMENTAL

3.4.1 Materials

Mulch treatments investigated herein are listed in Table 3.1.

Table 3.1. Properties and manufacturer of mulch films used in this study (2015-2018) [i].

Mulch	Color	Thickness, (µm)	Peak Load [ii],N	Elongation [b], %	Polymeric Constituents [iii]	Manufacturer
PLA/PHA	Black	37 ± 1.4	17 ± 0.5	246.0 ± 5.0	PLA/PHA blend	Experimental Film [iv]
BioAgri [v]	Black	29 ± 1.2	12 ± 0.6	295.0 ± 30.0	PBAT/starch blend	BioBag Americas, Inc., Dunedin, FL
Organix A.G., Black [vi]	Black	20 ± 0.7	9 ± 0.4	270.8 ± 8.3	PBAT/PLA blend	Organix Solutions, Maple Grove, MN
Organix A.G., White-on-black (WOB) [f]	Black, white on top	18 ± 1.3	12 ± 0.5	208 ± 10.0	PBAT/PLA blend	Organix Solutions
Organix A.G., Clear [f]	Clear, transparent	13± 1.0 [vii]	12 ± 0.4	215 ± 13.0	PBAT/PLA blend	Organix Solutions
Polyethylene (PE)	Black	40 ± 0.3	16 ± 0.6	567.5 ± 23.9	Linear low-density polyethylene	Filmtech, Allentown, PA

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[i] Errors reflect standard deviation.

[ii] Measurements were performed along machine direction of the films.

[iii] PLA, PHA and PBAT refer to polylactic acid, polyhydroxyalkanoate and polybutylene adipate terephthalate, respectively.

[iv] An experimental film with polymeric components consists of 68-71 wt % PLA and PHA prepared by Metabolix Inc., Cambridge, MA.

[v] Mater-Bi® grade EF04P. Bio360 was produced from DuBois Agrinovation, Saint-Rémi, Quebec, Canada and used in 2017 and 2018.

[vi] Prepared from ecovio® grade M2351 (BASF, Ludwigshafen, Germany)

[vii] Value is for mulch roll obtained and employed in 2017. For 2018, a new mulch roll was prepared and employed, with thickness of 18±1 µm. Other properties did not differ between years.

BioAgri, and Organix are black-colored BDMs prepared from Mater-Bi® and ecovio® feedstocks (BASF), respectively, possessing PBAT as their main component. PLA/PHA mulch was an experimental film prepared from a blend of polylactic acid (PLA) and polyhydroxyalkanoate (PHA). BioAgri, Organix and PLA/PHA were employed for all four years. In addition, two BDMs formed from the same ecovio® feedstock as used to prepare Organix were employed in 2017 and 2018: "White-on-Black" and "Clear", used in TN and WA, respectively. Mulches employed for the 2015 and 2016 field trials were provided in 2015 (1.22 m-wide rolls). Newly produced rolls of BioAgri and Organix were prepared in 2017 and employed for the 2017 and 2018 field trials, except that the Mater-Bi® -based mulch was Bio360, obtained from Dubois (Ottawa, Canada). To assist the reader, the 2017 and 2018 Bio360 films are referred to as "BioAgri." The 2015 and 2017 rolls used the same polymeric feedstocks and possessed the same roll width and film thickness. In 2017, a clear Organix mulch was added in WA whereas white-on-black (WOB) Organix mulch was added in TN, with white surface facing up and the black surface touching the soil. In 2018, a new Clear Organix mulch roll was prepared a greater thickness to increase its durability. The rolls of PLA/PHA, prepared in 2015, were used for all four years. Further information on the properties of the mulches is given in Table 3.1. Mulches were stored indoors (22°C) in the dark under low humidity conditions prior to use. Solvents (e.g. $CHCl_3$, $CDCl_3$) were "HPLC grade" and purchased from Fisher Scientific (Pittsburgh, PA, USA).

3.4.2 Methods

Environmental weathering of plastic mulches was conducted during field trials for specialty crop production from 2015 to 2018. Field trials were conducted on a 4-year field experiment (2015-2018) at the East Tennessee AgResearch and Education Center, Plant Sciences Unit in Knoxville, TN (35°52’52’’N, 83°55’27” W) and the Northwestern Washington Research and Extension Center in Mount Vernon, WA (48°43’24’’N,122°39’09”W) during the summer months (late May through early September). Eight mulch treatment plots were arranged in a complete randomized block design with four replications. Treatment plots were carried out consistently throughout four sampling years to avoid contamination across- mulch treatments. Field conditioning involved installation of the drip irrigation system and PE removal from the field plots. Dimensions of the plot and the spacing between the plots are described elsewhere (Ghimire et al., 2018). In general, per instruction from the manufacturer, the "shinier" surface of the films faced upwards. Mulches described in this paper were machine laid.

A ‘Cinnamon Girl’ pie pumpkin was used as the test crop for 2015 and 2016 at both locations. In 2017 and 2018, ‘Aristotle peppers’ and ‘Xtra-Tender 2171’ sweet corn were employed in TN in WA, respectively. Soil in TN field site has moderately well drained Shady-Whitwell complex soil while soil in WA field site has Skagilt silt loam soil. More detailed information on crop preparation and planting, fertilizer and irrigation, and weed assessment and management of all sampling years is provided elsewhere (Devetter et al., 2017; Moore & Wszelaki, 2019; Sintim et al., 2019). To determine the date for which the plant canopy formed, plant growth was monitored every week via photographs. Mulch film specimen were retrieved from the field on the week of crop harvest in TN and WA and analyzed for physicochemical properties. Machine and cross-machine (transverse) directions (MD and CD, respectively) were noted. Adsorbed soil and other plant debris were carefully removed by hand from the mulches so as not to compromise the integrity of the mulch material. BDMs were cleaned with a soft bristle brush to avoid rips and tears. Mulches were allowed to equilibrate in the laboratory for two days for conditioning prior to physical testing. Table 3.2 contains the schedule of cropping activities and weather parameters throughout the field trials.

Table 3.2. Field events and weather conditions during field trials at Knoxville, TN and Mount Vernon, WA (2015 to 2018).

	Knoxville, TN				Mount Vernon, WA			
Field events	**2015 [a]**	**2016 [a]**	**2017 [b]**	**2018 [b]**	**2015 [a]**	**2016 [a]**	**2017 [c]**	**2018 [c]**
Laying of mulches	29 May	14 Jun	23 May	24 May	26 May	25 May	19 May	17 May
Planting of crops	29 May	16 Jun	25 May	26 May	29 May	31 May	23 May	22 May
Formation of plant canopy [d]	10 Jul	8 Jul	3 Jul	3 Jul	1 Jul	20 Jul	30 Jun	2 Jul
Harvesting of crops [e]	17 Sep	12 Sep	21 Sep	6 Sep	16 Sep	21 Sep	26 Sep	26 Sep
Environmental parameters [f]								
Rainfall (mm)	354	138	439	234	90	190	56	46
Solar radiation (MJ/m^2) up to canopy formation	1062	583	852	854	960	1155	954	959
Solar radiation (MJ/m^2)	2570	1977	2401	2166	2455	2315	2692	2486
Relative humidity (%)	82	77	81	82	76	77	76	75
Daily degree (°C) up to canopy formation	861	654	957	1019	629	908	661	693
Daily degree (°C)	2792	2423	2887	2684	2000	1977	2168	2139
Wind speed (m/s)	2.3	1.2	1.3	0.9	1.7	1.7	1.6	1.6
Average, daily minimum air temperature (°C)	25	26	23	25	17	11	11	10
Daily air temperature, average (°C)	20	21	18	20	11	16	16	16
Daily air temperature, maximum (°C)	30	33	30	31	24	22	23	23
Soil temperature, bare ground (10 cm depth, °C) [g]	25	28	26	27	20	19	19	19

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[a] Test crop: pie pumpkin

[b] Test crop: bell pepper

[c] Test crop: sweet corn

[d] Determined from photographs taken weekly.

[e] For 2017 and 2018 and both sites, harvest did not occur on a single day, but over multiple collection dates. Dates included for harvest in 2017 and 2018 were final harvest dates. Peppers and corn were harvested at multiple days/cycles until crops reached mature green stage.

[f] Measurement of all environmental parameters were started during mulch laying and finished at final crop harvest, unless noted otherwise.

[7] Soil temperature was different underneath the mulches only during the growing season but not after when canopy had fully developed (Sintim et al., 2019).

Soil parameters were recorded by a data logger (EM50G; Decagon Devices, Inc., Pullman, WA, USA) with sensors (5TM; Decagon).

Weather data in TN were taken from University of Tennessee (UT) Plant Sciences Unit weather station (~10 m away from the field site) except for rainfall data which was collected from a weather station at Knoxville McGhee Tyson Airport, US National Oceanic and Atmospheric Administration, located 533 m from the field site. Degree days (DD) were calculated using Eq. 3.1 where $T_{max,i}$, $T_{min,i}$ and T_{base} refer to maximum and minimum temperature on day *i* over a 0 "base" temperature, *n* indicates the total number of days evaluated.

$$DD = \sum_{i=1}^{n} \left(\frac{T_{max,i}\, T_{min,i}}{2} - T_{base} \right) \quad \text{(Equation 3.1)}$$

Changes of the mulch film's color on surface facing the sun before and after weathering were measured through colorimetry using a Hunterlab color difference meter (Model No. 45/0-L MiniScan XE Plus, Reston, VA, USA). For the WOB film, the white surface (mulch surface facing up) was measured. Three color scales were determined: *L,* referring to lightness (0 = black, 100 = white), *a* to redness (< 0 = green, > 0 = red), and *b* to blueness (< 0 = blue, > 0 = yellow). Mulch film color was measured from each of four field plots twice with six replications. The total composite color change (ΔE value) was calculated (Eq. 3.2).

$$\Delta E = (\Delta L\hat{}2 \;\; + \Delta\, a\hat{}2 + \Delta\, b\hat{}2\,)^{0.5} \quad \text{(Equation 3.2)}$$

Mechanical properties were assessed through peak Load and elongation. Peak load (N) and percent elongation at maximum tensile stress in the machine direction (MD) for each mulch were determined before and after weathering using a Model 5567 Instron instrument (Norwood, MA, USA) employing ASTM D-5035 (ASTM, 2011). Six replicate samples for each mulch films (15.24

CD x 17.78 cm MD) were used to determine mechanical properties. A load cell of 10 kN and gage length of 2.54 cm was used instead of the recommended dimension (7.72 cm) by the standardized method, due to sample size limitations as explained in our previous paper (Hayes et al., 2017).

Proportions of PLA and PHA among the polymers in PLA/PHA film was assessed through ^{1}H-Nuclear Magnetic Resonance (NMR) analysis. BDM samples were analyzed using a Varian 400 MHz NMR spectrometer (Agilent, Santa Clara, CA USA), with pulse width of 90°. Mulch (~20 mg) was dissolved in deuterated chloroform (~800 µL) containing 1% tetramethylsilane as an internal standard. Polymeric composition of PLA/PHA was determined through comparing relative amounts of PLA and PHA and of the 3- and 4-hydroxybutyrate units for the latter. Spectral assignments employed were taken from a previous study (Dharmalingam et al., 2016).Adipic acid, terephthalic acid and 1,4-butanediol units were determined from PBAT-based BDMs using spectral peak assignments from a study (Herrera et al., 2002). ^{1}H NMR spectra from 8.1 (aromatic) to 2.33 ppm (-OCOCH$_2$) were used to determine terephthalate and adipate mole fractions in the analysis of composition and sequence distribution.

Gel permeation chromatography (GPC) was used to determine weight-average molecular weight (M_w) and polydispersity index (PDI) of polymeric constituents of PLA/PHA and BioAgri samples. Subsamples of ~20 mg (four) were dissolved in 5 mL of chloroform. The mixture was stirred, centrifuged and filtered to remove chloroform-insoluble particles (Hayes et al., 2017). Filtered solution (200 µL) was injected into 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 x 7.5 mm ID PL Gel mixed D column purchased from Agilent (Santa Clara, CA, USA).

Chloroform was used in the mobile phase at a flow rate of 0.8 mL/min and a run time of 15 min. Molecular weight values reported are based on polystyrene molecular weight equivalents (EasiVial PS-H, Agilent). The ELSD detector signal was corrected with response factors to account for the power law relationship between detector signal and concentration. Under the conditions employed, the GPC data reflects the contribution of PLA alone since the detector signal from PHA was relatively weak, and for BioAgri, PBAT is reflected due to low solubility of starch in chloroform.

Analysis of gel content (wt. % of polymeric constituents that form a gel-like cross-linked network) was carried out through Soxhlet extraction (Testing & Materials–ASTM, 2001). Mulch samples (~ 0.5 g) were placed in a cellulose thimble (50 mL capacity) and the latter was placed in a flask containing 200 mL of tetrahydrofuran (THF) and a magnetic stir. Extraction cycles of ~ 30-45 min were implemented continuously for three days. The percent extract of the polymeric constituents was determined according to Equation 3.3 (Beyler & Hirschler, 2002; Kijchavengkul, Auras, & Rubino, 2008).

$$\%\,Extract = \frac{W_s - W_d}{(f)\,W_s} \times 100 \quad \text{(Equation 3.3)}$$

where W_s is the initial weight of the specimen, W_d the weight of the unextracted solids, and f the mass fraction of polymeric constituents. The value of f was determined via thermogravimetric analysis (TGA) by equating the mass lost upon heating up to 600°C to polymers and to mass remaining at 600°C as consisting of fillers, colorant and other non-polymeric constituents. For BioAgri, f also excludes the starch component of Mater-Bi® since it is insoluble in THF (confirmed via TGA analysis of THF-soluble and insoluble fractions). For Organix, f excludes a heating stage that occurs at ~550°C; TGA analysis indicated that this unknown component was THF-insoluble.

For PLA/PHA, TGA analysis indicated the presence of residual solids (i.e., mass remaining at 600°C) in both THF-soluble and insoluble fractions, likely a result of covalent bonding between the polymers and the fillers. Therefore, f was determined via mass balances using polymer content of both THF-soluble and insoluble fractions via TGA. Gel content was calculated using Equation 3.4.

$$\%\, Gel\ content = 100 - extract \quad \text{(Equation 3.4)}$$

Infrared absorption spectra of surfaces exposed to sunlight were recorded using an IRAffinity-1 spectrometer (Shimadzu) equipped with a single reflection ATR system (MIRacle ATR, PIKE Technologies, Madison, WI, USA). Four replicates each of initial mulches and three samples from each mulch plots (surface exposed to the sun) were scanned between 4000 to 600 cm^{-1} using a spectral resolution of 4 cm^{-1} and 16 scans per spectrum. Spectral data were normalized by equating the integrated peak area of the entire spectrum to 1.0 (mean normalization).

Thermogravimetric Analysis (TGA) was carried out all plastic mulches initially and after agricultural weathering. Mulch samples (~ 2 mg) were analyzed using a Discovery TGA (TA Instruments, New Castle, DE, USA) at a heating rate of 10 °C/min from room temperature (25 °C) to 600 °C in an unsealed platinum sample pan under a nitrogen atmosphere.

3.4.3 Statistical Analysis

All data were subjected to analysis of variance using generalized linear mixed model (GLIMMIX) procedure in SAS (Statistical Analysis System Version 9.2 for Windows; SAS Institute, Cary, NC, USA) using a macro program (MMAOV; Saxton, 2010) to evaluate the effect of weathering on mulch films' physicochemical properties. Data were analyzed as a completely randomized block design with replication and factorial arrangement of treatments (location, mulch). Tukey's honestly significant difference (HSD) was used to test and compare treatment means at α = 0.05 for significant differences. However, because crops used in 2017 and 2018 differed from those used in 2015-2016, only mulch effect on physicochemical properties were assessed in 2017 and 2018 with crops-nested within the years in respective location.

3.5. RESULTS AND DISCUSSION

3.5.1 Environmental data

Table 3.2 lists the date for milestones during the field trials and environmental conditions at both locations (TN and WA). Environmental conditions differed between two field sites across all four years, consistent with what we observed in 2015 (Hayes et al., 2017). Rainfall, relative humidity (RH), air temperature (hence, DD) and soil temperature were observed to be higher in TN in all cropping years. Except for 2015, solar radiation was relatively higher in WA than TN and difference between sites was quite large. Moreover, total solar radiation in WA, from mulch laying until plant canopy formation (2016-2018), was 2-3 fold higher than the solar radiation throughout the entire cropping season in 2015. DD was higher in TN than WA in all cropping years except in 2016.

Extreme weather events occurred during the four-year study that may have contributed to temporal variability of physicochemical property changes across sampling years. In 2016, TN had an extensive heat and drought period (e.g., lower rainfall), while in 2017, WA experienced a drought and TN had a two-day period of heavy precipitation that accounted for ~30% of the total rainfall during the trials. In 2017, yellow nutsedge (*Cyperus esculentus* L.) became a major problem in TN while using bell peppers as a test crop. The weed penetrated through the BDMs and PE mulch, increasing the number of rips and tears (Moore and Wszelaki, 2019). As a result, glyphosate (Roundup®, Bayer, Leverkusen, Germany) was sprayed thoroughly before mulch laying in 2018 to reduce weeds. During a heavy rain event (e.g., 2017 in TN), pesticides could possibly have leached out of the soil and have become adsorbed to the mulches. Wind speed was more consistent across all years in WA and higher than in TN, except for 2015. In the environment, the diurnal hot-cold cycles, action of animals and friction from rainwater and wind movement can break up the surface of mulch films which can have drastic impact on the mechanical integrity of the films (Andrady, 2015).

3.5.2 Change of color

Color change, denoted by ΔE (Eq. 1), reflects mainly an increase of *L* (i.e., decrease of blackness) and to a lesser extent a decrease of *b* (i.e., transition from blue to yellow). ΔE values are significantly different between mulch type ($p<0.0001$) and location ($p=0.0323$), but not years (Fig. 3.1).

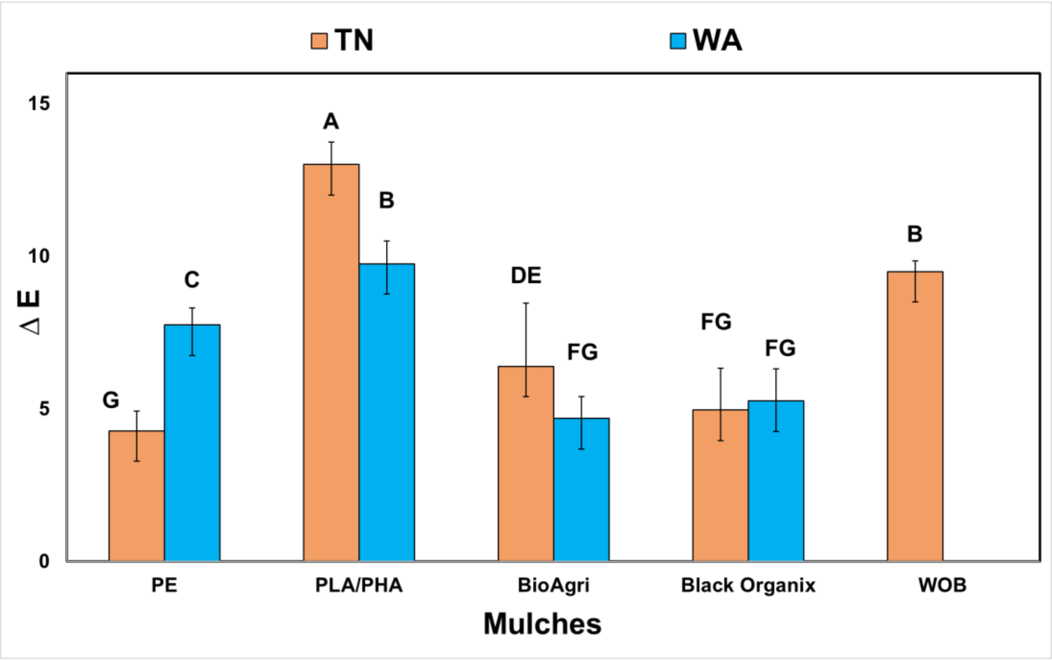

Figure 3.1. Effect of weathering on the change of mulch color (ΔE) before and after weathering in TN and WA. Error bars reflect standard error and means that do not share common capital letters reflect statistically significant difference. Data is pooled mean value of ΔE of 2015 to 2018. Note: The white surface of the White-on-Black Organix mulch was laid in the field facing up and exposed to sunlight.

Change of mulch film color is attributable to photodegradation (e.g. photobleaching of carbon black colorant via exposure to UV radiation) and soil particles that strongly adhere to the weathered film and cannot be removed by the cleaning procedure (Rudnik et al., 2008, Shogren & Hochmuth, 2004). Photooxidation leads to breaking of the polymer chains, producing free radicals that induce depolymerization, which further results to deterioration of mechanical properties (Yousif & Haddad, 2013). Light-induced photobleaching can increase yellowness and whiteness, resulting to uneven discoloration and chalking of the film surface (Lendlein & Sisson, 2011), which explains the large error bars of Fig. 1. As mulch weakens, colorant can be leached away by rainwater due to contributing factors such as wind, soil-mulch contact and other minor components in the air/ water (e.g. agrochemicals) (Hayes et al., 2017). ΔE is generally higher in WA than TN (Table 3.2). An exception to this rule is PE, which underwent a greater color change in WA, perhaps due to higher soil adhesion. Among the mulches, however, ΔE was highest for PLA/PHA. ΔE increased slightly with increasing year for PLA/PHA which may be due to successive ageing during long-term storage. Moreover, PLA/PHA was the only one of the mulches that used the same rolls (prepared in 2015) across four year. The PBAT-based black films, BioAgri and Organix, underwent similar extents of color change, except for BioAgri in TN, which experienced a higher ΔE. Perhaps the difference is attributable to the differences in manufacturing process between the mulches or to the minor components therein (e.g., starch and PLA in Mater-Bi® and ecovio®, respectively). Between two Organix mulches used in TN, ΔE was observed to be higher for WOB compared to (black) Organix.

3.5.3 Change of mechanical properties

Change of mechanical properties were assessed through peak load and elongation. With the exception of PLA/PHA, the peak load of three commercially available PBAT-based BDMs and PE in both locations significantly decreased as a result of weathering ($p<0.0001$) (Fig. 3.2). The increase of peak load for PLA/PHA upon weathering, at a higher extent in WA, can be attributed to formation of cross-links between the polymers and the $CaCO_3$ nanofiller on exposure to solar radiation, as explained previously (Hayes et al., 2017).

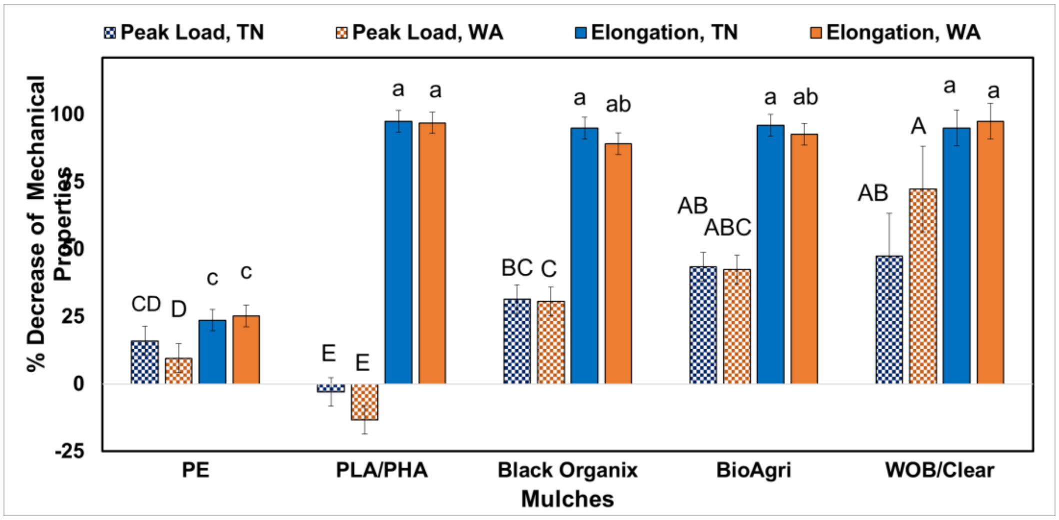

Figure 3.2. Effect of weathering on % decrease of peak load and elongation at maximum tensile stress in the machine direction. Means are pooled mean value of mulches throughout the cropping season (2015-2018) retrieved from two field sites (TN, WA). Error bars reflect standard error and means that do not share common capital and lower-case letters (for peak load and elongation, respectively) reflect statistically significant difference.

Consistent with this explanation was the observation that inorganics were present in the THF-soluble fraction during gel content analysis, demonstrating the strong association between the inorganics (mainly $CaCO_3$) and the polyesters. Although the change of peak load was not statistically different between two locations (p=0.09), changes were slightly more pronounced in TN than WA and the trend was consistent throughout the cropping years. The other BDMs share the same major polymeric component, PBAT, and possess similar peak load and elongation values initially (Table 3.1). However, among the black films, Organix underwent a greater decrease of peak load than BioAgri. This trend is partially attributable to the higher adipate and lower terephthalate for Organix compared to BioAgri, measured by NMR (discussed below). Among the ecovio®-based BDMs, the clear film underwent the greatest loss of peak load, followed by WOB/Clear and then black (Figure 3.2), a trend also observed for other PBAT mulches that differed in color (Kijchavengkul et al., 2008). In other studies, mulch films consisting of PBAT-based blends (e.g., with starch or PLA) performed as well as conventional PE mulch in terms of agronomic performance but underwent a reduction of maximum tensile strength and elongation on strawberry and grapevine farms (Bilck et al., 2010; Touchaleaume et al., 2016).

Mulches became brittle upon weathering, as confirmed by the strong decrease of elongation, which was significant for all mulches (p<0.0001), particularly for BDMs (> 90% decrease; Fig 3.2). PE at both locations had a slight decrease on its elongation (25-35%; Fig 3.2). Other studies found PBAT-based mulch films underwent a significant decrease of mechanical properties, shown through cracks, rips, tears and holes (Kijchavengkul et al., 2010; Muthuraj et al., 2015). The embrittled condition of mulches eventually led to disintegration in the soil within two to four weeks and enhanced in an environment with high RH conditions (Kijchavengkul et al., 2008). Elongation loss was greater for TN that for WA, a trend that can be attributed to higher air and soil temperature and RH in TN; but, the difference was not statistically significant (p=0.83). However, studies have also identified UV radiation exposure to be the most influential factor for the loss of BDMs' mechanical properties (e.g. reduction of elongation at break) rather than higher temperature and water content (Kijchavengkul et al., 2008, Beyler & Hirschler, 2002). Beside crosslinking due to UV photo-degradation (Norrish Type I reaction) and chain scission due to hydrolysis and Norrish type II photodegradation also promotes embrittlement and reduced tensile strength of films. No significant trend across years was observed.

NMR was performed to determine the change of PLA and PHA content relative to each other for the PLA/PHA mulch film. The PLA content underwent a slight but significant increase (i.e., PHA underwent a decrease) in both locations (p=0.0001) and across all years (p=0.0002) due to weathering (Fig. 3.3).

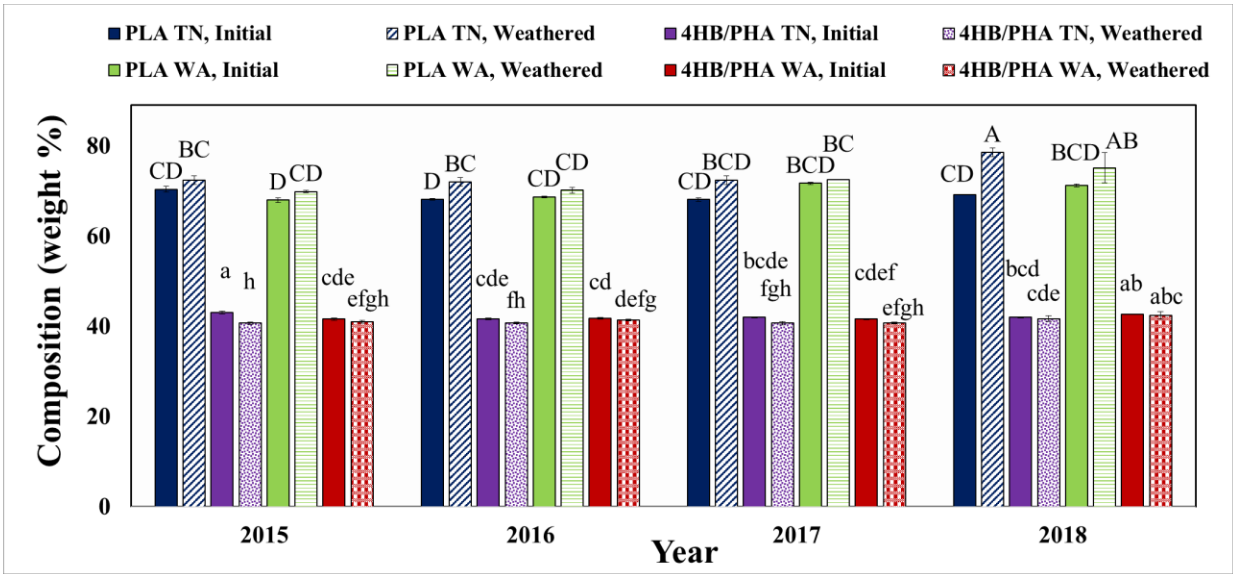

Figure 3.3. Effect of weathering on polylactic acid (PLA) mass fraction among the polymers and 4-hydroxybutyrate (4HB) monomer units among polyhydroxyalkanoate (PHA) of PLA/PHA film before and after weathering in TN and WA from sampling year 2015 to 2018 (NMR analysis). Error bars reflect standard error and means that do not share common capital and lower-case letters (employed for % PLA and % P4HB among PHA, respectively) reflect statistically significant difference.

3.5.4 Change of polymeric composition of PLA/PHA

The susceptibility of PLA to thermal degradation becomes higher when temperature gets closer to its glass transition temperature (T_g) of 60-65°C, where the structure of PLA transitions from a highly crystalline structure to an amorphous state (Beyler & Hirschler, 2002). Amorphous regions are preferentially degraded upon hydrolytic attack than the corresponding semi-crystalline regions (Andrady, 2015; Zhang & Thomas, 2011; Scott, 2002). Such a high temperature is uncommon for ambient soil or air temperature in TN or WA. However, the film's actual temperature is often higher than either the soil or air temperature due to radiative heat transfer (El-Shaikh & Fouda, 2008). Weathering of mulches can also proceed by oxidative processes that are often accelerated by temperature and oxidation agents (e.g. O_2) (Andrady, 2015; Beyler & Hirschler, 2002). The higher air and soil temperatures in TN (by ~4^{o}C and ~6-7^{o}C, respectively; Table 3.2) likely contributed the greater loss for TN compared to WA. The greater extent of amorphous structure at higher temperature will allow the lower molecular weight PHA molecules to be selectively leached away and the more labile ester bonds of PHA to be selectively cleaved. The greatest change occurred in 2018 at both sites, which can be attributed to deterioration occuring during the three years of storage for the film. The percentage of poly-4-hydroxybutyrate (P4HB), a major fraction among PHAs (with the other major fraction being poly-3-hydroxybutyrate, P3HB), decreased slightly due to weathering for all years and both locations, especially in TN, suggesting the 4HB ester bonds are more labile than those of 3HB.

A higher adipate (and lower terephthalate) content within PBAT would be expected to lead to a greater loss of mechanical properties due to hydrolysis occurring on susceptible adipate ester groups to a greater extent than on terephthalate ester groups (Kijchavengkul et al., 2010). To determine if such a compositional difference may have caused the greater loss of peak load for BioAgri (Mater-Bi®) compared to Organix (ecovio®), NMR analysis was employed. The analysis showed that BioAgri possessed a slightly but significantly higher adipate content (27%) than Organix (26%) (*p*=0.0007), thereby supporting the proposed hypothesis.

3.5.5 Change of molecular-weight related properties

Weight-averaged molecular weight (M_w) was determined via GPC for the PLA component of PLA/PHA and the PBAT component of BioAgri before and after weathering (Figure 3.4.A).

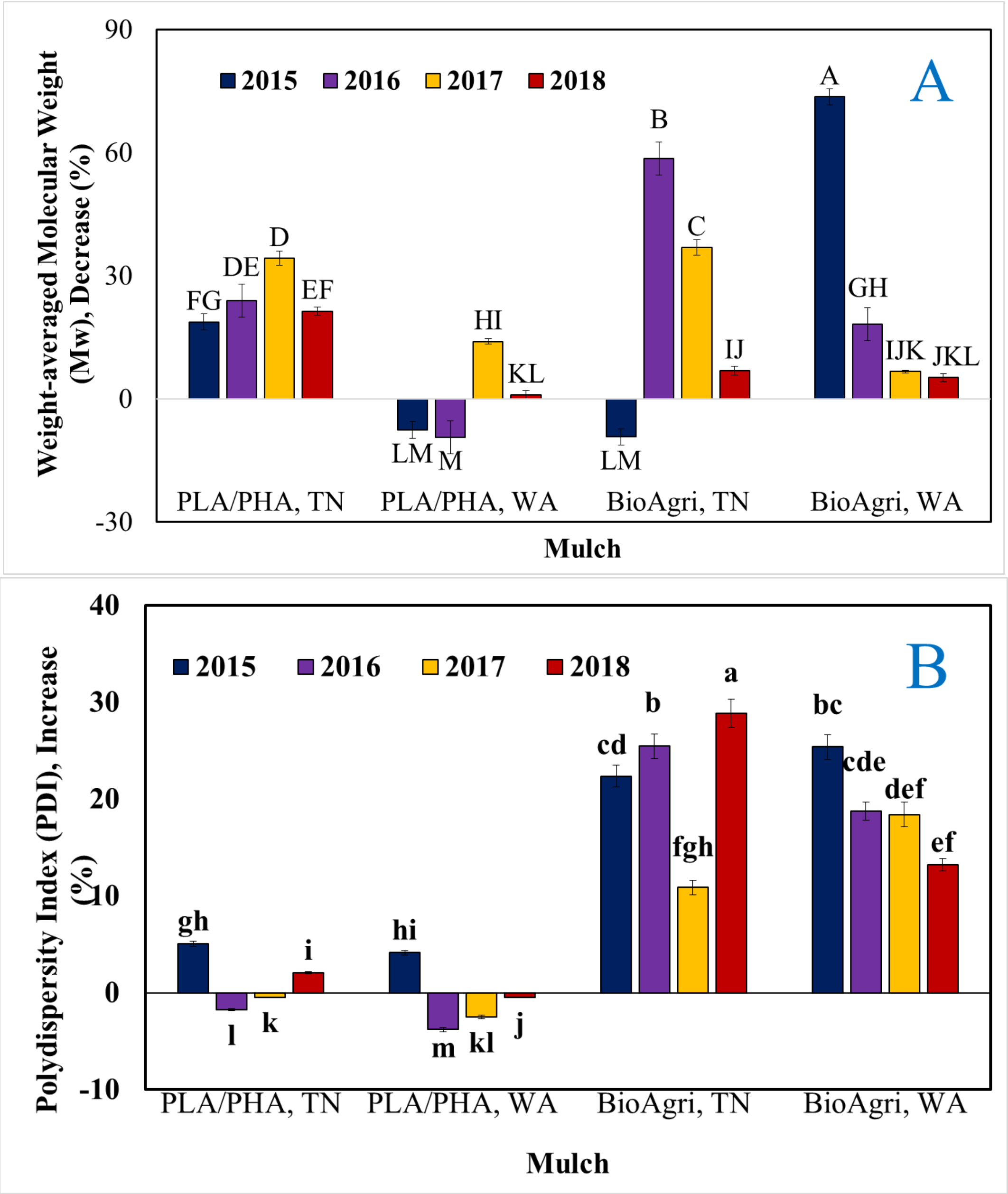

Figure 3.4. Effect of weathering on change of (A) weight-averaged molecular weight (M_w) and (B) polydispersity index (PDI) of the PLA component PLA/PHA and of PBAT component of BioAgri after weathering in TN and WA (2015 to 2018; GPC analysis). Error bars reflect standard error and means that do not share common letters reflect statistically significant difference.

The decrease of M_w for PLA/PHA's is significantly higher for TN compared to WA, likely a result of the former's higher soil and air temperature, resulting in enhanced photodegradation and subsequent thermo-oxidation, therefore to reduction of molecular weight (Brodhagen et al., 2015; Kijchavengkul et al., 2008). The decrease of M_w for PLA/PHA increased slightly with years between 2015 and 2017 in TN, which may reflect the aging of the mulch roll prepared in 2015 during storage. But in 2018, the M_w decrease due to weathering was slightly lower than for 2017, perhaps due to the use of a different mulch roll PLA/PHA in 2018 compared to the other years. M_w increased for PLA/PHA during weathering in WA in 2015-2016, which is likely attributable to the selective removal of lower molecular polymers via leaching (Hayes et al., 2017; Dharmalingam et al., 2016). The largest decrease of M_w for PLA/PHA in WA occurred in 2017, a year where WA experienced drought period and received higher solar radiation than TN up to plant canopy formation (Table 3.2). The increase of peak load, as well as increase of M_w of the mulch after weathering, can be attributed to loss of lower-M_w polymer molecules via leaching (Hayes et al., 2017, Li et al., 2014, Copinet et al., 2004). For BioAgri, the change of M_w varied between -5% and 60% (Fig. 4A). There are no consistent trends between years or location, nor does the change correlate with solar radiation moisture, or any environmental data. PDI values (Figure 3.4.B), whose increase often serves as an indication of depolymerization, significantly increased due to weathering for both PLA/PHA and BioAgri across four years, to a greater extent observed on BioAgri than PLA/PHA ($p<0.0001$) (Fig. 3.4 B). There are no trends of PDI changes in both locations across years.

Norrish Type I photooxidation rearranges the polymeric chains, leading to crosslinking and formation of insoluble gel (Hayes et al., 2017; Stloukal et al., 2012). To confirm the occurrence of cross-linking, the effect of weathering on the gel content of the mulches' polyester constituents was performed for the 2017 field season (Figure 3.5).

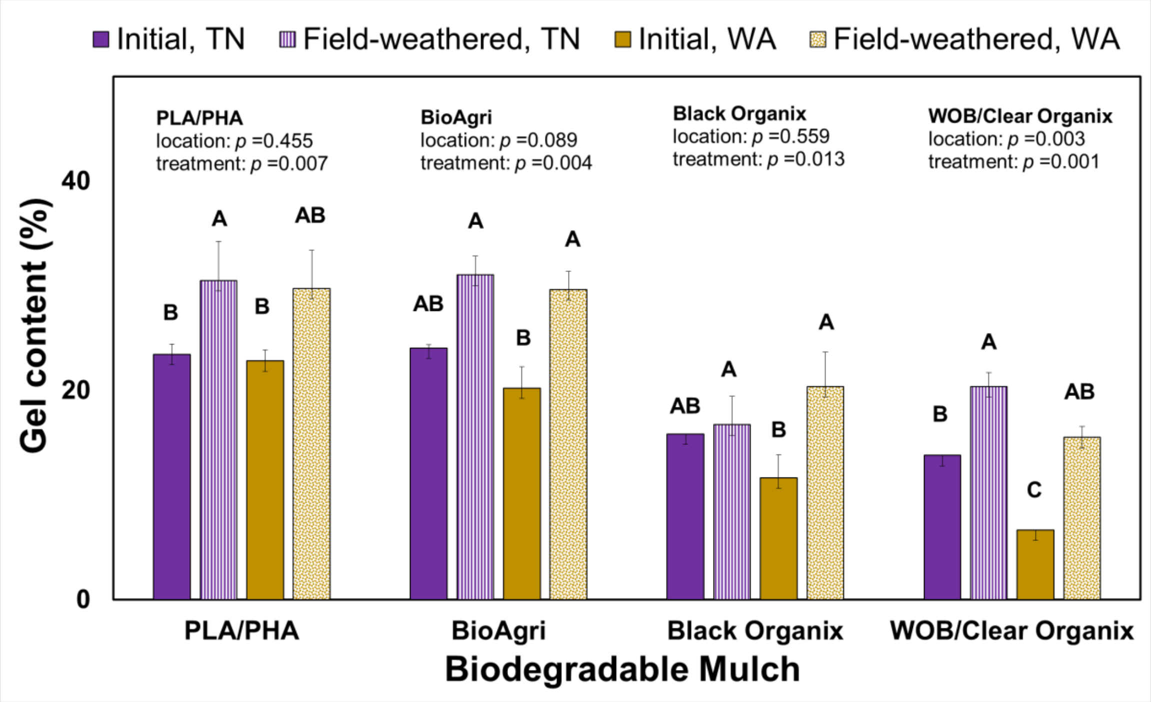

Figure 3.5. Effect of weathering on gel content of polymeric components of PLA/PHA, BioAgri and Organix after weathering in TN and WA in 2017 (Eqs. 3-4). Values for Organix reflect solely PBAT. Error bars reflect standard error and means that do not share common letters for each mulch reflect statistically significant difference. Note: Statistical analysis were performed for each mulch separately, with location and treatment as factors.

Gel content of PLA/PHA significantly increased from 23% to 30% in TN and 22% to 29% in WA after weathering (p=0.007). In the same manner, for BioAgri, gel content significantly increased from 24% to 31% in TN and 20% to 29% in WA (p=0.004) (Fig. 3.5), respectively. There were no location effects on gel content of PLA/PHA and BioAgri. Among the three ecovio®-based BDMs, a significantly higher increase of gel content occurred in Clear Organix mulch. The gel content increase for black Organix in TN was significantly lower in TN (15% to 16%) than for WA (11% to 20%) (p=0.013). Gel content % of WOB increased significantly from 13% to 20% in TN, an extent slightly less than for Clear Organix in WA, from 6% to 15% (p=0.001). Studies showed that additives such as UV stabilizers (e.g., carbon black colorant that was used herein) and nanofillers can inhibit photooxidation and reduce cross-linking (Hayes et al., 2017; Beyler & Hirschler, 2002). However, the results demonstrate that the additives do not completely inhibit cross-link formation. Increased gel content can correlate to higher content of aromatic constituent of the material (Bregg, 2006). However, the higher terephthalate content of PBAT for Organix (24%) compared to BioAgri (23%) (via NMR analysis, as described above) is inconsistent with the higher gel content increase for BioAgri.

3.5.6 Change of surface chemistry of mulch films

Functional groups that are associated with polymeric components of BDMs can be studied to determine qualitative changes on the surface chemistry of mulch films before and after weathering, providing information on the type of surface reactions that may occur, such as the relative extent of hydrolysis and photodegradative reactions. FTIR spectra for weathered mulches (2016-2018) were not significantly different than those already published for 2015 (data not shown) (Hayes et al., 2017). Figure 3.6 compares the effect of color and location on the FTIR spectra for the ecovio®-based Organix films during the 2017 cropping season. Spectral band assignments are given in Table 3.3.

Table 3.3. Peak assignments for FTIR-ATR analysis of Organix (Figure 6). Values obtained from Hayes et al., 2017.

Wavenumber (cm^{-1})	Contribution
3000-3700	OH stretch
2956, 2920, 2876, 2846	C-H stretch
1712	C=O stretch (polyester)
1456, 1410, 1390	-CH_2- bend
1268, 1252, 1166, 1118, 1102, 1082	C-O stretch (polyester)
1076-1000	OH bend
874	C-H stretch

There were differences between the spectra of the weathered Organix mulches (1800- 800 cm^{-1}; 3600- 600 cm^{-1}; Fig 3.6).

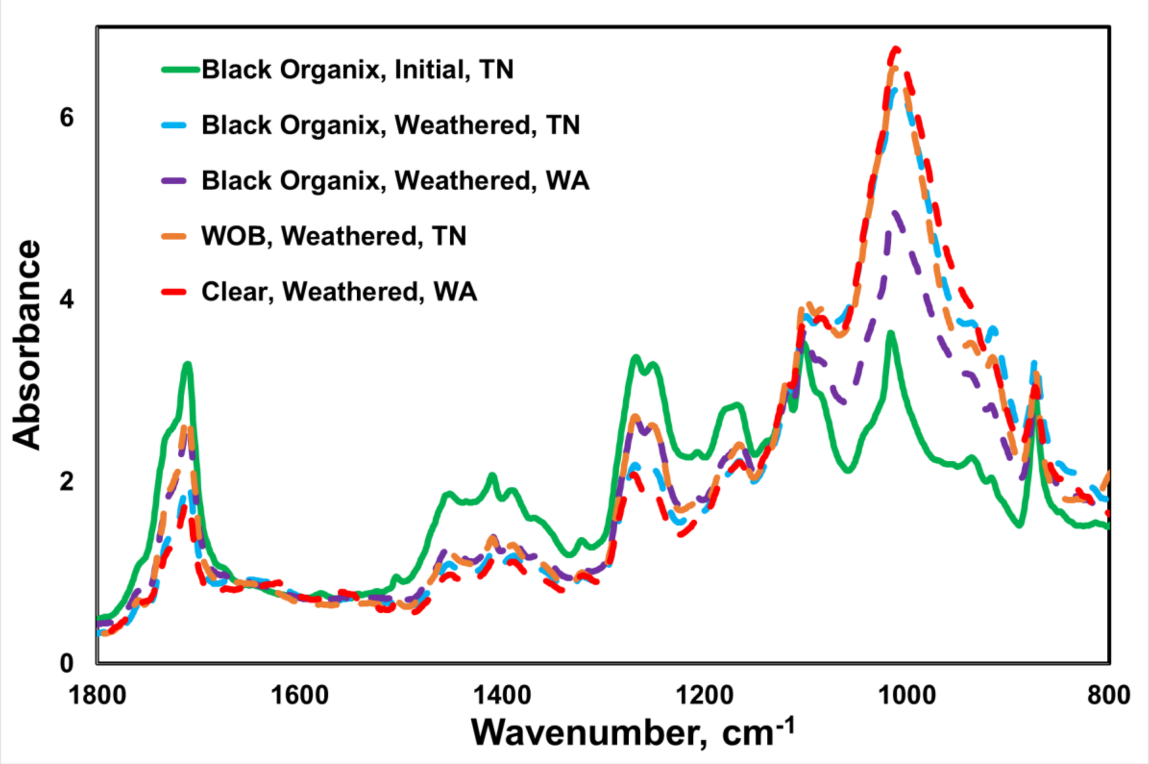

Figure 3.6. Changes in chemical bonding of Black, WOB, and Clear Organix mulches before and after weathering in 2017 (FTIR-ATR analysis). Similar trend was observed in 2018. Note: Initial Black Organix (TN, WA), WOB (TN) and Clear (WA) presents share similar FTIR spectra.

Both Black and WOB Organix in TN underwent more substantial spectral changes due to weathering than black Organix in WA. However, Clear Organix in WA underwent a significant change of spectra, comparable to the changes observed for weathering in TN. Peak intensities of COO stretching (1750–1650 cm^{-1}) and C-H$_2$ bend (1450-1300 cm^{-1}) decreased, whereas C-O stretching (1127– 800 cm^{-1}), that indicates hydrolysis occurring on mulch surfaces increased (Hayes et al., 2017; Kijchavengkul et al., 2010). As observed in our previous study (Hayes et al., 2017), at 1636 cm^{-1}, a broad minor band formed for all Organix films, to higher extent for black Organix, which can be attributed to C=C stretching due to Norrish-Type II chain scission. Photodegradation also increased the peak of an out-of-plane C=C bending (1002 cm^{-1}) for PBAT, to greater extent for Clear and WOB Organix. As confirmed by other studies, higher ambient air temperature, RH and higher exposure of solar radiation in TN contributed to more pronounced spectral changes of BDMs, through photodegradation and hydrolysis of ester linkages (Hayes et al., 2017; Copinet et al., 2004).

3.5.7 Change of thermal stability

TGA measures the mass loss of the plastic material as the temperature is slowly and steadily increased (10°C/min for this study). Figure 3.7 presents the TGA and differential thermograms (DTGs) of BioAgri, PLA/PHA and Organix before and after weathering in 2016.

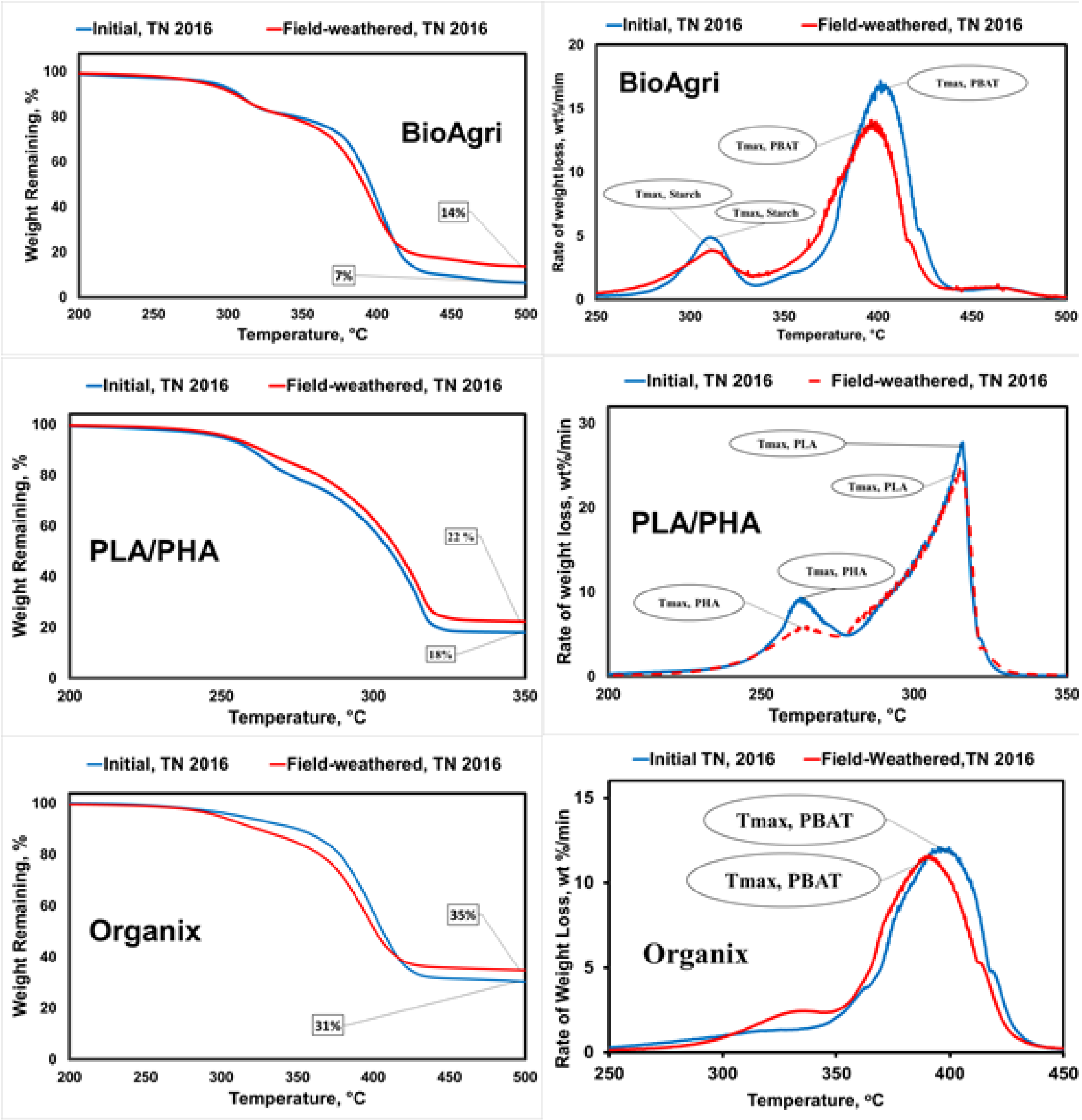

Figure 3.7. Representative thermograms (A, C and E) and differential thermograms (DTG; B, C and F) from thermogravimetric analysis (TGA) of (A,B) PLA/PHA, (C,D) BioAgri and (E,F) black Organix before and after weathering in 2016 at Knoxville, TN.

Weight (%) remaining at 550°C of all BDMs increased (Figs. 3.7A,C, and E), where residual materials are likely to be inorganic constituents, soil particles, and gels generated via cross-linkage between polymer molecules (Hayes et al., 2017).

Weathering lowered temperatures of main heating stages of PHA and PLA (PLA/PHA), and starch (BioAgri) and PBAT (BioAgri and Organix), easily observable by the DTGs (Figs. 3.7 B,D, and F). Figures 3.8.A,.B display the maximum and temperatures onset (T_{max} and T_{onset}, respectively) of PLA for PLA/PHA and PBAT for BioAgri and Oxganix mulches, respectively, averaged across all four years. Weathering generally led to a decrease of T_{max} for all mulches, consistent with the observed decrease of M_w (Fig. 3.5.A); but, the extent of the decrease was not statistically significant. The decrease of T_{max} was greater for TN (statistically significant for BioAgri and black Organix), likely due to the higher temperatures and rainfall (Table 3.2). The only exception to the decrease of T_{max} was for PLA/PHA in WA, as would be expected due to the high resistance of PLA to degradation for temperatures far below T_g. There was little or no difference in the decrease of T_g between the black, WOB and clear Organix mulches for a given location.

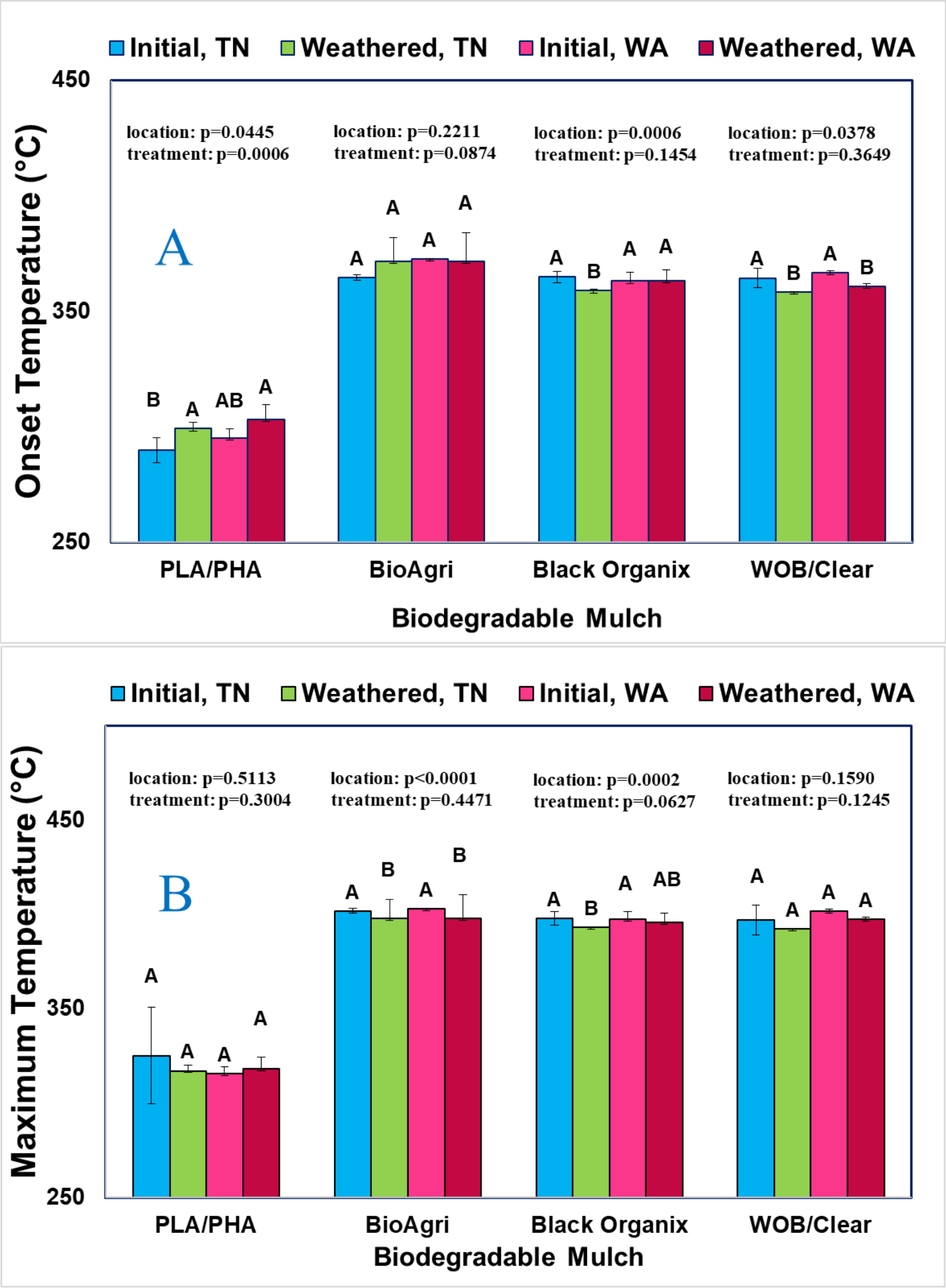

Figure 3.8. Effect of weathering on onset (T_{onset}) and maximum (T_{max}) temperatures of PLA (PLA/PHA) and PBAT (BioAgri, Black and WOB/Clear Organix) after weathering in TN and WA (2015-2018). Error bars reflect standard error and means that do not share common letters reflect statistically significant difference for each mulch. Note: Statistical analysis were performed for each mulch separately, with location and treatment as factors.

The change of T_{onset}, representing the lowest molecular weight polymers from amorphous morphological regions, differed in trend between mulches. While ecovio®-based BDMs underwent a decrease of T_{onset} (except for black Organix in WA, which experienced a minimal change of T_{onset}), a similar trend to what was observed for T_{max}, T_{onset} increased for PLA/PHA at both locations and for BioAgri in TN (Fig. 3.8B). Although the increase of T_{onset} was only significant for PLA/PHA in TN, the trend was consistent for the two BDMs across all years (data not shown). This trend is likely attributable to selective depolymerization or leaching away of lower MW and lower crystalline regions of the polymer. PLA's rate of hydrolysis for thermal degradation of its ester linkages increased due to weathering, which can be attributed to warmer temperature and higher RH or can be also contributed by PHA's low resistance to thermal degradation (Copinet et al., 2004; Rudnik, 2008; Bregg, 2006).

3.6. CONCLUSIONS

The main objective of this study was to investigate the degradation of BDMs determined through assessment of physicochemical properties before and after weathering. To do so, three PBAT-based BDMs and an experimental PLA/PHA BDM were tested to assess degradation during specialty crop production (e.g. pie pumpkin, sweet corn, bell peppers).

Our study shows that mulch degradation greatly depends on the mulch type and oftentimes influenced by climatic conditions, particularly higher or lower solar radiation, temperature, or rainfall. Major changes on physical properties and mechanical integrity of BDMs occurred to a greater extent in TN, a region with climatic conditions which may favor faster degradation than WA (e.g. higher rainfall and soil and air temperatures).

Changes of the physicochemical properties vary minimally between years despite of climate variability between years, such as higher or lower solar radiation, temperature or rainfall experienced in one of the sampling locations. In some instances, physicochemical properties changes were greater in TN than in WA, presumably due to the higher temperature (air and soil) in TN since the solar radiation in WA was higher. Major physicochemical properties were observed more in TN than WA. There were higher elongation (%) decrease, greater FTIR spectral changes and greater decrease of T_{max} (TGA) for TN mulches compared to WA. Higher solar radiation in WA resulted in greater gel content increase in WA compared to TN. Changes of properties were greater for an experimental PLA/PHA film, than for commercially available BDMs, particularly in TN: ΔE, increased peak load, increased PLA% (decreased PHA%,) and higher extent of M_w decrease. The changes are likely due to PLA/PHA's greater sensitivity toward temperature, especially when the temperature approaches T_g.

For PBAT-based BDMs, which had similar initial physical properties (thickness, peak load, elongation, “L” colorimetric parameter) and thermal stability (T_{max}) but differed in the chemical properties of their polymeric feedstocks (e.g. higher adipate/terephthalate ratio for BioAgri than for Organix), physicochemical changes differed. ΔE was higher for BioAgri than Organix and differences can be attributable to differences between minor components and film processing conditions between the two. Decrease of peak load and increase of gel content was greater for BioAgri compared to Organix. While T_{onset} increased for BioAgri, suggesting leaching out of lower molecular weight molecules out of the film or they are more readily degraded, T_{onset} Organix decreased, indicating depolymerization of lower-molecular weight PBAT molecules. Comparing 3 PBAT-based BDMs that differed only in color (black Organix, WOB and Clear), Clear Organix was more susceptible to degradation, followed by WOB and by black (e.g., changes in FTIR spectra, peak load decrease and higher gel content). As carbon black (used in higher % in black Organix) can act as a photostabilizer, these may explain more FTIR spectral changes, greater peak load decrease and higher gel content in Clear and WOB than black Organix mulch though greater changes on T_{onset} and T_{max} occurred for Black Organix than Clear Organix in WA.

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4. EFFECT OF ENVIRONMENTAL WEATHERING ON BIODEGRADATION OF BIODEGRADABLE MULCHES UNDER AMBIENT SOIL AND COMPOSTING CONDITIONS

A version of this chapter was submitted to the Journal of Science of the Total Environment and is titled as follows:

Marife Anunciado, Douglas G. Hayes, Larry C. Wadsworth, Christina Danielle Cowan Banker, Jose Liquet, Jennifer DeBruyn. Effect of Environmental Weathering on Biodegradation of Biodegradable Mulches Under Ambient Soil and Composting Conditions

In the study, I and Larry Wadsworth were responsible for analyzing physicochemical data. Christina Danielle Cowan Banker conducted the biodegradation experiment in the soil for Naturecycle and Organix mulches while I performed the BioAgri and PLA/PHA soil biodegradation experiments. I performed all composting experiments and interpreted the results and prepared the manuscript. Jose Liquet performed the microbial assessment and interpret the results. Jennifer DeBruyn provided technical inputs and Douglas Hayes conceived, directed the research and edited the manuscript, and serves as the senior author.

4.1 ABSTRACT

The numerous benefits provided by plastic mulches such as the ability to reduce weeds, limit evaporative loss of water from the soil, control soil temperature that is conducive for plant growth and increase of crop productivity led to a wide utilization of plastic mulches around the world. Disposal of conventional polyethylene (PE) mulch films is an ongoing problem due to lack of biodegradability and laborious retrieval. Concerns for PE films' sustainability has led to development of biodegradable mulches (BDMs) films that can be tilled into the soil or retrieved back and sent to a composting facility after crop harvest. Yet, the impact of environmental weathering on BDMs' biodegradation after their useful life-stage is not known. Environmental weathering during field trials in TN field site resulted to depolymerization and mechanical weakening of mulches (Section 3.6). Yet some mulches showed to underwent cross-linking due to photodegradation (Section 3.5). Aforementioned processes may enhance or hinder biodegradation.

This study evaluated the effects of environmental weathering on biodegradation of BDMs. Biodegradation of agriculturally weathered and unweathered BDMs were tested in soil and compost using standardized laboratory tests (ASTM D5988 and D5338). An experimental polylactic acid and polyhydroxyalkanote film (PLA/PHA), and three polybutyrate (PBAT)-enriched BDMs were used. For biodegradation in soil (ASTM D5988), CO_2 evolution was measured over 12 months and BDM fragments recovered from soil were evaluated for chemical properties at several different durations.

Biodegradation of PLA/PHA in the soil was greater for weathered than unweathered PLA/PHA mulches. However, for PBAT-based mulches, extent of biodegradation due to weathering was

different. The M_w of all BDMs decrease and all polymeric components of four BDMs (PLA, PHA, PBAT, starch) had a decrease of thermostability upon biodegradation in the soil. The relative proportion of PHA (%) among the polymeric components decreased for the experimental BDM during biodegradation, indicating preferential utilization of PHA over PLA by microbes. Under composting conditions an unweathered PBAT-enriched mulch film resulted in more CO_2 evolution than its weathered counterpart. Bacterial and fungal abundance determined using quantitative polymerase chain reaction (PCR) of ribosomal ribonucleic acid (rRNA) genes revealed significant differences of abundances between weathered and unweathered BDMs and among biodegradable mulch treatments.

4.2. INTRODUCTION

The expanding use of plastic mulch for agricultural production of specialty crops has enabled farmers to increase crop production and profitability; however, a major drawback of their use is the poor environmental sustainability associated with their disposal, particularly for conventional polyethylene (PE) mulch films that are resistive to degradation (Kyrikou & Briassoulis, 2007; Rujnić-Sokele & Pilipović, 2017; Shah et al., 2008). Recovery of plastic mulch from agricultural fields after crop harvest is not an economically feasible practice due to labor and disposal costs and plastic debris that forms due to environmental weathering often remains in the soil or nearby water as litter (Chiellini & Solaro, 1997).

Biodegradable mulches (BDMs) have been prepared as alternative to PE mulches to address the ongoing problem of plastic waste accumulation in the environment (Rujnić-Sokele & Pilipović, 2017; Brodhagen et al., 2015; Miles et al., 2017). BDMs provide similar benefits to specialty crop production as PE mulches but are designed to be tilled into the soil, where they will biodegrade (Malinconico, 2017; Touchaleaume et al., 2016). Although biodegradable polymers used as BDM ingredients such as polybutylene adipate terephthalate (PBAT) are more expensive than PE, BDMs provide savings to farmers through avoidance of laborious and costly removal of PE mulches (Touchaleaume et al., 2016; Hayes et al., 2019).

A new standard was recently released, with requirements for soil biodegradation, chemical composition, physical characteristics and ecotoxicity, European Standard EN 17033: Plastics-Biodegradable mulch films for use in agriculture and horticulture. According to EN 17033, BDMs should biodegrade in soil within 2 years, utilizing a standardized testing method such as ASTM D5988 (EN 17033, 2018). Composting is considered as a sustainable alternative for waste management disposal (Rujnić-Sokele & Pilipović, 2017; Vieyra et al., 2015; Gomez & Michel, 2013). According to the standard ASTM D6400, a plastic can be claimed to be biodegradable under industrial composting conditions (i.e., “compostable”) if it meets specified criteria on mineralization, deterioration and ecotoxicity compliance (ASTM D 6400, 2004). One criterion is 90% biodegradation under composting condition in 3 months using a standardized lab test (ASTM D5338)’ that simulates industrial composting operations (ASTMD5338, 2003). Despite compostability certification for some BDMs, the National Organic Program assessment of biodegradation of BDMs is based on laboratory testing in soil. For this reason, uncertainty arises that the testing environment may not be representative of environmental condition in the field (Miles et al., 2017).

Soil biodegradation tests conducted in the lab may not effectively simulate biodegradation in the field due to the inability to simulate environmental conditions in the field (e.g. pH, moisture, oxygen content, and temperature) and changes of inherent characteristics and properties of the polymers due to weathering Kijchavengkul et al, 2008; Brodhagen et al., 2015, Krueger et al., 2015; Elsawy et al., 2017; Rudnik, 2008; Kijchavengkul et al., 2008; Kijchavengkul et al., 2010). Biodegradation is very much dependent on the degradation environment, which is highly variable due to differences in soil ecosystems and microbial communities (Dharmalingam et al., 2015; Barragan et al., 2016; Morro et al., 2019); but, only one study has evaluated the impact of weathering on biodegradation, and this study focused upon nonwoven geotextiles that underwent artificial weathering, and the latter’s impact on compostability (Hablot et al., 2014]. An important gap in knowledge is the impact of environmental weathering during the BDMs’ service life on the BDMs’ biodegradability in soil and under composting conditions. Upon deployment of BDMs in the field, weathering agents (e.g. soil moisture, temperature, UV radiation) cleave polymeric constituents of the mulch films (Copinet et al., 2004; Hablot et al., 2014). Deterioration of mechanical properties of films, e.g., embrittlement, is attributable to hydrolysis and photodegradation (Hayes et al., 2017; Li et al., 2004). Both depolymerization and embrittlement can enhance biodegradation of BDMs due to availability of weaker and lower-weight constituent of polymers for microbial utilization. On the other hand, ultraviolet (UV) light exposure can result in to crosslinking and increased gel content (Hayes et al., 2017; Kijchavengkul et al, 2008) (and Chapter 3 of this dissertation). Photochemical reactions create free radicals that can catalyze the formation of polymer networks via cross-linking (Norrish Type I reaction) that may be recalcitrant to biodegradation. Particularly susceptible to photodegradation are polymers with higher aromatic content such as PBAT (Kijchavengkul et al, 2008, Tosin et al., 2019]. 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.

This paper investigates, under laboratory conditions, the biodegradability of 4 BDMs composed of PBAT, starch, PLA and PHA as their polymeric constituents, and specifically examines the impact of environmental weathering during the BDMs’ service life (to produce green peppers in Knoxville, TN, USA). In addition, the changes of chemical properties for the BDMs and soil microbial communities were studied throughout time-course of biodegradation in soil to better understand the underlying mechanisms of biodegradation.

4.3. OBJECTIVES

Most published reports have focused on mulch degradation during its useful-life stage in the field. After cropping season and after mulch use, weathering essentially influence how biodegradation proceeds thereafter. The assessment of the physicochemical properties of BDMs after its useful-life stage is of utmost importance as BDMs are intentionally plowed in the field after cropping season, to biodegrade in the soil. The objective is to determine how agricultural weathering affects biodegradation of BDMs in the soil and under composting conditions, either favoring biodegradation or hindering it using standardized lab tests of biodegradation in the soil and in compost. Our hypothesis is that biodegradation of BDMs will be enhanced due to embrittled condition and depolymerization of BDMs that results from weathering. This paper investigates the biodegradation of 4 BDMs with PBAT, PLA and PHA as major polymeric composition under laboratory conditions. This study examines how weathering impacted the biodegradation of BDMs at the end of its useful life. Both weathered and unweathered BDMs were tested in both biodegradation studies under soil and under composting conditions. In addition to CO_2 evolution for all experiments, chemical properties of the BDMs were studied throughout the time-course of biodegradation in soil, and microbial abundances assessed after biodegradation in the soil.

4.4. MATERIALS AND METHODS

4.4.1 Materials

Four black BDMs were used for the biodegradation tests: an experimental PLA/PHA film and three PBAT-based films, namely BioAgri (BioBag Americas, Inc., Dunedin, FL, USA); Organix (Organix Solutions, Maple Grove, MN, USA) and Naturecycle (Custom Bioplastics, Burlington, WA, USA). A cellulosic (paper) mulch (WeedGuardPlus, Sunshine Paper Co., Aurora, CO, USA) was employed as a positive control. In addition, environmentally weathered forms of clear and white-on-black versions of Organix, using the same polymeric feedstock as black Organix, were investigated. Further information of the mulches and their physicochemical properties, both before and after weathering, are provided in Table 4.1.

Test soil was a sandy loam (Shady-Whitwell complex soil), collected from the University of Tennessee (UT) East Tennessee Research and Education Center, Knoxville, TN, USA on May 2017 and Sept 2018 (for biodegradability testing of Organix and Naturecycle, and PLA/PHA and BioAgri, respectively), and stored in the lab at room temperature (22°C) for a period not exceeding 4 days prior to the initiation of the biodegradation experiments. For soil testing, a 2-mm sieve was used to remove large particles (e.g. roots and gravel) and manually inspected for other debris to ensure a homogenous mixture. Soil had a C:N ratio ranging from 10:1 to 13:1 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%, within the required range for ASTM D5988.

Registered organic compost, made of yard and food scraps, was provided by Cedar Grove Composting (Everett, WA, USA) and sieved to less than 8 mm particle size. Inert materials were manually removed (e.g. glass and stones). Compost was placed in storage bags, with lids slightly open, so that aerobic conditions would remain and moisture would not decrease below 55%. Compost was then kept in a cool and dry place prior to the start of the experiment and turned and shaken twice every week to maintain good aeration, which 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 (CO_2 level of 6, as described in the test kit instructions). The moisture content of the compost was not adjusted since initial moisture content was maintained at the required range (50% to 60%) during storage.

Several properties of the compost were evaluated. To determine total dry solids via gravimetry, compost samples were dried in the oven at 105°C for 10 h until constant mass was achieved. The amount of volatiles was determined gravimetrically after calcination at 550°C in an electric furnace. 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 for the resultant supernatant. Total C and N was determined on oven-dried compost samples by combustion (Flash EA 1112 NC Soil Analyzer; Thermo Fisher Scientific Inc., Waltham, MA, USA).

Solvents (e.g., chloroform, deuterated $CDCl_3$, 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 ($Mg_{1.8}Fe^{2+}_{0.9}Al_{4.3}SiO_{10}(OH)_2*4H_2O$), 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 (Schneider et al., 2012)

4.4.2 Methods

The extent of biodegradation (%) of environmentally weathered BDMs, retrieved from the field after cropping season, was compared to unweathered mulch counterpart. Environmental weathering took place for all mulches during green pepper production in Knoxville, TN, except for clear Organix, which took place during sweet corn production in Mount Vernon, WA. Weathering occurred during June-Sept, 2017. Further details on the weathering environment and procedure are provided in Chapter 3 of this dissertation. BDMs were cleaned through soft-bristle brush and cut into 1 cm^2 for soil biodegradation study and 4 cm^2 for biodegradation under composting condition.

The testing of biodegradability of BDMs in soil was carried out according to a standardized test method (ASTM D5988, 2012). Tightly sealed 16 oz mason jars (Item # 10500, Ball® Corporation, Westminster, CO, USA) served as incubators. Jar lids were mounted with 20 mm gray butyl stoppers (Millipore Sigma, Burlington, MA, USA) to enable sampling of the air headspace for CO_2 determination. Jars contained soil (50 g) and small (1 cm^2) pieces of mulch (500 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 kept at 27°C. Soil moisture loss was also retained by periodically adding deionized water to the soil when the moisture lost was > 1%, as determined gravimetrically by analysis on a weekly basis.

An infrared gas analyzer (LI-820, LiCor, Lincoln, NE, USA) was used to measure the CO_2 evolution in the headspace of the jars versus time. CO_2 concentrations were calculated based on a calibration curve using gas standards containing CO_2 concentrations of 1000 ppm, 1 vol%, and 5% in N_2 (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 room air to purge the jars of accumulated CO_2.

Each series of biodegradability tests involved the use of 4 BDM treatments (weathered and unweathered versions of two mulches) and three controls: soil that contained unweathered

WeedGuardPlus (WGP; positive control) a blank (soil only), and a technical control (air only). Each BDM treatment and control were replicated thrice.

The percentage of biodegradation was calculated according to the procedure described in ASTM D5988. The net CO_2 evolution resulting from microbial assimilation was determined by measuring the average CO_2 produced for a given mulch treatment and time, and subtracting the CO_2 measured for the controls. Biodegradation was determined by comparing the net CO_2 evolution to the theoretical maximum CO_2 that would be produced, the latter based on the mulches' carbon content as measured by elemental analysis (Hayes et al., 2017), given in Table 4.1.

Table 4.1. Biodegradable mulch films employed in this study and their physicochemical properties before and after agricultural weathering [i].

Major Polymeric Component/ Mulch [ii]	T_{max} [iii], °C	Gel content %	Peak Load [iv], N	Elongation [i], %	Carbon, % [v]	Manufacturer
PLA, PHA - PLA/PHA						
Initial	309 ± 1.2	22 ± 0.9	16 ± 2.0	202 ± 31.0	47 ± 0.10	Experimental Film [vi]
Weathered	310 ± 3.1	31 ± 1.8	13 ± 4.0	5 ± 1.0	44 ± 0.60	
PBAT - BioAgri [vii]						
Initial	401 ± 2.9	24 ± 0.3	11 ± 1.5	320 ± 104.0	58 ± 0.16	BioBag Americas, Inc., Dunedin, FL
Weathered	400 ± 0.1	31 ± 3.7	6 ± 1.3	9 ± 4.0	46 ± 4.76	
PBAT - Organix A.G., Black [viii]						
Initial	561 ± 5.6	31 ± 0.1	9 ± 0.8	237 ± 16.0	51 ± 0.12	Organix Solutions, Maple Grove, MN
Weathered	563 ± 3.5	33 ± 2.7	6 ± 0.8	13 ± 3.0	48 ± 0.73	
PBAT - Organix A.G., White-on-black (WOB) [v]	392 ± 2.3	23 ± 0.1	9 ± 0.3	215 ± 10.0	51 ± 0.12	Organix Solutions
Initial	390 ± 1.7	34 ± 1.3	7 ± 0.9	9 ± 2.0	48 ± 0.73	
Weathered						
PBAT - Organix A.G., Clear [v]						
Initial	396 ± 3.0	13 ± 0.1	12 ± 1.2	207 ± 3.0	51 ± 0.12	Organix Solutions
Weathered	396 ± 1.5	31 ± 1.0	9 ± 1.1	8 ± 2.0	48 ± 0.73	
Copolyester - Naturecycle						
Initial	NA[ix]	NA [vii]	10 ± 1.2	310 ± 102	55 ± 0.28	Custom Bioplastics, Burlington, WA
Weathered			10 ± 2.0	8 ± 1.8	52 ± 0.73	
Cellulosic - WeedGuardPlus						
Initial [vii]	NA [vii]	NA [vii]	61 ± 17.0	7 ± 1.1	46 ± 0.90	Sunshine Paper Co., Aurora, CO

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[i] Samples were taken during weathering in 2017 (Jun-Sept); field trials in Knoxville; pepper as test crop (Mount Vernon for clear Organix; sweet corn), discussed in Chapter 3 of this dissertation.

[ii] PLA, PHA and PBAT refer to polylactic acid, polyhydroxyalkanoate and polybutylene adipate terephthalate, respectively

[iii] Temperature (°C) where rapid and maximum thermal degradation occurs.

[iv] Measurements were performed along machine direction of the films.

[v] Values obtained from Hayes et al., 2017.

[vi] An experimental film with polymeric components consists of 68-71 wt % PLA and PHA prepared by Metabolix Inc., Cambridge, MA.

[vii] Mater-Bi® grade EF04P. Bio360 was produced from DuBois Agrinovation, Saint-Rémi, Quebec, Canada and used in 2017 and 2018.

[viii] Prepared from ecovio® grade M2351 (BASF, Ludwigshafen, Germany)

[ix] Only initial (unweathered) Weedguard was employed; "N/A" = no analyses were performed.

For microbial abundance assessment, DNA extraction and quantitative PCR (qPCR) with three replications were performed. Randomly sampled soil from jars used for CO_2 evolution were analyzed for DNA extractions using the 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 fluorescence was quantified using Synergy H1 hybrid plate reader (BioTek, Winooski, VT, USA). Extracted DNA was stored at -20°C. qPCR was done in replicates using Femto Bacterial and Fungal DNA quantification kits (Zymo Research, Irvine, CA, USA) to quantify 16S rRNA and internal transcriber space (ITS) region genes as a proxy for bacterial and fungal abundance in our samples, respectively. We followed manufacturer's instructions and used CFX Connect Real-Time PCR Detection System (Bio Rad, Hercules, CA, USA) to perform the reactions. All qPCR standard curves had R-squared of ≥98%.

A laboratory scale respirometric system was constructed to measure aerobic biodegradation of BDMS based on the system described in Hablot et al. (2014) that resembles a commercial scale industrial composting facility (ASTM D5338, 2016). A circuit was formed for each testing jar, consisting of the following: 1) Air supply (house air removed of its CO_2 content by passage through a series of three packed 621 kPa columns (28.9 cm height x 6.6 m inner diameter) of Drierite^{TM}, 2) air humidification system, consisting of the bubbling of CO_2-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) 1.9-L capacity composting jar (Fillmore Container, Lancaster, PA, USA), and the outlet air flow from the jar was sent to a 5) CO_2 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^{TM} packed columns, was retained in an incubator controlled at 58^{o}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 and to avoid mulch adhesion between jar walls and the mixture and prevent clogging of the air inflow, which occurred near the bottom of the jars. The composting media consisted of mature compost (400 g), vermiculite (50 g) and BDM (9 g of 2 cm x 2 cm pieces), which were thoroughly mixed by hand to ensure a homogeneous mixture and good mulch-compost contact. Vermiculite was added to ensure aeration and retention of compost moisture.

CO_2 concentrations were determined through measuring free $Ba(OH)_2$ remaining in solution in the traps via titration with 0.05 N HCl solution and phenolphthalein serving as an indicator. Evolved net CO_2 production was calculated through subtraction of the evolved CO_2 from the controls. Percent biodegradation was calculated similarly to that described above for biodegradability in soil and as specified in ASTM D5338.

Either 12 or 18 composting jars were incubated simultaneously for a series of tests, consisting of two or four BDM treatments (e.g., weathered vs unweathered versions of one or two BDMs), a positive control (unweathered WeedGuardPlus) and a blank (compost + vermiculite), with three replicates per treatment. All biodegradation experiments were conducted for 90 days.

Changes of molecular weight, polymer composition, surface chemical changes and thermal properties were measured during the time course of biodegradation in the soil. Nine replication jars for each of weathered and unweathered PLA/PHA (PLA – major polymeric constituent) and weathered and unweathered BioAgri (PBAT-based) were added to the corresponding CO_2

evolution (ASTM D5988) experiments described above, and therefore underwent identical environmental conditions as those employed for CO_2 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 x 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 x g) for 1 min and filtered (1.0 µm and 0.2 µm). Filtrate (200 µL) 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 BioAgri, since starch is insoluble in chloroform (verified by TGA analysis), GPC results are for PBAT only.

The ^{1}H-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 800 µL of $CDCl_3$ containing 1% tetramethylsilane as an internal standard (Acros, Geel, Belgium). Spectral assignment for PLA and PHA are given in our previous paper (Dharmalingam et al., 2016). Each sample was performed with two replications.

Thermogravimetric analysis (TGA) was carried out on PLA/PHA and BioAgri throughout time-course degradation using a Discovery TGA (TA Instruments, New Castle, DE USA) at a heating rate of 10 °C/min from room temperature (25 °C) to 600 °C in an unsealed platinum sample pan under a nitrogen atmosphere.

4.4.3 Statistical Analysis

Three replications for each mulch (PLA/PHA, BioAgri, Organix, Naturecycle) treatment were used for analysis of variance (ANOVA) to calculate statistical difference on the average final cumulative percent of carbon evolved or rate of biodegradation (%). Differences between treatment means of final cumulative biodegradation were performed using Tukey Kramer HSD analysis at significant difference level of α = 0.05. The statistical analyses were performed using JMP statistical program version 14 (SAS Institute Inc., CaryCary, NC, U.S.).

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 (R-Core-Team, 2018).

4.5. RESULTS AND DISCUSSION

4.5.1. Effect of environmental weathering on biodegradable mulches

Weathered mulches used in field trial in 2017 (clear Organix in WA and all other BDMs in TN) were retrieved to determine effect of environmental weathering on biodegradation of BDMs 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, polymeric composition, and gel content %) (Section 3.6). The change of physicochemical properties due to weathering was slightly influenced by the composition of the BDMs. All mulches, except the experimental PLA/PHA mulch, contain the same major polymer, PBAT. PLA/PHA underwent greater degradation in TN. However, due to the formation of chemical bonds between PLA, PHA and the filler used ($CaCO_3$) via solar radiation, demonstrated by an increase of peak load and gel content, biodegradation may be inhibited. All PBAT-based BDMs (BioAgri, Organix, Naturecycle) were affected by weathering; but, the change of physicochemical properties varied quite significantly between mulches (Chapter 3.6). Naturecycle was a mulch employed for the field studies and this biodegradation study; however, the change of its physicochemical properties were not monitored to the same extent as BioAgri and Organix. Therefore, the impact of weathering on Naturecycle was not reported in Chapter 3. Physicochemical properties changed to a greater extent (e.g. peak load decrease, increased gel content and thermal stability) for BioAgri than Organix, as observed in Table 4.1 and described in Chapter 3. Among the three Organix mulches, clear Organix underwent more degradation during weathering than black and white-on-black Organix films (e.g. FTIR spectra changes, gel content, peak load decrease), 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 (Section 2.2.1.4) such as the black and white-on-black Organix BDMs.

4.5.2. Effect of environmental weathering on CO_2 evolution: biodegradation in the soil

CO_2 evolution continuously increased during the initial phase of biodegradation, then eventually reached a plateau phase at ~100 days for PLA/PHA, BioAgri and Organix and ~200 days for Naturecycle (Figure 4.1).

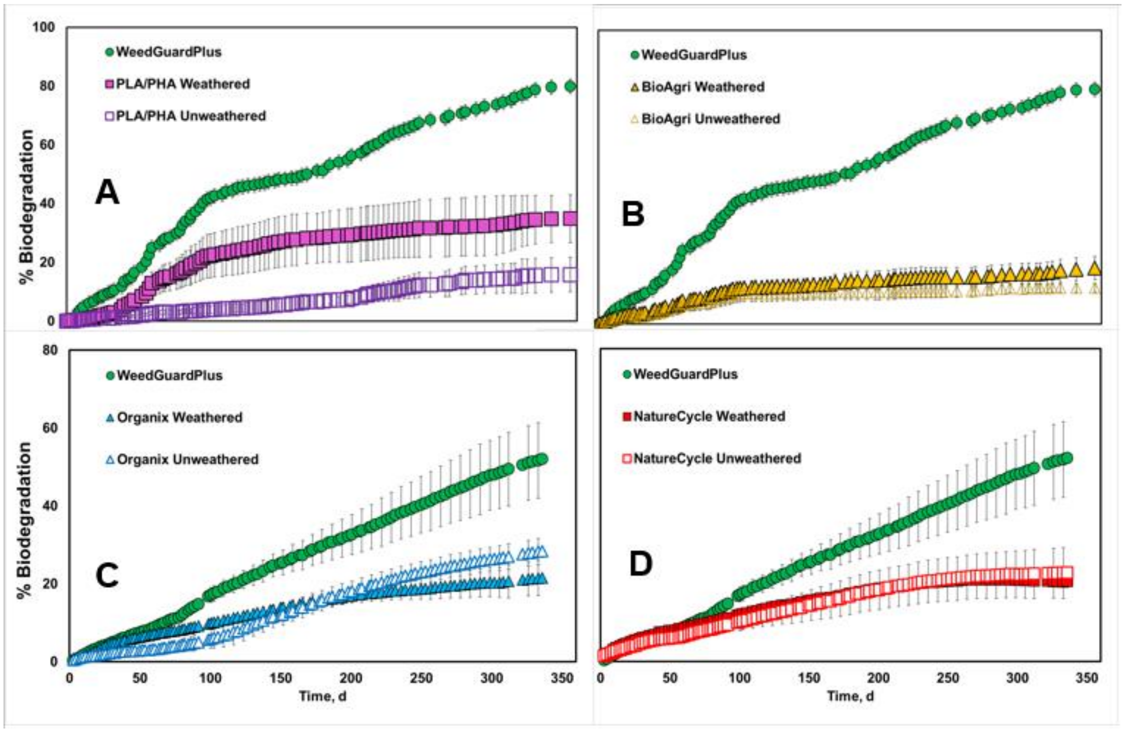

Figure 4.1. Cumulative mineralization of weathered and unweathered BDMs during 365 days of soil incubation, per the test conditions of ASTM D5988. Error bars reflect standard deviation. Data for Figs A and B, and C and D, were taken simultaneously; as a result, the time course of biodegradation for WeedGuardPlus is identical between Figs A and B and between C and D. Errors bars reflect standard deviation of 3 replication treatment jars except for WeedGuardPlus in Figures C and D, which involves 2 replicates.

As for WGP, the positive (cellulosic) control, CO_2 also continually increased, reaching 80% biodegradation for the first run (involving PLA/PHA and BioAgri; 10 mo) and 55% biodegradation for the second run (Organix and Naturecycle; 11 mo); but, a plateau was not reached for the latter run. For the second run, only 2 replicates were used for WeedGuardPlus due to one replicate being excluded. The latter produced CO_2 values that were in disagreement with the other two replicates after 4 mo. (We noticed a crack in the jar of concern and that the moisture content may not have been optimal as a result.) PLA/PHA underwent a lag phase during the initial 2-4 weeks, more prominently for unweathered than weathered mulch. Weathering enhanced the biodegradation of PLA/PHA and Naturecycle and BioAgri PBAT-based BDMs in the soil but not for Organix, for which unweathered mulch underwent higher biodegradation that weathered mulch. PLA/PHA produced the highest CO_2 rate and extent of biodegradation among the BDMs (Figure 4.1 A). Weathered PLA/PHA achieved 35% biodegradation after soil-incubation for 365 d, whereas unweathered PLA/PHA underwent only 16% biodegradation. Other studies achieved higher biodegradation during soil incubation for PLA-based plastics than our results using PLA/PHA but the other studies employed longer incubation times. A PLA/PHA resin was able to reach 70% biodegradation in 660 days (Gomez and Michel, 2013) whereas powder and pellets made from a PLA/starch blend attained 90% biodegradation, equal to that for cellulose, in 276 days (Tosin et al., 2019). PLA/PHA mulch fragments introduced into the biodegradation chambers in our study (1 cm^2 pieces) were larger in size compared to the cited studies. We did not use any methods for size reduction, such as mechanical grinding, to ensure the absence of artifacts in the physicochemical properties of the mulches. Larger particle size and surface area may be a contributing factor to a slower microbial colonization on mulch surfaces utilizing carbon in mulch as an energy source. The same study also found out that at nano size level, biodegradation of a biodegradable polymer will take 15-20 days to reach full biodegradation (Tosin et al., 2019). Among PBAT-based mulches, BioAgri yielded lower biodegradation than Organix and Naturecycle. Unweathered BioAgri (Figure 4.1B) reached only 12% biodegradation while weathered attained 19%. Unweathered Organix (Figure 4.1C) had a slightly higher biodegradation (25%) than weathered (20%); likewise, unweathered Naturecycle's biodegradation (22%) was only slightly higher than weathered Naturecycle (20%) (Figure 4.1D). The greater embrittlement for weathered BDMs compared to unweathered (e.g., lower % elongation; Table 4.1) may have aided depolymerization during the biodegradation process. In a recent study, both photodegradation and microbial action (e.g. *Bacillus subtilis)* significantly increased the biodegradation of BDMs (PBAT and PBAT/PLA) (Morro et al., 2019). In another study, PBAT-based mulch (1x1 cm^2 pieces) with minor biobased components (e.g. PLA, starch) mineralized in soils even with poor organic matter content: about 40-50% after 350 days, a value slightly higher than we found in our study (Touchaleaume et al., 2016). Depending on the microorganisms in the soil, aerobic or anaerobic environmental conditions, biodegradation can occur faster when organic materials are present in sufficient amounts to increase microbial activity and facilitate faster biodegradation of plastics (Kimura et al., 1994). Biodegradation varies in soil as a result of significant taxonomical differences between microbial populations and the polymeric materials (Nishioka et al., 1994). It could be that soil used in our study may not host diverse communities of microorganisms essential for biodegradation as a study found that morphology of fungi and actinomycetes on degraded surface films were dependent on the type of film samples and soil (Eya et al., 1994).

Microbial abundance assessment was determined through total bacterial and fungal abundances. Microbial abundance was measured for soil samples obtained from the biodegradability experiments after their completion in two separate soil experiments by qPCR using 16SrRNA gene and ITS region copies, respectively. As shown in Figure 4.2A, bacterial abundance was significantly different among BDMs (*p*=0.0303) for soil biodegradation experiment A (WGP A, PLA/PHA and BioAgri, Soil A) but not for soil biodegradation experiment B (WGP B, Organix, Naturecycle, Soil B).

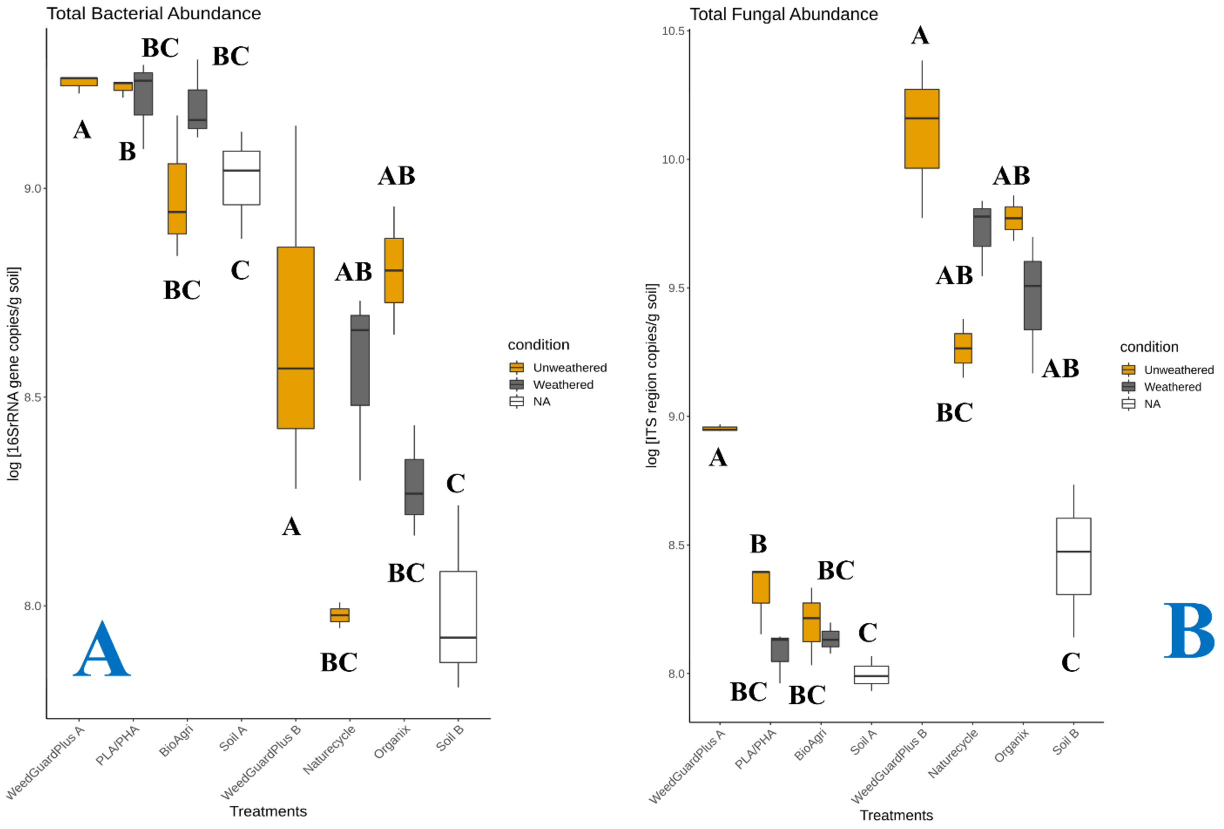

Figure 4.2. Microbial abundance of bacterial (A) and fungal (B) communities after biodegradation in the soil (12 mo). Soil samples were taken from the jars used for the experiments of Figure 4.1 after completion of the experiment

Yet for soil biodegradation experiment B, interaction of treatment (weathered, unweathered) and BDMs yielded a significant difference for bacterial abundance (*p*=0.0031). Mean bacterial abundance of soil A was significantly higher than that for Soil B. Weathered mulches significantly induced a higher bacterial abundance than unweathered mulch counterparts, particularly for Naturecycle, and only to a lesser extent for BioAgri and PLA/PHA. Greater bacterial abundance for weathered than unweathered BDMs is consistent with the higher biodegradation of weathered PLA/PHA, BioAgri and Naturecycle, with the differences being less for the latter mulch (Figure 4.1). The study of Chinaglia et al. (2018) suggests that maximum biodegradation rate at a molecular level can be achieved when all available microorganisms in the soil are utilizing the same carbon source and are fully engaged in biodegradation. The embrittled condition of weathered BDMs makes them more susceptible to undergo disintegration during the biodegradation process. Moreover, size reduction occurs more rapidly, providing more surface area for microbial communities to degrade. However, an opposite trend was observed for Organix mulch, indicating a relatively greater bacterial abundance on unweathered BDMs than weathered mulch. This result is consistent with the early plateau phase (180 days) reached by weathered Organix whereas unweathered BDMs continued to undergo biodegradation and achieved higher extent of biodegradation in the soil after 350 days. Biodegradation varies in soil and a significant taxonomical difference for the microbial population affects microbial utilization, the extent of which depends on the polymeric material (Nishioka et al., 1994). Chemical complexity, observed by a higher M_w of polymers, decreases biodegradation due to incapability of large molecules to penetrate through microbial cell (Kijchavengkul et al., 2010). When polymers possess more molecular branching, enzymatic reactions by microorganisms can be sterically hindered (Hatzinger & Kelsey, 2005).

Fungal abundance was significantly different among BDMs for soil experiments A ($p<0.0001$) and B ($p=0.0009$), but not within mulch treatments (unweathered vs. weathered) (Fig 4.2B). Yet interaction of BDM product and BDM treatments (weathered, unweathered) had a significant effect on fungal abundance for soil biodegradation experiment B ($p=0.0235$). Mean fungal abundance of soil associated with biodegradation of Naturecycle and Organix were similar to WGP A and WGP B, which were significantly higher than for BioAgri and PLA/PHA. BDMs employed in soil biodegradation experiment A had a fungal abundance lesser than WGP A but higher than that of fungal abundance in the control (Soil A). For soil biodegradation experiment B, fungal abundance of BDMs was lower than WGP B but higher than Soil B. A study on PBAT-based film showed that the higher adipate-to-terephthalate (A/T) ratio can give rise to greater amounts of amorphous regions available for microorganisms, will therefore results in faster enzymatic degradation (Zumstein et al., 2018). Our results, inconsistent with the previous study (Zumstein et al., 2018), showed a slightly higher biodegradation for Organix than BioAgri, which had a lower A/T ratio than BioAgri (Chapter 3.5). This can be attributed to differences in minor polymeric constituents used or techniques employed during mulch film processing. Uniquely observed for Organix, both fungal and microbial communities were higher on unweathered than weathered mulch. Filamentous fungal communities have been reported to extensively colonize PBAT film surfaces and directly contribute to accelerated degradation in soil (Zumstein et al., 2018; Koitabashi et al., 2012).

4.5.3. Effect of environmental weathering on biodegradable 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 4.3. Biodegradation under composting conditions was relatively higher than biodegradation in the 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% (Figure 4.3).

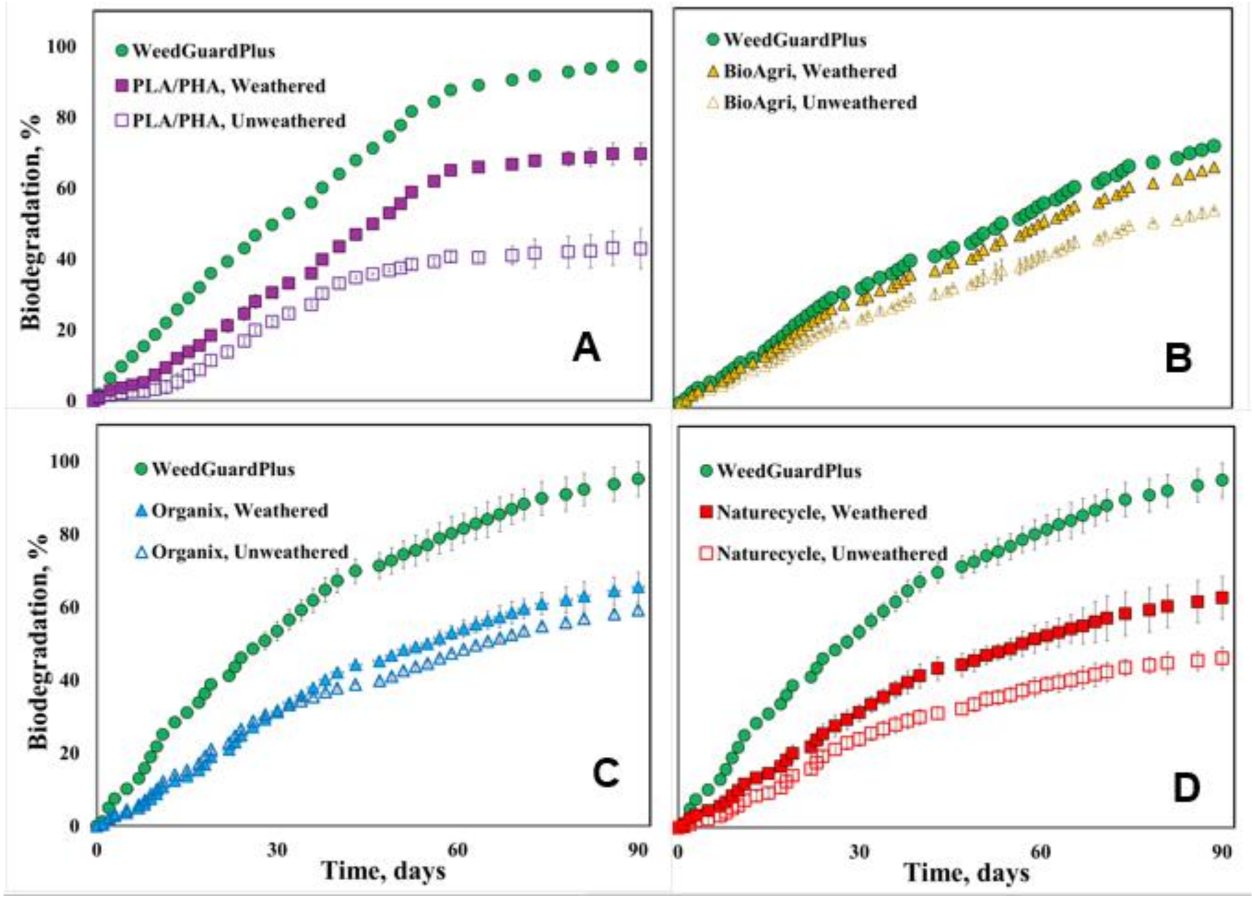

Figure 4.3. Cumulative mineralization of weathered and unweathered BDMs (PLA/PHA, BioAgri, Organix and Naturecycle) during 90 days of biodegradation under composting conditions, per the test conditions of ASTM D5338. Error bars reflect standard deviation and error bars for some data points appear smaller than markers. Data for Figs C and D were taken simultaneously; the time course of biodegradability for WeedGuardPlus is identical between the two subfigures.

The (%) 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 after composting, respectively (Figure 4.3A). In another study (Castro et al., 2017), PLA films that were slightly thinner than PLA/PHA mulch (22, 31, and 34 µm) and were incorporated into compost as smaller sized fragments (1 cm^2), achieved 47%-68% biodegradation in compost

(3 mo), values higher than achieved for unweathered PLA/PHA mulch in our study (Table 3.1). Although both studies employed the same temperature, 58°C, our study used a yard and food scrap-based compost whereas the previous study used a manure-straw-based compost, suggesting the two studies may differ in the C:N ratio, quality and characteristics of the compost. PLA is susceptible to both biotic and abiotic hydrolysis, both of which break down the polymer chain into lactic acid oligomers or monomers that are easily assimilated by microorganisms (Castro et al., 2017). Biodegradation of PLA is greatly affected by temperature (T) and the closer the environmental T is to the glass transition temperature (T_g) or above it, the faster the biodegradation rate (Rujnić-Sokele & Pilipović, 2017). During composting, T 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. Microbial polymerases that utilize PHA can biodegrade solid PHA/PHB blends into water-soluble monomers and oligomers, utilizing carbon as nutrient source within cells (Bregg, 2006). A study showed biodegradation of PHA occurred to the same extent of cellulose during 660-d soil incubation (Gomez & Michel, 2013).

In our study, a lag phase occurred for PLA/PHA during biodegradation under composting conditions, but not for the other BDMs. The lag phase lasted for ~2 wk, a shorter time than the lag phase observed during biodegradation in the soil (~45 days). The lag phase of weathered mulches was 8 d, shorter than for unweathered mulch, 15 d. In a study using PBAT films, the lag phase lasted longer (>3 wk) than what was observed in our study (Castro et al., 2017). The biodegradability of PLA was at 86% despite a lag phase of 21 days (Leejarkpai et al., 2011). Biodegradation of PLA in compost showed smaller size of films (1 cm^2) biodegraded faster than ground PLA resins and PLA film (5 cm^2). However, the effect of plastic particle size on the duration of the lag phase was not studied (Funabashi et al., 2009).

The biodegradation vs. time profiles of weathered and unweathered BioAgri under composting conditions were almost identical to WGP, and unlike PLA/PHA, there was no lag phase observed (Figure 4.3B). Biodegradation of WGP was lower for this study compared to other experiments of Figure 4, and this may be attributable to the fact that compost material was not validated at the optimal maturity index level (active compost), as verified through Solvita test kits. However, for the other composting experiment, compost maturity was confirmed. Nevertheless, weathering had significantly enhanced biodegradation of weathered BioAgri than unweathered mulches, and to a greater extent in the compost than soil. In high-moisture environments, microbial hydrolysis increases, 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 (Kijchavengkul et al., 2010; Herrera et al., 2002). High susceptibility of PBAT to hydrolysis (for BioAgri and other PBAT mulches used in the study) may also explain the similar biodegradation (%) of the cellulosic material (%) to PBAT-based BDMs in compost than in soil.

In contrast to BioAgri, biodegradation of weathered Organix and Naturecycle BDMs was much higher than for unweathered BDMs (Figures 4.3 C and D). In addition, although the % biodegradation achieved for all three weathered PBAT-based BDMs were similar (near 60%, 3 mo), the % biodegradation for BioAgri was closer to that achieved by the cellulosic control (WGP). Among PBAT-based BDMs, BioAgri underwent greater physicochemical changes due to weathering than Organix and Naturecycle. The weakened structure of weathered PBAT-based

BDMs (e.g. peak load decrease, increased gel content and decreased M_w) discussed in Section 3.6) enhanced the susceptibility to hydrolysis during biodegradation in compost. The higher adipate/terephthalate (A/T) ratio for BioAgri than for Organix, as indicated via NMR (Chapter 3) may also play a role. The degradation rate of PBAT is significantly enhanced by higher temperature of the degradation media and higher A/T ratio (Herrera et al., 2002). Highly amorphous regions (e.g. adipate regions) are more likely to serve as the sites of hydrolytic reactions (Kijchavengkul et al., 2010). 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 on increased gel content (%) (Figure 3.5). The higher extent of gel content increased observed in Organix compared to BioAgri (also a PBAT-based BDM), also indicative of higher degree of crosslinks, may explain the trends of lower biodegradation of weathered Organix than unweathered Organix mulches. Differences in the films' preparation, and minor and inorganic components may be factors that make BioAgri more susceptible to degradation among the PBAT-based BDMs. Naturecycle, the additional BDM used for biodegradation study was discussed in lesser detail to avoid disclosing proprietary information of the molecular structure of the product, per the manufacturer's request.

Colorants, particularly carbon black, which acts as a photostabilizer, inhibit photodegradation of mulches. As discussed in Chapter 3, three varieties of Organix that differed in color (black, white-on-black and clear) underwent changes in physicochemical properties that differed from each other. The three weathered Organix mulches were compared for their inherent biodegradability using the ASTM D5338 standardized test. All weathered Organix mulches generally underwent similar time courses of biodegradation (Figure 4.4). However, the clear film underwent the slowest rate of biodegradation, despite undergoing the greatest extent of embrittlement (e.g. loss of elongation), loss of tensile strength and greatest extent depolymerization due to weathering, as discussed previously (Figure 4.4).

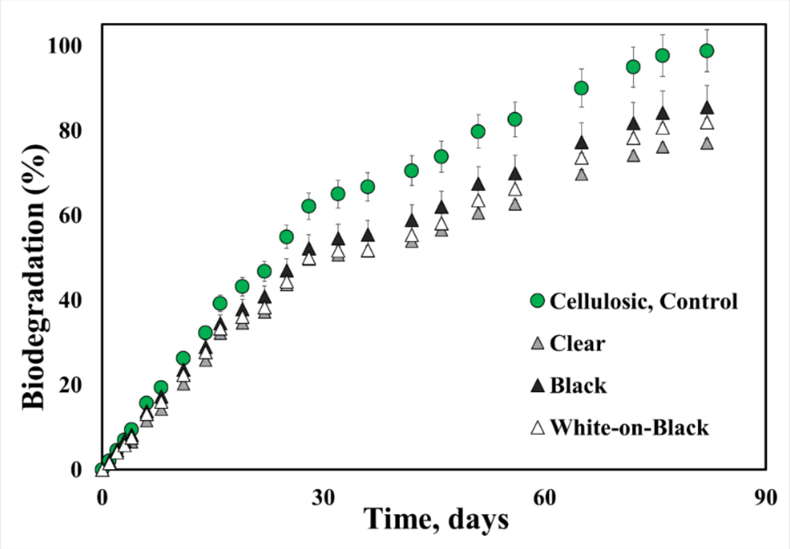

Figure 4.4. Cumulative mineralization of different colored-Organix BDMs after agricultural weathering during 90 days of biodegradation under composting conditions. Error bars reflect standard deviation and error bars for some data points appear smaller than markers.

The clear film underwent cross-linking to greater extent that black and WOB (Norrish type-I reaction) via photodegradation (Figure 3.5), which explains this result. In addition, the slightly lower biodegradation observed for WOB compared to black Organix is consistent with the higher gel content for WOB. Carbon black as a photostabilizer may have inhibited photodegradation, leading to a lower gel content for black Organix (Kijchavengkul et al., 2008). Up to ~30 d, biodegradation of both black and WOB Organix were nearly equal; but, WOB eventually slowed in its biodegradation rate relative to the other two Organix BDMs, as evidenced by a plateau in CO_2 production.

4.5.4. Changes of biopolymer composition of PLA (PLA/PHA) and PBAT (BioAgri) in the soil

The PLA and PHA composition of both weathered and unweathered BDMs throughout the time-course of biodegradation was determined through NMR. Results shows a consistent increase of PLA (i.e., decrease of PHA) for weathered and unweathered PLA/PHA mulch vs. time, but with the change being significantly higher for the unweathered mulch ($p<0.0001$; Figure 4.5).

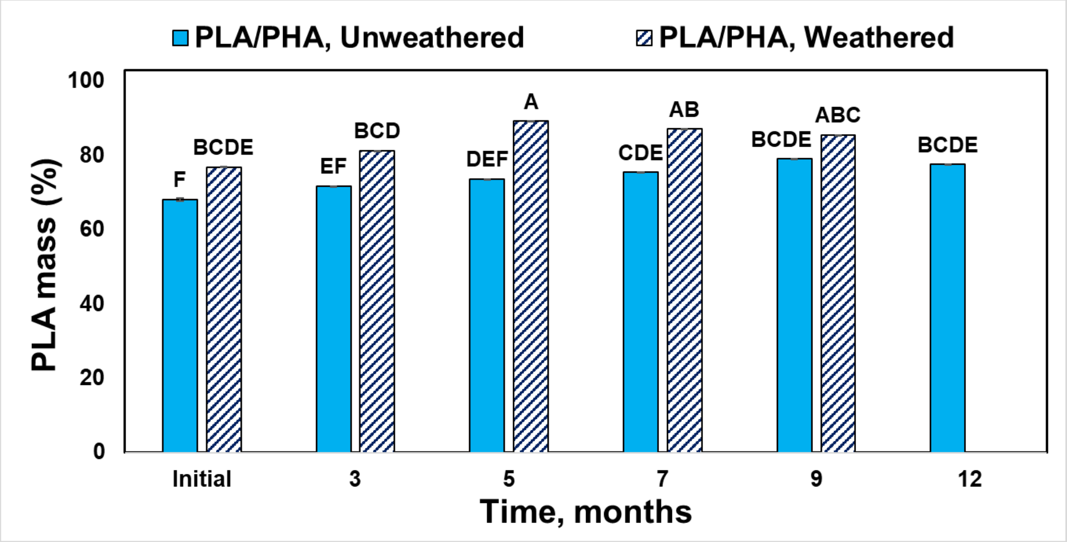

Figure 4.5. Effect of time-course biodegradation on PLA/PHA composition (%) before and after 365 days of soil incubation (via NMR). Error bars reflect standard error and means that do not share common capital letters reflect statistically significant difference. Time course of biodegradation is given in Figure 4.1A. No data was taken for weathered PLA/PHA at 12 mo due to excessive degradation of the mulch fragments.

This result clearly indicates that PHA was preferentially utilized over PLA as a carbon source by soil microorganisms. Similarly, during the time-course of biodegradation for a meltblown

PLA+PHA nonwoven mulch in compost-enriched soil, %PLA also increased (Dharmalingam et al., 2015; Dharmalingam et al., 2016). No further NMR analyses were conducted after 12 mo, due to complete disintegration.

Gel permeation chromatography was performed for PLA/PHA and BioAgri during the time course of biodegradation in soil and results are given in Table 4.2 and Figure 4.6.

Table 4.2. Change of weight-averaged molecular weight (M_w) and polydispersity index (PDI) of PLA and PBAT during time-course of biodegradation for PLA/PHA and BioAgri (GPC analysis) [a,b]

	M_w		PDI	
	Unweathered	Weathered	Unweathered	Weathered
PLA/PHA, Control	228.59 ± 0.71 A	198.73 ± 2.07 AB	1.76 ± 0.00 BC	2.28 ± 0.00 A
PLA/PHA, 3 months	171.41 ± 1.60 ABC	150.23 ± 2.18 BCD	1.61 ± 0.02 CDE	1.78 ± 0.01 AB
PLA/PHA, 5 months	137.26 ± 0.95 BCDE	121.16 ± 5.87 CDE	1.53 ± 0.02 DE	1.50 ± 0.02 E
PLA/PHA, 7 months	145.80 ± 9.83 BCD	123.64 ± 3.61 CDE	1.58 ± 0.00 CDE	1.71 ± 0.08 BCD
PLA/PHA, 9 months	97.79 ± 2.07 E	112.29 ±1.44 DE	1.71 ± 0.06 BCDE	1.66 ± 0.05 CDE
PLA/PHA, 12 months	128.14 ± 31.44 CDE	133.74 ± 8.83 BCDE	1.63 ± 0.06 CDE	1.73 ± 0.03 BCDE
BioAgri, Control	297.6 ± 17.31 A	172.71 ± 9.58 A	2.36 ± 0.07 BC	3.74 ± 0.51 A
BioAgri, 3 months	98.44 ± 8.48 B	153.05 ± 20.55 B	1.71 ± 0.03 C	2.61 ± 0.47 ABC
BioAgri, 5 months	93.89 ± 7.25 B	125.77 ±14.62 B	1.75 ± 0.11 BC	2.75 ± 0.33 AB
BioAgri, 7 months	97.79 ± 2.02 B	79.83 ± 8.99 B	1.90 ± 0.12 BC	2.24 ± 0.06 BC
BioAgri, 9 months	78.53 ± 25.84 B	110.02 ± 11.66 B	2.05 ± 0.15 BC	2.42 ± 0.17 BC
BioAgri, 12 months	80.39 ± 14.33 B	80.93 ± 32.58 B	1.99 ± 0.09 BC	2.43 ± 0.52 BC

[a] Error bars reflect standard error and means that do not share common capital letters reflect statistically significant difference; [b] time course of biodegradation given in Figures 1 A and B.

M_w consistently decreased throughout time-course biodegradation, but to a greater extent for PBAT in BioAgri than for PLA in PLA/PHA ($p<0.0001$). M_w decreased significantly after 3 mo for both weathered and unweathered BioAgri, but to a greater extent for the former ($p<0.0001$). With regard to the decrease of M_w vs. time, the minimum M_w for unweathered and weathered BioAgri occurred after 7 mo and 9 mo, respectively ($p<0.0112$; Figure 4.5). During surface erosion, microorganisms consume the polymer's monomeric and oligomeric regions near the mulch surface that assimilate into the cellular membrane to be further degraded by intercellular enzymes. This trend is noticed through a slow reduction of molecular weight during the early stage of biodegradation due to random scission of the polymeric chain at the start of hydrolysis (Kijchavengkul & Auras 2008; Seppälä & Malin, 1994). PLA/PHA, however, underwent a different trend than that observed from BioAgri. Unlike BioAgri, PLA/PHA has a high T_g (60-65^{o}C) and the soil temperature (27^{o}C) is far below T_g, thereby inhibiting thermal degradation. There was no significant difference for the M_w changes between weathered and unweathered PLA/PHA ($p=0.0872$). M_w reached it minimum value for both PLA/PHA mulches in 9 mo ($p<0.0001$). Other studies of molecular weight decrease for PLA during biodegradation used PLA sheets and films 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 (Rudeekit et al., 2008; Nakayama et al., 1994).

Table 4.2 also displays the changes of PDI for BioAgri and PLA/PHA during the time-course of biodegradation in soil. An increase of PDI was observed for BioAgri that was significantly greater for the weathered than the unweathered form ($p<0.0001$). No changes of PDI were observed for PLA/PHA vs. time ($p=0.3944$). Major PDI changes were only observed during the first three months of biodegradation. PDI values for PLA films, nonwoven geotextiles and blends studied in compost and in soil were found to increase during biodegradation (Rudeekit et al., 2008; Nakayama et al., 1994) (Dharmalingam et al., 2015).

The trends for onset (T_{onset}) and rapid degradation (T_{max}) temperatures of PLA (PLA/PHA) were consistent (Figure 4.6 A). There was an increase of both temperatures from 0-3 mo and then a decrease to about 8 mo, and a leveling off after in 9 mo. This trend of increased of M_w at an early stage is likely due to either abiotic leaching or biotic selection for lower molecular weight polymers. At this stage, microbes could be slowly becoming colonized ("situated" or established) on the mulch surface. A study also found this trend over time course degradation of a compost-enriched soil study (Dharmalingam et al., 2016) where M_w increased during the early stage but then decreased subsequently. The decrease of both temperatures after 3 mo was consistent with M_w decrease (Figure 4.6). Correlating T_{max} to M_w, a T_{max} decrease can reflect depolymerization (M_w decrease) while an increase can indicate leaching out of lower-weight oligomers/monomers (M_w increase). The same trends were similarly observed for PBAT of BioAgri (Figure 4.6 B). There was a slight increase of T_{onset} and T_{max} until 3 mo, and subsequently a decrease until 9 mo, after which it also levelled off through 12 mo.

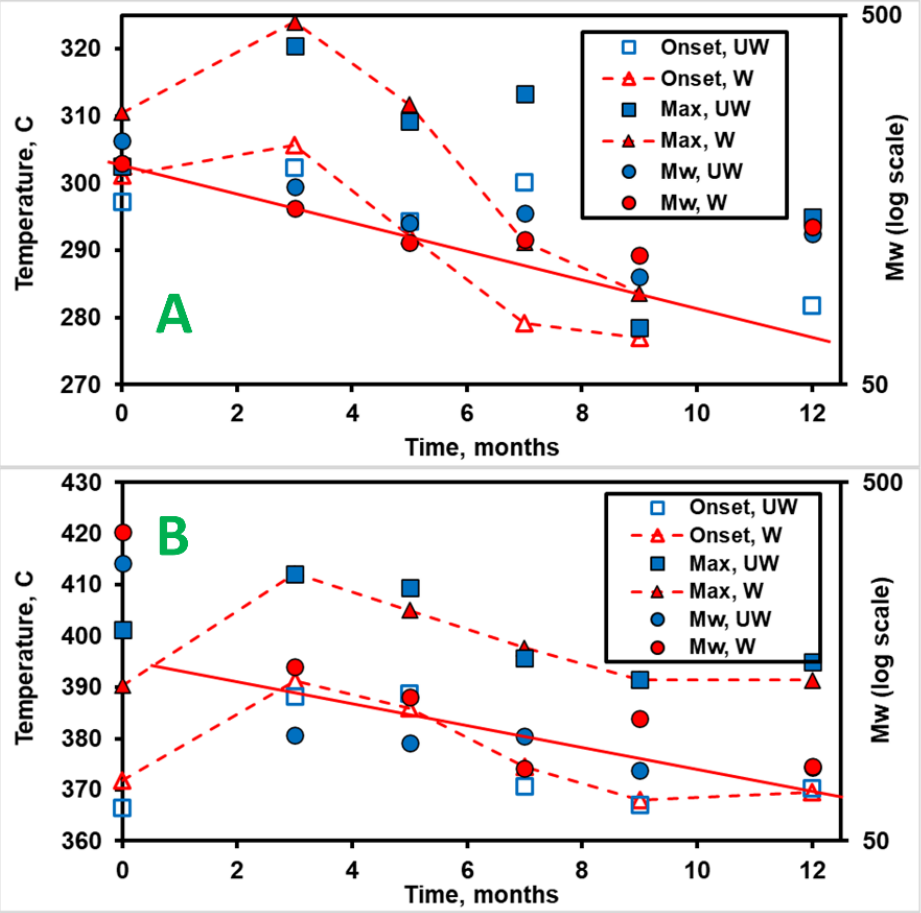

Figure 4.6 Changes of thermal degradation temperatures ($T_{onset,\ max}$) and weight-averaged molecular weight of PLA/PHA and BioAgri during 365 days of soil incubation.

Figure 4.7 presents the thermograms (*left*; TGA) and differential thermograms (*right*; DTG) of BioAgri and PLA/PHA throughout the time course of degradation.

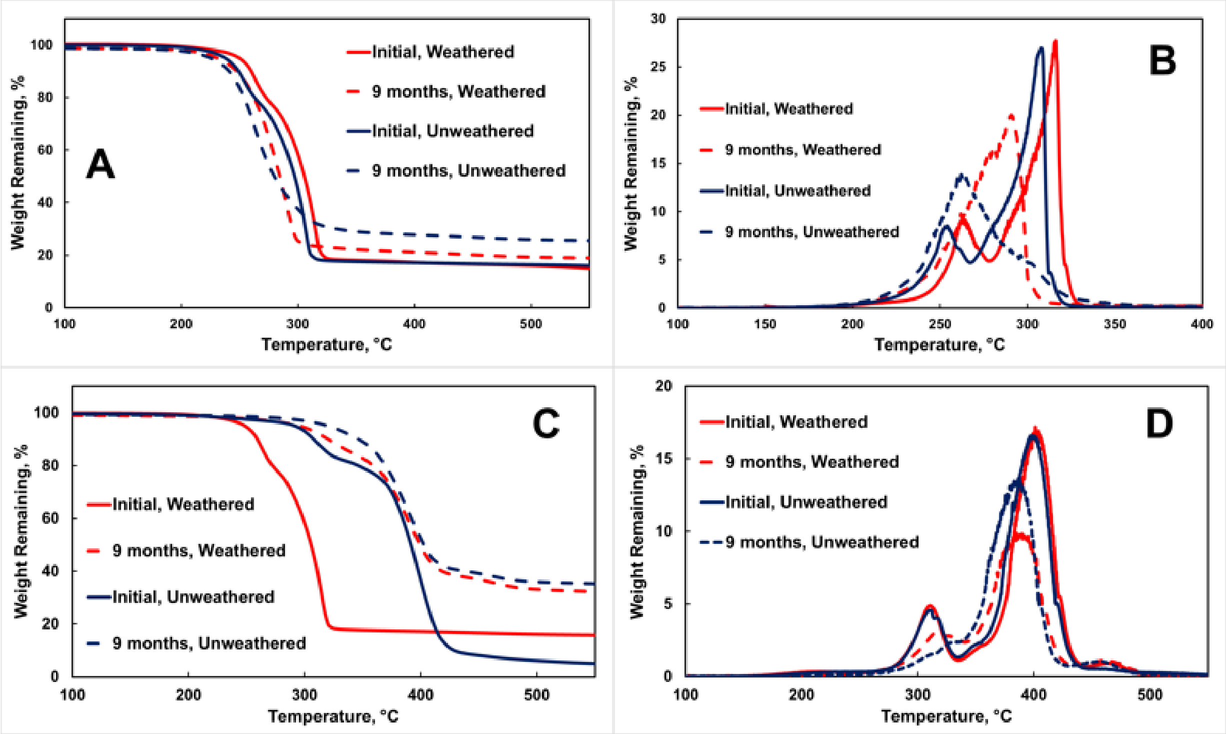

Figure 4.7. Change of TGA thermograms of PLA/PHA (Figures 1A and 1B) and BioAgri (Figures 1C and 1D) during the time-course of biodegradation in soil. CO_2 evolution profiles are given in Figures 1A and B.

As the polymeric materials are slowly and steadily heated at 10°C/min up to 600°C, polymers are vaporized and residual materials at 600^{o}C are mainly inorganic components, soil particles adhered to mulch, and gels (Hayes et al., 2017). Weight (%) remaining at 600°C of all weathered and unweathered BDMs increased after 9 mo in the soil (Figures 4.7. A and C). Mulches underwent a shift to lower temperatures for the main heating stages, representing PHA and PLA for PLA/PHA and starch and PBAT for BioAgri, an indication of thermal stability decrease. The temperature shifts were greater for weathered mulches (Figure 4.7). The presence of one composite heating stage for PHA and PLA heating stages in the DTGs after 9 mo of biodegradation in the soil is likely due to convolution of the two individual heating stages (Figure 4.7 B). For BioAgri, TGA data shows a significant decrease of starch concentration vs. time for both weathered and unweathered BDMs, indicating preferential utilization of starch over PBAT as a carbon source (Figure 4.8).

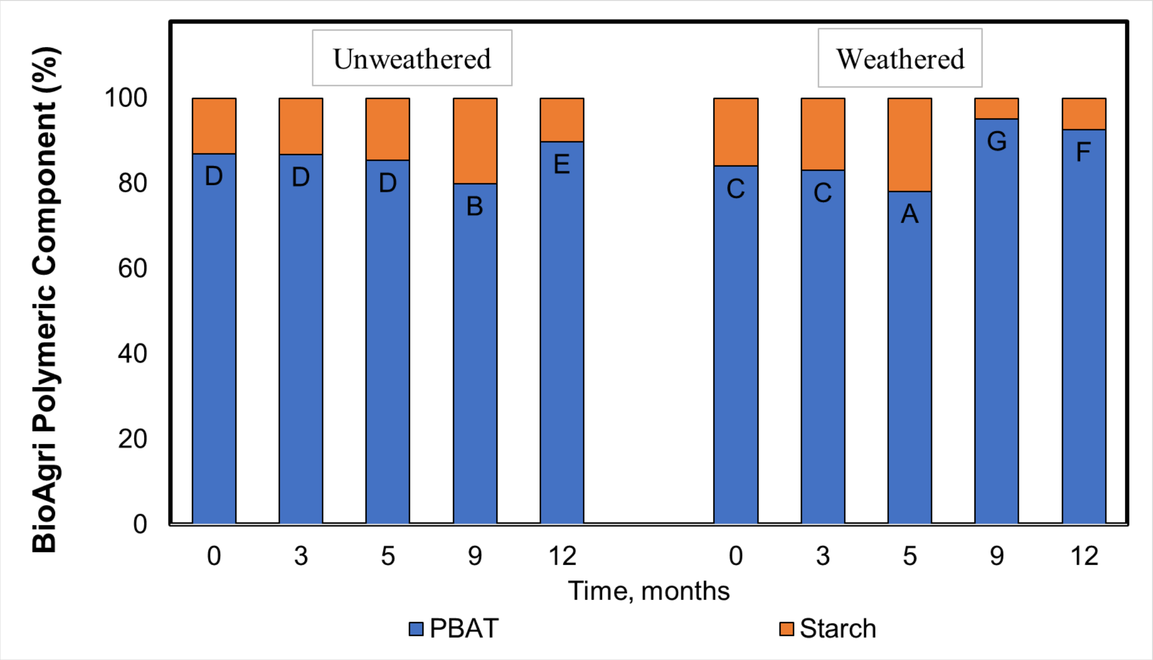

Figure 4.8. Change of relative polymeric composition of (A) PLA/PHA and (B) BioAgri BDMs during and BioAgri during the time-course of biodegradation in soil. CO_2 evolution profiles are given in Figures 1A and B.

4.6. CONCLUSIONS

Biodegradation of BDMs in the soil and compost depend upon the polymeric components of mulch. Weathering enhanced the rate of biodegradation for BDMs in the soil and increased the extent under composting conditions. The extent of biodegradation of BDMs during 365-days of soil incubation from highest to lowest was in the following order: PLA/PHA > BioAgri > Naturecycle > Organix. This order occurred for both weathered and unweathered mulches. Although crosslink formation was observed to increase due to agricultural weathering through increased gel content, weathered BDMs underwent biodegradation to a greater extent than unweathered BDMs in both soil and compost. Three Organix mulches that differed in color (black, white-on black, clear), having the same ecovio® feedstocks, underwent a significant increase of gel content due to weathering. Clear Organix mulch had the greater gel content (%) and also a slightly lower rate and extent of biodegradation under composting condition, especially as time increased. Under composting conditions, the order of biodegradation was PLA/PHA > Organix > Naturecycle > BioAgri. The enhanced rate of biodegradation for weathered mulches can be attributed to depolymerization (via a Norrish Type 1 photodegradative chain scission reaction and hydrolysis) and embrittlement, as observed via FTIR and GPC. The increased embrittlement will lead to more rapid size reduction during biodegradation in soil, hence, increasing the surface area exposed to the microorganisms. Microbial abundances were significantly higher on weathered than unweathered BDMs. Except for Organix, microbial activity was influenced more by bacterial communities than fungal communities.

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5. EFFECT OF AGEING ON THE STRUCTURAL INTEGRITY OF BIODEGRADABLE MULCHES

A version of this chapter will be submitted to a journal and will be titled as follows:

Marife B. Anunciado, Douglas G. Hayes, Larry C. Wadsworth, Annette L. Wszelaki, Jenny Moore, Shuresh Ghimire, Carol Miles. Effect of Ageing on the Structural Integrity and Performance of Biodegradable Mulches

I collected and analyzed the data and prepared the first draft of the chapter. Percent soil exposure (PSE) provided by Jenny Moore and Dr. Annette Wszelaki (Department of Plant Sciences, University of Tennessee, Dr. Shuresh Ghimire (College of Agriculture, Health and Natural Resources, University of Connecticut) and Dr. Carol Miles (Department of Horticulture, Washington State University) contributed to the work and were added to the manuscript but were not presented in this dissertation Drs. Douglas G. Hayes and Jennifer DeBruyn also provided edits and suggestions to improve the technical writing quality.

5.1 ABSTRACT

Biodegradable plastic mulches (BDM) provide benefits to specialty crop production to a similar extent as PE mulches. Prior to useful-life stage of mulches, it is critical that the structural integrity of BDMs is retained, particularly during storage, to ensure desired effectiveness and performance of BDMs in the field. Changes in physicochemical properties of BDMs due to ageing were investigated during 3-years of indoor storage in two laboratories (in the dark; 22^{o}C). Several measurements were performed once a year (Spring): mechanical properties (peak load and % elongation at maximum tensile stress), Fourier Transform Infrared (FTIR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, gel permeation chromatography (GPC) and thermogravimetric analysis, (TGA).

Indoor ageing of BDMs led to a small but significant decrease of peak load and elongation throughout storage. Changes of chemical properties and thermal stability were observed for two commercially available PBAT-based BDMs: BioAgri (Biobag Americas, Dunedin, FL, USA) and Organix (Organix Solutions, Bloomington, MN, USA, to a higher extent for the former). GPC results showed weight-averaged molecular weight (M_w) of PLA/PHA BDMs decreased significantly after 2 years of storage, whereas polydispersity index (PDI) significantly increased, indicating depolymerization. Changes of FTIR-ATR spectra during ageing reflect that hydrolysis occurred. NMR results showed PLA % for PLA/PHA mulch significantly increased during storage (via NMR and TGA). Unintentionally, BDMs can degrade even under ideal storage conditions. BDMs are manufactured and designed to last until the end of cropping season; but, BDMs are susceptible to degradation due to ageing while at storage prior to their useful life-stage. It is

important to know the maximum amount of time that BDMs should be stored to maintain and their physicochemical integrity to ensure that agricultural production is not compromised.

5.2. INTRODUCTION

Plastic mulches improve plant growth, crop yield and productivity due to modification of soil microclimate, conservation of water, suppression of weeds and elimination of soil amendments and the need for mechanical weeding (Lalitha et al., 2010; Zhang et al., 2019; Ming & Chen, 2020; Hayes et al., 2019; Kyrikou & Briassoulis, 2007). To resolve the ongoing accumulation of nonbiodegradable plastic waste resulting from conventional polyethylene (PE) mulches, biodegradable mulches (BDMs) were developed (Kasirajan & Ngouajio, 2012). BDMs are equivalently beneficial to specialty crop production as PE mulches (Brodhagen et al., 2015; Devetter et al., 2017; Ghimire et al., 2018; Miles et al., 2017; Moore & Wszelaki, 2019; Rujnić-Sokele & Pilipović, 2017). However, use of BDMs promotes sustainable cultivation through reduction of soil contamination by residual PE fragments (Scarascia-Mugnozza et al., 2006).

The machinery and procedure employed for mulch laying and insertion of holes for plant seeding can affect the degradation (e.g., abrasion, rips and tears due to the layer's guide wheels) (Ghimire et al., 2018; Zhang et al., 2019). Mechanical properties (e.g. tensile strength and elongation at break) are an important consideration in preparing BDMs not only for mechanical installation in the field but also to achieve satisfactory performance of BDMs during their useful life (Briassoulis & Giannoulis, 2018). BDMs are processed with mechanical properties and optimized chemical composition that enable them to last throughout the cropping season while retaining their ability to biodegrade (Briassoulis, 2004; Emadian et al., 2017; Vroman & Tighzert, 2009; Nogueira & Martins, 2019).

Mulch films deteriorate mechanically and chemically during their service life due to physical and chemical reactions attributed to weathering that results in bond scission and subsequent chemical transformation (discussed in Chapter 3, Shah et al., 2008). Degradation of plastics is a long-term process driven by ultraviolet (UV) irradiation, elevated temperatures, mechanical forces (e.g., wind) and loss of additives as a result of leaching (Emadian et al., 2017; Kasirajan & Ngouajio, 2012; Krueger et al., 2015). Under temperature stress, polymeric components can undergo depolymerization and susceptible functional groups (e.g. -OH, C-O-C) can undergo substitution reactions or cleavage (Rudnik, 2008c; Vroman & Tighzert, 2009). Temperature also directly affects other abiotic and biological chemical reactions that can induce thermo-oxidation in the polymers (Brodhagen et al., 2015). Crops can also impact the degradation of mulch films due to emergence of plants (e.g. seedlings, flower, fruits), weed proliferation, fruit set weight and pressure (e.g. pie pumpkin) and extensive vine growth on top of mulches (Ming & Chen, 2020; Ghimire et al., 2018; Moore et al., 2019). Hence, the degree of degradation can be affected by complex processes that involve approaches for laying, climatic conditions, and cropping systems (Moreno et al., 2017; Yin et al., 2019).

Due to their high market price, BDMs are often not perceived as an economical alternative to PE; however, when overall operational costs (e.g. labor costs) are counterbalanced by increased sustainability (i.e., reduction of plastic pollution in soil), then farmers can envision the transition from use of PE to BDMs (Marí et al., 2020; Velandia et al., 2020). Hence, farmers may consider storing BDMs for future use for subsequent cropping seasons to reduce operating costs, though it is never suggested by manufacturers. Some studies evaluated the degradation of aged mulches for a shorter period (12 months) and one-time assessment during one crop cycle (Mosnáčková et al., 2019; Scarascia-Mugnozza et al., 2006; Souza et al., 2019). Limited research has assessed the deterioration of BDMs during storage, moreover, the timeframe for which BDMs' integrity remains. This gap in knowledge has motivated us to pursue the objectives listed in the next section.

5.3. OBJECTIVES

BDMs are designed and manufactured with mechanical properties (e.g. tensile strength > 10 N and > 200% elongation in the machine direction) and structural integrity that discourage degradation prior to deployment for specialty crop production. When physicochemical properties are altered, agronomical performance of mulches can be compromised, leading to reduced crop yield and increased weed proliferation. The duration and conditions of BDM storage are therefore critical decisions for a farmer to consider to ensure retention of the desired physicochemical properties. The objective of this research is to understand how physical properties of mulch films change during indoor storage across years because of ageing. BDM rolls were kept under ideal indoor storage conditions for three years in two separate laboratories in TN and WA. Physical properties were determined each year, from 2015 to 2018. In addition, chemical properties of the BDMs were also studied to determine changes on the molecular structure.

5.4. EXPERIMENTAL

5.4.1 Materials

Mulches investigated for ageing are listed in Table 5.1 along with their physical properties and major polymeric components.

Table 5.1. Properties and manufacturer of mulch films used in this study (2015-2018) [1].

Mulch [2]	Thickness, (μm)	Peak Load, N [3]	Elongation, %	Polymeric Constituents [4]
PLA/PHA	37 ± 1.4	17 ± 0.5	246.0 ± 5.0	PLA/PHA blend
BioAgri [5]	29 ± 1.2	12 ± 0.6	295.0 ± 30.0	PBAT/starch blend
Organix A.G., Black [6]	20 ± 0.7	9 ± 0.4	270.8 ± 8.3	PBAT/PLA blend
Naturecycle [7]	57 ± 1.8	8 ± 0.3	244 ± 7.0	Starch/coployester blend
Polyethylene (PE)	40 ± 0.3	16 ± 0.6	567.5 ± 23.9	Linear low-density polyethylene

Mulch rolls were prepared and provided to us in the winter or early spring of 2015 and kept in storage until the summer of 2018 (3 years); however, replacement rolls of Naturecycle were provided to us in 2016 (1 year storage data) and these were employed for the remainder of the experiment. However, no Naturecycle mulch samples were available for physical testing in 2018. In Solvents (e.g. $CHCl_3$, $CDCl_3$) were "HPLC grade" and purchased from Fisher Scientific (Pittsburgh, PA, USA).

5.4.2 Methods

Mulches were stored indoors under controlled conditions in laboratories at Knoxville, TN and Mount Vernon, WA. Average temperatures under controlled condition in TN was 22°C ± 2.1 (2015), 18°C ± 2.0 (2016), 21°C ± 1.9 (2017) and 18°C ± 2.0 (2018). Under WA storage condition,

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[1] Errors reflect standard deviation. All mulches were black, except WGP which is a reddish-brown mulch.

[2] BioAgri was provided by BioBag Americas, Inc. (Palm Harbor, FL, USA); Naturecycle by Custom Bioplastics (Burlington, WA, USA); Organix by Organix Solutions (Maple Grove, MN, USA); WeedGuardPlus by Sunshine Paper Co., (Aurora, CO, USA); Experimental Product PLA/PHA by Filmtech Corporation (Allentown, PA, USA and PE by Filmtech Corporation (Allentown, PA, USA), respectively.

[3] Measurements were performed along machine direction of the films.

[4] PLA, PHA and PBAT refers to polylactic acid, polyhydroxyalkanoate and polybutylene adipate terephthalate, respectively.

[5] Mater-Bi® grade EF04P

[6] Prepared from ecovio® grade M2351 (BASF, Ludwigshafen, Germany)

[7] The mulch rolls prepared in 2015 degraded prematurely; therefore, the manufacturer provided new mulch rolls in 2016 that were monitored for the remaining two years of the study.

average temperatures were 19°C ± 2.1 (2015-2016) and 21°C ± 1.4 (2017-2018), respectively. Relative humidity was slightly higher in TN; around 56% ± 13.0 (2015-2016) and 47% ± 9.2 (2017-2018), than WA at 32% ± 8.7 (2015-2016) to 39% ± 10.4 (2017-2018).

Samples were taken off the mulch rolls in the early spring, with machine direction (MD) and cross machine direction (CD) identified. Tests were performed on mulch film (MD) each year every spring prior to each cropping season from 2015 to 2018.

Mechanical property assessment was determined through peak load (N) and percent elongation (%) at maximum tensile stress tests. Mechanical properties were analyzed using mulch samples taken along machine direction (MD) using standardized throughout 3-year storage using through Model 5567 Instron instrument (Norwood, MA, USA) employing ASTM D-5035. Six replicate samples for each mulch films (15.24 CD x 17.78 cm MD) were used to determine mechanical properties. A load cell of 10 kN and gage length of 2.54 cm was used instead of the recommended dimension (7.72 cm) by the standardized method, due to sample size limitations, as explained in our previous paper (Hayes et al., 2017).

^{1}H-Nuclear Magnetic Resonance (NMR) analysis was performed on PLA/PHA experimental film using a Varian 400 MHz NMR spectrometer (Agilent, Santa Clara, CA USA), with pulse width of 90°. Mulch (~20 mg) was dissolved in deuterated chloroform containing 1% tetramethylsilane as an internal standard (~800 µL). The polymeric composition of PLA/PHA, i.e., the relative amounts of PLA and PHA and of the 3- and 4-hydroxybutyrate units for the latter, were quantified.

Gel permeation chromatography (GPC) was used to determine weight-average molecular weight (M_w) and polydispersity index (PDI) of polymeric constituents of PLA/PHA and BioAgri samples. Subsamples of ~20 mg (four) of BDM were dissolved in 5 mL of chloroform. The mixture was stirred, centrifuged, and filtered to remove chloroform-insoluble particles. Filtered solution (200 µL) was injected into 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 x 7.5 mm ID PL Gel mixed D column purchased from Agilent (Santa Clara, CA, USA). Chloroform was used in mobile phase at a flow rate of 0.8 mL/min and a runtime of 15 min. Molecular weight values reported are based on polystyrene molecular weight equivalents (EasiVial PS-H, Agilent). The ELSD detector signal was corrected with response factors to account for the power law relationship between detector signal and concentration. Under the conditions employed, the GPC data reflects the contribution of PLA alone since the detector signal from PHA was relatively weak, and for BioAgri, PBAT is reflected due to low solubility of starch in chloroform.

Fourier Transform Infrared (FTIR) Spectroscopy was used to analyze infrared absorption spectra of surfaces exposed to sunlight using an IRAffinity-1 spectrometer (Shimadzu) equipped with a single reflection ATR system (MIRacle ATR, PIKE Technologies, Madison, WI, USA). Table 5.2 lists the spectral assignments.

Table 5.2. Peak assignments for FTIR analysis of plastic mulches. Source: Hayes et al., 2017.

Wavenumber	Mulch [8]	Contribution
3000-3700 [2]	PE (weathered), BioAgri, NC	OH stretch
2914, 2848	PE	C-H stretch
2994	PLA/PHA	C-H stretch
2956, 2920, 2876, 2846	PLA/PHA, BioAgri, NC, Organix	C-H stretch
1746	PLA/PHA	C=O stretch
1712 [3]	BioAgri, NC, Organix	C=O stretch (polyester)
1460	PE	C-H bend
1456, 1410, 1390	BioAgri, NC, Organix	$-CH_2-$ bend
1448	PLA/PHA	CH_3 bend
1356, 1384	PLA/PHA	-CH- deformation
1304, 1264	PLA/PHA	C=O bend
[1268, 1252, 1166, 1118, 1102, 1082]	BioAgri, NC, Organix	C-O stretch (polyester)
1180, 1128, 1082	PLA/PHA	C-O stretch
1076-1000	PLA/PHA, BioAgri, NC, Organix	OH bend
874	PLA/PHA, BioAgri, NC, Organix	C-H stretch
728	BioAgri, NC, Organix	$(CH_2)_4$ bend
716	PE	C-H rocking mode

Four replicates each of initial mulches and three samples from each mulch plot (surface exposed to the sun) were scanned between 4000 to 600 cm^{-1} using a spectral resolution of 4 cm^{-1} and 16 scans per spectrum. Spectral data were normalized by equating the integrated peak area of the entire spectrum to 1.0 (mean normalization).

Thermogravimetric Analysis (TGA) was carried out on all plastic mulches initially and after agricultural weathering. Mulch samples (~ 2 mg) were analyzed using a Discovery TGA (TA Instruments, New Castle, DE, USA) at a heating rate of 10 °C/min from room temperature (25 °C) to 600 °C in an unsealed platinum sample pan under a nitrogen atmosphere.

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[8] Abbreviations: PE = polyethylene; PLA = polylactic acid; PHA = polyhydroxyalkanoate; WGP = WeedGuardPlus (cellulosic) and NC = Naturecycle.

Experimental Location, Design and Planting and Agronomic Performance Assessment (2015-2018). Field trials were conducted on a 4-year field experiment (2015-2018) at the East Tennessee AgResearch and Education Center, Plant Sciences Unit, in Knoxville, TN, USA (35°52’52’’N, 83°55’27”W) and the Northwestern Washington Research and Extension Center in Mount Vernon, WA, USA (48°43’24’’N,122°39’09”W). Eight mulch treatment plots were arranged in a complete randomized block design with four replications and carried out consistently throughout four sampling years to avoid cross contamination among mulch treatments. More information on the plot designs were described elsewhere [Moore et al., 2019]. Mulches described in this paper were machine laid. In general, per instruction from the manufacturer, the "shinier" surface of the films faced upwards. The field trials as described above were employed in the assessment of ageing on mulch performance in 2015 and 2016 only. In 2017 and 2018, a non-replicated field trial was added in TN (peppers) and WA (pie pumpkin) to compare field weathering of mulches obtained in 2015 that was stored for two years.

A ‘Cinnamon Girl’ pie pumpkin was used as the test crop for 2015 and 2016 at both locations. In 2017 and 2018, ‘Aristotle peppers’ was employed in TN whereas WA utilized the same ‘Cinnamon Girl’ pumpkin used in 2015 and 2016. ‘Xtra-Tender 2171’ sweet corn was in WA, respectively. More detailed information on crop preparation and planting, fertilizer, and irrigation, and weed assessment and management of all sampling years is provided elsewhere [Ghimire et al., 2018; Sintim et al., 2019].

Agronomic performance of mulch was assessed through mulch deterioration ratings using percent soil exposure (PSE) technique. Mulch deterioration was visually evaluated and recorded by the same person twice a month over the cropping years. Assessment starts by monitoring the center row of each plot (1-meter width). PSE value of 0% represents a completely covered-soil (intact mulch), whereas 100% represents fully exposed-soil (Moore et al., 2019).

5.4.3 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and group means differences were compared using Tukey honestly significant difference (HSD) test at 5% level of significance. The statistical analyses were performed using JMP statistical program version 14 (SAS Institute Inc., SAS Campus Drive, NC, U.S.).

5.5. RESULTS AND DISCUSSION

Change of mechanical properties were assessed through peak load and elongation. Peak load of BDMs and PE determined under standard test conditions, at 1-year intervals (2015 to 2018) are shown (Figure 5.1). Under storage conditions in TN (Figure 5.1 A), all mulches except for Naturecycle and PLA/PHA underwent a decrease of peak load, whereas all mulches in WA underwent peak load decrease (Figure 5.1 B).

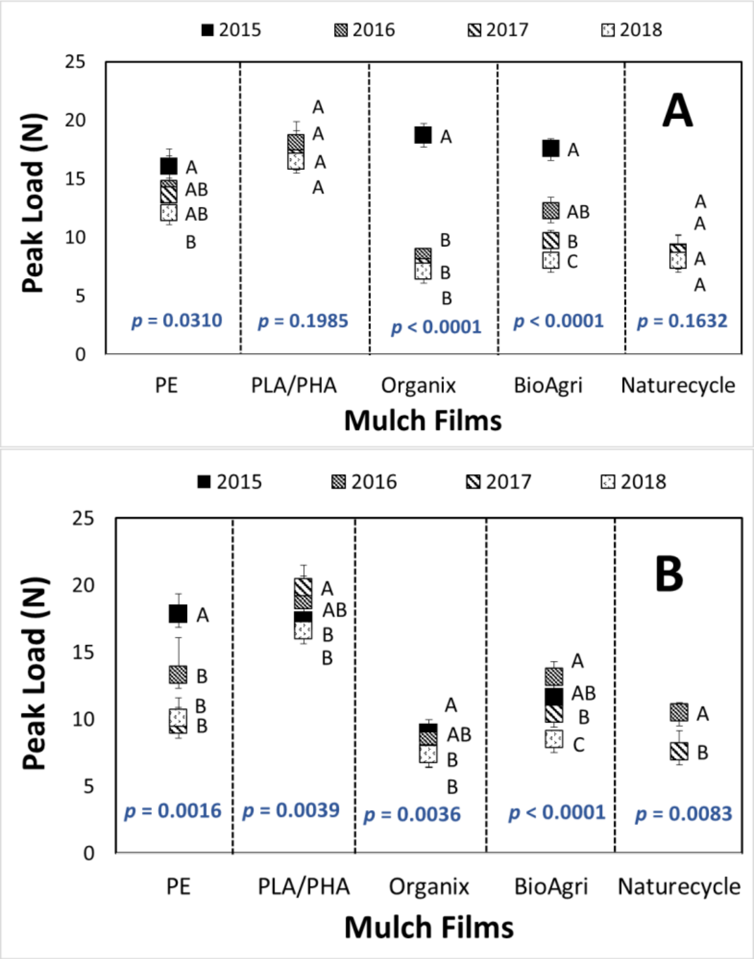

Figure 5.1. Change of peak load of PE and BDMs during 3-year storage in (A) TN and (B) WA. Note: Measurements were performed along machine direction of the films. Error bars represent standard deviation and letters represent treatment group means. Naturecycle (obtained in 2016) were only stored for a year (2016-2017) in WA.

Peak load of PLA/PHA slightly (but statistically insignificantly) increased in TN during storage; but, such a small difference may be attributable to variability in the sampling from mulch rolls. PE underwent a significant decrease of peak load in TN (p=0.0310) and WA (p=0.0016) in 2018 relative to 2017. BioAgri had a significant reduction of peak load between two storage locations, at 2017 in TN (p<0.0001) and 2018 in WA (p<0.0001) compared to the previous year (2016) (Figure 1B). Peak load of Organix significantly decreased in both locations after 1 yr of storage in TN (p<0.0001) and 2-yr storage in WA (p=0.0036) compared to the previous year (2016). PLA/PHA in WA underwent a decrease of peak load in 2017 relative to 2015 (p=0.0039). For Naturecycle, there was no significant decrease of peak load throughout 3-year storage in TN, whereas in WA, one year of storage (p=0.0083) caused a significant decrease of peak load.

Elongation of PE and BDMs stored in TN and WA (2015-2018) is plotted in Figure 5.2. Of all mulches used, only PE and Organix in TN (Figure 5.2A) and Naturecycle in WA (Figure 5.2 B) did not undergo any significant decrease over the 3-year period in TN and 1-year storage in WA, respectively.

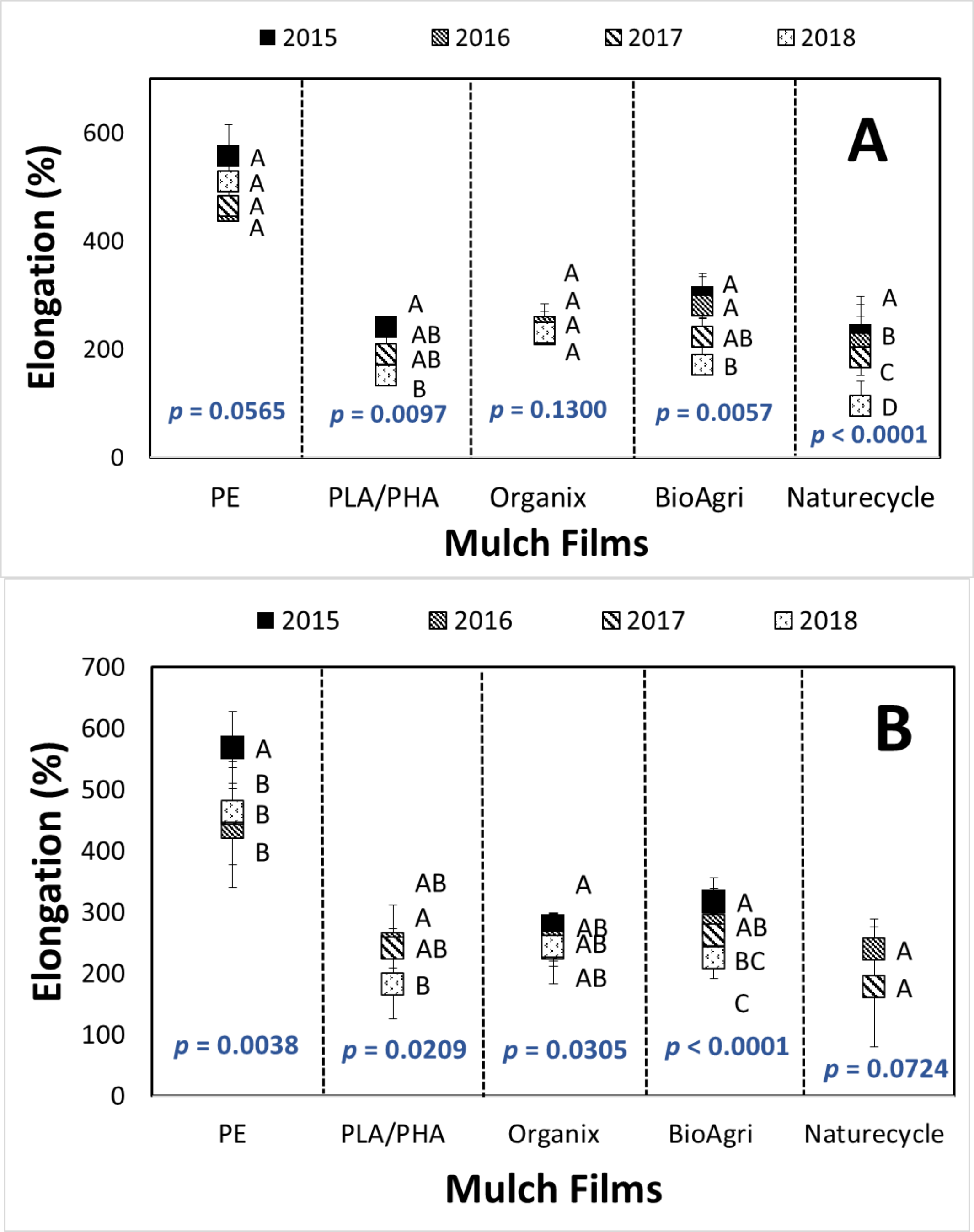

Figure 5.2. Changes of elongation of mulches during 3-year storage in TN (A) and WA (B). Note: Measurements were performed along machine direction of the films. Note: Naturecycle (obtained in 2016) were only stored for a year (2016-2017) in WA. Error bars represent standard deviation and letters represent treatment group means.

PE in WA, however, underwent decreased elongation after 1 year of storage (Figure 5.2. B; p=0.0038). Unlike PE, Organix had only a slight decrease of elongation in WA (p=0.0305). The elongation of PLA/PHA significantly decreased in TN (p=0.0097) and WA (p=0.0209) in 2018 relative to 2015. Naturecycle's elongation was not significantly reduced during one-year storage (2016-2017) in WA (p=0.0724). BioAgri underwent a significant decrease of elongation in TN in 2018 (p=0.0057) relative to 2015 with a similar decrease occurring earlier in WA: 2017 relative to 2015 (p<0.0001).

Weight-averaged molecular weight (M_w) and polydispersity index (PDI), analyzed through gel permeation chromatography (GPC), were determined during the 3-year indoor storage period (2015-2018) (Table 5.3).

Table 5.3. Change in molecular weight-related properties of the PLA component of PLA+PHA and the PBAT components of BioAgri storage mulches during 3 years of ageing under ideal conditions (Gel Permeation Chromatographic [GPC] analysis).

BDMs, Location	Year	Mw, kDa	PDI, SD
PLA/PHA, TN	2015	249 ± 30.00 D	1.51 ± 0.01 FG
	2016	268 ± 56.86 BC	1.52 ± 0.01 EF
	2017	287 ± 2.46 AB	1.84 ± 0.06 B
	2018	160 ± 5.22 F	1.67 ± 0.02 C
PLA/PHA, WA	2015	267 ± 10.28 CD	1.507 ± 0.01 G
	2016	313 ± 1.56 A	1.53 ± 0.03 E
	2017	302 ± 3.56 AB	1.86 ± 0.01 A
	2018	202 ± 19.49 E	1.65 ± 0.09 D
		location: $p < 0.0001$	**location: $p < 0.71$**
		year: $p < 0.0001$	**year: $p < 0.0001$**
		interaction: $p < 0.0076$	**interaction: $p < 0.0001$**
BioAgri, TN	2015	254± 5.75 CD	2.18 ± 0.07 C
	2016	255 ± 1.40 BC	2.02 ± 0.01 EF
	2017	221 ± 2.83 E	2.62 ± 0.02 B
	2018	138 ± 2.76 G	1.72 ± 0.12 G
BioAgri, WA	2015	244 ± 0.00 D	2.11 ± 0.00 D
	2016	295 ± 3.78 A	2.05 ± 0.03 DE
	2017	293 ± 3.02 B	3.12 ± 0.28 A
	2018	160 ± 1.19 F	1.84 ± 0.06 F
		location: p < 0.0001	**location: p < 0.0001**
		year: p < 0.0001	**year: p < 0.0001**
		interaction: p < 0.0001	**interaction: p < 0.0001**

M_w = weight-averaged molecular weight, PDI = polydispersity index; error bars represent standard deviation; values are based on 2 replicates. Error bars represent standard deviation and letters represent treatment group means.

M_w changes of PLA/PHA were significantly different between two storage locations ($p<0.0001$) and across years of storage ($p<0.0001$). M_w initially increased after 1-year to 2-year of indoor storage for both TN and WA. However, M_w significantly decreased after 3 years of storage in both locations. The same trends observed during biodegradation study (Chapter 4.5) also indicated an increase of molecular weight initially but followed a continuous decrease thereafter. As shown in Table 5.3, PDI significantly increased after 2 years of storage ($p<0.0001$) but not significantly different between storage locations ($p=0.71$).

For BioAgri, M_w significantly decreased in 2017 at TN and at a later storage time in WA (3-year). Decrease of M_w was significant between storage locations ($p<0.0001$) and across years ($p<0.0001$). PDI also showed a significant pronounced increase for both locations in 2017. PDI changes of BioAgri were also significant in two locations and across years of storage ($p<0.0001$).

Evidence from Fourier Transform Infrared – attenuated total reflectance (ATR-FTIR) analysis suggests minor deterioration of all BDMs (e.g., decrease in carbonyl stretching, C-O, and C-H stretching region, slight increase of OH stretch) as shown in Figure 5.3.

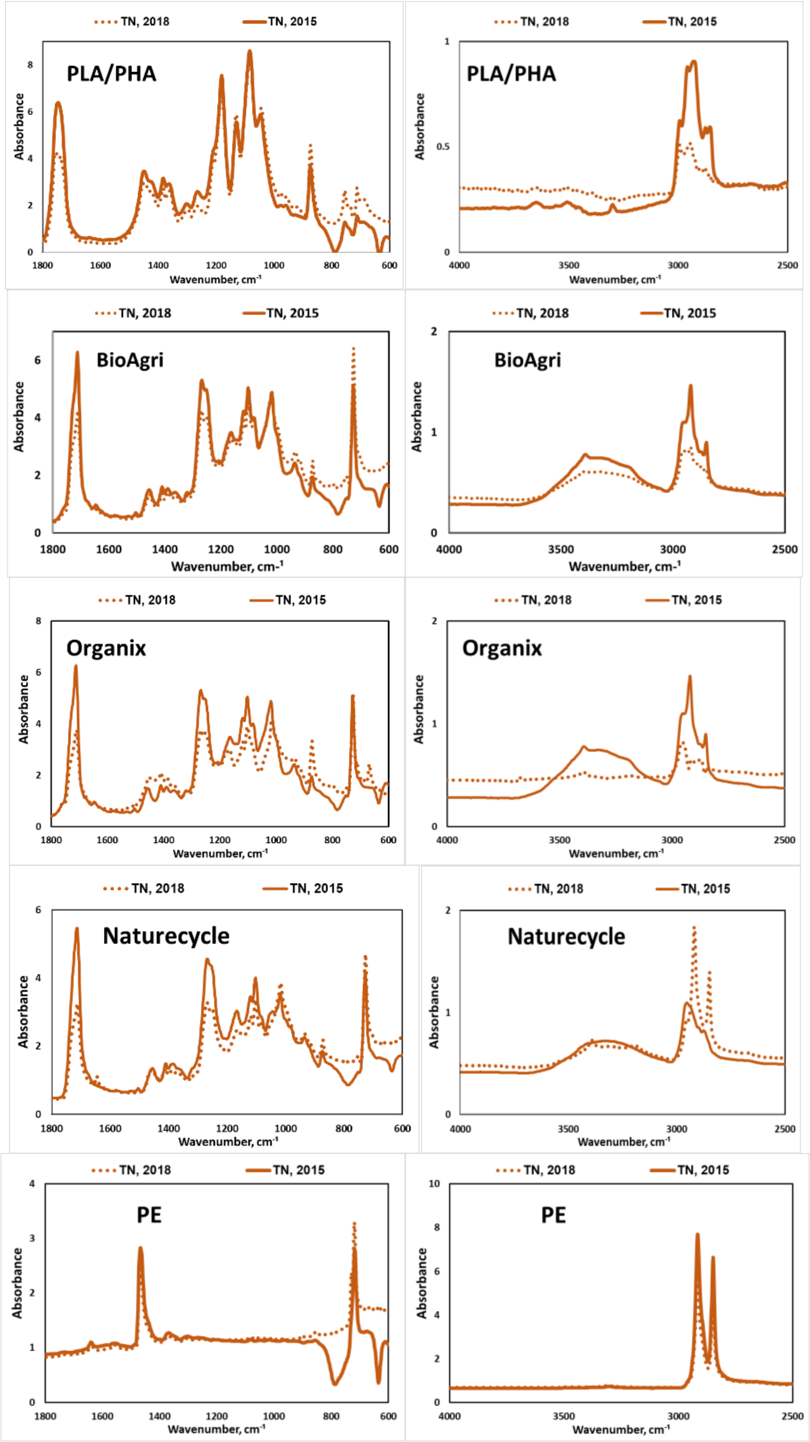

Figure 5.3. Changes on FTIR-spectra of mulches during 3-year storage in TN. FTIR spectra in WA reflects similar changes observed in TN.

BDMs under storage at WA underwent the same trends of FTIR changes as depicted in Figure 5.3 for TN. For PBAT-based BDMs, most spectral changes occurred after 2-year of indoor storage. The C=O and C-O stretching regions (1750 – 1700 cm^{-1}) of BioAgri, decreased after 2-year indoor storage. In the case for Organix, most major spectral changes were observed for WA stored mulches after 2 years (2017). The -OH and $(CH_2)_4$ bending regions increased for Organix mulch in WA but not in TN. However, Organix in both locations underwent decrease of -CH_2- bending, C-O and C-H stretching regions. Spectral changes such as increased peaks of -OH and -CH_2 and decrease of peaks of $(CH_2)_4$, C-H, -C-O, CH_2 and C=O were observed on Naturecycle. For PLA/PHA, pronounced spectral changes occurred after 1-year of indoor storage at both locations: decrease of peak intensity for ester-related bonds (C=O stretch, CH_3 bend, -CH- deformation, C=O bend and C-O stretch) and increase of intensity for C-H stretching bands, -OH bending and -OH stretching regions. Increase of -OH stretching regions may be due to formation of free -OH endgroups. The small peak (1650 cm^{-1}, spectra not shown) may represent the carbonyl stretch for free COOH (end) groups. PE, however, underwent no major spectral changes except for a slight increase on intensity of -C-H rocking peaks.

Changes in the polymeric composition of PLA/PHA throughout 3-year indoor storage time-course were determined through nuclear magnetic spectroscopy (NMR). Table 5.4 shows the change of %PLA among the biopolymers (i.e., 100% - %PHA) and 4PHB/PHA composition (%) vs. time for PLA/PHA in both storage locations.

Table 5.4. Change in composition of PLA+PHA mulch during 3-year storage (NMR analysis)

Storage samples	Wt % PLA	Wt% p4HB/PHA
TN, 2015	71 ± 0.700 [b]	43 ± 0.300 [a]
TN, 2016	68 ± 0.002 [d]	42 ± 0.002 [a]
TN, 2017	68 ± 0.400 [d]	42 ± 0.100 [a]
TN, 2018	69 ± 0.001 [c]	42 ± 0.500 [a]
WA, 2015	68 ± 0.300 [d]	42 ± 0.500 [a]
WA, 2016	69 ± 0.002 [c]	42 ± 0.002 [a]
WA, 2017	72 ± 0.100 [a]	42 ± 0.300 [a]
WA, 2018	72 ± 0.100 [a]	44 ± 0.001[a]
p-value	location: p<0.0001 year: p<0.0001	location: p=0.1209 year: p=0.1953

A slight but significant change was observed for PLA % (p<0.0001) vs. time: a decrease during storage in TN (after 1 yr), and an increase during storage in WA (after 2 yr). However, no significant difference was observed for the mass fraction of poly-4-hydroxybutyrate among the PHA at either location (p=0.12) and throughout the 4-year storage period (p=0.20).

Differential thermograms (DTG) of BDMs during 3-year indoor storage in TN and WA are shown in Figures 5.4.

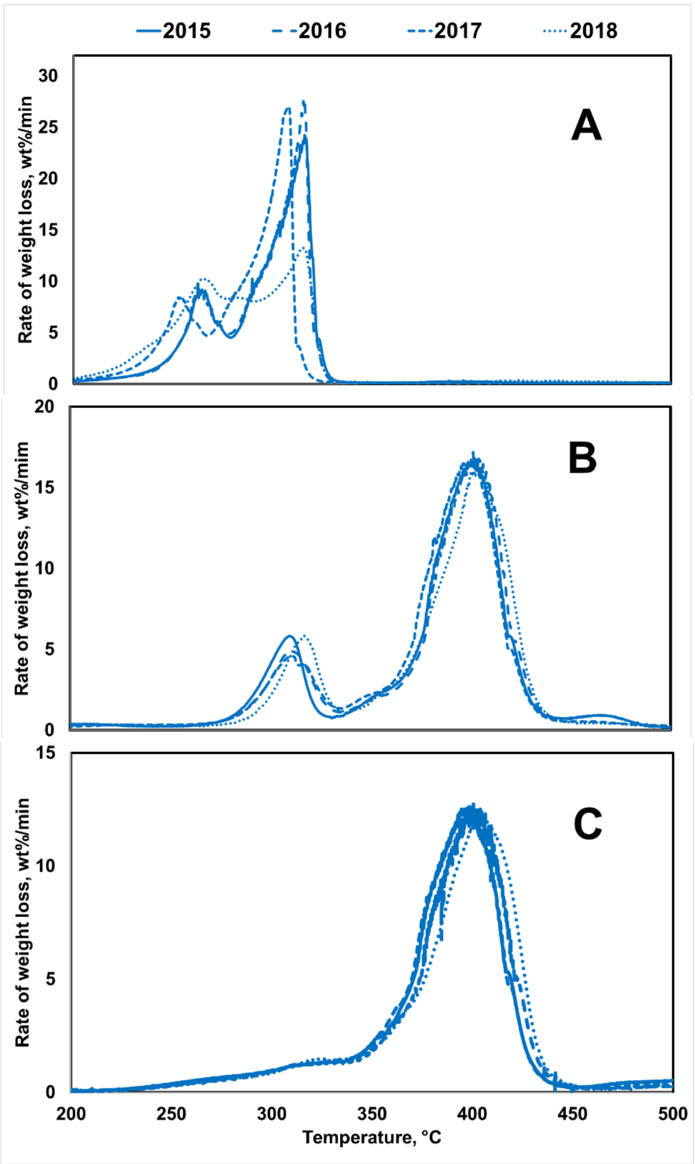

Figure 5.4. Changes of differential thermogram (DTG) of PLA/PHA (A), BioAgri (B) and Organix (C) during 3-year storage in TN. Thermogram of BDMs from WA reflect the same thermal changes.

The two heating stages of PLA/PHA (that slightly overlap in 2018), at 270°C and 320°C, represent PHA and PLA (Figure 5.4.A). The thermograms and DTGs shows a difference in the thermal stability of PLA/PHA mulches upon storage, with major changes occurring for PHA after one year of storage in TN and WA. On the other hand, the PLA heating stage in WA slightly shifted to higher temperature but only after one year of indoor storage. Both major heating stages of BioAgri, occurring at 325°C (starch) and 400°C (PBAT), increased slightly in mass% and shifted towards higher temperature throughout storage, particularly for starch after 1-year of ageing, for both TN and WA. The increase of the heating stage for PBAT, however, did not initiate until after 3 years of storage. As shown in Figure 5.5., the PBAT content slightly but significantly increased, an indication that there is a loss of lower molecular weight polymers from the BDM, particularly the starch constituent.

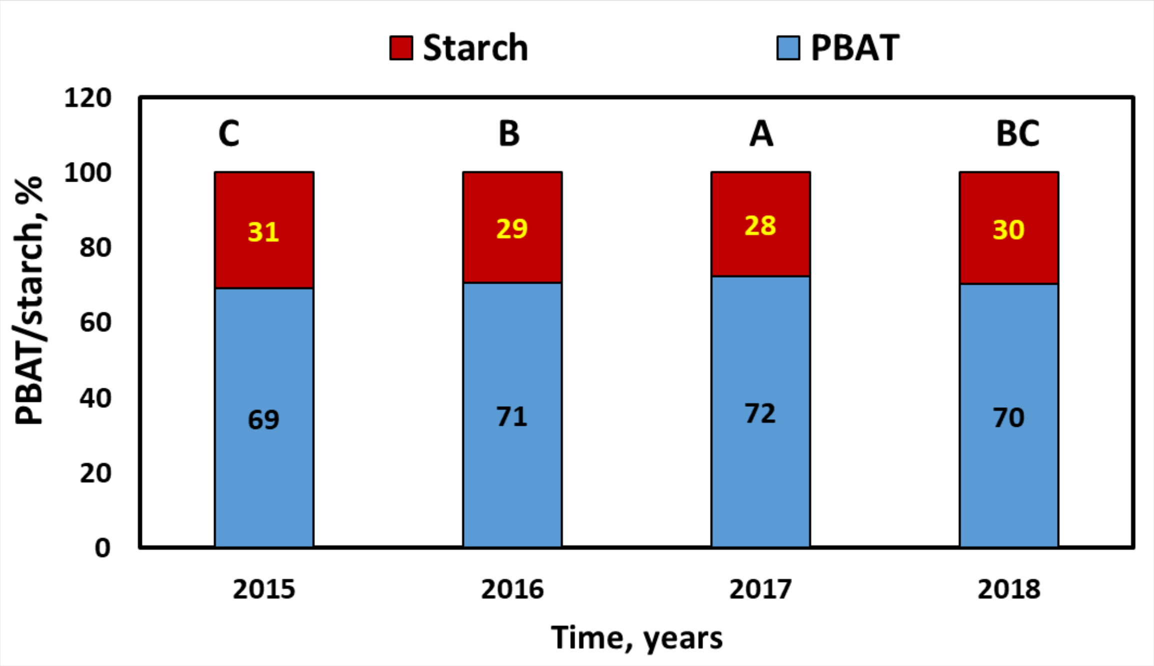

Figure 5.5. Change in PBAT/starch composition (%) of BioAgri during 3-year storage (Thermogravimetric analysis).

In contrast to BioAgri, DTGs for Organix reflect only minor changes during ageing at both storage locations, with the only significant change occurring at the end of the third year in 2018 (Figure 5.4.C). T_{onset} for the PBAT component of BioAgri slightly decreased after 1 year of storage in both locations, from 365°C to 363°C in TN and 368°C to 367°C in WA (Table 5.5).

Table 5.5. Change in degradation temperatures of BDMs during 3-year storage.

Polymeric Component, BDM	Storage Location	Years	T onset (°C)	T max (°C)
PBAT, BioAgri	TN	2015	365.0 ± 0.4 CD	400.9 ± 1.4 C
		2016	364.9 ± 0.4 CD	402.8 ± 1.0 BC
		2017	366.4 ± 2.9 C	401.2 ± 3.0 BC
		2018	363.2 ± 8.1 D	403.0 ± 0.4 BC
PBAT, BioAgri	WA	2015	367.7 ± 0.4 BC	400.3 ± 1.5 C
		2016	366.6 ± 0.4 BC	401.2 ± 1.0 C
		2017	388.8 ± 1.0 A	409.5 ± 1.0 A
		2018	369.8 ± 2.1 B	405.6 ± 1.5 B
p-value			**location: $p < 0.0001$** **year: $p < 0.0001$** **interaction: $p < 0.0001$**	**location: $p = 0.0076$** **year: $p < 0.0001$** **interaction: $p < 0.0001$**
PBAT, Organix	TN	2015	364.8 ± 0.6 BC	396.4 ± 0.1 C
		2016	365.2 ± 2.9 AB	400.1 ± 3.3 B
		2017	362.9 ± 1.0 CD	395.4 ± 0.6 C
		2018	360.2 ± 0.5 D	393.7 ± 3.1 A
PBAT, Organix	WA	2015	362.1 ± 2.0 CD	395.8 ± 0.4 C
			364.6 ± 1.5 BC	400.9 ± 3.8 AB
			361.7 ± 1.1 D	395.4 ± C
			366.5 ± 4.1 AB	401.9 ± 3.1 AB
p-value			**location: $p = 0.0002$** **year: $p < 0.0001$** **interaction: $p = 0.51$**	**location: $p = 0.67$** **year: $p < 0.0001$** **interaction: $p = 0.03$**
PLA, PLA/PHA	TN	2015	285.3 ± 1.4 AB	316.4 ± 1.2 AB
		2016	286.9 ± 0.9 AB	315.4 ± 3.1 BC
		2017	297.2 ± 1.4 A	309.9 ± 1.2 CD
		2018	290.7 ± 2.3 A	308.9 ± 0.7 D
PLA, PLA/PHA	WA	2015	291.6 ± 2.3 AB	314.7 ± 3.1 BCD
		2016	283.5 ± 3.7 AB	317.2 ± 2.7 AB
		2017	256.4 ± 2.6 B	310.6 ± 2.7 BC
		2018	302.7 ± 1.0 A	321.9 ± 2.0 A
p-value			**location: $p = 0.17$** **year: $p = 0.08$** **interaction: $p = 0.02$**	**location: $p = 0.02$** **year: $p = 0.0001$** **interaction: $p < 0.0001$**

T_{max} for BioAgri PBAT component in both storage, on the other hand, significantly increased to higher temperature in TN (400°C to 403°C) and WA (400°C to 406°C) from 2015 to 2018 ($p<0.0001$). For PBAT in Organix, a slight increase of T_{onset} were observed in TN (364°C to 365°C) and WA (362°C to 364°C) after 1-year of storage; but, a significant decrease of T_{onset} was observed in TN (365°C to 360°C) and an increase of T_{onset} in WA (362 °C to 366°C) ($p<0.0001$). T_{max}, however, consistently increased for both locations throughout the 3-year storage duration ($p=0.03$). T_{onset} of PLA consistently increased during 3-years of storage (2015-2018), from 285°C to 291°C in TN and 288°C to 310°C in WA. In contrast, T_{max} for PLA underwent a significant decrease from 316 °C to 309°C in TN (2015 to 2018) and an increase from 315 °C to 322°C in WA (2015-2018) ($p<0.0001$).

Agronomic performance (e.g. weed suppression) was assessed through percent soil exposure (PSE). PSE differed between years in TN (2015 vs. 2018) and WA (2017 vs. 2018), as shown in Figure 5.6.

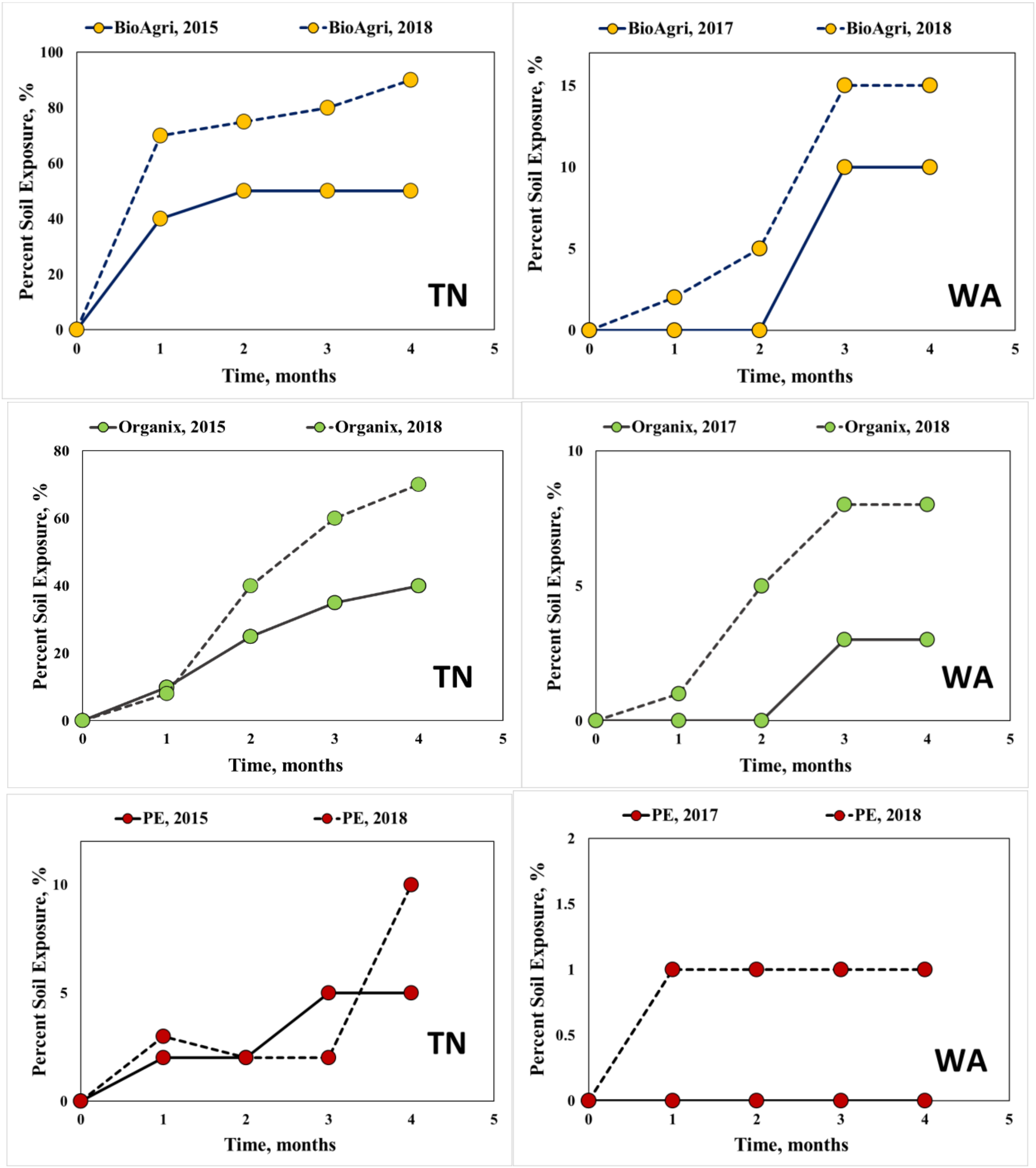

Figure 5.6. Percent soil exposure (PSE) of BDMs and PE utilizing peppers in TN (2015 vs. 2018) and pie pumpkin in WA (2017 vs. 2018). Note: Naturecycle was used in TN field only, so data is not presented. Data (unpublished) were collected and kindly provided to me by Dr. Annette L. Wszelaki, Plant Sciences Department, University of Tennessee and Dr. Carol Miles, Horticulture Department, Washington State University.

PSE rate had consistently increase in both field sites throughout the cropping season. For Naturecycle (provided by manufacturers in 2016, only used in TN field site), change of PSE was relatively similar between the two years, achieving 80% of PSE after the cropping season. Decrease of PSE occurred to a higher extent and earlier compared to other PBAT-based mulch, BioAgri and Organix. PSE of BioAgri in TN increased from 40% in 2015 to 90% in 2018 and slightly increase from 10% in 2017 to 15% in WA in 2018. For Organix, a significant increase of PSE occurred in TN from 40% to 80% (2015 to 2018) and 3% to 8% in WA (2017 to 2018). PSE of PE increase from 5% to 10% whereas WA had a slight increased from 0% to 1% PSE. Except for PE, ageing impacted the integrity of BDM; thus, greater PSE decrease occurred with increased duration for BDMs. The impact of deterioration during storage on PSE is greater in TN than WA, which may be attributable to the more extreme environmental conditions in TN (e.g. high soil and air temperature, soil moisture, per Section 3.6). As mulches loses mechanical properties, their ability to sustain environmental conditions throughout cropping season can be compromised (Briassoulis, 2004; Emadian et al., 2017; Vroman & Tighzert, 2009).

5.6. CONCLUSIONS

The degradation of BDMs and conventional PE during 3 years of indoor storage was studied. Temperature and RH in TN and WA labs, where storage took place, were favorable and nearly identical. BDMs significantly lost mechanical strength and elongation throughout the 4-year period, and even after one year of storage. Depolymerization occurred during storage, reflected by M_w decrease and PDI increase, slightly for BioAgri but to a greater extent for PLA/PHA. FTIR spectra suggest that hydrolysis occurred for BDMs as storage time increased, particularly for PLA/PHA experimental mulch film. NMR analysis showed significant increase of PLA % for the latter BDM, indicating that loss of lower weight oligomers or monomers may have occurred. Thermal stability decreased for BioAgri and Organix, and for PLA/PHA to a greater extent. Even under ideal storage conditions, inherent deterioration of mulch cannot be avoided and prevented. Agronomical performance, assessed through percent soil exposure (PSE), resulted in a significant increase of PSE for the next cropping season. The impact of ageing led a slight decrease of physical properties of BDMs and more variability on changes of chemical properties.

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6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Use of plastic mulches in agriculture is an essential tool for farmers to an increased yield and better specialty crop production. PE mulches, commercially available worldwide, do not biodegrade and end up in a landfill after field-use, or are stockpiled on farm due to the absence of recycling options. Use of biodegradable polymers as mulch component will help reduce accumulation of waste in the landfill. In contrast to PE mulches, biodegradable mulches (BDMs) returns into the environment as a biomass, releasing other products (e.g. CO_2, water and minerals) through microbial action. BDMs provide agronomic benefits to farmers to the same extent as PE.

After polymers' initial life stage (e.g. sourcing and feedstock preparation), plastic mulch films are manufactured appropriately to attain desired physicochemical properties (e.g. high tensile strength and elongation) needed for their useful-life stage. Chapter 5 discussed how these physicochemical properties can change during storage (3-year) prior to field-use, thereby demonstrating the need for farmers to carefully consider mulch storage conditions and duration During their useful-life stage in specialty crop production, mulch films provide benefits: weed reduction, soil moisture retention and soil temperature modulation. At the same time, exposure to environmental factors (e.g., solar radiation and fluctuations in soil moisture, soil, and ambient air temperature) induces degradation of mulch material. Chemical transformations that occur during agricultural weathering cause embrittlement of mulch films, resulting to soil incorporation of mulch's biodegradable components in the soil. Chapter 3 discussed how these physicochemical properties change and differ due to environmental weathering conditions (TN and WA).

When mulches are biodegradable (i.e., BDMs), they can be tilled into the soil or composted. Weathering during mulch films' service life has a strong impact on biodegradability. Chapter 4 discussed how biodegradation can be enhanced or inhibited by weathering and underlying factors of biodegradation. In general, one life-stage has an implication to another life stage of mulch film's life cycle, and all are interconnected and reflects how the overall performance of mulch during crop production.

The first research chapter (Chapter 3) focused on degradation of BDMs through agricultural weathering during field trials conducted during the summer in locations representing two diverse climatic conditions over a 4-year period. Mulch products investigated were composed of PE, PLA, PHA, PBAT and their blends (e.g. with starch). Even though different crops were used and climate varied between years, mulch deterioration (e.g., loss of mechanical properties) did not vary extensively between years. Peak load and elongation at maximum tensile stress significantly decreased due to weathering, particularly for PBAT-based BDMs; yet, trends differed between the PBAT-based mulch products. The observed decrease of thermostability and molecular weight indicates degradation of BDMs in both locations on a molecular level. Despite the occurrence of extreme climactic events (e.g. drought and intense rainfall), their impact on physicochemical properties of BDMs was minor. There are some secondary factors that may slightly affect the durability of mulches in the field: mulch color, temperature, polymeric composition of the films as well as fillers and other minor components. PLA can serve as a viable component of a polymeric blend serving as a BDM feedstock; but, it can be susceptible to degradation in the field particularly in places with higher temperatures.

The second research chapter (Chapter 4) evaluated how weathering during field-use impacted microbial utilization of BDMs in soil and compost. Biodegradation of BDMs in soil and under composting conditions was significantly enhanced by weathering. Biodegradation varied between BDMs and polymeric composition. An experimental BDM (PLA/PHA) had higher biodegradation than other (PBAT-based) BDMs, both in soil and compost. The extent and rate of biodegradation for BDMs was higher under composting conditions than in soil.

The third research chapter (Chapter 5) evaluated how ageing affected the structural integrity of BDMs. Even when mulches were kept under favorable storage conditions during a three-year duration (2015-2018), loss of mechanical properties, particularly decrease of %elongation, occurred. Agronomical performance assessment showed an increase of percent soil exposure (PSE) in the succeeding years, indicating the deterioration of physicochemical properties of BDMs negatively impacted their structural integrity in the field during specialty crop production. Thus, it is critical that the maximum amount of time allowable for storage to ensure that mechanical functionality be retained so that mulches' performance during their service life is not compromised.

6.2. RECOMMENDATIONS

There are limitations for this study. The following recommendations for future study address the limitations:

- Field trials only covered a 3-month period, which will not be relevant if mulch films will be intended for multiple crop rotation. Hence, a study on mulch durability should be performed using a cropping system that assesses long-term trends of degradation in the field.
- There should be a rigorous assessment on the ability of crops and canopy cover to intercept solar radiation, thereby reducing the amount of sunlight received by the BDM films and as a result minimizing photodegradation of BDMs. Changes of physicochemical properties due to weathering did not differ between years between locations, hence, the crop effect may be a minor, secondary factor that influences degradation of BDMs. Yet, it is still important to evaluate and predict crops' influence on degradation of BDMs
- Visual tools such as SEM micrographs would have been a helpful indicator of microbial activity on mulch surfaces and form biofilms.
- Future work should include ecotoxicological assessment to evaluate ecosystem functions before and after biodegradation of mulch in the soil as a new carbon input and other constituents (e.g. inorganics).
- Considering that mechanical properties decrease during storage, it is important to perform microbial abundance tests to assess degradation possibly associated with microbial activity.

VITA

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