Degradation, Efficacy, and Environmental Impact of a Sprayable Degradable Plastic Mulch

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Abstract

Mulching is the practice of spreading any material (natural or synthetic) over the soil surface to enhance soil microclimatic factors that ultimately decrease crop-growing time and increase crop yield. Polyethylene (PE) sheets are typically used as agricultural mulch, with over one million tonnes used annually. Unfortunately, this practice has led to deleterious effects, particularly associated with the fate of the used PE, which cannot be readily reused and may lead to microplastics accumulating in the soil. An alternative technology is the use of biodegradable plastic mulches (BPM), and a relatively new idea is the use of sprayable BPM for their ease of application, and inherent customisability. Although the work presented in this thesis focuses on one particular novel sprayable polymer, a polyester-urethane-urea (PEU), the totality of work is intended to represent a template for the holistic evaluation of any new BPM prior to their widespread use. Presented within are a series of studies that interrogate the PEU's water savings efficacy, the PEU's effect on plant growth, the PEU's effect on soil health, its degradation and biodegradation in a variety conditions, and its environmental impact.

A full factorial, on-soil degradation study in a temperature-controlled glasshouse revealed that the PEU was effective as a water saver on a variety of soil types, and that the most important factors controlling its degradation rate were soil moisture content, and soil type. In this study, enhanced CO_2 emissions were observed on soils treated with the PEU which was the first piece of evidence that it was properly biodegrading.

'Soil type' is a nebulous term within which a variety of factors are contained, and so to elucidate what properties of soil were more important in controlling the PEU degradation rate, a series of experiments was conducted. It was found that soil pH was the most important soil characteristic in controlling PEU degradation, with a lower pH leading to more rapid degradation. It was also found that the soil microbial community colonised the

PEU and its composition played a role controlling the rate of degradation, provided there were sufficient nutrients to allow the community to thrive.

A liquid chromatography-mass spectrometry analysis was carried out on the degradation media of the PEU in sterile water and two different soils. Results revealed the identity of the primary degradation intermediates. When degraded in a soil medium, the primary abiotic degradation intermediates were not present, indicating that soil microbes were metabolising or modifying them in some way. This was another piece of information that the PEU was properly biodegrading. Some of the identified degradation products at very high concentrations inhibited the germination of plant seeds.

The effect of the PEU treatment on the soil microbial community composition was interrogated in a cropping system. It was found that the PEU treatment did not alter the soil microbial community's functioning for a variety of enzymes, but it did change the relative abundances of bacterial, archaeal, and fungal taxa. The most important finding was that the PEU treatment increased the relative abundance of plant growth promoting microbes, which could be important for increasing crop productivity, though further study is needed to confirm this.

Lastly, a tomato growth study was undertaken to determine the PEU's effect on crop yield, but, the study did not provide conclusive results. It was however observed that after almost one year of degradation, there was no recoverable PEU film in half of the experimental units, which was another piece of evidence indicating biodegradation.

Throughout the studies, a substantial reduction in molecular weight (to less than 10% of the initial M_n and less than 2% of the initial M_w) was observed. This taken together with the identification of low molecular weight degradation intermediates, mineralisation to CO_2 and the complete absence of PEU under well-fertilised soil degradation conditions (quantified via GPC measurements yielding no detectable PEU) give strong evidence of biodegradation as the primary process of degradation of the PEU polymer. Overall, the

PEU represents a promising mulch technology with the potential to replace the benefit provided by PE while subverting the deleterious environmental consequences.

Declaration

This thesis is an original work of my research and contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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Publications during enrolment

Chapter 2:
Borrowman, C. K., Johnston, P., Adhikari, R., Saito, K. & Patti, A. F. Environmental Degradation and Efficacy of a Sprayable, Biodegradable Polymeric Mulch. *Polym. Degrad. Stab.* (2020).

Chapter 4:
Borrowman, C. K. *et al.* LC-MS analysis of the degradation products of a sprayable, biodegradable polyester-urethane-urea. *Polym. Degrad. Stab.* (2020).

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes two original papers published in peer reviewed journals and one submitted publication. The core theme of the thesis is the holistic understanding of a novel polyester-urethane-urea biodegradable mulch. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Chemistry under the supervision of Antonio F. Patti, Kei Saito, and Raju Adhikari.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of *Chapter 2, Chapter 3, Chapter 4, Chapter 5, Chapter 6* my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2	Environmental Degradation and Efficacy of a Sprayable, Biodegradable Polymeric Mulch	Accepted	75% concept design, all glasshouse work, all lab work, all data analysis, manuscript writing and editing	1) Priscilla Johnston, help with experimental design, editing manuscript 10% 2) Raju Adhikari, concept development and editing 5% 3) Kei Saito, concept development and editing 5% 4) Antonio F. Patti, concept development and editing 5%	N
3	Hydrolytic and Long-term Environmental Soil Degradation of a Sprayable, Polyester-urethane-urea Polymer	Prepared as Manuscript	85% concept design, all outdoor set-up and maintenance, all lab work, all data analysis, manuscript writing and editing	1) Raju Adhikari, concept development and editing 5% 2) Kei Saito, concept development and editing 5% 3) Antonio F. Patti, concept development and editing 5%	N
4	LC-MS analysis of the degradation products of a sprayable, biodegradable	Accepted	70% concept design, all lab work, most data analysis, manuscript writing and editing	1) Mark Bücking, concept development and editing 5% 2) Bernd Göckener, concept development	N

	polyester-urethane-urea			help with data analysis, editing 10% 3) Raju Adhikari, concept development and editing 5% 4) Kei Saito, concept development and editing 5% 5) Antonio F. Patti, concept development and editing 5%	
5	Sprayable Biodegradable Polyester-urethane-urea Mulching Treatment Increases Abundance of Plant Growth Promoting Microbes (PGPM) in Soil	Prepared as Manuscript	75% concept design, all glasshouse work, all lab work (sequencing outsourced), most data analysis, manuscript writing and editing	1) Karen Little, concept development help with data analysis, editing 10% 2) Raju Adhikari, concept development and editing 5% 3) Kei Saito, concept development and editing 5% 4) Antonio F. Patti, concept development and editing 5%	N
6	A comparative Plant Growth Study of a Sprayable degradable Polyester-urethane-urea mulch and two commercial plastic mulches	Submitted	80% concept design, all glasshouse work, all lab work, all data analysis, manuscript writing and editing	1) Karen Little, concept development and editing 5% 2) Raju Adhikari, concept development and editing 5% 3) Kei Saito, concept development and editing 5% 4) Antonio F. Patti, concept development and editing 5%	N

**If no co-authors, leave fields blank*

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Cuyler Borrowman

Student signature: **Date:**

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Antonio F Patti

Main Supervisor signature: **Date:**

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If any future PhD candidates happen to be reading this, I promise it never felt like I was getting it right, but I'm so happy I got it done. The Imposter Syndrome is real and it is mean, but you got this.

Table of Contents

1. Introduction

1.1 Background

According to Prosdocimi et al.[1], a mulch is any non-living material placed on the soil surface used to protect the soil surface from erosion, conserve water, and enhance plant growth. The practice of applying mulch, 'mulching', has long been used to alter the microclimates of soils and increase crop yield by increasing soil temperature[2], suppressing weed growth[3,4], enhancing soil water capacity[5,6], decreasing soil water evaporation[3,4,7,8], and reducing the effects of pests[9–11]. Many types of natural mulch materials have been used, including rock fragments[12], organic forest debris[13], wheat straw[6], wood strand and organic hydromulches[14], but they suffer a number of drawbacks. In particular, the availability and quality of natural mulch materials are highly variable, their deployment can be laborious, and they can also slow soil warming thus lowering crop productivity, which opposes one of their primary purposes, which is to enhance crop productivity[15].

It wasn't until the 1950s that concept of using plastic for mulching was first studied and implemented by Dr. Emmert at the University of Kentucky[15]. Using plastic for mulching is often a cost effective alternative to organic mulching[16] that shares many of the advantages of other mulching materials, with some additional benefits. For instance, Schonbeck studied the weed suppression capacity of organic (plant material), paper, and plastic mulches and found that plastic mulch was most effective at suppressing weed growth[17]. In another study Schonbeck et al. compared black plastic mulch with three types of paper mulch and hay mulch and found that soil temperature was increased most and for the greatest duration with black plastic mulch[2]. An important downside to organic mulches is that they can actually increase pest populations by providing a habitat for pests, which often vector diseases, and thus organic mulch may cause disease to spread through crops more quickly[18] whereas coloured plastic mulches can reduce pest populations[9]. Additionally, plastic mulches provide greater resistance to extreme weather conditions. For example, they are less easily blown from the soil surface by large winds than organic mulches due to their comparatively large mass, and they are highly resistant to erosion; whereas inclement weather can cause organic mulches (composed of many low mass pieces of plant

matter) to degrade quickly or blow away completely[19]. Waggoner et al. studied the relationship between several soil microclimatic factors (i.e. soil water evaporation, nutrient leaching, soil temperature, and crop yield) and different mulching materials in a variety of conditions and determined that polyethylene (PE) plastic film was most effective[20]. Due to the many advantages of plastic mulch, its use has become ubiquitous. In their 2016 review article, Steinmetz et al. found that the most common agricultural mulch base is PE[19]. It has been estimated that 700,000-1,000,000 tons/annum, and over 800,000 hectares of land each year are covered by plastic mulch[21–23]. PE degrades extremely slowly in the environment, in fact, Briassoulis et al. used UV light and heat to artificially age by several decades a PE film with added pro-oxidants, and after allowing the pre-aged film to further degrade over 7.5 years in soil they found that the film was mostly intact with only some mechanical degradation[24]. The abundant use of PE based mulching films, combined with their recalcitrance, poses several significant concerns.

PE and similar nondegradable plastic mulch films are single or limited use materials that must be disposed after use, and they comprise a significant amount of the total plastic waste stream (20 vol% worldwide)[21]. There are three primary disposal methods for nondegradable plastic mulches: landfilling, recycling, and incineration. Recycling of plastic mulch film is a relatively 'environmentally friendly' disposal fate, but unfortunately plastics can only be recycled when in a relatively 'clean' state, which is about 5% contaminants by weight, but plastic mulch film that comes out of the field is typically excessively dirty and frequently exceeds 50% contaminants by weight (soil, plant matter, moisture, and other pollutants).[25] For the plastic mulch that comes out of the field clean enough to be recycled, the recycling process still requires collection, compaction, and transport of the plastic waste which is both laborious, costly and resource intensive. These complications mean that large amounts of plastic mulch film end up being disposed of via landfilling and incineration.

Incineration of plastic mulch poses its own environmental risks. Often the most cost effective disposal method for plastic mulch films available to a grower is simply to stockpile used

mulch film on-site and burn it. Unfortunately, the incineration process emits high amounts of greenhouse gases (CO_2), and creates carcinogenic and ozone destroying compounds such as polycyclic aromatic compounds (PAC) and CO[26]. Worse still, if plastic mulch is not incinerated at sufficiently high temperatures then dioxins are formed at high levels[19], and because plastic mulch incineration is typically carried-out on-site this provides a source for toxic compounds to enter the food chain at its origin.

When plastic mulches are not recycled or incinerated they necessarily must be landfilled. Landfilling presents its own environmental problems for a number of reasons. For one, there is a finite amount of landfill space available, so it is irresponsible to continually fill landfills with used plastic mulch. Once in a landfill, plastic mulches will be exposed to conditions unfavourable to degradation (low oxygen and low sunlight), thus taking longer to degrade. Furthermore, PE sheets have been shown to adsorb pesticides while in agricultural soil, and then serve as a source of pesticide pollution to groundwater once in a landfill[27] as the adsorbed pesticides slowly leach away.

In addition to the disposal problems, nondegradable plastic mulches have several other environmental concerns. Many plastic mulch films have additives mixed in with the polymer during production. Plasticizers (phthalic esters, commonly referred to as phthalates) are common additives in PE which are typically bound within the polymer matrix by weak physical interactions, and have been shown to leach into soil[28] and be taken up by plants (crops)[29,30]. Pro-oxidants, including TiO_2[31] and various transition metal stearates[32], are also commonly added to PE mulch and then accumulate in the field over repeated applications[33]. The use of pro-oxidants in nondegradable plastic mulches such as PE is especially problematic because they may encourage the formation of smaller pieces of plastic[34,35], termed ‘microplastics’, which can be ingested by, and harmful to soil invertebrates[36,37].

1.2 Biodegradable plastic mulches

Despite the environmental problems posed by the use of plastic mulches, their agricultural productivity benefits cannot be ignored. The United Nations predicts increasing food and water insecurity in the coming years, and the agricultural sector, which currently accounts for

approximately 70% of global freshwater consumption,[38] will have to become more productive while using less water, so it is clear that the practice of using plastic mulch cannot be abandoned[39–41]. Fortunately, many of the problems posed by nondegradable plastic mulches may be overcome with the use of biodegradable plastic mulches (BPMs).

In order for a material to be biodegradable, according to ASTM International (formerly The American Society for Testing and Materials), it needs to degrade into CO_2, H_2O, small inorganic compounds and biomass, and leave no visually distinguishable residue nor toxic residue[42]. Additionally, degradation should occur over timescales similar to other known compostable materials, and strictly speaking degradation should be done through the action of naturally occurring microbes.[42] A plastic mulch that meets these standards would not have the same environmental concerns regarding disposal as its nondegradable counterparts. By definition, there would be no need for disposal as all trace of the mulch would be gone, either mineralized or converted into biomass. This alleviates much of the stress posed to biota by the formation and presence of microplastics, eliminates the problem of pesticides or other toxic compounds being adsorbed and reemitted into the environment, and prevents the formation of volatile toxic emissions during incineration.

Recently, in 2017, the European Committee for Standardization published a standard entitled, "Plastics – Biodegradable mulch films for use in agriculture and horticulture – Requirements and test methods".[43] This document includes, for the first time, standardized definitions which delineate differences between biodegradation, degradation, disintegration and photodegradation with respect to BPM. According to this document, for a BPM to be classified as biodegradable, it must achieve a 90% conversion of its organic carbon into CO_2 (when compared with a reference material such as cellulose) in a time period no longer than 24 months. This must be accomplished within strictly defined incubation conditions and with a well-defined soil, as stipulated in the document. The standard also provides guidelines for volatile solids content, heavy metal levels, material properties and ecotoxicity requirements. The ecotoxicity tests are put in place

to “investigate possible adverse effects caused by the material of the mulch film and residues as intermediates (degradation products),” and they include testing on the effects on plant life, soil invertebrate life, and the soil microbial community.

1.2.1 Polymer Biodegradation Process

The biodegradation process of a BPM proceeds in general through a series of steps, beginning with the colonisation of the polymer film by microbes (bacteria, archaea, and fungi). After colonisation, microbes begin to excrete exoenzymes capable of hydrolysing susceptible moieties in the polymer backbone. Exoenzymes depolymerise the polymer backbone into increasingly smaller pieces (oligomers and monomers, collectively ‘degradation intermediates’), and eventually the polymer degradation intermediates become small enough to be taken up by nearby microbes. Once taken up, microbes utilize the degradation intermediates as a source of energy and carbon. The ultimate fate of the polymer degradation intermediates is mineralisation to CO_2 (or CH_4 under anaerobic conditions), inorganic N (NH_3 and NO_3^-) in cases when N is present in the polymer, and incorporation into microbial biomass[44].

The degradation process described above proceeds via one of two main mechanisms: surface erosion or bulk erosion[45]. Surface erosion occurs due to the inability of water to penetrate into the bulk of the polymer through its amorphous regions, so polymer breakdown proceeds from the polymer surface into the bulk. Enzymatic hydrolysis is the primary depolymerisation mechanism in surface erosion. This is contrasted by bulk erosion, in which water is able to percolate through the polymer bulk. In bulk erosion, abiotic hydrolysis takes place throughout the polymer bulk, as the name would suggest, and that process competes with the enzymatic degradation occurring on the polymer surface. In other words, under both mechanisms surficial breakdown is occurring, but when bulk erosion dominates, the diffusion rate of water through the polymer bulk is greater than the rate of enzymatic hydrolysis on the polymer surface.

Polymer breakdown is faster under a bulk erosion mechanism because of the depolymerisation occurring across the polymer film’s cross section, and it is characterised by a rapid initial reduction in molecular weight. Surface erosion, conversely, is characterised by a slow

initial reduction in molecular weight until a point where the majority of the polymer is available to enzymatic attack, at which point the molecular weight reduction proceeds rapidly (Figure 1).

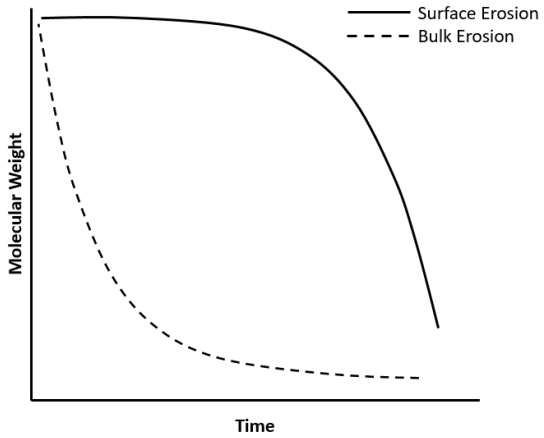

Figure 1. Time profile of polymer molecular weight for surface and bulk erosion biodegradation mechanisms. Figured adopted from Kijchavengkul et al. 2008[45].

Environmental factors understood in the literature to affect the rate of biodegradation include availability of water, acidity/alkalinity of the surrounding environment, and composition of the surrounding microbial community[44,45]. Water is important both because it can cleave the polymer backbone at hydrolysable groups and because it supports microbial activity. Higher temperatures yield higher reaction rates, and that typically gives faster biodegradation[46,47], although in some cases higher temperature counterintuitively decreases rate of biodegradation[48]. Of course, as temperatures get too high microbial activity is inhibited, and biodegradation slows[46]. The acidity or alkalinity of the environment in which a polymer biodegrades is important both because it can provide optimal (or sub-optimal) conditions for enzymatic reactions to take place, and because abiotic hydrolytic reactions can be catalysed in the presence of an acid or base[46,49]. Different microbial taxa excrete enzymes capable of attacking different moieties in the polymer backbone, so both the chemical identity of the polymer and the presence of different microbial species in the surrounding environment will be important in controlling the rate of degradation of a given polymer[50].

1.3 Biodegradable Polymer Degradation Studies

1.3.1 Important Considerations

When evaluating a BPM, there are several aspects that need to be considered, and they can be broadly classified as follows: factors that affect its biodegradation rate, the BPM's efficacy under a variety of conditions, its short and its long-term effects on soil health, and its degradation products mobility. These categories, and the interplay between them, provide a holistic picture of the 'impact' of a large-scale implementation of a new BPM.

An understanding of how different environmental factors affect biodegradation rate informs a user of a new BPM on how long to expect the BPM to remain in their soil. This knowledge, and knowledge on the efficacy of that BPM's performance under different environmental conditions would be very powerful for the user. Is a BPM as effective during times of low rainfall as times with plenty of rain? Does a soil high in microbial activity cause it to breakdown too quickly to be useful? Could a thinner (and therefore cheaper) BPM film achieve the same results, or is a thicker BPM necessary? These are important aspects to understand from a BPM user's perspective.

All mulching materials alter the soil microclimate (this is of course a mulch's purpose) to ultimately yield a faster growing and more productive crop. With conventional PE mulch this is accomplished simply by retaining moisture in soil by preventing evaporation from the soil surface, blocking weed growth if the mulch is black, and by retaining heat in the soil. A BPM has those same effects on the soil microclimate[51,52], but because BPMs degrade they have the additional effect of adding matter to the soil. This in effect makes them behave both as a mulch and as an organic soil amendment, and both effects will have an impact on the soil microbial community. The matter added to soils by BPMs, small oligomers and monomers (degradation intermediates), can act as a nutrition and carbon source to soil microbes[44]. In fact, they must be taken up by soil microbes in order to be mineralised or incorporated into the soil microbial biomass, but it is possible that certain degradation intermediates could have a deleterious effect on some soil microorganisms, or on other plant or invertebrate life. In fact, if some parts of a BPM are slow to degrade and microplastics form, that could have deleterious effect on soil invertebrates[36,37]. It is important to understand BPM

polymeric composition and the degradation intermediates a BPM adds to the soil before being mineralised, and more crucially, how those degradation intermediates affect the soil microbial community, and plant growth. Furthermore, it is important to determine degradation intermediates' mobility in soil. If the degradation intermediates are highly mobile, and can leach through the soil profile then it is possible that those intermediates can end up contaminating ground water and transporting throughout the environment.

1.3.2 Overview of Common and Key Characterisation Techniques

There are a variety of analytical techniques and methods that are commonly employed to understand a BPMs efficacy, degradation rate and extent over time. There are also common techniques used to understand a BPMs fate in the environment as it breaks down. In this section a brief overview of a sampling of these techniques is given.

1.3.2.1 BPM Film Biodegradation Characterisation

Standards organizations such as ASTM International, the European Committee for Standardization, and the International Organization for Standardization (ISO) have developed methods for determining biodegradability of polymeric (plastic) materials under different conditions (aerobic and anaerobic)[42,43,53–56]. These techniques involve the burial of a piece of the BPM being tested in soil contained within vessels with well-defined composting conditions, a CO_2 free air supply, and a CO_2 trap to measure total CO_2 evolved. Under anaerobic conditions the procedure is similar, but there is no oxygen source, and instead of measuring only CO_2, total gaseous carbon evolved is measured (both CO_2 and CH_4). These techniques are commonly employed[57–61] and are an important part of understanding BPM biodegradation, but they have their limitations. For one they miss any volatile N emissions (N_2O, NH_3, and NO_x) for BPM that have N in their composition. They do not measure immobilized carbon, which is carbon that becomes incorporated in the soil microbial community, and they also do not measure other volatile forms of carbon (low molecular weight alcohols for example). They also take no measure of degradation intermediates that remain in the soil and are yet to be mineralised, and importantly give no mechanistic information on

biodegradation. Using only a standard biodegradation test method is not sufficient for understanding a new BPM product.

A common experimental methodology used to measure the biodegradation of a new BPM is to take a piece and bury it in a soil (either in a lab or in the field), then remove it from the soil at various time points and characterise the film itself. Typically, when a standard method measuring evolved CO_2 (termed a respirometric measurement) from a BPM is not used, gravimetry is used on the BPM film to quantify extent of degradation[47,61–66]. This can be useful, but it also has issues. It is difficult to ensure complete collection of BPM fragments from soil, and to ensure a 'clean' film with no contaminants is recovered.

Scanning electron microscopy (SEM) is used to visualize degradation. "Before and after" images, or in some cases a time series of images of a BPM being degraded in soil are taken to look for visual signs of degradation such as pitting, fissuring, and cracking[24,67,68]. This is also sometimes used to search for evidence of fungal colonisation of a BPM[69].

Differential scanning calorimetry (DSC), and in some cases thermogravimetric analysis (TGA) are used to track changes to the phase of polymeric materials over time. Changes to amorphous and crystalline phases, as well as changes to glass transition temperature (T_g) or melting temperature (T_m) may be observed[24,58,59,68,70]. These are useful in giving some mechanistic information, for example, which phase of a BPM is degraded first, and how long it takes for that degradation to begin.

Tensile strength testing can be used to analyse the reduction of mechanical properties of polymeric film over time, and asses for early signs of degradation[24,68,71].

Infrared spectroscopy (IR) can be used to track changes in functional groups over time[59,67,72–75]. Both Solid State and solution ^{13}C nuclear magnetic resonance spectroscopy (NMR) and solution ^{1}H (NMR) may also be used to monitor changes in functional groups and forms of carbon over time[76]. This is useful for gaining a mechanistic understanding of the degradation process, and to understand what chemical transformations a BPM is undergoing as it degrades.

Electron spectroscopy for chemical analysis (ESCA) has also been used to determine how the elemental composition of BPM films changes at varying depths in the film over time[77].

Gel permeation chromatography (GPC) is a technique used to determine average molecular weight of a polymer. Both the number average (M_n, the molecular weight of the modal polymer chains) and weight average (M_w, the weighted average of all polymer chains) molecular weight are determined. This is an important technique for the characterisation of polymer degradation because it does not just quantify polymer lost (like gravimetric or respirometric measurments), but it also gives insight to the rate of polymer main chain scissions. It is a very commonly used technique[59,68,78–80].

One interesting technique developed by Martin-Closas and colleagues is an ordinal scale to qualify degradation extent of BPM using visual observations[81] such as how many cracks or pits the BPM has per unit area, or evaluating how thoroughly a BPM covers the soil over which it was originally placed. This is potentially of great practical use, provided it is validated across multiple BPM and in a variety of environments.

Recently, Nelson et al. employed an accelerated soxhlet extraction coupled with quantitative ^{1}H-NMR to quantify the residual level of BPM in mulched soils.[82]

1.3.2.2 Techniques to determine Environmental Fate and Effects on Soil Health

In comparison to the multitudes of studies characterising the biodegradation of BPM, there are comparatively few that investigate the environmental fate of the BPM degradation intermediates, or look into the impacts on soil health. The likely cause for this discrepancy is threefold:

1. It is very difficult to track the fate of BPM degradation intermediates well without expensive isotopic labelling.
2. BPMs are developed to replace the environmentally problematic PE mulch, and so it is pre-supposed that if a BPM degrades it must be environmentally benign.

3. It is time consuming to run longitudinal studies investigating the impacts on soil health of a BPM. Whereas it takes comparatively less time to vary formulation parameters and composite blends to create new BPM.

Nonetheless there are some examples in the literature to draw on[83]. The most common technique is to radiolabel part of the polymer with ^{14}C, or less commonly ^{3}H, and set up an experiment in a biometer flask (Figure 2)[84,85] to track the radioactivity. In a biometer flask the BPM under investigation is added to soil and a steady, CO_2-free air source flows through the flask. The air flows out past an activated charcoal plug to capture any volatiles, and then through a CO_2 trap to capture any CO_2 produced. The soil, charcoal plug, and CO_2 trap are then analysed for radioactivity.

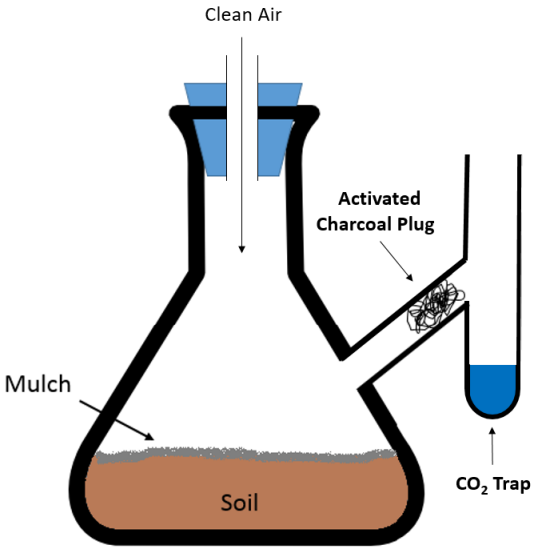

Figure 2. Cartoon schematic of a biometer flask.

A simpler set up commonly used is to degrade labelled BPM in a sterile saline solution inoculated with different enzymes[86–88], although this misses the impact of in-soil degradation. In both cases the uptake by plants of degradation intermediates is not measured, but this can be accounted for by using a plant pot uptake experiment[29,30].

In a plant pot uptake experiment, BPM is applied to a soil and a plant is grown, usually from seed or seedling to maturity. The plant is then harvested and either analysed for radioactivity in experiments using a radio-labelled BPM, or by gas chromatography coupled to mass spectrometry (GC-MS) to look for tracer molecules in experiments without radiolabelling. Recently, a new

technique using ^{13}C labelled BPM and Nano Secondary Ion Mass Spectrometry (NanoSIMS) and Cavity Ring Down Spectroscopy (CRDS) has been employed to determine how much of the BPM ends up incorporated in microbial biomass[89].

The use of liquid chromatography coupled with mass spectrometry (LC-MS) to look at degradation intermediates of polymeric materials has shown promise[90,91], but very few studies have utilized this methodology in a soil matrix[92,93], and there are no examples in the literature of this methodology being used to investigate BPM degradation intermediates.

1.3.3 Review of Biodegradable Polymers

There have been several varieties of biodegradable polymers, and biodegradable co-polymers of different chemical identity synthesized and investigated. Add to that the many different polymer blends and composites created using a wide variety of natural fibres and waste materials at different mixing ratios, and the permutations of BPM films becomes quite high. In this section the most common biodegradable polymers are reviewed.

Poly(lactic acid) (PLA) is commonly used in biodegradable plastic mulch films. It is a thermoplastic with a high tensile strength and high elastic modulus. It can be moulded into a film easily on standard plastic processing equipment and therefore is viewed as an important alternative to PE[94]. It is composed of a repeating ester unit (Figure 3), and it has an environmental degradation time in the range of 1-2 years, although it can degrade slower or faster depending on the specific conditions in which it is placed. Unfortunately the PLA homopolymer is stiff[94] which causes tearing problems during application of a mulch film, which reduces its efficacy[95]. PLA is a polyester, and it commonly degrades primarily via hydrolysis of the ester bonds.

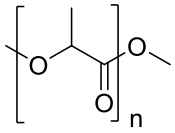

Figure 3. Chemical structure of poly(lactic acid) homopolymer.

Soil burial, and compost burial studies investigating the degradation of BPM based on PLA, PLA-XXX copolymers, and PLA-natural material composites are abundant[58,63,73,96,97].

Polyhydroxyalkanoates (PHA) are a class of polyesters synthesized by a variety of bacterial species as a form of energy storage[98]. There have been over 80 different distinct monomers identified. The most commonly studied PHAs are polyhydroxybutyrate (PHB)[65,67,73], and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)[59,62,99] (Figure 4). In addition to those predominantly studied PHAs, there has been significant work done on a variety of other PHAs, and PHA-natural material composites[57,74,100,101], though none have been used commercially as mulches.

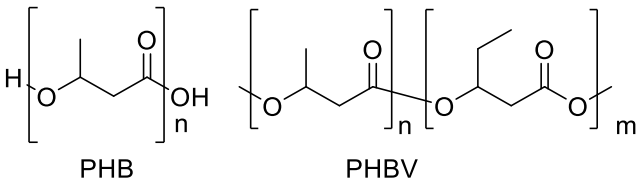

Figure 4. Common polyhydroxyalkanoates chemical structure.

Polycaprolactone (PCL) is another commonly used biodegradable polyester (Figure 5) used as BPM[48,62,80]. Due to its compatibility with a variety of other polymeric materials, easy-to-process characteristics (low melting point in particular), and low cost[102] it is commonly used as a component in co-polymers[75], and in blends with a variety of natural materials including starch[103], wood fibres and microcrystalline cellulose[104], rice husks[105], egg shells[70], and acorn nuts[106].

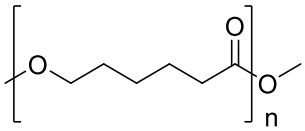

Figure 5. Chemical structure of polycaprolactone.

A class of polyesters based on 1,4-butanediol have also been developed as BPM. The biodegradation of polybutylene adipate-*co*-terephthalate (PBAT)[67], polybutylene succinate-co-adipate (PBSA) [48], and polybutylene succinate (PBS)[62,97,107] has been studied in soil burial experiments (Figure 6).

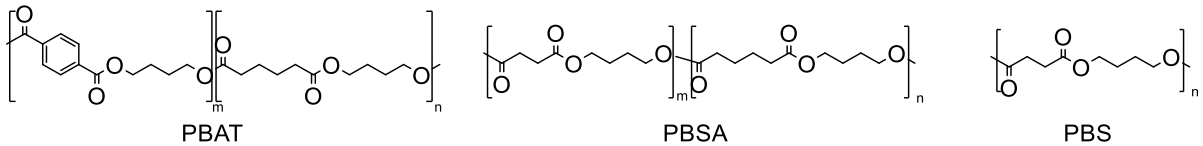

Figure 6. Chemical structure of common 1,4-butanediol based polyesters.

1.2.3 Review of Sprayable Biodegradable Polymers

The above overviewed biodegradable polymers all have shown promise as BPM, but because they are available as preformed films, they also share common drawbacks. For one, they do not have the same excellent mechanical strength properties as PE, which causes issues during the application process[95], in particular tearing which reduces their efficacy. They also can be difficult, laborious, and costly to apply, and especially so in horticultural contexts[108].

One solution for overcoming these issues that has been proposed is the development of a sprayable BPM. Using a sprayable mulch should be easy to implement as Adhikari et al. point out – the practice of spraying a solution, including solutions containing film forming polymers, is a common agricultural practice[95]. Spraying the mulch also eliminates many application problems associated with pre-formed films. Immirzi et al. outlined that using a spray-gun to apply mulch in a greenhouse setting is much less labour intensive than using preformed film as mulch, which necessarily requires measuring, cutting and placement of the film[108]. A sprayable BPM differs from a preformed BPM in its interaction with the soil. A sprayable BPM will form strong physical, and potentially chemical, interactions with the soil, and will draw its strength from its interaction with the soil surface, as opposed to only from interaction with itself. This is an important difference as it could cause differences in degradation and efficacy.

There has been limited work done in the development of sprayable BPM, and even less work done investigating the biodegradation and environmental impacts of these films. In this section, the sprayable BPM that have been developed will be reviewed.

Protein hydrolysate (PH) from waste from the leather industry has been investigated for its potential as a sprayable BPM[109,110]. It has been prepared as a mixture with polyethylene vinyl acetate (PEVA), which is not a biodegradable polymer, and polyethylene glycol, which is a biodegradable polymer[111] (Figure 7). In follow up work, the mulching efficacy and biodegradation of the PH-PEG blend was investigated[51]. It was found that the mulch provided similarly effective performance as PE mulch, and that 2 months after the PH-PEG blend was tilled into the soil, only 5

wt% of the originally applied mulch remained (determined gravimetrically). It is not known by the author why these materials are not available commercially yet. It could be that they are too expensive to produce at scale or that there isn't a sufficient supply of PH available.

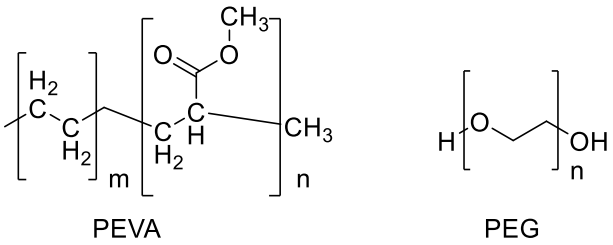

Figure 7. Chemical structure of PEVA and PEG.

Polysaccharide based sprayable BPM have also been investigated using polysaccharides from guar gum and locust bean gum[112], chitosan[113], and sodium alginate[108]. The efficacy of the chitosan based sprayable BPM on weed suppression was investigated and it was found to be effective for approximately 2 months, after which the mulch film began to degrade allowing weeds to grow through. The weed suppression efficacy, and in-soil biodegradation of the sodium alginate based sprayable BPM was investigated, and it was found that it was effective at suppressing weed growth, and degraded at a similar rate to crystalline cellulose (the gold standard for biodegradability). No efficacy nor biodegradation testing was conducted on the sprayable BPM based on guar gum and locust bean gum.

Lastly, there has been work done investigating polydimethylsiloxane (PDMS) as a sprayable BPM[3,4] (Figure 8). PDMS has not shown to be biodegradable strictly speaking, but they are hydrolysable and the primary degradation intermediate, dimethylsilanediol, is taken up and degraded by soil microbes.[84] It was found to be effective in conserving water, suppressing weed growth, and increasing crop yield all while also showing good material properties.

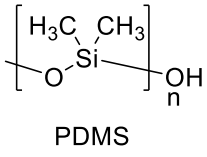

Figure 8. Chemical structure of PDMS.

It is clear that sprayable BPM technologies show promise, though none have been brought to market at the time of writing. Also, there have been few (only three) studies investigating the biodegradation of these materials, and in those studies only a limited set of measurements were taken (BPM weight loss or CO_2 evolved) which, as discussed, is not sufficient for developing a holistic understanding of how these materials can impact the environment[51,108,109].

Currently the biggest challenge to sprayable BPM is in forming a continuous surface covering to ensure complete efficacy, and in preventing the losses in mechanical integrity of the mulch with too rapid degradation, which also reduces their efficacy[95,114]. In an attempt to address these issues, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) sought to develop a sprayable BPM that could reliably form a continuous soil surface coverage, and with greater control over degradation rate.

1.4 CSIRO's Polymer

CSIRO recently developed a sprayable BPM based on PCL[115]. It is a random block copolymer synthesized from polycaprolactone (PCL) diol, isophorone diisocyanate (IPDI), dimethylolpropionic acid (DMPA), and ethylene diamine (EDA) (Figure 9) that is suspended in water. It has repeating ester, urea and urethane linkages, and thus is a polyester-urethane-urea and will be referred to as PEU in this work.

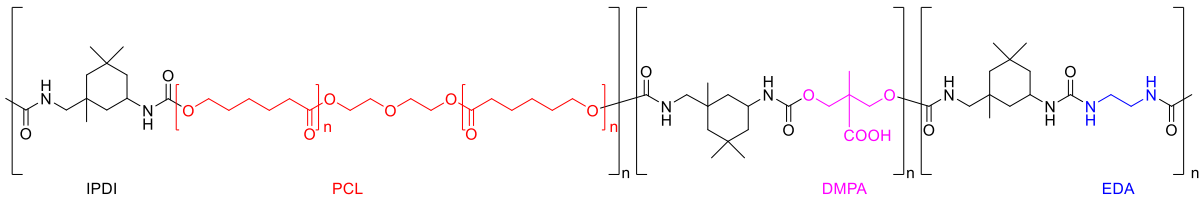

Figure 9. Representative structure of PEU with individual components highlighted.

In addition to the PEU, the aqueous suspension includes Methocel® (Dow, methylcellulose) as a biodegradable viscosity modifier, and carbon black as a biodegradable pigment. This novel sprayable BPM is the focus of the work presented in this thesis.

1.4.1 Polymer Synthesis and Characterisation in Brief

The PEU used was synthesized using a two-step method as described by Adhikari et al.[115] In brief, a PCL based polyester-urethane pre-polymer was synthesized by reacting anhydrous PCL diol

and IPDI under a N_2 atmosphere. DMPA was then added to the reaction mixture, followed by an EDA chain extender. The reaction mixture was left to react until all of the isocyanate had reacted (as confirmed by Attenuated Total Reflectance Infrared Spectroscopy, ATR-IR). Methyl cellulose and carbon black were added to the mixture to adjust the viscosity and to provide pigmentation. The final polymer formulation contained 20 wt% polymer solids.

ATR-IR of PEU shows a strong ester peak at 1700 cm^{-1} from the ester groups in the PCL segment, and the carbamate shoulder peak at 1650 cm^{-1}. PEU has a low temperature glass transition temperature due to the soft segment PCL around -50ºC and broad melting endotherms above 200ºC associated with the ordering of hard segments. PEU reveals a unimodal, almost symmetrical peak with a slight bias to lower molecular weights and the polydispersity is approximately 2.0 from GPC.

1.4.2 Research Hypothesis and Objectives

The work presented in this thesis focuses on the applications of one sprayable BPM based on PEU developed by CSIRO. The objectives of this work were to:

1. Investigate differences in water savings efficacy and biodegradation rate of PEU under a variety of environmental conditions (Chapter 2).
2. Understand the magnitude of the importance of individual environmental parameters (temperature, soil pH, soil microbial community composition, and soil particle size) on PEU degradation rate (Chapter 3).
3. Determine the identity of the most common degradation intermediates of the PEU. Understand their mobility in soil, and measure their potential phytotoxicity (Chapter 4).
4. Investigate the impacts of the application of PEU mulch on soil health and on the soil microbial community (Chapter 5).
5. Determine how agricultural productivity (specifically tomato growth), and agricultural water use may be affected through the application of PEU mulch to an active cropping system (Chapter 6).

These objectives were addressed through a series of studies:

1. A glasshouse study investigating the effect of sunlight, soil moisture, soil type and polymer pigmentation on the degradation and water savings efficacy of the PEU. (Chapter 2)
2. A series of lab controlled hydrolytic incubations interrogating the effect of temperature, soil pH, soil microbial community composition, and soil particle size on the rate of hydrolytic degradation. (Chapter 3)
3. A LCMS study to identify the primary abiotic and soil degradation intermediates of the PEU. (Chapter 4)
4. An investigation into the effects of the PEU on the soil microbial community composition, relative abundance of plant growth promoting microbes, and soil microbial community's function. (Chapter 5)
5. A glasshouse tomato growth study using commercial conditions comparing the efficacy of the PEU with two commercially available PE films. (Chapter 6)

The following chapters address these objectives. It is hypothesized that the PEU is biodegradable, has no adverse environmental effects, and supports plant growth and soil health by conserving water and supporting the soil microbial community.

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Chapter 2. Environmental Degradation and Efficacy of a Sprayable, Biodegradable Polymeric Mulch

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Abstract

Polyethylene (PE) and other non-degradable plastics are used in vast quantities as agriculture mulch to help protect the soil surface, conserve water, and improve soil microclimatic factors. Unfortunately, their continued use poses several environmental problems, so an environmentally friendly solution needs to be found. The use of biodegradable plastics in place of conventional PE is one well studied solution, and here we investigate how different controlled environmental conditions affect the water conservation efficacy, and rate of biodegradation of a novel, biodegradable, sprayable polycaprolactone based polyurethane mulch. The effect of soil moisture content, sunlight, soil type, and polymer pigmentation are investigated using several different characterization techniques and measurements. It was found that the polymer studied is effective at conserving soil moisture, and that it biodegrades at different rates via a bulk erosion mechanism. The rate at which it degrades mostly depends on the soil type to which it is applied, and the moisture content of that soil. This was confirmed using soil CO_2 emissions, polymer mass loss, polymer molecular weight reduction, and scanning electron microscopy. Results are discussed.

Introduction

Plastic mulches have been used since the 1960s[1] to protect the soil surface from erosion and to enhance soil microclimatic factor, ultimately improving plant growth. Estimates indicate that 700,000-1,245,000 tons/annum of plastic mulch is used, which covers up to 20,000,000 hectares of land.[2,3,4] Polyethylene (PE) plastic mulch is a preferred material due to its high tensile strength, resistance to degradation, and customisability.[5] However, PE mulch does not degrade[6] and so it must be disposed of after use, generating a large, environmentally deleterious, waste stream for the farmer. Only a small portion of plastic mulch can be recycled due to contamination issues,[7] and so most plastic mulch ends up being incinerated or landfilled, both of which present environmental problems.[8,9,10] An additional problem posed by the accumulation of non-degradable plastic is their ability to form small bits of plastic, known as microplastics,[11] which is of concern because microplastics have been shown to be deleterious to terrestrial invertebrates,[12,13] which ultimately leads to a worsening of soil health.

The use of biodegradable polymers (plastics), that is polymers that degrade to CO_2, H_2O, CH_4, inorganic compounds and biomass in approximately 12 months while leaving no visible, nor toxic residue,[14] are a suitable and widely studied solution to the problems posed by nondegradable plastic mulches.

Currently commercially available biodegradable mulches suitable for agriculture are available as preformed films only. Adhikari et al. suggested developing a sprayable biodegradable mulch film as a unique solution to overcome the technical problems (mechanical properties suitable for application, durability and retaining properties when wet) involved with using preformed biodegradable mulches, while maintaining the same benefits.[15]

Immirzi et al. identify that using sprayable mulch is much less labour intensive for horticultural practices,[16] and by repurposing existing equipment to spray a biodegradable polymer mulch a farmer may realize the same efficiencies. It has also been highlighted that sprayable mulch draws its strength

from its interaction with the soil surface, and does not require mechanical application – during which tearing frequently occurs.[15]

Despite growing research interest into the concept of sprayable biodegradable polymeric mulches, there is no commercially available product to date. Biodegradable liquid mulch that is applied via pouring has been developed (patented technology, base component is cellulose), although it is cost prohibitive for large scale operations ($80,000 ha^{-1}).[17,18] Some biodegradable sprayable mulches based on hydrolyzed proteins,[19,20] polysiloxane (Guilspare ®)[21], sodium alginate[16], and blends of natural polysaccharides with additives[22,23] have been developed and field tested for mechanical strength and radiometric properties, and have shown promise in terms of their water savings, but these have not been made available commercially at the time of writing. Furthermore, little is known how different environmental conditions may affect the performance of these biodegradable materials, and that is an important knowledge gap as environmental conditions vary widely.

To fill this technological vacancy, and to provide a sustainable and practical mulching solution for the future, the Commonwealth Scientific and Industrial Research Organization (CSIRO) has developed a polycaprolactone (PCL) based sprayable, polymer formulation for agricultural mulching designed to be biodegradable. It is composed of PCL soft segments and polyurethane hard segments. It is a random block polyurethane copolymer synthesized from polycaprolactone (PCL) diol, isophorone diisocyanate (IPDI), dimethylolproprionic acid (DMPA), and ethylene diamine (EDA) (a representative structure is displayed in Figure *10*) that is suspended in water. This study seeks to understand the polymer's degradation and its extent of biodegradation. The manner in which the polymer affects soil physico-chemical properties over time was also investigated and this is paramount should the technology be commercialized. The work presented here seeks to understand how different environmental variables may affect the rate of the polymer's overall degradation, and water savings efficacy.

Materials and Methods

Study Design

A full factorial experimental design was used to investigate the effects of moisture level, light level, polymer pigmentation, and soil type on the degradation of the polymer. Two moisture levels, nominally 'high' and 'low'; two light conditions, sunlight and no light; two pigmentations, unpigmented and pigmented with carbon black; and three soils were used. Using these four variables (one ternary and three binary variables) yields 24 unique sets of conditions.

$$2^3(binary\ variables) * 3^1(ternary\ variable) = 24(unique\ condition\ sets) \quad (1)$$

The study ran from July 11, 2017 to November 30, 2017 for a total of 142 days with data being collected at five different time points, T_0-T_4. T_0 was taken two days after the polymer was applied, on July 13, 2017. T_1, T_2, T_3, and T_4, were taken at 4, 8, 14, and 20 weeks after T_0, respectively.

Materials

Soils were chosen to have distinct physicochemical properties. Soils were sourced from three different agricultural sites in Victoria, Australia: Echuca (a vertosol), Seville (a dermosol), and Ouyen (a tenosol). Table 1 gives information on the soil moisture levels used in this study, and Table **2** describes each soils characteristics. Additional soil characteristics are given in the supplementary material (Table S1.).

Table 1. Field water holding capacities of each soil, and the percentage saturation used in this study.

	100% Water Saturation	*Average % Saturation*	
Soil	(gH2O/100gSoil)	Low (%)	High (%)
Echuca (vertosol)	67	42	62
Seville (dermosol)	53	46	63
Ouyen (tenosol)	27	43	77

Table 2. Soil characteristics.

	Echuca	*Seville*	*Ouyen*
Source Type	Agricultural	Grazing Paddock	Agricultural
Soil Type	Vertosol	Dermosol	Tenosol
pH	7.01	5.53	6.87
Organic Matter (OM), %	2	6.7	0.2
C:N	9.18	17.86	2.97
Sand, %	31.6	56.4	92.8
Silt, %	12.8	23.5	0.2
Clay, %	50.6	8.4	3.7

The polymeric material used in this study was an aqueous suspension of a PCL based polyurethane developed by CSIRO (Figure *10*). The solution was 20% by weight polymer solids, and 0.65% by weight Methocel ® (methylcellulose) as a biodegradable viscosity modifier. The pigmented polymer version also contained 4% by weight carbon black.

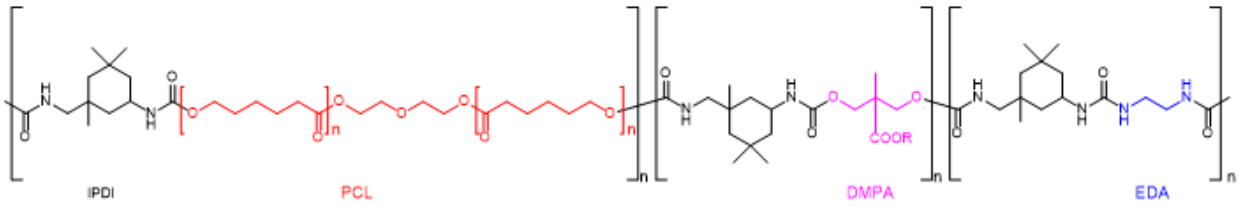

Figure 10. Representative structure of the biodegradable sprayable polymer used in this study

To extract the polymer from the soil, ≥99.9% tetrahydrofuran (THF) (Sigma) was used. To prepare polymer for gel permeation chromatography (GPC) analysis, the polymer was dissolved in a 4.34g L^{-1} LiBr in *N,N*-Dimethylacetamide (DMAc) (≥99.9%, Sigma) solution.

Study Set Up

In typical soil degradation studies of polymeric materials, a polymeric film is buried in soil and a combination of its physical, chemical, and mechanical properties are characterized over time.[24–28] This approach was not suitable for this study firstly because the polymer's degradation behavior in conditions similar to its intended use (on the soil surface) was of interest, and secondly, due to the

inherent differences between a preformed film and a sprayed film that cures in-situ. This polymer, cured in-situ, will develop contact with the soil surface that would be disturbed and could not be recreated if it were removed and replaced at each time point, so therefore destructive sampling was required.

This study was carried out in a temperature controlled glasshouse (19-25ºC) using 5 cm radius (surface area of 78.5 cm^2), 10 cm high polypropylene pots. A sufficient number of pots (480) for a quadruply replicated full factorial experiment with destructive sampling at five time points were filled with soil. Pots were filled with soil to the same height, to control for surface area, and because surface area was being controlled for, masses of soil added to each pot varied. 225 g, 312 g, and 421 g of Seville, Echuca, and Ouyen soil were added per pot respectively.

Field water holding capacities were experimentally determined for each soil type. After all pots had been filled with soil, sufficient water was added to reach the 'high' or 'low' moisture levels (Table 1), and then polymer solution was applied to the soil surface via syringe.

In addition to the 480 polymer containing pots, 24 control pots were set up with only soil and no polymer. These were all maintained at either the high or low moisture level, and were used to determine the polymer's efficacy in maintaining soil moisture via comparison with the polymer containing pots. They were also used as controls to determine how the polymer treatment influenced greenhouse gas (GHG) emissions from the soil.

Soils were not brought to the same percent soil water saturation due to practical constraints. It was found that using a percent soil water saturation greater than those given in Table 1 would lead to water leaching out of the base of the pot.

Pots that were designated to be exposed to no light were shaded from the sun by placing a perforated cardboard sheet across the tops of the pots.

Pigmented or non-pigmented polymer suspension was applied to the soil surface at a targeted polymer solution loading of 1 kg m^{-2}, or 200 g m^{-2} of solid polymer. This translates to 6.4 g of polymer solution

(1.3 g solid polymer, ~0.5mm thickness) per pot. Due to variability in the application rate, approximately 6-7 g of polymer solution per pot was applied on average.

Polymer was applied via 50 mL syringe with no needle, as opposed to via spraying, due to difficulties in controlling the amount of polymer delivered through spraying at low volumes. This resulted in a polymer with a typical thickness of 500-1000 μm depending on soil type and amount of polymer delivered. During polymer application, the polymer solution pooled on the surface regardless of application method (several spraying methods were trialed, but none had sufficient control over the amount delivered) and cured overnight.

Watering

In order to both maintain a relatively constant soil water saturation, and to mimic environmental conditions, pots were watered at least twice weekly, with no more than 5 days and no fewer than 2 days passing between watering events. The total mass of the soil, pot, polymer film, and water was known for each soil type at each moisture level, so to maintain the correct percent soil water saturation (Table 1), pots were simply weighed and topped up with water to reach the expected total mass. In this way, water lost from each treatment type was tracked.

Water was added by gently pouring the required mass onto the soil-polymer surface. Water first pooled on the surface before being pulled into the soil bulk by gravity. The length of time this process took depended upon the soil type, moisture level (high or low), and extent of polymer degradation. Water infiltration time decreased as amount of surface polymer imperfections (cracks, pits, polymer being pulled away from the edges) increased.

Polymer Sampling

As previously stated, polymer sampling occurred at five time points: T_0, T_1, T_2, T_3, and T_4. T_0 two days after polymer application to ensure that the polymer was completely cured, and T_1, T_2, T_3, and T_4 occurred 4, 8, 14, and 20 weeks after T_0, respectively.

After GHG collection, the polymer and top 2-3 cm of soil were collected. This was done by using the polymer and soil to a depth of ~ 3 cm, which was separated from the rest of the pot by the GHG collection chamber (Figure 11). This ensured that similar areas of polymer and soil were collected between pots, and avoided any edge effects of potentially accelerated degradation where the polymer met the pot. In addition, soil samples were collected at a lower depth (3 cm to the bottom of the pot) from a subset of pots to check for the presence of small oligomers. The basal area of the measuring chamber was circular with a radius of 2.4 cm, meaning that ~24% of the total polymer applied, or a maximum 290-340 mg of polymer could have been collected.

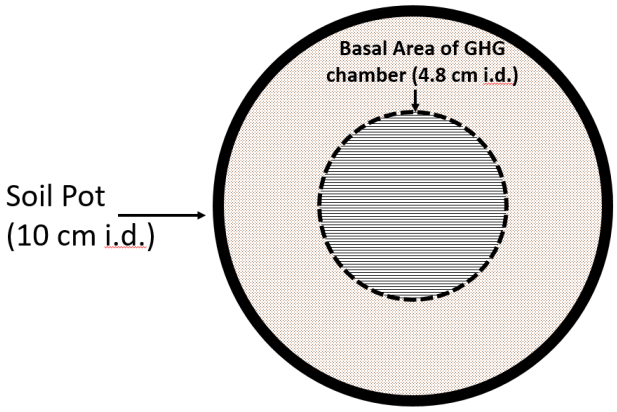

Figure 11. Diagram of where the GHG measuring chamber was placed in the soil pot. Polymer sample was recovered from the inside of the dotted line (n = 4 per treatment group, per time point).

To extract the polymer from the soil, 10 mL of THF was added and samples were then shaken for 20 hours on a horizontal shaker. Samples were then centrifuged at 3200 g for 12 minutes at 20°C, and the supernatant was transferred to clean centrifuge tubes and dried at ambient conditions. When all of the THF had evaporated, the remaining polymer was weighed, and then samples were flushed with N_2, capped, and stored in the freezer until used for further analysis.

Greenhouse Gases

Full details available in the SI. Method adapted from van Zwietten et al.[29]

Gel Permeation Chromatography (GPC)

Full details available in the SI.

Scanning Electron Microscopy (SEM)

Full details available in the SI.

Thermogravimetric Analysis (TGA)

Full details available in the SI.

CHN analysis of Soil

Full details available in the SI.

Results and Discussion

Water Conservation

Pots were watered at least twice weekly, and water loss was determined gravimetrically. At each watering event, pots were brought back to the starting mass (soil + water + polymer + pot) at the given moisture level by adding water. Any mass loss between watering events was attributed to water lost due to evaporation.

Figure 12 displays the cumulative water added (which is equal to the water lost) over time to pots containing Seville soil, based on different conditions. Data for other soils is given in SI (Figures S2 and S3).

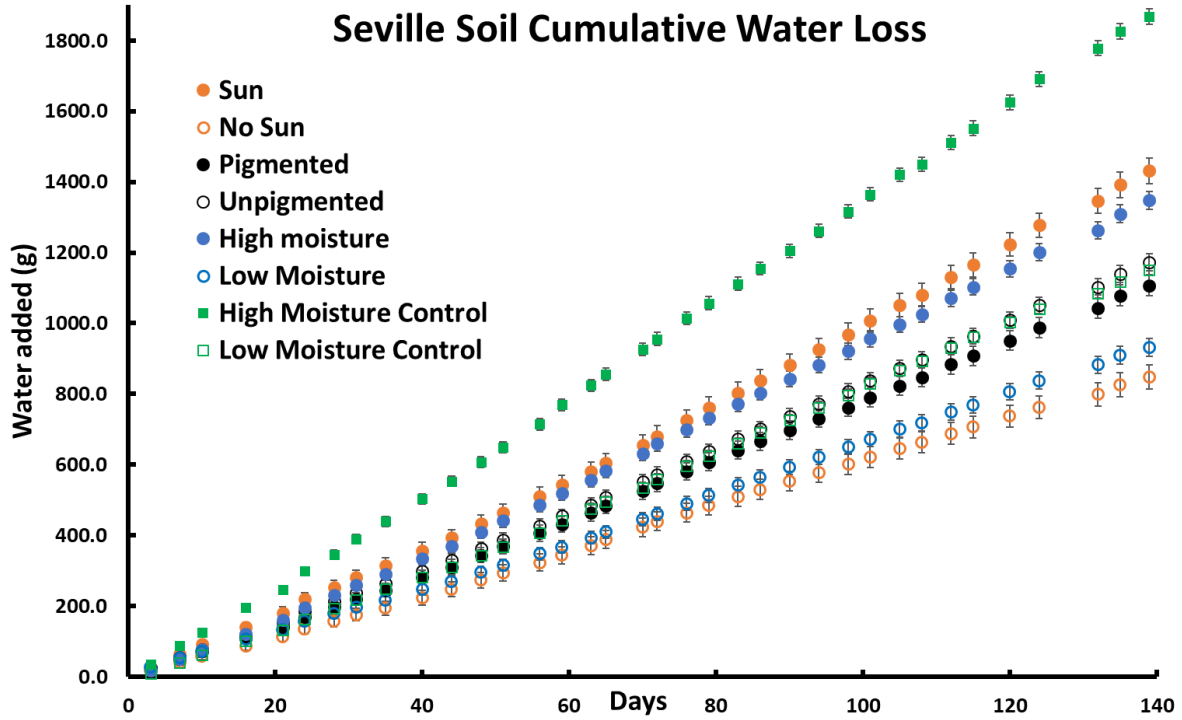

Figure 12. Cumulative water loss over time to Seville soils based on environmental condition. Data points are means ± standard deviation.

Compared to the highest water losing treatment group, control pots (pots with no polymer mulch) lost 436g, 1415g, and 524g of water for Seville, Echuca, and Ouyen soils, respectively, over the course of the study. This indicates that the polymer successfully reduced water evaporation from the soil surface.

More water evaporated from pots exposed to sunlight compared to the unexposed ones (which were covered by a cardboard sheet), which indicates that either additional heat is absorbed by the soil, or reduced air circulation over the unexposed pots inhibited the rate of evaporation. It is likely both effects are at play.

A higher percent saturation in soil gives a higher water vapour pressure, which means water will evaporate faster at higher soil water saturations. As expected, the high moisture pots required more water to maintain their moisture level than the low moisture pots. This indicates that as soil moisture decreases, the mulches water retention efficiency increases.

The difference in water added to pots with and without pigmentation is small but statistically significant. In the case of Seville soils, unpigmented polymer treated pots lost more water than the

corresponding pigmented pots. This is due to weed growth in Seville soils, which provided an additional water loss process (transpiration) not present in Echuca and Ouyen soils (Figure 13).

Water loss in Ouyen and Echuca soils show similar trends as in Seville soils. One exception was noted for the pigmented polymer treated soils which lost slightly, but significantly, more water (65g) than those treated with unpigmented polymer. This might be due to the black pigment absorbing extra heat, causing water to evaporate at a slightly higher rate.

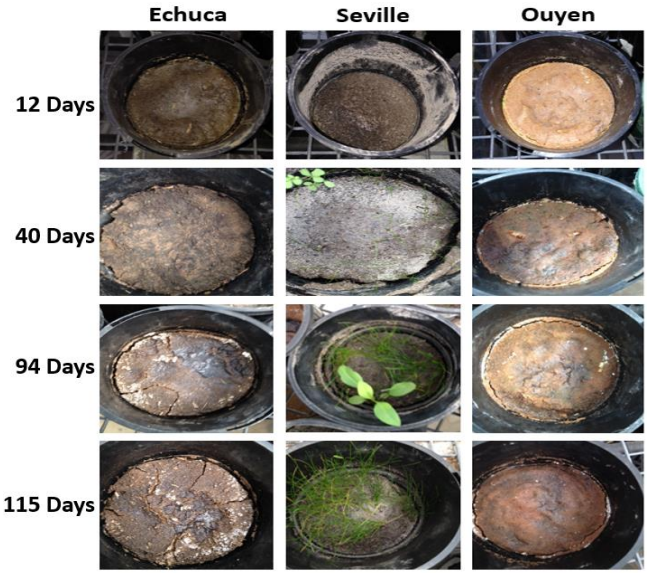

Figure 13. Picture of Echuca, Seville, and Ouyen (E, S, and O) soils treated with unpigmented polymer (U), exposed to sunlight (Y), and maintained at high moisture conditions (8).

Polymer Degradation

A suite of techniques monitored polymer degradation. CO_2 emissions were used as an indicator of biodegradation, and polymer mass loss was used to assess bulk erosion. GPC was used to follow the chemical breakdown of the polymer chains, SEM was used to obtain information on the polymer morphology and how that evolved as the polymer degraded. TGA was used to monitor the polymer's thermal stability as it degraded. Finally, CHN analysis of the soil immediately underneath the polymer was analyzed to determine if the polymer mulching treatment adds organic matter to the soil as it degrades.

Greenhouse Gases

Under each set of environmental conditions, there were higher CO_2 emissions from polymer treated soils than non-treated control soils (Figure 14). N_2O and CH_4 emission rates were unaffected by the mulching treatment. The CO_2 emission rates found on the soils in this study, were in the order of 300-500 mg CO_2 m^{-2} h^{-1} (depending on the conditions set), which are in line with previous reports for bare soils.[30]

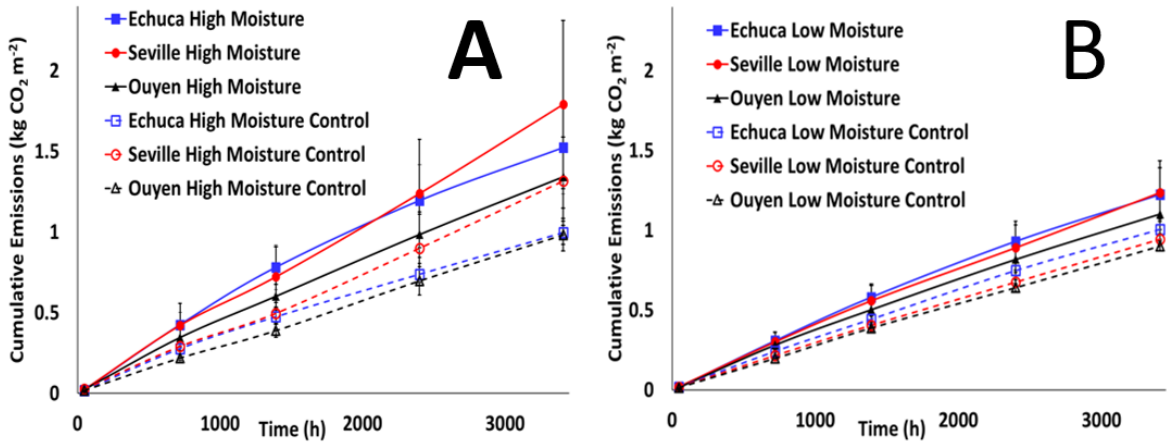

Figure 14. Cumulative CO2 emissions from soils maintained under A) high moisture conditions, and B) low moisture conditions.

Overall, higher CO_2 emissions were observed from the polymer-treated pots. Increased CO_2 emissions indicated either that soil microbes in the polymer treated soils respire at a higher rate than those in the unmulched soils, or that there is a larger microbial community present. A more active microbial community would suggest more favourable conditions for respiration (i.e. more moisture, increased temperature, optimal pH, etc.) and a larger population would be caused by the presence of additional energy and carbon sources for the community to grow (i.e. the polymer mulch). As the polymer mulch does in fact help conserve soil moisture, which provides the microbes more favourable respiration conditions, a more active community in the polymer mulched soils cannot be ruled out, but the polymer is certainly providing an additional food and energy source. Others have demonstrated that PCL based polymers are susceptible to enzymatic degradation, and mineralize completely into CO_2[31,32], and it has been demonstrated that similar PCL based polyester-urethane-ureas are broken down enzymatically.[33] These findings, in addition to the mass loss and polymer

molecular weight reduction observations displayed here suggests that this polymer mulch is truly biodegrading and being mineralized to CO_2, although to confirm this further study is required.

To further confirm that the enhanced CO_2 emissions were not simply a more active community mineralizing the already present SOM, a carbon mass balance was performed using the CO_2 emissions from the Ouyen soil (which is only 0.2% OM, refer to Table 2). In each pot of Ouyen soil there was a maximum amount of 0.5 g C, which would be emitted as a maximum 1.83 g CO_2. A greater amount (0.7 g C, 2.55 g CO_2) of carbon was emitted than was present as SOM, and therefore we can conclude explicitly that microbes were using the polymer as a carbon source.

In terms of condition specific effects on the rate of greenhouse gas emissions from the soils studied here, moisture content is the most important factor controlling CO_2 emission rate. Again, there was no difference in emission rates of N_2O and CH_4 between polymer treated and unmulched soils.

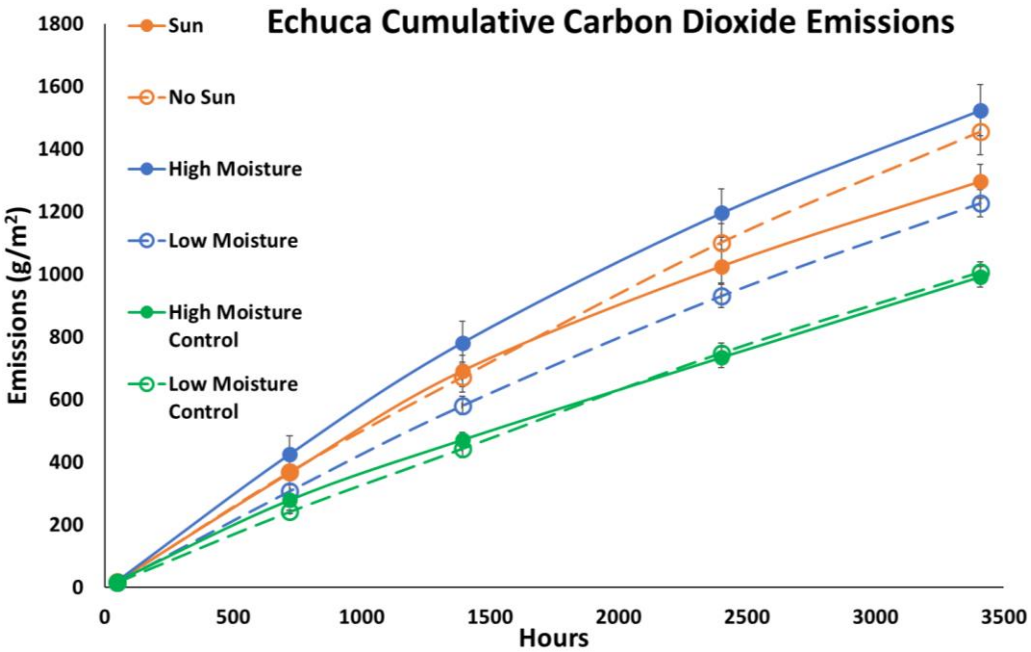

Figure 15. Cumulative CO2 emissions from Echuca soils based on environmental condition.

Figure 15 shows that Echuca soils with more moisture (blue solid trace) emit more CO_2. Similar trends were observed on Ouyen and Seville soils (available in the SI, Figures S4 and S5). As mentioned above, the soils that were not exposed to sunlight actually retained more water, and so the enhanced CO_2 emissions from those soils is an artefact of soil moisture content. Polymer pigmentation did not affect the rate of CO_2 emissions.

Mass Loss

There are several competing mass loss processes occurring as the polymer degrades. The primary mass loss process is the polymer breakdown into smaller particles and subsequent metabolization by the soil microbial community into presumably CO_2, H_2O, biomass, and other inorganic compounds such as nitrate. Other mass loss processes could include the irreversible sorption of small polymer particles to soil matter, and the leaching of polymer molecules down the soil's vertical profile and thus not being extracted.

Approximately 24% of the total applied polymer (290-340 mg undegraded) was sampled (Figure 11). The recovered polymer was weighed after being extracted, cleaned and dried, to give an approximation of how much mass was lost since application. The uncertainty (see error bars in Figure 16) in the mass loss measurements is high in this study for several reasons. Firstly, difficulty controlling the application rate caused a range of polymer mass (1.2 – 1.4 g) to be applied to the pots due to difficulty in controlling the polymer film thickness during the application. Secondly, the soil surfaces to which the polymer was applied were not perfectly uniform. This contributed to the variation in the polymer thickness across the soil surface, with thicker regions forming in troughs and thinner regions forming on peaks, so depending on the topography of the soil surface there could be great variation in the amount of polymer recovered from the area designated in Figure 11.

Despite this uncertainty, mass loss was still a useful measurement as it gave a broad picture of the extent of the polymer's degradation, with greater mass loss indicating more degradation. Figure 16 shows polymer mass loss over time per soil type.

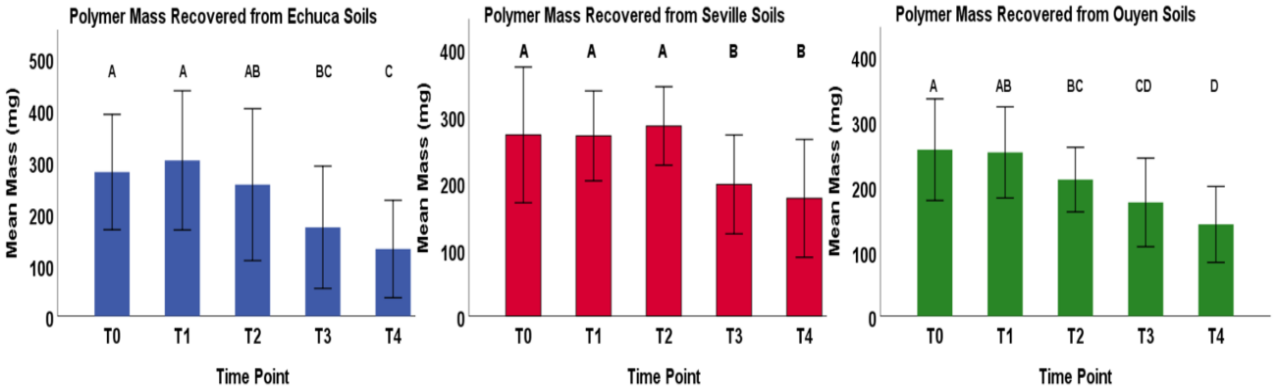

Figure 16. Average polymer mass recovered per time point from each soil type. Letter designations above columns specify statistically homogeneous subsets.

The polymer mass loss on Ouyen soil showed the strongest trend. This is essentially due to less uncertainty - the sandiness of Ouyen soil (see

Table 2) provided the most topographically flat surface, which allowed the polymer to cure at a relatively more consistent thickness across samples than on the other soil types. Due to the large uncertainty associated with the recovered polymer mass, it was not possible to determine how any of the specific conditions (moisture level, light level, polymer type) affected mass loss.

GPC Results

The changes to polymer molecular weight (M_w, M_n) over time will be discussed in the following section. Figure 17 summarizes the changes to M_w and M_n, according to soil type. Note that no polymer fragments were detected in the soil sampled beneath the top 3 cm by GPC (detection limit of 575 Da).

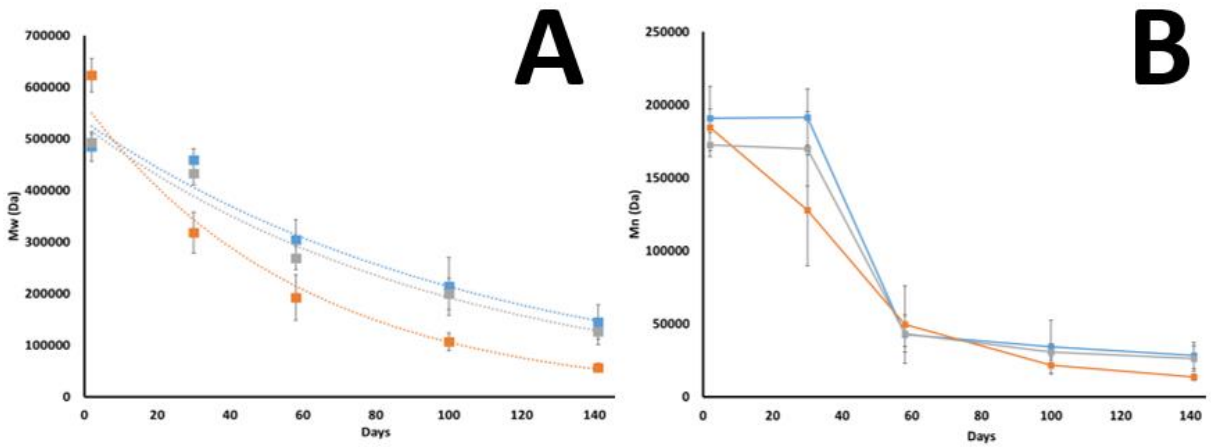

Figure 17. Orange, grey and blue data is on polymer recovered from Seville, Ouyen and Echuca soils, respectively. A) M_w time series for each soil type, error bars are ±1 standard deviation. Dotted lines are exponential lines of best fit. B) M_n time series for each soil type, error bars are ±1 standard deviation. Lines are to guide the eye only.

The exponential fits to the data in Figure 17 can be used to calculate the polymer's hydrolysis half-life on each of the soils using:

$$t_{\frac{1}{2}} = \frac{\ln(2)}{\lambda} \quad (2)$$

Where $t_{1/2}$ is the hydrolysis half-life and λ is the exponential coefficient of x taken from the fits' equations. The calculated half-lives of the polymer on Echuca, Seville, and Ouyen soil is 77.0 ± 1.6 days, 40.8 ± 0.4 days, and 69.3 ± 1.2 days respectively.

M_w and M_n of polymer recovered from Echuca and Ouyen soils does not significantly decrease between 0 and 30 days, and after day 30 there is a continuous exponential decrease in M_w for the duration of the study (Figure 17, blue data). This indicates that the onset of degradation is delayed for the polymer on these soils. Degradation of polymer recovered from Seville soil on the other hand begins without delay, with a drastic reduction in M_w evident after 30 days. M_n of the polymer on each soil type is reduced by a factor of roughly four during the first 58 days, after which a gradual linear decrease was observed.

Soil physicochemical properties clearly play an important role in the rate of polymer degradation. Given the soil physicochemical properties of the three soils studied here (

Table 2), the most likely factors causing the enhanced degradation on the Seville soil are soil pH, soil organic matter (SOM), and soil morphology as these characteristics are substantially different between Seville and the other two soils. Hydrolysis of ester, urea, and carbamate bonds can be catalyzed under acidic or basic conditions. SOM plays an important role in supporting microbial activity, which plays an important role in catalyzing the lysis of long polymer chains via secreted exoenzymes, and in the final biodegradation steps through the uptake of small oligomers to be used for energy and ultimately mineralized.[34–36] Further targeted study of each of these factors is necessary

to elucidate which soil characteristics are most important at controlling the rate of polymer biodegradation.

The degradation pattern shown in the M_w data, i.e. a continuous, exponential like decrease in M_w, is evidence that "bulk erosion" is the predominant degradation mechanism this material undergoes. Bulk erosion, which opposes "surface erosion," is characterized by random hydrolytic scissions of the hydrolytically labile functional groups, which in this case are carbamates, ureas, and esters.[37] It is well understood that ester groups are more hydrolytically labile than urethanes,[38] and Chapman has shown that all else being equal, urea groups hydrolyze before carbamates.[39] However, it is also well understood that the exoenzymes secreted by soil microbes play an important role in polymer degradation in soil[27,40–44], especially so in polyester urethanes[45], and affect the degradation kinetics of the polymer. In any case, because the polymer is degrading the primary site and mechanism of degradation will be hydrolysis of the ester linkages within the PCL soft segment of the polymer. This is the case because esters are the most susceptible to hydrolysis, the most numerous hydrolytically labile functional group within the polymer, and because water will readily percolate through the amorphous soft segment (as opposed to the crystalline hard segments), thus reaching the ester linkages earlier and more frequently.

The presence or absence of light played a small but statistically significant role in the rate of M_w reduction for polymers on Ouyen and Echuca soils, with polymers degraded in the absence of light degrading slightly faster (data not shown). The polymer also degraded faster under high moisture conditions on Echuca soil only. Pigmentation, or lack thereof, played no significant role in the rate of polymer degradation on any soil.

SEM Results

SEM micrographs of polymer recovered from each soil type 2 days (T_0) after application and 142 days (T_4) after application are displayed in Figure 18. The T_0 images show how the polymer would look in its 'natural,' hydrated state. It is composed of many small bubbles and red blood cell shaped

structures when hydrated, reminiscent of a hydrogel[46], which makes sense as the polymer behaves somewhat like a hydrogel, holding up to 350% of its weight in water (data not shown). Note the difference in topography of the polymer recovered from different soils, and especially how the polymer recovered from Seville soil is 'rougher' and covered in deep pits. This difference in morphology could explain why the polymer began degrading immediately on Seville soils while there was a delay on the other soils. Additional experiments are underway to interrogate this possibility.

The micrographs captured on the heavily degraded, T_4 polymer samples are shown in a dehydrated state. Nonetheless, there is evidence of the polymer degrading, as much of the fine surface detail shown in the T_0 images has been lost through the controlled weathering of the polymer. Additionally, there is evidence of the polymer film recovered from Echuca soil stretching, tearing, and pitting throughout the T_4 image, and there is a clear rip in the polymer film recovered from Seville soils toward the top of the image.

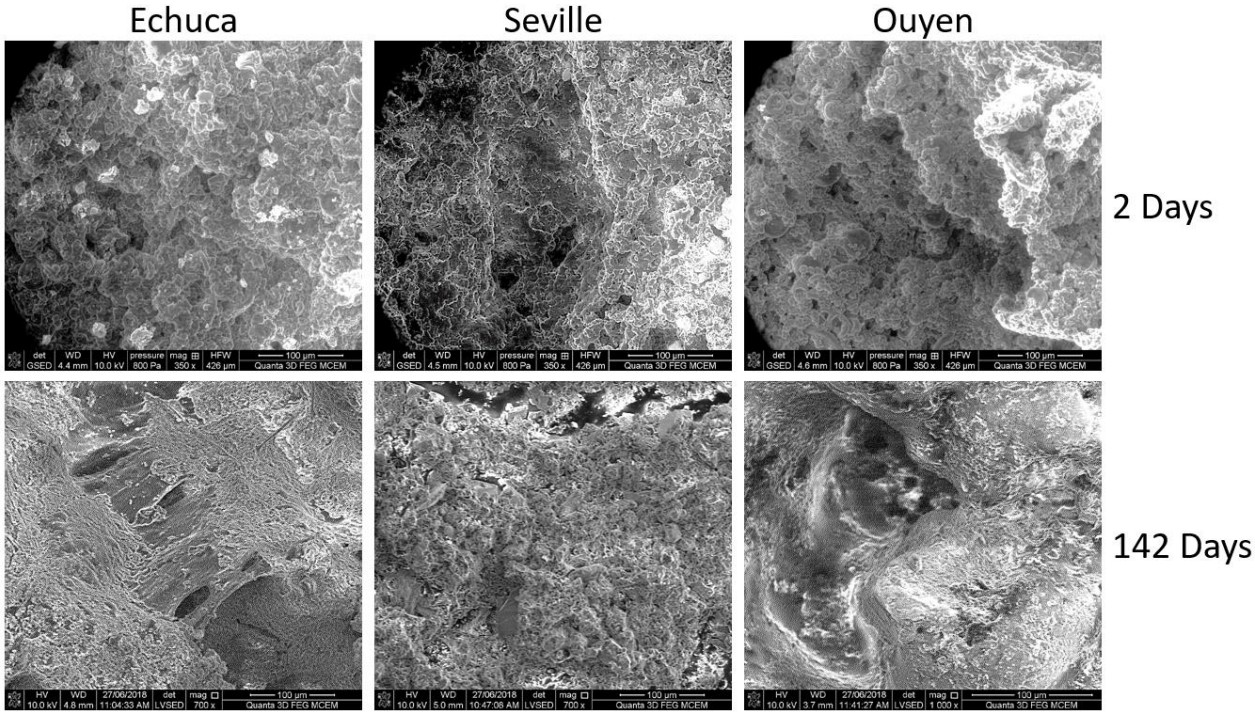

Figure 18. SEM micrographs of polymer recovered from each soil type after 2 days and 142 days degrading on the soil surface.

TGA Results

Figure 19 gives a representative TGA thermogram for a polymer sample recovered from soil and also follows changes in the onset temperature of thermal degradation throughout the trial. The onset of thermal degradation of the polymer was determined using STARe software (see SI) and it occurred in one continuous step beginning near 350 °C, indicating that the polymer existed as a single phase. The one-step thermal degradation profile was observed throughout the duration of the study, suggesting that the degraded polymer did not phase separate. As expected, the onset temperature of degradation decreased gradually as the polymer degraded and its molecular weight decreased. Additionally, the thermal stability of the polymer on Ouyen and Echuca soils did not change between the first two time points, which correlated well with the GPC data that showed a delayed onset in degradation to the polymer on those soils.

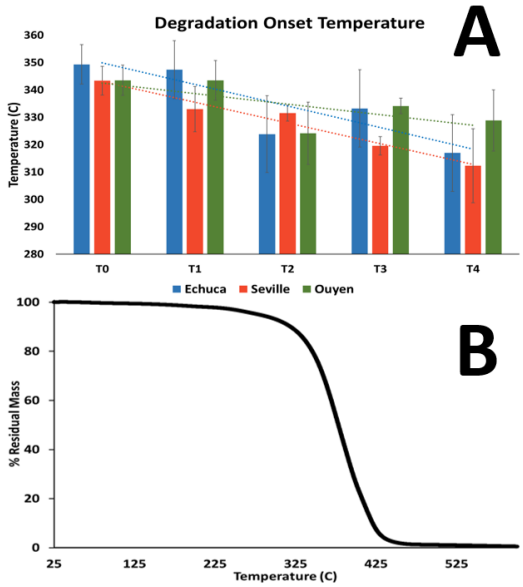

Figure 19. A) Degradation onset temperature of polymer recovered from each soil type. Dotted lines are lines of best fit through the data to guide the eye, and B) Example thermogram of the polymer recovered from soil.

Soil CHN Analysis

The soils were analyzed for Carbon, Hydrogen and Nitrogen content, before application of the polymer, and at the conclusion of the study in order to determine if the mulching treatment was adding

organic matter to the soil. The Hydrogen levels in the soil did not change, but the C:N ratio did increase in Ouyen and Echuca soils, from 0.37±0.009 to 7.12±0.69 for Ouyen soils and from 5.01±0.48 to 5.74±0.19 for Echuca soils. No statistically significant difference was observed on Seville soil, which could be due to its already high organic matter content. The increase in C:N ratio is further evidence that the polymer is degrading into smaller oligomers and small organic molecules, which may be available to the soil microbes to metabolize. This evidence, along with the enhanced CO_2 emissions on polymer treated soils suggests that microbes are metabolizing the polymer. This is a potentially important finding as it indicates that the polymer mulching treatment could be providing nutrients to the soil.

Conclusions

The sprayable, biodegradable polymer studied here was found to be effective at conserving soil moisture on three different soils, and under a variety of environmental conditions. Strong evidence, in the form of weight loss data, TGA, GPC analysis, and SEM micrographs demonstrated that the polymer is degrading on each soil type and under all environmental conditions studied. Furthermore, enhanced CO_2 emissions on soils treated with the polymer showed that soil microbes were able to utilize the polymer as a carbon and energy source, and that the polymer is biodegrading. Further long-term studies and real field conditions will be needed to elucidate how the polymer is affecting the soil microbial community.

Moisture content was the most important environmental variable studied in controlling the polymer's rate of biodegradation. This is an important finding because it can inform users on how long they could expect the polymer to perform under their specific environmental conditions.

The polymer biodegraded via a bulk erosion mechanism, and that biodegradation occurred fastest on soil from Seville, Australia. Soils are a complex mixture of minerals, organics, bacteria, archaea and fungi and are composed of particles of varying sizes. Due to this complexity it is difficult to deconvolute which characteristics are most important for controlling the rate of biodegradation, but

due to the differences in the soils studied here, some strong candidates have emerged. Soil pH, percent soil organic matter, and polymer morphology (based on soil particle size) all could be important in controlling the rate of polymer biodegradation, and likely all contribute in some way. Further study will be necessary to elucidate the effect of each characteristic.

This study demonstrated that under controlled glasshouse conditions the polymer shows promise as a replacement to polyethylene and other non-degradable plastics used as agricultural mulch, but also raises some questions. The polymer biodegraded extensively, but not completely, over the course of 5 months on the soil surface while maintaining its water conservation efficacy. In a real world setting the polymer would be tilled into the soil at the end of its use. Further study in an outdoor environment would be necessary to establish whether tilling can potentially accelerate biodegradation at the end of the useful lifetime of the polymer.

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Chapter 3. Hydrolytic and Long-term Soil Degradation study of a Sprayable, Polyester-urethane-urea

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Key Words: Sprayable polymer, Hydrolytic Degradation, Mulch, On-soil Degradation

Abstract

The use of conventional plastic mulch is an essential agricultural practice for increasing crop yield and improving water use efficiency, but their continued use presents environmental problems, and leads to a decrease in agricultural productivity. As biodegradable plastic mulches are a technological solution to this issue, it is important to understand how different environmental factors and application rates will affect the rate at which they degrade in nature. In this work, a series of lab scale hydrolytic degradation experiments were conducted to determine how different soil characteristics (pH, microbial community composition, and particle size) affected the degradation rate of a sprayable polyester-urethane-urea (PEU) developed as a potential biodegradable replacement to conventional non-degradable plastic mulches. This was coupled with long-term, outdoor, soil degradation studies to build a picture of important factors that can control the rate of PEU degradation. It was found that temperature and acidity were the most important factors controlling the rate of PEU degradation, with increasing temperature and decreasing pH leading to faster degradation. Other important factors that affected the rate of PEU degradation were the composition of the soil microbial community, the loading rate of PEU on soil, and the amount of PEU-soil contact.

Introduction

Nondegradable plastic mulch has emerged as a major source of single use plastic waste and pollution due to its widespread use (over 1 million tons/year) and inefficient removal from the field[1,2]. At the same time, this technology is essential to maintain crop yields and conserve agricultural water usage[3–7], while it is also deleterious to soil health and soil biota[8–10]. So, with a projected increase in global food demand under increasing water stress[11–13] it is critical that plastic mulch continues to be used, but in an environmentally safe way.

The use of biodegradable plastics is a viable alternative to nondegradable plastic mulch[14]. A relatively new field that has drawn interest is the use of sprayable, biodegradable polymeric mulches because of their inherent customisability and ease of application in variety of situations (ridge-furrow systems, horticultural systems, greenhouses and potted crops)[15].

In the literature, there have been many studies reported on the degradation of preformed biodegradable polymer films in different media, and with different formulation parameters[16,17]. It is well understood that the higher the degree of crystallinity within a biodegradable polymer, the slower the rate of biodegradation, and that typically with increasing polymer chain length and branching, the biodegradation rate will decrease. Other important formulation factors include glass transition temperature (T_g) and melting temperature (T_m) both of which relate to polymer chain flexibility or conformational freedom, and the consensus is lower T_g or T_m correlates with faster biodegration[18,19]. All of these factors (degree of crystallinity, chain flexibility and conformational freedom) relate to water infiltration into the polymer network, which in turn facilitates abiotic hydrolysis reactions and substrate access for relevant enzymes.

There has also been an abundance of work published on different environmental factors' (temperature, soil type), biotic (polymer degrading microbes and enzymes) and abiotic factors' (hydrolysis at varying pH, oxidation) effect on polymer biodegradation[20,21]. Contrary to the intuitive assumption, increased temperature does not always result in an increased rate of biodegradation, and in fact can decrease rate of biodegradation[22]. However, in most cases higher temperature

equates to a higher rate of degradation[20,23] until a point at which microbial activity is inhibited, and then high temperatures can cause biodegradation to cease.

Acidity or alkalinity also play an important role in controlling the rate of biodegradation, and each polymer will react differently in different pH conditions. This is both because acidic and basic conditions can catalyse abiotic hydrolysis of hydrolysable moieties, and because different pHs providing optimal (or suboptimal) conditions for enzymatic reactions[20,24].

Microbial action is often considered the most important factor controlling polymer biodegradation. Through polymer surface colonisation, the excretion of enzymes (exoenzymes) which can breakdown a variety of moieties, and the uptake of small oligomers, microbes are involved throughout the degradation process[17]. Many microbes native to the soil environment have been identified as biodegradable polymer degraders, but this varies between soil types and polymer type[16], and there are few studies that specifically investigate the relationship between soil type and polymer degradation[25,26]. Soils with a greater proportion of organic matter are more likely to accommodate favourable conditions for degradation due to their higher abundance of microbes, given that there is adequate water, nutrients, and temperatures.

To date there have been a number of studies performed on the development and efficacy of novel, biodegradable, sprayable mulches for agriculture. Giaccone *et al*. developed a sprayable mulch based on deacetylated chitosan mixed with polyglycerol and cellulosic fibres, and studied its efficacy on weed suppression.[27] Sartore and colleagues have developed and studied the efficacy of sprayable mulches based on protein hydrolysate (PH) blended with other biodegradable polymeric components (polyethylene glycol, poly(ethylene) vinyl acetate, lignin)[28,29]. Schettini *et al*. have done work developing sprayable mulches based on polysaccharides and PH, and evaluating their efficacies and material properties[30,31]. There has been some work done in the development of sprayable polysiloxane mulches, and the evaluation of their efficacy (water conservation, enhancement on crop yield, suppression of weed growth), material properties, and effect on soil

temperature[32,33]. Immirzi *et al*. developed a sodium alginate based sprayable mulch and conducted a thorough investigation into its material properties[34].

Of these studies, only three (Sartore *et al*. 2016, 2018, and Immirzi *et al*.)[28,29,34] have published the polymer degradation testings: Sartore and colleagues performed polymer degradation testing in water, and measured degradation by polymer mass loss alone; Immirzi and colleagues performed a standard biodegradation test (ASTM D5988)[35] in which a polymer film is buried in soil and the CO_2 evolved is measured.

Sprayable polymeric mulches and preformed polymeric mulches have very different interactions with the soil to which they are applied. Sprayable mulches derive much of their strength from their interaction with the soil, and form physical (and perhaps chemical) interactions with the soil that is absent with preformed polymeric mulches. This difference could cause differences in degradation behaviour between the two types of mulch. Because of this soil-polymer interaction, and the large variety of soil types to which a sprayable biodegradable polymer could be applied, it was of interest to gain an understanding of the relative importance certain soil characteristics play in affecting degradation behaviour and rate.

In addition to the array of different soils to which a sprayable polymer could be applied, application strategy, land management practice and the presence of inclement weather all could play an important role in the rate of degradation of a sprayable biodegradable polymer. The same polymer applied at different loadings may degrade at different rates, and the soil microbial communities' ability to degrade a particular polymer structure across multiple applications may change. Also, whether a sprayable polymer film has its contact with the soil disturbed by inclement weather could impact the rate of degradation.

Adhikari *et al*.[36], with the Commonwealth Scientific and Industrial Research Organisation (CSIRO), have developed a sprayable, degradable polyester-urethane-urea (henceforth referred to as PEU) for use as an agricultural mulch. In previous work, we have studied its degradation on the soil surface under a variety of environmental conditions, and observed noticeable variability in the rate

of its degradation predominantly due to the soil type to which the polymer was applied. Based on the characteristics of those soils, three parameters stood out as the possible causes for the different rates of degradation: pH, soil organic matter (SOM) content, and differences in the microscopic shape the polymer took on each soil type. It was of interest to deconvolute the importance of each of these factors on PEU degradation.

Here, through a series of controlled laboratory experiments, the first systematic study showing the impacts of soil pH, soil microbial community, and polymer microscopic shape on the hydrolytic degradation of a sprayable, biodegradable polymer is presented. Additionally, the impacts of some external factors (application loading, multiple applications, and soil-polymer disturbance) that may influence degradation rate were investigated via long-term, outdoor soil degradation trials.

Materials and Methods

Soil

Soil was obtained from three locations in Victoria, Australia: a grazing paddock in Seville; a well-tilled, active commercial tomato farm in Echuca, and an active, well-tilled wheat farm in Ouyen (Table 3). It was collected from the top 30 cm at each location and was air-dried and sieved < 2 mm prior to being set up in pots.

Table 3. Soil Characteristic

	Seville	*Echuca*	*Ouyen*
Soil Type	Dermosol	Vertosol	Tenosol
Electrical Conductivity, dS/m	0.43	0.1656	0.06164
pH	5.53	7.01	6.87
% Organic Matter	6.7	2.0	0.2
C:N	17.86	9.18	2.97
Sand, %	56.4	31.6	96.1
Silt, %	28.5	10.8	0.2
Clay, %	8.4	55.6	3.5

Polymer Mulch

A sprayable, polyester-urethane-urea (PEU) developed by Adhikari *et al.*[36] with the Commonwealth Scientific and Industrial Research Organisation (CSIRO) was used in this study. The main constituents of the PEU are polycaprolactone (PCL) which makes up >70 wt% of the PEU and isophorone diisocyanate (IPDI) which makes up 25 wt%. The rest of the PEU is constituted of the dimethylolpropionic acid (DMPA) and ethylene diamine (EDA) as a chain extender (Figure 20). The formulation was an aqueous suspension (20 wt% PEU solids) with Methocel® as a biodegradable viscosity modifier and carbon black as a biodegradable pigmentation.

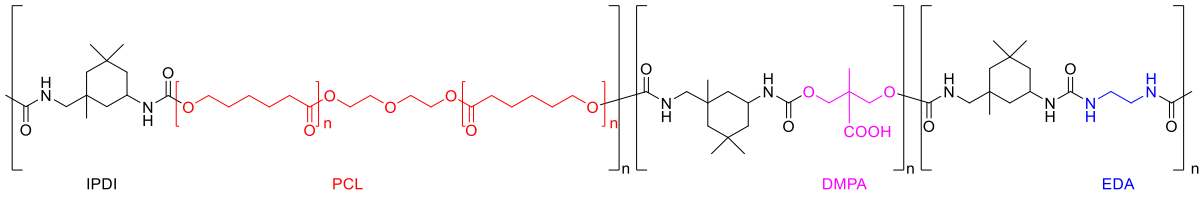

Figure 20. Representative structure of the PEU.

The suspension was drop cast into a film on a polytetrafluoroethylene (PTFE) plate. The resulting film's thickness was 1 mm, and it was cut into 10 mm x 50 mm strips, with a weight of 116 ± 19 mg. Additionally, PEU films were formed on each field moistened soil (Table 3), and then were cut into strips with the same dimensions.

Incubation Media

NaOH (Sigma) and HCl (37%, Sigma) were used with ultra-high purity water to form pH 9, and pH 5 incubation media, respectively. Ultra-high purity water was used as pH 7 incubation media. LB broth (pH 7), prepared by Monash University School of Biological Sciences Media and Prep Services, was used as incubation media for degradation experiments using soil microbial extracts as inoculants. All media was sterilised by autoclaving (121 °C, 15 psi for 30 minutes).

Hydrolytic Degradation Experiments

PEU films were placed in sterile vessels, and incubated in a variety of different media (pH5, pH 7 and pH 9, as described above) such that they could be destructively sampled in triplicate at four

times over a period of 60 days. PEU films formed on PTFE plates were sterilised by UV irradiation for 10 minutes on each side. Films were then placed in pH 5, pH 7, and pH 9 solutions and stored at room temperature (23 °C) to determine the effect of pH on PEU degradation, and an additional set of films were incubated in pH 7 solution at 40 °C to determine the importance of temperature on PEU degradation.

To determine the impact of the shape PEU takes when applied to different soils, films were formed on three soil types (Table 3), removed and then soil particles were gently removed via ultrasonication for 5 minutes and manual agitation. Films were then air dried at room temperature and sterilised by exposure to UV irradiation – 10 minutes of exposure to each side. Gel permeation chromatography (GPC) was conducted on films before and after sonication, and UV irradiation to ensure there was no change in polymer M_w and M_n from hydrolytic reactions or UV induced cross-linking. Scanning electron microscopy (SEM) was used to visualise the different morphologies the polymer films' took when formed on different soils.

The effect of the soil microbial community on polymer degradation was determined by incubating sterilised (by UV irradiation as described above) PEU films formed on PTFE in LB broth (pH 7) inoculated with soil microbial extracts from each soil (Table 3). Soil microbial extractions were performed using an adapted method originally described by Riis *et al*.[37] In brief, soil was agitated in sterile pH 7 phosphate buffered saline (PBS) for 10 minutes followed by five minutes of ultrasonication, and this process was repeated two additional times giving a total agitation time of 30 minutes, and a total ultrasonication time of 15 minutes. Soil solutions were then left undisturbed for five minutes to allow the heavy particles (sand and coarse silt) to settle, and after the five minute settling period an aliquot of the supernatant was transferred to the appropriate vessel containing LB broth and polymer film. No centrifugation was done to ensure the extraction captured both bacteria and fungi. To ensure sterility and adequate oxygenation of the incubation media, vessels were topped with a cotton plug soaked in 70% ethanol, and loosely capped to slow evaporation. Vessels

were stored at room temperature (23 °C) in an active fume hood. Additional ethanol was added to the cotton plugs three times weekly to ensure the cotton plug was always near saturation.

Long-term outdoor degradation experiments

To determine how different application loadings, multiple applications of the PEU to the same soil, and a disturbance of the soil-polymer contact impact its degradation, a long-term outdoor degradation experiment was carried out from 24/09/2018 to 03/02/2020. Average monthly temperature and total monthly rainfall is plotted in Figure 21. It was conducted in soil pots (24 cm inner diameter, 23 cm depth) filled with 8 kg of Seville soil (Table 3), and replicated five times. The soil was brought to 65% of the soil's experimentally determined field capacity, then allowed to degrade in an outdoor environment exposed to the natural weather. Mulching application was either at a rate of 0.5 kg m^{-2} (0.1 kg m^{-2} solid PEU) or 1.0 kg m^{-2} (0.2 kg m^{-2} solid PEU), and PEU film sampling was carried out as follows with treatment codes given in **bold**:

- 0.5 kg m^{-2} sampled after 275 days (**0.5**)
- 1.0 kg m^{-2}
    - Sampled after 275 days (**1.0**)
    - Disturbed after application via mechanical ripping and mixing. Sampled after 275 days (**Disturb**)
    - Reapplied at 1.0 kg m^{-2} loading after 275 days. Sampled at 497 days (**Reapply**)
    - Mechanically tilled into the soil after 275 days of degradation. Sampled at 497 days (**Till**)

Pots were watered regularly during periods when there was no rain. Sampled PEU films were characterised by GPC.

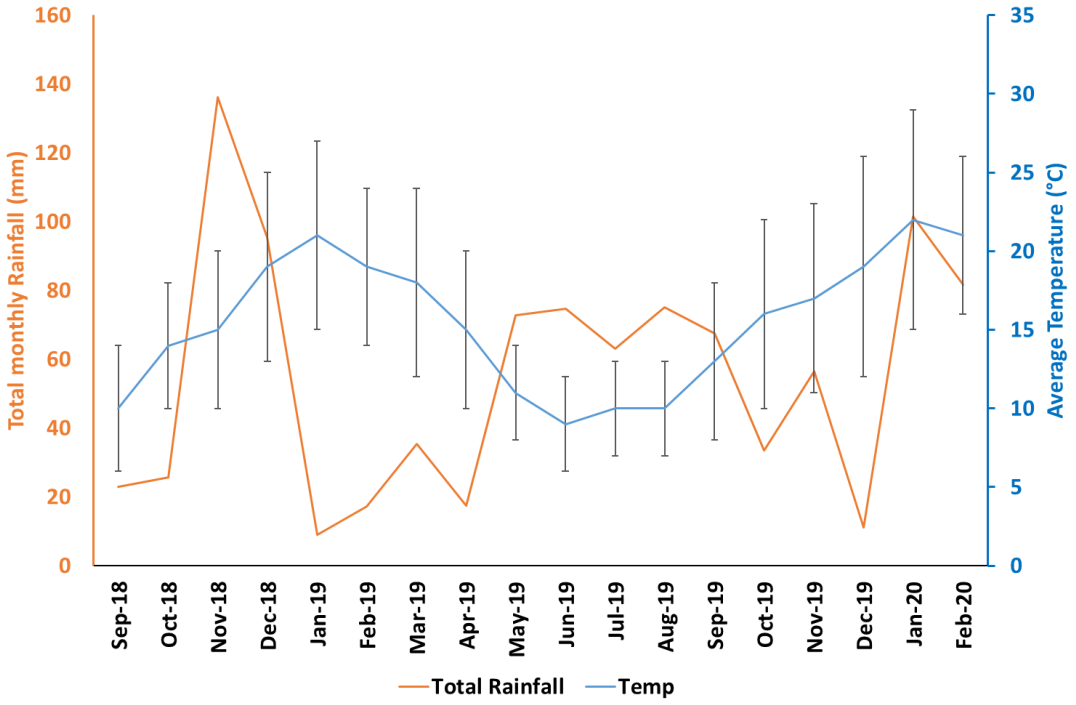

Figure 21. Average monthly temperature and total monthly rainfall over the studies duration. Error bars are ± 1 standard deviation.

Characterization

Gel Permeation Chromatography

Gel permeation chromatography was performed on a Shimadzu system equipped with a CMB-20A controller system, an SIL-20A HT autosampler, an LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, an RDI-10A refractive index detector, and 4X Waters Styragel columns (HT2, HT3, HT4, and HT5, each 300 mm × 7.8 mm^2, providing an effective molar mass range of 100-4 × 10^6). Samples were dissolved in DMAc containing 4.34 g L^{-1} LiBr, at a concentration of 1-2 mg mL^{-1}. The columns were calibrated with low dispersity polystyrene (PS) standards ranging from 575 – 3,242,000 g mol^{-1}. DMAc containing 4.34 g L^{-1} LiBr was used as an eluent at a 1 mL min^{-1} flow rate and 80 °C. M_n and M_w were evaluated using Shimadzu LC Solution software.

Scanning Electron Microscopy (SEM)

SEM micrographs were obtained using the secondary electron detector in a ThermoScientific FEI Quanta 3D FEGSEM. The SEM was operated under low vacuum imaging conditions to mitigate

sample charging issues. Operating conditions were as follows: 6 nA beam current, 20 kV accelerating voltage, 50 Pa chamber pressure, ~5 mm working distance.

Statistical Analysis

Statistical analyses were performed in Microsoft Excel 2016, and IBM SPSS Statistics 25. Excel was used for data organisation and processing, preliminary clean-up, outliers testing (Grubbs' test), and normalisation. SPSS was used for conducting ANOVAs to determine statistical differences between treatment groups with significance level set at $\alpha \leq 0.05$.

Results

Hydrolytic Degradation

A brief discussion of the expected points of breakdown in the PEU (Figure 20) backbone is warranted. The PEU will degrade abiotically via hydrolysis of the repeating ester bonds in the PEU's PCL soft-segment and the repeating urea and urethane moieties in the hard-segment. According to the literature, it can be expected that the esters will hydrolyse an order of magnitude faster than the urethanes and ureas, and urea groups will hydrolyse faster than the urethane groups[38–40]. Given the preponderance of ester links (prevalent in 70 wt% of the PEU, refer to materials and methods) and their enhanced rate of hydrolysis, it can be assumed that these will hydrolyse in the greatest quantity, especially so in the early stages of degradation. In fact, in Chapter 4 evidence of these ester hydrolysis reactions is given. In terms of biotic degradation, it is understood that fungi are the primary microbes responsible for degrading polyurethanes via excretion of ureases, esterases and proteases[41,42]. The sum of these abiotic hydrolytic reactions, and enzymatically catalysed hydrolytic reactions (where applicable) will be the primary cause for reduction in the molecular weight of the PEU.

As the polymer backbone is lysed, there will be an increasing abundance of carboxyl and amino groups, which are produced from the hydrolytic reactions of esters, ureas and urethanes. These can be susceptible to enzymatic deamination[43] and decarboxylations[44], but these reactions will have a minor effect on PEU molecular weight in comparison to the main chain scissions.

Soil alkalinity and acidity varies greatly[45], with soil pH measured as low as 4 and as high as 10 just in soils sampled in Australia. Due to this variation, it was important to understand how pH impacts the degradation of the PEU, and it was found that the degradation rate of PEU was increased in acidic conditions (Figure 22). Alkaline conditions slowed the rate of degradation compared to a neutral pH. Given the PEU structure, it is most likely that the abiotic hydrolysis of the ester bonds was acid catalysed while conversely the alkaline conditions had a protective effect on abiotic hydrolysis.

Evidently temperature played an extremely important role controlling the rate of PEU breakdown (Figure 22), with PEU films incubated at 40 °C showing the fastest rate of degradation.

There was little evidence of PEU mass loss in all treatment conditions except for those incubated under elevated temperature (Figure 22). At 40 °C the PEU film had an M_w of 30 kDa and 15 kDa after 28 and 60 days of degradation, respectively. These M_w correlated with mass loss of ~5% and ~40%, and so it can be surmised that PEU oligomers are not small enough to become water soluble and diffuse away from the PEU film until some threshold molecular weight less than 30 kDa but greater than 15 kDa is achieved.

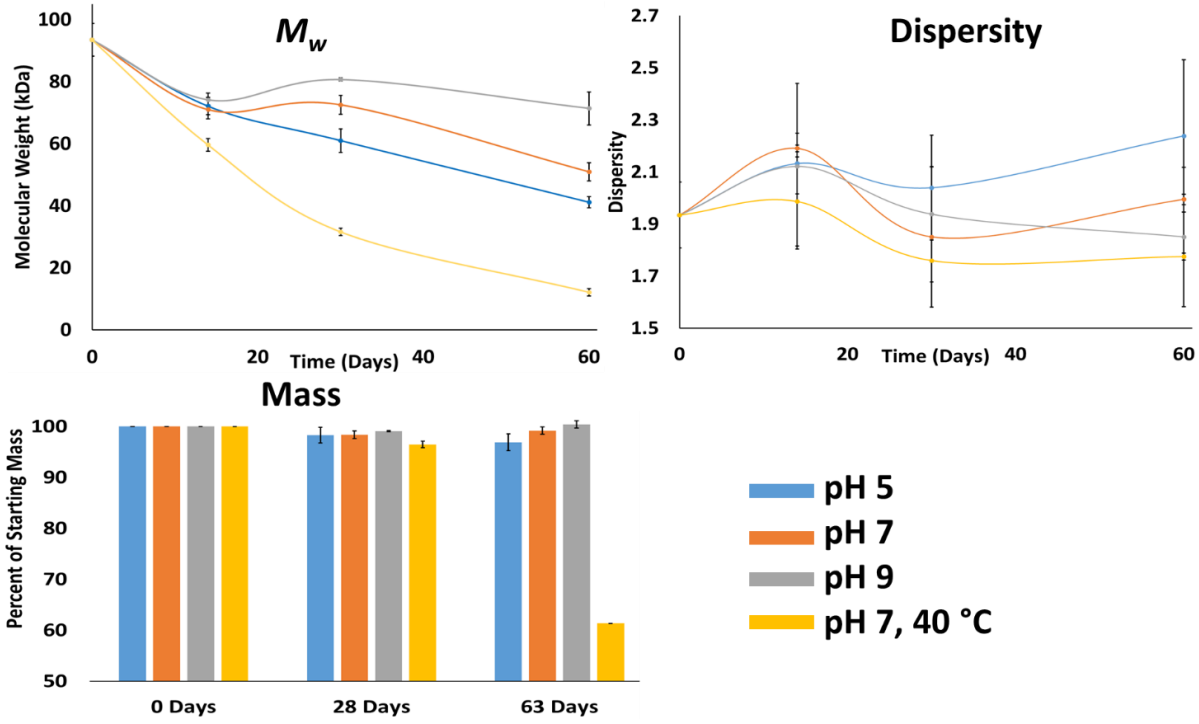

Figure 22. The effect of pH on PEU degradation. Incubations carried out at 23 °C unless otherwise specified. Lines are shown only to guide the eye, and do not represent lines of best fit. Error bars are ± one standard deviation.

By incubating PEU film strips in a buffered nutrient broth inoculated with soil microbial extracts, the effect of the soil microbial community on PEU degradation was demonstrated (Figure 23). After 60 days of incubation there was no difference in the extent of PEU degradation between any of the soil microbial extracts. It should be noted that any soil microbial extraction method cannot extract the entire soil microbial community, but the method used here has been previously validated as highly effective compared to other methods[37]. Interestingly the PEU film incubated in the presence of the microbial extract from Echuca soil degraded at a faster rate over the first 28 days. It is possible this trend would have continued if the nutrient broth had been replaced throughout the study to ensure adequate nutrient availability to the microbes because it is likely after 60 days the microbial community had consumed most of the resources available in the nutrient broth. This would be an interesting follow up study, but regardless it can be concluded that the soil microbial community plays a role in controlling the rate of PEU degradation, although the effect is of lesser magnitude than that of acidity.

The mass of the PEU film appears to have increased over the course of the degradation study, but this can be attributed to the colonisation of the PEU film by microbes or perhaps adhesion of small, suspended soil particles. It is interesting that the PEU film colonised by the largest microbial community (in terms of biomass on the film, Seville Soil Extract, Figure 23) was not degraded the most rapidly, which highlights the importance of the composition of the microbial community degrading the PEU, rather than just its size.

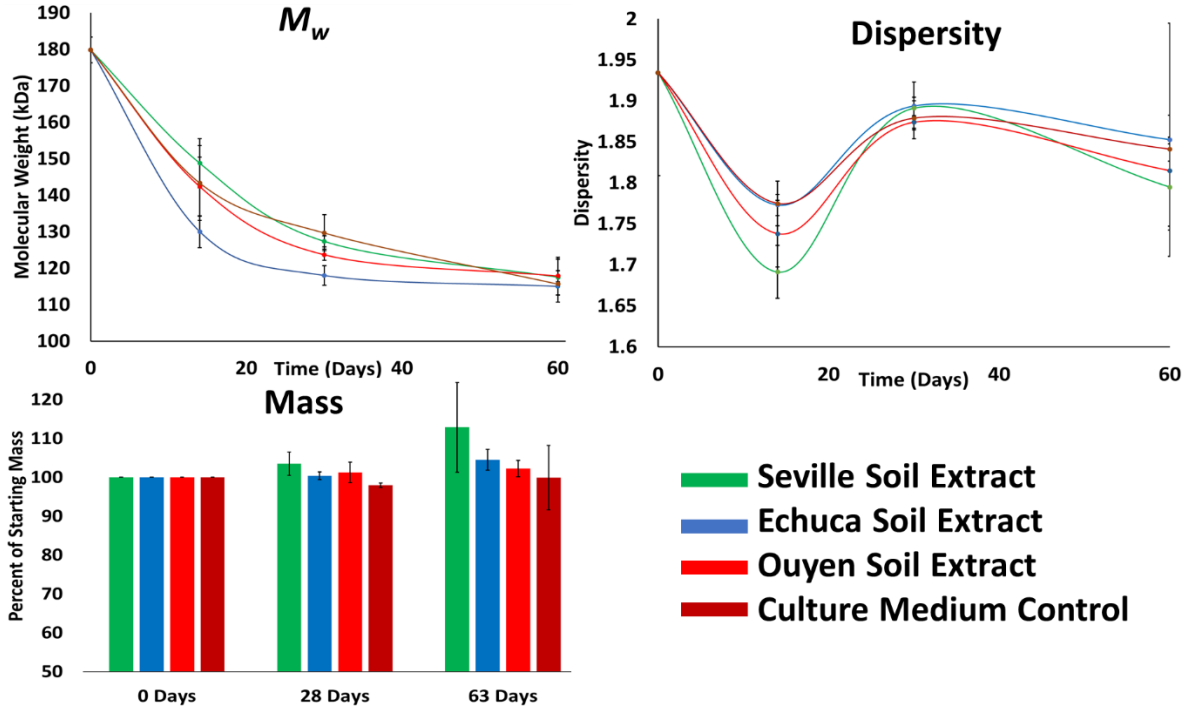

Figure 23. The effect of the soil microbial community on PEU degradation. Lines are shown only to guide the eye, and do not represent lines of best fit. Error bars are ± one standard deviation.

The last group of hydrolytic experiments conducted were to determine the importance of the microscopic shape of the PEU film on the rate of degradation. Soils will vary widely in their mineralogy and particle size distribution. It is commonly understood that clay particles are <0.002 mm in diameter, silt particles are between 0.002 mm and 0.06 mm diameter, and sand particles are greater than 0.06 mm in diameter[46], and the soils used here had large variation in the distribution of these three classes of particles (Table 3). The films formed on these different soils did have slightly different microscopic shape (Figure 24), but evidently that did not play a role in controlling the rate of PEU degradation (Figure 25). An unexpected finding here was the difference in PEU molecular weight immediately after application (Figure 25, M_w and M_n). The molecular weight (M_w) of the PEU formed on PTFE, Seville soil, Echuca soil, and Ouyen soil was 180 ± 4 kDa, 113 ± 4 kDa, 130 ± 1 kDa, and 123 ± 3 kDa, respectively. A possible explanation for these differences is that after application to the soil, the PEU film immediately hydrolysed to different extents.

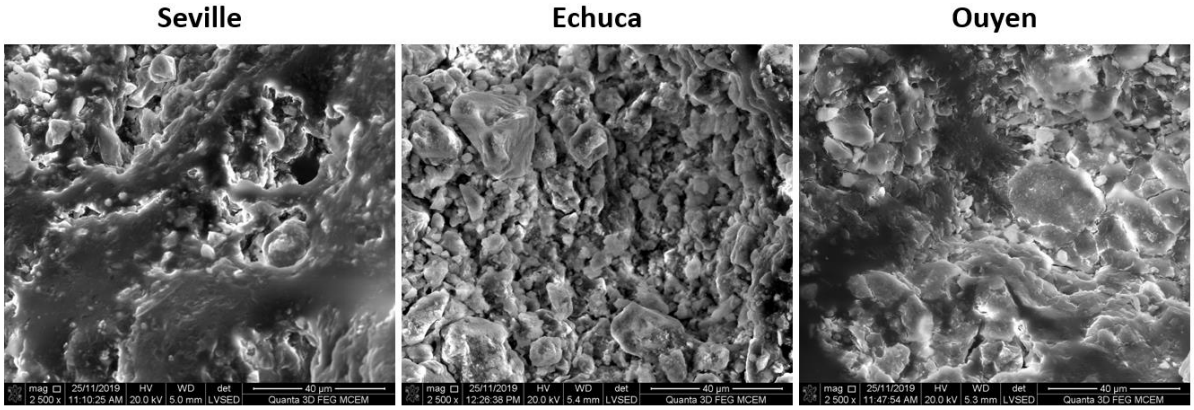

Figure 24. SEM micrographs of the PEU film formed on each soil type.

Regardless, after 14 days of degradation the PEU film on each soil had degraded to the same extent, and over the 60 day study the soil formed PEU films degraded at a rate of 800 ± 140 Da day^{-1}, which was slower than the rate of PEU film formed on PTFE (1300 ± 20 Da day^{-1}). Some mass loss was observed, but this is more likely attributed to soil particles being freed from the PEU matrix during degradation than actual PEU film mass loss.

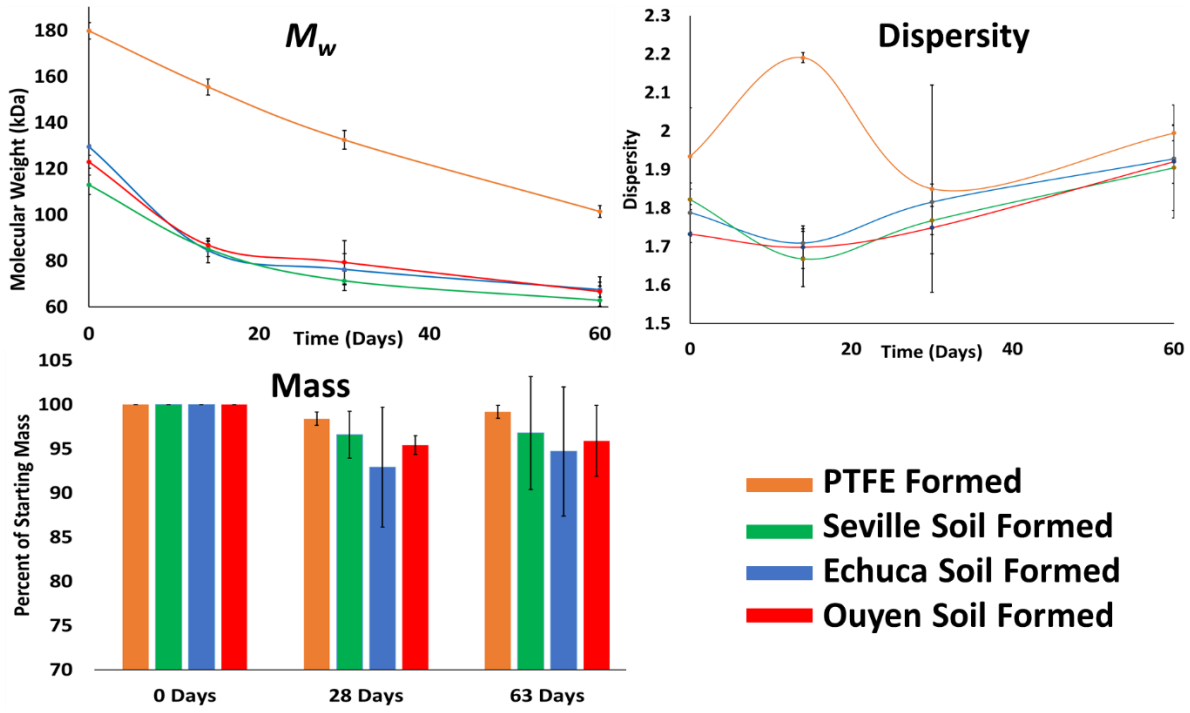

Figure 25. The effect of PEU shape on PEU degradation. Lines are shown only to guide the eye, and do not represent lines of best fit. Error bars are ± one standard deviation.

Long Term Degradation

The impact of PEU loading, repeated applications and a disturbance of the soil-polymer interface was investigated over a period of nearly 500 days (

Figure 26). After 275 days in soil degradation, PEU applied at 0.5 kg m^{-2} degraded more extensively than PEU applied at 1.0 kg m^{-2} (4500 ± 1000 Da and 25500 ± 8400 Da, respectively). It took twice as long for the 1.0 kg m^{-2} application to degrade to the same extent as the 0.5 kg m^{-2} loading, and this was an expected finding. An unexpected finding was that the PEU film which was mechanically disturbed immediately after curing, degraded faster than the PEU which was left undisturbed on the soil surface (Disturbed vs 1.0,

Figure 26). It was thought that by disturbing the soil-polymer interface it would slow the colonisation of the PEU by soil microbes, but evidently that was not the case. The disturbed PEU film had two surfaces directly exposed to the soil medium, which could explain the faster degradation rate.

The molecular weight of tilled PEU (Till), that is PEU which was treated in the same manner as 1.0 kg m^{-2} during the first 275 days of degradation and then was thoroughly mixed through the soil to further degrade, was reduced by the same amount as the 0.5 kg m^{-2} PEU. This finding conflicts with the finding in Chapter 6, where there was no evidence of the PEU after nearly 12 months of on-soil degradation. This suggests that the additional fertiliser (as used in Chapter 6) is necessary to bring the biodegradation process to completion.

It is interesting that the reapplied PEU degraded to a greater extent in a shorter time than PEU applied to previously unmulched soil (Reapply vs 0.1,

Figure 26) despite similar temperature and rainfall (Figure 21). This finding suggests some kind of conditioning effect, where the soil's capacity to degrade the PEU increased due to its previous presence in the soil.

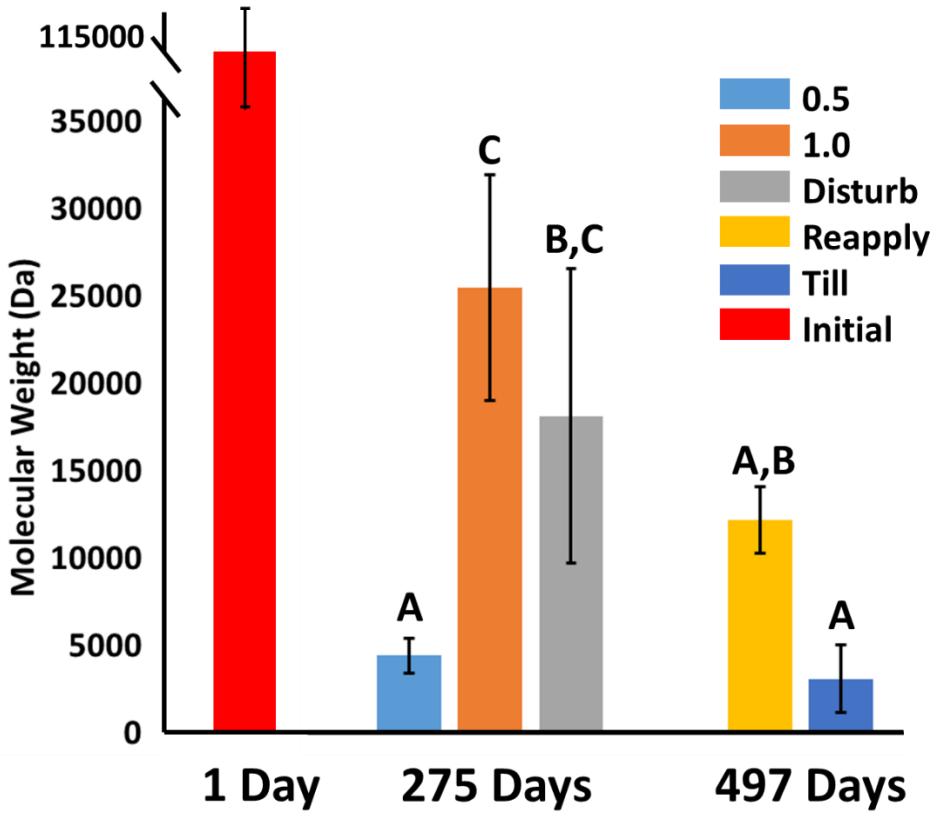

Figure 26. Molecular weight of PEU after outdoor degradation under a number of different degradation scenarios.

Conclusions

A series of laboratory based hydrolytic experiments, complemented with outdoor soil degradation studies have revealed several important factors controlling the degradation rate of a sprayable, biodegradable PEU mulch. It was determined that temperature had the largest effect on the rate of hydrolytic degradation, followed by acidity, with increasingly acidic conditions yielding faster degradation rates.

The soil microbial community composition affected the rate of PEU degradation, but apparently only under conditions where there were sufficient resources available for the community to grow. A follow-up study would be necessary to understand the importance of different nutrients' availability on microbial degradation of PEU. The microscopic shape the PEU film took when formed on different soils had no effect on its rate of degradation.

The PEU loading was a significant factor controlling the rate of degradation, and PEU that was mixed through the soil degraded faster than PEU that remained undisturbed on the soil surface. This could be important for PEU application in the field because gusting winds and heavy precipitation

that may increase PEU-soil contact (either by blowing soil onto the PEU film surface, or by pushing PEU film into the soil bulk) would increase its rate of degradation.

The factors studied here can be used to help predict the rate at which the PEU will degrade in different environments using information easily available to a grower (soil pH, seasonal temperatures) and can help guide a grower's decision in how much PEU to apply. Synergistic effects between these factors were not investigated, and this could be an important area of further study.

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Chapter 4. LC-MS analysis of the degradation products of a sprayable, biodegradable polyester-urethane-urea

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Abstract

Biodegradable polymers must degrade completely to CO_2, H_2O, small and non-toxic molecules (e.g. NO_3^- and NH_3/NH_4^+), and biomass on a similar timescale to classically compostable materials (3-12 months). More importantly, the degradation intermediates - the compounds that form as a polymeric material breaks down in the environment, before being mineralized or bio-assimilated also need to be non-toxic. Here, for the first time, the identity of the degradation intermediates formed from the breakdown of a sprayable, biodegradable polyester-urethane-urea was investigated using a liquid chromatography mass spectrometry (LC-MS) system. This was accomplished by degrading the polymer in abiotic aqueous media for varying lengths of time and in soil media for 57 days, and analyzing the degradation media for polymer degradation intermediates. It was found that during degradation, monomers and short oligomers were formed containing amino, alcohol and carboxylic acid moieties. Interestingly, the most prominent degradation products formed during abiotic

degradation (6-hydroxy hexanoic acid, and its oligomers) were not detectable when the polymer was degraded in a soil environment. Gel permeation chromatography confirmed that the polymer's molecular weight was substantially reduced during the degradation studies, but the presence of polymer fragments >1000 Da in the soil indicated that there would be an ongoing release of 6-hydroxy hexanoic acid and its oligomers. Taken together this suggests that those molecules were rapidly bio-assimilated by the soil microbial community.

Introduction

Plastic (polymeric) mulch films are used in agriculture in great quantity, with estimates ranging from 700,000-1,245,000 tons applied annually to over 20 million hectares of land.[1–3] They are used to conserve soil moisture by providing a physical barrier to prevent water from evaporating, to alter soil microclimatic factors such as soil temperature, prevent weed growth and even to reduce pest populations all of which ultimately lead to higher crop yields, earlier in the growing season.[4–8] As the world population grows to over 10 billion by 2050[9] and food security continues to be an ever increasing problem[10] an increased usage rate of plastic mulch will be necessary to continue to meet food demand while preserving water security. Unfortunately, the plastic mulch typically used is non-degradable, or extremely slow to degrade polyethylene (PE), which has been thoroughly discussed throughout the literature as environmentally deleterious, and harmful to future soil productivity.[11–14] Evidently, an alternative must be used – biodegradable polymeric mulch films. There has been extensive research done on many different biodegradable polymeric formulations based on a variety of materials[15–20], but widespread adoption of biodegradable technologies has not yet occurred. Cost[21] and achieving suitable mechanical properties for the duration of the growing season[22] in biodegradable films remain the biggest challenges to the technology, but Adhikari et al. suggest that the use of a sprayable polymer may help overcome these challenges.[21] Sintim and Flury point out that the degradation products of a biodegradable plastic mulch must not be toxic, persistent, and ideally

should be entirely consumed by soil microorganisms or added to the soil organic matter (SOM) pool, as a benign carbon source.[23]

A sprayable, biodegradable polyester urethane-urea, [24] has been developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia for use as an agricultural mulch, but little is known on what happens to the polymer as it breaks down. In this study, the degradation behaviour and identification of degradation products of this sprayable biodegradable polymer were investigated using liquid chromatography coupled to a high-resolution mass spectrometer (HRMS). This is accomplished by degrading the polymer i) abiotically in sterile water at elevated temperature, ii) enzymatically in phosphate buffered saline (PBS) inoculated with different enzymes, and iii) in two soil types. Matrices from each set of degradation tests were analyzed.

Although significant work has been reported using mass spectrometry on biodegradable polymeric materials, the majority of that work focuses on characterizing the polymer itself, or the residual polymer films after degradation[25], and there are currently no standard test methods that utilize mass spectrometry to characterize polymer degradation modes [26]. Comparatively few mass spectrometric studies have been carried out analyzing the degradation media itself[25], and of those even fewer have been carried out using soil as a degradation medium. Rankin et al.[27], and Washington et al.[28] are two examples describing mass spectrometric characterization of polymers degraded in a soil matrix, and in both cases the studied polymers were low molecular weight (M_n = 3,000 Da) perfluorinated polyesters. Recently, Zumstein et al.[29] used isotopic ratio mass spectrometry to analyze the evolved CO_2 from a polybutylene adipate terephthalate film buried in soil, but did not identify degradation products. There have been some studies utilizing mass spectrometry to identify degradation products of polymeric materials containing repeating ester, urethane and/or urea bonds similar to the polymer studied here[30–32] (Figure 27), but none of these studies were carried out in a soil matrix. Furthermore, all work previously done has been carried out on preformed films, and not on sprayable polymer formulations. Therefore, herein for the first time the characterization of the degradation products of

a sprayable, biodegradable polymer by mass spectrometric techniques, are reported, including degradation products of a soil-biodegraded polyester-urethane-urea based polymer.

Figure 27 shows a representative structure of the polymer used in this study. By weight percent, the polymer is constituted of ~ 25% IPDI, 72% PCL, and 2.5% EDA, and 0.5% DMPA.

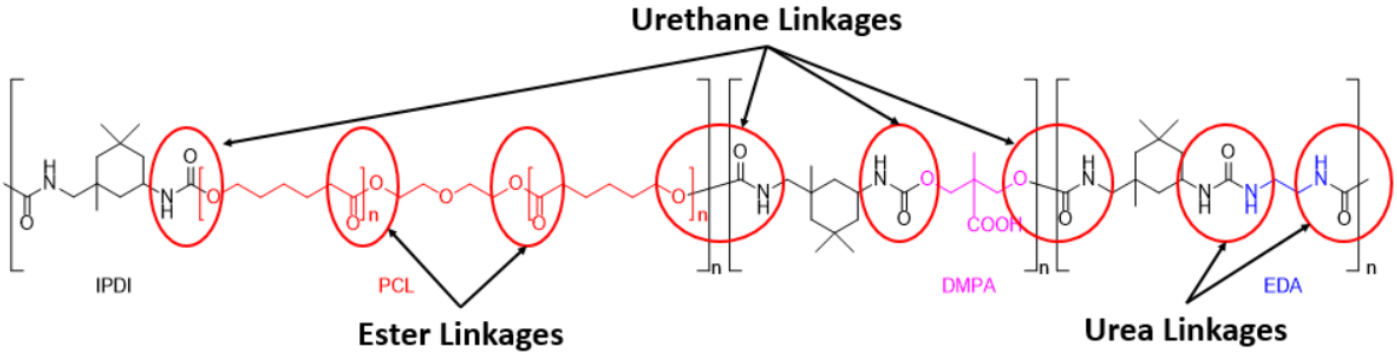


Figure 27. Representative structure of polyester urethane-urea used in study.

Experimental Section

Materials and Methods

All chemicals used for polymer synthesis were of reagent grades and obtained from Sigma: polycaprolactone diol (PCL, Mn ~1250), isophorone diisocyanate (IPDI, 98%), 2,2-Bis(hydroxymethyl)propionic acid (DMPA), ethylene diamine (EDA), and triethyl amine (TEA). Sodium dodecyl sulphate (SDS, Sigma), Methocel™ (Dow), and carbon black (CB, Sigma) were used in the polymer suspension as a surfactant, viscosity modifier, and pigment, respectively. Abiotic degradation experiments were carried out in MilliQ water. Enzymatic degradation experiments using urease (from *Canavalia ensiformis*, Sigma) and esterase (from *Bacillus subtilis*, Sigma) were carried out in phosphate buffered saline made with NaH_2PO_4 (Sigma), Na_2HPO_4 (Sigma) and MilliQ water. Enzymes were buffered at their optimum pH which was 7 and 7.5 for urease and esterase, respectively. Enzyme activity was quantified using 4-nitrophenyl acetate (Sigma), and urea (Sigma) as substrates for the esterase and urease, respectively. Urea was detected as ammonia after hydrolysis via reaction with Nessler's Reagent (Sigma). 4-nitrophenol (Sigma), and $(NH_4)_2SO_4$ (Sigma) were used as standards in the enzyme activity quantification. The reference soils were obtained from the

Fraunhofer Institute for Molecular Biology and Applied Ecology in Schmallenberg, Germany. Some of their characteristics are given in Table 4. The soils have been designated as 'Low Organic Matter' (LOM) or 'High Organic Matter' (HOM) based on their specifications, relative to each other. Polymer was extracted from the soil using dimethylformamide (>99.8%, Sigma), and the efficiency of this extraction was validated (data not shown). Liquid chromatography (LC) solvents used were ultra high purity water, LC-MS grade methanol (>99.95%, TH Geyer, Chemsolute®), LC-MS grade formic acid (no exact purity provided, Fisher Scientific), ammonium acetate (>99.0%, Sigma Aldrich).

Table 4. Soil characteristics

Designation	Texture	Sand	Silt	Clay	Organic Carbon	Total N	pH	WHC	Microbial Biomass
		%	%	%	%	g/kg		g/kg	mg/kg
LOM	Sandy Loam	74.0	19.8	6.2	0.93	0.92	5.71	293	198
HOM	Silt Loam	22.1	52.8	25.1	3.02	4.42	6.03	697	488

Polymer Synthesis

The polymer used in this study was synthesized using the two step method as described by Adhikari et al.[24] In brief, a PCL based polyester-urethane pre-polymer was synthesized by reacting anhydrous PCL diol and IPDI under a N_2 atmosphere. DMPA was then added to the reaction mixture, followed by an EDA chain extender. The reaction mixture was left to react until all of the isocyanate had reacted (as confirmed by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy, ATR-FTIR), giving a final M_w and M_n of 120 kDa and 40 kDa respectively (as measured by GPC). The final polymer formulation contained 20 wt% polymer solids. Methyl cellulose and carbon black were added to the final mixture to adjust the viscosity and provide pigmentation. Polymer solution was drop-cast into film onto polytetrafluoroethylene (PTFE) plates and dried overnight in a vacuum oven at 40°C. The resulting film thickness was approximately 0.1mm. 1x5 cm strips (100.11±0.16mg) of polymer film were prepared for degradation studies.

Degradation Experiments

Hydrolytic Degradation (Abiotic)

Polymer films prepared earlier were immersed in 10mL of MilliQ water in glass vials. The glass vials were capped and placed in a 100°C oven in order to enhance the rate of degradation, and ensure no microbial growth nor enzymatic activity. Sufficient replicates were set up so that vials could be destructively sampled in triplicate after 19 hours, 140 hours, and 304 hours.

Additionally, three vials were set up in the same manner, in a sterile environment, but were incubated in a 35°C oven for 52 days. This lower temperature trial was used for comparison of the degradation products formed under the unrealistically high 100°C accelerated conditions.

Immediately after removal from the oven, polymer film residue was removed from the incubation medium (MilliQ H_2O) and dried in an oven at 35°C for further characterization, and the incubation medium was saved for HPLC-mass spectrometric analysis.

Enzymatic Hydrolytic Degradation

In enzymatic hydrolytic degradation experiments, pre-formed polymer films were immersed in phosphate buffered saline (PBS), inoculated with either urease from *C. ensiformis* or esterase from *B. subtilis*, and incubated in an oven at 35°C for 34 days. Polymer films were prepared in the same way as the hydrolytically degraded samples, except the film was cut into 10.05±0.16 mg squares (approximately 70 mm x 70 mm). PBS of different concentration and pH was necessary to provide optimal enzyme activity conditions for the two enzymes.[33,34]

0.2M pH 7 PBS was prepared, and inoculated with 3 mg mL^{-1} urease. Urease solutions were stored at 4°C in the dark for up to 10 days before being discarded, and fresh urease solution made. Urease activity was determined experimentally (data not shown) for freshly prepared, and 10 days stored urease solutions and was found to be 83.8±6.9 U mg^{-1} and 91.0±7.5 U mg^{-1}, respectively. These differences were not found to be statistically significant by the Student's t-test.

1.5 mL of the urease solution, and one 10 mg square of the polymer film (equivalent to 37.7 U urease mg polymer^{-1}) were placed in a 2 mL glass vial, and incubated in a 35°C oven. Incubation solutions were shaken at minimum five times daily. Urease solution was removed from the polymer film and replaced with 'fresh' urease solution every 72 hours. Removed urease solution was immediately stored at 4°C for liquid chromatography-mass spectrometric (LC-MS) analysis.

At the end of the 34 day incubation period (10 urease solution replacements), the residual polymer film was rinsed three times with ultra high quality (UHQ) water, and then placed in a 35°C oven to dry overnight. The films were then cooled to 4°C in a fridge, and their mass was measured. Films were then stored at 4°C until further characterization.

Esterase solutions were stored and prepared in the same manner as described for urease. 0.05M pH 7.5 PBS was prepared, and inoculated with 0.667 mg mL^{-1} esterase. The activity of freshly prepared, and 10 days stored esterase solutions was experimentally determined (data not shown) and found to be 325±90 U mg^{-1} and 287±43 U mg^{-1}, respectively. These differences were not found to be statistically significant by the Student's t-test. This meant approximately 32.6 U esterase mg polymer^{-1} was present.

Polymer film incubations were performed using the same conditions, and sampling procedures as described above for urease incubations.

Soil Degradation

Two soil types LOM and HOM were used in this study. Soils were set up in 9 cm ID by 8 cm tall polypropylene pots. Soil was filled to the same depth (6 cm) in order to control for surface area, and because each soil had a different bulk density this resulted in different total masses of soil being used (300 g LOM, and 230 g HOM per pot). LOM and HOM soils were maintained at 70% and 63% of field WHC, respectively.

Soils were wetted to the WHCs described previously and then the polymer solution was applied by syringe at a loading of 1 kg m^{-2}, or 6.5 g pot^{-1} (1.3 g solid polymer), and immediately after application

the liquid polymer suspension was mixed within the top 2 cm of soil. This mixing was done to maximize polymer-soil contact in order to increase the rate of degradation. Pots were then incubated in a 35°C oven for 57 days, being weighed and topped up with water daily in order to maintain 70% or 63% field WHC for LOM and HOM, respectively.

At the end of the incubation period, the top 3 cm of soil-polymer matrix (or soil only in the case of control pots) was removed and leached with excess water, and the leachate was saved for LC-MS analysis. The following 3 cm of soil (soil from 3 to 6 cm from the surface) in the pot was then removed and leached with excess water, and this leachate was also saved for LC-MS analysis. The top 3 cm of soil-polymer matrix (or soil only in the control pots) was then extracted with DMF. The DMF was then evaporated, leaving behind polymer residue to be characterized. Note that polymer was directly applied to the top 2 cm of soil only, so an additional 1 cm of soil was collected to ensure that there was no direct polymer contamination in the 3-6 cm soil fraction.

Characterization

Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid chromatography coupled to a high-resolution mass spectrometer (LC-MS) was performed on incubation media of the abiotically and enzymatically hydrolyzed polymer films, and on the leachate recovered from the different soil layers at the termination of the soil-polymer incubation period.

An Acquity UPLC system (Waters) was coupled with a Q-Exactive Plus Orbitrap MS (ThermoScientific). The LC column used was a BEH C18, 100 x 2.1 mm, 1.7µm from Waters. The following parameters were used: 20µL injection volume, 0.35mL/min flow rate, 55° C column temperature, 15° C sample temperature, and both positive and negative ionization mode were measured with an Electrospray Ionization (ESI) source.

MS properties were as follows: 50-750 m/z range, 70,000 resolution, 200 ms injection time, and both FullMS and All-Ion Fragmentation (AIF) data were collected, with the AIF being collected using a stepped normalized collision energy (NCE) of 35, 60 and 80.

The LC solvent program is given in Table 5 where A and B are Water/MeOH (95/5 v/v) + 0.1 % formic acid and MeOH + 0.1% formic acid, respectively, for positive ionisation mode. In negative ionisation mode the same solvent system was used except formic acid was replaced by 2mM ammonium acetate.

Table 5. Liquid Chromatography Solvent Program

Time (min)	A (%)	B (%)	Curve
Initial	100	0	Initial
10	0	100	6
13	0	100	1
15	100	0	1

Gel Permeation Chromatography (GPC)

See supporting material for run conditions.

Scanning Electron Microscopy (SEM)

See supporting information for details on operating conditions.

Mass Loss

The residual polymer film mass was measured for enzymatically degraded samples. Films were recovered from incubation media, rinsed three times with MilliQ water, and dried overnight in a 30°C oven before being weighed. Mass loss measurements were not taken on abiotically degraded samples because the highly degraded samples were extremely waxy and adhesive. Accurate measurement was difficult.

Toxicity Testing

To determine if there any of the identified degradation products were toxic, each of their structures were inputted into two different toxicity predicting structure-activity relationship (SAR) programs. The Toxicity Estimation Software Tool (TEST) developed by the United States Environmental Protection Agency (USEPA)[35] and the OSIRIS property explorer developed by Thomas Sander from Idorsia Pharmaceuticals.[36] Using TEST, identified molecules were checked for bioaccumulation factor (BAF), developmental toxicity (DT), mutagenicity (Mut), and rat oral LD50 (ROLD50). In addition the certainty of these predictions was broken into three classifications – certain, semi-certain and uncertain based on the mean absolute error (MAE), and concordance of the predictions. MAE is the error between predicted values for similar chemicals to the tested chemical and their actual value, and concordance is the fraction of all compounds that are predicted accurately (i.e. experimental results match predicted results). Predictions were deemed **certain** when MAE <10% or concordance >0.8; **semi-certain** when 10%< MAE <50% or 0.4< concordance < 0.8; and **uncertain** when MAE >50% or concordance <0.4 or there were less than 4 cases to compare with.

Using the OSIRIS software identified molecules were checked for Mut, tumorigenicity (Tum), irritant (Irr), and reproductive effect (RE). No certainty tests for these predictions were given.

Phytotoxicity Testing

To determine phytotoxicity of some of the identified molecules, a germination trial was carried out using 6-hydroxy hexanoic acid, isophorone diamine, and total polymer hydrolysate (TPH) on radish seeds (*Raphanus sativus*), cress seeds (*Lepidium sativum*), and lettuce seeds (*Lactuca sativa*).

The germination trial was carried out following the procedure as described by Mosse et al.[37] In brief, the appropriate amount of degradation product was dissolved in sterile H_2O and then 2mL of the solution was applied to filter paper in a petri dish. Ten of the appropriate seeds were then added, and the petri dish was stored in the dark in a temperature controlled incubator at 25 C for 10 days.

Deionized water was used as a control. Degradation products were applied at two levels based on a polymer field rate of 1 kg m^{-2}:

(1) Assuming half of the polymer was hydrolyzed to its constituents and entirely remained at the soil surface where seeds germinate. And

(2) Assuming one tenth of the polymer was hydrolyzed to its constituents and entirely remained at the soil surface where seeds germinate.

Through these tests germination, defined as seed radicle length ≥ 5mm, was assessed daily. Total percentage germination, mean time to germination (MTG) and germination index (GI) were determined. MTG was calculated as follows:

$$MTG = \sum \frac{n \times d}{N}$$

Where *n* is the total number of seeds germinated between scoring intervals, *d* is incubation time in days, and *N* is tot number of seeds germinated in the treatment. GI was calculated after 48 h as follows:

$$GI = \frac{G_s}{G_c} \times \frac{L_s}{L_c} \times 100$$

Where G_s and G_c are the number of seeds germinated in the treatment group and control group respectively, and L_s and L_c are the radicle length of the seeds in the treatment group and control group respectively. GI is expressed as a percentage of control.

Statistical Analysis

Where necessary, statistical testing of experimentally determined means was undertaken using either Microsoft Excel 2016, or IBM SPSS Statistics 25. The statistical tests used were One-way ANOVAs followed by Tukey's Honestly Significant Difference, or the Student's T-test assuming unequal variances.

Results and Discussion

In the context of understanding how the polymer might behave in degradation, some structural features warrant consideration (Figure 27). The polymer is assembled by first reacting isophorone diisocyanate (IPDI, in black) with polycaprolactone diol (PCL, in red, average M_n ~ 1250 Da), resulting in an IPDI end-capped polyester. 2,2-Bis(hydroxymethyl)propionic acid (DMPA, pink) is then added to the reaction mixture, and shortly after ethylene diamine (EDA, blue) is added as a chain extender. As IPDI covalently bonds to each of the other reactants during the synthesis, thus forming the repeating urethane and urea structure, any breakdown products which are not simply the liberated monomeric reagents (for example, varying lengths of PCL) are expected to include an isophorone moiety, but will eventually mineralize to CO_2, NO_3^- and NH_4^+.

The primary sites of hydrolytic degradation are the ester groups in the PCL soft segment, the urethane groups formed between IPDI's isocyanates and the alcohols from PCL or DMPA, and the ureas formed between IPDI's isocyanates and the amines from EDA. According to the literature, under abiotic conditions at 70°C, the rate of ester hydrolysis is an order of magnitude greater than that of urethanes[38–40], and ureas hydrolyze before urethanes[41]. Therefore, 6-hydroxy hexanoic acid (6HHA), the monomeric unit of the PCL polymer, as well as 6HHA dimers, trimers and oligomers of 5-6 6HHA units linked together were predicted, based on the size of the PCL diol used in the polymer's preparation.[42]

Other polymer fragments that form should contain IPDI bonded to 6HHA, EDA and in some cases DMPA. At longer degradation times, a large proportion of urethane bonds were expected to hydrolyze, leaving isophorone diamine (IPDA). During enzymatic hydrolysis by esterase, it is expected that the ester bonds will be preferentially hydrolyzed. Enzymatic hydrolysis by urease should result in the preferential hydrolysis of the urea groups, which proceeds in parallel with the abiotic hydrolysis of the labile ester bonds.

When in the soil environment, it is expected that in addition to the hydrolysis of the ester, urea, and urethane groups, secondary and tertiary reactions will occur. These further reactions could include

abiotic organic modifications (Fischer esterifications), redox chemistry[43,44], enzyme catalyzed deaminations[45] and decarboxylations[46], to name a few. It is understood that degradation by fungi, and their exoenzymes is the predominant degradation mechanism of polyurethanes in the soil environment[47,48].

Abiotic Hydrolytic Degradation

GPC results of the polymer degraded at 100°C indicated that random, hydrolytic chain scissions occurred continuously, reducing both the M_w and M_n of the polymer according to first-order reaction kinetics (Figure 28). By fitting exponential functions through the data it was possible to determine hydrolysis half-life for the polymer at 100°C, and this was found to be 77.0 ± 6.6 hours. This half-life is very short, but of course does not hold much practical value as temperatures in the field will never reach anything close to 100ºC. After 33 days incubation at 35ºC the M_w and M_n were only reduced to 57,000 ± 5,000 Da and 3,600 ± 3,000 Da (initially M_w and M_n of 120 kDa and 40 kDa), respectively.

During the first 20 hours of degradation the weight average molecular weight (M_w) is rapidly halved, while there is a delayed onset to the reduction of the number average molecular weight (M_n). This correlates with first order rate kinetics – the likelihood of the random chain scissions occurring in the largest polymer molecules (in the soft segment) is greater, thereby halving the molecular weight of those molecules, and replenishing the pool of the numerically most abundant molecular weight molecules (M_n). As the polymer molecules are randomly hydrolyzed, M_w and M_n converge (Dispersity, Đ, approaches 1) due to the increased probability of random scissions occurring initially on larger polymer molecules. Figure 29 displays the molecular weight distribution of the residual polymer film over time. Note that the initial film's M_w is evenly distributed, and after 19 hours of degradation there is an asymmetry, indicative of a greater proportion of lower M_w polymeric chains.

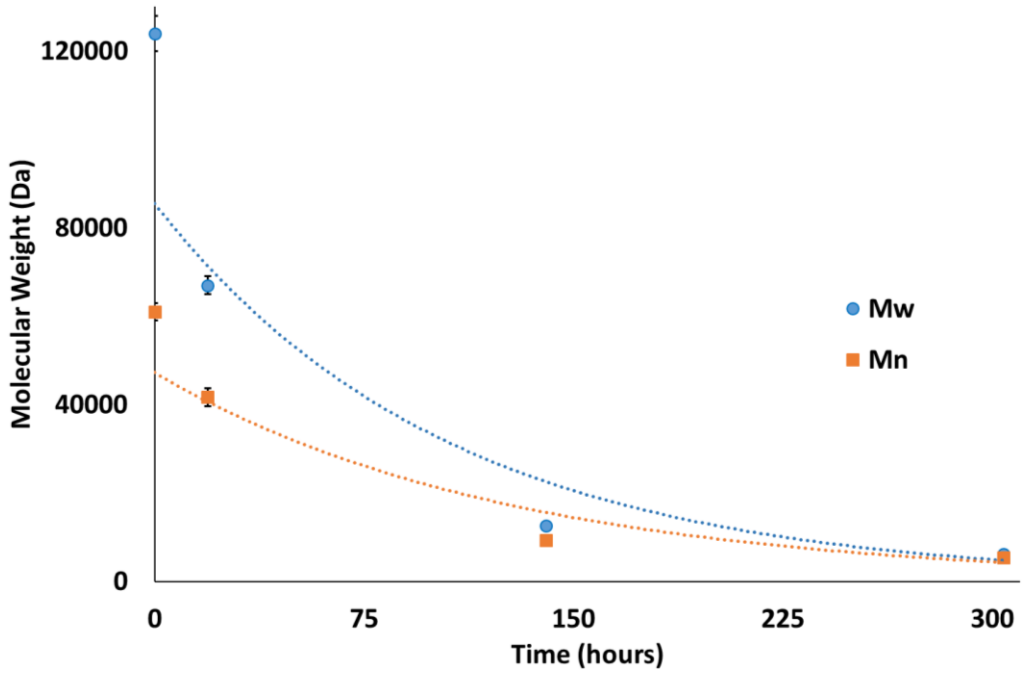


Figure 28. Molecular weight vs. time of abiotically hydrolyzed polymer films at 100°C.

By examining the LC-MS data, it was possible to deduce the relative abundance of the low molecular weight oligomers and their identities.

Figure 30 shows the total ion current-chromatograms obtained in FullMS mode in both positive ionization mode (PIM) and negative ionization mode (NIM) from the degradation media of abiotically hydrolyzed samples. The chromatograms have been background subtracted using solvent blanks, and the final 5 minutes have been omitted as all they contained was noise. Peaks represent individual degradation products. Mass spectra associated with each peak were analyzed to determine the identity of the degradation products. For example, analysis of the peak at a retention time of 4.3 minutes in the NIM chromatogram produced the mass spectrum displayed in Figure 31.

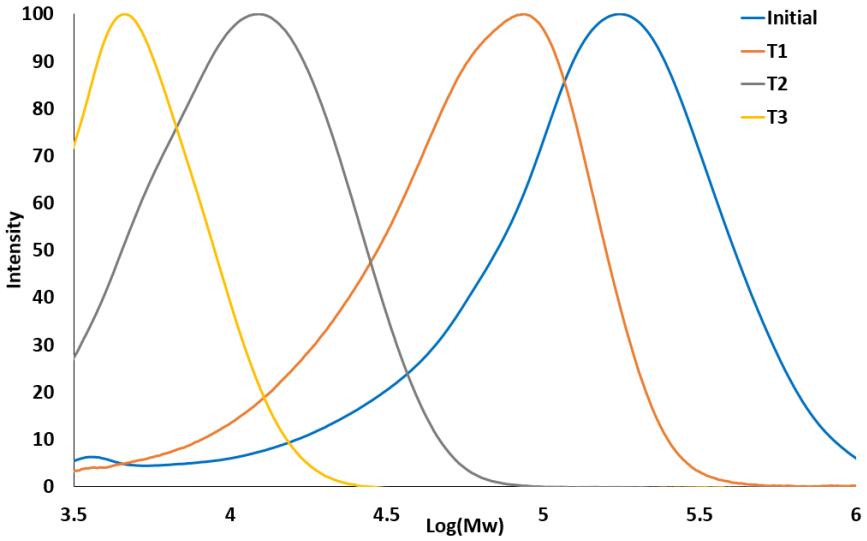


Figure 29. Evolution of polymer molecular weight distribution vs degradation time at 100°C.

The different coloured traces in Figure 30 are the chromatograms produced from differing degradation times. It can be seen that as degradation time increases, so do the relative amounts of degradation products at each peak. The one exception to this rule is the peak in the NIM chromatogram at 8.85 minutes, which has been identified as the surfactant used in preparing the sprayable polymer formulation, sodium dodecyl sulphate (SDS). The SDS peak decreases at increasing degradation times, and this is because SDS degrades at high temperature.[49]

Figure 30 also displays the chromatograms obtained from the degradation media of samples hydrolyzed for an extended period of time at 35°C. Under these conditions the relative amounts of degradation products are greatly diminished, which is expected and indeed a positive finding, as this polymer is intended to survive under agricultural conditions for up to 6 months. Furthermore, the degradation products from the 35°C incubation identified were no different than those found under the higher temperature incubation.

The other peaks invariably increase at increasing degradation times which is evidence that given enough time, the polymer does abiotically degrade into small oligomers and monomers. In a biotic matrix these small molecules would be taken up by microbes, used for energy and mineralized or converted to biomass.[50–52] In unpublished data from this work, it was shown that the application of

this polymer to certain soils increased the rate of microbial respiration (as determined by evolved CO_2).

In Figure 31 the mass fragment at m/z of 245.13858 is a deprotonated 6HHA dimer, the less abundant fragment at m/z 131.07076 is the deprotonated 6HHA monomer (Table 6), which likely formed in the ion source (in-source fragmentation) when subjected to the high temperatures and potentials.

By analyzing each peak or group of peaks individually within each chromatogram a thorough understanding of what degradation products form was obtained and indirectly where the polymer is most susceptible to hydrolytic degradation. Table 6 and Table 7 list the major components identified in the NIM and PIM chromatograms, respectively.

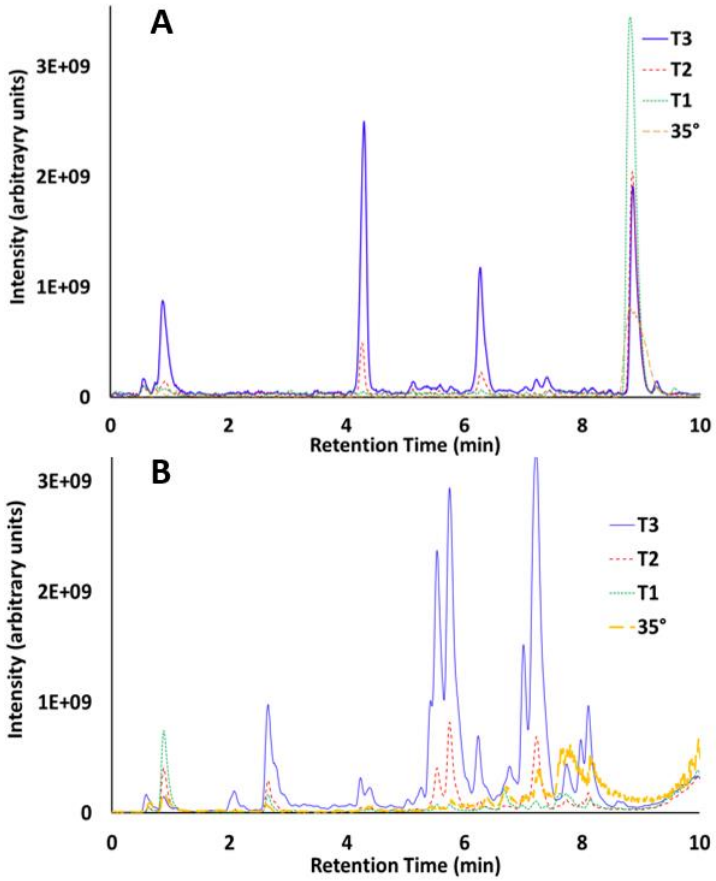


Figure 30. Background-subtracted chromatograms obtained in FullMS mode A) Negative ionisation mode, and B) Positive ionisation mode from abiotically hydrolysed samples. Last 5 minutes omitted.

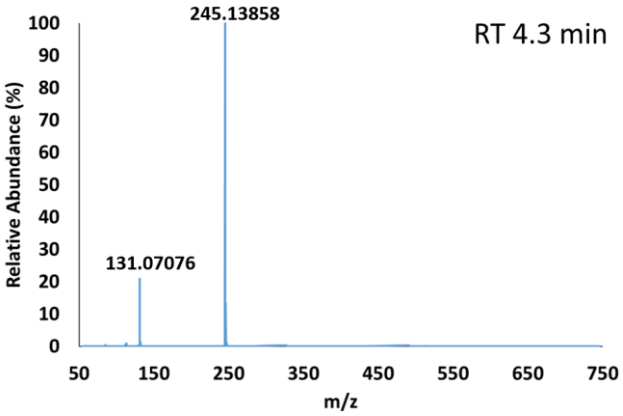
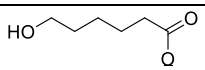
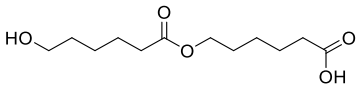
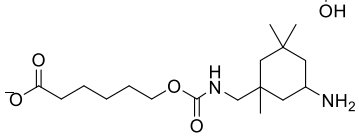
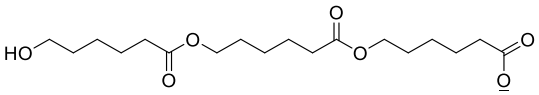
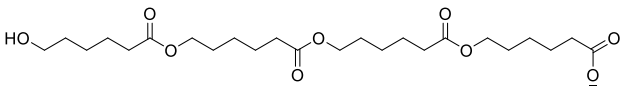
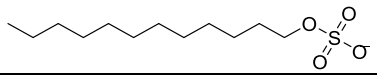


Figure 31 Mass spectrum of the peak at 4.3 minutes retention time from the NIM chromatogram (Figure 4A).

Table 6. Identified molecules in NIM chromatogram obtained from the abiotic hydrolysis of the polymer film.

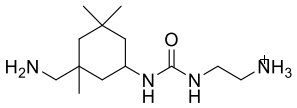
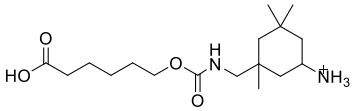
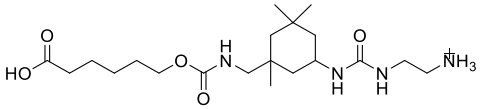
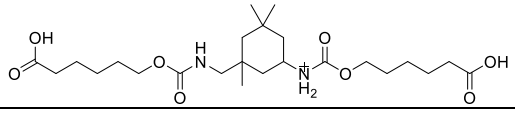
Retention Time (minutes)	Exact Mass to Charge Ratio (m/z)	Molecular Formula $[M\text{-}H^+]^-$	Identity	Molecule $[M\text{-}H^+]^-$
0.88	131.07076	$C_6H_{11}O_3$	6HHA	
4.3	245.13855	$C_{12}H_{21}O_5$	6HHA dimer	
5.13-5.72	327.22797	$C_{17}H_{31}O_4N_2$	NH_2-IPDI-6HHA[a]	
6.27	359.20668	$C_{18}H_{31}O_7$	6HHA trimer	
7.39	473.27406	$C_{24}H_{41}O_9$	6HHA tetramer	
8.85	265.14708	$C_{12}H_{25}SO_4$	Sodium dodecyl sulphate[b]	

[a] One of two possible isomers
[b] Surfactant used during polymer synthesis to stabilise suspension.

Note that the structures given in Table 6 and Table 7 that contain IPDI are one of two possible isomers (except 6HHA-IPDI-6HHA) because it is very difficult to distinguish which urethane linkage hydrolyzed, and it is likely that both isomers would be present. Each of these isomers may interact differently in the LC column, which may cause a broadening or splitting of their elution peaks.

Many of the compounds identified (Table 6 and Table 7) are as hypothesized - varying lengths of PCL oligomers, and fragments containing IPDI bound to 6HHA and/or EDA. What is interesting is that even at the shortest degradation time (19 hours) and temperature (35 °C) there are degradation products being formed from the hydrolysis of the urethane linkages, for example the NH_2-IPDI-EDA or NH_2-IPDI-6HHA which eluted at 2.21 and 5.14 minutes, respectively. However, these products are only being formed in relatively small quantities (Figure 30). Isophorone diamine (IPDA) the product of both urethane linkages being hydrolyzed from a single IPDI, is only found in the most degraded (304 hours) samples.

Table 7. Identified molecules in PIM chromatogram obtained from the abiotic hydrolysis of the polymer film.

Retention Time (minutes)	Exact Mass to Charge Ratio (m/z)	Molecular Formula $[M+H^+]^-$	Identity	Molecule $[M+H^+]^-$
0.88	102.12784	$C_6H_{16}N$	Triethyl amine (TEA)[a]	
2.21	257.22364	$C_{13}H_{29}ON_4$	NH_2-IPDI-EDA[b]	
5.14	329.24365	$C_{17}H_{33}O_4N_2$	NH_2-IPDI-6HHA[b]	
6.17	415.29169	$C_{20}H_{39}O_5N_4$	EDA-IPDI-6HHA[b]	
7.77	487.30179	$C_{24}H_{43}O_8N_2$	6HHA-IPDI-6HHA	

[a] Catalyst used during polymer synthesis.
[b] One of two possible isomers

Enzymatic Degradation

Hydrolysis of polymer films by urease and esterase was carried out in phosphate buffered saline (PBS) of pH 7.0 and pH 7.5, respectively. Degradation was carried out over the course of 34 days at

35 °C, with enzyme solution being replaced every 2-4 days. Unfortunately, LCMS data didn't yield any useful information due to large signal-to-noise ratio (SNR). Perhaps because the enzyme solutions were replaced so frequently there wasn't enough opportunity for degradation products to accumulate to a detectable level in the degradation media.

There was however evidence that degradation did occur. Figure 32 shows the remaining mass of the residual polymer films following 33 days of enzymatic degradation. The presence of urease had no significant impact on polymer degradation over that time period. In addition, the slightly alkaline esterase buffer (pH 7.5) catalysed faster degradation than the neutral urease buffer. Finally, the esterase treatment was found to have degraded the polymer films the most, at a statistically significant level (α=0.032). It should be noted that GPC results of the recovered films did show a reduction in M_w, but no reduction in M_n and no significant difference between treatments.

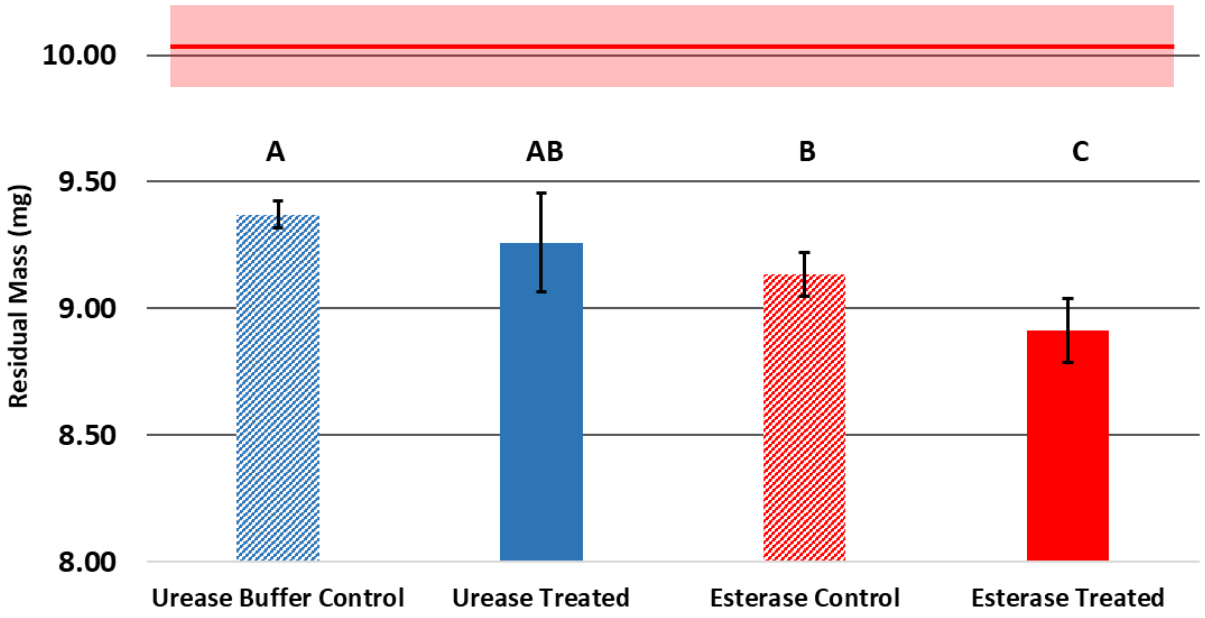


Figure 32. Remaining mass of enzymatically degraded polymer films. Error bars are ± 1 standard deviation. Red line and shaded area is the initial mass ± 1 standard deviation, respectively (10.07 ± 0.16 mg). Letters above each treatment indicate statistically different means as determined by one-way ANOVA and Tukey's Honestly Significant Difference (HSD).

Other evidence for degradation of the films can be seen in SEM micrographs of the recovered polymer films (Figure 33). The films exposed only to buffer show little evidence of degradation, whereas there is some evidence of degradation in the urease and esterase treated films. In the urease treated films

there appears to be selective degradation occurring, leaving behind small 'islands' of undegraded polymer, and in the esterase treated films small pits have begun to form.

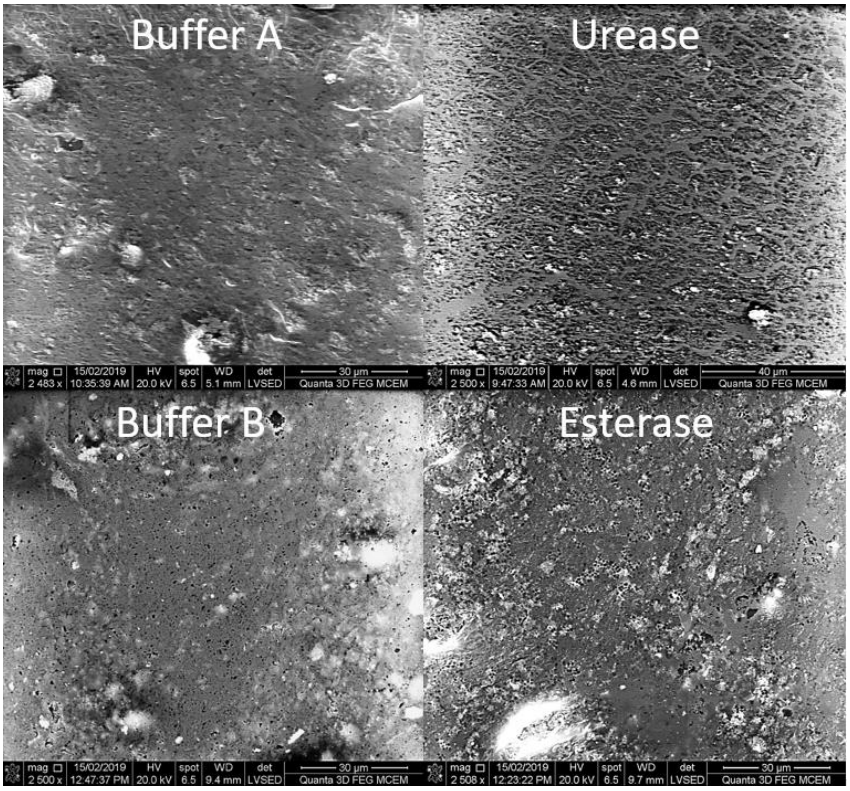


Figure 33 SEM micrographs of the polymer film recovered from enzymatic degradation experiments.

Soil Degradation

The GPC results showed that the Mw was reduced to 39,000 ± 6,000 Da and 21,000 ± 8,000 Da for LOM and HOM soils (initially 120 kDa), respectively. Mn was reduced to 12,000 ± 1,100 Da and 7,400 ± 1,400 Da for LOM and HOM soils (initially 40 kDa), respectively. Both of these differences were found to be statistically different as determined using the Student's T-test assuming unequal variances. Interestingly, the molecular weight distribution showed a trimodal and bimodal profile for LOM and HOM soils, respectively (Figure 34). Note that the two polymer molecular weight modes in HOM soil are the same as the two lower polymer molecular weight modes in the LOM soil. This indicates that the polymer has degraded more rapidly in the HOM soil, as there are no high molecular weight fragments present. This molecular weight distribution is very different to the comparatively uniform, single molecular weight mode in the abiotically hydrolysed samples. Apparently, polymer fragments reach some threshold molecular weight (around 15,000 Da), and then are rapidly degraded,

and this process repeats itself at a lower molecular weight. In the case of polymer recovered from HOM soil, all of the high Mw polymer fragments have already been degraded, perhaps due to the larger microbial community. In this manner polymer fragments from the largest molecular weight mode will rapidly degrade into the intermediate molecular weight mode, and again towards the lowest molecular weight mode. Further study is required to investigate this phenomenon. When applied in a field situation, the polymer would be applied as a surface covering, and not mixed through the soil, which would result in a slower degradation than observed here, but not necessarily in a different molecular weight distribution.

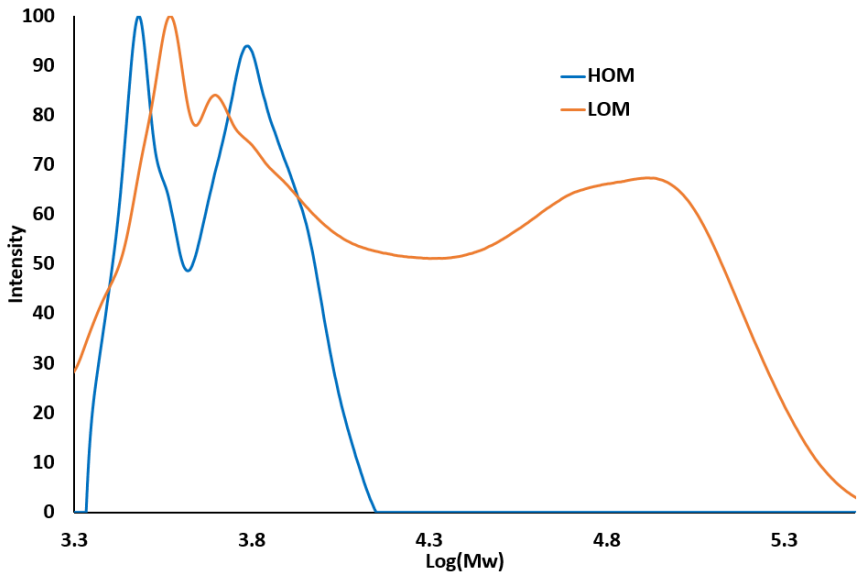


Figure 34 Molecular weight distribution of residual polymer recovered from the soils.

Figure 35 displays the PIM and NIM chromatograms obtained from the leachates of both soils. The first important observation is that the chromatograms are much more complex than the ones obtained during abiotic hydrolysis. This increased complexity is due to the much more complex polymer breakdown occurring in the soil matrix. These chromatograms have been background subtracted, or in other words have had the chromatograms of soil only controls subtracted, and therefore what is left is entirely due to the presence of the polymer.

The next important observation is that the soil collected from 3-6 cm below the surface, or the soil that has not had polymer applied directly to it, does indeed have some polymer degradation products present. This is evidence that as the polymer degrades and water soluble products form, they are

pulled through the soil's vertical profile by successive watering events. This has potentially important implications for polymer degradation products contaminating ground water, depending on how mobile those degradation products are and the physicochemical properties of the soil. Further study is required to determine how far the polymer degradation products may leach, and in what quantity. The polymer was applied to the top 2 cm of soil only, and soil was collected in two fractions: 0-3 cm and 3-6 cm, thereby minimizing the likelihood of polymer directly contaminating the 3-6 cm fraction. The third important observation is that although the relative quantities of the degradation products vary between soils, the same major peaks are present in both LOM and HOM soils.

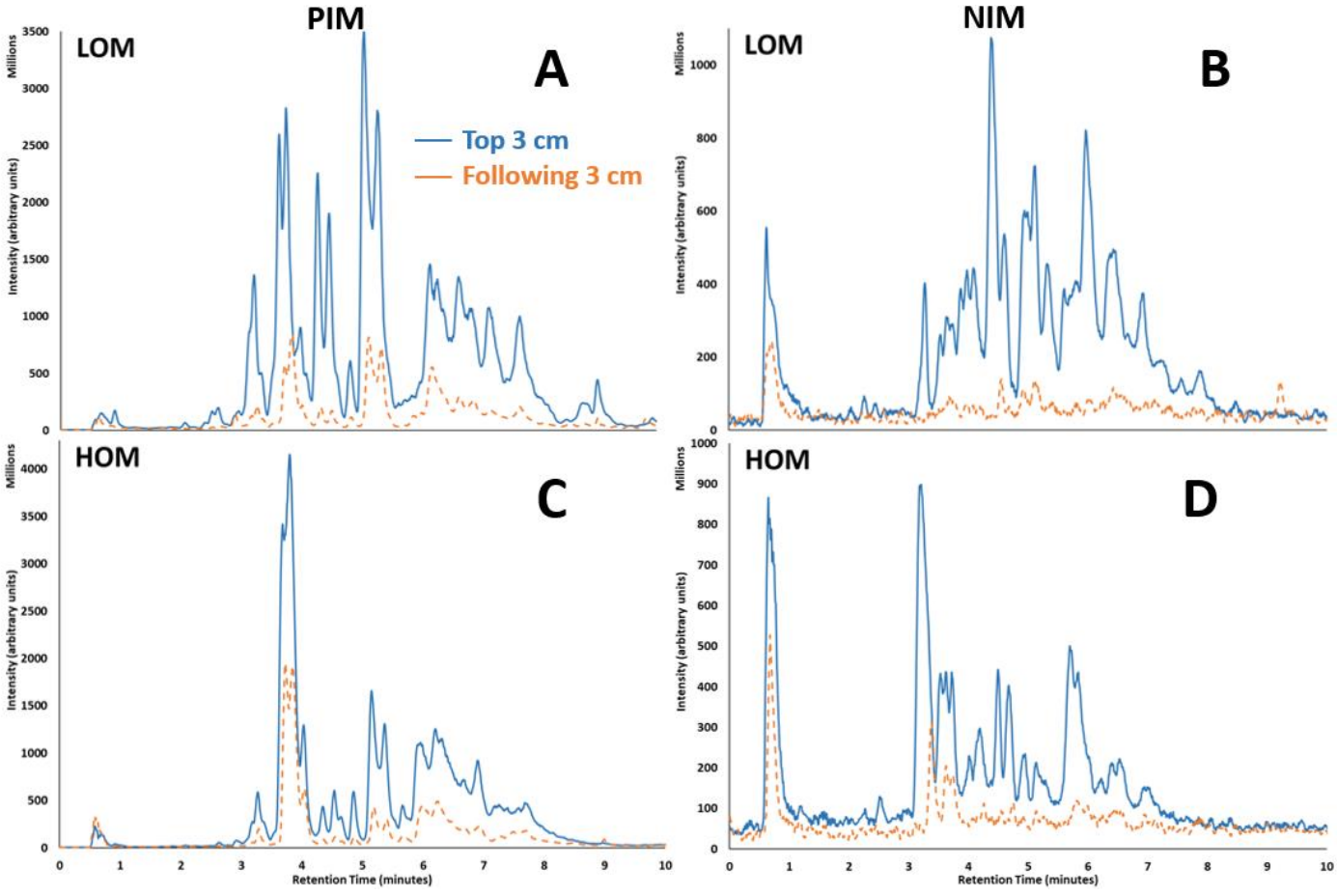


Figure 35 Chromatograms obtained from leachate from soils. A and B are the chromatograms obtained in PIM and NIM from the leachate from LOM soil, respectively. C and D are the chromatograms obtained from the leachate from HOM soil, respectively. Blue traces in all chromatograms are from the top 3 cm of the soil, and orange traces in all chromatograms are from 3-6 cm of the soil.

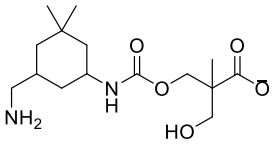
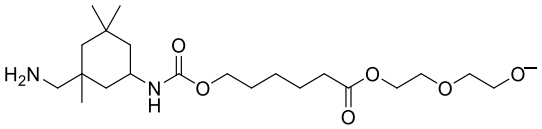
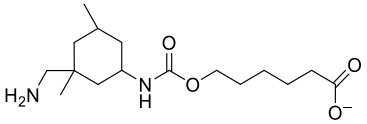
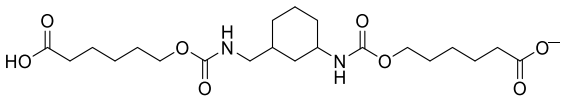
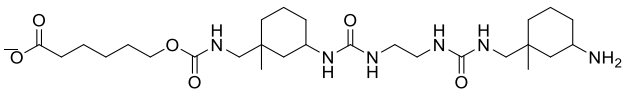
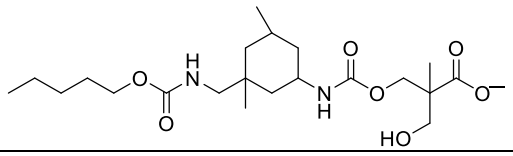
Each peak was analyzed in the same manner as the chromatograms obtained from the abiotically hydrolyzed samples, and where necessary AIF spectra were consulted to provide additional evidence

for the identifications made. It is important to note that in addition to the identifications made, there were many unidentified mass fragments observed in the chromatograms (Figure 35). This is in part because of the many permutations of reactions that can occur in rich matrices like soils. Abiotic hydrolysis of ester, carbamate, and urea bonds, will compete with enzymatically and pH catalysed hydrolysis processes. Within the soil there will be expansive suite of enzymes secreted by bacteria, archaea, and fungi[53] such as general hydrolases, lipases[54,55], decarboxylases[46], and demethylases[56–59]. Also present in soil are an assorted range of humic substances[60] that may react with existing functional groups on the polymer, as well as with newly generated functional groups (i.e. carboxylic acids and alcohols from hydrolysis of esters) forming complex equilibria.[44] The soil mineralogy (mineral speciation) will provide different surfaces to which degradation products may adhere, and for other chemistry to occur, and transition metals present can facilitate redox chemistry.

The result of this complexity is that comprehensive identification of many of the species present would require extensive detailed studies due to the inherent difficulty in isolating products and predicting the many possible reactions that may occur, both degrading the polymer and transforming the degradation products. Despite this complexity, several prominent degradation products directly linked to the added polymer were identified. Table 8 and Table 9 list the compounds identified in either (or both) soils' leachate, and the unidentified compounds that were found in the leachate from both soils.

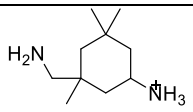
Most of the degradation products identified were found in both soils which is a good indication that the polymer is susceptible to biotic and abiotic degradation across a variety of soil types. The main difference between the two soil types is the size of the microbial community and the quantity of humic substances. These two factors play an important role in polymer degradation because organic carbon, a proxy for soil organic matter (SOM), increases microbial respiration. It is not understood why some species were only identified in NIM or PIM, but not both.

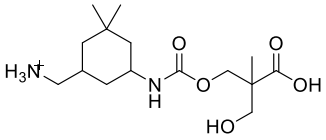
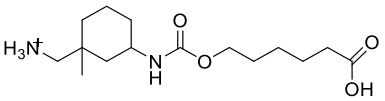
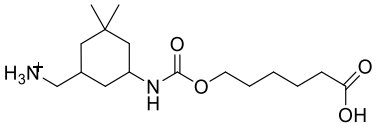
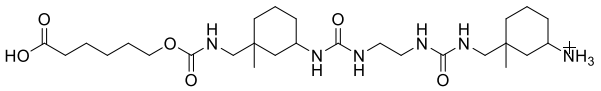
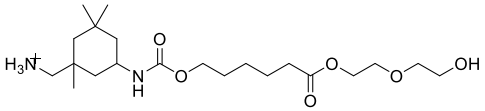
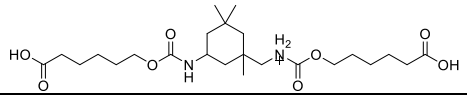
Table 8. Identified compounds and unidentified from NIM chromatograms from both HOM and LOM soils.

Soil	Retention Time (min)	Exact Mass to Charge Ratio (m/z)	Molecular Formula $[M-H^+]^-$	Identity	Molecule[a,b]
LOM, HOM	3.24	297.14392	Undetermined	Undetermined	Undetermined
LOM, HOM	3.52	373.15961	Undetermined	Undetermined	Undetermined
LOM	3.57-3.70	315.19095	$C_{15}H_{27}O_5N_2$	NH_2-IPDI-DMPA demethylated[c,d]	
LOM, HOM	3.94	401.19081	Undetermined	Undetermined	Undetermined
LOM, HOM	4.60	415.20660	$C_{21}H_{41}O_6N_2$	NH_2-IPDI-6HHA-diglycol-OH[c,d]	
LOM, HOM	4.93	313.21184	$C_{16}H_{29}O_4N_2$	NH_2-IPDI-6HHA demethylated[c]	
LOM, HOM	5.12	443.23784	$C_{21}H_{35}O_8N_2$	6HHA-IPDI-6HHA demethylated three times	
LOM, HOM	5.70-5.82	655.36407	Undetermined	Undetermined	Undetermined
LOM, HOM	5.70-5.82	579.34833	Undetermined	Undetermined	Undetermined
LOM, HOM	6.35	553.36925	$C_{27}H_{49}O_6N_6$	6HHA-IPDI-EDA-IPDI-NH_2 demethylated four times[c,d]	
HOM	6.53	429.25842	$C_{21}H_{37}O_7N_2$	6HHA-IPDI-DMPA Demethylated once and decarboxylated once[c]	

[a] Molecules shown which have been demethylated, decarboxylated, or hydrolysed to form an amine are one possible isomer. It is not possible to distinguish which group was demethylated, decarboxylated or hydrolysed.
[b] Ionised moieties may be one of several possible sites of ionisation.
[c] One possible isomer
[d] Identified in both NIM and PIM chromatograms

Table 9. Identified compounds and unidentified from PIM chromatograms from both HOM and LOM soils.

Soil	Retention Time (min)	Molecular Ion Mass (Da)	Molecular Formula	Identity	Molecule[a,b]
LOM	0.91	171.18530	$C_{10}H_{23}N_2$	IPDA	
LOM, HOM	3.25	186.14854	Undetermined	Undetermined	Undetermined

LOM, HOM	3.60-3.90	317.20647	$C_{15}H_{29}O_5N_2$	NH2-IPDI-DMPA demethylated[c,d]	
LOM, HOM	4.30-4.60	301.21161	$C_{15}H_{29}O_4N_2$	NH_2-IPDI-6HHA demethylated twice[c]	
LOM, HOM	5.10-5.40	315.22722	$C_{16}H_{31}O_4N_2$	NH_2-IPDI-6HHA demethylated[c]	
LOM, HOM	6.15-6.35	555.38557	$C_{27}H_{49}O_6N_6$	6HHA-IPDI-EDA-IPDI-NH_2 demethylated four times[c,d]	
LOM, HOM	6.24	417.22244	$C_{21}H_{41}O_6N_2$	NH_2-IPDI-6HHA-diglycol-OH[c,d]	
LOM	7.70	487.30048	$C_{24}H_{43}O_8N_2$	6HHA-IPDI-6HHA	

[a] Molecules shown which have been demethylated, decarboxylated, or hydrolysed to form an amine are one possible isomer. It is not possible to distinguish which group was demethylated, decarboxylated or hydrolysed.
[b] Ionised moieties may be one of several possible sites of ionisation.
[c] One possible isomer
[d] Identified in both NIM and PIM chromatograms

Many of the identified molecules had similarities to those hypothesized. All of the fragments originated primarily from the polymer backbone, including IPDI, with some further modification in the form of demethylations, and decarboxylations. The decarboxylations was not particularly surprising as there is evidence in the literature for soil decarboxylases[46], but the demethylations are more surprising. Demethylases are known to be excreted from lignin degrading fungi[56,57], but typically these enzymes demethylate methyl groups attached to an ether bond. There is however some evidence in the literature for enzymatic demethylation of methyl groups attached to an alkyl chain[58]. Isolation and identification of the enzyme or enzymes demethylating the methyl groups attached to the cyclohexane ring on the polymer could potentially be of interest. The molecular ion identified to be NH_2-IPDI-6HHA-diglycol-OH should be comparatively rare because typically one would expect the diglycol group from the PCL diol reactant to result in more 6HHA residues removed from the terminus of the PCL diol polymer.

Another important observation comes from the molecules that were not found in the soil samples, but might have been expected. In particular, none of the 6HHA oligomers were identified in either soil type. The two possible explanations are 1) the 6HHA oligomers are suitably small and water soluble

to be taken up by soil microbes and metabolised, or 2) the 6HHA oligomers are highly mobile in the soil medium and simply leach through the soils' profile and therefore are undetected. Likely both processes compete, but the presence of relatively high (>1000 Da) molecular weight fragments indicates that there would be an ongoing release of 6HHA and 6HHA oligomers, so their absence indicates they are rapidly bioassimilated. It has also been observed that microbes in soils treated with the polymer respire at a higher rate which further indicates that these oligomers (and perhaps other small water soluble degradation products) are able to be metabolised by soil microbes.

Toxicity Testing

Results from the SAR software tests are displayed below. TEST software predictions are given in Table S2 and OSIRIS software predictions are given in Table S3. Most of the identified degradation products are safe across the interrogated metrics. IPDA containing compounds have bioaccumulation potential due to their relative lipid-solubility, and many of the compounds were predicted to be a developmental toxicant, but with limited certainty. It should be noted that this polymer will be applied at low levels, and that these degradation products are simply intermediates prior to mineralisation (Table 8 and Table 9). In any case, this preliminary screening indicates that experimental testing on the in-soil accumulation, bioavailability, and toxicity on some of the degradation products should be carried out. TEA (triethylamine), a catalyst used during polymer synthesis, is the only identified compound confirmed experimentally to have mutagenicity, and tumorigenicity.

Seed Germination Studies

The effect on the germination of three species of seeds (radish, cress, and lettuce) of two of the identified degradation products (6-hydroxyhexanoic acid, 6HHA and isophorone diamine, IPDA) and total polymer hydrolysate was investigated at two levels. IPDA at both levels completely inhibited the germination of all species of seeds. 6HHA also had an inhibitory effect on seed germination and

interestingly TPH had the smallest inhibitory effect on seed germination, only preventing some germination at the high loading (Figure 36).

Cress, the most robust seed tested, had only a small (~ 24 h) but significant increase in MTG at the highest loading of TPH (Figure 37). Lettuce, the most sensitive seed tested, had an increase in MTG of about 60 h at the highest loading of MTG. GI confirmed that all treatments had an inhibitory effect on germination (Figure 38), but the smallest inhibitory effect was observed with TPH treated seeds at the lowest level tested.

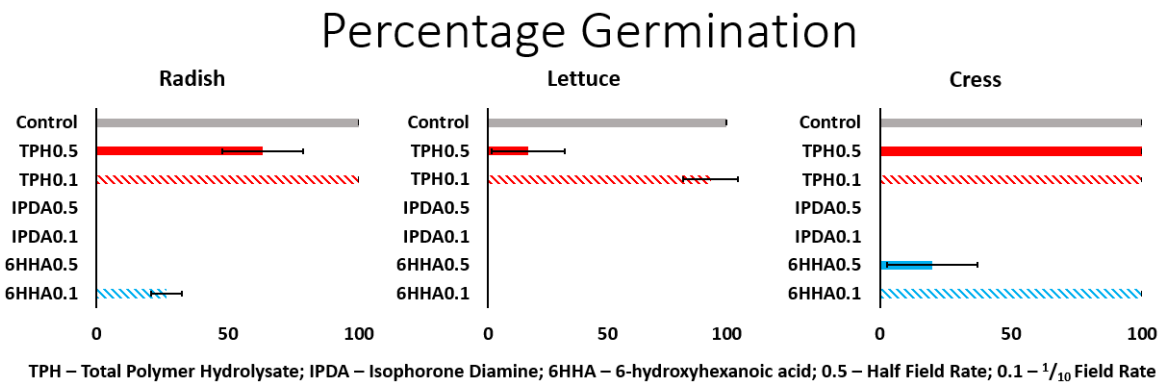


Figure 36. Percentage germination of each seed species in the presence of different degradation products. Error bars are ± 1 standard deviation.

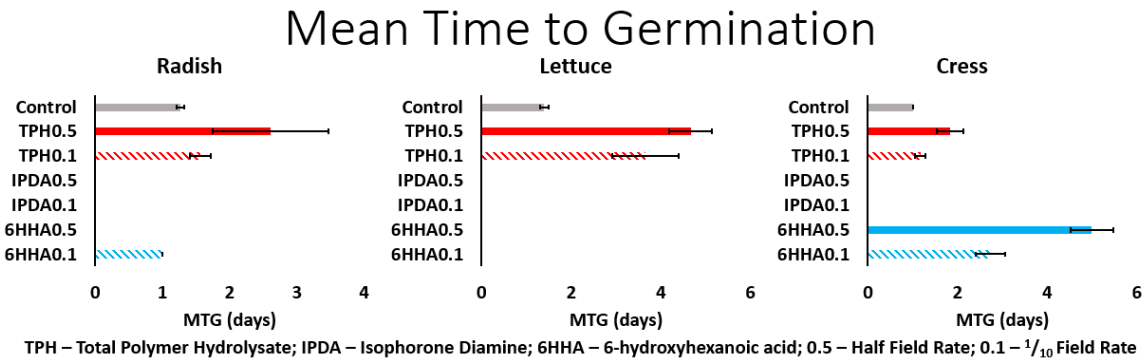


Figure 37. Mean time to germination of each seed species in the presence of different degradation products. Error bars are ± 1 standard deviation

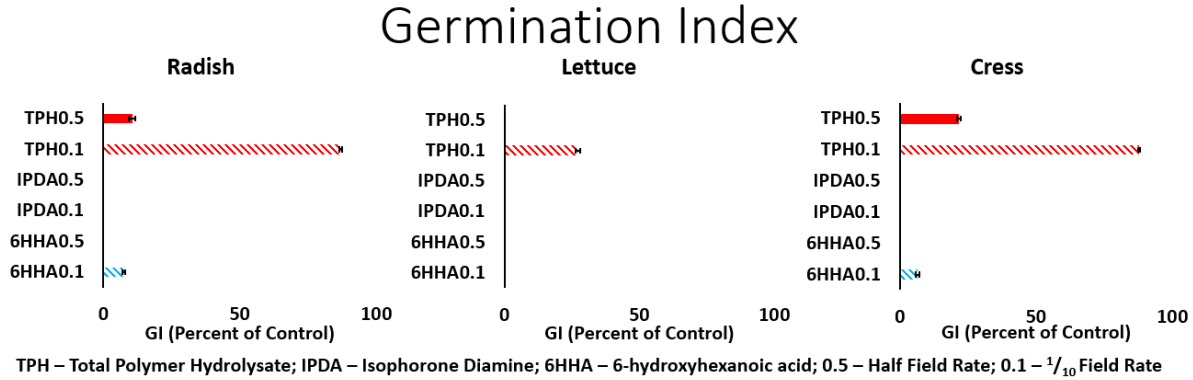


Figure 38. Germination index of each seed species in the presence of different degradation products. Error bars are ± 1 standard deviation.

To understand these seemingly contradictory findings it must be understood that the TPH will contain a range of compounds, and lower absolute quantities of both 6HHA and IPDA than are present in the single degradation product solutions shown here. This is because the hydrolysis process is incomplete and both 6HHA and IPDA will predominantly be covalently bound to other polymer constituents. While the complete and partial germination inhibition shown by the individual degradation products are cause for caution, it must be understood that even the $^{1}/_{10}$ field rate loadings is much higher than ever expected to be present in the field. Polymer degradation takes place over many months, degradation intermediates will be present at varying stages of mineralization, and their water solubility will cause their dispersal through the soil vertical profile, and each of these factors means the actual concentrations of any individual degradation product in the field will be much lower than those tested here.

This laboratory germination test is a good starting point and indicates a need for further testing.

Conclusions

Many degradation products of a sprayable, biodegradable polymer were successfully identified utilizing a liquid chromatography system coupled to a HRMS. In an abiotic hydrolytic medium (UHQ water) at 100ºC for approximately 13 days the polymer degraded extensively, reaching an M_w of less than 5% of the initial M_w. When incubated abiotically at 35ºC for 33 days, the polymer was much less degraded, reaching an M_w of about 50% of the initial M_w. Identified degradation products were all the result of random, hydrolytic chain scissions along the polymer backbone, at hydrolysable sites (esters, carbamates, and ureas).

In the enzymatic degradation study there was evidence of enzymatic polymer degradation in the form of polymer film mass loss and differences in the polymer films surface morphology after degradation.

No degradation products were identified in this study, perhaps due to the constant replacement of degradation medium preventing detectable levels of polymer degradation products to accumulate.

In soil degradation experiments, in two different soils, the identified degradation products were the result of random main chain scissions and further enzymatic modifications. These modifications included decarboxylations and demethylations, and there is some evidence for enzymes demethylating aliphatic methyl groups in the literature.[46,56–59] Also of note is that the polymer degradation products showed some limited mobility moving down the soils' vertical profile. This movement would be the result of discrete watering events where the water acted like the liquid phase of a column and the soil as stationary phase. The absence of what should be the most abundant degradation products, 6-hydroxy hexanoic acid and its 2-5 unit oligomers, gives evidence that soil microbes are either able to utilize such molecules for their energy and carbon needs, or that those molecules have been modified and were not identified in our analysis. The mobility of polymer degradation products was demonstrated in this study and should be considered in all studies of synthetic polymers applied to soils.

Acknowledgements

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Chapter 5. Sprayable Biodegradable Polyester-urethane-urea Mulching Treatment Increases Abundance of Plant Growth Promoting Microbes (PGPM) in Soil

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Key Words: Sprayable biodegradable mulch, Enzyme Activity Assay, Plant Growth Promoting Microbes, Soil Microbiome

Abstract

Polyethylene (PE) mulch is an important technology for increasing productivity and conserving water in the agricultural industry. Unfortunately, its continued use worsens crop productivity in the long term while also creating a large single-use plastic waste stream whose disposal has environmental consequences. A well investigated alternative, replacing PE mulch with biodegradable plastic mulches (BPM), is becoming increasingly popular. One understudied aspect of the widespread implementation of biodegradable plastics in the terrestrial environment is the impact those plastics, and their degradation intermediates, have on the soil microbial community and soil health in general. In particular, to date there has been no investigation into the impact of BPM on plant growth promoting microbes (PGPM), a subset of soil microorganisms that support plant growth through a variety of mechanisms. The work presented in this paper, investigates the impact of a sprayable, biodegradable polyester-urethane-urea (PEU) mulch newly developed by the Commonwealth Scientific and Industrial Research Organisation on the health, and microbial community composition of an agricultural soil. By means of a tomato crop system under controlled

greenhouse conditions, changes to the composition of the soil microbial community were monitored and changes to soil enzyme activities involved in nutrient cycling were measured. Particular attention was given to impacts on the relative abundance changes in PGPM. The PEU mulch reduced the abundance of a small number of taxa, but also provided an environment in which some taxa, which were comparatively rare in initial and unmulched soils, thrived. Importantly, the relative abundances of the PGPM *Azospirillum*, *Noviherbaspirillum*, *Exophiala*, *Phoma*, *Chaetomium* and *Clonostachys* all increased in soils treated with PEU mulch. Principal coordinates analysis revealed that the PEU film and PEU treated soil microbial communities' composition were most similar although still significantly different, while the PEU films' microbial community differed the most from the initial soil's microbial community. These results indicate that from an agricultural productivity and an environmental safety standpoint the use of PEU mulch is preferable to PE, and could provide additional plant growth benefits by increasing the abundance of PGPM.

Introduction

Mulching is the practice of spreading a material over the soil surface to enhance a number of soil microclimatic factors, ultimately yielding a faster growing and more productive crop. Polyethylene (PE) sheets are a commonly used mulching material in ridge-furrow systems because of their ability to reliably increase crop yield while also conserving water by acting as a physical barrier thereby preventing evaporation[1–5]. Unfortunately, PE is slow to degrade[6] which necessitates its removal from the field at the end of the growing season, thus creating a large single-use plastic waste stream. Furthermore, the use of PE mulch has been shown to create microplastics in the field when it breaks into smaller, non-degradable peices[7,8] which are deleterious to soil invertebrate health[9,10]. The use of PE mulch has become widespread, with over 1.2 million tons of PE mulch being used in China alone in 2011, and the continued use of PE mulch has been shown to worsen crop productivity over time[11].

The use of biodegradable plastic mulch as an alternative to PE would maintain the productivity benefits gained through using plastic mulch, while avoiding the environmental and long-term

productivity consequences. A wide variety of different biodegradable polymers and biodegradable polymer blends have been developed and evaluated for their efficacy in conserving water, increasing crop yield, and importantly their rate and extent of degradation[12–19].

Biodegradable polymers are inherently different to nondegradable polymers because they will breakdown in the environment. According to ASTM International, for a material to be considered biodegradable it must break down completely into CO_2, H_2O, CH_4, small inorganic compounds and biomass in approximately 12 months while leaving behind no visible nor toxic residue[20,21].

Polymers are large molecules and will not mineralise in a single step, but rather will first break down into oligomers and monomers (collectively, 'degradation intermediates') before being taken up by microorganisms and mineralised[22,23]. As these degradation intermediates enter the environment they will impact the microbial community[24], and this impact is important to understand before a new mulching technology is used broadly.

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) have recently reported developing a sprayable biodegradable polymer for use as an agricultural mulch[25]. The polymer, a polyester-urethane-urea (referred to as PEU henceforth), is the subject of the work presented in this paper.

There have been several studies to date investigating the changes to the soil microbial community brought about by the presence of biodegradable plastics[26–31]. In only one study (Meng et al.[31]) is the entire soil bacterial/archaeal community (BAC) monitored with high-throughput DNA sequencing, but the fungal community is not sequenced, and in that case the authors were interrogating the effect of biodegradable plastic seedling trays on the soil microbial community, not a mulch. More commonly, selective techniques such as colony forming unit counting[32], or techniques that only monitor general shifts in the composition of the soil microbial community (such as phospholipid fatty acid analysis) are used. These techniques are very useful; however, it is well understood that one hallmark of a healthy soil is a highly diverse soil microbial community[33,34]

and so it is also important to understand how the microbial diversity is impacted by the use of biodegradable plastic mulches.

Soil health is commonly defined as the capacity of a soil to support plant and animal productivity, maintain or enhance water and air quality, and function as a living system[35]. A vital component of this is a soil's capacity to support microbial diversity, activity and nutrient cycling capability[33,36]. Given that mulching impacts the soil microclimate, it will also impact the soil microbial community[37]. A healthy soil has a diverse microbial community which is adaptable to changing conditions (environmental conditions, land management practices, different cropping systems) while maintaining function, which is typically measured via soil enzyme assays[38]. The more diverse a soil microbial community, the greater the likelihood that there will be redundancy in the functions performed by different microbial taxa. Plant growth-promoting microbes (PGPM) compromised of both plant growth promoting bacteria (PGPB) and plant growth promoting fungi (PGPF) have been proven to provide beneficial services to plants with which they interact. These services can include improved seed germination rates, hardier seedlings, increased disease resistance, increased atmospheric N_2 fixation, increased root or shoot size, and plant pathogen control[39]. It was of particular interest to determine if BPM increased the abundance of PGPM, thereby providing valuable knowledge to the agricultural community[37].

The aims of the study were to investigate:

1. If the PEU mulch changed the relative abundance of PGPM in a selected soil.
2. If the PEU mulch treatment impacted only the soil microbial community composition in close proximity to the PEU film, or throughout the soil profile.
3. Whether any changes in the diversity of the soil microbial community altered their ability to cycle nutrients though enzymatic transformation.

Materials and Methods

Tomato plants were grown in greenhouse conditions with and without the PEU mulch. The soil microbial community's (fungal, bacterial, and archaeal taxa) DNA was extracted, amplified and

sequenced at three time points. The time points were before the tomato plants were transplanted into the growing pots, at tomato harvest (4.5 months after transplanting), and six months after harvest (Figure 39). Furthermore, the soil was sampled at two depths: the surface soil (0-2 cm) which was in closest proximity to the PEU mulch, and the bulk soil (2-15 cm). The PEU film itself was also sampled. In this way, the interplay between the microbial community that colonised the PEU film itself, and the soil microbial community across the soil's profile could be investigated.

Study Set-Up

Twenty-four free-draining, polypropylene pots with a 24 cm internal diameter and a 23 cm height were filled with 8 kg of air-dried soil (Vertosol, collected from a well-tilled commercial tomato farm in Echuca, Australia) sieved at < 2 mm (Figure 39). Six of the pots were immediately watered with tap water to 70% of the experimentally determined field capacity, and then left to equilibrate for 48 hours before being sampled (Initial soil). To the other eighteen, tomato seedlings (grown from seeds obtained from a commercial tomato farm) were transplanted into the centre of the pots. Also, in each pot, two Falcon® 50 mL centrifuge tubes, with their bases removed, were buried. These were used to water the tomato plants to mimic sub-surface drip irrigation. All of the remaining eighteen pots were then watered with tap water to 70% of field capacity.

Twelve of the pots were mulched with liquid PEU while the other six were left as unmulched controls. The PEU was composed primarily (~70 wt%) of a polycaprolactone soft segment, with repeating carbamate and urea linkages throughout the hard segment. The liquid PEU formulation was a 20% solids (by weight) aqueous suspension, and it was applied directly to the soil surface by syringe at a loading of 1 kg m^{-2} (200g solid PEU m^{-2}). Tomato plants were placed in a temperature controlled greenhouse with a 16-8 h day-night cycle with mean temperatures of 26°C and 16°C, respectively (temperature ranged from 25-31°C during the day and 14-18°C at night). Full spectrum high intensity discharge (HID) lights were used, with the illumination level ramped from 0-30 klux during the 24 h period.

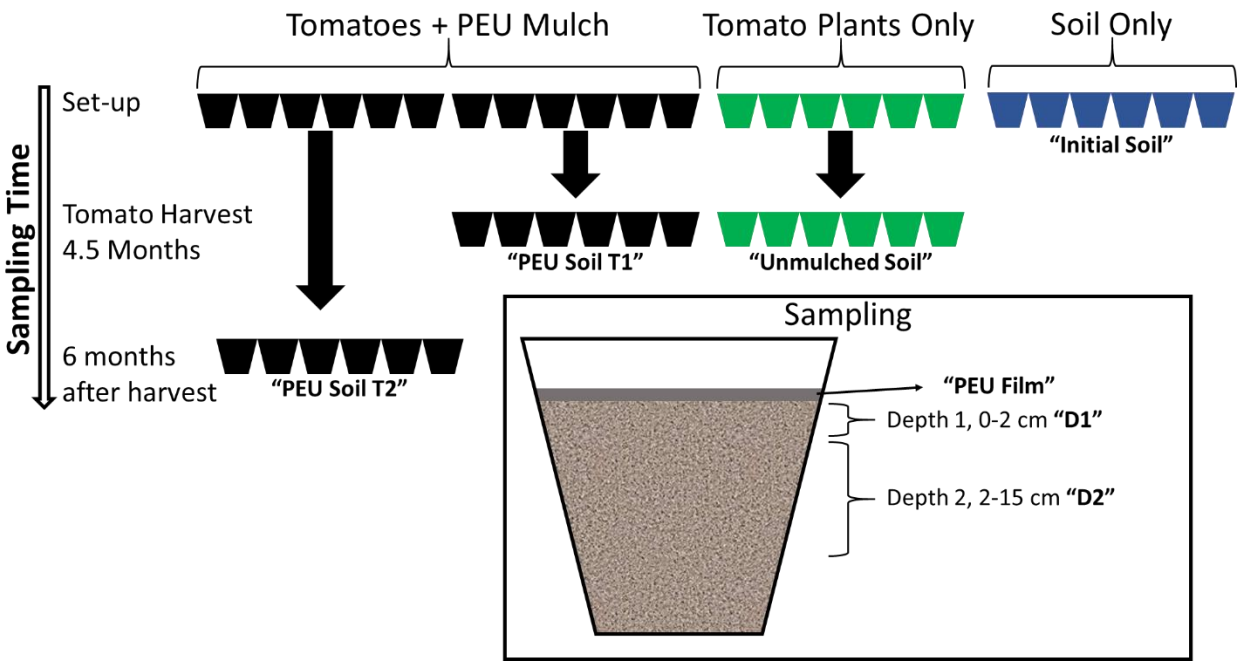

Figure 39. Allocation of the pots to each treatment, sampling times and depths.

Pot Maintenance and Sampling

During the growing period, the pots were watered three to four times per week using the buried centrifuge tubes, and fertiliser was applied as an aqueous solution through the centrifuge tubes once weekly for the first 12 weeks of the study. The fertiliser program used was adapted from the management plan used on the tomato farm from which the soil and tomato seeds were obtained, and is confidential. For reference, a typical tomato farm fertiliser program can be found in the Australian Processing Tomato Grower Report [40].

The tomatoes were grown to maturity over a period of five months after which six (out of twelve) PEU treated pots' soil and residual film were sampled, as well as all of the six unmulched pots. The remaining six PEU treated pots were maintained in greenhouse conditions for a further 6 months (in the absence of a tomato plant) to allow the PEU film to continue to degrade before the soil and PEU film were sampled.

The tomato shoots were removed from all 18 pots by separation from the roots using secateurs. Six of the PEU treated pots and all of the unmulched pots were sampled. From PEU mulched pots, PEU film was sampled by peeling the film off the soil surface and gently brushing away loosely adhered

soil particles. The soil was sampled at two depths: the first depth was the soil directly below the PEU film, nominally the top 2 cm, and the second depth was bulk soil, nominally 2-15 cm (Figure 39).

The remaining six PEU treated pots, with the tomato shoots removed, were left in the greenhouse for a further 6 months, being watered in the same way as before harvest, and then sampled in the same way as described previously. The remaining six PEU treated pots were maintained in greenhouse conditions for a further 6 months (in the absence of a tomato plant) to allow the PEU film to continue to degrade before the soil and PEU film were sampled.

In total, there were nine soils or films (replicated 6 times) from which DNA was extracted and sequenced. These were as follows:

- Initial soil with no tomato plant nor fertilisation (Initial Soil)
- Unmulched soil, at tomato harvest, 0-2 cm (Unmulched Soil D1)
- Unmulched soil, at tomato harvest, 2-15 cm (Unmulched Soil D2)
- PEU treated soil, at tomato harvest, 0-2 cm (PEU Soil T1 D1)
- PEU treated soil, at tomato harvest, 2-15 cm (PEU Soil T1 D2)
- PEU film at tomato harvest (PEU Film T1)
- PEU treated soil, six months after tomato harvest, 0-2 cm (PEU Soil T2 D1)
- PEU treated soil, six months after tomato harvest, 2-15 cm (PEU Soil T2 D2)
- PEU film 6 months after tomato harvest (PEU Film T2).

Soil Analysis

Physicochemical

Soil pH and electrical conductivity (EC) were determined using a 1:5 (m/m) soil:water suspension method[41]. In brief, soil and water were mixed in a 1:5 mass:mass ratio, agitated for 1 hour and then allowed to settle for 30 minutes. The supernatant's EC was first measured using an EC meter (Hach, sensION+ EC5) and then the pH was measured using a pH meter (TPS, WP-80). Soil nitrate and ammonium were determined using methods described previously[42,43]; in brief, soil and 2 M KCl

were mixed at a 1:2.5 (m/m) ratio and agitated for 20 minutes. The soil-KCl slurry was then centrifuged at 4200 rpm for 10 minutes before being analysed colorimetrically. For nitrate determination, an aliquot of the supernatant was mixed with a VCl_3 (97%, Sigma), N-(1-Napthyl)ethylene diamine dihydrochloride (NED, >98%, Sigma), and sulphanilamide (≥99%, Sigma) reagent and colour was developed overnight before measurements were taken at 540nm on a multiplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). Ammonium determination was carried out by first mixing an aliquot of the KCL supernatant with a reagent composed of sodium nitroprusside (Sigma), sodium salicylate (≥99.5%, Sigma), sodium citrate (Sigma), and sodium tartrate (≥99%, Sigma). This reagent solution was then mixed with an alkaline sodium hypochlorite solution (reagent grade 6-14% active Chlorine, Sigma), the colour allowed to develop for 2 hours, and then measurements were taken at 650 nm on a multiplate reader (Multiskan™ GO Microplate Spectrophotometer).

Enzyme Activity Assays

All enzyme assays were measured colorimetrically (Multiskan™ GO Microplate Spectrophotometer). Each soil enzyme assay followed the same general procedure: creation of a soil-water solution, creation of substrate-buffer solution, mixing and incubation of buffered soil and substrate solutions (in triplicate, with both soil and substrate controls) and finally measurement. Soil solutions were made by mixing soil and water at a 1:50 (m/m) ratio for 1 hour in all assays except the lipase assay, which was 10 min.

The acid phosphatase and β-glucosidase assays were adapted from a method described by Allison and Jastrow[44]. Substrates for acid phosphatase and β-glucosidase were *p*-Nitrophenyl Phosphate (Sigma), and *p*-Nitrophenyl β-D-glucopyranoside (≥98%, Sigma), respectively. Substrate solutions were made up in pH 7 3-(N-Morpholino)propanesulfonic acid (MOPS, ≥99.5%, Sigma) buffer, then mixed with soil solution and incubated at room temperature for 2 hours while shaking. NaOH (≥97%, Sigma) solution was added to terminate the reactions and then measurements were taken at 400 nm. Calibration curves were constructed using *p*-Nitrophenol (≥99%, Sigma) standards.

The lipase assay was adapted from the method described by Margesin et al.[45] The substrate solution used was *p*-Nitrophenyl palmitate (Sigma) diluted in isopropanol (≥99.5%, Sigma) with sodium deoxycholate (≥97%, Sigma) as an emulsifier. The soil solution was made by equilibrating soil and warmed (30 °C) pH 7.25 phosphate buffered saline at a 1:50 (m/m) ratio. The soil solution and substrate solution were then mixed and incubated for 10 minutes at 30 °C, centrifuged at 4200 rpm for 3 minutes, and then measured at 400 nm.

The urease assay was adapted from the method described by Kandeler and Gerber[46]. The substrate for the urease assay was urea, which was hydrolysed to ammonia and converted to ammonium which was then detected. The soil solution (soil: water 1:10 m/m) was mixed with substrate solution (urea in pH 5 acetate buffer) and incubated for 5 hours while shaking. The reaction was terminated by adding 4 M KCl and then ammonium determination was carried out as described above.

DNA extraction, amplification and sequencing

DNA extraction was carried out using the ZymoBIOMICS *Quick*-DNA Fecal/Soil Microbe prep kit using a modified version of the manufacturer's procedure as follows. Bead beating was performed at 6 m s^{-1} for two 60 s cycles with a 180 s delay between cycles (MPBio Fast Prep 24). A volume of 400 µL of 100% ethanol was added to the lysis solution immediately prior to loading extracts onto the spin column. DNA quantity and purity were checked using a NanoDrop™ Lite spectrophotometer (Thermo Scientific) and a Qubit ® Fluorometer. To validate the extraction efficiency, a ZymoBIOMICS community standard (D6310) and a negative extraction control were also run. Samples were normalised to 5 ng μL^{-1} in nuclease free high purity water.

For bacteria and archaea, the V3 and V4 regions of the 16S rRNA were amplified using the universal primers 515Fmod (5'-CCTACGGGNGGCWGCAG-3') and 806Rmod (5'-GACTACHVGGGTATCTAATCC-3')[47]. For fungi, the internal transcribed spacer (ITS) region was amplified using the ITS 86F (5'-GTGAATCATCGAATCTTTGAA-3') and ITS 4R (5'-TCCTCCGCTTATTGATATGC-3') primer sets[48]. Polymerase chain reaction (PCR) was performed in triplicate using 2.5 µL of forward and reverse primer, 2.5 µL of template DNA, 12.5 µL of Q5®

Hot Start High-Fidelity 2X Mastermix (NEB), and 5 µL of sterile, nuclease free, high purity water. The PCR run conditions were as follows: 95°C initial denaturation for 3 minutes; then 25 cycles of denaturation (95 °C for 30 s), annealing (55 °C for 16S rRNA and 52 °C for ITS for 30 s), and extensions (72 °C for 30 s); followed by a final extension of 72 °C for 5 min. PCR was validated using a 1.5% agarose TAE gel, then triplicate runs' amplicons were pooled and purified. Amplicons were purified using AMPure XP beads (Beckman Coulter), followed by two washes with 80% ethanol and finally they were resuspended in 10 µL of high purity, nuclease free water.

Amplicons were then indexed with unique i7 and i5 primers. Index PCR was prepared using 2 µL of target amplicons, 2 µL i5 index primer – 8 base index (1.0 µM), 2 µL of i7 index primer – 8 base index (1.0 µM), 10 µL of Q5® Hot Start High-Fidelity 2X Mastermix (NEB), and 4 µL of high purity, nuclease free water. Index PCR run conditions were as follows: 95°C initial denaturation for 3 minutes; then 25 cycles of denaturation (95 °C for 30 s), annealing (57 °C 30 s), and extensions (72 °C for 30 s); followed by a final extension of 72 °C for 5 min. Index PCR products were verified using a gel as described above to ensure the indexed adapters had attached (index PCR products should appear 50 base pairs larger than amplicons). Index PCR products were then purified using AMPure XP beads (Beckman Coulter), followed by two washes with 80% ethanol and finally they were resuspended in 15 µL of 10 mM Tris buffer (pH 8.5). The concentration of the index PCR libraries was checked with a Qubit ® Fluorometer.

The index PCR libraries were then combined in equimolar amounts, and a 4 nM metagenome pool was prepared in high purity, nuclease free water. An aliquot of the metagenome library was denatured in freshly prepared NaOH then diluted in hybridization buffer (HT1) to make a 20 pM meta genome pool. This was then mixed with PhiX v3 control library (Illumina) and diluted in HT1 buffer for a final concentration of 8 pM metagenome library and 1 pM control library.

The metagenome library was finally sequenced on an Illumina MiSeq platform using a V3 600 cycle sequencing kit (Illumina).

Raw fastq files were quality filtered using DADA2 and phyloseq packages. The *filterAndTrim()* function was used for filtering and trimming the amplicon sequence variants (ASVs), with parameters *truncLen=c(237,202), maxEE=c(1,1),* and *trimLeft=c(26,22)* for 16S ASVs. The ITS ASVs were quality filtered in the same manner but with the following parameters: *truncLen=c(217,200), maxEE=c(2,2),* and *trimLeft=c(23,23).* 16S rRNA ASVs' taxonomy were annotated using a SILVA database (database file *silva_nr_v132_train_set.fa.gz),* and ITS ASVs' taxonomy were annotated using a UNITE database (database file *h_general_release_dynamic_02.02.2019.fasta.gz).*

Statistical Analysis

Three α-diversity (in-sample diversity) indices were used to understand the microbial communities' distribution in samples. Hill and co-authors[49] provide an excellent discussion on which α-diversity indices are suitable for describing microbial diversity. Based on their recommendations and the information sought the following indices were chosen: Simpson's diversity index (taxa evenness), the Berger-Parker diversity index (taxa dominance), and the Chao-1 diversity index (taxa richness). Statistical analyses were performed in PAleontological STatistics (PAST) V2, Microsoft Excel 2016, and IBM SPSS Statistics 25. Excel was used for data organisation, preliminary clean-up, outliers testing (Grubbs' test), and normalisation. Raw counts were converted to relative abundance and then normalised using an arcsine transformation in Excel. SPSS was used for conducting ANOVAs to determine statistical differences between treatment groups with significance level set at $\alpha \leq 0.05$. Principal coordinates analysis (PCoA), and canonical correspondence analysis (CCA) were performed in PAST, as well as the subsequent variance testing: permutational multivariate analysis of variance (PERMANOVA).

Results and Discussion

Bacterial and Archaeal Community Composition

Firmicutes (10.8%), *Actinobacteria* (23.2%), and *Proteobacteria* (25.7%) were the most common phyla present (Figure 40). This phyla abundance distribution is within the range reported in soils[50],

but typically *Acidobacteria* would be more abundant while *Firmicutes* would be less so. In the presence of a cropping system (tomato plants, fertiliser and regular watering), which was all treatment groups excluding the initial soil, the abundance of phylum *Firmicutes* was reduced (from 29% to 10.8% ± 4.9%) and the abundance of phylum *Chloroflexi* increased (from 2.8% to 13.2% ± 2.4%). The *Proteobacteria* relative abundance increased by 21% on the PEU films six months after tomato harvest.

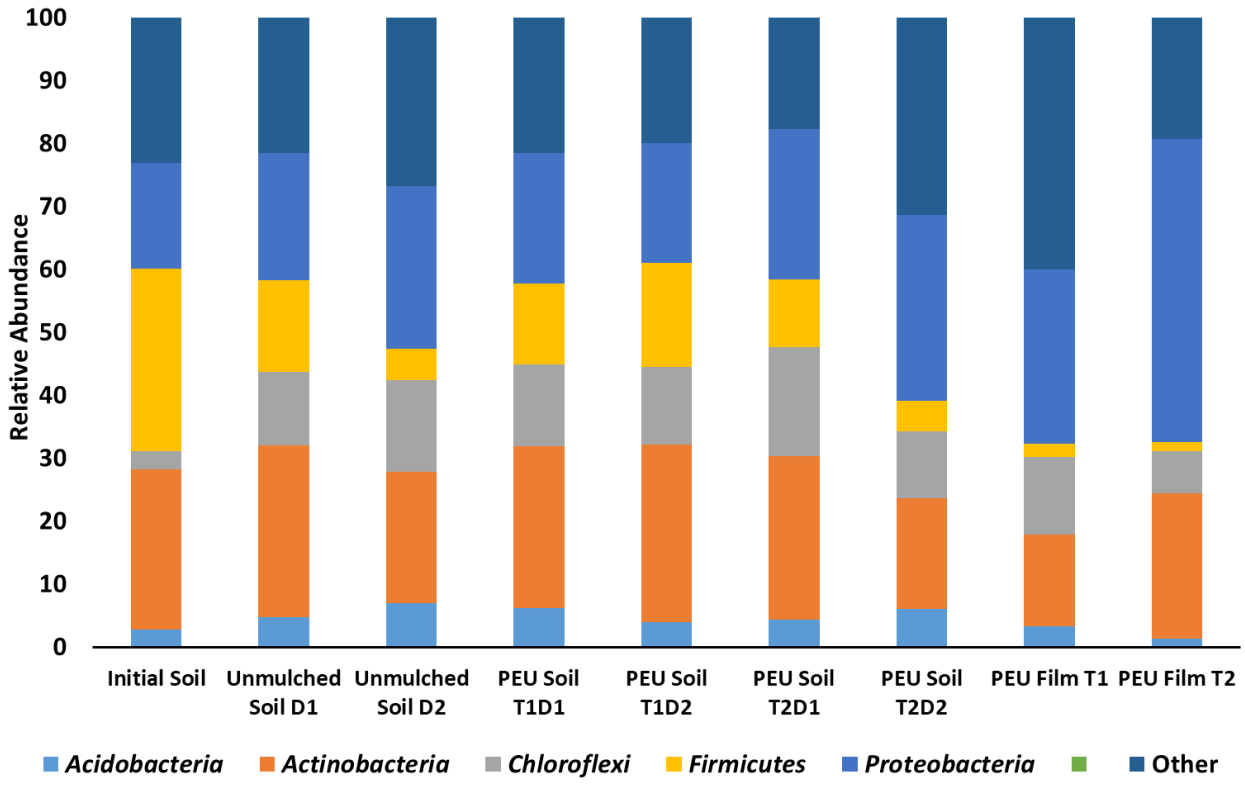

Figure 40. Relative abundance of the bacterial and archaeal phyla in each treatment. Phyla with less than maximum 4% relative abundance categorised as 'Other'.

While Simpson's diversity was relatively high across the PEU treated soils, unmulched soils and PEU film (Figure 41, >0.99), it was significantly lower in the initial soil. The BAC evenness was decreased on the PEU films, but this was not statistically significant. This correlates well with the Berger-Parker index, which shows a significantly increased dominance of the most abundant taxa in the following order: initial soil > PEU Films > all other soils. This was expected as the presence of plants, which excrete various organic molecules, have been shown to increase microbial diversity (and therefore decrease the dominance of the most abundant taxon) [34]. The Chao-1 diversity shows

that the initial soil had a lower richness. Taken together, this shows that the presences of PEU films does not alter soil BAC diversity despite the PEU film itself being colonised by a less diverse BAC. An explanation for this could be that the PEU provides a carbon source that is only accessible to, or more easily utilised by a small subset of organisms, thus reducing competition and allowing a less diverse community to flourish in a less competitive environment.

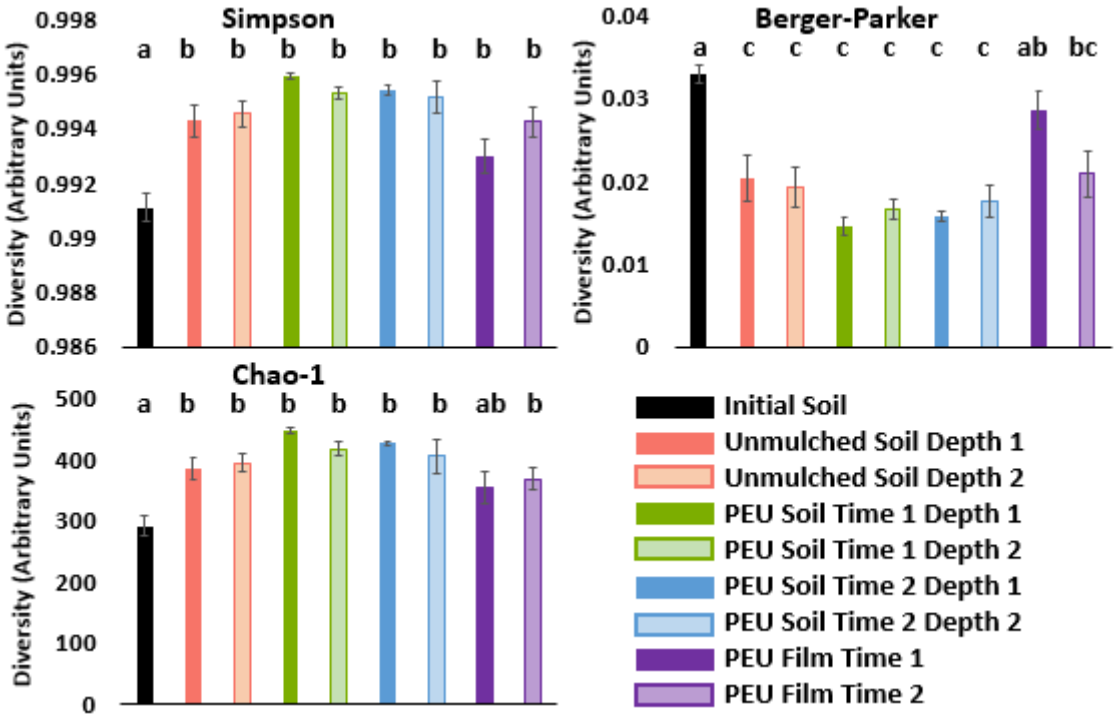

Figure 41. α-Diversity indices in different treatment groups BAC composition at the genus level. Letters above columns indicate statistically homogeneous subsets (as determined by Tukey's HSD), and error bars are ± one standard error.

To gain an understanding of the specific taxonomical compositional changes to the BAC, a heat map was constructed of the 30 most abundant bacterial and archaeal genera (Figure 42). Interestingly, the dominant taxa in the initial soil (*Gaiellales* unclassified genus, *Tumebacillus*, *Planococcaceae* unclassified genus, and *Candidatus Udaeobacter* for example) were, in general, well represented in other soils but had very small populations on the PEU films. Likewise, the dominant taxa on the PEU film (*Blastococcus*, *Azospirillum*, and *Noviherbaspirillum* for example) had very small populations in the initial soil, and were more abundant in PEU mulched soils than in unmulched soil. This is an important finding because *Azospirillum* are well known biological

nitrogen fixers and PGPB[51], and *Noviherbaspirillum* are suspected PGPB based on genes that encode for enzymes that contribute to the nitrogen fixation process[52].

Many taxa with small relative abundances in the initial soil had substantial communities in all, or most, other soils (*Nitrososphaeraceae* unclassified genus, *Acidobacteria* unclassified genus, and *Pseudarthrobacter*). All of this taken together shows that the PEU treatment did not have a negative impact on soil microbial diversity and the presence of plant roots, or fertiliser was more important in increasing BAC diversity than mulching treatment. . This suggests that the PEU created an environment suitable for a subset of taxa to thrive either through altered physicochemical factors or by providing a nutrition source available only to that subset of taxa.

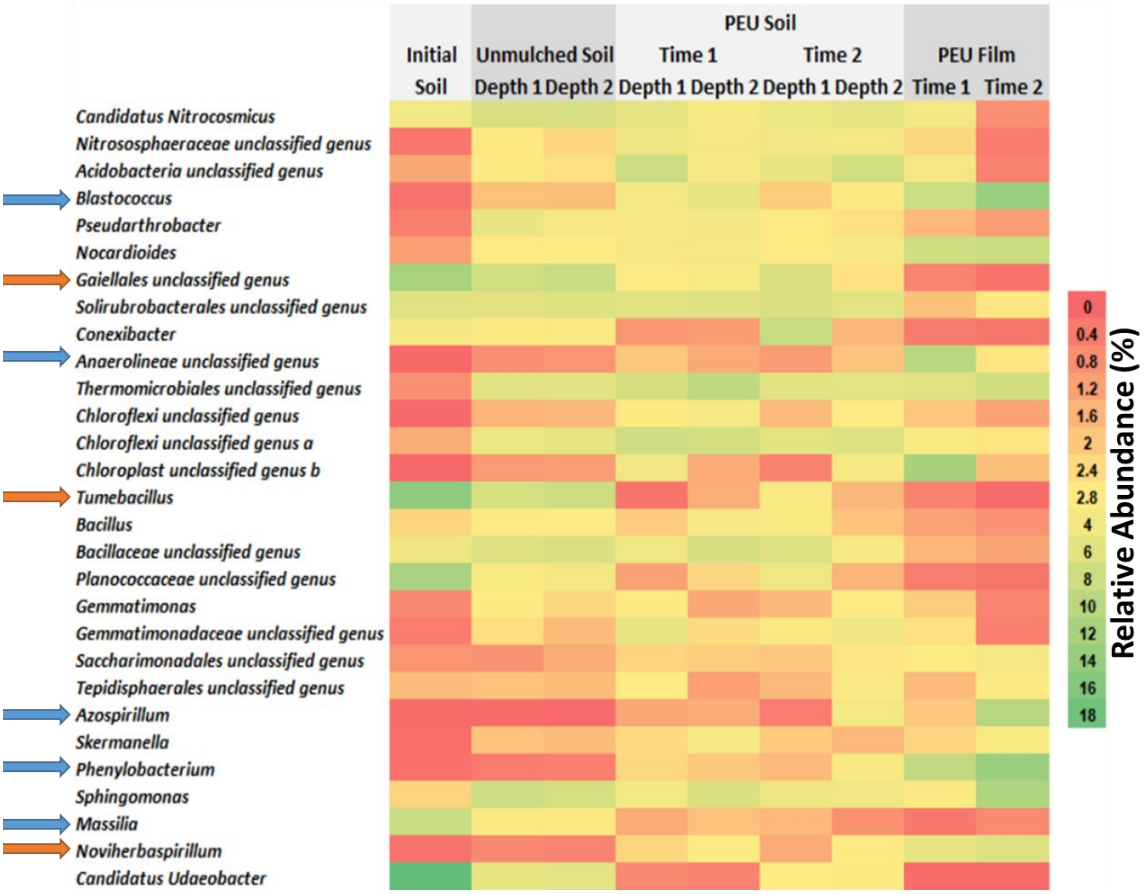

Figure 42. Heat map of the top 30 most abundant bacterial and archaeal genera based on treatment group. Blue arrows indicate genera whose abundance was enhanced by the presence of PEU mulch, and orange arrows indicate genera whose abundance was diminished by the presence of PEU.

To understand how the different treatment groups' BAC composition related to each other (β-diversity), a principal coordinates analysis (PCoA) was conducted using Bray-Curtis similarity distances to generate an ordination plot (Figure 43). Soils from the same treatment group, but

different depths, were combined into the same category (for example PEU Soil T1 D1 and PEU Soil T1 D2 were combined to PEU Soil T1) due to a lack of statistical difference in β-diversity between the sampling depths. There was the greatest distance and therefore the biggest difference between PEU film BAC communities and initial soil communities. Clearly, the unmulched soils' BAC community composition shifted from the initial (before transplanting) conditions and application of the PEU treatment shifted the BAC community composition further from initial conditions towards the BAC community composition of the PEU film itself. The BAC communities in the PEU treated soil did not shift in a statistically significant way during the six months after tomato harvest (PEU Soil T1 and PEU Soil T2). PERMANOVA was conducted to determine which treatment groups were significantly different (Table 10). With the exception of the PEU treated soils at each sampling point, each treatment group was statistically significantly different.

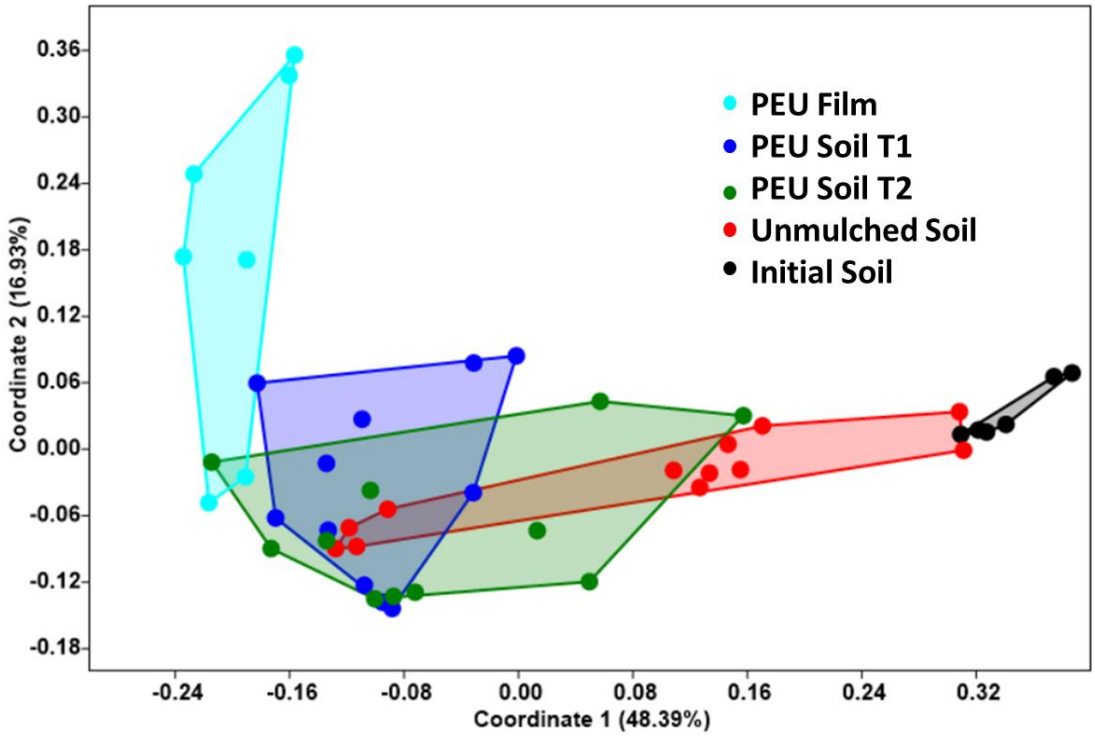

Figure 43. Principal coordinates analysis of bacterial and archaeal genera by treatment group. Polygons are to guide the eye only.

Table 10. PERMANOVA of the BAC to identify significant differences between the relative abundance of the microbial community. * indicates a significant difference.

	Initial Soil	Unmulched Soil	PEU Treated Soil Time 1	PEU Treated Soil Time 2	PEU Film
Initial Soil		0.0014*	0.0002*	0.0001*	0.001*
Unmulched Soil	0.0014*		0.0232*	0.0282*	0.0001*
PEU Treated Soil Time 1	0.0002*	0.0232*		0.1889	0.0042*
PEU Treated Soil Time 2	0.0001*	0.0282*	0.1889		0.001*
PEU Film	0.001*	0.00018	0.0041*	0.001*	

Fungal Community Composition

The fungal community in all samples was dominated by the phylum *Ascomycota* (78.2%), with *Basidiomycota* (11.9%) being the second most abundant phylum present in all treatment groups (Figure 44). There were no clear trends in the distribution of fungal phyla between treatment groups, although the phylum *Mortierellomycota* was significantly enhanced (from 0.5% ± 0.1% to 1.6% ± 0.1%) in PEU treated soils at the second sampling time point compared to the other treatment groups.

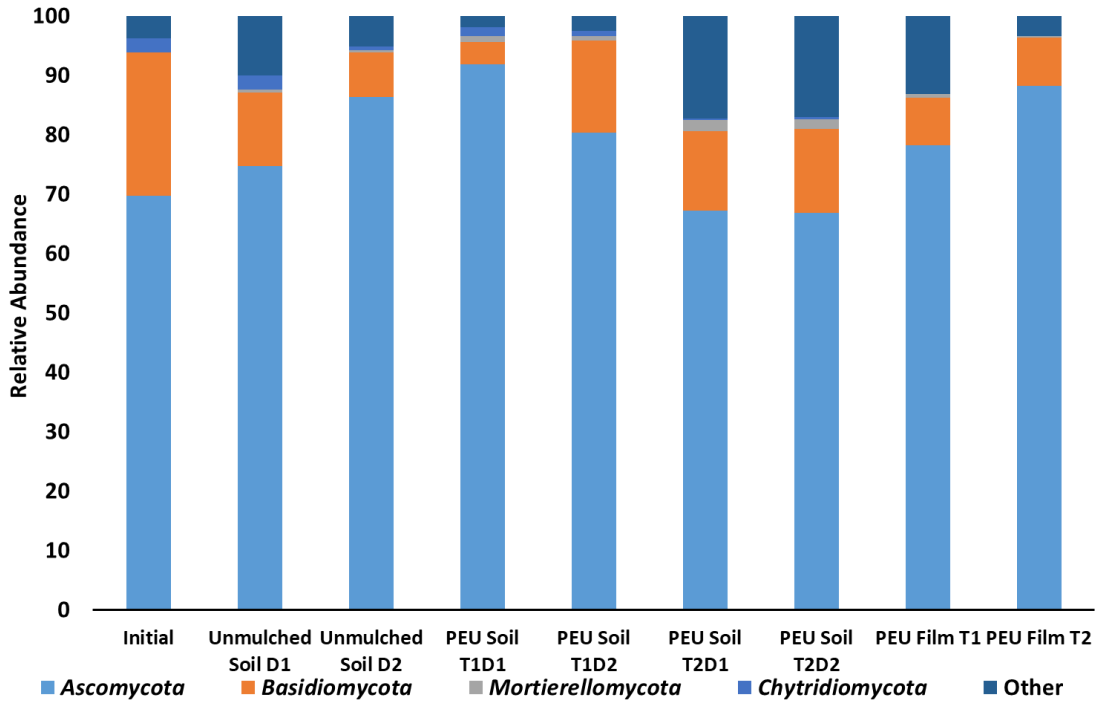

Figure 44. Relative abundance of fungal phyla in each treatment. Phyla with less than maximum 0.5% relative abundance categorised as 'Other'.

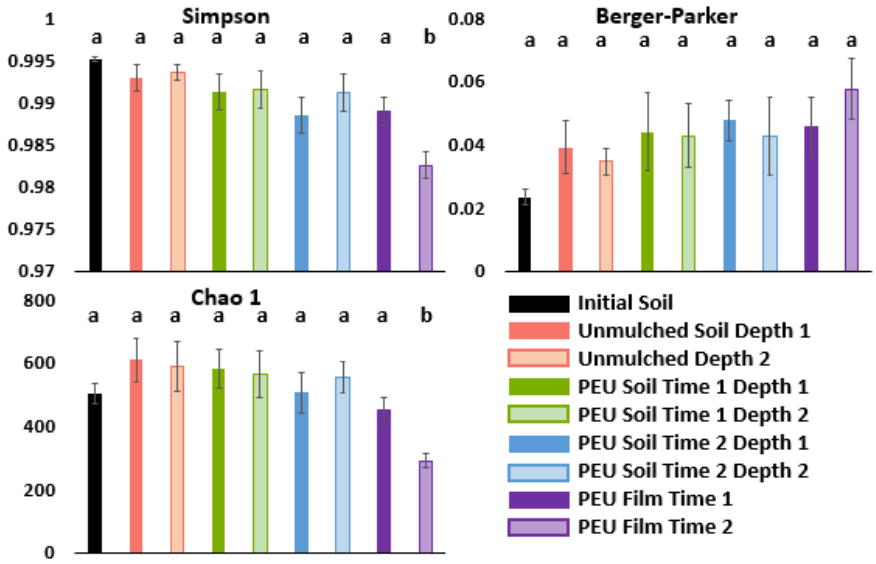

Figure 45. α-Diversity indices in different treatment groups' fungal community composition at the genus level. Letters above columns indicate statistically homogeneous subsets (as determined by Tukey's HSD), and error bars are ± one standard error.

Similar to the BAC α-diversity, all fungal treatment groups had a high evenness (Simpson's diversity index > 0.98, Figure 45), and lacked a particularly dominant single taxon, with no treatment group being more or less dominated by a single taxon (Berger-Parker index, Figure 45). In terms of taxa richness, only the PEU films six months after harvest (PEU Film Time 2) had significantly fewer taxa present.

As with the BAC, a heat map was generated to visualise the relative abundance differences between treatment groups of the top 30 most abundant fungal genera (Figure 46). There were fungal genera that preferentially colonised the PEU film, which was an expected finding, as it is understood from the literature that fungi are the primary degraders of polyurethanes[53]. There were, however, no fungal taxa which colonised only the PEU film. Any taxa which thrived on the PEU film also had a substantial presence in PEU treated soils.

Exophiala, *Phoma*, *Chaetomium* and *Clonostachys* are all understood to be PGPF[39], and the abundance of each was enhanced in the presence of PEU treatment. *Exophiala* are known to increase shoot growth, *Phoma* are known to increase crop yields, *Chaetomium* are known to stimulate germination, and *Clonostachys* are known to suppress plant pathogens and increase

seedling vigour[36,39]. *Phoma* and *Exophiala* had particularly small communities in both the initial and unmulched soils, but substantial communities in all of the PEU films and PEU treated soils. Although there were no detected taxa that colonized only the PEU film, there were taxa whose abundance through at least the top 15 cm of soil were greatly enhanced by the presence of the PEU film (*Chaetomium* and *Mortierella* for example). Interestingly, the genera *Exophiala*, and *Chaetomium* both had substantially larger abundances in the soil adjacent to PEU film (PEU Soil Depth 1, both time points) compared to the lower soil fractions. Clearly, those particular taxa are greatly impacted by the presence of PEU mulch, and their enhanced relative abundance near the soil surface strongly suggests those organisms can utilize the PEU film as a nutrition source, though further targeted study would be needed to confirm this assertion.

There were several genera (*Trichoderma*, *Agaricus*, and to a lesser extent *Arachnomyces*) with substantial community sizes in the initial and unmulched soils whose abundance was reduced in PEU treated soils. In fact, those particular genera (*Trichoderma*, *Agaricus*, and *Arachnomyces*) were almost completely absent on the PEU film itself.

This significant reduction of certain fungal taxa is in contrast to the absence of effect of the PEU treatment on the distribution of the BAC community across the treatment groups (Figure 42). The results of this study do not provide evidence as to whether the reduction in community size of particular fungal taxa in the presence of PEU treatment is due to toxicity caused by the PEU and its degradation intermediates, or the out-competition of those taxa by rival taxa due to a competitive advantage granted by the PEU treatment. Further targeted study in this area should be undertaken to elucidate which effect is at play.

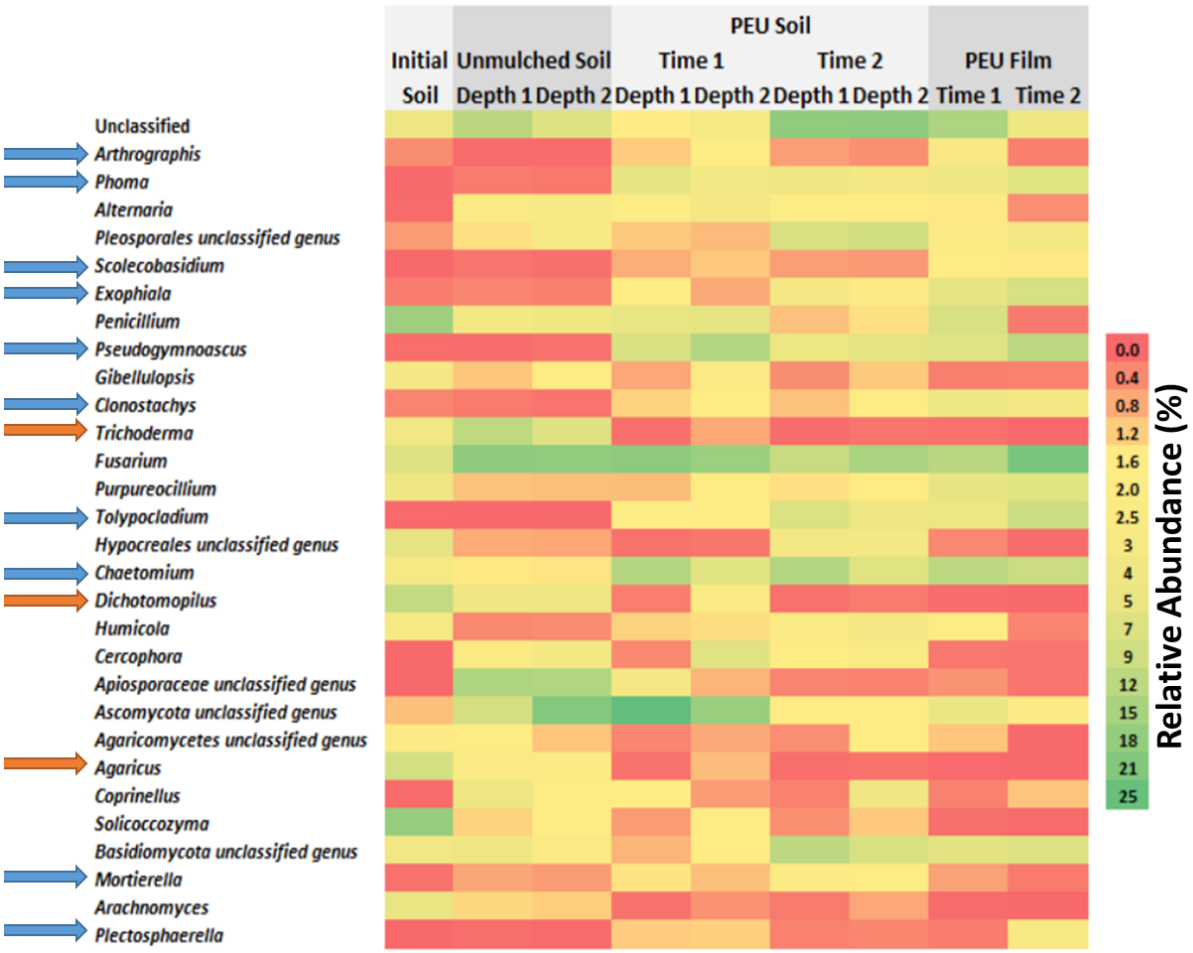

Figure 46. Heat map of the top 30 most abundant fungal genera based on treatment group. Blue arrows indicate genera whose abundance was enhanced by the presence of PEU mulch, and orange arrows indicate genera whose abundance was diminished by the presence of PEU.

There was no statistical difference between fungal communities' composition at different depths and so soils from the same treatment group but different depths were combined into the same category (for example PEU Soil T1 D1 and PEU Soil T1 D2 were combined to PEU Soil T1) for the purposes of PCoA to simplify the ordination plot and analysis (Figure 47). There was a large overlap in the β-diversity between the PEU treated soil at the two sampling times. The unmulched soils had the highest similarity to the initial soils, while the PEU treated soils clustered closer to the PEU films' fungal community confirming that the fungal composition is indeed distinct within each treatment group. Analysis by PERMANOVA indicated that the fungal communities' composition between all treatment groups was significantly different from each other (Table 11).

Emadian et al.[24] in 2016 published a review that covered, among other information, isolated microorganisms shown to degrade PCL (the primary component of the PEU, see materials and

methods), and none of those were represented in the 30 most abundant bacterial and archaeal taxa found here. The identified PCL degrading microorganisms were isolated from PCL samples in different media other than that used here (different soils, compost, fresh or salt water), but nonetheless it is of note that those particular taxa were not amplified by the presence of the PEU film in this work. Also, Barratt et al. and Cosgrove et al. in 2003[54,55] showed that fungi are the predominant microbes that degrade polyester-urethanes, and identified *Penicillium* spp. amongst others to be the most common degraders. Here *Penicillium* spp. were among the most 30 abundant genera, but they were not most abundant on the PEU film, and there were other taxa with much larger abundances on PEU films. Evidently, both the chemical structure of the polymer being degraded, and the degradation medium used are important in controlling which taxa are present and which proliferate in both soil and BPM. Given the large variety of biodegradable polymers being used and developed, the assortment of natural fibres that can be used in polymer blends, and the many degradation environments available, it is likely that the current literature only covers a very small subset of degrading microbes. It is not surprising that the most abundant taxa identified in this work have not been found in other literature given the unique chemical structure of the PEU and degradation medium used.

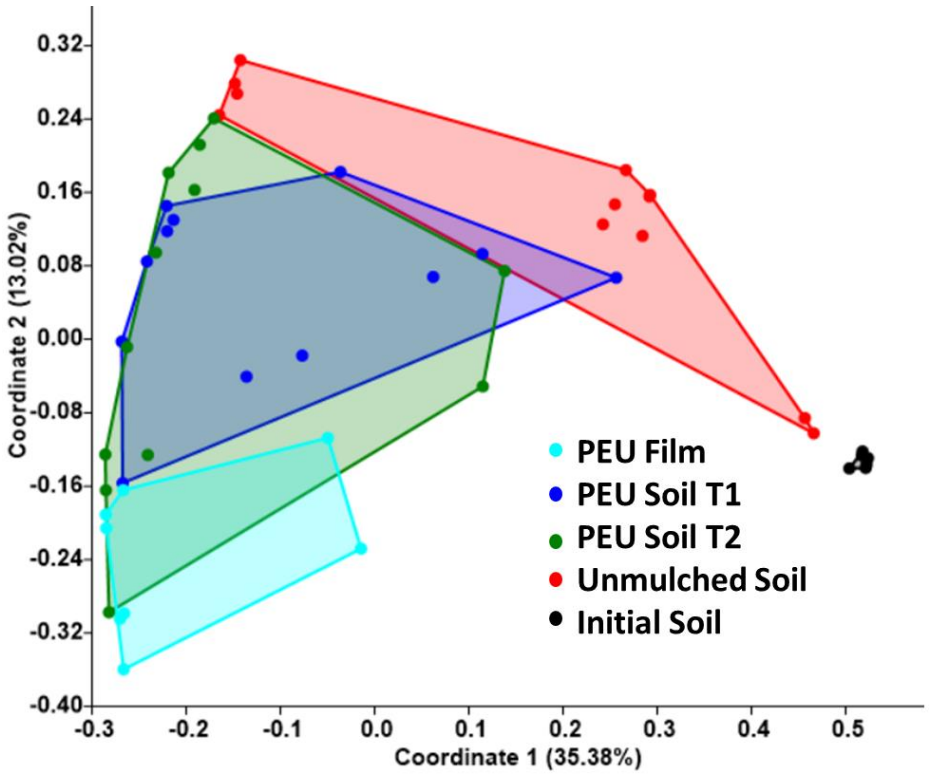

Figure 47. Principal coordinates analysis of fungal genera by treatment group. Polygons are to guide the eye only. * indicates a significant difference.

Table 11. PERMANOVA results for the fungal community.

	Initial Soil	Unmulched Soil	PEU Treated Soil Time 1	PEU Treated Soil Time 2	PEU Film
Initial Soil		0.0012*	0.0002*	0.0001*	0.0003*
Unmulched Soil	0.0012*		0.0013*	0.0001*	0.0002*
PEU Treated Soil Time 1	0.0002*	0.0013*		0.062	0.0001*
PEU Treated Soil Time 2	0.0001*	0.0001*	0.062		0.0022*
PEU Film	0.0003*	0.0002*	0.0001*	0.0022*	

Soil Physicochemical Properties and Microbial Taxa Distribution

The soil pH significantly increased for all treatments from initial conditions, and this was likely due to one highly alkaline component of the fertiliser program used. Soil moisture was highest in the initial soils and the inorganic forms of nitrogen decreased significantly in all of the treated soils compared to the initial conditions. This decrease was likely due to the uptake of nitrate and ammonium by the tomato plants. PEU film was excluded from this analysis because it did not make physical sense to measure the same physicochemical properties in a meaningfully similar way as the soil.

Table 12. Soil Physicochemical Properties. Values are mean ± one standard error, and the superscript letter refers to statistically homogeneous subsets as determined by Tukey's HSD.

	pH	EC (µS/cm)	Moisture (%)	Nitrate (mg/kg)	Ammonium (mg/kg)
Initial Soil	5.17±.03[a]	99±6[a]	40±4[a]	30±3[a]	44±6[a]
Unmulched Soil Depth 1	6.2±0.3[b]	120±21[a]	32±8[a,b]	9±2[b]	9±3[b]
PEU Soil T1	6.9±0.1[b]	160±16[a]	28±6[b]	7±2[b]	2.8±0.4[c]
PEU Soil T2	7.0±0.1[b]	160±16[a]	28±5[b]	13±2[b]	1.2±0.4[c]

Canonical correspondence analysis (CCA) was used to understand how soil pH, EC, moisture, nitrate and ammonium (Table 12) influenced the total soil microbial community composition (Figure 48).

There was a high degree of overlap between PEU treated soils (Figure 48). It can be seen that the higher level of inorganic N played a role in shaping the microbial community in initial soils, while a higher EC, and pH played a role in shaping the microbial communities in PEU treated soil. Soil moisture was highest in initial soils, but it did not correlate with the microbial community composition.

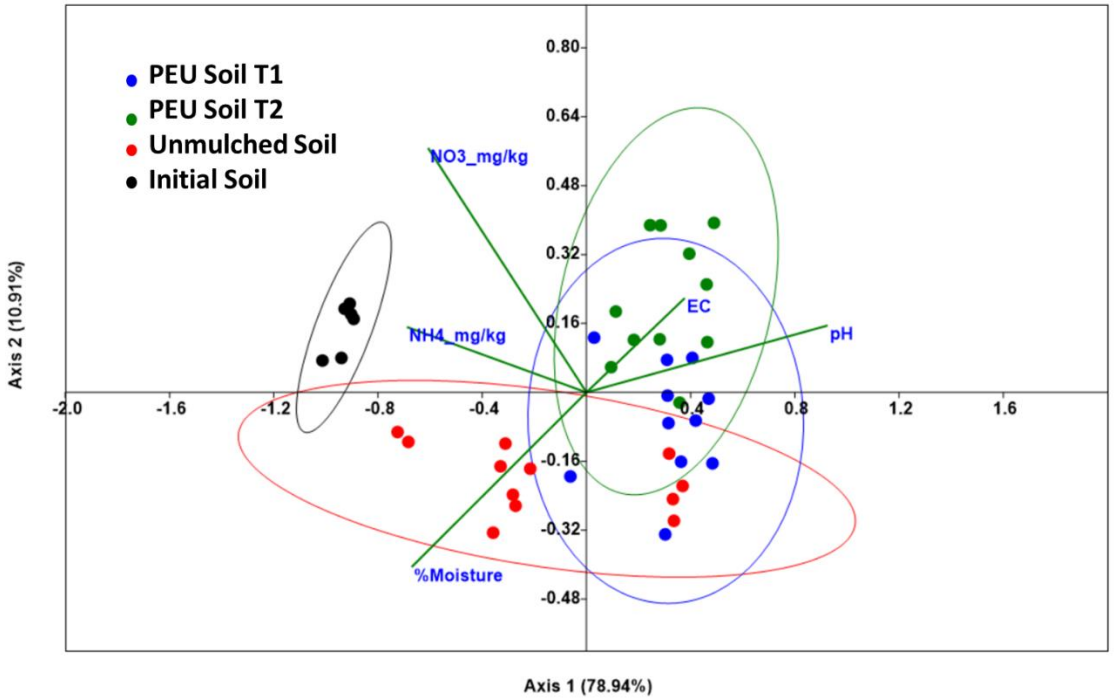

Figure 48. Canonical correspondence analysis of the total soil microbial community composition. Ellipses are 95% confidence intervals for the given treatment group.

Soil Enzyme Assays

In addition to the change in soil microbial composition caused by the presence of PEU, it was of interest to identify any functional changes provided by the community. To assess this, soil enzyme assays were carried out to determine the activity of β-glucosidase, acid phosphatase, urease, and lipase (Figure 49).

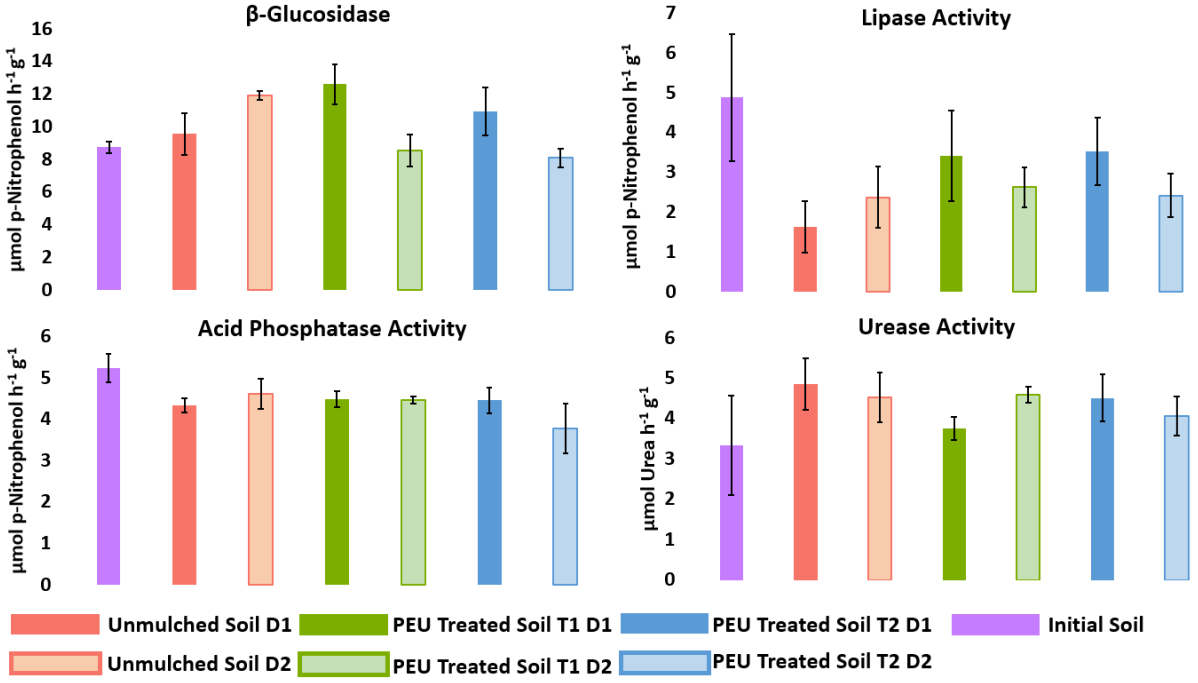

Figure 49. Soil enzyme assays. Error bars are ± one standard error.

The enzyme activities were statistically similar across all treatment groups, indicating that the PEU treatment did not impact the overall microbial capacity to carry out these enzymatic reactions, which are important in the C, P, and N cycles. It was thought that the added substrate for lipases and ureases enzymes (the urea and ester linkages in the PEU) would cause an upregulation of those enzymes, so this was an unexpected finding. It is possible that the amount of substrate wasn't sufficiently increased to cause a change in the enzyme activities. Further, targeted studies would be of interest to elucidate explicitly if BPM can condition an upregulation in relevant enzyme production. The enzyme activities measured in the soil used here were all within typically reported ranges[30,44,45,56–60].

Conclusion

The changes to the soil microbial community from the application of a novel, sprayable, biodegradable PEU were investigated. Microbial DNA was extracted and sequenced from soils at two depths: the top 2 cm of soil, which was closest to the PEU films, and from 2-15 cm which was further from the PEU film. The PEU film itself was also sampled. It was found that the BAC and fungal diversity increased across all treatment groups from the initial community, and the presence of the PEU film did not reduce microbial diversity. There were no significant differences in the soil microbial communities' composition between the two soil depths sampled. The microbial community composition between the initial soil and PEU film differed the most. The PEU treated soils microbial community composition shifted away from that observed in the initial soil and more closely resembled the microbial community composition on the PEU film. The abundance of the PGPM *Azospirillum*, *Noviherbasperillum*, *Exophiala*, *Phoma*, *Chaetomium* and *Clonostachys* all were increased in soils treated with PEU. This is an important finding, and one that warrants further study. If these results are replicated in the field, then the use of the PEU as mulch could prove beneficial in increasing crop yield by supporting PGPB and PGPF populations, and provide a strategy for reducing fertiliser inputs. Given that the PEU film did not present any detrimental effects to the soil microbial community nor its function, and in fact increased the abundances of several PGPM in soil, its use would be preferential to PE mulch both from an agricultural productivity and an environmental safety standpoint.

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Chapter 6. A comparative Plant Growth Study of a Sprayable degradable Polyester-urethane-urea mulch and two commercial plastic mulches

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Abstract

The agricultural practice of spreading polyethylene (PE) sheets over the soil surface as a mulch is a common, global practice that aids in conserving water, increasing crop yields, suppressing weed growth and decreasing growing time. However, these PE sheets are used for only a single growing season, and their use comes with environmental consequences including the accumulation of microplastics in soils which are deleterious to soil invertebrates, and cause decreases in crop productivity over time. In order to maintain the crop productivity afforded by PE mulching while avoiding the environmental downsides, the use of biodegradable polymer technologies has begun to be explored. Here the efficacy of a newly developed (by the Commonwealth Scientific and Industrial Research Organization, CSIRO), water dispersible, sprayable degradable polyester-urethane-urea (PEU) based polymer was compared to that of two commercial PE mulches in a greenhouse tomato growth trial. The water savings efficacy, effect on plant growth, and effect on some soil characteristics were studied. It was found that the PEU provided similar water savings to the commercial PE mulches, while showing no deleterious effects on plant growth. Due to the inherent differences between growing plants under carefully controlled conditions in a greenhouse and growing plants in the field, the results here should be taken as preliminary indications that the sprayable, biodegradable PEU shows promise as a replacement for PE mulch, and warrants further study under true field conditions.

Keywords: sprayable polymer mulch, tomato growth, greenhouse study

Introduction

Single use, non-degradable plastic waste is a global problem, and the agricultural sector is a major contributor [1,2]. The conventional practice of spreading polyethylene (PE) mulch over the soil surface in ridge-furrow cropping systems is beneficial from a crop productivity and water conservation perspective, but detrimental from a sustainability and environmental pollution perspective [3–9]. In 2011 in China alone over 1.2 million tons of single use PE mulch was used covering nearly 20 million ha of cropland [1]. With this practice employed worldwide, it is clear that this creates a large environmental burden, and it has been shown that this practice is a source of microplastics in agricultural fields [10–12]. This is a concerning finding because microplastics have been shown to be deleterious to terrestrial fauna, and eventually decrease crop productivity [13,14]. With United Nations models predicting an increase in food and water insecurity [15–17], it is vital that this practice is not abandoned, but rather modified using sustainable and environmentally benign technologies. Biodegradable polymers as an alternative to PE are one solution that has been investigated thoroughly [18–25] and an emerging branch to this field is that of sprayable, biodegradable polymers given their simple application and easy customisability [26–34]. By harnessing this technology, the benefits of plastic mulching can be realised without the consequences associated with plastic waste.

The Commonwealth Scientific and Industrial Research Organization (CSIRO) has reported the development of a new sprayable, biodegradable polyester-urethane-urea (PEU) mulch for this purpose, and should its use become widespread, information on its performance and environmental behaviour is necessary. In other work (unpublished at the time of writing), it has been shown that the PEU is effective at conserving soil moisture in several different soils and over a range of environmental conditions, but in the absence of plants. It is important to evaluate the PEU's water conservation efficacy when a crop is grown, as well as its effects on plant growth and soil properties.

To accomplish this, a tomato growth study investigating the impacts of this novel, degradable PEU mulch on water conservation, plant growth, and soil chemistry was undertaken and results compared with two commercially available plastic mulches, black polyethylene and transparent oxo-degradable polyethylene. In particular, the study sought to ensure the use of the sprayable PEU did not impede plant growth. To ensure the study provided realistic and applicable insights, it was carried out in pots in a greenhouse using soil, tomato seeds, and a fertiliser program sourced from an active, commercial tomato farm in Echuca, Victoria, Australia to mimic field soil conditions.

Materials

Soil (Vertosol) was collected from a well-tilled, commercial tomato farm in Echuca, Australia (36°09'18.9"S 144°38'50.6"E) prior to the growing season at a depth of approximately 20cm. The soil was air dried and sieved at 2 mm. A representative subsample of the soil was analysed for a range of key soil physicochemical properties by the Environmental Analysis Laboratory at Southern Cross University (Table 13). Tomato seeds were obtained from Kagome® Australia and grown to seedlings for three weeks in a commercial seed raising mix. The seeds were of the same variety used on the farm from which the soil was obtained.

Mulches used include a commercial black polyethylene (PE); a commercial, transparent, slotted (perforated by repeating slits in the centre of the film) oxo-degradable polyethylene (OPE); and a sprayable, water dispersible, biodegradable polyester-urethane (PEU) developed by Adhikari et al, CSIRO[26,35].

Table 13. Soil Characteristics.

Characteristic	*Vertosol*
Ca^{2+}, mg/kg	1675
Mg^{2+}, mg/kg	524
Na^{+}, mg/kg	140

K^+, mg/kg	58
P(Colwell), mg/kg	65
NO_3^-, mg/kg	26.1
NH_4, mg/kg	3.7
Electrical Conductivity, dS/m	0.17
Total C, %	1.12
Total N, %	0.12

Fertiliser used included urea (analytical grade, Sigma), $CaCl_2 \cdot 2H_2O$ (Sigma), anhydrous $ZnCl_2$ (Sigma), commercial Super Phosphate (RICHGRO), Sulphate of Potash (RICHGRO), and Boron (Manutec).

Chemicals used in soil characterisation experiments include KCl (Sigma), N-(1-Napthyl)ethylene diamine dihydrochloride (NED, >98%, Sigma), sulphanilamide (≥99%, Sigma), vanadium(III) chloride (97%, Sigma), potassium nitrate (≥99%, Sigma), hydrochloric acid (Sigma), sodium salicylate (≥99.5%, Sigma), sodium citrate dihydrate (Sigma), sodium tartrate dibasic dihydrate (≥99%, Sigma), sodium nitroprusside (Sigma), ammonium sulphate (≥99%, Sigma), and anhydrous sodium hydroxide (≥98%, Sigma).

Tomato Growth Trial Conditions and Maintenance

The tomato growth trial consisted of four treatment groups in total, of which three were plastic mulches (PE, OPE, and PEU) and one was an unmulched control group (C). Treatments were replicated 6 times in 24 cm internal diameter, free-draining polypropylene (PP) pots (one plant per pot) set-up in a temperature-controlled greenhouse with a mean day-night temperature of 26°C and 16°C, respectively (temperature ranged from 25-31°C during the day and 14-18°C at night) . Pots were set up underneath full spectrum high intensity discharge (HID) lights set to a 16-8 hour day-night cycle, and the illumination level was ramped from 0-30 klux during the cycle.

PP pots were filled with 8 kg of soil and brought to 50% of the soil's experimentally determined field capacity by adding 2.1 L of tap water. To mimic the typical on-site practice, subsurface drip irrigation, two Falcon® 50 mL centrifuge tubes, with their bases removed, were inserted into the soil on either side of the pot. These were capped at all times except when watering the tomato plants.

Tomato seedlings were then transplanted from seed-raising mix into the centre of each pot, one per pot. Finally, mulching treatments were applied to the soil surface by being cut to the appropriate dimensions in the case of the preformed plastic mulches (NPE and OPE), and by being applied as a liquid suspension at a loading of 1 kg m^{-2} via syringe (20% solids by weight) in the case of the sprayable, biodegradable polyester-urethane-urea (PEU). The sprayable PEU cured into a film over the course of 24 hours. Figure 50 shows the pots immediately after set-up was completed.

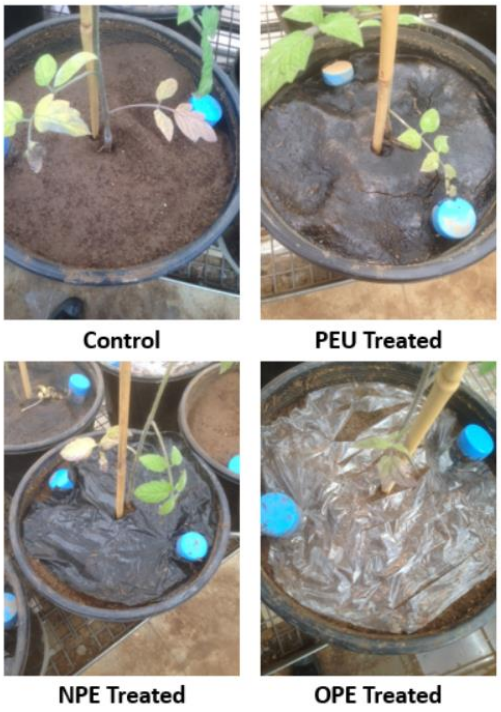

Figure 50. The final tomato pot set up with mulching treatments applied.

During the growing period, the plants were watered 3-4 times per week, and after each watering event the pots were repositioned randomly. Watering was done by removing the caps from the buried centrifuge tubes, and pouring water directly into the tubes, where it would then run into the

soil at a depth of approximately 10 cm. The amount of water added was determined gravimetrically in the following way: the initial total mass of pot, plant, soil and water was known for each pot, and any mass loss was assumed to be due to evaporation. The mass of water lost from each pot between watering events was recorded, in order to assess the water-savings efficacy of the PEU in comparison to commercial products. Fertiliser was applied once weekly for the first 12 weeks post-transplant according to the fertiliser program used at the commercial farm from which the soil and tomato seeds were obtained (fertiliser program is confidential, a typical tomato farm fertiliser program can be found in the Australian Processing Tomato Grower Report [36]). Fertiliser was applied as an aqueous solution to mimic the sub-surface fertigation. The plants were grown to maturity and harvested 136 days post-transplanting.

At maturity, fruit was picked and characterised, and the remaining plant mass (roots plus shoots) was weighed first as fresh and then as dry weights after 24 hours of oven drying at 105°C. Residual PEU was characterised by gel permeation chromatography (GPC), and soil from each pot was analysed for pH, electrical conductivity (EC), nitrate, and ammonium.

Plant Sampling and Characterisation

The growth of the tomato plant was characterised by measuring the plant height periodically over the first 50 days of the study, counting the number of flowers that formed and the number of fruits that developed. The mature fruit number, and type of visible defects (blossom end-rot, and discolourations) on each fruit were recorded, and as previously stated, at maturity the mass (fresh and dry) of the whole plant excluding the fruit was determined.

The juice of the fruit from each plant was characterised by homogenising whole fruit from each plant in a mortar and pestle, and then measuring the pH and sugar content (Brix). Fruit pH was measured using a pH metre (TPS, WP-80), and fruit Brix was measured by dropping a small amount of the filtered juice onto a refractometer (Livingston, BRIXREF113).

Soil Sampling and Characterisation

After harvest of the mature plants, the soil was sampled at two depths, 0-2 cm and 2-12 cm. After sampling, the soil was air-dried before being characterised.

Soil pH and electrical conductivity (EC) were determined using a 1:5 (m/m) soil: water suspension ratio [37]. In brief, 20 g of soil and 100 g of DI water were agitated for 1 hour then allowed to settle for a further 30 minutes. The EC of the supernatant water was first measured using an EC meter (Hach, sensION+ EC5), after which the pH of the supernatant water was measured using a pH metre (TPS, WP-80).

Soil nitrate and ammonium were determined using previously described methods [38,39]. In brief, 5 g of soil was agitated in 12.5 mL of 2M KCl for 20 minutes, then centrifuged at 4200 rpm for 10 minutes before being analysed colorimetrically. For soil nitrate determination, an aliquot of the KCl supernatant was mixed with a reagent containing VCl_3 and Griess reagent (NED and sulphanilamide in water) and colour was allowed to develop overnight at room temperature, measurement was carried out on a multiplate reader at 540 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). For soil ammonium determination, an aliquot of the KCl supernatant was added to an aliquot of sodium nitroprusside reagent (including sodium salicylate, sodium citrate, and sodium tartrate) after which an aliquot of alkaline sodium hypochlorite was added. The colour developed for 2 hours before being measured on a multiplate reader at 650 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). Soil nitrate and soil ammonium quantities were determined using sodium nitrate and ammonium sulphate standards, respectively.

Polymer Characterisation

Residual PEU film from was collected at tomato harvest, and then allowed to degrade for a further six months. PEU was characterised by gel permeation chromatography (GPC) on a Shimadzu system equipped with a CMB-20A controller system, an SIL-20A HT autosampler, an LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, an RDI-10A

refractive index detector, and 4X Waters Styragel columns (HT2, HT3, HT4, and HT5, each 300 mm × 7.8 mm^2, providing an effective molar mass range of 100-4 × 10^6). Samples were dissolved in dimethylacetamide (DMAc) containing 4.34 g L^{-1} LiBr, at a concentration of 1-2 mg mL^{-1}. The columns were calibrated with low dispersity poly(methyl methacrylate) (PMMA) standards ranging from 1,500 – 1,500,000 g mol^{-1}. DMAc containing 4.34 g L^{-1} LiBr was used as an eluent at a 1 mL min^{-1} flow rate and 80 °C. M_n and M_w were evaluated using Shimadzu LC Solution software

Data Analysis

All data was analysed using Microsoft Excel 2016, IBM SPSS Statistics 25, or a combination of both. Data clean up (means calculations, outlier testing, and formatting) was carried out in Excel. To determine statistical significance, one-way ANOVA tests were carried out with Tukey's Honestly Significant Difference post-hoc testing in SPSS. Significance level was set at $\alpha < 0.05$.

Results and Discussion

Water Conservation Efficacy

Water loss was determined gravimetrically at each watering event. Figure 51 shows the mass of water lost on average from each mulching treatment over the duration of the 136 day study. As expected, unmulched control pots lost the most water, 35.8 ± 3.4 kg, over the study duration compared to 31.5 ± 2.1 kg, 30.0 ± 1.7 kg, and 28.0 ± 4.3 kg for PEU, NPE and OPE, respectively. There was no significant difference in water lost from PEU mulched and NPE mulched pots, and OPE mulched pots lost the least water. The OPE mulch was slotted, so this finding was unexpected, but the OPE plants initially grew slower (see Figure 53), which possibly caused by a reduced rate of evapotranspiration. Also, the OPE mulch did not have a black pigmentation (Figure 50), which could have caused the soil to remain cooler, thus slowing soil water evaporation rate.

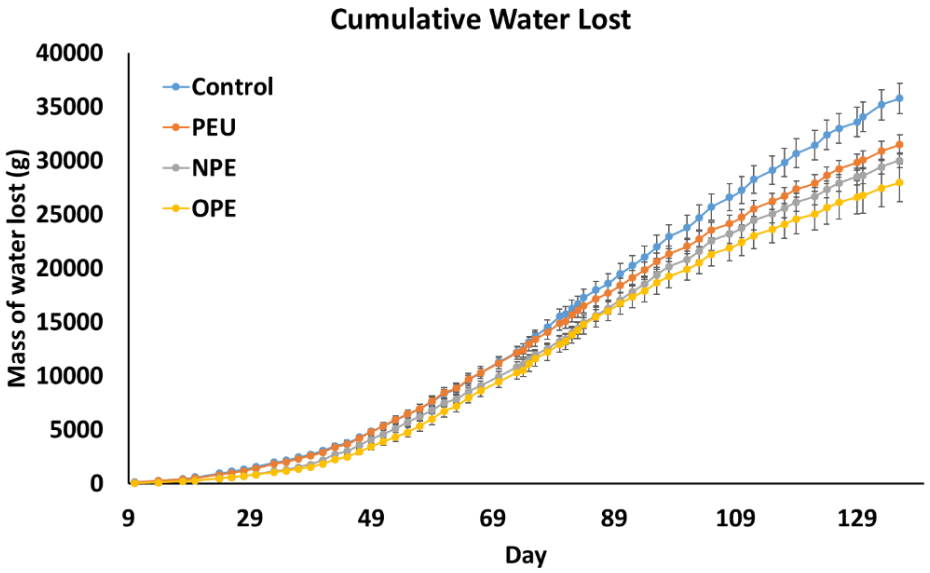

Figure 51. Water loss from the different mulching treatments during the trial. Error bars are ± one standard deviation.

Soil Analysis

Soil from each pot was analysed at two depths (0-2 cm and 2-10 cm) for pH, electrical conductivity (EC), nitrate, and ammonium (Figure 52). These are typically measured characteristics to understand soil health. Quantifying soil nitrate and ammonium was of particular interest because the PEU material contained N (in both the repeating carbamate and urea functional groups) and it would have been interesting if that N ended up in the soil in an inorganic form, easily accessible to plants.

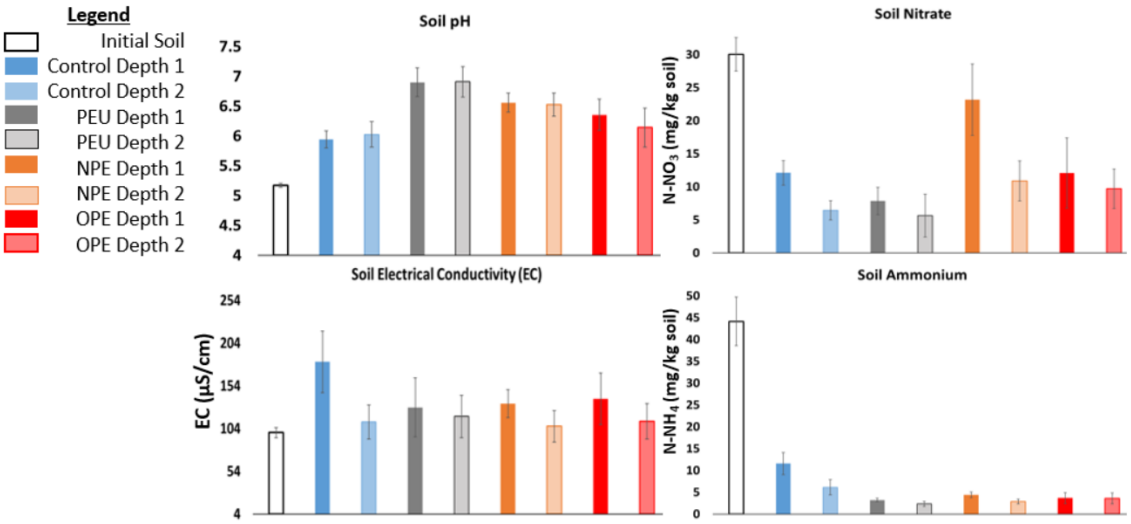

Figure 52. Soil Physicochemical Properties. Depth 1 is sampled from the top 0-2 cm of soil and Depth 2 is sampled from the following 2-10 cm of soil. Data is mean (n=6) ± one standard error.

There was no change in soil salinity (as measured by EC) in any of the mulching treatments compared to the salinity of the initial soil. Soil pH rose significantly for all treatment groups, including the control, the likely cause of this was the fertiliser program, which was slightly alkaline (pH > 8) due to one highly alkaline component (pH > 11 when in solution). No differences based on sampling depth were observed. Both soil nitrate and soil ammonium decreased significantly from the initial conditions in all treatments, which was expected as the tomato plants take up and use both chemical forms of nitrogen. If the PEU did act as a source for inorganic N, the analysis here did not reveal any differences. Further study with under a simpler system would be needed to determine whether no inorganic N is released from the PEU, or if some inorganic N is released and rapidly assimilated by any plants present. Across the mulching treatments, higher levels of ammonium and nitrate were observed in the soil sampled nearest the surface. This finding is intuitive as the root density is low at the soil surface (less opportunity for inorganic N to be taken up by the plants), and due to the watering method used (sub surface), there would be little opportunity for these chemicals to leach down the soil profile as they would do with above ground irrigation methods. This trend was common in all treatment types although none of the differences between treatments were statistically significant. From a soil physicochemical perspective, the PEU mulch performed comparatively to conventional, commercially available plastic mulches.

Plant Growth Analysis

Growth of the tomato plants was monitored by measuring their height periodically over the first 50 days of the trial (Figure 53), and by measuring their wet and dry mass at harvest. Over the first 50 days the plants mulched with the sprayable PEU or NPE showed increased growth than those not mulched, or mulched with OPE, however these differences were not statistically significant.

In terms of total plant mass, there was very little variation in wet or dry mass between treatment groups. Interestingly the plants grown in unmulched conditions had the largest average wet mass, but this was not statistically significant, and after oven drying the difference in masses between

treatment groups was negligible. This data gives some preliminary evidence that the sprayable PEU does not create any adverse impacts on plant growth.

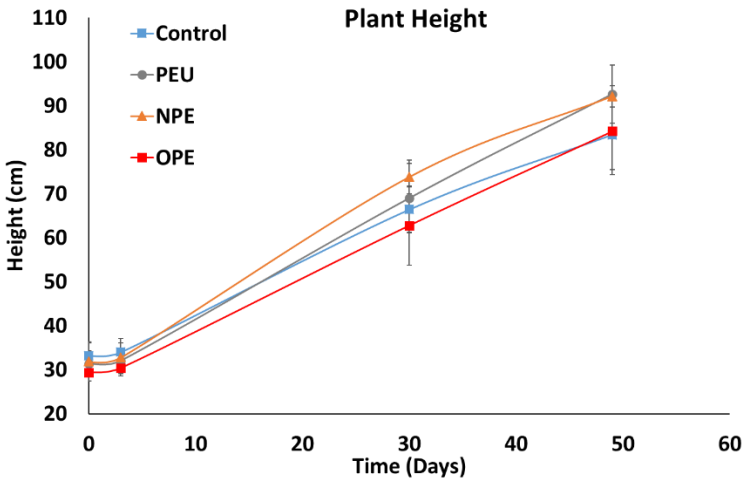

Figure 53. Time series of plant height. Error bars are ± one standard error.

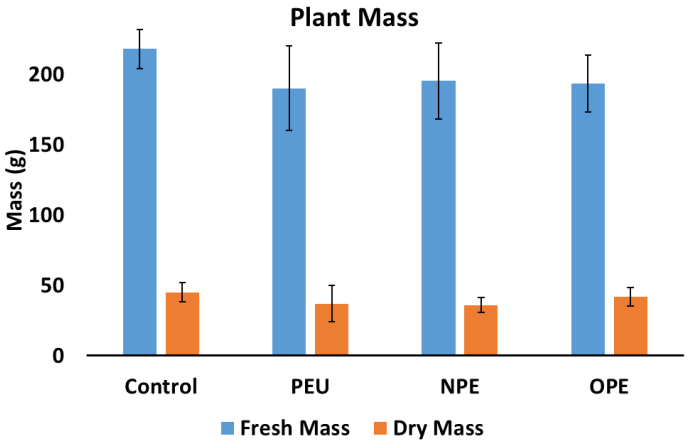

Figure 54. Wet and dry tomato plant mass. Error bars are ± one standard deviation.

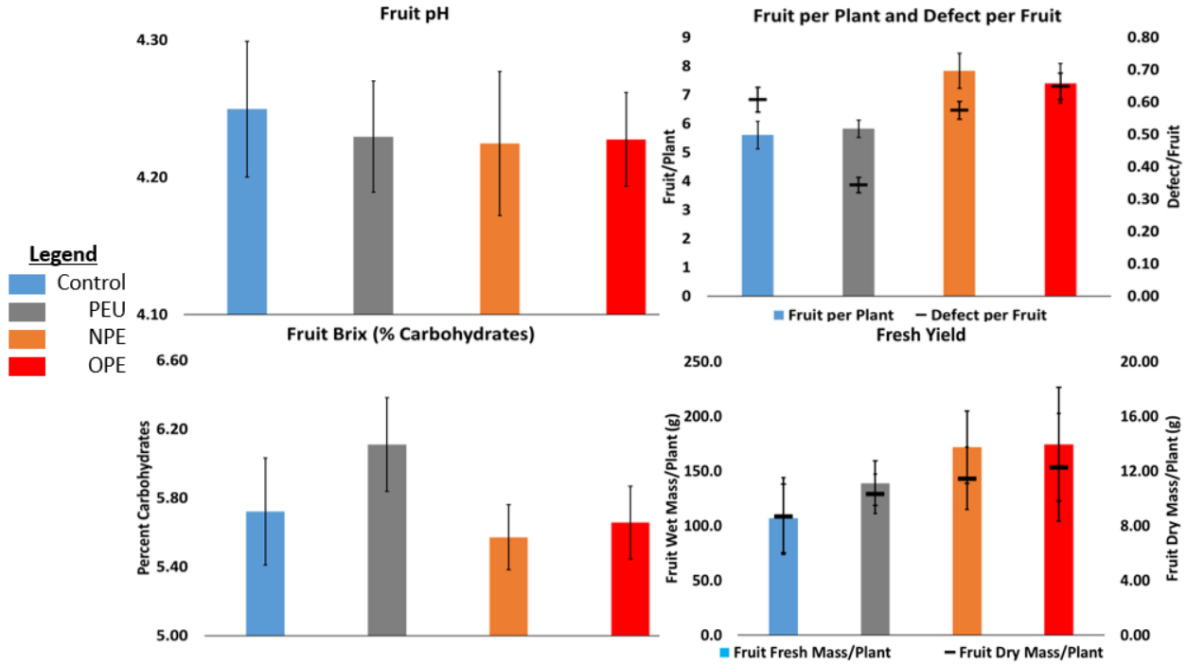

Figure 55 Fruit Characteristics. Data displayed are mean ± one standard error.

Fruit Brix, fruit pH, and fruit defects were measured because these are important parameters for both tomato growers and tomato processors (Figure 55). There were no statistically significant differences in any measured fruit characteristic between treatment groups, which contradicts much of the literature that shows that plastic mulching increases crop yield [7,8]. These findings are likely evidence of a limitation of this study, or greenhouse pot trials in general; perhaps using larger pots, or increasing the replication number would have revealed statistically significant trends, and allowed for increased fruit growth. In any case, this can be taken as further preliminary evidence that the application of the sprayable PEU does not cause large, detrimental effects to plant growth, but further study is necessary to determine if there truly are no adverse effects.

The large number of defects per fruit (ranging from 0.4 - 0.65 defects per fruit) is noteworthy. A preponderance of the fruit defects were blossom-end rot (BER), which is caused in part by a calcium deficiency[40]. Given that the growth conditions used in this study (soil, tomato cultivar, and fertiliser program) were identical to those used in an active tomato farm, this large incidence of BER indicates that a large scale field trial is necessary to provide optimal growing conditions for the tomatoes.

Tracking the number of flowers per tomato plant over time is another way to gain an understanding of how different treatments affect plant health. Figure 56 displays the average number of flowers per plant per treatment group. Each mulching treatment increased the number of flowers per plant compared to no mulch, but the only statistically significant increase compared to control was the sprayable PEU mulched plants. However, both OPE and NPE mulched plants were statistically higher than control at the $\alpha < 0.10$ level.

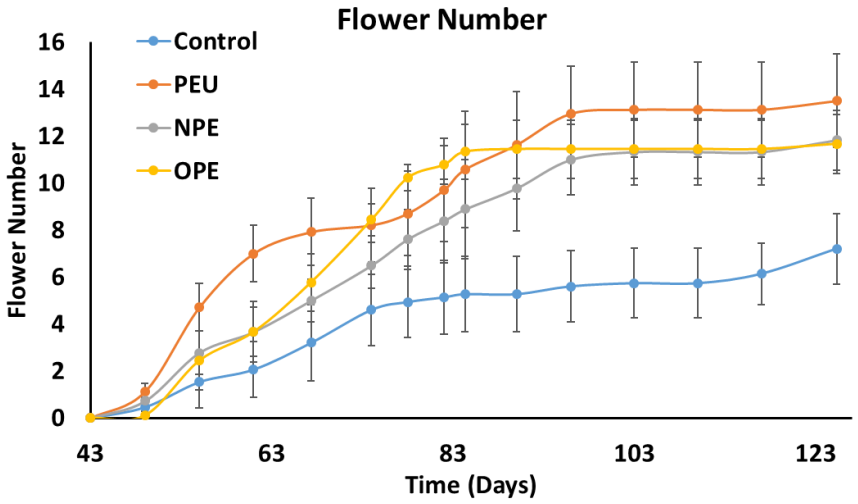

Figure 56. Flower number per tomato plant. Error bars are ± one standard error.

Polymer Degradation

The PEU degraded extensively over the course of the growing period and a further six months, as measured by GPC (Figure 57).

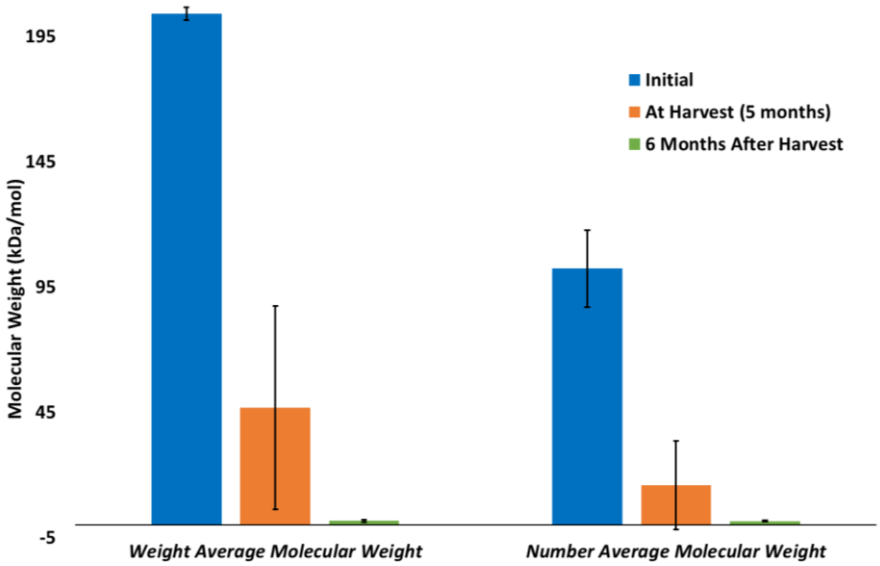

Figure 57. Molecular weight change of PEU over the course of the study.

The large error in molecular weight observed at harvest (orange bars, Figure 57) is due to some films degrading nearly completely (i.e. M_w < 1500 Da) and some films remaining more or less intact though degraded significantly (M_w between 30-90kDa). A possible cause for this disparity comes from the method of application. The soil surface is inherently uneven, and with a liquid mulch formulation the PEU pools at low points thus creating small portions of relatively thicker film which would degrade more slowly. After a further 6 months of on-soil degradation, only three pots had residual PEU to be characterised by GPC. This data helps demonstrate that PEU is effective at conserving soil moisture despite degrading extensively while being used.

Conclusions

The effects of two commercial plastic mulches and a novel, sprayable, biodegradable polyester-urethane-urea mulch on certain soil physicochemical properties, water conservation, and tomato plant growth were investigated. Enhanced water savings were observed in plants treated with mulch of any kind, with no differences in the water savings between mulching treatments. The PEU caused no negative impacts on any plant growth measurement nor on any measured fruit characteristic, while degrading significantly over the course of the tomato growth period.

The limitations of pot trials are evident in the data presented here. There was no enhanced crop yield for plants grown in mulched soil, which was to the contrary of expectations, and there was a high incidence of fruit defects in all treatment groups. There were however more flowers put out by mulched plants, and an apparently higher yield of fruit, just not statistically significantly so. These data further highlight that a study conducted in larger pots or in the field likely would have produced statistically significant results.

This study did demonstrate that sprayable, biodegradable polymers can perform similarly to conventional non-degradable plastic mulches without causing adverse effects to soil nor plant health, and serves as a starting point for further study. This work should be followed up with a field trial conducted under commercial growth conditions to validate inconclusive findings and confirm the technology's performance under real field conditions.

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Chapter 7. Conclusions and Future Directions

Overview

The practice of mulching, spreading material over the soil surface with the intention of shortening crop growth time and increasing crop yield, is essential to society.

This statement may seem hyperbolic, but the global population is expected to grow past ten billion in the next three decades[1], resource scarcity (particularly freshwater, of which agriculture already accounts for 70% of global consumption[2]) is expected to increase[3] and the climate will become increasingly unpredictable: all of which contribute to the ongoing and intensifying problem of feeding the world's population[4,5]. Mulching is a technology that provides the dual benefit of increasing the crop yield while also requiring less water[6,7]. Mulching is not the only essential agricultural technology, of course, but it is one essential component in the suite of agricultural productivity and efficiency strategies available that will help address the globe's ongoing food and water security challenges.

The most reliable mulching material, in terms of consistency of supply and performance with minimal variability, has historically been polyethylene (PE)[8]. Unfortunately, in recent years it has become evident that the ongoing use of PE is not sustainable. Not only does PE mulch represent a large (1 million+ tons per annum) single-use plastic waste stream, but its ongoing use also worsens soil health and agricultural productivity in the long term[9,10], particularly by creating microplastics[11,12] which accumulate in the soil and are deleterious to soil invertebrates[13,14]. Clearly, an alternative must be used which can provide the benefits of PE while avoiding the environmental and agricultural consequences, and biodegradable plastic mulches (BPM) are that alternative. BPM, by design, degrade in the environment into CO_2, H_2O and other small inorganic compounds[15] thereby avoiding the creation of waste and microplastics. To date, there have been several commercial BPM, all available as preformed films, but investigators have identified that these products are laborious and costly to apply[16], and due to their lesser mechanical strength compared to PE they can be difficult to apply in the field, especially without tearing[17]. As a result, it has been suggested that a sprayable, BPM would overcome these issues. If the agricultural sector is to

undergo a paradigm shift with respect to mulching technology, then it makes sense to utilise technologies that will provide the necessary service with the greatest ease. The spraying of solutions is a common agricultural practice, hence the spraying of mulch in many cases will not require large capital investment for growers, but rather a repurposing, or modification of existing equipment. A further advantage provided by sprayable BPM is that their application rate is inherently customisable: their application rate can be tailored to suit the needs of the particular context under which they are being used. This advantage does come with a caveat: it is difficult to obtain uniform film thickness via spraying, as was demonstrated throughout the work presented in this thesis.

The work presented in this thesis has all been conducted on a novel polymer (plastic) formulation developed by Adhikari et al.[18] to be a sprayable, BPM. It is an aqueous suspension of a polycaprolactone (PCL) based polyester-urethane-urea (PEU) with methylcellulose as a biodegradable viscosity modifier, and typically carbon black as a biodegradable pigment. Although the work focuses on the PEU, the information sought and the methodologies used were carefully selected to serve as a template for the evaluation of future BPM of any kind. The work contained within this thesis is not important because of the precise plastic studied, although should this plastic be commercialised then the findings will be of significant value. The work is important because it represents a holistic approach to understanding a new BPM.

It is of note that the work presented borrows from many fields of study, and was a true interdisciplinary endeavour. Polymer chemistry, soil science, molecular biology, and crop science were all crucial components to the work presented here, and indeed each field should be considered when evaluating any novel biodegradable plastic intended for widespread use.

Considerations and Conclusions

There are several important pieces of information that should be understood before a new BPM is used on a large scale. Firstly, the efficacy of the new material in terms of water savings efficiency and crop yield should be understood, and ideally under a range of environmental conditions or

cropping systems. If the BPM is not effective, there is no need to pursue further testing or use until its performance is improved.

Equally important to understanding the efficacy of a new BPM, is understanding its degradation rate under a variety of environmental conditions and the products resulting from its degradation. If there are particular conditions that cause too rapid (which could limit efficacy) or too slow (which could cause accumulation of the BPM) degradation then this should be understood, and accounted for when determining the application loading of a BPM. Of course, altering the loading of a BPM is only possible with sprayable formulations, as preformed films are constrained to the specific thickness at which they are manufactured.

Once the efficacy and the degradation rate of a BPM are established under a range of conditions, other impacts of its use should be considered. Because BPM are large molecules, they will not mineralise into CO_2, H_2O and small inorganic molecules in a single step, but rather will break down into degradation intermediates first. Understanding the identity, and importantly, the toxicity of the intermediates is important. If the degradation intermediates are toxic to plant life, microbial life or animal life then the use of the BPM should be seriously reconsidered.

Finally, the effect of the application of the BPM to the soil microbial community should be understood. One aspect of soil health is a diverse microbial community[19,20]. A diverse microbial community is more adaptable to changing conditions, and has a greater chance for functional redundancy. For example, if a change in climate causes some microbial taxa to die off, a more diverse soil microbial community will have a greater chance that other taxa can provide the same functional service previously provided by the now absent taxa. Particular attention should be paid to the impact of a BPM on plant growth-promoting microbes (PGPM), a subset of microorganisms that provide services that enhance plant growth in some manner. If a BPM is impairing the soils ability to maintain a large community of PGPM, then its use should be reconsidered.

The standard *Plastics - Biodegradable mulch films for use in agriculture and horticulture - Requirements and test methods* recently created by the European Committee for Standardization[21] is

excellent in its coverage of the important aspects of biodegradation and ecotoxicity that need to be investigated on any novel BPM. By design, it falls short in discussing the efficacy of a novel BPM, and it may need to be expanded substantially in the future to accommodate sprayable BPM which are absent from its current iteration.

The objectives of the work presented in this thesis were to 1) design a set of studies that can adequately measure the above outlined considerations, and 2) evaluate the sprayable PEU mulch based on those studies. The studies and conclusions were as follows:

- Chapter 2 was a full factorial greenhouse study investigating the impact of sunlight, soil moisture content, soil type, and polymer pigmentation on its degradation rate and water savings efficacy. It was found that soil type and moisture level were important in controlling the degradation rate, while sunlight and polymer pigmentation did not change the degradation rate in a detectable way. The PEU mulch's water savings efficacy ranged from 30-50% compared to no mulch.
- Chapter 3 was a follow-up study to Chapter 2, to understand what factors within 'soil type' were responsible for controlling the PEU degradation rate. This was coupled with an outdoor soil degradation study to determine how different environmental factors and PEU loadings would affect degradation. pH was the most important factor controlling the rate of PEU degradation, and the soil microbial community composition played a smaller role in controlling the rate of PEU degradation.
- Chapter 4 was a liquid-chromatography mass-spectrometry (LCMS) study to identify the degradation intermediates formed during abiotic hydrolysis and in-soil degradation of the PEU mulch. The primary degradation products identified in sterile conditions were monomers and oligomers of PCL, but interestingly they were not present at all when the PEU was degraded in the soil environment, which indicated their consumption or modification by soil microbes. Two of the identified degradation products, and total PEU hydrolysate was used in a seed germination study to determine if they presented any toxicity

towards plants. At high concentrations (equivalent to half of a typical PEU application instantaneously degrading into its constituents and being present in proximity of germinating seeds), the degradation products did inhibit seed germination.

- Chapter 5 focused on the impact of PEU mulching on the soil microbial community. PEU was applied to soils in a cropping system (tomatoes) and at various times the soil and PEU film was sampled, the metagenome sequenced, and soil enzyme activity assays were conducted. It was found that the PEU mulch had no detrimental effect on the soil microbial community composition nor on the soil enzyme activities measured. Importantly, the presence of PEU mulch increased the relative abundance of several PGPM, especially plant-growth promoting fungi (PGPF).
- Chapter 6 was a comparative plant growth study between the PEU mulch and two commercially available PE mulches, one being standard non-degradable PE and one being an oxo-degradable PE. No detrimental effects to plant growth nor to soil health were observed, and the PEU proved to be slightly less effective at conserving water than the PE mulches, but more effective than no mulching treatment.

Limitations

The primary limitations in this work are born from variability in soils, and the scale of the studies conducted.

The term ‘soil’ encompasses a complex mixture of interrelated components. Mineralogy, particle size distribution, soil organic matter, inorganic nutrients, the presence of ionic species, pore size and the soil microbial community all vary individually both in time and in space. Add to that the different environments soils may exist and land management practices available and the variability becomes substantial. It is of course not possible to exhaustively study how any BPM performs and degrades in all soils, and it is difficult to perform such testing on even a majority of soils because of time, budget, and accessibility constraints. The methodologies used here can be generalised to any soil type, but not the specific results.

The second limitation is with the scale of the studies used. Most of the work done here was under controlled conditions and all of the soil experiments were conducted in pots. This is of value, but it needs to be validated with true field studies. Due to the cost associated with executing a field study there was no opportunity for such a study within this thesis. This limitation is particularly evident in the plant growth study (Chapter 6), where there was no observed increase in the tomato yield in pots treated with PE mulch, despite ample literature evidence that there should have been[6,7]. Two possible explanations for this behaviour could be that the plants had sufficient resources regardless of treatment type, or because the plants' productivity was constrained due to the size of the pots being used.

One other key limitation to this work was that no direct evidence of biodegradation was collected. There was plenty of indirect evidence of biodegradation presented:

- Enhanced CO_2 emissions on PEU treated soils (Chapter 2)
- Rapid and extensive reduction in PEU molecular weight (Chapter 2, Chapter 3, Chapter 4, and Chapter 6)
- A lack of detectable residual PEU film in a number of replicates after 12 months on-soil degradation in a cropping system (Chapter 6)
- The primary degradation intermediates in a sterile environment were not detectable in a soil medium (Chapter 4)

Relating the findings of this thesis, with these limitation in mind, to the hypothesis of this thesis, the PEU was effective at conserving water and did not hinder plant growth while maintaining a healthy soil capable of supporting a diverse microbial community with an elevated abundance of PGPM. It was inconclusive whether the PEU underwent complete biodegradation; although there was strong evidence to suggest it did, and a brief follow-up study should confirm this evidence.

Taking a broader view of the PEU's compliance with standards, in particular the standard *Plastics – Biodegradable mulch films for use in agriculture and horticulture – Requirements and test methods*, further standard testing would need to be executed to ensure it meets all

requirements.[21] To speculate on its compliance, based on the data presented in this thesis, and with the caveat that a sprayable BPM falls outside of the scope of the standard in its current form, it seems likely that it would meet the required 90% conversion of organic matter to CO_2 in 24 months' time, but it may fail the seed germination ecotoxicity test outlined in the standard.

Future Directions

From the discussed limitations, arise several avenues for future study that will be especially important if the PEU is commercialised. The execution of a true, split-plot field trial to determine the PEU mulch's effect on crop yield, water conservation, and true environmental degradation would be a crucial next step for this technology. With sufficient funds, this could be carried out on several soil types in order to add understanding of the PEU's performance and degradation on more soils.

As a follow up to the work investigating the impact of the PEU mulch on the soil microbial community (Chapter 5) it would be interesting to carry out a plating study to discover which specific microbial species are capable of directly degrading and utilizing the PEU and its degradation intermediates as a carbon and energy source[22].

The synthesis of several of the identified degradation intermediates, and their inoculation into a closed soil environment to determine if microbes can consume them would make an interesting study and important follow-up study. This study would involve CO_2 and volatile organic emissions capture, LCMS analysis of the soil, and soil total carbon analysis to determine the ultimate fate of the degradation intermediates. This would close the biodegradation picture because it has already been shown that the PEU films' molecular weight reduces extensively over a period of 5 months (Chapter 2), and that low molecular weight degradation intermediates form (Chapter 4). So demonstrating that these low molecular weight intermediates can be metabolized by microbes would show the complete process from large macromolecule to increasingly small oligomeric and monomeric components, to complete mineralisation.

Finally, it would be of interest to use a ^{14}C radiolabelled PEU mulch to track the environmental transportation potential for the PEU carbon. This could be accomplished using a biometer flask[23,24] in a closed system with a growing plant. The CO_2 and volatile emissions would be captured, the soil leachate would be collected, and the soil and plant tissue would be sampled. The radioactivity in each set of samples would be measured to learn the environmental fate of the PEU and its degradation products.

Final Remarks

To conclude, the work in this thesis represents a holistic investigation into the performance, degradation, and environmental impact of a BPM. The PEU mulch studied here certainly shows promise in terms of its degradation, performance and role in shaping the soil microbial community to support a larger abundance of PGPM. There are a few follow-up studies, in particular a field study, that would be useful to carry out before the PEU mulch is used commercially. Ultimately, a techno-economic assessment of this PEU should be carried out, but that was outside of the scope of this thesis.

As society moves towards a greener economy with more sustainable practices, the ideal BPM would be sprayable and perform similarly to the PEU studied here, but would be in large part composed of renewable feedstocks sourced from what are now considered to be waste streams.

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Appendices

Appendix 1. Environmental Degradation and Efficacy of a Sprayable, Biodegradable Polymeric Mulch Supplementary Material

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Number of pages: 7

Number of Figures: 5

Number of Tables: 1

Table S1. Additional soil characteristics.

	Echuca	*Seville*	*Ouyen*
Ca, mg/kg	1675	268	259
Mg, mg/kg	524	190	66
Na, mg/kg	140	592	56
K, mg/kg	58	28	56
P, mg/kg	123	17	26
NO3, mg/kg	26.1	5.1	4.3
NH3, mg/kg	3.7	6.2	2.7
Electrical Conductivity, dS/m	0.17	0.43	0.06
Total C, %	1.12	3.84	0.10
Total N, %	0.12	0.22	0.03

Greenhouse Gases

Soil greenhouse gas emissions were collected at each of the 5 sampling times using a static chamber method, in which a chamber of known volume and basal area (152 cm^3 and 15.2 cm^2, respectively) is pressed into the soil surface (in this case soil and polymer surface) and allowed to accumulate emitted gases for 20 minutes. The accumulated gases were then extracted from the chamber with an airtight syringe and introduced into pre-evacuated 12 mL Exetainer ® vials through a septum. This process is visualized in Figure S1. Vials were then analyzed for CO_2, N_2O, and CH_4 on an Agilent 7890A gas chromatograph (GC), fitted with a Gerstel MultiPurpose Sampler (MPS) autosampler.

Gel Permeation Chromatography (GPC)

Gel permeation chromatography was performed on a Shimadzu system equipped with a CMB-20A controller system, an SIL-20A HT autosampler, an LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, an RDI-10A refractive index detector, and 4X Waters Styragel columns (HT2, HT3, HT4, and HT5, each 300 mm × 7.8 mm^2, providing an effective molar mass range of 100-4 × 10^6). Samples were dissolved in DMAc containing 4.34 g L^{-1} LiBr, at a concentration of 1-2 mg mL^{-1}. The columns were calibrated with low dispersity polystyrene (PS) standards ranging from 575 – 3,242,000 g mol^{-1}. DMAc containing 4.34 g L^{-1} LiBr was used as an eluent at a 1 mL min^{-1} flow rate and 80 °C. M_n and M_w were evaluated using Shimadzu LC Solution software.

Scanning Electron Microscopy

SEM micrographs were obtained using the secondary electron detector in a ThermoScientific FEI Quanta 3D FEGSEM. Polymer samples from time points T0, and T4 were collected from outside the area designated by the dotted line in Figure 11, with care being taken to gently loosen as much soil as possible. T0 samples, which had not been degraded and therefore were very sturdy and elastic,

were placed in deionized water and sonicated for half an hour, gently brushed with a spatula to remove any loose soil, and dried in a vacuum oven overnight. Samples collected at T4 were substantially degraded, and had lost a lot of their elasticity and structural integrity and could not survive sonication, so were simply gently brushed with a spatula to loosen soil particles and then sprayed with compressed N2 to blow off any loosely bound particles and allowed to air dry. All samples were coated with a thin layer of Au prior to imaging.

The SEM imaging conditions also had to be altered based on how thoroughly the polymer was degraded. Environmental SEM (ESEM), a technique where a small amount of water vapour is present in the imaging chamber in order to reduce sample charging effects and to observe the sample in its natural state, was used to image samples recovered at T0. ESEM imaging conditions are as follows: 12 pA beam current, 10 kV accelerating voltage, 800 Pa chamber pressure, ~5 mm working distance.

For degraded samples (T4) ESEM was not effective in generating high quality images, so instead low vacuum mode was used, in which a lesser amount of water vapour than in ESEM is present in the imaging chamber. Low vacuum SEM imaging conditions were as follows: 6 nA beam current, 10 kV accelerating voltage, 50 Pa chamber pressure, ~5 mm working distance.

Thermogravimetric Analysis (TGA)

All thermogravimetric analysis was performed on a Mettler Toledo TGA 2 STARe System. All runs were performed from 25 °C to 600 °C with a heat ramp of 10 °C min^{-1} under N_2 gas. Determination of thermal degradation onset temperatures was accomplished using the Mettler Toledo STARe software.

CHN Analysis of Soil

Soil samples were analyzed for total carbon, hydrogen and nitrogen at the beginning of the study and after its conclusion using a Perkin Elmer Series II CHNS/O 2400 system in CHN mode. The top

five cm of soil from polymer treated samples, with the residual polymer film removed, was collected and analyzed at the end of the study.

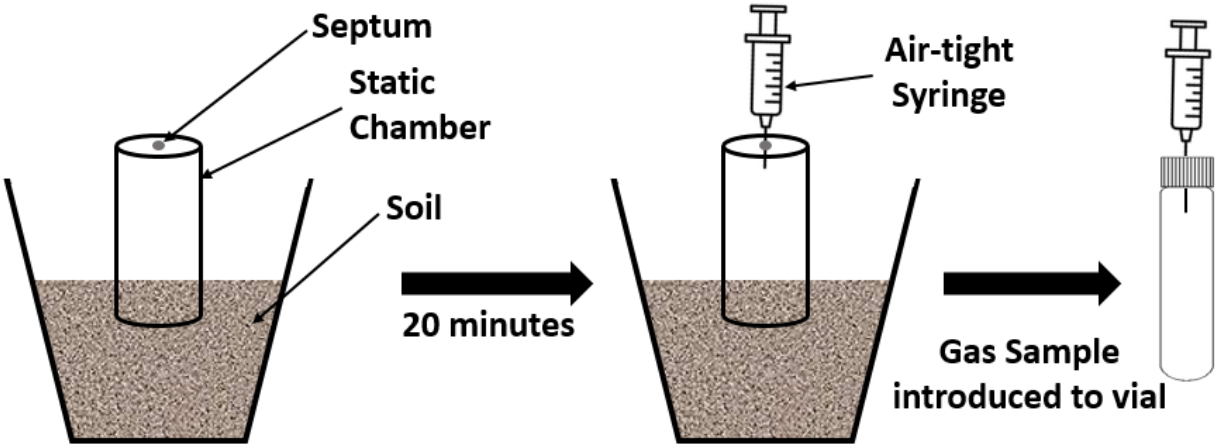

Figure S1. Greenhouse gas sampling procedure.

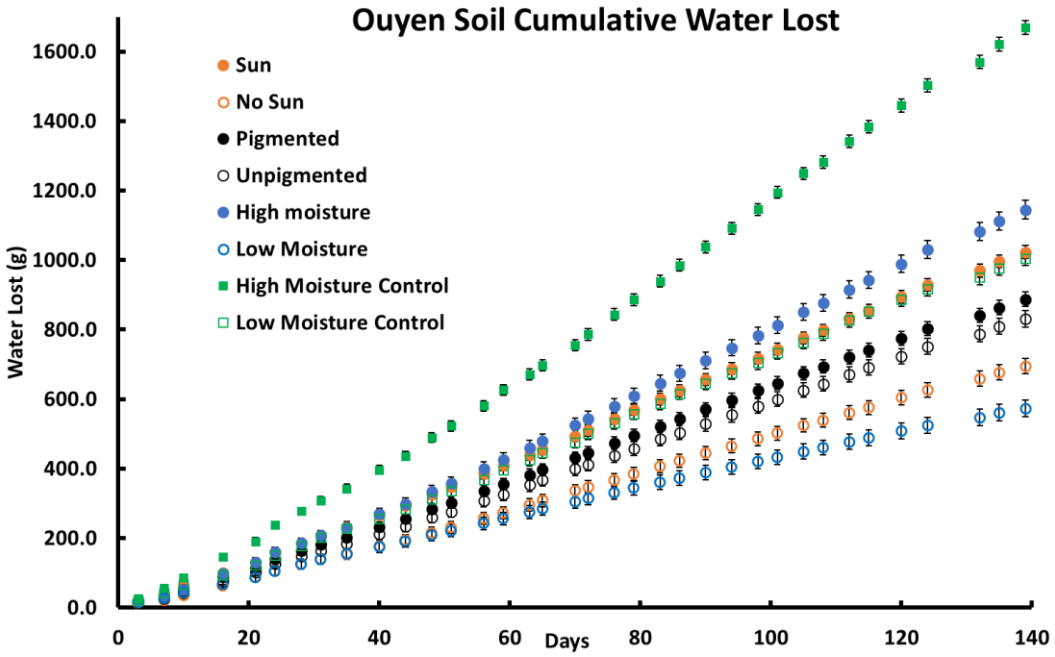

Figure S2. Cumulative water loss over time to Ouyen soils based on environmental condition. Data points are means ± standard deviation.

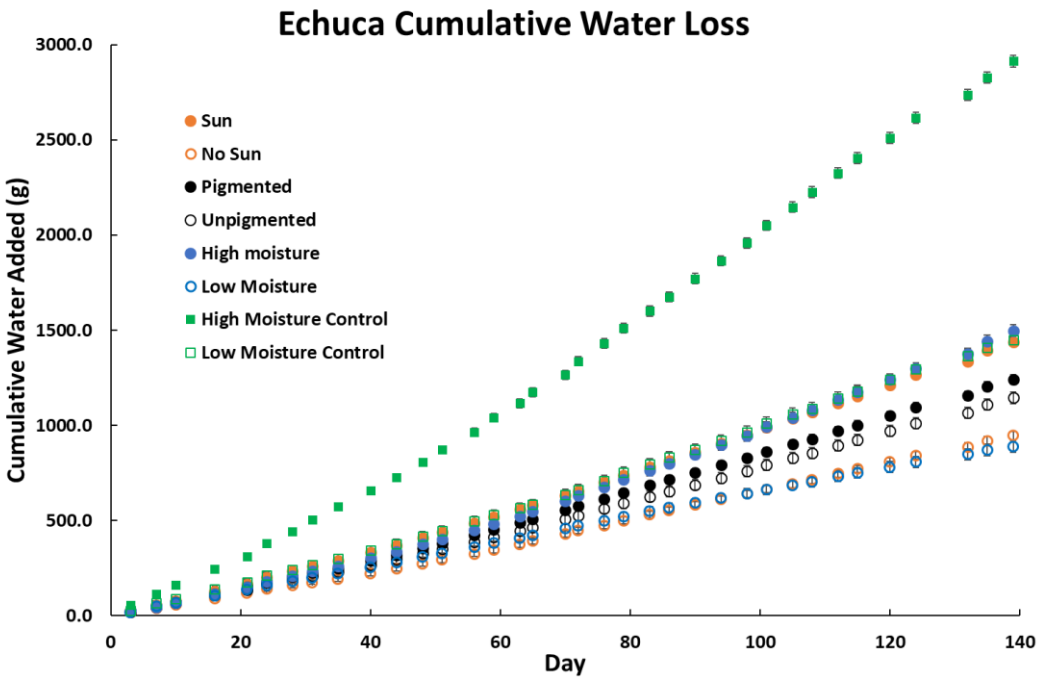

Figure S3. Cumulative water loss over time to Echuca soils based on environmental condition. Data points are means ± standard deviation.

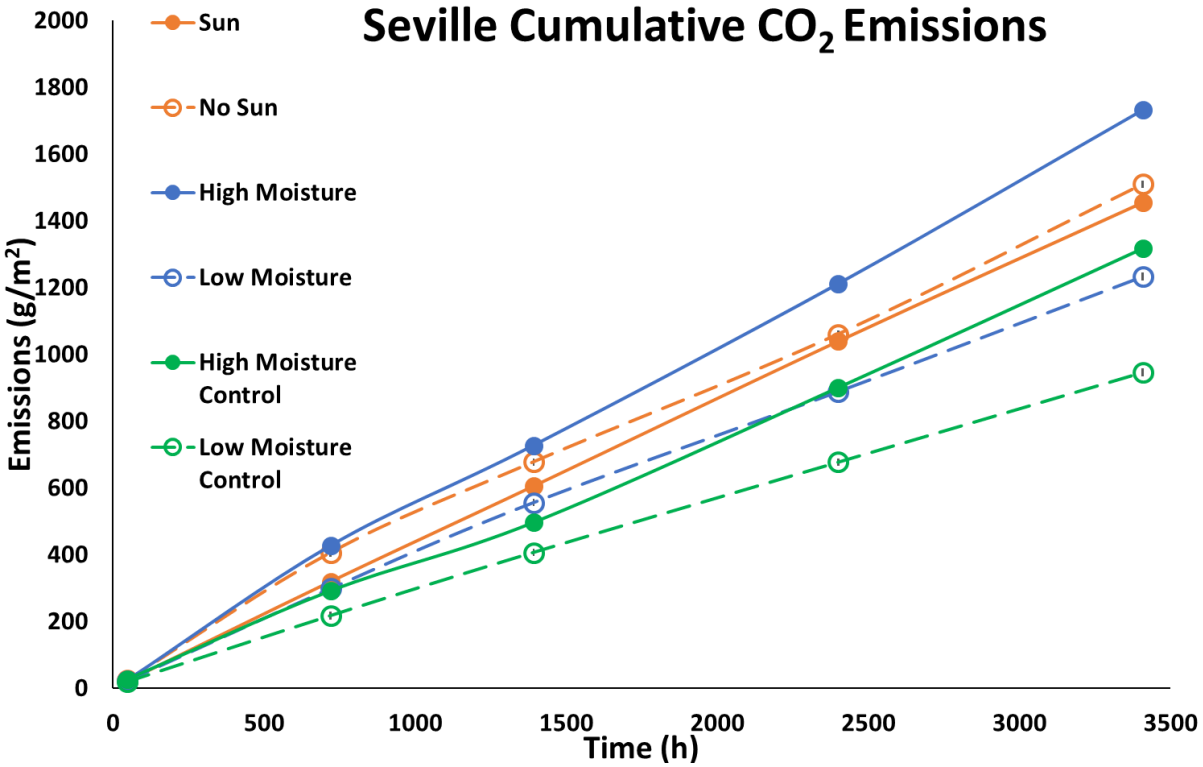

Figure S4. Cumulative CO_2 emissions from Seville soils based on environmental condition.

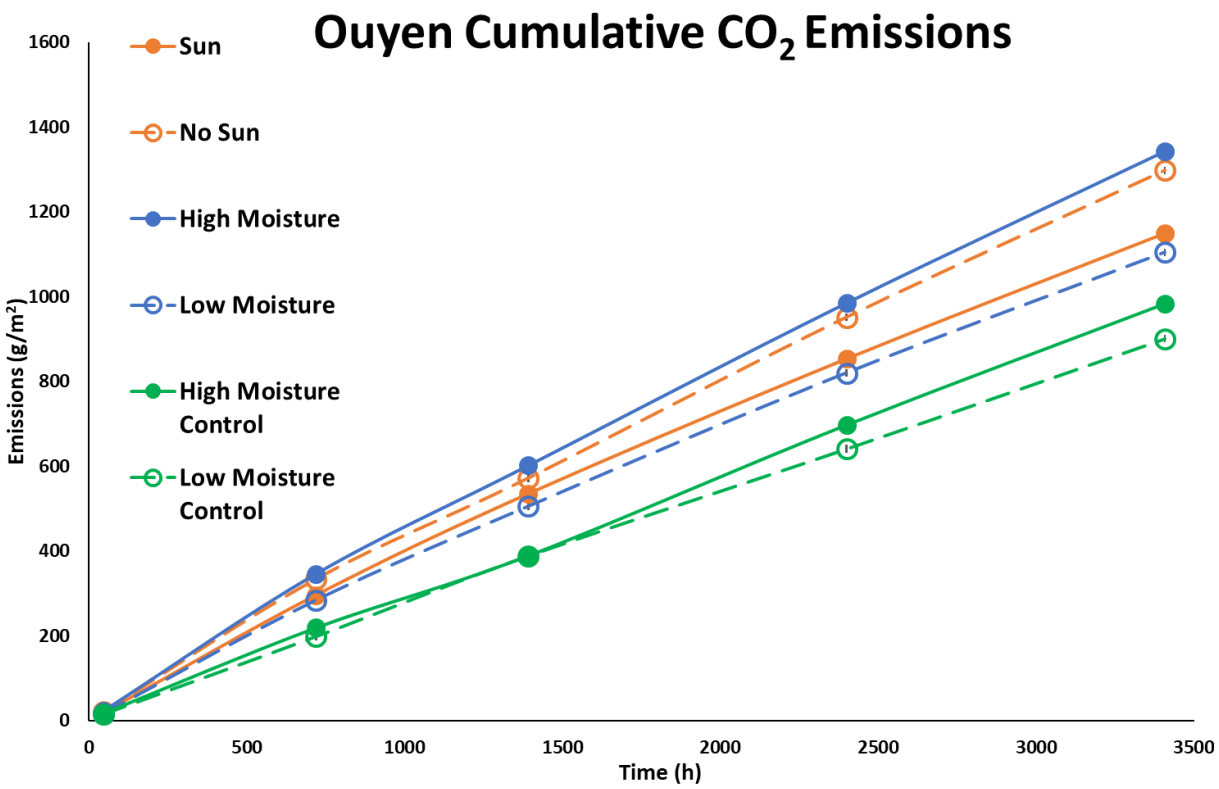

Figure S5. Cumulative CO_2 emissions from Ouyen soils based on environmental condition.

Appendix 2. LC-MS analysis of the degradation products of a sprayable, biodegradable polyester-urethane-urea Supplementary Material

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Number of pages: 3

Number of Figures: 0

Number of Tables: 2

Experimental Section

Polymer Synthesis

The polymer used in this study was synthesized using the two step method as described by Adhikari et al.[24] In brief, a PCL based polyester-urethane pre-polymer was synthesized by reacting anhydrous PCL diol and IPDI under a N_2 atmosphere. DMPA was then added to the reaction mixture, followed by an EDA chain extender. The reaction mixture was left to react until all of the isocyanate had reacted (as confirmed by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy, ATR-FTIR), giving a final M_w and M_n of 120 kDa and 40 kDa respectively (as measured by GPC).

The final polymer formulation contained 20 wt% polymer solids. Methyl cellulose and carbon black were added to the final mixture to adjust the viscosity and provide pigmentation.

Gel Permeation Chromatography (GPC)

Recovered polymer was analyzed by gel permeation chromatography (GPC) to determine molecular weight reduction of the polymer itself. GPC was performed on a Shimadzu system equipped with a CMB-20A controller system, an SIL-20A HT autosampler, an LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, an RDI-10A refractive index detector, and 4X Waters Styragel columns (HT2, HT3, HT4, and HT5, each 300 mm × 7.8 mm^2, providing an effective molar mass range of 100-4 × 10^6). Samples were dissolved in dimethylacetamide (DMAc) containing 4.34 g L^{-1} LiBr, at a concentration of 1-2 mg mL^{-1}. The columns were calibrated with low dispersity polystyrene (PS) standards ranging from 575 – 3,242,000 g mol^{-1}. DMAc containing 4.34 g L^{-1} LiBr was used as an eluent at a 1 mL min^{-1} flow rate and 80 °C. M_n and M_w were evaluated using Shimadzu LC Solution software.

Scanning Electron Microscopy (SEM)

SEM micrographs were obtained using a ThermoScientific FEI Quanta 3D FEGSEM operated under low vacuum mode (to help diminish sample charging) with the following parameters: chamber pressure 100 Pa, 20 kV accelerating voltage, 0.85 nA beam current, and 1 µs dwell time.

Results and Discussion

Table S2. Toxicity predictions from TEST software. BAF is bioaccumulation factor, DT is developmental toxicity, Mut is mutagenicity, and ROLD50 is rat oral LD50

Chemical	BAF	Certainty	DT	Certainty	Mut	Certainty	ROLD50 (mg/kg)	Certainty
6HHA	1.25	certain	Toxicant	semi-certain	Negative	certain	3430	semi-certain
6HHA Dimer	2.34	certain	Non-toxicant	semi-certain	Negative	certain	9451	certain
6HHA Trimer	1.1	certain	Non-toxicant	semi-certain	Negative	certain	5916	certain
6HHA Tetramer	0.93	semi-certain	Toxicant	uncertain	Negative	certain	9237	certain

IPDA	9.28	uncertain	Toxicant	uncertain	Negative	certain[a]	1156	certain
IPDA-6HHA	13.7	uncertain	Toxicant	uncertain	Negative	certain	1857	certain
SDS	0	N/A	Toxicant	semi-certain	Negative	certain	1594	certain[a]
TEA	1.57	certain[a]	Non-toxicant	uncertain	Negative	certain	461	certain[a]
IPDA-EDA	4.43	uncertain	Toxicant	uncertain	Negative	uncertain	940	certain
6HHA-IPDA-EDA	0.34	uncertain	toxicant	uncertain	Negative	certain	1928	uncertain
6HHA-IPDA-6HHA	1.35	uncertain	toxicant	uncertain	Negative	certain	73.36	certain
IPDA-6HHA demethylated twice	4.25	uncertain	Toxicant	uncertain	Negative	certain	1206	certain

[a] Indicates predictions validated by experimental evidence

Table S3. Toxicity predictions from OSIRIS software.

Chemical	Mutagenicity	Tumorigenicity	Irritant	Reproductive Effect
6HHA	No	No	Yes	No
6HHA Dimer	No	No	Yes	No
6HHA Trimer	No	No	Yes	No
6HHA Tetramer	No	No	Yes	No
IPDA	No	No	Yes	No
IPDA-6HHA	No	No	Yes	No
SDS	No	No	No	No
TEA	Yes[a]	Yes[a]	Yes[a]	No
IPDA-EDA	No	No	Yes	No
6HHA-IPDA-EDA	No	No	No	No
6HHA-IPDA-6HHA	No	No	No	No
IPDA-6HHA demethylated twice	No	No	No	No

[a] Indicates predictions validated by experimental evidence.